SUMMARY REPORT

OF THE EXTERNAL PEER REVIEW OF THE DRAFT

TOXICOLOGICAL PROFILE FOR

ETHYLENE GLYCOL

Submitted to:

The Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, MS F-32
Atlanta, GA 30333

Submitted by:

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QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by ATSDR. ATSDR confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled ATSDR's selection criteria. Reviewers conducted the review according to a charge prepared by ATSDR and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to ATSDR

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SECTION I

PEER REVIEWERS' SUMMARY COMMENTS

SUMMARY COMMENTS RECEIVED FROM

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CHAPTER 1. PUBLIC HEALTH STATEMENT

This section is well written. The stated objectives of the Public Health Statement have been met.

Suggest that summary of birth defects note that large amounts of ingested ethylene glycol results in defects in experimental animals.

Other minor changes / suggestions are written in red on the pages of the manuscript.

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

This section is generally well written.

As noted in this section, the developing fetus is "particularly sensitive" to ethylene glycol administered orally. One issue that is not adequately addressed is whether developmental effects are observed at doses that are or are not maternally toxic. The studies on developmental effects discussed beginning on page 12, and on pages 16 should include descriptions of the presence or absence of maternal effects such as weight gain, food consumption, and/or clinical signs. Subsequent statements in the Toxicological Profile note that development effects are observed at doses that do not cause maternal toxicity, but there is little, if any, discussion of these endpoints in this and subsequent sections of the document. It is important for the reader to understand whether the chemical is, or is not, a selective developmental toxicant, adversely effecting embryonic or fetal development at doses that do not adversely affect the mother.

Section 2.2 Summary of Health Effects (page 9, line 29f) is inconsistent with discussions in other sections of the document regarding the time course of development of metabolic acidosis. Although it is recognized that time course information has some overlap, given that the metabolic acidosis is the result of the buildup of ethylene glycol metabolites (primarily glycolic acid), it is probably more accurate to include this clinical endpoint in the second stage of toxicity. This is consistent with other areas of the document (e.g., Section 3.5.2, page 91, line 26f). Suggested changes in wording to this section are written in the text.

Other minor changes / suggestions are written in red on the pages of the manuscript.

CHAPTER 3. HEALTH EFFECTS

This chapter is generally well written. Specific comments are provided below. Other minor changes / suggestions are written in red on the pages of the manuscript.

Sections 3.2.1.6 and 3.2.2.6 Developmental Effects

As discussed above for Section 2.0, one issue that is not adequately addressed is whether developmental effects are observed at doses that are or are not maternally toxic. The studies on developmental effects should include descriptions of the presence or absence of maternal effects such as weight gain, food consumption, and/or clinical signs. The summary statement on page 62 (line 25) states that "there is a substantial database demonstrating development toxicity at ethylene glycol doses that are not maternally toxic. However, there is no discussion of these endpoints in the preceding summaries of individual studies that would support this conclusion. It is important for the reader to understand whether the chemical is, or is not, a selective developmental toxicant, adversely effecting embryonic or fetal development at doses that do not adversely affect the mother.

Section 3.4 Toxicokinetics

This section is well written.

Page 69 lines 13-19. State whether the skin was shaved prior to application.

Page 71 lines 9-10. Do you mean that the urinary excretion of glycolic acid increased as percent of dose, with increasing doses?

Page 71 lines 31-34: Please state the purpose of the sucrose administration.

Figure 3-3: Please define the difference between the heavy arrows and the light arrows. Also, it would be helpful to note on the figure the rate limiting steps, since these are often referred to in the text. The line leading to acetate needs an arrow.

Other minor changes / suggestions are written in red on the pages of the manuscript.

