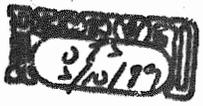


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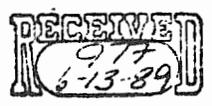


CHEMICAL MANUFACTURERS ASSOCIATION

Geraldine V. Cox, Ph.D.
Vice President-Technical Director

*FYI-OTS-0589-0692 INITIAL
Sequence A*

May 5, 1989



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The Honorable William K. Reilly
Administrator
Environmental Protection Agency
401 M Street, SW, Room W1200
Washington, DC 20460

Dear Mr. Reilly:

The Chemical Manufacturers Association makes public all final reports developed from research projects that it administers. The following reports, recently completed, are enclosed.

ETHYLENE GLYCOL: COMPARISON OF PHARMACOKINETICS AND MATERIAL BALANCE FOLLOWING SINGLE INTRAVENOUS, ORAL AND CUTANEOUS ADMINISTRATIONS TO MALE AND FEMALE SPRAGUE-DAWLEY RATS

DEVELOPMENTAL TOXICITY EVALUATION OF ETHYLENE GLYCOL ADMINISTERED BY GAVAGE TO CD-1 MICE: DETERMINATION OF A "NO OBSERVABLE EFFECT LEVEL" (NOEL)

Sincerely yours,

Geraldine V. Cox

Enclosures

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OTS
OFFICE

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BUSHY RUN RESEARCH CENTER

R.D. 4, Mellon Road, Export, Pennsylvania 15632

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Project Report 51-543

TITLE: Ethylene Glycol: Comparison of Pharmacokinetics and Material Balance Following Single Intravenous, Oral and Cutaneous Administrations to Male and Female Sprague-Dawley Rats

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SPONSOR: Ethylene Glycol Program
Chemical Manufacturers Association
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CMA REFERENCE NUMBER: EG-3.0-PK-BRRC

DATE: March 24, 1989

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Project Report 51-543
BRRC Number 86-81-70704
88 Pages
March 24, 1989

Ethylene Glycol: Comparison of Pharmacokinetics
and Material Balance Following Single Intravenous,
Oral and Cutaneous Administrations to Male and Female Sprague-Dawley Rats

Sponsor: Chemical Manufacturers Association

* * * * *

SUMMARY

The material balance determinations of [1,2-¹⁴C]-labeled Ethylene Glycol (EG) have been conducted in the Sprague-Dawley rat following intravenous, peroral and percutaneous doses of 10 or 1000 mg EG/kg body weight. Expired ¹⁴CO₂ was the major metabolite and urine was the secondary route of excretion for all three routes of administration at the 10 mg EG/kg dose level. Similar recoveries were obtained for both sexes following intravenous or peroral doses. In comparison with the 10 mg/kg dose, a dose-dependent shift in the proportion of radioactivity found in urine and ¹⁴CO₂ was observed following a dose of 1000 mg EG/kg by the intravenous and peroral routes. At the high dose, urine was the major excretion route for the radioactivity, while ¹⁴CO₂ was produced at a much-reduced level. Further definition of the dose-dependent change in excretion routes was attempted by administering additional peroral doses of 400, 600 and 800 mg/kg to both sexes. There was a clear progression across dose levels from high ¹⁴CO₂ elimination levels at the low dose to reduced levels at the high dose in both sexes. Changes in urinary ¹⁴C excretion with increasing dose were not as clearly defined due to the inherent individual variation with rats for inconsistent interval urinary elimination. This dose-dependent pathway shift was not observed following the 1000 mg/kg percutaneous doses of either undiluted or 50% (w/w) aqueous EG. Instead, similar radioactivity levels to those for the low intravenous and low percutaneous (undiluted) dose studies were found for high dose animals, indicating that much less of the applied EG dose (approximately 22-36%) had crossed the skin than was absorbed following the peroral 1000 mg EG/kg dose (approximately 83% of the administered dose). ¹⁴CO₂ accounted for the largest recovery fraction of radioactivity for all percutaneous dose groups and urine was the secondary elimination pathway. For all three routes of administration, individual tissue recoveries did not indicate any accumulation of ¹⁴C in a particular organ or tissue following single doses of EG.

Pharmacokinetic data for both unchanged EG and the total radioactivity in plasma demonstrated marked differences in EG plasma profiles vs. those for its metabolites. The unchanged EG data for the intravenous and peroral routes demonstrated a first-order kinetic behavior (dose-linear) between the 10 and 1000 mg/kg dose levels for the disappearance of EG from the plasma. This was evident from AUC_{0-∞} values which were roughly dose-proportional. Following intravenous administration, dose-independent relationships were seen in the values obtained for the total clearance of EG, mean residence time, apparent volume of distribution at steady state, the terminal half-life, the total amount of EG excreted in the urine to 24 hr post-dosing, and the renal and metabolic clearance values. These parameters all demonstrated a first-order plasma time-course which was not apparent from the excreta profiles, for which a dose-dependent relationship existed (total urinary ¹⁴C and ¹⁴CO₂ were not constant between dose levels). Increases in urinary ¹⁴C-glycolate were also observed when the intravenous or peroral doses were increased from 10 to 1000 mg EG/kg, indicating that metabolism makes a substantial contribution to the terminal disposition phase of the plasma disappearance curves for this higher dose level. The percutaneous pharmacokinetic data suggested a different absorption pattern from the considerable amount (29-36%) of an undiluted EG dose which penetrated skin as unchanged EG when left in place over the course of an occluded 96-hr exposure. Slightly different results were observed for shorter exposure times, as approximately 9-13% of the applied radioactivity from undiluted EG doses placed on the skin was absorbed by the end of the first 6 hr after dosing. Also, ¹⁴C continued to enter the plasma despite removal of any unabsorbed dose from the surface of the skin at the end of this 6-hr period. In contrast to the results for undiluted EG doses, a 50% aqueous dose of EG applied to skin for 6 hr was not appreciably absorbed (1.1-1.3% of the dose) before it was removed from the skin. In summary, we have shown that ethylene glycol by three different routes demonstrates apparent first-order pharmacokinetic behavior for the disposition in and the elimination from the plasma but that dose-dependent changes occur for the elimination of metabolites in the urine and as ¹⁴CO₂ after single doses for the intravenous and peroral, but not the percutaneous, routes.

INTRODUCTION

Ethylene glycol is an important industrial chemical which is commercially produced by the aqueous hydrolysis of ethylene oxide. Its principal uses are as a component in antifreeze for cooling and heating systems, in hydraulic brake fluids, as a solvent for boric acid and borates in electrolytic condensers, as a diluent and solvent for paint formulations, and in the production of synthetic ester materials in the plastics and fabric industries. It has also been used as a solvent for pharmaceuticals, food additives, cosmetics, inks and lacquers (Casaratt and Doull, 1986). Limited information is available on the fate of EG in mammalian species. Marshall (1979; 1982) has investigated the pharmacokinetics of EG in Fischer 344 rats following intravenous administration. Similar studies have been conducted in other mammals (Gessner *et al.*, 1961; McChesney *et al.*, 1971; Martis *et al.*, 1982). These reports indicated that a substantial portion of the dose is eliminated as ¹⁴CO₂. Only a few studies have evaluated the fate of EG in rats (Gessner, *et al.*, 1960, 1961, strain unspecified; McChesney, *et al.*, 1971, strain unspecified; Marshall, 1979; 1982) and none of these studies were

conducted via the percutaneous route. It was therefore the primary purpose of the present study to investigate the fate of EG by comparing the intravenous, oral and percutaneous routes in separate material balance and pharmacokinetic studies.

OBJECTIVE

The overall objective of this investigation was to determine the radioactive material balance and pharmacokinetics of ^{14}C -labeled ethylene glycol in Sprague-Dawley rats following intravenous, oral and cutaneous administration. The tissue distribution and material balance were determined separately because of the substantial generation of carbon dioxide as a metabolite of ethylene glycol. The information from this investigation should provide the basis for a more definitive and quantitative interpretation of other general toxicological studies with ethylene glycol, particularly those conducted with Sprague-Dawley rats. In general, this data should also help in the evaluation of potential human health effects which may be produced by this chemical when used in situations involving different major routes of exposure.

METHODS

Test Animals

Adult male Sprague-Dawley rats weighing approximately 275-350 g (10-11 weeks old) and females weighing 180-250 g (10-11 weeks old) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Upon arrival at the laboratory, the rats were examined by the BRRC clinical veterinarian and found to be in generally good health. The animals were acclimated to the laboratory environment for at least 5 days prior to the administration of dose and each animal was uniquely identified by toe clipping. Prior to use in the study, animals were housed in groups of 3-4 in plastic shoe box cages. Those animals selected for study were transferred to individual Roth-type metabolism cages, designed for the separate collection of urine, feces and expired CO_2 . The animals were acclimated to the experimental environment for approximately two days prior to test material administration. Animals were maintained throughout the study in rooms which were continuously monitored for humidity and temperature and which had a 12-hour photoperiod and approximately 10-15 air changes per hour. Municipal drinking water (Municipal Authority of Westmoreland County, Greensburg, PA) and pelleted food (Agway Certified Rodent Chow) were available ad libitum throughout the study.

Test Material

Two separate lots of [1,2- ^{14}C]-Ethylene Glycol were obtained from Sigma Chemical Company (St. Louis, MO). The first was purchased for the material balance studies (lot no. 037F9207) and had a specific activity of 3.6 mCi/mmol and a radiopurity of 99.2%. The second lot was purchased for the pharmacokinetic studies (lot no. 077F9209) and had a specific activity of 6.0 mCi/mmol and a radiopurity of > 98%. Unlabeled EG (lot no. PG-35227, Polyester Grade) was obtained with a purity of 100.0% (by wt. %) from the Union Carbide Ethylene Oxide/Glycol Division in Hahnville, LA. The dosing

solutions used in these studies contained both ^{14}C -EG and unlabeled EG and were prepared in amounts sufficient to produce 0.5% and 50% (w/w) solutions. These solutions were prepared as follows: 1) dissolved in 0.9% saline (CAS Nos.: NaCl, 7647-14-5; and water, 7732-18-5) for intravenous studies; 2) dissolved in water for oral studies; and 3) dissolved in water (or applied as undiluted EG) for percutaneous studies. Target doses of 10 and 1000 mg EG/kg body weight were chosen for both the material balance and pharmacokinetic studies with a dose volume of about 2.0 ml/kg. Dosing solution analysis was conducted by gas chromatography for quantification of chemical concentration; the dosing solution analysis results are contained in Appendix C.

**Description of
Physico-Chemical
Properties:**

CAS No.:	107-21-1
Chemical Formula:	$\text{C}_2\text{H}_6\text{O}_2$
RTECS No.:	KW2975000
Synonyms:	1,2-Ethanediol; Ethane-1,2-diol; Ethylene Alcohol; Ethylene Dihydrate; Glycol Alcohol; Monoethylene Glycol: Tebcol
Physical State:	Slightly viscous liquid; very hygroscopic
Molecular Weight:	62.07
Specific Gravity:	1.1135 at 20°C
Boiling Point:	197.6°C (760 mmHg)
Vapor Pressure:	0.06 mmHg at 20°C
Viscosity:	21 centipoise at 20°C
Flash Point:	240°F
Solubility:	Water: completely soluble at 25°C

Dosing Solution Preparation and Test Material Delivery

Intravenous Administration

The target doses (10 and 1000 mg/kg) selected for ^{14}C -EG intravenous administration were administered in physiological (0.9%) saline solution with a target volume of about 2.0 ml/kg. The dose was delivered via the tail vein for the material balance studies and via an indwelling jugular cannula for the pharmacokinetics determinations which was surgically implanted about 48 hr prior to dose administration. A minimum of 5 μCi of the radioactivity was given to each animal for both dose groups and the ^{14}C concentration was checked prior to and immediately following dose administration by liquid scintillation counting of replicate aliquots. The EG concentrations were confirmed by GC analysis after dosing and the amount of individual dose delivered was determined gravimetrically (weighing the syringe before and after dosing) at the time of administration.

Peroral Administration

The target doses for ^{14}C -EG gavage administration were the same as those selected for the intravenous route injections (10 and 1000 mg/kg). Each dose was delivered in water with a target volume of about 2.0 ml/kg. A minimum of

5-10 μCi of the radioactivity in both dose groups was given to each animal. The ^{14}C concentration was confirmed prior to and immediately following dosing and the amount of individual dose delivered was determined gravimetrically (weighing the syringe before and after dosing) at the time of administration. The EG concentrations in the dosing solutions were confirmed analytically by GC following dose administration.

Percutaneous Application

For the cutaneous portion of the study, the ^{14}C -EG dosing solutions were prepared as either: 1) a 50% water solution administered in a target volume of 2.0 ml/kg; or 2) as undiluted material (10 and 1000 mg/kg). The dose levels selected for percutaneous administration were the same as those selected for the intravenous route (10 and 1000 mg/kg), with an additional dose level administered as a 50% aqueous solution (at 1000 mg/kg) to investigate cutaneous exposure to an automotive antifreeze mixture. Sufficient radioactivity was targeted for this dosing solution to deliver about 15-25 $\mu\text{Ci}/0.2$ ml of the tracer to a 200 g animal. The dose solution was applied to approximately a 1 cm^2 area in the interscapular region of the back using a syringe. The volume of the dose delivered was determined gravimetrically by weighing the syringe before and after delivery. The application site was occluded with polyethylene film which was held in place with waterproof adhesive tape to minimize oral ingestion from grooming and maximize skin penetration. Permeability of polyethylene to EG over a 48-hr period was examined prior to use. This film/tape was covered with a Spandex[®]-type rodent jacket produced by a local supplier and the test material was left in place for 48 hours during this probe study.

Preliminary Studies: Tail Vein vs. Jugular Vein Cannula Trial for Administration of IV Pharmacokinetic Doses

The route of injection for intravenous material balance studies was via the lateral tail vein but injection via the indwelling jugular vein cannula was a preferred route for the pharmacokinetics studies. Therefore, the effect of the route of injection on ^{14}C distribution following intravenous dose administration was compared in order to assess the appropriate method for administering the IV dose for the pharmacokinetic studies. Four rats had cannulae surgically implanted using the procedure described in the pharmacokinetics method section of the protocol. Two rats per route (tail vein or cannula injection) were dosed with a 0.9% saline solution of ^{14}C -EG. Blood was drawn at 0.5, 1 and 2 minutes after dosing and quantified as described in the pharmacokinetics study design. In addition, the potential for the cannula tubing to absorb ^{14}C -test material was analyzed by combustion of the tubing upon termination of the experiment.

Chemical Analysis

The analytical phase of the ethylene glycol (EG) pharmacokinetics and material balance studies included dose solution, plasma and urine analyses. Concentrations of EG in the dosing solutions were measured by capillary gas chromatography (GC) with flame ionization detection (FID). Ethylene glycol in selected pooled plasma samples from the pharmacokinetics studies was derivatized with phenylboronic acid to form a cyclic phenylboronate ester and then analyzed by capillary GC coupled to a Mass Selective Detector (MSD). The

identification of EG and several known metabolites in selected urine samples from the material balance studies was accomplished using high performance liquid chromatography (HPLC) with refractive index detection. In addition, the proportion of unchanged ^{14}C -EG to that of major labelled metabolites in the urine samples was quantified using a radioactivity flow monitor in series with the refractometer. The details of the analytical methods and results are contained in Appendix C.

Material Balance Study Design

The following design applies generally for each route of administration in the determination of material balance. Twelve rats (six per sex) were placed individually into Roth-type glass metabolism cages for two days prior to dose administration. The six rats per sex per dose group were selected for chemical treatment based on uniformity of body weight from a pool of at least eight rats per sex which were acclimated to the laboratory for at least five days.

On the first day of the test, four of the six acclimated rats per sex were selected for dosing on the basis of uniformity of body weight and general health status. Following EG administration by the pertinent route, urine and feces were collected at 12-, 24-, 36-, 48-, 72-, and 96-hr intervals post-dosing. The urine samples were collected in flasks cooled to dry ice temperature and were stored frozen (approximately -80°C) upon completion of radioactivity determinations until the comparative analysis of parent compound and metabolites was conducted. Air flow through the glass metabolism cages was approximately 500 ml/min. Expired $^{14}\text{CO}_2$ was trapped during 12-hr intervals through 48 hr post-dosing and 24-hr intervals thereafter using solutions of 2-methoxyethanol: ethanolamine (7:3) at room temperature. The feces and CO_2 samples collected were stored at approximately -20°C until analyzed.

Ninety-six hours after administration of the EG dose, the animals were anesthetized with methoxyflurane and exsanguinated via cardiac puncture (terminal blood sample). Cage washes were collected to quantify radioactivity for inclusion in the balance. For all administration routes, the pelt was removed from the carcass and the following tissues were collected:

- | | |
|-----------|--------------------|
| 1. liver | 4. fat (perirenal) |
| 2. kidney | 5. lung |
| 3. brain | 6. testes |

Prior to removal of the pelt, the dosed area of skin from the percutaneous study animals was removed and weighed in order to quantify unabsorbed EG at the dose site. Skin segments from two remote areas (ventral surface and sacral regions) were also taken to estimate the systemic distribution of EG to the skin. The skin from both the dose site and directly surrounding it (1 cm from edge of dose site) was pulverized at liquid nitrogen temperatures in a Spex Freezer Mill (Metuchen, NJ) and an aliquot from each sample was counted directly in a suspension of 10 ml Aquasol-2 $\text{\textcircled{R}}$ (New England Nuclear) and 6 ml water.

For all of the material balance studies, radioactivity from skin samples removed from the pelt at the two remote sites was quantified after tissue oxidation. Radioactivity was also determined following tissue oxidation of

isolated organs, aliquots of homogenized carcass (33% w/w in distilled water), feces (33% w/w in distilled water), and red blood cells from the final collection. Radioactivity recovered from the dose site and from the occlusive devices used to cover the dose site was also quantified. Tissue oxidation was conducted in an OX-300 Biological Materials Oxidizer (R. J. Harvey Instrument Corp., Hillside, NJ) and the $^{14}\text{CO}_2$ derived from combustion of these tissues was quantified by liquid scintillation spectrometry.

Pharmacokinetics Study Design

The design which follows applies generally for each route of administration, with some exceptions which are noted in the individual sections of the pharmacokinetics results. Approximately 48 hr prior to administration of the dose, twelve rats (six per sex) were prepared for surgical implantation of an indwelling jugular cannula under methoxyflurane anesthesia using a modification of the method of Harms and Ojeda (1974). The animals for the percutaneous study were lightly clipped using electric clippers in approximately a 4 x 6 cm area in the shoulder region of the back prior to surgery. Following recovery from surgery, the animals were returned to the Roth-type glass metabolism cages to complete the acclimation prior to dosing. Food was removed approximately 15 hr prior to dose administration for the peroral studies.

On the first day of the test, four rats per sex with acceptable (within normal limits) hematocrit values and patent cannulae were selected and the dose was administered as described in the previous section on Administration of Test Material. Blood samples were collected via the indwelling jugular cannula at 0 (pre-administration for the determination of hematocrits), 0.5, 1, 2, 4, 8, 12, 18, 24, 36, 48, 72, and 96 hours post-dosing for the peroral and percutaneous study designs. Blood samples were collected at 0 (pre-administration for the determination of hematocrits), 5, 15 and 30 min and 1, 2, 4, 8, 12, 18, 24, 36, 48, 72 and 96 hr post-dosing for the intravenous study design. Approximately 0.15 ml was drawn at each time interval and immediately placed in heparinized capillary tubes, sealed using Seal-ease® tube sealer, and then centrifuged in an Adams Autocrit Centrifuge (Clay Adams, Parsippany, NJ). The spun capillary tubes were scored and broken at the plasma/packed cell interface. The plasma was then expressed from the tubes into a tared scintillation vial and the plasma weight recorded. An aliquot of plasma was added to a 10 ml volume of Aquasol-2® counting scintillant with shaking and then counted by liquid scintillation spectrometry. The remaining plasma volume was pooled in equal volumes with plasma from other animals in the dose group for parent compound quantification by GC separation with Mass Selective Detection (GC/MSD). Radioactivity was determined from tissue oxidation of red blood cells from all collection intervals in an R. J. Harvey Biological Materials Oxidizer (Hillside, NJ) and quantitation of the $^{14}\text{CO}_2$ derived from combustion of these tissues was by liquid scintillation spectrometry.

Approximately 6 hr following the administration of the percutaneous dose, the occlusive device was removed and any unabsorbed test material was washed from the dose site with water-wetted, cotton-tipped applicators. The device was then replaced and left on the animal for the duration of the 96-hr exposure. The applicators were saved in order to quantify any unabsorbed dose.

Urine and feces were collected at 12-, 24-, 36-, 48-, 72- and 96-hr intervals post-dosing. The collection flasks for urine samples were cooled to dry ice temperature and were assayed for ^{14}C following collection; the remaining volumes were stored at approximately -20°C . Feces were frozen at approximately -20°C immediately after collection. Air flow through the glass metabolism cages was approximately 500 ml/min. Expired $^{14}\text{CO}_2$ was trapped at 12-hr intervals through 48 hr post-dosing and 24-hr intervals thereafter using solutions of 2-methoxyethanol: ethanolamine (7:3) at room temperature; $^{14}\text{CO}_2$ samples were stored under cold temperatures until analyzed.

Ninety-six hours after administration of the EG dose, the animals were anesthetized with methoxyflurane and exsanguinated via cardiac puncture (terminal blood sample). Carcasses were stored at approximately -20°C and were not assayed for radioactivity. For the percutaneous study, the dosed area of skin together with the skin around the periphery (approximately 1 cm from edge of the dose site) was separated from each animal using blunt dissection techniques. The skin samples were pulverized at liquid nitrogen temperatures in a Spex Freezer Mill (Metuchen, NJ) and aliquots were counted directly in a suspension of 10 ml Aquasol-2 $\text{\textcircled{C}}$ and 6 ml water.

Metabolite Profiling and Preliminary Identification

The relative proportions of unchanged ^{14}C test chemical to that of major metabolites found in the plasma and urine were examined over a 48-hr period following percutaneous administration of ^{14}C -EG. Selected urine samples were subjected to liquid chromatography separation with ^{14}C quantification via an in-line radioactivity flow monitor (Model 7150 Trace II, Packard Instrument Co., Downers Grove, IL). Selection of interval samples for analysis of the relative proportions of metabolites was based on the concentration of radioactivity determined initially by liquid scintillation spectrometry and the size of urine volumes collected. The HPLC identification work was confined to only the major metabolites present in urine. Determinations were done by direct comparison of retention times for the observed peaks with those determined for both standard solutions of ^{14}C -EG and non-radiolabeled EG and the major metabolites using the on-line radioactivity detector and a refractive index detector (Waters Associates, Milford, MA) where appropriate. The GC method for derivatization of plasma samples was modified from a previously-developed method while the capillary GC separation conditions were developed at BRRC (see Appendix C).

Pharmacokinetic Description

Semilogarithmic plots of both total radioactivity and unchanged EG concentrations in plasma vs. time provided the basis for the pharmacokinetic data analysis in this study. These pharmacokinetic descriptions of the fate of ^{14}C -EG were evaluated using RSTRIP (Fox and Lamson, MicroMath, Inc., Copyright 1987) to derive optimum parameter estimates for the plasma data. Mean plasma ^{14}C concentrations (μg Equiv/g) were used initially to fit one-, two- or three exponential equations for which elimination and transfer of ^{14}C -EG between compartments were assumed to be first-order processes. The parameters which were estimated using mean plasma concentration values included: the rate constant of absorption, k_a (except IV doses); the rate constant for initial disposition, α ; the rate constant for terminal

disposition, β ; the rate constant of elimination, k_e ; the volume of distribution at steady state, V_d^{SS} ; and the half-lives ($t_{1/2}$) of absorption and elimination. Other pharmacokinetic terms which were calculated and used in the succeeding tables have been footnoted to explain their mathematical derivation.

RESULTS: MATERIAL BALANCE STUDIES

For each of the individual material balance studies conducted by three administration routes, a summary of the mean body weights at study initiation and the amount of radioactive dose delivered to each animal is presented in Table 1. Mean body weights (± 1 S.D.) were summarized for all groups in the study: values of 280.18 ± 1.85 g for males and 215.50 ± 8.52 g for females were calculated from group mean body weights for the seven material balance studies conducted. In addition, Table 1 shows that uniform radioactive doses were administered to both sexes when ^{14}C doses were normalized for body weight in each study.

The recovery of radioactivity in CO_2 and urine is summarized in Figures 1-6. Mean summary data (including organs, carcass, cage wash, etc.) for each of the individual material balance studies are contained in Tables 4-15.

Intravenous Route: Material Balance Results

The data obtained for the disposition of radioactivity (mean ± 1 S.D.) following the intravenous injection of ^{14}C -EG in either a 10 or 1000 mg/kg body weight dose are summarized, respectively, in Tables 4 and 5; individual animal data are presented in Appendix A and mean animal data (itemized for all intervals of collection) are presented in Appendix B. The data for the disposition of radioactivity in the individual tissues and the carcass are summarized in Table 6. The following were the major features of the data obtained in this segment of the investigation and will be described in the succeeding paragraphs:

1. The expiration of $^{14}\text{CO}_2$ was the major metabolite after a 10 mg/kg IV dose, while urine was the secondary excretion route;
2. Urinary ^{14}C excretion was the principle elimination route following a 1000 mg/kg IV dose, with less of the dose expired as $^{14}\text{CO}_2$.
3. More of the administered ^{14}C dose was recovered in the tissues and carcasses of low dose animals than for the high dose animals.

The recovery of radioactivity for the 10 mg/kg intravenous dose (Table 4) amounted to $90.0 \pm 2.4\%$ ($n = 4$) for male animals and $85.4 \pm 4.7\%$ ($n = 4$) for female animals. For males, the major excretion route was via $^{14}\text{CO}_2$ exhalation (41.2%) while lesser amounts were excreted in the urine (27.3%) and in the feces (2.9%) after the 96-hr collection period. Figures 1 and 2 depict cumulative percent recovery plots over 96 hr for $^{14}\text{CO}_2$ and urine. Another 18.6% of the dose for males was recovered in tissues (8.5%), the carcass (9.9%) and in cage washes (0.2%). Results for females were very similar to

male rat results at this dose level: 43.7% was recovered in expired $^{14}\text{CO}_2$, 25.1% was excreted in the urine, and 2.0% was recovered in feces over the 96-hr collection period (Figures 1 and 2). Slightly less of the radioactive dose was recovered in tissues taken from females (5.3%) vs. males, while very similar ^{14}C amounts were found in the carcass (9.2%) and in cage washes (0.2%). The slightly lower recoveries in urine and in the tissues for females compared to males account for most of the difference observed in total recoveries.

There was a dose-dependent shift in the route of excretion for radioactivity following the 1000 mg/kg intravenous dose (Table 5), with $^{14}\text{CO}_2$ expiration following an apparent inverse relationship to dose. Overall, most of the radioactive dose was excreted in the urine (Figure 2), with lesser amounts recovered as expired $^{14}\text{CO}_2$ (Figure 1). Also, less of the dose was found in tissues and carcass than was observed for the 10 mg/kg dose. There were no substantial differences in excretion routes or amounts between the two sexes at this higher dose (Figure 5B). Total recovery of radioactive dose for the male animals was $92.2 \pm 1.1\%$ ($n = 3$) while $88.1 \pm 5.1\%$ ($n = 4$) of the dose was recovered from female animals. A total of 52.5% of the administered dose was excreted in the urine of male animals while lesser quantities were eliminated as expired $^{14}\text{CO}_2$ (28.2%) and in the feces (2.8%). Recovery values for tissues (3.8%) and the carcass (4.7%) were relatively lower than those recoveries in the low dose animals but the cage wash value was similar (0.2%). Recovery values from females were similar to those for males (Figure 5). In contrast to male values, female recovery values were more variable, but much of this variation occurred in the 0-12 hr collection period for urine, feces and $^{14}\text{CO}_2$ fractions. However, it is typical that data from this collection interval displays the greatest amount of individual variation compared to later intervals, particularly when large percentages of the dose are excreted in the urine, because the animals can be quite variable in their voiding frequency.

Individual tissue recovery data for the 10 mg/kg intravenous dose are itemized in Part A of Table 6; the corresponding data for the 1000 mg/kg dose are presented in Part B of this Table. In the tissue recoveries for the 10 mg/kg intravenous dose, the male data exhibited pertinent recovery percentages for liver, carcass and pelt. Tissue/plasma ratios of greater than 1.0 were calculated for liver, kidney, lung and pelt, indicating a greater presence of radioactivity in these tissues than would be accounted for by perfusion alone. Tissue/plasma ratios of less than 1.0 were calculated for brain, fat, testes and carcass in males. Female tissue data indicated similar percentages of the dose to those for males, except for the pelt, which was a lower percentage for females.

For the 1000 mg/kg intravenous dose, those percentages of the dose which were recovered in both sexes for liver, kidney, lung, carcass and pelt were reduced to approximately half of the percentage values found for the lower dose animals. However, the tissue/plasma ratios remained relatively consistent in both sexes at this higher dose for liver, kidney, lung, and pelt. Furthermore, the total amount of ^{14}C in the individual tissues (as $\mu\text{g Eq/g}$) was approximately two orders of magnitude greater following 1000 mg EG/kg compared to 10 mg EG/kg (Table 6), as would be expected for a difference in body burden after a 100-fold higher dose is administered.

Peroral Route: Material Balance Results

The data obtained for the disposition of radioactivity (mean \pm 1 S.D.) following the peroral administration of ^{14}C -EG in either a 10 or 1000 mg/kg body weight dose are summarized, respectively, in Tables 7 and 8 and presented graphically in Figures 3 and 4. The individual animal data are presented in Appendix A and mean animal data (including all intervals of collection) are presented in Appendix B. The data for the disposition of radioactivity in the individual tissues and the carcass are summarized in Table 9. The data for three additional peroral dose levels, 400, 600 and 800 mg/kg body weight, are summarized in Table 10 while excreta comparisons are presented in Figure 6. The individual and mean data for these animals are summarized, respectively, in Appendices D and E.

The main features of this portion of the data were quite consistent with the intravenous material balance results. That is:

1. Expired $^{14}\text{CO}_2$ was the major metabolite following peroral 10 mg/kg doses, with urine the secondary ^{14}C excretion route;
2. Excretion of ^{14}C -labeled metabolites in the urine became the predominant mode of elimination of ^{14}C -EG-derived radioactivity as the dose was increased to 1000 mg/kg.
3. Intermediate peroral dose levels of 400, 600 and 800 mg/kg resulted in transition relationships for $^{14}\text{CO}_2$ vs. urinary ^{14}C elimination; thus, $^{14}\text{CO}_2$ expiration decreased as dose was increased and the amount of urinary ^{14}C output was directly related to magnitude of the dose.
4. Lesser amounts of ^{14}C residue were recovered in tissues/carcass for the high dose animals than for the low dose animals.

For the 10 mg/kg dose groups, a total of $93.4 \pm 2.1\%$ of the administered dose was recovered for male animals and $91.8 \pm 3.4\%$ was recovered in female animals. Expired $^{14}\text{CO}_2$ (Figure 3) represented the major excretion route in both males (42.2%) and females (47.9%). Figure 5A shows that lower percentages of the administered dose were excreted in the urine (26.2% for males; 25.5% for females) and in the feces (2.9% for males; 2.8% for females) when compared to the expired $^{14}\text{CO}_2$ values. As was observed for the 10 mg/kg intravenous dose recoveries, there were no clear differences between the sexes in either the routes of excretion or the magnitude of dose percentage recovered in excreta (Figure 5). Slightly more of the dose was recovered in the urine (Figure 4) of females than of males and comparatively less radioactivity was present in tissues from females (5.7%) than in tissues from males (10.1%) at 96 hr after dose administration. However, the differences in tissue recoveries resulted from the lower percentage of the dose being recovered in pelts for females than for males. This apparent decrease in recovery is not considered to indicate any biological significance. Carcass and cage wash recovery values were virtually the same for both sexes.

A dose-dependent shift in routes of excretion, similar to that observed with the 1000 mg/kg intravenous dose, occurred when EG was administered perorally at 1000 mg/kg (Figure 5). Overall, $83.2 \pm 7.2\%$ of the dose was recovered for male animals and $83.3 \pm 3.5\%$ for females (Table 8). The urine (Figure 4) was the major route for radioactive dose elimination in both sexes (42.7%, males; 35.0%, females), while lower percentages of the dose were found as expired $^{14}\text{CO}_2$ (27.3%, males; 28.2%, females). Fecal elimination accounted for additional low percentages of the dose (2.4%, males; 4.4%, females). Relative to the other intervals of collection, the recovery for excreta samples was most variable in the 12-24 hr interval, particularly for the collection of female urine samples (Table 8). This variation accounted for much of the apparent difference between mean total percentages for urine over 96 hr post-dosing for both males and females. However, a large percentage of the dose was recovered in cage wash samples for females (with a large variation as well) and much of this apparent difference in urine recoveries can be accounted for in this fraction. Comparatively lower recoveries than for the low peroral dose were observed for tissues and carcass from this high dose group (Tables 7 and 8) and this was again consistent with the magnitude of the differences between the high and low intravenous dose groups (Tables 4 and 5).

Individual tissue recovery data for the 10 mg/kg peroral dose are presented in Part A of Table 9; the corresponding data for the 1000 mg/kg dose are presented in Part B of this table. For the 10 mg/kg peroral dose, the larger tissue recovery percentages were observed in the male data for liver, carcass and pelt. Tissue/plasma ratios of greater than 1.0 were calculated for liver, kidney, lung and pelt, indicating a greater presence of radioactivity in these tissues than would be accounted for by perfusion alone. Tissue/plasma ratios of less than 1.0 were calculated for brain, fat, testes and carcass in males. Tissue data from females indicated similar percentages of the dose, except for the pelt, which was higher for males.

Data for the 1000 mg/kg dose indicated that there was a lower percentage of administered radioactivity present overall, when compared to low dose tissue recoveries, which was approximately two-fold lower when expressed as a percentage of the administered dose. The tissue/plasma ratios were slightly elevated from lower dose values for the liver, kidney, lung, carcass and pelt of males and the kidney, lung, carcass and pelt of females; the ratio for the liver was comparable for females between the high and low peroral doses. This was again consistent with intravenous study results since the difference in $\mu\text{g EG/g}$ values between individual tissues for the two dose levels was approximately two orders of magnitude higher as the dose was increased 100-fold.

Peroral Route (Intermediate Dose Levels): Partial Material Balance Results

The data obtained for the disposition of radioactivity (mean data \pm 1 S.D.) following the peroral administration of ^{14}C -EG at 400, 600 and 800 mg/kg body weight doses are summarized in Table 10 and presented graphically in Figure 6. Individual animal data are presented in Appendix A and mean animal data (with all intervals of collection included) are presented in Appendix B.

To further define the range for dose-dependent changes in peroral dose route excretion patterns, samples of urine and expired $^{14}\text{CO}_2$ were collected from male and female animals for 96 hr post-dosing following peroral doses of 400 and 600 mg/kg body weight; the final dose was not selected until results from these dose levels had been evaluated. For male animals, the largest fraction of the dose was recovered as expired $^{14}\text{CO}_2$ (Figure 6A) in both the 400 mg/kg dose ($38.8 \pm 0.8\%$) and the 600 mg/kg dose ($34.0 \pm 1.5\%$). Both of these recoveries were of similar magnitude to $^{14}\text{CO}_2$ recoveries for the 10 mg/kg dose ($42.2 \pm 0.3\%$ for males, Table 7), with the amount expired inversely related to the administered dose. Lower mean percentages of administered dose were recovered for male urine samples (Figure 6B) than for $^{14}\text{CO}_2$, making these recoveries consistent with excreta samples recovered for the 10 mg/kg peroral dose. However, the mean recoveries exhibited large standard deviations, particularly in the 0-12 hr collection interval, for both doses (Table 10). Male urine recovery totals for the 0-96 hr collection period were $20.5 \pm 9.4\%$ (individual %: 26.1, 19.5, 7.7 and 28.8%) for the 400 mg/kg dose and $25.8 \pm 10.2\%$ (individual %: 40.1, 21.7, 25.2 and 16.1%) for the 600 mg/kg dose (Figure 6B). With this variation, it was only possible to say that urine recoveries for males at these two dose levels were more comparable to those observed for the 10 mg/kg dose ($26.2 \pm 2.1\%$, Table 7) than to the 1000 mg/kg dose ($42.7 \pm 7.1\%$, Table 8).

Excreta data for females from the 400 and 600 mg/kg peroral doses presented a different profile which was less distinct than that for males (Figure 6). Urine and expired $^{14}\text{CO}_2$ recoveries were very similar at the 400 mg/kg level, with $38.0 \pm 7.6\%$ (individual %: 44.1, 43.6, 27.8 and 36.7%) of the dose recovered as ^{14}C in the urine (Figure 6B) and $39.4 \pm 1.0\%$ of the dose eliminated as expired $^{14}\text{CO}_2$ (Figure 6A). Urinary ^{14}C excretion was slightly larger than $^{14}\text{CO}_2$ over 96 hr for the female 600 mg/kg dose groups, but the variation of individual urine recoveries again complicated the interpretation of the data. A total of $37.1 \pm 17.0\%$ of the dose (individual %: 17.4, 28.4, 50.6 and 52.0%) was recovered in the urine, while $32.8 \pm 3.4\%$ was expired as $^{14}\text{CO}_2$. Thus, the $^{14}\text{CO}_2$ elimination data appeared to indicate that females in the 400 mg/kg dose group expired roughly the same percentage of the dose as the 10 mg/kg females ($47.9 \pm 0.8\%$, Table 7) while the 600 mg/kg females eliminated the dose as $^{14}\text{CO}_2$ similar to the 1000 mg/kg females ($28.2 \pm 2.1\%$, Table 8). However, urine ^{14}C data indicated a greater similarity to the 1000 mg/kg females, which displayed a recovery percentage of $35.0 \pm 13.2\%$ (Table 8). Further examination of the variation in this high dose group revealed individual recovery percentages of: 47.2, 41.2, 16.1 and 35.0% of the dose in urine. The 10 mg/kg female urinary recoveries were far less variable ($25.5 \pm 3.8\%$). Therefore, although it might be concluded from the female urine data that a dose-dependent shift in excretion routes occurs at doses exceeding 400 mg/kg perorally, it can be seen that the similarities in male urinary ^{14}C data for the 400 and 600 mg/kg doses make it difficult to establish this dose range conclusively (Figure 6). Because of this absence of a clear distinction between the 400 and 600 mg/kg dose, it was concluded that the last peroral dose would be 800 mg/kg for both sexes.

Results for the recovery of radioactivity in urine and expired $^{14}\text{CO}_2$ following an 800 mg/kg peroral dose are summarized in Part C of Table 10. For male animals, a total of $26.7 \pm 6.4\%$ of the dose was recovered in the urine (Figure 6B), while $30.1 \pm 2.1\%$ was expired as $^{14}\text{CO}_2$ (Figure 6A). Female

animals excreted $41.0 \pm 5.1\%$ of the ^{14}C dose in the urine and $32.1 \pm 2.3\%$ as expired $^{14}\text{CO}_2$ at this dose level. For males, the individual animal values for urine recoveries were: 24.4, 20.4, 26.6 and 35.5%; female individual urine recovery values were: 40.2, 42.1, 34.6 and 47.0%. The majority of the male values were similar to individual values at the 10 mg/kg peroral dose: 29.2, 24.4, 25.9 and 25.2%. Taken together with the slightly higher recovery of $^{14}\text{CO}_2$ at 800 mg/kg, the excreta profile again appeared to be more similar to the low peroral dose excretion patterns, as was true for the 400 and 600 mg/kg peroral dose in males (Figure 6). At the highest dose level tested in females, more of the radioactive dose was eliminated in the urine (35%) than in the expired $^{14}\text{CO}_2$ (28%) and similar differences between excretion routes were observed in data for the female 800 mg/kg peroral group. Thus, for male rats, a dose-dependent shift in urinary excretion routes appeared to occur between dose levels of 800-1000 mg/kg body weight. In females, this shift was more difficult to pinpoint but appeared to occur between 10-400 mg/kg. However, due to the degree of variability encountered in these determinations, it would be difficult to call this a true sex difference.

Percutaneous Route: Material Balance Results

The data obtained for the disposition of radioactivity (mean \pm 1 S.D.) following the percutaneous application of ^{14}C -EG in undiluted 10 or 1000 mg/kg body weight doses or a 50% aqueous 1000 mg/kg body weight dose are summarized, respectively, in Tables 11, 12 and 13; individual animal data are presented in Appendix A and mean animal data (including all intervals of collection) are presented in Appendix B. The data for the disposition of radioactivity in the individual tissues and the carcass are summarized, respectively, in Tables 14 and 15.

The major findings of the percutaneous study segment of this investigation were:

1. Undiluted doses of either 10 or 1000 mg/kg resulted in the expiration of $^{14}\text{CO}_2$ as the major metabolite, with smaller amounts of ^{14}C being excreted in the urine, and most of the applied dose being recovered from the materials used to occlude the dose site;
2. An aqueous 50% w/w dose, applied at 1000 mg/kg, showed similar results to those for undiluted EG percutaneous doses, with $^{14}\text{CO}_2$ again the predominant elimination route and the majority of the applied dose recovered in occlusion materials;
3. Relatively the same recovery values for tissues and excreta were found for each cutaneous dose group, regardless of application conditions.

Following an application of an undiluted 10 mg/kg EG, $31.5 \pm 2.1\%$ of the administered dose penetrated male skin and was systemically-available while $31.5 \pm 17.2\%$ of the dose penetrated female rat skin (Table 11). The balance of the recovered dose was contained in the occlusion materials (used to prevent oral ingestion of the dose) and in the skin removed from the dose site, which had been thoroughly rinsed at study termination to remove any unabsorbed radioactive dose (part B of Table 11). This dose site recovery fraction amounted to 17.4% of the dose in males and 10.9% in females,

with 9.9% recovered in the rinsed skin of males and 2.9% found in female skin samples. The largest fraction of dose which penetrated male skin was expired as $^{14}\text{CO}_2$ (Figure 1, 14.0%). Lesser amounts of ^{14}C were excreted in the urine (Figure 2, 6.7%) and in the feces (1.1%); most of the dose was excreted after 24 hr post-dosing. Another 9.7% of the dose was recovered in tissues, carcass and cage wash for the males. Similar results for distribution of the absorbed dose were observed for excretion and tissue distribution in female rats. Again, the largest excretion fractions were in expired $^{14}\text{CO}_2$ (13.1%) and urine (8.2%), with greatest amounts excreted after 24 hr post-dosing (Figures 1 and 2); another 1.1% of the dose was eliminated in the feces. Overall, this dose penetrated male and female skin about equally (with greater variation for the female animals) and was subsequently eliminated with similar percentages in the individual fractions. However, the overall recovery of radioactivity following an undiluted percutaneous dose of 10 mg EG/kg body weight (Table 11) was $48.9 \pm 5.0\%$ for male animals and $42.4 \pm 19.2\%$ for female animals. Further extraction of the occlusion appliances for the dose site yielded only minor additional percentages (0.2 - 0.5%) of the radioactive dose. This final recovery result would appear to support the conclusion that the dose delivered to the site of application was subject to losses which were unaccounted for and lead to an overestimation of the radioactive dose applied in this group of animals.

The 1000 mg EG/kg percutaneous dose of undiluted EG showed similar penetration and excretion patterns for both sexes to those observed for the 10 mg/kg percutaneous dose (Table 12). Expired $^{14}\text{CO}_2$ (Figure 3) was also the major elimination route at this dose level (14.4%, males; 11.4%, females), followed by ^{14}C excretion in the urine (Figure 4, 8.1%, males; 7.6%, females) and in the feces (0.6%, males; 1.4%, females). Tissue and carcass distribution, and recoveries in cage washes accounted for the balance (12.6%, males; 8.8%, females) of the overall recovery of absorbed radioactivity for this dose group. A total of $35.7 \pm 10.7\%$ of the applied dose penetrated the skin of male rats, while $29.1 \pm 2.7\%$ penetrated female rat skin over the 96-hr exposure period. The remainder of the applied radioactive dose was recovered from the occlusive coverings, rinses of the dose site, and in the skin from the site (part B of Table 12). A total of $48.2 \pm 9.4\%$ for males and $55.6 \pm 2.2\%$ for females of the applied dose was found in these fractions. In total, $83.8 \pm 4.1\%$ of the dose for males and $84.7 \pm 2.0\%$ for females was recovered at the termination of this segment of the study and there were no substantial differences in the skin penetration of this applied dose between sexes.

Similar tissue and excreta recoveries to those for the two undiluted doses were obtained following the application of a 50% aqueous EG solution at a dose of 1000 mg/kg body weight (Table 13). The amount of radioactive dose which penetrated skin was $22.1 \pm 4.0\%$ for males and $25.8 \pm 2.7\%$ for females from this 50% aqueous solution. Of the total absorbed dose amounts, the largest excretion fraction was again in the expired $^{14}\text{CO}_2$ (Figure 3). Males were slightly lower in the amount of $^{14}\text{CO}_2$ expired (5.9%) in comparison to females (9.3%) (Figure 3), but urine radioactivity levels (Figure 4) were virtually identical (4.6%, males; 4.4%, females). Fecal ^{14}C elimination was minimal after this application for both males (0.6%) and females (0.5%). Values for recovery in tissues, carcass and cage wash were almost identical between the sexes as well (11.0%, males 11.6%, females). Again, as with the

high dose of undiluted EG, the bulk of the recovery was obtained in occlusion materials and dose site (rinsed) skin: 59.2%, males; 56.8%. Overall, $81.2 \pm 3.8\%$ for males and $82.7 \pm 4.5\%$ for females was recovered from the total radioactivity applied. Taken together, these results demonstrate that there were no substantial differences in the penetration of skin by a 50% EG solution between male and female rats. However, somewhat lower recoveries in tissues and excreta were generally found for this dosing application than was true for the undiluted 1000 mg/kg application. These results are thus consistent with the lower effective EG concentration (500 mg/kg) which was applied in the 50% dose.

The distribution of radioactivity to tissues and carcass following three different percutaneous dosing regimens of EG (Tables 14 and 15) showed similar disposition profiles to those observed for the intravenous (Table 6) and peroral doses (Table 9). For the 10 mg/kg undiluted percutaneous dose (Part A, Table 14), approximately 1.0% of the administered dose was recovered in the livers of male and female rats and the recovery in carcass and the pelt exceeded 7.0% of the dose for both sexes. Tissue to plasma ratio values of greater than 1.0 were calculated for liver, kidney and pelt in both sexes and for lung tissue in female rats; lung tissue in male rats had a tissue/plasma ratio of slightly less than 1.

At the 1000 mg/kg undiluted dose (Part B, Table 14), notable percentages of administered dose were recovered for carcass and pelt and the tissue/plasma ratios exceeded 1.0 for liver, kidney, lung and pelt in both male and female rats. Recoveries for the 50% aqueous dose (Table 15) were only slightly different than those obtained for the two undiluted dose groups. Only carcass and the pelts contained more than 1.0% of the applied dose and a tissue/plasma ratio greater than 1.0 was calculated only for liver and the pelt in both males and females. Minor concentrations of radioactivity were recovered in fat tissue samples of both sexes and in the testes of male rats for all three percutaneous dosing regimens, indicating that this exposure route for EG does not tend to result in any appreciable accumulation in these two tissues following a single dose exposure. Taken together, these tissue distribution results are consistent with the overall metabolism and excretion patterns for this chemical and do not indicate any specific accumulation potential for a particular organ or tissue following a single percutaneous dose of EG.

RESULTS: PHARMACOKINETICS STUDIES

The pre-study body weight and dose administration summaries for the pharmacokinetics dosing groups are contained in Table 2.

Also, prior to the intravenous studies, a probe study on intravenous injection was conducted. Comparison of the results for relative distribution of ^{14}C after intravenous injection via the two points of entry are presented in Table 3. No substantial differences in ^{14}C distribution/elimination from the plasma were seen. Therefore, the animals selected for the intravenous pharmacokinetics studies were injected with an EG dose via the cannula since this injection route did not result in any substantially different distribution of EG than for a tail vein injection of the dose. In addition, only negligible amounts of ^{14}C were associated with the cannula tubing, indicating that EG was not absorbed into the tubing during injection.

The results from the pharmacokinetics phase of this investigation will be described in the following order: 1) initial pharmacokinetic parameter estimation from plasma radioactivity concentrations; 2) results of the analytical determinations for unchanged EG in plasma; and 3) pharmacokinetic parameter estimations and bioavailability calculations using plasma EG analytical concentration data. These data are presented in this order to show the differences in plasma pharmacokinetic profiles which resulted for uptake, distribution and elimination of unchanged EG vs. metabolites present in the plasma. The data have been presented graphically in Figures 7-20 and the pharmacokinetic parameter estimates are summarized in Tables 16-32.

Intravenous Route: Pharmacokinetics Results

The pharmacokinetic parameter estimates (expressed as mean values) which were obtained from plasma radioactivity data collected following the intravenous injection of ^{14}C -EG at either a 10 or 1000 mg/kg body weight dose, are summarized, respectively in Tables 16 and 17. Individual animal data for these studies are presented in Appendix D and mean animal data from which group mean plasma values were obtained for these parameter estimations are presented in Appendix E.

The disappearance of radioactivity from the plasma following a 10 mg/kg IV dose followed a biphasic elimination pattern (Figure 7 and 8) which was described using a two exponential equation. Males and females showed remarkably similar plasma ^{14}C concentration vs. time profiles for both the distribution and elimination phases. The initial distribution (α) phase for plasma radioactivity in both sexes (Table 16) was very brief with a plasma disappearance half-life value on the order of 1.5-2.6 minutes for both sexes.