Section 3.5 Mechanisms of action

Section 3.5.2 (Mechanisms of toxicity) needs rewording and clarification. Page 79 lines 19-22 are awkwardly written, and should incorporate lines 11-14 from page 80. The paragraph could be better written, for example, as "There are three main effects responsible for the toxicity of ethylene glycol: increased osmolal gap, metabolic acidosis, and formation of calcium oxalate crystals. Several lines of evidence suggest that metabolites of ethylene glycol and responsible for these effects. First, there is a latent period before the symptoms of acidosis appear; second, there is no correlation between observed toxicity and ethylene glycol blood concentration; and third, inhibition of ethylene glycol oxidation prevents toxicity (Jacobsen and McMartin 1986)."

Page 79 line 24: The authors state "In the initial stages after ingestion...", which raises the question of whether ethylene glycol has caused clinical toxicity following inhalation or dermal absorption. If there are no reports in the literature of toxicity following inhalation or dermal exposure, the authors should state this. If toxicity can occur via routes other than ingestion, the above sentence needs to be changed.

Page 79 lines 14-15: This sentence is out of place and doesn't seem to belong here.

Page 79 lines 15-17: It was stated on page 71 lines 9-10 that saturable metabolism is the reason for glycolic acid accumulation; however, this sentence states that it is "because it is a substrate for lactic dehydrogenase and/or glycolic acid oxidase." Also, figure 3-3 states that glyoxylic acid is the substrate for lactate dehydrogenase and/or glycolic acid oxidase. Please clarify so that the information on page 71 matches that on page 79 and figure 3-3.

Page 79 line 32: Do you mean methanol instead of mannitol?

Page 80 line 19: The sentence states that lactic acid is a metabolite of ethylene glycol. However, lactic acid is not mentioned as a metabolite in section 3.4.3 as a metabolite, although lactate may be formed when pyruvate is converted to lactate generating NAD. The authors should determine whether lactate is in fact a metabolite and provide a reference or alter the sentence accordingly.

Page 80 lines 19-20: This statement repeats the information in lines 8-11 and should be deleted.

Page 80 lines 26-30: These sentences are out of place and appear to belong in the paragraph that starts on line 33.

Other minor changes / suggestions are written in red on the pages of the manuscript.

Section 3.12.2 Identification Needs – Epidemiology and Human Dosimetry Studies (page 104, line 31f)

The sentence beginning with "Populations **likely to show effects** of ethylene glycol include individuals exposed through dermal contact with ethylene glycol-containing automobile antifreeze....." (emphasis added) is misleading and not consistent with available data discussed elsewhere in the document that demonstrates that individuals with limited exposures are not likely to experience adverse effects. This is particularly true for the identified populations of "individuals exposed through dermal contact with ethylene glycol-containing automobile antifreeze and individuals who live near hazardous waste sites..." It seems that the intent of this section and specific sentence is to identify potential populations to include in future epidemiological studies. Accordingly, a rewording aimed at this purpose is suggested in the text.

Section 3.12.2 Identification Needs – Absorption, Distribution, Metabolism, and Excretion (pages 106 and 107)

There are no data available addressing the kinetics of *in vivo* ethylene glycol in humans following dermal exposure. I have added a statement suggesting that these types of studies be conducted (page 107, line 27).

Sections 4, 5, 6, 7, and 8

These sections are well written and organized. No major corrections or revisions are recommended.

One minor correction should be made on page 120, line 20: "suggest" should be changed to "suggests". This is also noted in red on the original document.

Appendices A, B, and C

These sections appear to be complete and well written. No changes or revisions are recommended.

SUMMARY COMMENTS RECEIVED FROM

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Chapter 1 - Public Health Statement

Page 2, Line 12: "Once in your body, most of the ethylene glycol is broken down into other more toxic chemicals and excreted in the urine".

- The tone of this chapter is appropriate

- The major headings are answered adequately and the summary statements are consistent and supported by facts. I would suggest wording change on page 5, line 3 to "Ethylene glycol and its effects can be measured in blood and urine". No clinical hospital laboratory can measure ethylene glycol's metabolites. Suggest including regional poison center phone number (1-800-222-1222) under the "where can I get more information?' heading (page 6, line 8).

- Scientific terms are appropriate.