This initial disposition phase was so rapid, in fact, that it was difficult to adequately describe the plasma disappearance at this dose level using the RSTRIP analysis program. In addition, a transient increase in ^{14}C concentration was present in the plasma curves for both sexes between 1-4 hr post-dosing which could not be described well by RSTRIP. Relative to the initial phase, a much longer disposition (β) phase occurred at this dose level which resulted in elimination half-life values of 26.6 hr for males and 18.9 hr for females. These longer half-life values are consistent with the extensive metabolism which occurs for EG and may reflect delayed elimination of the radioactive dose due to the slower, rate-limiting steps in the metabolic process.

The parameter estimates for the 1000 mg/kg intravenous dose, summarized in Table 17, indicate similar pharmacokinetic profiles for both males (Figure 7) and females (Figure 8). There was a rapid initial disposition (α) phase, although not as rapid as that observed following the 10 mg/kg intravenous dose, and this was again followed by a prolonged terminal disposition (β) phase in both sexes. However, the overall differences in pharmacokinetic behavior between the 10 and 1000 mg/kg dose levels may be attributed to the poor fit of the lower dose plasma curve obtained in the RSTRIP analysis. The distribution half-life values following the high dose were 3.9 and 2.7 hr, respectively, for males and females. Disposition half-life values were considerably longer than values for the initial phase: 37.0 hr for males and 31.6 hr for females. Transition from the α to β phases of these curves began at approximately 30-60 min after injection of the dose in both sexes and lasted until approximately 24 hr post-dosing when the linear portion of the elimination phase became distinct.

Peroral Route: Pharmacokinetics Results

The pharmacokinetic parameter estimates (expressed as mean values), obtained from plasma radioactivity collected following the peroral administration of ^{14}C -EG as either a 10 or 1000 mg/kg body weight dose, are summarized, respectively in Tables 18 and 19; individual animal data for these studies are presented in Appendix D and mean animal data from which group mean plasma values were used for these parameter estimations are presented in Appendix E.

The plasma radioactivity concentrations vs. time following a 10 mg/kg peroral dose are presented graphically in Figures 9 and 10. Absorption of this dose was moderately rapid, with the peak plasma ^{14}C concentrations occurring at 4-5 hr for both sexes. The half-life of absorption values were very similar for either sex, with absorption in the females being slightly faster ($t_{1/2}$ of 2.2 hr) than for males ($t_{1/2}$ of 2.7 hr) after a 10 mg/kg peroral dose (Table 18). Following this absorption phase, the disappearance of radioactivity from the plasma followed a biexponential mathematical description which had a rapid initial (α) phase through the first 6-15 hr after dosing and a much slower terminal (β) phase which lasted until termination of the exposure at 96 hr post-dosing. Initial (α) and terminal (β) disposition phases were remarkably similar for both sexes, with a 5.2-5.3 hr half-life value calculated for the initial disposition (α) phase and a 38.4-39.8 hr half-life value computed for the terminal (β) phase of the plasma ^{14}C disappearance curve. This longer terminal phase could indicate extensive metabolism for the administered chemical and was comparable to the long elimination phase observed for the 10 mg/kg intravenous dose. In addition, area under the curve (AUC) values were quite comparable through 96 hr post-dosing with AUC_{96} values from the low intravenous dose (Table 16), indicating the similarities between these two routes for the initial and terminal disposition phases for radioactivity (EG and metabolites) following a 10 mg/kg dose.

An extremely rapid absorption phase characterized the plasma uptake of the 1000 mg EG/kg peroral dose in both male and female animals (Figures 9 and 10). Peak plasma concentrations were achieved in both sexes in about 1.1 hr following this dose and very rapid absorption half-life values were calculated (Table 19, 13.0 min, males, 33.3 min, females). The disappearance of radioactivity from the plasma was again well-described with a biexponential equation, just as it was in the 10 mg/kg peroral dose. The initial (α) phase for this clearance from the plasma was roughly similar, both between sexes ($t_{1/2}$: 7.5 hr, males; $t_{1/2}$: 4.9 hr, females) and in comparison with the same phase in the plasma curves for the 10 mg/kg peroral dose. However, the terminal disposition (β) phase was different for males ($t_{1/2}$: 60.2 hr) compared to the females ($t_{1/2}$: 32.0 hr) and this resulted in a greater contribution from the AUC_{96} to the male plasma AUC_{∞} value (17.6% of male AUC_{∞} value vs. 7.4% for females). There was a disproportionate increase for both sexes in the AUC_{∞} values between the 10 and 1000 mg/kg peroral plasma curves which was lower than would be expected for the 100-fold increase in dose (42.9-fold increase, males; 41.9-fold increase, females). Overall, the comparison of ^{14}C pharmacokinetic parameters between these two dose levels indicates that the total radioactivity (e.g., metabolites) at the higher dose may be handled in a slightly different manner than at the low dose.

Peroral Route (Intermediate Dose Levels): Pharmacokinetics Results

The pharmacokinetic parameter estimates (expressed as mean values), obtained from plasma radioactivity data collected following the peroral administration of ^{14}C -EG at 400, 600 or 800 mg/kg body weight dose, are summarized, respectively in Table 20. Individual animal data for these studies are presented in Appendix D and mean animal data from which group mean plasma values were used for these parameter estimations are presented in Appendix E. The plasma analysis for these dose levels was carried out using blood samples collected only at 24, 36, 48, 72 and 96 hr post-dosing. These blood sampling times were selected in order to avoid the disruption of $^{14}\text{CO}_2$ collection in the first 24 hr of the study (during which the bulk of the $^{14}\text{CO}_2$ is expired) and to define the elimination (β) phase of the plasma ^{14}C concentration vs. time curves. Thus, no attempt was made to define the absorption and initial disposition phases of the plasma curves for these doses.

Very similar pharmacokinetic parameters were derived from the plasma ^{14}C data collected following the peroral administration of a 400, 600 or 800 mg EG/kg dose (Table 20). When the plasma ^{14}C concentrations from 24-96 hr for the 10 and 1000 mg/kg dose groups were evaluated using RSTRIP (Figures 11 and 12), the slopes calculated for the β phase of these curves were strikingly similar to the slopes derived for the intermediate doses. Consequently, the terminal rate constants and the corresponding half-life values were virtually the same for both males (Part A of Table 20) and females (Part B) across all five dose levels. These terminal half-life values (obtained from 24-96 hr plasma time points) showed remarkable agreement, ranging from 28-31 hr for males and 26-33 hr for females. The similarity in these elimination half-life values indicates that elimination is independent of dose in this range for both males and females (Figures 11 and 12). Also, the calculated $\text{AUC}_{24-\infty}$ values were roughly proportional to the administered dose. Figure 13 graphically represents the linear relationship observed when $\text{AUC}_{24-\infty}$ is plotted as a function of the dose as further evidence that the processes which regulate the elimination of EG are following first-order relationships; the line drawn was derived from regression analysis of all data points except for the 1000 mg/kg data, which does not appear to be proximal for either sex to the line which can be drawn for the other dose levels. This plot is shown only to illustrate the relationship of the peroral AUC values to the administered dose and does not include standard deviations since the values were derived from mean plasma data.

Percutaneous Route: Pharmacokinetics Results

Pharmacokinetic parameter estimates (expressed as mean values) were obtained from plasma radioactivity data collected in the percutaneous studies over a 96-hr period post-dosing. Following the application of undiluted ^{14}C -EG in a 10 or 1000 mg/kg dose or as a 50% aqueous solution (1000 mg/kg), the test material was kept in contact with the skin for a 6 hr duration. A wash-off at 6 hr post-dosing was conducted as stipulated in the study protocol. These data are summarized in Tables 21, 22 and 23. The individual animal data for these studies are presented in Appendix D and mean animal data from which group mean plasma values were obtained for these parameter estimations are presented in Appendix E.

The absorption of an undiluted 10 mg/kg dose of ^{14}C -EG through skin of rats and the subsequent uptake and disappearance of radioactivity from the plasma (Figures 14, males, 15, females) were appropriately described with a biexponential equation which assumed first-order transfer processes (Table 21). Differences in both plasma uptake following skin penetration and in elimination were observed between the sexes at this dose level. Female rats achieved peak plasma concentrations at approximately 11.1 hr after dose application while male plasma concentration maxima did not occur until about 24 hr post-dosing. Furthermore, differences between the sexes could be found in the calculated absorption rate constants and their corresponding half-life values ($t_{1/2}$: 6.1 hr, males; $t_{1/2}$: 2.0 hr, females) and in the terminal rate constants and corresponding half-life values (50.4 hr, males; 48.2 hr, females). This difference was supported by the finding that approximately 9% of the dose was recovered in excreta and washed skin of the male animals in this study while 12% was found for females in these same fractions (data from Appendix E).

The disappearance of radioactivity from the plasma following an undiluted, percutaneous dose application of 1000 mg EG/kg was well-described by a two exponential equation with first-order transfer processes assumed (Figures 14, males, and 15, females). Absorption was faster than elimination from the plasma at this dose level. A similar absorption phase to that for the 10 mg/kg percutaneous dose (Table 21) was apparent for this high dose plasma curve, particularly with regard to penetration of the skin in females vs. males (Table 22). As was seen at the low dose, the peak plasma concentration (C_{max}) for females occurred in about half of the time (13.2 hr post-dosing) than it took for maximum levels to be achieved in the males (24.4 hr). These C_{max} values were also dose-proportional to the C_{max} values from the 10 mg/kg dose. While the magnitudes of these C_{max} values were roughly equivalent, the data indicate that a more rapid time course took place for skin penetration in females than in males. This conclusion is supported by the shorter absorption half-life for females (2.1 hr) compared to males (6.7 hr). Despite this apparent difference in the absorption time course, the elimination of the dose from the plasma was very similar for both sexes and was much slower than the absorption phase ($t_{1/2}$ for males: 65.4 hr; $t_{1/2}$ for females: 70.1 hr). Similar percentages of the applied radioactivity were recovered for either sex in excreta and washed skin: 13% for males and 12.5% for females (data from Appendix E).

Comparison of AUC_{96} values for the 1000 mg/kg percutaneous dose with those of the 10 mg/kg percutaneous dose (Table 21) indicated a dose-proportional increase in AUC to the last time point measured for both sexes. This comparison is consistent with a first-order behavior but a large contribution from the AUC_{0-6} is evident in the 1000 mg/kg dose group. However, because of the variation which is common in percutaneous plasma curves, this portion of the AUC may contribute substantially to an overestimation of the AUC_{96} , particularly for the female plasma curve in which the variation is quite evident. Overall, these data taken together with the low percutaneous dose information suggest that EG is absorbed through female skin in a slightly faster initial time-course than for males. In addition, it should be remembered that the majority of the dose was removed and rinsed from the dose site at 6 hr post-dosing. This implies that slightly larger amounts of an acute dose may be absorbed through female skin in the first 6 hr after exposure than for male skin. However, over the 96-hr study interval, roughly the same total amounts were absorbed and eliminated.

There was a difference in the time-course for skin penetration between the undiluted doses and the results for the 50% aqueous EG dose when applied at 1000 mg/kg (Table 23). Because virtually none of the dose penetrated, the plasma samples analyzed for both males and females were not quantifiable over the 96-hr experiment and these data could therefore not be modeled using RSTRIP. On the other hand, the summary of recovery data given in Table 23 clearly shows that only 1.1 - 1.3% of the applied radioactivity penetrated skin as a result of the 6-hr contact period with this dose. This clearly indicated that contact with a 50% aqueous dilution of EG does not pose an extensive absorption potential, even over 6 hr under occlusive conditions.

RESULTS: PLASMA GC/MSD ANALYSIS FOR UNCHANGED ETHYLENE GLYCOL

Results for the GC/MSD analysis of pooled plasma samples from the pharmacokinetics studies are summarized in detail in Appendix C. Selected plasma samples were pooled in equal volumes following collection, derivatized with phenylboronic acid (PBA), and analyzed for the cyclic phenylboronate ester of EG using capillary GC with a Mass Selective Detector (MSD). Unchanged EG was identified in these plasma samples using a Selective Ion Monitoring (SIM) technique with the MSD and was quantitated in comparison to PBA-derivatized 1,3-propylene glycol used as an internal standard. These EG concentration results were then used in RSTRIP analysis for the disappearance of EG from the plasma vs. time. Results from the GC/MSD analyses were obtained from all dose groups except for the undiluted 10 mg/kg percutaneous study (due to unchanged EG concentrations which were below the limit of detection) and for the 50% aqueous percutaneous dose (radioactive concentrations were not quantifiable).

Plasma Pharmacokinetics of Unchanged Ethylene Glycol

The results for the pharmacokinetic parameter estimates which were calculated for the 10 and 1000 mg/kg intravenous plasma EG concentrations from GC/MSD analysis are summarized, respectively, in Tables 24 and 25. For the 10 mg/kg intravenous dose, EG concentrations were only quantifiable up to 8 hr for males and only to 4 hr for females (Table 24). Disappearance of the unchanged EG from plasma was well-described by a monoexponential equation with a rate constant of elimination which indicated a rapid clearance from the plasma at this dose level (Figures 16 and 17). Elimination half-lives were similar between the sexes (0.83 hr, males; 1.18 hr, females) and both were of short duration (Table 24). The unchanged EG from a 1000 mg/kg dose was also cleared rapidly from the plasma in a monoexponential manner for both sexes, displaying an elimination rate which was similar to that for the low dose plasma curve (Table 25). A half-life of elimination value of 1.98 hr for males and 1.73 hr for females was computed from the data for these plasma curves. In addition, AUC_{∞} values for both sexes were dose-proportional to AUC_{∞} values for the 10 mg/kg plasma data, indicating that EG is probably cleared from the plasma in a first-order manner for either dose level. This conclusion is supported by the plots which contrast the disappearance from plasma of both unchanged EG and radioactivity concentrations as a function of time for both males (Figure 16) and females (Figure 17). The unchanged EG plasma concentrations decreased rapidly in the first 0-12 hr for low dose animals and in the first 24 hr post-dosing for the high dose animals, while radioactivity concentrations disappeared from plasma much more slowly.

Peroral plasma concentrations of unchanged EG vs. time are presented in Figures 18 (males) and 19 (females); the corresponding pharmacokinetic parameter estimates are summarized in Tables 26 and 27. Extremely rapid absorption of EG occurred following peroral intubation for both dose levels administered and in both sexes. The absorption rate constants were an order of magnitude higher than the rate constants of elimination in all cases, and the derived absorption half-life values were on the order of 10-20 min for the 10 mg/kg dose (Table 26) and 12-27 min for the 1000 mg/kg dose (Table 27). For the 10 mg/kg peroral dose, the absorption and elimination of unchanged EG was again well-described by a monoexponential fit of the data with first order transfer processes. The elimination rate was slower than the rate of absorption and these elimination phases displayed half-life values of 1.42 hr for males and 2.48 hr for females (Table 26). The last quantifiable time point was at 12 hr post-dosing for males (Figure 18) and 18 hr post-dosing for females (Figure 19), making the overall clearance of unchanged EG from the plasma a comparatively rapid process. The uptake and elimination from the plasma following the 1000 mg/kg peroral dose was also well-described by a two-exponential fit of the data with first order transfers assumed. The absorption rate constants from these data (Table 27) were of the same order of magnitude as those for the 10 mg/kg peroral dose (Table 26), with correspondingly rapid absorption half-life values (0.44 hr, males; 0.20 hr, females). In addition, the elimination rates and their corresponding half-life values were of similar duration between dose levels and for both sexes. Taken together with the fact that the calculated C_{max} and $AUC_{0-\infty}$ values were also dose-proportional between dose levels for each sex, a linear (concentration-related) pharmacokinetic relationship for unchanged EG was apparent for these two peroral dose levels.

The samples collected for the evaluation of unchanged EG plasma concentrations from the 400, 600 and 800 mg/kg peroral dose groups were not quantifiable at the time points selected and could not be used for pharmacokinetic parameter estimation. This resulted from provisions in the study design to collect blood samples only at 24, 36, 48, 72 and 96 hr post-dosing. Earlier sampling times were not selected in order to minimize possible losses of $^{14}CO_2$ samples during collection caused by the frequent opening of the cages to draw blood samples. However, the data described previously for unchanged EG following the 10 and 1000 mg/kg doses bracket these intermediate levels and clearly demonstrated the EG in unchanged form is cleared from the plasma during 18-24 hr period post-dosing. Therefore, the absence of quantifiable levels for unchanged EG at time points beyond 24 hr after dosing is consistent for the 400, 600 and 800 mg/kg peroral doses.

Percutaneously-applied doses of undiluted EG resulted in quantifiable levels of unchanged EG in the plasma for only the 1000 mg/kg male and female animals (Table 28). As for the peroral dose groups, a biexponential equation was sufficient to describe the skin penetration and elimination from the plasma of unchanged EG for a 1000 mg/kg application. The absorption rate constants for unchanged EG concentrations and the analogous ^{14}C concentrations (Table 22) were of similar magnitude, indicating that EG is probably being absorbed in the unmetabolized form through the skin. Further evidence for absorption of essentially unchanged EG through the skin was observed in urinalysis data for this high dose (presented in a subsequent section of this report). However, the elimination of EG from the plasma displayed a faster time-course than was apparent for radioactivity in this dosing application (Figure 20). This elimination rate was faster than the

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rate of elimination for radioactivity (Table 22) by a factor of about 3 for males and by a factor of almost 10 for the females. However, C_{max} and t_{max} values were roughly similar to these values for the radioactive plasma data for a 1000 mg/kg undiluted dose (Table 22) and to the t_{max} value from the 50% aqueous percutaneous dose (Table 23). These similarities further support the conclusion that EG is probably penetrating the skin in its unchanged form.

Bioavailability of Unchanged Ethylene Glycol

The results for bioavailable dose (unchanged EG) were derived from data for the non-systemic dosing routes (peroral and percutaneous) and are summarized in Table 29. These calculations were based on the $AUC_{0-\infty}$ values derived from plasma concentration curves for unchanged EG (Tables 24-28). Overall, EG has virtually complete peroral bioavailability for both sexes at the two dose levels measured; this was confirmed in the absolute bioavailability calculated from total radioactivity for these doses (Part B of Table 32). Also, while the intermediate peroral doses were not quantifiable for unchanged EG, it can probably be assumed that $AUC_{0-\infty}$ values for these doses would also indicate a virtually complete peroral bioavailability. This outcome is consistent with the rapid absorption and elimination of EG apparent from the peroral plasma curves and indicates that most, if not all, of an orally ingested dose is taken up and systemically distributed. Percutaneous bioavailability was lower than it was for peroral doses, with a 1000 mg/kg undiluted EG dose being 22.5% bioavailable for males and 16.4% bioavailable for females over a 6 hr exposure to the dose with a wash-off step included. Similar bioavailability values were obtained for total radioactivity from undiluted percutaneous doses (Part B of Table 33). The agreement between the sexes is reasonable here and suggests that a substantial amount of an EG dose will penetrate skin unchanged over the course of a 6-hr exposure, despite removal of any unabsorbed dose at the end of this period.

RESULTS: ANALYSIS OF URINE SAMPLES BY HPLC

The HPLC analysis of selected urine samples from the high dose (1000 mg/kg) studies resulted in detectable levels of EG, as measured by the refractometer, for all routes of administration. Furthermore, measurable amounts of glycolic acid and/or oxalic acid were also present, and were qualitatively identified by comparison of retention times with metabolite standards. Generally, the levels of EG and metabolites in urine samples from the low dose (10 mg/kg) studies could not be detected by the refractometer. However, retention times for the radioactive peaks in these samples were compared to those for characterized peaks from the high dose studies to determine metabolite profiles. EG and its proposed metabolites (oxalate, glyoxalate, glycolaldehyde and glycolate) had distinct, reproducible retention times under the stated operating conditions. Although the glycolaldehyde and glycolic acid peaks were not resolved to baseline, there was a 0.4 minute difference in their retention times which permitted their identification when present at quantifiable levels. This resolution had not been achieved with the HPLC method used by Marshall (1982). Verification experiments indicated that glycolaldehyde and glycolic acid could still be separated when the acid was present in fifty-fold excess. The minimum detectable level of glycolaldehyde was approximately 5 μ g injected.

Table 30 contains the mean values from urinalyses results for animals from the intravenous, oral and percutaneous studies; individual animal results are contained in Appendix C. The results are expressed as the mean percentages of total peak radioactivity for all of the quantifiable peaks in a run. Overall, ^{14}C -labelled ethylene glycol, glycolic acid and/or oxalic acid accounted for the majority of the detectable radioactivity in the urine samples from all dose routes. The occurrence of radioactive peaks that could not be associated with EG or known metabolites was relatively rare and may have been due to peak spreading. No glycolaldehyde or glyoxylic acid was detected in any samples. In general, the individual urinalyses show that the proportion of unchanged ^{14}C -EG to that of labelled metabolites was fairly consistent among animals in a given dose group (cf. Appendix C).

The urinary excretion of ^{14}C for the high (1000 mg/kg) and low (10 mg/kg) dose intravenous studies, as noted in the study by Marshall (1982), indicated that a dose-dependent increase in the amount of glycolic acid was observed. Approximately 65% of the radioactivity in the high dose urines at 12 hours was due to unchanged ^{14}C -EG and the remaining 35% was accounted for by glycolic acid. In the low dose urine samples, ^{14}C -EG comprised about 93% of the detectable radioactivity at 12 hours. A slight increase in the amount of urinary glycolic acid (45%) was observed at 24 hours in the high dose samples for both sexes. In addition, the male urine samples from the 1000 mg/kg dose contained a significant percentage (17%) of oxalic acid at this collection interval.

The ^{14}C content of urine samples from the high (1000 mg/kg) and low (10 mg/kg) dose oral studies was similar to that for the IV studies. The majority of the radioactivity (92-96%) in the low dose samples was due to ^{14}C -EG at both 12 and 24 hours post-dosing. An increase in urinary glycolic acid was observed in the high dose oral samples relative to the low dose. However, in comparison to the high dose intravenous results, the percentage of glycolic acid (approximately 24% at 12 hours) was slightly lower. Oxalic acid accounted for less than 8% of the total peak radioactivity in urines from the high and low dose studies. In comparison to the intravenous and oral results, the most notable differences in the percutaneous urine samples were the greater percentages of urinary ^{14}C -EG at the 1000 mg/kg dose level (87-100%), and the lack of detectable oxalic acid in any samples. At the 50% aqueous 1000 mg/kg dose level (effectively 500 mg/kg), exposure to the test chemical was terminated after 6 hours; urinary ^{14}C -EG accounted for 100% of the detectable radioactivity through 36 hours. These results indicate that measurable evidence for metabolism of ^{14}C -ethylene glycol, based on measurements in the urine, did not occur following percutaneous exposure.

DISCUSSION

The results from this investigation have provided evidence for the following conclusions:

1. The disposition of ethylene glycol following either intravenous or peroral routes was essentially the same, with similar percentages recovered in tissues and excreta for a given dose by both routes and for either sex (cf. Figures 5).

2. The dose-dependency described in prior studies for the disposition and excretion of intravenous ethylene glycol doses was confirmed in the present study and extended for the same doses when administered perorally.
3. There was an absence of dose-dependent changes in disposition and elimination following the percutaneous application of ethylene glycol.
4. The apparent absence of any dose-dependent pharmacokinetic patterns, which were observed in the plasma concentration vs. time curves, may be explained by the metabolism of ethylene glycol.
5. With the exception of slightly faster absorption rates for skin penetration by the radioactive dose in female rats than for males, no clear sex differences in the overall uptake, metabolic rate or elimination of EG were observed for any route of administration in this study.

Several general features regarding the disposition of total radioactivity were apparent in the material balance portion of these experiments. A generally higher percentage of the dose was found in tissues and carcass from the low intravenous and peroral doses and is probably related to the extensive metabolism of EG. The observed higher tissue/plasma values for liver, kidney and lung are consistent with metabolism and excretion of EG by the routes indicated in the disposition data. Also, the greatest variation in excretion recovery values occurred in the first 12 hr after dosing, particularly for the urine recoveries. Although 96 hr post-dosing was selected for the period of excreta collection, virtually all of the ¹⁴C which was voided in the urine and most of the dose fraction expired as ¹⁴CO₂ was excreted in the first 24 hr after dosing for the intravenous and peroral studies. This collection period was selected to follow the design used by Marshall (1982) in the collection of excreta following intravenous dosing with ethylene glycol. However, the 24-96 hr collection period had the greatest significance for the percutaneous dose applications, during which the largest component of penetration of EG was occurring from 12-24 hr post-dosing. For the most part, the elimination of these percutaneous doses took place over the final 24-96 hr of the experiment.

Similar disposition patterns to those reported by Marshall (1982) for intravenous EG doses of 20, 200 and 1000 mg/kg were observed for approximately the same intravenous doses in the study reported here. Furthermore, this pattern was also shown to be true for the peroral material balance recoveries (Figure 5). In fact, EG by the peroral route was extremely rapidly absorbed and, as presented in the pharmacokinetics results (Table 29), essentially showed a complete oral bioavailability when compared with analogous intravenous doses. Accordingly, for both of these routes the dose-dependent changes in excretion routes and amounts agreed with the prior results of Marshall (1982) who reported an increased excretion of ¹⁴C in urine with increases in dose, along with a concomitant decrease in expired ¹⁴CO₂. The urine analytical results from this study also concur with Marshall's report of increasing glycolate concentrations in the urine as dose is increased. The output of glycolate in the urine (as a % of the recovered dose) only increased approximately 10-fold when the intravenous dose was increased from 10 to 1000 mg/kg in our study (cf. Appendix C). Similar

increases in glycolate concentrations were detected in urine samples collected during the first 24 hr after peroral doses of EG, thus confirming that, within the dose range tested, peroral doses of EG followed similar elimination patterns to those for intravenous EG doses.

The dose-dependent changes in routes and rates of excretion were further examined for the peroral route of administration. Doses of 400, 600 and 800 mg/kg were administered perorally, based on the following rationale: The selection of the 10 and 1000 mg/kg dose levels for the present study was based on previous intravenous dose levels of 20, 200, 1000 and 2000 mg/kg used by Marshall. Also, the 100-fold difference in dose levels was selected to examine a first-order (dose-proportionate) vs. dose-dependent nature in EG pharmacokinetics. Evaluation of doses intermediate to the 10 and 1000 mg/kg dose levels was attempted using 200 mg/kg increments for higher doses from the 200 mg/kg intravenous dose data available from the Marshall paper. This seemed to be a reasonable approach, since the excretion data from this prior 200 mg/kg intravenous dose was virtually identical to the amounts excreted in urine and expired $^{14}\text{CO}_2$ for either a 20 mg/kg dose (Marshall, 1983) or the 10 mg/kg intravenous dose in the present investigation. Taken together, these data effectively defined 200-1000 mg/kg as the dose range for which no pharmacokinetic information by the peroral route existed in the rat.

In the attempt to define a "break point" for the dose-dependent shift in routes of excretion, it appeared that the data followed more of a gradual transition, particularly for expired $^{14}\text{CO}_2$, than an actual "break point" for this change. The amounts of radioactivity excreted in the urine following peroral doses of 400 and 600 mg/kg (Table 10) indicated a similar disposition to the lowest dose (10 mg/kg) for male urine data (cf. Figure 6). Conversely, the expired $^{14}\text{CO}_2$ levels decreased steadily as the dose was increased. Thus, there was no clear-cut shift in excretion routes as compared to the 1000 mg/kg dose, which showed increased urinary ^{14}C output and decreased $^{14}\text{CO}_2$ expiration. Consequently, the 800 mg/kg dose was administered to narrow the range further. These data confirmed a transition in the anticipated dose-dependent shift from more to less $^{14}\text{CO}_2$ elimination as dose was increased. Equivalent amounts of the dose were excreted by either route for males and slightly more of the dose was excreted in urine for females. Therefore, due to the individual variation in the data which is inherent for interval urinary sample collection, it was only possible to define the transition as occurring between 10 and 400 mg/kg for the females and between 800 and 1000 mg/kg for the males. Given the nature of the dose-dependent changes in the overall metabolism of EG, it is reasonable to expect that this shift will be more of a gradual transition than a well-defined break in the dose-response curves. This has been described for a case in which the elimination of a drug occurs by a combination of saturable (or Michaelis-Menten) kinetics and a parallel apparent first-order process (Gibaldi and Perrier, 1975). Thus, the drug exhibits $t_{1/2}$ values which are independent of dose at dose levels where the K_m is not exceeded. However, as the capacity-limited process (e.g., metabolism) is overwhelmed, the rate of the parallel first-order elimination process increases proportionately to the increase in dose. These parallel processes appear to be responsible for the overall elimination of EG.

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Penetration of the skin by EG did not follow this same dose-dependent behavior between the 10 and 1000 mg/kg doses. Instead, percutaneous absorption followed apparent first-order relationships, as indicated by the dose-proportionate increases in AUC values. This resulted in tissue and excreta recoveries which were similar, regardless of the dose level applied. Following an application of the undiluted EG doses, the absorption process was slow over the initial 24 hr of the experiment. This was indicated by substantially lower recovery values in excreta (as percent of dose) than for the other two routes. In addition, virtually all of the urinary radioactivity voided in the initial 24 hr after applying the 1000 mg/kg dose was excreted as unchanged ^{14}C -EG. Thus, the urinary elimination of either undiluted percutaneous EG dose (10 or 1000 mg/kg) resembled the excretion profiles exhibited by the 10 mg/kg intravenous dose. This evidence indicates that the slow absorption of EG through the skin acted to limit the overall exposure to EG. This was further illustrated by the differences observed between the balance and pharmacokinetics studies following a 50% aqueous dose of EG. In the balance studies, the absorption of a 50% aqueous dose left in place for the entire 96-hr collection period amounted to 22-26% of the dose applied. However, plasma ^{14}C levels following the same dose in the pharmacokinetic studies (washed off the application site 6 hr after dosing) were not quantifiable for both males and females. Thus, the time-course for skin penetration appeared to be remarkably slower for a 50% aqueous dose than for an undiluted one.

The recovery of dose was much lower for the 10 mg/kg balance study than for the other two percutaneous dose levels. This was not a conclusive finding since data from the pharmacokinetic study at this dose level indicated a higher overall recovery of dose from the occlusion materials (cf. Table 23) than was found in the corresponding balance study for this dose. Relatively the same amount of radioactivity was applied to rat skin in this second study and, while these recoveries were obtained at 6 hr after dose application in this second study (due to a provision to wash off the dose at that point), approximately 87-94% of the dose was recovered in the occlusion materials. This large loss in the apparent recovery for the balance study may be related to small errors in the weighing and delivery of the very small dosing volumes (2-2.5 μl) applied in this study. Such losses could have resulted in an overestimation of delivered dose. It therefore seems that much of the missing fraction for this 10 mg/kg dose may have been lost prior to, if not during, the 96-hr exposure period in the balance study.

A summary of the pharmacokinetic parameter estimation information for each route of administration studied is presented in Tables 31-33. Data for both unchanged EG and total radioactivity plasma concentrations are presented in these tables to contrast the differences in EG pharmacokinetic behavior vs. that for its metabolites. The unchanged EG data for the intravenous route (Table 31, Part A) demonstrate a first-order kinetic behavior (dose-linear) between the 10 and 1000 mg/kg dose levels. This is evident from AUC₀ values which are roughly dose-proportional. Furthermore, dose-independent relationships can be seen in the values for the total clearance of EG ($\text{Cl}_{\text{total}}^{\text{EG}}$), the mean residence time (MRT), the apparent volume of distribution at steady state (V_d^{SS}), the terminal half-life ($t_{1/2}^{\text{S}}$), the total amount of EG excreted in the urine (U_{EG} , quantitated to 24 hr post-dosing), and the renal ($\text{Cl}_{\text{renal}}^{\text{EG}}$) and metabolic ($\text{Cl}_{\text{non-renal}}$) clearance values. Taken together, these parameters all demonstrate an

apparent first-order plasma time-course (Figures 16 and 17). This is not apparent in the excreta profiles, for which a dose-dependent relationship exists for total radioactivity: e.g., U_{∞} and $^{14}CO_2^{\infty}$ (as percentages of administered dose) changed in magnitude as the dose level was increased (Part B of Table 31). Marshall (1982) made similar conclusions regarding the absence of any dose-dependency in the clearance of ^{14}C from plasma. Marshall also found this apparent dose-independence in plasma data and concluded that this was the direct result of a compensatory renal clearance of ^{14}C -labeled glycolate in conjunction with a decrease in expired $^{14}CO_2$ output. Increases in urinary ^{14}C -glycolate were observed in the present study when the dose was increased from 10 to 1000 mg/kg EG, indicating that metabolism makes a substantial contribution to the terminal disposition phase of the plasma disappearance curves for the higher dose level. This difference can be readily seen in the plasma curves for unchanged EG following intravenous doses (Figures 16 and 17). This was particularly true for the 10 mg/kg plasma radioactivity curves (Figures 7 and 8), for which an apparent increase in concentration can be observed in the 2-8 hr portion of the curve. This apparent influx of radioactivity may represent an entry of metabolites derived from EG into the plasma, since EG was shown to be so rapidly cleared ($t_{1/2}$ of 50-70 min).

Further evidence for this dose-independent behavior in plasma pharmacokinetics was observed for the peroral administration of EG (Table 32). Again, AUC_{∞} was roughly dose-proportionate between the high and low dose levels, while apparent Cl_{total}^{EG} , MRT, apparent V_d^{SS} , $t_{1/2}^B$, U_{∞}^{EG} and the apparent renal and non-renal clearance values were all independent of dose for this administration. The remarkable similarities of peroral to intravenous parameter estimates are consistent with the nearly 100% bioavailability calculated for the same doses. The rapid disappearance of unchanged EG vs. radioactivity concentrations can be readily viewed in the plot for simultaneous curves for these data (Figures 18 and 19). Furthermore, the dose-dependency for urine and $^{14}CO_2$ excretion can be readily seen in the values calculated for U_{∞} and $^{14}CO_2^{\infty}$ (Part B of Table 32). The dose-independent behavior for AUC_{∞} , MRT and $t_{1/2}^B$ in these radioactivity data suggested that the total radioactivity profile was still first-order in the plasma following peroral doses, despite this dose-dependent shift in excretion routes for radioactivity. For the undiluted dose percutaneous studies (Table 33), dose-proportionality could not be compared for the unchanged EG data (Part A) since the 10 mg/kg dose EG concentrations were not quantifiable. However, the high dose data show that the fate of EG in plasma had virtually the same time-course as the radioactivity did in this study. This was readily confirmed in the similar values calculated for absolute bioavailability when either unchanged EG plasma data (Table 29) or total radioactivity data (Table 33) are compared. The EG terminal half-life ($t_{1/2}^B$) values were of the same magnitude as the corresponding values for radioactivity data. These similar time-courses can be readily appreciated in the simultaneously plotted radioactivity curves for EG and radioactivity concentrations (Figure 20). Dose-proportional characteristics were evident as well from the radioactivity data in this table (Part B) for the AUC_{∞} , U_{∞} and $^{14}CO_2^{\infty}$ values. There were longer terminal $t_{1/2}$ values obtained using the high dose data than for the low dose data, but these values were still of the same general magnitude between doses. A similar increase in the mean residence time was noted between doses as well. However, these inconsistencies were probably related to the incomplete definition of the terminal phase in these

plasma curves (Figures 14 and 15). There is a large contribution to the AUC_∞ value from the AUC_{0-∞} estimation resulting from the shallow slope for this phase in the curve. Since this portion of the AUC_∞ is determined from AUC_{0-∞} + terminal slope, apparent disproportionalities have resulted. Given the variability normally found in percutaneous plasma data, and the fact that the terminal phase of these plasma curves did not span at least three half-lifetimes, these data are not unreasonable.

Marshall (1982) postulated that the reasons for both the dose-dependent shift in ¹⁴C excretion routes and the apparent absence of a dose-dependency in the plasma clearance curves following large intravenous doses of EG are related to a limited glycolate metabolism and excretion of this acid into the urine. Glycolate has been shown to be an important metabolite in the biotransformation of EG by several investigators (McChesney, et al., 1971; Richardson, 1973; Clay and Murphy, 1977) and this intermediate is readily converted to glyoxylate at low concentrations. Marshall suggested that the rate-limiting step in the metabolism of EG at high doses was the conversion of glycolate to glyoxylate. Additionally, in low concentrations the conversion of glycolate to glyoxylate and then glyoxylate to oxalic acid are mediated by the same enzyme, glycolic acid oxidase, and glycolate and glyoxylate are competitive inhibitors under *in vitro* conditions (Richardson and Tolbert, 1961). Glycolate has a greater affinity for this enzyme than does glyoxylate and there thus exists a great potential for capacity-limited accumulation of glyoxylate following high doses of EG. At the same time, because this enzymatic step is rate-limiting, a compensatory, parallel elimination process (e.g., renal) becomes the dominant excretion route at high doses. Conversely, the presence of oxalate in urine, which was seen in the present study principally after low doses of EG (cf. Table 30), can be explained by this inhibition, which would act to limit oxalate production at higher doses. However, because the parallel urinary excretion process is probably first-order, the contribution to plasma total radioactivity levels from intermediate metabolites derived from these saturable metabolism pathways is probably negligible. For this reason, the effect of such a capacity-limited process on the dose-related pharmacokinetic profile would not be apparent. Accumulation of glyoxylate probably results in a toxic consequence for the animal. Evidence for this possibility was presented by Richardson (1973), who demonstrated that a partial hepatectomy increased the acute toxicity of glyoxylate but decreased the toxicity of ethylene glycol and glycolate when rats were fed these chemicals. Thus, the evidence presented in the study reported here appears to support this capacity-limited shift to glycolate excretion in the urine for both the intravenous and peroral routes.

In summary, we have shown that ethylene glycol given by three different routes demonstrates apparent first-order pharmacokinetic behavior for the disposition in and the elimination from the plasma. At the same time, dose-dependent changes occur in the excretion of ¹⁴C into the urine and ¹⁴CO₂ expiration after single doses for the intravenous and peroral, but not the percutaneous, routes. In general, while limited conclusions can be drawn from the plasma radioactivity concentration data, more reliable conclusions about the fate of EG can be made from the definitive plasma uptake and elimination data obtained for unchanged EG in this study. In addition, the HPLC analytical results from this investigation have gone further in resolving the possible presence in urine of glycoaldehyde from one of its metabolic precursors, glycolate. The glycoaldehyde moiety was resolved from glycolate by our method but the presence of this chemical species still was not detected at

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levels above the stated limit of detection (cf. Appendix C). It appears from our data in Sprague-Dawley rats and that of Marshall (1982) in Fischer 344 rats that the dose-dependent changes in EG metabolism are a direct result of alterations in the metabolic patterns of EG and compensatory urine clearance of glycolate. Thus, the presence of glycolate in urine increases with dose and does not result in dose-dependent changes in the plasma pharmacokinetic profile for EG. These results are consistent with clinical reports of metabolic acidosis when EG is ingested by humans (Clay and Murphy, 1977).

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Table 1

Summary of Animal Body Weight Means and Radioactive Dose
Delivered for Ethylene Glycol Material Balance Studies With
Male and Female Sprague-Dawley Rats

	Study Initiation Body Weight (g)	Radioactive Delivered Dose	
		¹⁴ C Administered (μ Ci Per Rat)	Normalized Dose (Per 0.2 Kg BW ^a)
I. Intravenous Administration			
A. Low Dose^b			
Males	281.25 \pm 1.50	22.16 \pm 0.20	15.76 \pm 0.08
Females	214.25 \pm 5.60	16.85 \pm 0.53	15.72 \pm 0.15
B. High Dose^c			
Males	281.50 \pm 4.80	14.73 \pm 0.45	10.46 \pm 0.22
Females	233.00 \pm 14.90	12.00 \pm 0.72	10.30 \pm 0.04
II. Peroral Administration			
A. Low Dose^b			
Males	282.25 \pm 2.06	16.12 \pm 0.65	11.42 \pm 0.39
Females	208.25 \pm 0.96	11.95 \pm 0.18	11.48 \pm 0.17
B. High Dose^c			
Males	280.50 \pm 6.03	11.01 \pm 0.01	7.70 \pm 0.17
Females	208.75 \pm 2.63	8.31 \pm 0.12	7.96 \pm 0.03
III. Percutaneous Administration			
A. Undiluted Low Dose^d			
Males	280.50 \pm 0.37	32.50 \pm 0.50	23.17 \pm 0.50
Females	211.00 \pm 1.15	23.90 \pm 0.80	22.96 \pm 0.66
B. Undiluted High Dose^e			
Males	277.5 \pm 0.6	24.35 \pm 0.57	17.55 \pm 0.38
Females	218.5 \pm 4.1	19.33 \pm 0.24	17.70 \pm 0.23
C. 50% (w/w) Aqueous High Dose^c			
Males	277.75 \pm 9.25	19.41 \pm 0.63	13.98 \pm 0.09
Females	214.75 \pm 2.99	15.06 \pm 0.11	14.03 \pm 0.12

^a Radioactive dose delivered (in μ Ci) \times (0.2 kg + body weight in kg);
presented to demonstrate equivalency of radioactive dose administered between
sexes.

^b Low dose was 10 mg EG/kg body weight administered in 0.5% water solution.

^c High dose was 1000 mg EG/kg in 50% (w/w) water solution.

^d Low dose was 10 mg EG/kg applied undiluted.

^e High dose was 1000 mg EG/kg applied undiluted.

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APPENDIX CINTRODUCTION

The analytical phase of the ethylene glycol (EG) pharmacokinetics and material balance study included dose solution, plasma and urine analyses. Concentrations of EG in the dosing solutions were measured by capillary gas chromatography (GC) with flame ionization detection (FID). Ethylene glycol in selected pooled plasma samples from the pharmacokinetics studies was derivatized with phenylboronic acid to form a cyclic phenylboronate ester and then analyzed by capillary GC coupled to a Mass Selective Detector (MSD). The identification of EG and several known metabolites in selected urine samples from the material balance studies was accomplished using high performance liquid chromatography (HPLC) with refractive index detection. In addition, the proportion of unchanged ^{14}C -EG to that of major labelled metabolites in the urine samples was quantified using a radioactivity flow monitor in series with the refractometer. The following sections contain a summary of the analytical methods and results.

MATERIALS

A sample (1 quart) of Polyester Grade Ethylene Glycol (CAS No. 107-21-1) was received at BRRC on February 18, 1987 from Union Carbide Corporation, Hahnville, LA. The material was a clear, slightly viscous liquid and was labelled with the identification number PC-35227, UCC B35227 12/86 4407. The chemical was given the BRRC No. 50-74. Aliquots of the sample were transferred to a scintillation vial for analytical use as needed. The chemical was stored at ambient temperatures throughout the course of the study.

Two shipments of [1,2- ^{14}C]-ethylene glycol were received at BRRC from Sigma Chemical Company, St. Louis, MO. The first shipment arrived on March 24, 1987 and included three aliquots which were given the BRRC Nos. 50-160A, B, C. The second shipment was received on September 2, 1987 and also included three aliquots which were given the BRRC Nos. 50-411A, B, C. Related correspondence stated that the radiochemical purity of the shipments was 99.2% and 98.0% respectively. The samples were stored in the freezer except during dose solution preparation.

The reagents used in the plasma analyses were all obtained from Aldrich Chemical Company, Milwaukee, WI. The internal standard, 1,3-propanediol, had a stated purity of 98%. The derivatization reagent was phenylboronic acid in 2,2-dimethoxypropane. Acetonitrile (HPLC grade) was obtained from Burdick and Jackson Labs, Muskegon, MI.

Several known metabolites of EG were used as standards in the HPLC analysis of urine samples from the material balance studies. Glycolaldehyde monomer was purchased from Sigma Chemical Co., St. Louis, MO. Glyoxylic acid, glycolic acid, oxalic acid, and glycolaldehyde dimer were obtained from Aldrich Chemical Co., Milwaukee, WI. Sulfuric acid (Reagent A.C.S.), used in the preparation of the HPLC mobile phase, was a product of Fisher Scientific Co., Fairlawn, NJ. Monophase® S, obtained from Packard Instrument Co., Downers Grove, IL, was used as the scintillation cocktail in the radioactivity flow monitor.

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METHODS

GC Analyses

Dosing solution analyses were conducted on a Hewlett Packard 5880 GC equipped with a Carbowax 20M fused silica capillary column and a flame ionization detector. The operating conditions are listed in Table 1. Dosing solutions were prepared in physiologic saline (0.9% NaCl) for the intravenous studies, and in Millipore® water for the oral and dermal studies. The stability of the test chemical in both matrices when stored frozen was measured. Stock standard solutions of EG were prepared in the same solvent as the dose solution being evaluated. Dilutions of the stock standard were then prepared in acetonitrile. Based on concentration, weighed aliquots of the dosing solution were also diluted in acetonitrile to fall within the linear concentration range of the standard curve. Pre- and post-dosing aliquots were analyzed to ensure that the solutions had remained homogeneous throughout the dosing period. The concentration of EG in each aliquot was obtained from the equation for the standard curve developed by linear regression. Pre and post-dosing values were averaged. The injection volume for standards and samples was 1 µl.

Plasma samples were chosen for analysis by the study director based on sample availability. At selected time intervals, plasma aliquots of equal volume from each animal per dose group were pooled and the total weight was recorded. The pooled plasma samples were stored in the freezer until analysis. The method used for the determination of ethylene glycol in plasma was a modification of the derivatization procedure reported by Flanagan, *et al.* (1987). In this procedure, ethylene glycol reacts with phenylboronic acid to form the cyclic phenylboronate ester. An internal standard, 1,3-propanediol, which also forms a phenylboronate ester, is added to the reaction mixture to improve the reliability of the assay. Both derivatives can then be measured by gas chromatography.

In our application, phenylboronic acid (120 or 40 mmol/L) in 2,2-dimethoxypropane was prepared as needed. The internal standard, 1,3-propanediol, was used as a solution in acetonitrile. Both solutions were refrigerated between analyses. Plasma standards ranging from 2000 to 1 µg/ml ethylene glycol in control plasma were prepared as needed and stored frozen between uses. Appropriate standards were derivatized at each analysis in an attempt to bracket the range of expected EG concentrations in the study samples. Sample or standard (100 µl) was pipetted into a 700 µl Microtainer® Brand Tube (no additive). Internal standard solution (200 µl) and derivatization reagent (100 µl) were then added using a Gilson Pipetman®. The tube was tightly capped, and the contents were vortex-mixed (30 sec.) and centrifuged at 14,600 RPM for 4 minutes (Eppendorf 5412 centrifuge). A portion of the supernatant was transferred to a 0.1 ml conical glass autosampler vial for analysis. Plasma blanks were injected between samples to minimize the possibility of carryover. The injection volume for standards, blanks and samples was 1 µl.

The plasma analyses were conducted on a Hewlett Packard 5890 GC equipped with a bonded phase DB-1 (J & W Scientific, Rancho Cordova, CA) fused silica capillary column. Concentrations of the cyclic phenylboronate derivatives of ethylene glycol and 1,3-propanediol were measured using a Hewlett Packard 5970

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Mass Selective Detector (MSD) operated in a Selective Ion Monitoring (SIM) mode. The chromatographic conditions were developed at BERC since the original method of Flanagan, et al. (1987) was optimized for packed column analysis. The GC/MSD operating conditions are listed in Table 2. A calibration curve was generated for each analysis by plotting the response ratio (peak area) vs. the amount ratio of the derivatized ethylene glycol/1,3-propanediol in the plasma standards. Amounts of ethylene glycol in the plasma samples were then derived from this curve.

HPLC Analyses

All urinalyses were conducted on a Waters Associates High Performance Liquid Chromatographic (HPLC) system. The system consisted of a WISP 710B, an Automated Gradient Controller, a Model 6000A solvent pump, a Differential Refractometer model R401, and a QA-1 Data Integrator. In addition, a Packard Trace II® Radioactivity Flow Monitor was connected in series with the refractometer and was used for ¹⁴C-detection. The Trace II® was equipped with a 0.5 ml homogeneous cell in which the HPLC mobile phase and the scintillation cocktail were mixed in a 1:6.5 ratio. Based on the work done by Marshall (1982), an Aminex HPX-87H anion exchange column (BioRad Laboratories, Richmond, CA) was used to separate EG from its oxidative metabolites. The operating conditions are listed in Table 3.

Standard solutions of ethylene glycol in urine were prepared at various concentrations to produce a range of 10 to 250 µg EG depending upon injection volume. In addition, aqueous solutions of EG and its oxidative metabolites (oxalic acid, glyoxylic acid, glycolaldehyde and glycolic acid) were prepared separately and in combination to establish retention times by refractive index detection. A solution of ¹⁴C-EG in either urine or water was used to verify the identity of the radioactive EG peak in study urine samples.

The urine standards and samples selected for analysis were centrifuged (Beckman Microfuge S) to remove visible debris. An aliquot (200 or 225 µl) of the supernatant was then pipeted into a 500 µl Microcentrifuge tube which was placed into an ice water bath. A sufficient amount of 3M perchloric acid was added to the sample to produce a total volume of 250 µl. The tubes were capped, and the contents vortexed briefly and centrifuged (Eppendorf 5412) at 14,600 RPM for 3 minutes. A portion of the supernatant was transferred to a Polyspring® insert in an HPLC vial for analysis. Injection volumes were based on the concentration of radioactivity in each urine sample. If necessary, samples were re-injected at higher volumes to obtain quantifiable levels of radioactivity. Peaks with a maximum CPM value equal to twice the average background CPM value were considered quantifiable. The Area-CPM values for all quantifiable peaks in a given run were summed, and the levels of EG and metabolites were expressed as percentages of the total radioactivity.

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RESULTS & DISCUSSION

GC Analyses

The GC analysis of standard and dosing solutions containing EG produced a single peak which was well-resolved from the solvent front. The detector response to standard concentrations ranging between 2000 and 50 µg/ml EG in acetonitrile was linear and reproducible. The limit of sensitivity for the assay was approximately 0.01 µg EG per µl injected.

Table 4 contains a summary of results for the concentration verification analyses of the EG dosing solutions. The actual concentrations of the solutions ranged between 96.0 and 109.2% of nominal values. Pre- and post-dosing analyses indicated that the solutions had remained homogeneous throughout the dosing period for all dose administrations. The mean concentration of EG in the dose solutions from all of the pharmacokinetics and material balance studies was 102.5 ± 4.1% of nominal. EG was shown to be stable in saline or water for at least 54 and 26 days respectively when stored frozen.

Preliminary attempts to quantitate the levels of unchanged EG in selected pooled plasma samples from the pharmacokinetics studies were conducted. Quantifiable levels of EG could be detected through a majority of the collection intervals in all studies except the intravenous low dose. Samples from the 50% aqueous and low dose cutaneous studies were not analyzed because the low plasma radioactivity levels indicated that EG would be undetectable. The limit of sensitivity for the assay was imposed by the presence of a carry-over peak (approximately 0.5 ng/µl apparent EG) which was observed in the plasma blanks analyzed between samples. According to Flanagan *et al.* (1987) this peak could be eliminated by the addition of hydrochloric acid (0.2% v/v) to the derivatization reagent. However, this modification did not have the desired effect in the capillary analysis of the derivatized plasma samples. It is also possible that the high sensitivity of the MSD contributed to the persistent detection of the peak. Comparative analysis of standards derivatized with or without acidified reagent resulted in similar quantitation and better peak shapes when the acid was not used. Therefore, although the carry-over phenomenon could not be completely eliminated, it was minimized by the injection of blanks between all samples.

Figure 1 illustrates the chromatographic response for ethylene glycol phenylboronate and the phenylboronate derivative of the internal standard, 1,3-propanediol. The derivatives displayed good mass spectral characteristics with detectable molecular ions at M/Z 148 and 162 respectively. The EG peak had a retention time of approximately 3.7 minutes, and the internal standard eluted at 5.5 minutes. Concentrations of EG in plasma standards used in the analyses ranged between 2000 and 1 µg/ml. However, standard curves were generated over no more than a fifty-fold range of concentrations to insure a linear response at all levels. The recovery of EG from plasma was approximately 85-90% relative to aqueous standards.

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The levels of EG in pooled plasma samples from the intravenous high dose studies are presented in Table 5. In both the male and female groups, plasma concentrations of EG decreased with time and were quantifiable through the 24

hour collection interval. In comparison to mean radioactivity concentrations measured in the plasma samples, the proportion that was due to unchanged parent chemical ranged from 103.3 to 0.19% with one exception. The high recovery (140%) in the 0.5 hour sample from the male study may have been due to the pooling of unequal plasma volumes from each animal. Levels of EG in the low dose intravenous samples decreased to less than 0.1% of the nominal radioactivity concentration at 8 hours and could not be measured beyond that point.

The analysis of pooled plasma extracts from the high dose oral studies resulted in EG levels which ranged from 111.8 to 0.35% of the mean plasma concentrations of radioactivity. In general, plasma concentrations of EG increased slightly or plateaued through 45 minutes and then decreased thereafter. Levels of EG were quantifiable through the 18 hour collection interval. The results are presented in Table 6. Table 7 contains a summary of results for plasma analyses from the male and female low dose oral studies. Measurable levels of EG were detected through 48 hours in both sexes, however some of the later samples had EG peaks which resembled the carryover in the blanks. These samples are indicated in parentheses. Overall, the pattern of EG distribution observed in the oral high dose studies was duplicated in the low dose samples.

Quantifiable levels of EG were detected in all but one of the analyzed plasma samples from the high dose percutaneous studies. Unchanged EG accounted for the greatest percentage (98%) of measured total radioactivity in the 4 hour sample. However, the highest concentration of plasma EG occurred 8 hours after dosing. Generally, the plasma levels of EG increased through 8 hours and then gradually declined over the remainder of the time-course for the cutaneous samples. Table 8 contains a summary of results for plasma analyses from the male and female studies.

HPLC Analyses

The HPLC analysis of standard solutions of EG in urine produced a single peak with a retention time of 11.8 minutes. The response of the refractometer was linear and reproducible over a range of 10-1000 µg EG injected, although 250 µg EG was generally the highest standard used in the analyses. The limit of sensitivity for the assay was approximately 1 µg EG injected. An experiment in which urine samples with detectable EG levels were spiked with additional known amounts of EG indicated that the recovery of EG from the column averaged 98.6%.

EG and its proposed metabolites (oxalate, glyoxalate, glycolaldehyde and glycolate) had distinct, reproducible retention times under the stated operating conditions. Although the glycolaldehyde and glycolic acid peaks were not resolved to baseline, there was a 0.4 minute difference in their retention times which permitted their identification when present at quantifiable levels. This resolution had not been achieved with the HPLC method used by Marshall. Verification experiments indicated that glycolaldehyde and glycolic acid could still be separated when the acid was present in fifty-fold excess. The minimum detectable level of glycolaldehyde was approximately 5 µg injected. A representative chromatogram of EG and metabolites is included in Figure 2.

Detection of ^{14}C -EG using the Trace II $\text{\textcircled{R}}$ Radioactivity Flow Monitor (in-series with the refractometer) produced a single peak with a retention time of 12.2 minutes. The response of the radioactivity monitor to ^{14}C -EG in spiked urine standards was linear and reproducible. When compared to a liquid scintillation counter (LSC), the counting efficiency of the radioactivity flow monitor was 103.4%. The average background radioactivity, calculated from analyses of blank urine samples, was 176 Max-CPM. Twice background was established as the minimum value for a peak (EG or metabolite) to be considered quantifiable.

The HPLC analysis of selected urine samples from the high dose (1000 mg/kg) studies resulted in detectable levels of EG, as measured by the refractometer, for all routes of administration. Furthermore, measurable amounts of glycolic acid and/or oxalic acid were also present, and were qualitatively identified by comparison of retention times with metabolite standards. Generally, the levels of EG and metabolites in urine samples from the low dose (10 mg/kg) studies could not be detected by the refractometer. However, retention times for the radioactive peaks in these samples were compared to those for characterized peaks from the high dose studies to determine metabolite profiles. Due to the close proximity of the glycolaldehyde and glycolic acid peaks, an additional experiment was done to verify the identity of this peak in a study urine sample. Equivalent amounts of either glycolaldehyde or glycolic acid spikes were added to two aliquots of urine with a detectable metabolite peak to determine which chemical produced a qualitative increase in metabolite peak height. As shown by retention time comparisons, the identity of the metabolite peak was confirmed as glycolic acid.

Tables 9 through 12 contain the urinalyses results for individual animals from the intravenous, oral and percutaneous studies. The results are expressed as percentages of total peak radioactivity for all of the quantifiable peaks in a run. Overall, ^{14}C -labelled ethylene glycol, glycolic acid and/or oxalic acid accounted for the majority of the detectable radioactivity in the urine samples from all dose routes. The occurrence of radioactive peaks that could not be associated with EG or known metabolites was relatively rare and may have been due to peak spreading. No glycolaldehyde or glyoxylic acid was detected in any samples. In general, the individual urinalyses show that the proportion of unchanged ^{14}C -EG to that of labelled metabolites was fairly consistent among animals in a given dose group.

Table 13 contains a summary of the urinary excretion of ^{14}C for the high (1000 mg/kg) and low (10 mg/kg) dose intravenous studies. As noted in the study by Marshall (1982), a dose-dependent increase in the amount of glycolic acid was observed. Approximately 65% of the radioactivity in the high dose urines at 12 hours was due to unchanged ^{14}C -EG and the remaining 35% was accounted for by glycolic acid. In the low dose urines, ^{14}C -EG comprised about 93% of the detectable radioactivity at 12 hours. A slight increase in the amount of urinary glycolic acid (45%) was observed at 24 hours in the high dose samples for both sexes. In addition, the male urine samples contained a significant percentage (17%) of oxalic acid at this collection interval. Representative chromatograms from the female intravenous high dose study are included in Figure 3.

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The ^{14}C content of urine samples from the high (1000 mg/kg) and low (10 mg/kg) dose oral studies is summarized in Table 14. The majority of the radioactivity (92-96%) in the low dose samples was due to ^{14}C -EG at both 12 and 24 hours post-dosing. An increase in urinary glycolic acid was observed in the high dose oral samples relative to the low dose; however, in comparison to the high dose intravenous results, the percentage of glycolic acid (approximately 24% at 12 hours) was slightly lower. Oxalic acid accounted for less than 3% of the total peak radioactivity in urines from the high and low dose studies.

HPLC analysis of urinary ^{14}C after percutaneous administration of 10, 500 or 1000 mg/kg ^{14}C -ethylene glycol indicated that unchanged parent chemical accounted for 87-100% of the detectable radioactivity. The results are summarized in Table 15. In comparison to the intravenous and oral results, the most notable differences in the percutaneous urine samples were the greater percentage of urinary ^{14}C -EG at the 1000 mg/kg dose level, and the lack of detectable oxalic acid in any samples. At the 500 mg/kg dose level, exposure to the test chemical was terminated after 6 hours; urinary ^{14}C -EG accounted for 100% of the detectable radioactivity through 36 hours. These results indicate that measurable metabolism of ^{14}C -ethylene glycol did not occur following percutaneous exposure.

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Table 1

Ethylene Glycol: Gas Chromatographic Operating Conditions
for Dose Solution Analyses

Chromatograph	Hewlett Packard 5880A
Detector	Flame Ionization
Column*	Carbowax 20M (50 m x 0.31 mm I.D. - fused silica capillary)
Oven Temperature Profile	120°C - 0 min - 10°C/min - 200°C (180°C) - 2 min
Injector Temperature	200°C
Detector Temperature	250°C (225°C)
Carrier Flow Rate	60 ml/min Helium - Splitless injection (30 ml/min Helium - Split injection)
Column Flow Rate	1.0 ml/min Helium
Auxiliary Flow Rate	30 ml/min Helium
Hydrogen Flow Rate	30 ml/min
Air Flow Rate	~ 350-400 ml/min

*The dose solution analyses were transferred to a 25 m x 0.31 mm I.D. Carbowax 20M column midway through the study. The numbers in parentheses indicate the changes in chromatographic conditions used for the new column.

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Table 2

Ethylene Glycol: Gas Chromatographic Operating Conditions
for Plasma Analysis

Chromatograph	Hewlett Packard 5890 GC
Column	DB-1 (30 m x 0.31 mm I.D. - fused silica capillary; 1.0 μ m film thickness)
Oven Temperature Profile	140°C (0 min)-5°C/min-175°C (0 min)-25°C/min-225°C (1 min)
Injector Temperature	200°C or 225°C
Transfer Line Temperature	225°C
Detector	Hewlett Packard 5970 Mass Selective Detector (MSD)
Acquisition Mode	Selective Ion Monitoring (SIM)
Ions monitored (EG)	m/z 117, m/z 118, m/z 146, m/z 147, m/z 148
Carrier Flow Rate	~ 30 ml/min Helium
Column Flow Rate	~ 1.0 ml/min Helium

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APPENDIX D

Individual Animal Data

The data on the following pages represent summary pharmacokinetics data from individual animals in this study. These data are summarized according to dose groups and presented here to illustrate the individual plasma, RBC and excreta concentrations of the radioactive dose vs. time of collection. Individual totals for Total DPM and % of dose values may be slightly greater than sum of tissue values due to computer rounding of calculated numbers.

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PROJECT REPORT 51-591

TITLE: Developmental Toxicity Evaluation of Ethylene Glycol Administered by Gavage to CDO-1 Mice: Determination of a "No Observable Effect Level" (NOEL)

AUTHOR: R. W. Tyl, Ph.D., DABT

SPONSOR: Ethylene Glycol Program Panel
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DATE: April 5, 1989

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Developmental Toxicity Evaluation Of Ethylene Glycol
Administered by Gavage to CD-1 Mice:
Determination of a "No Observable Effect Level" (NOEL)

Ethylene Glycol Program
Chemical Manufacturers Association

* * * * *

ABSTRACT

Ethylene glycol (CAS No. 107-21-1) has been shown to be teratogenic when administered by gavage at high doses to CD rats (1250-5000 mg/kg/day) and CD-1 mice (750-3000 mg/kg/day). However, that gavage study did not establish a "no observable effect level" (NOEL) for mice since the lowest dose evaluated, 750 mg/kg/day, was an effect level for developmental toxicity. Therefore, this study was undertaken to define a NOEL for developmental toxicity of ethylene glycol in CD-1 mice by gavage. Timed-pregnant CD-1 mice were administered ethylene glycol by gavage on gestational days (gd) 6 through 15 at 0.0, 50.0, 150.0, 500.0, or 1500.0 mg/kg/day, 30 plug-positive females per group. The vehicle was deionized (Millipore®) water and the dosing volume was 10.0 ml/kg. Study females were weighed on gd 0, 6, 9, 12, 15 and 18, observed daily for clinical signs and evaluated for water consumption every three days, gd 0-18. At sacrifice on gd 18, females were evaluated for body weight, gravid uterine weight, liver and kidney (2) weight. Maternal kidneys were retained in fixative and maternal kidneys from the vehicle control group and top dose group were examined microscopically. Ovarian corpora lutea were counted and all uterine implantation sites evaluated for resorptions (early and late), dead and live fetuses. All live fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and variations.

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One to two pregnant females died or were sacrificed moribund in each group (except at 150.0 mg/kg/day) from reproductive tract bleeding. No females aborted or were removed from study. One to three dams across all groups delivered early. Pregnancy rate was approximately equivalent across all groups except for a slightly reduced rate (70.4% pregnant) at 0.0 mg/kg/day). There were fully resorbed litters at 50.0 mg/kg/day (one), 150.0 mg/kg/day (one), and 1500.0 mg/kg/day (two). A total of 19-24 litters were examined in each group. Maternal body weights and weight gain were unaffected by treatment. Maternal clinical signs were limited to those animals which died or were sacrificed moribund, and were not treatment related. Water consumption did not differ among groups for any interval evaluated. Maternal observations at scheduled necropsy indicated no treatment-related findings; observations on animals which died or were sacrificed moribund indicated blood in the stomach, reproductive tract and urinary bladder. Maternal organ weights at scheduled sacrifice did not exhibit any statistically significant differences among groups for terminal body weight, gestational weight gain, liver, kidney (2) or gravid uterine weight. There were no significant effects of treatment on the number of corpora lutea/dam, the number of total, nonviable or viable implants/litter or on sex ratio. Fetal body weights per litter were reduced only at 1500.0 mg/kg/day. There was no increase in the incidence of individual or total external or visceral malformations in any group relative to the vehicle control group. There was a statistically significant increase in the incidence of two (2) skeletal malformations in the 1500.0 mg/kg/day group and the incidences of pooled skeletal malformations and all malformations were significantly increased in this group as well. The incidence of total malformations was also significantly increased at 500.0 mg/kg/day. There were no significant increases in individual external or visceral variations, or in pooled external, visceral or skeletal variations or in total variations. The incidences of 23 skeletal variations were increased in the 1500.0 mg/kg/day group. One skeletal variation was also increased in incidence at 500.0 mg/kg/day. Examination of maternal kidneys from the vehicle control and top dose groups indicated no treatment-related histologic renal lesions and no oxalate crystals observed in the kidney sections examined in the top dose group.

In conclusion, exposure of pregnant CD-1 mice to ethylene glycol by gavage at 50.0, 150.0, 500.0 or 1500.0 mg/kg/day during organogenesis resulted in developmental toxicity in the absence of observable maternal toxicity at 500.0 and 1500.0 mg/kg/day. The "no observable effect level" (NOEL) for maternal toxicity was therefore 1500.0 mg/kg/day and the NOEL for developmental toxicity was 150.0 mg/kg/day by gavage. This study confirmed the developmental toxicity (including teratogenicity) by gavage at 1500.0 mg/kg/day from previous work, documented the absence of maternal renal toxicity at 1500.0 mg/kg/day and established a NOEL for ethylene glycol for developmental toxicity when administered by gavage in CD-1 mice.

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OBJECTIVES

The objective of this study was to determine the "no observable effect level" (NOEL) for developmental toxicity (including teratogenicity) of ethylene glycol when administered by gavage during organogenesis to pregnant CD-1 mice. Maternal toxicity was also evaluated.

INTRODUCTION

Ethylene glycol (EG, CAS No. 107-21-1) is a major industrial chemical, produced by hydrolysis of ethylene oxide and used in antifreeze and coolant mixtures for motor vehicles, in hydraulic fluids and heat exchangers and as solvent. Large amounts are used as a chemical intermediate in the production of ethylene glycol esters, ethers and resinous products, especially polyester fibers and resins (Rowe and Wolf, 1982).

The major route of EG metabolism is oxalic acid via glyoxylic acid, with the production of calcium oxalate crystals in the urine a typical finding in overdosed animals (and humans). The classic peroral toxicity profile in humans follows three stages. The first stage is observed within 30 minutes to 12 hours and involves central nervous system effects from unchanged ethylene glycol. The second stage, observed 12-36 hours after exposure, affects the cardiopulmonary system from acidosis and hypocalcemia from metabolites. The final stage is renal failure accompanied by oxaluria, and renal necrosis which includes dilation of proximal tubules, degeneration of the tubular epithelium and intratubular crystals (Rowe and Wolf, 1982).

Recent evidence has accumulated that repeated (or continuous) exposure to large amounts of EG results in developmental toxicity (including teratogenicity) in rats and mice. Price *et al.* (1984 a,b; 1985) have reported that exposure to EG by gavage during organogenesis in CD rats (0, 1250, 2500 or 5000 mg/kg/day) and CD-1 mice (0, 750, 1500 or 3000 mg/kg/day) resulted in reduced maternal weight and weight gain, and reduced gravid uterine weight in a dose-related manner. An increase in postimplantation loss (resorptions plus dead fetuses) was observed in rats at 5000 mg/kg/day only. Fetal weight was reduced at all doses in mice and at 2500 and 5000 mg/kg/day in rats. The percentage of malformed live fetuses per litter was increased at all doses in mice and at the mid and high doses in rats. The proportion of litters with one or more malformed fetuses was elevated at all doses in both species. The typical malformations observed were craniofacial defects and severe axial skeletal dysplasia in both species. Ethylene glycol was also evaluated in the so-called Chernoff assay where pregnant CD-1 mice were dosed by gavage at one dose (11,090 mg/kg, 10 ml/kg undiluted) on gestational days 7 to 14 (the day a copulatory plug was observed was designated gestational day 1) and allowed to litter; litters were examined on postnatal days 1 and 3 and discarded. Ten percent (5/50) of the dams died and 41% (15/37 dams) had viable pups. The number of live pups per litter at birth was reduced (and the number of dead pups per litter at birth was increased), and postnatal survival was reduced (40% versus 100%) relative to controls. Pup birth weight and pup weight gain were both reduced relative to controls (Schuler *et al.*, 1984). The authors gave EG high priority for further testing.

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In an NTP protocol for fertility assessment by continuous breeding (Gulati et al., 1984; Lamb et al., 1985), EG was administered in the drinking water at 0.0, 0.25, 0.5 or 1.0% during 98 days of cohabitation (one male:one female); litters were assessed at birth and discarded except for the last litter which was retained for offspring assessment. Exposure to EG resulted in a small but significant decrease in the number of litters per breeding pair, in the number of live pups per pair and in the live pup weight. A significant number of pups in the 1.0% dose group were born with distinct facial deformities. In the retained litters at this dose, the facial deformities were more obvious with age. These malformed animals also exhibited fused ribs and shortened nasal, parietal, and/or frontal bones of the skull. When pups from the high dose group were raised to adulthood (with continued exposure to EG) and mated, they exhibited decreased mating and fertility indices relative to controls handled in the same manner, but no effects were observed on litter size, pup weight or sex ratio (Gulati et al., 1984). The authors deemed EG a "weak reproductive toxicant, but a potential teratogen" (Gulati et al., 1984, p. 15).

All of the above studies involved exposure to EG via the gastrointestinal route (by gavage or dosed drinking water) and all reported developmental toxicity including malformations in both CD₀ rats (Price et al., 1984b; 1985) and CD₀-1 mice (Price et al., 1984a; 1985; Schuler et al., 1984; Gulati et al., 1984; Lamb et al., 1985).

The study by Price et al. (1984a; 1985) did not define a NOEL in CD₀-1 mice since the lowest dose evaluated, 750 mg/kg/day, resulted in reduced fetal body weight and increased fetal skeletal malformations. The study by Schuler et al. (1984) employed only one dose, 11,090 mg/kg/day by gavage. The study by Gulati et al. (Gulati et al., 1984; Lamb et al., 1985) identified 0.5% EG (approximately 800 mg/kg/day in the drinking water) as a NOEL.

The present study was therefore undertaken to determine the NOEL for EG, administered by gavage to CD₀-1 mice during organogenesis, for maternal and developmental toxicity (including teratogenicity). The protocol duplicated, as closely as possible, the procedures followed by Price et al. (1984a; 1985).

MATERIALS AND METHODS

Chemical and Dosage Formulation and Analyses

One quart container of polyester grade ethylene glycol test material (CAS Number 107-21-1, BRRC Sample Number 51-182, identified by the supplier as "PC-35227, UCC B35227 12/86 4407") was received from Union Carbide Corporation (Hahnville, LA) on May 12, 1988. The purity and stability were verified by analysis by Mr. Steven A. Guidry, Union Carbide Corporation, Taft Plant No. 1 Laboratory, Hahnville, LA. The test material was highly pure (essentially 100.0 weight percent). Deionized (Millipore[®]) water (CAS No. 7732-18-5) was used as the vehicle.

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The dosing solutions were prepared as follows. The appropriate amount of EG was weighed and mixed with deionized (Millipore®) water (CAS No. 7732-18-5) to make appropriate weight/volume solutions of the test chemical in vehicle at a dosing volume of 10.0 mg/kg/day based on each animal's most recent body weight. The four (4) dosage levels were 50.0, 150.0, 500.0 and 1500.0 mg/kg/day (5.0, 15.0, 50.0 and 150.0 mg/ml, respectively). The vehicle control group received deionized (Millipore®) water alone (0.0 mg/kg/day). The resulting solutions were stirred prior to each use. These doses, dosing volumes and procedures were chosen to duplicate the middle dose employed in the EG gavage developmental toxicity study with CD-1 mice (Price et al., 1984a, 1985) and three (3) doses below the low dose of that study (750 mg/kg/day). In that study, both 1500 and 750 mg/kg/day produced reduced fetal body weight and increased incidence of fetal skeletal malformations and variations.

The dosing solutions were analyzed for content by the following procedure. Standard stock solutions were prepared of 5.0, 15.0, 50.0 and 150.0 mg/ml of EG. Appropriate dilutions of the stock solution were made (v:v) with acetonitrile to generate a standard curve of 150 to 500 ng/µl. For dosing solutions and standards, 1.0 µl aliquots were analyzed for EG. A Hewlett-Packard 5880 Gas Chromatograph was used for all analyses with flame ionization detection. The column was a fused silica capillary column, CARBOWAX® 20M, 25 M x 0.32 mm i.d. The carrier gas was ultra high purity helium. For all analyses, the actual concentration of each dosing solution was calculated by comparing the peak area of the sample to the peak area of the appropriate standard. Details of the formulations and analyses of the dosing solutions are presented in Appendix 1.

Mice

Two hundred thirty-three (233) virgin male and 232 female CD-1 [Crl:CD-1 (ICR)BR] outbred albino mice were received from Charles River Breeding Laboratories, Inc. (Kingston, NY) on June 13, 1988 and housed in animal Room 147 (the animals were 35 days old upon arrival, birth date May 9, 1988). They were quarantined for at least two weeks, during which time representative animals were subjected to fecal sampling, histologic examination of selected organs and to serum viral antibody examination. The results of the quality control tests indicated that these animals were in good health and suitable for use in this study. All mice received a unique number and were toe-clipped. The mice were housed, one to three per cage, separated by sex, in stainless steel wire-mesh cages (23.5 cm X 20 cm X 18 cm high) with food (Agway Certified Diet RMH-3200, Batch No. 5-16-88 W2; Agway, Inc., St. Marys, OH) and water (Municipal Authority of Westmoreland County, Greensburg, PA) available ad libitum throughout the study. All animals in quarantine and mating, and the study females received drinking water in bottles with stainless steel sipper tubes to reduce stress and to allow water consumption measurements. Paperboard (Deotized Animal Cage Board®, Shepherd Specialty Papers, Inc., Kalamazoo, MI) was placed beneath the cages and changed regularly. Animals were kept on a 12-hour photoperiod (0530-1730 hours for light phase) and room temperature and relative humidity were continuously recorded (Cole-Parmer Hygrothermograph® Seven-Day Continuous Recorder, Model No. 8368-00, Cole-Parmer Instrument Co., Chicago, IL). Temperature was maintained at 69-75°F and relative humidity at 50-58% throughout the in-life portion of this study.

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Mice were bred 1:1 (one female at least 20 g; one male at least 25 g) in cages as described above in room 147 and checked daily for vaginal copulation plugs. Each male was used only once in this study. The date a copulation plug was found was designated gestational day (gd) 0 (Hafez, 1970). Thirty (30) plug-positive females were randomly assigned to each of the experimental groups on gd 0 using a stratified randomization system based on body weight. The mating period was June 29-July 5, 1988 and gd 0 dates were June 30 through July 5, 1988. A total of 150 plug-positive females were used in this study. Plug-positive females were singly housed in stainless steel cages (23.5 cm X 20 cm X 18 cm high) in BRRC animal room 147 for the duration of the study.

All study females were administered the EG dosing solutions or the vehicle control (deionized water) on gd 6 through 15, July 6 through July 20, 1988. All animals were observed daily (gd 0-18) for any clinical signs of toxicity and were weighed on gd 0, 6, 9, 12, 15 and 18. Water consumption was measured every three days, gd 0-3, 3-6, 6-9, 9-12, 12-15 and gd 15-18.

The EG dosing solutions (in deionized water) or vehicle control solution were administered by gavage through an 18 gauge, 1.5-inch commercial ball-end stainless steel dosing needle (Perfektum®, Popper and Sons, New Hyde Park, NY) attached to a Hamilton Diluter/Dispenser (Hamilton, Reno, Nevada) with a 1000 µl glass syringe. The dosing volume was 10.0 ml/kg, based on the individual animal's most recent body weight.

Thoracotomy, Maternal and Fetal Examinations

All surviving study females were sacrificed by carbon dioxide asphyxiation on gd 18, July 18 through 23, 1988. The maternal body cavities were opened by midline thoracotomy. The gravid uterus, ovaries (including corpora lutea), cervix, vagina, and abdominal and thoracic cavities and organs were examined grossly. Ovarian corpora lutea of pregnancy were counted. Maternal liver, kidney (2) and gravid uterine weights were determined. Maternal kidneys were bisected (left longitudinally, right transversely) and retained in buffered neutral 10% formalin. Uteri were externally examined for signs of hemorrhage, removed from the peritoneal cavity and dissected longitudinally to expose their contents. Status of all implantation sites, i.e. live and dead fetuses, and resorption sites (early, late), was noted and recorded. Uteri from females that appeared nongravid were placed in a 10% ammonium sulfide solution for detection of early resorptions (Salewski, 1964).

All live fetuses were weighed, sexed and examined for external malformations including cleft palate, and variations. All live fetuses were also examined for thoracic and abdominal visceral abnormalities by modification of methods described by Staples (1974) and sex verified internally. One-half of the fetuses (even-numbered fetuses from litters with an even number of live fetuses, odd-numbered fetuses from litters with an odd number of live fetuses) were then decapitated and their heads were fixed in Bouin's solution for examination of craniofacial structures by sectioning methods modified from Wilson (1965; 1973) and van Julsingha and Bennett (1977). All of the fetuses in each litter (50% intact, 50% decapitated) were eviscerated, fixed in ethanol, processed for skeletal staining with alizarin red S (Dawson, 1926; Peltzer and Schardein, 1966), and examined for skeletal malformations and variations.

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Microscopic Examination of Maternal Kidneys

The fixed kidneys from dams surviving to scheduled sacrifice in the vehicle control and top dose groups were embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin. The veterinary pathologist for this study examined these kidney sections, including examination of the sections for detection of oxalate crystals in renal tubules, if present.

Statistical Analyses

The unit of comparison was the pregnant female or the litter (Weil, 1970). Results of the quantitative continuous variables (e.g., maternal body weights, organ weights, fetal weights, etc.) were intercompared for the four ethylene glycol groups versus the vehicle control group by use of Levene's test for equal variances (Levene, 1960), analysis of variance (ANOVA), and t-tests with Bonferroni probabilities. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated homogeneous variances, and the ANOVA was significant, the pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances (Brown and Forsythe, 1974) followed, when necessary, by the separate variance t-test.

Nonparametric data obtained following laparotomy were statistically treated using the Kruskal-Wallis test (Sokal and Rohlf, 1969) followed by the Mann-Whitney U test (Sokal and Rohlf, 1969) when appropriate. Incidence data were compared using Fisher's Exact Test (Sokal and Rohlf, 1969). Statistical evaluation of the incidence of fetal malformations and variations was performed with the unit of comparison the number of litters with one or more affected fetuses. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

Personnel

The determination of the NOEL of ethylene glycol for developmental toxicity in CD-1 mice when administered by gavage was conducted at Bushy Run Research Center (BRRC), Export, PA, for The Ethylene Glycol Program Panel, Chemical Manufacturers Association, Washington, DC. Sponsor's Representative was Dr. C. R. Stack. The BRRC personnel indicated below contributed to the completion of this study.

Dr. R. W. Tyl served as Study Director. Developmental toxicity personnel included Dr. T. L. Neeper-Bradley, R. R. Altman, T. R. Brownfield, B. L. Butler, M. A. Copeman, C. D. DeMann, D. L. Fait, L. C. Fisher, L. J. Fosnight, M. F. Kubena (Study Leader) and D. J. Tarasi. Analytical personnel included Dr. J. P. Van Miller and M. A. Vrbancic. The veterinary pathologist was P. E. Losco. The histology technicians were M. A. McGee, G. J. DiSalvo and M. S. Soehl. Quality Assurance personnel included L. J. Calisti, J. R. Bernard and J. H. Coleman. Animal care personnel were R. R. Altman, N. S. Bellich, P. E. Biondo, T. A. Christopher, J. DeNinno, G. W. Klingensmith, Jr., L. E. Lipko and E. J. Mika.

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The Final Report was prepared by Dr. R. W. Tyl with assistance from L. C. Fisher on data compilation and statistical analyses. The individual scientist reports were signed by the authors. The protocol and one amendment detailing the design and conduct of this study are presented in Appendix 6.

Storage of Records

All original data sheets for the present study are stored in the BRRC Archives along with all biological samples collected during the course of the study which remain the responsibility of BRRC. Work sheets and computer printouts which were generated in the statistical analysis of data are stored in the BRRC Archives. Copies of this report will be filed with the BRRC Archives as well as with the Ethylene Glycol Program Panel, Chemical Manufacturers Association, Washington, DC. In accordance with Good Laboratory Practice Standards, Environmental Protection Agency Toxic Substances Control Act (1983), all records, data and reports will be maintained in storage for a minimum of ten (10) years or for as long as the quality of the preparation affords evaluation, whichever is less.

RESULTS

The results of the analyses of the dosing formulations are presented in Table 1. The dosing solutions for all groups were homogeneous and stable for at least 21 days. They were formulated once and analyzed for content prior to the onset of dosing. All dosing solutions were 92.0-107.4% of the target concentrations, well within the specification of target concentrations \pm 10%. See Appendix 1 for details of formulation and analyses of the dosing solutions.

The distribution and fate of all animals on study are presented in Table 2. One to three females across all groups (except at 150.0 mg/kg/day) died or were sacrificed moribund. One (1) each at 0.0 and 50.0 mg/kg/day, three (3) at 500.0 mg/kg/day and two (2) at 1500.0 mg/kg/day died during the dosing period; one (1) at 50.0 mg/kg/day was sacrificed moribund during the dosing period. Of these, one female each at 500.0 and 1500.0 mg/kg/day had as the proximate cause of death technical dosing error, but these animals were so physically compromised by previously observed clinical signs (see below) that the decision was made by the Study Director to retain their data and not remove them from study. The cause of the deaths appeared to be loss of blood from bleeding from the reproductive tract (all but one of the females were pregnant; the one nonpregnant female was in the 500.0 mg/kg/day group). No females aborted or were removed from study. Two (2) females at 0.0 mg/kg/day, three (3) at 50.0 mg/kg/day, two (2) at 150.0 mg/kg/day, one (1) at 500.0 mg/kg/day and one (1) at 1500.0 mg/kg/day delivered early. Pregnancy rate was approximately equivalent across all groups (84.0-92.3%), except for the control group which had a 70.4% pregnancy rate (eight of 27 examined at scheduled sacrifice were not pregnant). Dams with fully resorbed litters included none (0) at 0.0 mg/kg/day, one (1) each at 50.0 and 150.0 mg/kg/day, none (0) at 500.0 mg/kg/day and two (2) at 1500.0 mg/kg/day. A total of 19-24 litters were examined in each group. All subsequent data and discussion are based on pregnant females only.

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Maternal body weights and weight gain are presented in Table 3. Body weights and weight gain were statistically equivalent across all groups at all time points and intervals measured. There were slight, but not statistically significant reductions in maternal weight for gd 12, 15 and 18, and similar reductions in maternal weight gain for gd 6-15 (treatment period) and for gd 0-18 (gestation period) at 1500.0 mg/kg/day.

Maternal clinical signs are presented in Table 4. There were no clinical signs which exhibited a dose-related incidence, or statistically significant increases. The one to two pregnant females which died or were sacrificed moribund across all groups (except at 150.0 mg/kg/day) exhibited a number of clinical signs prior to death/sacrifice; none of the other females exhibited any clinical signs (except for one dam at 50.0 mg/kg/day which exhibited red urogenital discharge).

Water consumption, expressed as grams/animal/day (Table 5) exhibited no significant changes in any treatment group at any interval evaluated. Individual maternal in-life data are presented in Appendix 2.

Maternal observations at scheduled necropsy did not differ significantly among any of the groups (Table 6). Necropsy observations on animals which died or were sacrificed moribund prior to scheduled laparotomy are presented in Table 7. There was blood observed in the stomach, vagina, vulva, and/or urinary bladder in all of the animals except for the two animals (one each at 0.0 and 50.0 mg/kg/day) which were too autolyzed to confirm the findings. The two females whose proximate cause of death was technician error are documented as punctured esophagus for the one at 500.0 mg/kg/day and as abscess in the subcutis and adhesion of the submandibular lymph node (indirect evidence of a punctured esophagus) for the one at 1500.0 mg/kg/day. Maternal organ weights at scheduled sacrifice are presented in Table 8. No parameters evaluated exhibited statistically significant differences in any treatment group relative to the control group. Liver and kidney weights (absolute or relative to body weight) were unaffected. Maternal terminal body weight (absolute and corrected for gravid uterine weight) and gestational weight change (weight change for gd 0-18, minus the gravid uterine weight) were slightly, but not significantly, reduced at 1500.0 mg/kg/day. Individual maternal data for necropsy and laparotomy are presented in Appendix 3.

Gestational parameters are presented in Table 9. There were no treatment-related significant differences among groups in the number of corpora lutea/dam, the number of total, nonviable (early or late resorptions or dead fetuses) or viable implantations/litter, or on sex ratio (percent male fetuses). The number of early resorptions and non-viable implants/litter were slightly increased at 1500.0 mg/kg/day, but these values were not statistically different from the values in the concurrent control group and are within the range of historical control values for these parameters in this species and strain in this laboratory. Fetal body weights per litter (all fetuses, males or females) were significantly reduced only at 1500.0 mg/kg/day relative to the vehicle control group. Individual fetal body weights are presented in Appendix 4A (with the fetal external findings).

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The incidence and frequency of fetal malformations and variations observed in this study are presented in Tables 10 (malformations) and 11 (variations). There was no statistically significant increase in the incidence of any individual external or visceral malformations, or of pooled external or visceral malformations. The incidences of two individual skeletal malformations, of pooled skeletal malformations and of total malformations were all significantly increased at 1500.0 mg/kg/day. The incidence of total malformations was also significantly increased at 500.0 mg/kg/day. The increased skeletal malformations were fusion of 2-12 thoracic arches and fusion of 2-12 ribs. In addition, a number of skeletal malformations occurred only at 1500.0 mg/kg/day, but the incidences were not statistically significantly different from those in the vehicle control group.

There were no statistically significant increases in any individual external or visceral variations, in variations by category (external, visceral or skeletal) or in total variations. The incidence of 24 skeletal variations (out of 134 different findings observed) exhibited statistically significantly different values in one or more treatment groups relative to the vehicle control group. Twenty-three (23) of these were significant increases in incidence for the 1500.0 mg/kg/day group, including increased incidences of poorly ossified and unossified cervical centra (four findings), poorly ossified and unossified thoracic centra (five findings), extra thoracic centrum and arch (one finding), poorly ossified lumbar centra (four findings), extra ribs (thoracic and lumbar, two findings), unossified bones of the forelimbs in the paws (proximal phalanges, one finding), poorly ossified sternbrae (three findings), extra ossification site between sternbrae (one finding), enlarged sagittal suture (one finding) and enlarged frontal fontanel (one finding). In addition, two (2) findings exhibited a significantly different incidence in groups other than the top dose group relative to those in the vehicle controls. They were an increased incidence of extra fourteenth rib on the first lumbar arch, bilateral, at 500.0 mg/kg/day (also observed at 1500.0 mg/kg/day), and a decreased incidence of poorly ossified frontal bone at 150.0 mg/kg/day. The incidence of pooled visceral and skeletal variations was 100% in all groups (one or more fetuses in all litters exhibited at least one finding, and in fact, all fetuses examined exhibited one or more skeletal variations) and the incidence of all variations was therefore also 100%. Malformations and variations by individual fetuses and by litters are presented in Appendix 4.

The results of the microscopic examination of maternal kidneys from the vehicle control and top dose animals are presented in Table 12. In kidneys from the top dose group (1500.0 mg/kg/day), there were no treatment-related histologic renal lesions and no oxalate crystals were observed in any of the kidney sections examined. Details are presented in Appendix 5.

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DISCUSSION

Previous work presented data that administration of ethylene glycol via the gastrointestinal route (gavage, dosed drinking water) at high doses resulted in developmental toxicity including teratogenicity in CDO rats (Price *et al.*, 1984b; 1985) and C57-1 mice (Schuler *et al.*, 1984; Price *et al.*, 1984a; 1985; Gulati *et al.*, 1984; Lamb *et al.*, 1985). In the gavage study with mice (Price *et al.*, 1984a; 1985) at 750.0 mg/kg/day (the lowest dose evaluated), fetal body weights per litter were significantly reduced and the percentage of malformed fetuses per litter and the proportion of litters with one or more malformed fetuses were increased. Only the number of litters with one or more skeletally malformed fetuses was increased at this dose; there was no increase in the incidence of litters with one or more externally or viscerally malformed fetuses. The incidences of fetuses with fused ribs and fused thoracic arches were the only specific skeletal malformation findings which appeared increased at this dose (no statistical tests were performed on individual findings). Fused ribs and fused thoracic arches were the only skeletal malformations observed in the present study with significantly increased incidences and only at 1500.0 mg/kg/day. Fused ribs also exhibited an increased incidence from nose-only exposure to ethylene glycol at 2500 mg/m³ (Tyl, 1988a). The mouse fetal skeleton is the most sensitive system to insult from ethylene glycol, exhibiting increased incidences at all doses employed by gavage (Price *et al.*, 1984a; 1985) and the only system which exhibited malformations from nose-only exposure to or cutaneous application of ethylene glycol (Tyl, 1988a; 1988b) and in the present study. Fused ribs have been demonstrated in CDO-1 mice exposed to immobilization stress (restraint) on gd 9 for 12 hours (both for 9:00 a.m.-9:00 p.m. and for 9:00 p.m.-9:00 a.m.; Chernoff *et al.*, 1987) so it appears that this skeletal malformation can be induced both by stress associated with restraint as well as by exposure to ethylene glycol per se.

Extra ribs (designated a fetal skeletal variation) were observed with significantly increased incidence at 500.0 and 1500.0 mg/kg/day ethylene glycol by gavage in the present study. Extra ribs (rudimentary, extra or bone island) were also observed in the nose-only versus whole-body study evaluating water aerosol or air (Tyl, 1987) and were observed in the ethylene glycol nose-only study (Tyl, 1988a) in all groups (with significantly increased incidences at 2500 mg/m³ nose-only for unilateral extra lumbar rib and at 2500 mg/m³ nose-only and 2100 mg/m³ whole-body for bilateral extra thoracic rib). Interestingly, extra ribs (rudimentary, extra or bone island) were not reported in the Price *et al.* study (1984a; 1985). The finding of extra ribs is consistent with both the effects of ethylene glycol in this laboratory and with restraint (Kavlock *et al.*, 1985; Beyer and Chernoff, 1986) on the fetal mouse skeleton.

In the present study, there were no significant indications of maternal toxicity at any dose evaluated. In the Price *et al.* study (1984a; 1985), maternal body weight was significantly reduced on gd 11, 15 and 17 (day of scheduled necropsy) and maternal weight gain was also significantly reduced for gd 6-15 (treatment period) and gd 0-17 (gestation period) at 1500.0 mg/kg/day. In the present study, maternal weights appeared slightly, but not significantly reduced at gd 12, 15 and 18 (day of scheduled necropsy) and weight gain appeared slightly but not significantly reduced for gd 6-15 and gd

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0-18 at 1500.0 mg/kg/day (Table 3). However, the indications of maternal toxicity in both studies (reduced maternal body weights and weight gain during and after the treatment period) exhibited the same pattern. Nose-only exposure of CD-1 mice (Tyl, 1988a) to 1000 and 2500 mg/m³ resulted in minimal maternal toxicity (increased kidney weight; absolute organ weight in both groups, relative organ weight only at 2500 mg/m³) consistent with ethylene glycol's known renal toxicity (e.g. Rowe, 1982). Maternal kidneys were not examined microscopically in the study by Price et al. (1984a; 1985). In a previous study at BRRC (Tyl, 1988b), administration of ethylene glycol by gavage at 3000.0 mg/kg/day resulted in treatment-related maternal renal lesions. In the present study, maternal kidneys from the 1500.0 mg/kg/day group were examined histologically, with no treatment-related lesions observed. The absence of histologic lesions is consistent with the lack of effect on water consumption and kidney weights.

In the present study, the "no observable effect level" (NOEL) for maternal toxicity was 1500.0 mg/kg/day and the NOEL for developmental toxicity from ethylene glycol administered by gavage was 150.0 mg/kg/day. The NOEL for maternal renal toxicity from ethylene glycol administered by gavage was established at 1500.0 mg/kg/day.

Currently available information on ethylene glycol-induced teratogenicity in mice (and rats) allows a conclusion that ingestion of large doses appears to be the major and possibly only route of administration to produce malformations. The present study has demonstrated that the NOEL for maternal toxicity is 1500.0 mg/kg/day and the NOEL for developmental toxicity of ethylene glycol when administered by gavage to CD-1 mice is 150.0 mg/kg/day.

CONCLUSIONS

Exposure of pregnant CD-1 mice to ethylene glycol during organogenesis by gavage at 50.0, 150.0, 500.0 or 1500.0 mg/kg/day produced developmental toxicity but no maternal toxicity at 500.0 and 1500.0 mg/kg/day. The "no observable effect level" (NOEL) for maternal toxicity was therefore 1500.0 mg/kg/day and the NOEL for developmental toxicity was 150.0 mg/kg/day by gavage. The results of gavage dosing of ethylene glycol confirmed the developmental toxicity findings at 1500.0 mg/kg/day observed in a previous study (Price et al., 1984a, 1985), and reported the absence of maternal renal lesions at this dose.

Reviewed and Approved by:

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Table 1
Analyses of Dosing Formulations

<u>Target Concentrations</u>		<u>Analytical Concentrations^b</u>	
<u>in mg/kg/day</u>	<u>in mg/ml^a</u>	<u>in mg/ml</u>	<u>% of Target</u>
1500.0	150.0	159.6	106.4
500.0	50.0	53.7	107.4
150.0	15.0	14.0	93.3
50.0	5.0	4.60	92.0
0.0	0.0	<MDL ^c	-

a Formulated in deionized (Millipore®) water for a dosing volume of 10.0 ml/kg.

b Dosing solutions were homogeneous and stable for at least 21 days, so they were formulated once and analyzed prior to use.

c Less than the minimum detection limit of 0.01 mg/ml.

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Developmental Toxicity of Ethylene Glycol
Administered by Gavage to CDO-1 Mice

Anatomic Pathology Report

REPORT

The kidneys of 30 maternal mice per group were examined microscopically from the control (0.0 mg/kg/day) and high (1500.0 mg/kg/day) dose groups of an ethylene glycol gavage developmental toxicity study.

The kidneys were collected at the time of sacrifice from sacrificed animals, and as soon as possible after death for mice which were found dead on study. Kidneys were bisected, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at approximately five microns and stained with hematoxylin and eosin. The frequency of histologic lesions was compared between the high dose group and the control group of mice sacrificed on schedule using the Fisher's exact test. The fiducial limit of 0.05 (two-tailed) was used as the critical level of significance.

The microscopic findings of the kidneys are listed in Tables 1 (animals sacrificed on schedule on gestational day 18) and 2 (animals which died or were sacrificed moribund during the study). The latter table also includes two control females and one high dose group female which were sacrificed before gestational day 18 because they had delivered their litters early. There were no kidney lesions found which were attributable to ethylene glycol gavage in any mice. The lesions seen were minimal or mild in all animals and consisted of degenerative or inflammatory changes typical of mice of this age and strain. Tubular basophilia, a regenerative change, was cited as occurring significantly less frequently in high dose group females than in controls. This difference is believed to be due to random biological variation and is a spurious finding. No oxalate crystals were seen in any kidneys.

Ethylene glycol was found to produce no lesions of the kidneys of maternal mice in this test system.

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DEVELOPMENTAL TOXICITY OF ETHYLENE GLYCOL ADMINISTERED BY GAVAGE
TO CD⁰-1 MICE: DETERMINATION OF A "NO OBSERVABLE EFFECT LEVEL" (NOEL)

PATHOLOGY PROTOCOL - FEMALE MICE

The following tissues were examined at necropsy and histologically for the high dose and control females with no significant lesions observed unless otherwise specified:

TOTAL BODY ²	ADIPOSE TISSUE ²	PERITONEAL CAV ²	THORACIC CAV ²	PERICARDIAL CAV ²
ESOPHAGUS ²	STOMACH ²	STOMACH GLAND ²	STOMACH NON-GL ²	LIVER ^{1,2}
PANCREAS ²	INTESTINES ²	ADRENAL GL ²	HEAD ²	SPLEEN ²
DIAPHRAGM ²	EYES ²	OVARIES ²	CORPORA LUTEA ²	OVIDUCT ²
UTERUS ^{1,2}	CERVIX ²	VAGINA ²	VULVA ²	AMNIOTIC SACS ²
PLACENTAS ²	TRACHEA ²	TRACH/BRONC BIF ²	LUNGS ²	KIDNEYS ^{1,2}
URETER ²	URINARY BLADDER ²	GALL BLADDER ²		

¹ = Organ weights collected, ² = examined at necropsy only, unless gross lesions present.

Grade codes:

1=MINIMAL, 2=MILD, 3=MODERATE, 4=MARKED, 5=SEVERE, P=PRESENT
()=FOCAL, (())=MULTIFOCAL, NO PARENTHESES=DIFFUSE

Micro diagnosis prefix codes:

@ = NEOPLASM, B = BENIGN, M = MALIGNANT, @PN = PRE-NEOPLASTIC

MICRO+ indicates histologic confirmation of preceding gross diagnosis.

NOTE: STUDY DAYS ARE DEFINED FROM THE FIRST GESTATIONAL DAY 0 (STUDY DAY 1) TO THE LAST SACRIFICE DAY (STUDY DAY 23). THE STUDY DAY ON WHICH THE ANIMALS WERE SACRIFICED WILL VARY DEPENDING ON THE NUMBER OF MATING DAYS REQUIRED TO PRODUCE THE CORRECT NUMBER OF PLUG/SPERM-POSITIVE FEMALES. THE STUDY DAYS DO NOT CORRESPOND TO GESTATIONAL DAYS.

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Developmental Toxicity of Ethylene Glycol Administered by Gavage
To CDO-1 Mice: Determination of a "No Observable Effect Level" (NOEL)

PURPOSE

The purpose of this study is to determine the "no observable effect level" (NOEL) for developmental toxicity (including teratogenicity) of ethylene glycol when administered by gavage during organogenesis to pregnant CDO-1 Mice. Maternal toxicity will also be evaluated.

INTRODUCTION

Ethylene glycol (EG, CAS No. 107-21-1) is a major industrial chemical, produced by hydrolysis of ethylene oxide and used in antifreeze and coolant mixtures for motor vehicles, in hydraulic fluids and heat exchangers and as solvent. Large amounts are used as a chemical intermediate in the production of ethylene glycol esters, ethers and resinous products, especially polyester fibers and resins (Rowe and Wolf, 1982).

The major route of EG metabolism is oxalic acid via glyoxylic acid, with the production of calcium oxalate crystals in the urine a typical finding in overdosed animals (and humans). The classic acute peroral toxicity profile in humans follows three stages. The first stage is observed within 30 minutes to 12 hours and involves central nervous system effects from unchanged EG. The second stage, observed 12-36 hours after exposure, affects the cardiopulmonary system from acidosis and hypocalcemia from metabolites. The final stage is renal failure accompanied by oxaluria, and renal necrosis which includes dilation of proximal tubules, degeneration of the tubular epithelium and intratubular crystals (Rowe and Wolf, 1982).

Recently, evidence is accumulating that repeated (or continuous) exposure to large amounts of EG results in developmental toxicity (including teratogenicity) in mice. Price *et al.* (1984; 1985) have reported that exposure to EG by gavage during organogenesis in CDO-1 mice (0, 750, 1500 or 3000 mg/kg/day) resulted in reduced maternal weight and weight gain, and reduced gravid uterine weight in a dose-related manner. Fetal weight was reduced at all doses in mice. The percentage of malformed live fetuses per litter and the proportion of litters with one or more malformed fetuses were increased at all doses in mice. The typical malformations observed were craniofacial defects and severe axial skeletal dysplasia. No "no observable effect level" (NOEL) was determined in this study. Ethylene glycol was also evaluated in the so-called Chernoff assay where pregnant CDO-1 mice were dosed by gavage at one dose (11,090 mg/kg, 10 ml/kg undiluted) on gestational days 7 to 14 (the day a copulatory plug was observed was designated gestational day 1) and allowed to litter; litters were examined on postnatal days 1 and 3 and discarded. Ten percent (5/50) of the dams died and 41% (15/37 dams) had viable pups. The number of live pups per litter at birth was reduced (and the number of dead pups per litter at birth was elevated), and postnatal survival was reduced (40% versus 100%) relative to controls. Pup birth weight and pup weight gain were both reduced relative to controls (Schuler *et al.*, 1984). The authors gave EG high priority for further testing.

In an NTP protocol for fertility assessment by continuous breeding (Gulati et al., 1984; Lamb et al., 1985), EG was administered in the drinking water to CDB-1 mice at 0.0, 0.25, 0.5 or 1.0% during 98 days of cohabitation (one male:one female). These doses were approximately equivalent to 0, 400, 800 and 1600 mg/kg/day. Litters were assessed at birth and discarded except for the last litter which was retained for offspring assessment. Exposure to EG at 1.0% resulted in a small but significant decrease in the number of litters per breeding pair, in the number of live pups per pair and in the live pup weight. A significant number of pups in the 1.0% dose group were born with distinct facial deformities. In the retained litters at this dose, the facial deformities were more obvious with age. These malformed animals also exhibited fused ribs and shortened nasal, parietal, and/or frontal bones of the skull. When pups from the high dose group were raised to adulthood (with continued exposure to EG) and mated, they exhibited decreased mating and fertility indices relative to controls handled in the same manner, but no effects were observed on litter size, pup weight or sex ratio (Gulati et al., 1984). The authors deemed EG a "weak reproductive toxicant, but a potential teratogen" (Gulati et al., 1984, p. 15).

All of the above studies involved exposure to EG via the gastrointestinal route (via gavage or dosed water) and all reported developmental toxicity including malformations in CDB-1 mice (Price et al., 1984; 1985; Schuler et al., 1984; Gulati et al., 1984; Lamb et al., 1985).

The study by Price et al. (1984; 1985) did not define a NOEL since the lowest dose evaluated, 750 mg/kg/day by gavage, resulted in reduced fetal body weight and increased fetal skeletal malformations. The study by Shuler et al. (1984) employed only one dose, 11,090 mg/kg/day by gavage. The study by Gulati et al. (Gulati et al., 1984; Lamb et al., 1985) identified 0.5% (approximately 800 mg/kg/day in the drinking water) as a NOEL.

Therefore, the present study is undertaken to determine the NOEL for ethylene glycol, administered by gavage to CDB-1 mice during organogenesis for developmental (and maternal) toxicity. The protocol will duplicate, as closely as possible, the procedures followed by Price et al. (1984; 1985).

MATERIALS AND METHODS

Test Article

Liquid ethylene glycol (EG; 1,2-ethanediol; glycol; CAS No. 107-21-1) will be the test material. The test material, polyester grade EG, was supplied by Union Carbide Corp., Hahnville, LA. One quart of slightly viscous liquid in a brown bottle, identified as "PC-35227, UCC B35227 12/86 4407" was received on February 18, 1987, and received the Bushy Run Research Center (BRRC) Sample No. 50-74. A sample of this material was returned to UCC, South Charleston, WV, prior to its use in this study for confirmation of purity and stability. Pertinent chemical and physical properties of EG are presented in Appendix 1.

Purity and Stability

Purity and stability of the test chemical will be provided by the supplier.

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Handling Precautions

Precautions will be undertaken to avoid contact of the compound to the clothing, skin or eyes. Rubber gloves and safety glasses will always be used in handling this compound.

Cleaning Contaminated Equipment: Equipment contaminated with the test chemical will be cleaned with water.

Storage Conditions: The test material will be kept in a chemical storage area. Samples used for the study will be stored in amber glass bottles at room temperature. A reserve sample will be taken and kept at BRRC.

Animals

Two hundred (200) virgin female and the same number of virgin male Cr1:CD-1 (ICR) Br outbred albino mice, referred to as the CD-1 mouse will be ordered from Charles River Breeding Laboratories, Inc., Kingston, NY. Female mice will be approximately 35 days old (20-25 g) and male mice will be the same age (25-30 g) upon arrival. The CD-1 mouse was chosen by the Sponsor because of its use in a previous teratogenicity evaluation with ethylene glycol, which was administered by gavage (Price et al., 1984; 1985). In addition, the incidence and frequency of spontaneous malformations in the CD-1 mouse have been reported (Palmer, 1972; Perraud, 1976; Fritz et al., 1978; Kimmel et al., 1985).

Quarantine Period

All animals will be housed in BRRC animal room 147. The animals will be received at least two weeks before the initiation of the mating period. During the quarantine period the animals will be observed regularly for general health status and ability to adapt to the watering system. Females will be kept on water bottles, males on the automatic watering system. Body weights will be ascertained at least once prior to the mating period. Only mice in good health as certified by the attending veterinarian with a body weight at least 20 g (females) or at least 25 g (males) will be used for this study.

Quality Control

Quality control will be performed upon the receipt of the animals. The following quality control evaluation will be performed on five mice per sex for serum analyses and for fecal examination (zinc flotation, cellophane test and visual inspection), and three per sex for histopathologic examination:

<u>To be analyzed</u>	<u>Analysis for</u>
Blood	Possible viral antibodies.
Feces	Intestinal parasites.
Salivary gland, trachea, lung, liver, kidney, cervical lymph nodes, and nasal cavity	Histopathology.

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Husbandry

During the quarantine period, mice will be housed one to three per cage (same sex) in stainless steel wire-mesh cages mounted in steel racks in animal room 147. After mating, females will be housed one per cage in stainless steel wire-mesh cages mounted in steel racks also in room 147. Decontaminated paperboard (Shepherd Specialty Papers, Inc., Kalamazoo, MI) will be placed under each row of cages to collect solid and liquid excreta. All females will be maintained on water bottles (with stainless steel sipper tubes) to minimize stress. Water and food will be available ad libitum at all times. Upon initiation of the dosing regimen, all study animals will remain in Room 147. The light cycle in this room will be 12 hours light/12 hours dark (approximately 0530-1730 for light). Specific information on the cages (dimensions, etc.) will be included in the study data.

Animal Identification

Each mouse will be assigned a unique number and will be identified by a system of ear and/or toe clipping. The system used will be documented in the study records.

Temperature and Relative Humidity

Temperature and relative humidity will be recorded continuously for each animal holding room. Room temperature will be maintained at $22 \pm 3^{\circ}\text{C}$, and relative humidity will be maintained between 40% and 60%.

Food and Water

The water available to the animals will be tap water (Municipal Authority of Westmoreland County, Greensburg, PA). The incoming water will be analyzed for contaminants by the supplier and by the NUS Corporation (Pittsburgh, PA). The water quality analyses performed per EPA specifications include the 129 "priority" pollutants, which were identified in the Federal Register, Vol. 45, No. 98 Appendix D, Part 122. The food available to the rats will be Agway Prolab Certified Rodent Chow (Agway Inc., St. Marys, OH). The analyses of each feed batch for composition and for possible contaminants will be performed by the supplier, Agway Inc., Research Laboratories, Ithaca, NY. Results of water and food analyses will be reviewed by the Study Director.

Mating

Virgin female C57BL/6 mice (at least 20 g and seven weeks old) will be randomly mated to male mice (at least 25 g), on a one male to one female basis. Each male will be used once in this study. The observation of vaginal copulation plugs will be considered evidence of successful mating for mice. The day a copulation plug is observed will be designated gestational day (gd) 0 (Hafez, 1970). Plug-positive mice will be housed individually in clean cages.

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Experimental Protocol

- a. **Randomization and group assignments:** Successfully mated females will be assigned by stratified randomization by body weight to one of four EG treatment groups and the vehicle control group on gd 0. Each experimental group will consist of thirty (30) plug-positive female mice.
- b. **Route of administration:** As per the request of the Sponsor, the test article in solution (or vehicle only) will be administered by oral-gastric intubation (gavage).
- c. **Dosage selection, formulation and administration:** The appropriate amount of EG will be weighed and dissolved in deionized (Millipore®) water (CAS No. 7732-18-5) with the concentration determined by the following formula:

$$\text{concentration (mg/ml)} = \frac{\text{dose level (mg/kg)}}{\text{dose volume (ml/kg)}}$$

The dosage volume will be 10.0 ml/kg (the same as that employed by Price et al., 1984; 1985), based on the individual animal's most recent body weight. The resulting solution will be administered through a 18 gauge 1.5-inch commercial ball-end stainless steel needle (Perfektum®, Popper and Sons, New Hyde Park, NY) attached to a 1.0 cc disposable syringe. The exact amount of dosing solution given to each animal will be recorded and will be available to the Sponsor upon request. The dosage solutions will be prepared as necessary based on the stability data (see Section "d" below). Deionized (Millipore®) water is selected as the vehicle since the test chemical is fully soluble in it and it was the vehicle employed by Price et al. (1984; 1985).

d. **Chemical analyses:** Prior to the onset of this study, the appropriate dosage formulations in water will be evaluated for homogeneity and stability by BRRC. Based on this information, dosage solutions will be formulated at BRRC and analyzed at least once each time they are formulated. The procedures and results of these evaluations and the times for formulation and analysis will be included in the raw data and detailed in the final report.

e. **Frequency and length of administration:** The test article solution (or vehicle only) will be administered as a single daily dose by gavage for 10 days (gd 6 through gd 15).

f. **Doses of administration:** The target concentrations for the four (4) groups receiving ethylene glycol in deionized water will 50.0, 150.0, 500.0 and 1500.0 mg/kg/day. The vehicle control group will receive deionized water at 10.0 ml/kg (0.0 mg/kg/day).

g. **Maternal observations and measurements:** Mice will be weighed on gd 0, 6, 9, 12, 15, and 18. All animals will be observed daily throughout the study (acclimation to necropsy). In addition, animals may be additionally observed daily during the treatment period and from the end of treatment until the termination of pregnancy for clinical signs of toxicity, if warranted. Water consumption will be recorded at three-day intervals, for gd 0-3, 3-6, 6-9, 9-12, 12-15, and 15-18.

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h. **Maternal sacrifice and laparotomy:** Mice will be sacrificed on gd 18, euthanized by cervical dislocation. The maternal body cavities will be opened by a midline thoracolaparotomy. The gravid uterus, ovaries (including corpora lutea), cervix, vagina, and abdominal and thoracic cavities of each pregnant animal will be examined grossly. Ovarian corpora lutea of pregnancy will be counted, and maternal liver weight will be determined. Maternal kidneys (2) will also be weighed, bisected (left longitudinally, right transversely) and fixed in buffered neutral 10% formalin. Kidneys from high dose and control dams will be examined histologically. Maternal kidneys from other animals may be examined histologically at the request of the Sponsor at additional cost. The uterus with ovaries and oviducts attached will be removed from the abdominal cavity, weighed, and dissected longitudinally to expose the contents. All live and dead fetuses and resorption sites (early and late) will be recorded. Uteri from females that appear nongravid will be placed in a 10% ammonium sulfide solution for confirmation of pregnancy status (Salewski, 1964).

All live fetuses will be weighed, sexed externally, and examined for external malformations including cleft palate, and external variations. All live fetuses in each litter will be examined for thoracic and abdominal visceral abnormalities by modification of methods described by Staples (1974). One half of the live fetuses in each litter (odd numbered fetuses from litters with an odd number of live fetuses and even numbered fetuses from litters with an even number of fetuses) will be decapitated and their heads will be fixed in Bouin's solution for examination of soft tissue craniofacial structures by a free-hand sectioning method modified from Wilson (1965, 1973) and van Julsingha and Bennett (1977). All fetuses in each litter (50% intact, 50% decapitated) will then be eviscerated, fixed in ethanol, processed for skeletal staining with alizarin red S (Crary, 1962; Peltzer and Schardein, 1966), and examined for skeletal malformations and variations.

i. **Dead or moribund animals:** A gross necropsy will be performed on all animals not surviving to the scheduled sacrifice in an attempt to determine the cause of death. Pregnancy status will be determined, if possible, by examination of uterine contents or by staining apparent nongravid uteri with 10% ammonium sulfide. Maternal tissues will be preserved for microscopic examination in neutral buffered 10% formalin only as deemed necessary by the gross findings.

j. **Culled animals:** Animals not used in the study will be euthanized or used for methods development and training of the BRRC staff. Their fate will be fully documented.

Statistical Analysis

The unit of comparison will be the pregnant female or the litter (Weil, 1970). Results of the quantitative continuous variables (e.g. maternal body weights, organ weights, etc.) will be intercompared for the four treatment groups and one vehicle control group by use of Levene's test for equal variances (Levene, 1960), analysis of variance (ANOVA), and t-tests with

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Bonferroni probabilities. When Levene's test indicates homogeneous variances and the ANOVA is significant, the pooled t-test will be used for pairwise comparisons. When Levene's test indicates heterogeneous variances, all groups will be compared by an ANOVA for unequal variances (Brown and Forsythe, 1974) followed, when necessary, by the separate variance t-test.

Nonparametric data obtained following laparohysterectomy will be statistically evaluated using the Kruskal-Wallis test (Sokal and Rohlf, 1969) followed by the Mann-Whitney U test for pairwise comparisons (Sokal and Rohlf, 1969) when appropriate. Frequency data will be compared using the Fisher's Exact Test (Sokal and Rohlf, 1969). For all statistical tests, the fiducial limit of 0.05 (two-tailed) will be used as the critical level of significance.

Personnel Health and Safety

Normal safety precautions will be employed in the handling of the test article. The Sponsor will advise BRRC of any additional precautions that should be taken.

Protocol Alterations

Alterations of this protocol may be made as the study progresses. No major changes in the protocol will be made without the specific written request or consent of the sponsor. In the event that the Sponsor authorizes a protocol change verbally, such change will be made by BRRC. However, it then becomes the responsibility of the Sponsor to follow such verbal change with a written verification. All protocol modifications will be issued as amendments and will be signed by the Study Director and Sponsor.

Scheduling

- Animals arrive at BRRC: June 13, 1988
- Acclimatization period begins: June 13, 1988
- Mating period begins: June 29, 1988 (first gd 0 June 30, 1988)
- Treatment period begins (gd 6): July 6, 1988
- Sacrifice period begins (gd 18): July 18, 1988
- Approximate date of completion of fetal head and skeletal evaluations: October 28, 1988
- Submission of draft final report to Sponsor: November 11, 1988

Records and Data Retention

All records pertaining to this study will be permanently retained and placed in the BRRC Archives upon completion of the report. All data, including reports from this study, will be retained for at least ten (10) years after completion of the study in the BRRC Archives and will be made available for inspection upon request by authorized personnel of the Sponsor. An appropriate sample of the test article will be retained for ten (10) years following completion of the study.

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Good Laboratory Practice Compliance

The Bushy Run Research Center through the administration of a quality assurance program by the Good Laboratory Practices Committee and Quality Assurance Unit, assures compliance of all phases of toxicological studies with existing regulations and generally accepted good laboratory practices [EPA (TSCA), 1983].

Personnel

Study Director: R. W. Tyl, Ph.D., DABT,
Manager, Reproductive and
Developmental Toxicology

Attending Veterinary Pathologist: P. E. Losco, VMD, Diplomate ACVP

Pathologist: P. E. Losco, VMD, Diplomate ACVP

Histology Personnel: M. A. McGee, HT(ASCP), Supervisor

**Developmental Toxicology
Personnel:** B. L. Butler, AHT, AALAS Cert. II
M. A. Copeman, A.A., B.A.
D. L. Fait, AALAS Cert. II
L. C. Fisher, B.S.
M. F. Kubena, B.S., AALAS Cert. II
D. J. Tarasi, AHT, A.S., AALAS Cert. 1

Analytical Personnel: J. P. Van Miller, Ph.D., DABT, Manager
M. A. Vrbanic, B.A.

Animal Care: R. E. Altman

Quality Assurance Personnel: L. J. Calist, B.S., AALAS Cert. III,
Group Leader
J. R. Bernard, B.S.
J. H. Coleman, B.S., AALAS Cert. III

Other study team members to be determined.

Report

A report will be prepared upon completion of the study. The narrative portion of the report will include an abstract, introduction, materials and methods, results, discussion, conclusions and references. The report will also include mean maternal body weights of pregnant animals, mean number of corpora lutea per dam, total, viable and nonviable implantations, embryonic resorptions, pre- and postimplantation losses, fetal sex ratios, mean fetal body weights, and malformations and variations observed in this study. All means will be accompanied by standard deviations. The report will include the following tables and appendices:

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A.

Tables

- Summary of Analyses of Dosing Solutions
- Distribution and Fate of All Animals on Study
- Summary of Maternal Body Weight and Weight Changes
- Summary of Maternal Clinical Signs
- Summary of Maternal Water Consumption
- Summary of Laparohysterotomy Findings
- Summary of Histologic Examination of Maternal Kidneys (high dose and controls)
- Summary of Fetal External Examinations
- Summary of Fetal Visceral Examinations (including results from cranio-facial examinations)
- Summary of Fetal Skeletal Examinations

B.

- Good Laboratory Practices Compliance Statement
- Dates of Quality Assurance inspections and report to management of significant deviations from protocol

C.

Appendices

- Protocol and Amendments(s)
- Analyses of Test Chemical and Dosing Formulations
- Individual Maternal Data (in-life and necropsy)
- Individual Fetal Body Weights by Litter, Sex and Uterine Location
- Individual Malformations and Variations by Fetuses and Litters
- Anatomic Pathology Report

WPC/esk/1207P-3
05-03-88

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MATERIAL SAFETY DATA SHEET

EFFECTIVE DATE: August, 1985

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APPENDIX 1

Union Carbide Corporation urges the customer receiving this Material Safety Data Sheet to study it carefully to become aware of hazards of any of the product involved in the interest of safety for himself (1) notify your employees, agents, and contractors of the information on this sheet. (2) furnish a copy to each of your customers for the product, and (3) request your customers to inform their employees and customers as well.

I. IDENTIFICATION

PRODUCT NAME:	ETHYLENE GLYCOL		
CHEMICAL NAME:	Ethylene Glycol	CHEMICAL FAMILY:	Glycols
FORMULA:	$\text{HOCH}_2\text{H}_2\text{OH}$	MOLECULAR WEIGHT:	62.07
SYNONYMS:	EG; Glycol; 1,2-Ethanediol		
CAS #	107-21-1	CAS NAME	1,2-Ethanediol

II. PHYSICAL DATA

BOILING POINT, 760 mm Hg	197°C (387°F)	FREEZING POINT	-13°C (9°F)
SPECIFIC GRAVITY ($\text{H}_2\text{O} = 1$)	1.115 at 20/20°C	VAPOR PRESSURE at 20°C.	0.08 mm Hg
VAPOR DENSITY (air = 1)	2.1	SOLUBILITY IN WATER, % by wt.	100
APPEARANCE AND ODOR	Colorless liquid; mild odor.	EVAPORATION RATE (Butyl Acetate = 1)	0.01

III. INGREDIENTS

MATERIAL	%	TLV	HAZARD
Ethylene Glycol	100	50 ppm (125 mg/m ³ ceiling, for vapor and mist combined (ACGIH 1984-85). Union Carbide recommended ceiling is the same as ACGIH TLV.	Toxic Animal Teratogen (UCC classification)

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT	241°F, Tag closed cup, ASTM D 56 240°F, Cleveland open cup ASTM D 92		
FLAMMABLE LIMITS IN AIR, % by volume	LOWER	3.2 (Estimated)	UPPER 15.3 (Estimated)
EXTINGUISHING MEDIA	Apply alcohol type or all purpose type foams by manufacturers' recommended techniques for large fires. Use water spray, carbon dioxide or dry chemical media for small fires.		
SPECIAL FIRE FIGHTING PROCEDURES	Do not spray pool fires directly. A solid stream of water or foam directed into hot, burning liquid can cause frothing. Use self-contained breathing apparatus and protective clothing.		
UNUSUAL FIRE AND EXPLOSION HAZARDS	None		

EMERGENCY PHONE NUMBER • 1-800-UCC-HELP • This number is available days, nights, weekends, and holidays.

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V. HEALTH HAZARD DATA (ADVERSE HEALTH EFFECTS WHICH MAY OCCUR FROM OVEREXPOSURE.)**LEVEL AND SOURCE:**For vapor and mist combined: 50 ppm ceiling (ACGIH 1984-85). 718**EFFECTS OF AN ACUTE OVEREXPOSURE -**

SWALLOWING	May cause abdominal discomfort or pain, dizziness, malaise, lumbar pain, oliguria, uremia, and central nervous system depression. Severe kidney damage follows the swallowing of large volumes of ethylene glycol. May be fatal.
SKIN ABSORPTION	No evidence of adverse health effects from available information.
INHALATION	May cause irritation of the nose and throat with headache, particularly from mists. High vapor concentrations caused, for example, by heating the material in an enclosed and poorly ventilated workplace may produce nausea, vomiting, headache, and dizziness.
SKIN CONTACT	No evidence of adverse health effects from available information.
EYE CONTACT	Liquid, vapor, and mist, may cause discomfort in the eye with transient conjunctivitis. Serious corneal injury is not anticipated.

EFFECTS OF REPEATED OVEREXPOSURE

Inhalation of mist may produce signs of central nervous system involvement, particularly dizziness and nystagmus.

OTHER EFFECTS OF OVEREXPOSURE

Ethylene glycol has been shown to produce dose-related teratogenic effects in rats and mice when given by gavage or in drinking water at high concentrations. There is, however, no currently available information to suggest that ethylene glycol has caused birth defects in humans. Therefore, ethylene glycol is considered an animal teratogen.

Two chronic feeding studies, using rats and mice, have not produced any evidence that ethylene glycol causes dose-related increases in tumor incidence, or a different pattern of tumors compared with untreated controls. The absence of a carcinogenic potential for ethylene glycol has been supported by numerous in vitro genotoxicity studies showing that it does not produce mutagenic or clastogenic effects.

(continued)

HEALTH HAZARD DATA (Continued)

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EMERGENCY AND FIRST AID PROCEDURES:

SWALLOWING	If conscious, give two glasses of water and induce vomiting. Call a physician immediately.
SKIN	Remove contaminated clothing and flush skin with water.
INHALATION	Remove to fresh air. Call a physician if discomfort persists.
EYES	Immediately flush with water, and continue washing the eyes for several minutes.

NOTES TO PHYSICIAN

The principal toxic effects of ethylene glycol, when swallowed, are kidney damage and metabolic acidosis. Ethanol is antidotal, and its early administration may block the formation of nephrotoxic metabolites of ethylene glycol in the liver. Ethanol should be given intravenously, as a 5% solution in sodium bicarbonate, at a rate of about 10 ml ethanol per hour. A desired therapeutic level of ethanol in blood is 100 mg/dl. Hemodialysis may be required. Pulmonary edema with hypoxemia has been described in a number of patients following poisoning with ethylene glycol. The mechanism of production has not been elucidated, but it appears to be noncardiogenic in origin in several cases. Respiratory support with mechanical ventilation and positive end-expiratory pressure may be required.

VI. REACTIVITY DATA

STABILITY		CONDITIONS TO AVOID	None
UNSTABLE	STABLE		
--	X		
INCOMPATIBILITY (materials to avoid)		Normally unreactive; however, avoid strong bases at high temperatures, strong acids, strong oxidizing agents and materials reactive with hydroxyl compounds.	
HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS		Burning may produce carbon monoxide and/or carbon dioxide.	
HAZARDOUS POLYMERIZATION		CONDITIONS TO AVOID	None
May Occur	Will Not Occur		
--	X		

(continued)

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VIII. SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN MATERIAL IS RELEASED OR SPILLED	Wear suitable protective equipment. Small spills should be flushed with large quantities of water. Larger spills should be collected for disposal.
WASTE DISPOSAL METHOD	Incinerate in a furnace where permitted under appropriate Federal, State, or local regulations. At very low concentration in water, ethylene glycol is readily biodegradable in a biological wastewater treatment plant.

VIII. SPECIAL PROTECTION INFORMATION			
RESPIRATORY PROTECTION (specify type)	NIOSH approved breathing air equipment or NIOSH approved face mask with organic vapor cartridge and dust or mist pre-filter (not for use in fire fighting or in atmospheres with reduced oxygen content).		
VENTILATION	General (mechanical) room ventilation may be adequate if handled at ambient temperatures or in covered equipment. If ambient temperatures are exceeded or operations exist which may produce misting, local exhaust ventilation is needed.		
PROTECTIVE GLOVES	Rubber or polyvinyl chloride coated	EYE PROTECTION	Monogoggles/ Face Shield
OTHER PROTECTIVE EQUIPMENT	Eye bath and safety shower		

IX. SPECIAL PRECAUTIONS	
PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING	Do not take internally. Do not breathe mist. Avoid prolonged or repeated breathing of vapor. Avoid contact with eyes. Keep container closed. Use with adequate ventilation. Wash thoroughly after handling.
FOR INDUSTRY USE ONLY	

OTHER PRECAUTIONS

None

The opinions expressed herein are those of qualified experts within Union Carbide Corporation. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. Since the use of this information and of these opinions and the conditions of use of the product are not within the control of Union Carbide Corporation, it is the user's obligation to determine the conditions of safe use of the product.



BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632

Telephone (412) 733-5200

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PROTOCOL AMENDMENT #1

TITLE: Developmental Toxicity of Ethylene Glycol Administered by Gavage to CDB-1 Mice: Determination of a "No Observable Effect Level" (NOEL)

BREC PROJECT NUMBER: 87-81-60212

SPONSOR: Chemical Manufacturers Association
Ethylene Glycol Program
2501 M Street, NW
Washington, DC 20037

TESTING FACILITY: Bushy Run Research Center
RD 4, Mellon Road
Export, PA 15632
Attention: E. W. Tyl
(412) 733-5277

Reviewed and Approved by:
Bushy Run Research Center:

Rochelle W. Tyl 5/15/88

Rochelle W. Tyl, Ph.D., DABT Date
Study Director/Manager,
Reproductive and Developmental
Toxicology Section

Linda J. Calisti 5/17/88

Linda J. Calisti, B.S. Date
Group Leader, Good Laboratory
Practices/Quality Assurance

Fred R. Frank 5/17/88

Fred R. Frank, Ph.D. Date
Director

Sponsor's Representative:

Carol E. Stack 5/20/88

Carol E. Stack, Ph.D. Date
Manager, Ethylene Glycol Program

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The protocol is amended as follows:

1. Location of Protocol Change: MATERIALS AND METHODS, Test Article (page 3)

Description of Protocol Change:

From:

"Liquid ethylene glycol (EG; 1,2-ethanediol; glycol; CAS No. 107-21-1) will be the test material. The test material, polyester grade EG, was supplied by Union Carbide Corp., Hahnville, LA. One quart of slightly viscous liquid in a brown bottle, identified as "PC-35227, UCC B35227 12/86 4407" was received on February 18, 1987, and received the Bushy Run Research Center (BRRC) Sample No. 50-74. A sample of this material was returned to UCC, South Charleston, WV, prior to its use in this study for confirmation of purity and stability. Pertinent chemical and physical properties of EG are presented in Appendix 1."

To:

"Liquid ethylene glycol (EG; 1,2-ethanediol; glycol; CAS No. 107-21-1) will be the test material. The test material, polyester grade EG, was supplied by Union Carbide Corp., Hahnville, LA. One quart of slightly viscous liquid in a brown bottle, identified as "PC-35227, UCC B35227 12/86 4407", UCC order No. 631370 was received on May 12, 1988, and received the Bushy Run Research Center (BRRC) Sample No. 51-182. Pertinent chemical and physical properties of EG are presented in Appendix 1."

Rationale: The analysis of a sample of the original EG, BRRC Sample No. 50-74 by UCC, South Charleston, WV indicated that although the purity was still >99.99%, the ultraviolet results were not in compliance with international standards. Therefore a new shipment of EG was obtained from the same source with appropriate analysis on the date indicated, prior to the start of the study, for use in this study.

2. Location of Protocol Change: MATERIALS AND METHODS, (page 4)

Description of Protocol Change:

From:

"Animals

Two hundred (200) virgin female and the same number of virgin male Crl:CD-1 (ICR) Br outbred albino mice, referred to as the CD-1 mouse will be ordered from Charles River Breeding Laboratories, Inc., Kingston, NY. Female mice will be approximately 35 days old (20-25 g) and male mice will be the same age (25-30 g) upon arrival. The CD-1 mouse was chosen

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by the Sponsor because of its use in a previous teratogenicity evaluation with ethylene glycol, which was administered by gavage (Price et al., 1984; 1985). In addition, the incidence and frequency of spontaneous malformations in the CDO-1 mouse have been reported (Palmer, 1972; Perraud, 1976; Fritz et al., 1978; Kimmel et al., 1985).

Quarantine Period

"All animals will be housed in BRRC animal room 147. The animals will be received at least two weeks before the initiation of the mating period. During the quarantine period the animals will be observed regularly for general health status and ability to adapt to the watering system. Females will be kept on water bottles, males on the automatic watering system. Body weights will be ascertained at least once prior to the mating period. Only mice in good health as certified by the attending veterinarian with a body weight at least 20 g (females) or at least 25 g (males) will be used for this study."

To:

Animals

Two hundred twenty-five (225) virgin female and the same number of virgin male Crl:CDO-1 (ICR) Br outbred albino mice, referred to as the CDO-1 mouse will be ordered from Charles River Breeding Laboratories, Inc., Kingston, NY. Female mice will be approximately 35 days old (20-25 g) and male mice will be the same age (25-30 g) upon arrival. The CDO-1 mouse was chosen by the Sponsor because of its use in a previous teratogenicity evaluation with ethylene glycol, which was administered by gavage (Price et al., 1984; 1985). In addition, the incidence and frequency of spontaneous malformations in the CDO-1 mouse have been reported (Palmer, 1972; Perraud, 1976; Fritz et al., 1978; Kimmel et al., 1985).

Quarantine Period

"All animals will be housed in BRRC animal room 147. The animals will be received at least two weeks before the initiation of the mating period. During the quarantine period the animals will be observed regularly for general health status and ability to adapt to the laboratory animal care procedures. All mice will be kept on water bottles. Body weights will be ascertained at least once prior to the mating period. Only mice in good health as certified by the attending veterinarian with a body weight at least 20 g (females) or at least 25 g (males) will be used for this study."

Rationale: The number of animals ordered was changed from 200/sex to 225/sex prior to start of the study to optimize the chances of obtaining the appropriate number of plug-positive females within the designated mating period. In addition, all animals will be on water bottles rather than just the females, since mice in general have a difficult time adjusting to automatic watering systems and the amended procedure will minimize stress and maximize the number of animals which acclimatize successfully.

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3. Location of Protocol Change: MATERIALS AND METHODS, Experimental Protocol (page 6)

Description of Protocol Change:

From:

"c. Dosage selection, formulation and administration: The appropriate amount of EG will be weighed and dissolved in deionized (Millipore®) water (CAS No. 7732-18-5) with the concentration determined by the following formula:

$$\text{concentration (mg/ml)} = \frac{\text{dose level (mg/kg)}}{\text{dose volume (ml/kg)}}$$

The dosage volume will be 10.0 ml/kg (the same as that employed by Price et al., 1984; 1985), based on the individual animal's most recent body weight. The resulting solution will be administered through a 18 gauge 1.5-inch commercial ball-end stainless steel needle (Perfektum®, Popper and Sons, New Hyde Park, NY) attached to a 1.0 cc disposable syringe. The exact amount of dosing solution given to each animal will be recorded and will be available to the Sponsor upon request. The dosage solutions will be prepared as necessary based on the stability data (see Section "d" below). Deionized (Millipore®) water is selected as the vehicle since the test chemical is fully soluble in it and it was the vehicle employed by Price et al. (1984; 1985)."

To:

"c. Dosage selection, formulation and administration: The appropriate amount of EG will be weighed and dissolved in deionized (Millipore®) water (CAS No. 7732-18-5) with the concentration determined by the following formula:

$$\text{concentration (mg/ml)} = \frac{\text{dose level (mg/kg)}}{\text{dose volume (ml/kg)}}$$

The dosage volume will be 10.0 ml/kg (the same as that employed by Price et al., 1984; 1985), based on the individual animal's most recent body weight. The resulting solution will be administered through a 18 gauge 1.5-inch commercial ball-end stainless steel needle (Perfektum®, Popper and Sons, New Hyde Park, NY) attached to a Hamilton Diluter/Dispenser (Hamilton, Reno, Nevada). The exact amount of dosing solution given to each animal will be recorded and will be available to the Sponsor upon request. The dosage solutions will be prepared as necessary based on the stability data (see Section "d" below). Deionized (Millipore®) water is selected as the vehicle since the test chemical is fully soluble in it and it was the vehicle employed by Price et al. (1984; 1985)."

Rationale: A Hamilton Diluter/Dispenser will be used to measure and deliver the dosing solutions rather than a 1.0 cc disposable syringe to optimize accurate measurement and delivery of the correct volume of dosing solution to each animal, since the Diluter/Dispenser is calibrated in µl.

4. Location of Protocol Change: MATERIALS AND METHODS, Experimental Protocol
(Page 7)

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Description of Protocol Change:

From:

"h. Maternal sacrifice and laparotomy: Mice will be sacrificed on gd 18, euthanized by cervical dislocation. The maternal body cavities will be opened by a midline thoracolaparotomy. The gravid uterus, ovaries (including corpora lutea), cervix, vagina, and abdominal and thoracic cavities of each pregnant animal will be examined grossly. Ovarian corpora lutea of pregnancy will be counted, and maternal liver weight will be determined. Maternal kidneys (2) will also be weighed, bisected (left longitudinally, right transversely) and fixed in buffered neutral 10% formalin. Kidneys from high dose and control dams will be examined histologically. Maternal kidneys from other animals may be examined histologically at the request of the Sponsor at additional cost. The uterus with ovaries and oviducts attached will be removed from the abdominal cavity, weighed, and dissected longitudinally to expose the contents. All live and dead fetuses and resorption sites (early and late) will be recorded. Uteri from females that appear nongravid will be placed in a 10% ammonium sulfide solution for confirmation of pregnancy status (Salewski, 1964).

All live fetuses will be weighed, sexed externally, and examined for external malformations including cleft palate, and external variations. All live fetuses in each litter will be examined for thoracic and abdominal visceral abnormalities by modification of methods described by Staples (1974). One half of the live fetuses in each litter (odd numbered fetuses from litters with an odd number of live fetuses and even numbered fetuses from litters with an even number of fetuses) will be decapitated and their heads will be fixed in Bouin's solution for examination of soft tissue craniofacial structures by a free-hand sectioning method modified from Wilson (1965, 1973) and van Julsingha and Bennett (1977). All fetuses in each litter (50% intact, 50% decapitated) will then be eviscerated, fixed in ethanol, processed for skeletal staining with alizarin red S (Crary, 1962; Feltzer and Schardein, 1966), and examined for skeletal malformations and variations."

To:

"h. Maternal sacrifice and laparotomy: Mice will be sacrificed on gd 18, euthanized by carbon dioxide asphyxiation. The maternal body cavities will be opened by a midline thoracolaparotomy. The gravid uterus, ovaries (including corpora lutea), cervix, vagina, and abdominal and thoracic cavities of each pregnant animal will be examined grossly. Ovarian corpora lutea of pregnancy will be counted, and maternal liver weight will be determined. Maternal kidneys (2) will also be weighed, bisected (left longitudinally, right transversely) and fixed in buffered neutral 10% formalin. Kidneys from high dose and control dams will be examined

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histologically. Maternal kidneys from other animals may be examined histologically at the request of the Sponsor at additional cost. The uterus with ovaries and oviducts attached will be removed from the abdominal cavity, weighed, and dissected longitudinally to expose the contents. All live and dead fetuses and resorption sites (early and late) will be recorded. Uteri from females that appear nongravid will be placed in a 10% ammonium sulfide solution for confirmation of pregnancy status (Salewaki, 1964).

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All live fetuses will be weighed, sexed externally, and examined for external malformations including cleft palate, and external variations. All live fetuses in each litter will be examined for thoracic and abdominal visceral abnormalities by modification of methods described by Staples (1974) and the sex verified internally. One half of the live fetuses in each litter (odd numbered fetuses from litters with an odd number of live fetuses and even numbered fetuses from litters with an even number of fetuses) will be decapitated and their heads will be fixed in Bouin's solution for examination of soft tissue craniofacial structures by a free-hand sectioning method modified from Wilson (1965, 1973) and van Julsingha and Bennett (1977). All fetuses in each litter (50% intact, 50% decapitated) will then be eviscerated, fixed in ethanol, processed for skeletal staining with alizarin red S (Crary, 1962; Peltzer and Schardein, 1966), and examined for skeletal malformations and variations."

Rationale: Mice will be sacrificed by carbon dioxide asphyxiation rather than by cervical dislocation since the former procedure is more humane and consistent with euthanization procedures at BRRC. Also, during the visceral examination of all live fetuses, the sex of each fetus will be verified internally. This is the routine procedure at BRRC and is added to the protocol for completeness.

PATH/plh/1386P-1
05-13-88

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Pharmacokinetics and Disposition Studies with Ethylene Glycol for FYI-OTS-0589-0692

FROM: Leonard C. Keifer, Ph.D. *L. Keifer*
Chemist
Metabolism/Structure Activity Section
Toxic Effects Branch
Health and Environmental
Review Division (TS-796)

TO: Jackie Favilla
FYI Coordinator
Chemical Screening Branch
Existing Chemical Assessment Branch (TS-778)

THRU: Pauline Wagner *Pauline Wagner*
Section Chief
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Toxic Effects Branch
Health and Environmental
Review Division (TS-796)

I. INTRODUCTION

Ethylene glycol (EG) is a slightly viscous, very hygroscopic liquid with a boiling point of 197.6°C; it is miscible with water (Windholz, 1983).

The study reviewed below was conducted to compare the pharmacokinetics and material balance for EG following single doses administered via intravenous (iv) injection, gavage, and dermal application to male and female rats.

II. REVIEW OF STUDY

A. Study Identification: Frantz SW, Beskitt JL, Grosse CM, Jensen CB, Tallant MJ. 1989 (March 24). Ethylene Glycol: Comparison of Pharmacokinetics and Material Balance Following Single Intravenous, Oral and Cutaneous Administrations to Male

and Female Sprague-Dawley Rats. Performed by Bushy Run Research Center, Export, PA. Project Report 51-543.

B. Study Type: Absorption, Distribution, Excretion, Pharmacokinetics, Material Balance

C. Test Materials: Unlabeled polyester grade EG from Union Carbide (PC-35227, UCC B35227 12/86 4407) had a purity of 100.0%. [^{14}C]EG was received in two lots from Sigma Chemical Company: 1) lot no. 037F9207, specific activity 3.6 mCi/mmol, radiopurity 99.2%; 2) lot no. 077F9209, specific activity 6.0 mCi/mmol, radiopurity >98%.

D. Test Animals: Male and female Sprague-Dawley rats 10-11 weeks old were acclimated to the laboratory for at least five days and to individual Roth-type metabolism units for two days prior to dosing. Each animal in the pharmacokinetics phase of the study was fitted with a jugular vein cannula prior to acclimation to the metabolism units. Groups of four male and four female animals were used for each phase of the study.

E. Dosing Regimen: Groups of animals received single doses of EG via iv injection, oral gavage, or dermal application at nominal doses of 10 or 1000 mg/kg. In addition groups of animals received single oral gavage doses of EG of 400, 600, or 800 mg/kg. Animals dosed dermally were dosed on an area of 1 cm² of clipped skin with undiluted EG or with a 50% aqueous solution of EG covered with an occlusive cover for 6 hours after application.

Dosing solutions were analyzed before and after dosing using a Hewlett Packard 5880 gas chromatograph (GC) with a Carbowax 20M fused silica capillary column and a flame ionization detector (FID).

F. Methods:

1. Material Balance Study: Following administration of EG by the appropriate route, animals were placed in individual metabolism units equipped for the separate collection of urine, feces, and expired CO₂. Urine and feces were collected at 12, 24, 36, 48, 72, and 96 hours post-dosing. Expired $^{14}\text{CO}_2$ was trapped during 12-hour intervals through 48 hours post-dosing and then at 24-hour intervals until termination of the collection period. Urine samples were stored at -80°C and $^{14}\text{CO}_2$ samples were stored at -20°C until analyzed.

At the end of the 96-hour (four days) collection period animals were killed and the following tissues and fluids were collected: blood, liver, kidney, brain, perirenal fat, lung, testes, pelt, dosed site (from dermal application groups), and residual carcass. Aliquots of tissues were

oxidized using an OX-300 Biological Materials Oxidizer (R. J. Harvey Instrument Corp.) prior to quantification of ^{14}C via liquid scintillation counting (LSC, instrument not identified).

2. Pharmacokinetics Study: Blood samples were drawn via the indwelling jugular vein cannula immediately before and at 0.5, 1, 2, 4, 8, 12, 18, 24, 36, 48, 72, and 96 hours following administration via either the oral or dermal route. Blood samples were collected at 0 (pre-administration), 5, 15, and 30 minutes and 1, 2, 4, 8, 12, 18, 24, 36, 48, 72, and 96 hours following administration of the iv dose. Radioactivity was determined in aliquots of plasma and red blood cells (following oxidation) using LSC. Equal portions of plasma from each animal in each group at each time interval were pooled for determination of unchanged EG.

Approximately 6 hours following the application of the dermal doses, the occlusive covering was removed and any unabsorbed test compound was washed from the site of application with a water-wetted cotton applicator. At the end of the collection period the skin at the site of application was removed from the animals. The coverings, washes, and skin were analyzed for radioactivity.

Urine and feces were collected at 12, 24, 36, 48, 72, and 96 hours post-dosing. Expired $^{14}\text{CO}_2$ was trapped during 12-hour intervals through 48 hours post-dosing and then at 24-hour intervals until termination of the collection period. Urine samples were stored at -80°C and $^{14}\text{CO}_2$ samples were stored at -20°C until analyzed.

3. Metabolite Profiling and Preliminary Identification: Relative proportions of unchanged ^{14}C -EG and metabolites in urine were determined using high performance liquid chromatography (HPLC, Waters Associates) with a refractive index (Waters Associates) and an in-line radioactivity flow monitor (Packard Instruments). Unchanged EG in plasma was determined using GC of the phenylborate ester derivatives using a Hewlett Packard 5890 GC with a bonded phase DB-1 fused silica capillary column and a Mass Selective Detector (MSD) operated in the Selective Ion Monitoring (SIM) mode.

4. Pharmacokinetic Description: Semilogarithmic plots of both total radioactivity and unchanged EG concentrations in plasma vs. time and the RSTRIP (Fox and Lamson, MicroMath Inc.) software were used to estimate the pharmacokinetic parameters. Parameters estimated were the following: the rate constant of absorption, k_a (except iv doses); the rate constant of initial disposition, α ; the rate constant for terminal disposition, β ; the rate constant

of elimination, k ; the volume of distribution at steady state, V_d^{ss} ; and the half-lives ($t_{1/2}$) of absorption and elimination.

G. Reported Results:

1. Material Balance Study:

a. IV Route: In rats dosed via iv injection with EG at 10 mg/kg the major route of excretion of the ^{14}C was in the expired air as $^{14}\text{CO}_2$ (approximately 41(M) - 44(F)% of the dose in 96 h) with the majority of the radioactivity expired in the first 12 h (30(M) - 32(F)% of the dose). Lesser amounts were excreted in the urine (25(F) - 27(M)% in 96 h). A total of 15(F) - 19(M)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The total recovery of radioactivity ranged from 85(F) to 90(M)%.

There was a dose dependent shift in the route of excretion of ^{14}C in the rats dosed with 1000 mg/kg EG. The major route of excretion of the ^{14}C at the high dose was in the urine (45(F) - 52(M)% of the dose in 96 h) with the majority of the radioactivity excreted in the first 12 h (39(F) - 44(M)% of the dose). Lesser amounts were excreted in the expired air as $^{14}\text{CO}_2$ (approximately 28(M) - 29(F)% of the dose in 96 h). A total of approximately 9(M,F)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The total recovery of radioactivity ranged from 88(F) to 92(M)%.

The tissue/plasma ratios for liver, kidney, and lung at both dose levels and pelt at the low (M) and the high dose (M,F) were greater than one.

b. Oral Route (Low and High Doses): At the low oral dose of 10 mg/kg EG the major route of excretion of the ^{14}C was in the expired air as $^{14}\text{CO}_2$ (approximately 42(M) - 48(F)% of the dose in 96 h) with the majority of the radioactivity expired in the first 12 h (30(M) - 35(F)% of the dose). Lesser amounts were excreted in the urine (approximately 26(M,F)% in 96 h). A total of 16(F) - 22(M)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The total recovery of radioactivity ranged from 92(F) to 93(M)%.

Excretion of ^{14}C in the rats dosed with 1000 mg/kg EG was primarily in the urine (35(F) - 43(M)% of the dose in 96 h) with the majority of the radioactivity

excreted in the first 12 h (approximately 31(M,F)% of the dose). Lesser amounts were excreted in the expired air as $^{14}\text{CO}_2$ (approximately 27(M) - 28(F)% of the dose in 96 h). A total of 11(M) - 16(F)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The total recovery of radioactivity was approximately 83(M,F)%.

The tissue/plasma ratios for liver, kidney, lung, and pelt (male) at the low dose were greater than one. At the high dose the tissue/plasma ratios were greater than one for the liver, kidney, lung, pelt, and residual carcass (male).

c. Oral Route (Intermediate Doses): Routes of excretion of ^{14}C at the intermediate oral dose levels were as follows:

<u>Dose (mg/kg)</u>	<u>Urine (%)</u>	<u>Expired $^{14}\text{CO}_2$ (%)</u>
400	21(M)	39(M)
	38(F)	39(F)
600	26(M)	34(M)
	37(F)	33(F)
800	27(M)	30(M)
	41(F)	32(F)

d. Dermal Route: At the low dermal dose of 10 mg/kg EG the major route of excretion of the ^{14}C was in the expired air as $^{14}\text{CO}_2$ (13(F) - 14(M)% of the dose in 96 h) with the majority of the radioactivity expired in the period of 24-96 h (7(F) - 10(M)% of the dose). Lesser amounts were excreted in the urine (7(M) - 8(F)% in 96 h). A total of 9(F) - 10(M)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The absorbed dose was approximately 31.5(M,F)% of the applied dose. The amount of radioactivity recovered from the skin at the site of application ranged from 3(F) to 10(M)%. The total recovery of radioactivity ranged from 42.5(F) to 49(M)%.

At the high dermal dose of 1000 mg/kg EG the major route of excretion of the ^{14}C was in the expired air as $^{14}\text{CO}_2$ (12(F) - 14(M)% of the dose in 96 h) with the majority of the radioactivity expired in the period of 24-96 h (6(F) - 8(M)% of the dose). Lesser amounts were excreted in the urine (7.5(F) - 8(M)% in 96 h). A total of 9(F) - 13(M)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The absorbed dose was

29(F) - 36(M)% of the applied dose. The amount of radioactivity recovered from the skin at the site of application ranged from 1.5(M) - 2(F)%. The total recovery of radioactivity ranged from 84(M) to 85(F)%.

For the dermal dose of 1000 mg/kg 50% aqueous EG the major route of excretion of the ^{14}C was in the expired air as $^{14}\text{CO}_2$ (6(M) - 9(F)% of the dose in 96 h) with the majority of the radioactivity expired in the period of 24-96 h (5(M) - 8(F)% of the dose). Lesser amounts were excreted in the urine (4(F) - 4.6(M)% in 96 h). A total of 11(M) - 11.5(F)% of the dose of radioactivity was recovered in the tissues examined, the residual carcass, and the cage washings. The absorbed dose was 22(M) - 26(F)% of the applied dose. The amount of radioactivity recovered from the skin at the site of application ranged from 4(F) - 5(M)%. The total recovery of radioactivity ranged from 81(M) to 83(F)%.

The tissue/plasma ratios for liver, kidney, lung (female), and pelt at the low dose were greater than one. At the high dose the tissue/plasma ratios were greater than one for the liver, kidney, lung, and pelt. For the 50% aqueous EG dose the tissue/plasma ratios for liver and pelt were greater than one.

2. Pharmacokinetics Study: The following text table summarizes the pharmacokinetic parameters estimated for total radioactivity for all dosing routes. The column headed " $k_a t_{1/2}$ " presents the half-lives for absorption, the column headed " $\alpha t_{1/2}$ " presents the half-lives for the initial disposition phase, the column headed " $\beta t_{1/2}$ " present the half-lives for the terminal disposition phase, and the column headed "Time to C_{max} " presents the time required for maximum concentration in plasma to be reached. Under the dermal route the column headed " $k_e t_{1/2}$ " presents the half-lives for elimination and the column headed "Lag time" presents the time delay until radioactivity reached the systemic circulation.

<u>Route</u>	<u>Dose (mg/kg)</u>	<u>$k_a t_{1/2}$</u>	<u>$\alpha t_{1/2}$</u>	<u>$\beta t_{1/2}$</u>	<u>Time to C_{max}</u>
IV	10	0	1.48m(M)	26.6h(M)	0
		0	2.62m(F)	18.9h(F)	0
	1000	0	3.88h(M)	37.0h(M)	0
		0	2.72h(F)	31.6h(F)	0