Chapter 2 – Relevance to Public Health

- I agree with the staging of effects. Regarding inhalation MRL's (page 16) it should be noted that systemic toxicity via inhalation has rarely been described. I would place all human studies (on page 17) at the beginning of the section. Human data should be presented before animal data. While human data is good for acute exposure, animal data is necessary for repeated exposure.

- I believe that the animal effects are crucial to include because animals are frequently exposed and are often the index case due to pet exposure.

- Exposure conditions have been adequately described.

Chapter 3 – Health Effects

Section 3.2:

Human Study Toxicity

- Human studies that were adequately designed were identified in the text
- Author's conclusions were appropriate and accurately reflected.
- Appropriate NOAELs and LOAELs were identified (if applicable).
- Statistical results are not emphasized in this text, nor is there a need for extensive statistical analysis. The cancer sections have adequate statistical references.

Animal Study Toxicity

- Adequately designed animal studies were identified in the text.
- Animal species selected were appropriate.
- Author's conclusions of these studies appear to be appropriate and accurate.
- Appropriate NOALs and LOAELs were identified.
- Toxicities of various forms are not relevant and not really described.
- Statistical testing was minimally referenced and is appropriate.

Specific Suggestions

Page 40; Lines 4-5: "The American Association of Poison Control Centers (AAPCC) reported 17 fatalities in 2005 due to ethylene glycol ingestion."

Reference: Lai MW, Klein-Schwartz W, Rodgers GC, et al. 2006. 2005 Annual Report of the American Association of Poison Control Centers' National Poisoning and Exposure Database. Clinical Toxicology. 44:803-932

Page 53; Line 1: The normal value for an osmolal gap in humans is 10 to 15 mOsm per kg H_2O. An elevated osmolal gap is associated with ethylene glycol in the blood with an increase of 2 mOsm/kg H_2O for every 0.1 grams/L (1/6 mmol/L) of ethylene glycol.

Page 53; Line 53; Line10-11: add –Leukocytosis can be expected shortly after acute ethylol glycol exposure.

LSE TABLES

- The LSE tables and figures are completely and self-explanatory and is clearly explained.
- I would agree with the categorization of "less serious" and serious.
- Values of MRLs are justified.
-

Evaluation of Text

- Study limitations have been discussed
- Key endpoints have critically evaluated
- When appropriate, bottom line statements were made
- Conclusions are appropriate
- Adequate attention (specifically with NOAEL) was generally given to LSE charts. Figure 3-2 gives consistent data and is very readable.
- Animal data has been used to support possible human effects. This is especially true for Table 3-3 whereupon no dermal human effects were studied but rabbit/mouse studies are listed (primarily as NOAEL).

Section 3.4

- There is adequate discussion regarding absorption, distribution, metabolism and excretion of ethylene glycol. However the beginning of Section 3.4, 1.2 (page 97; line 22) the first line should be changed to "Direct evidence of rapid oral absorption of ethylene glycol..."
- The paragraph on inhalation exposure (absorption) should include Dr. Wezorek's case report: Inhalation of aerosolized ethylene glycol from an automobile heater resulted in a blood ethylene glycol level of 28mg/dl

Reference: Wezorek C, Hodgman M, Dean B. 1995. Inadvertent ethylene glycol inhalation resulting in a toxic level (Abstract). J Toxicol Clin Toxicol. 33: 553

- Page 73; line 9: I would add that the half life of ethylene glycol during Fomepizole therapy is 11 to 14.75 hours.
-

Reference: Baud FJ, Galliot M, Astier A. 1988. Treatment of ethylene glycol poisoning with intravenous 4-methylpyrazole. N Engl J Med 319: 97-100

- Major organ/tissue storage factors were identified as per physiologic parameters. I suggest including autopsy/forensic data from R. Baselt's text: Ethylene glycol appears to concentrate in the brain, liver and kidney.

Reference: Disposition of Toxic Drugs and Chemicals in Man. 2002. Editor: RC Baselt. Biomedical Publications, Foster City, California 6th Edition: 406-409

- All applicable metabolic parameters have been presented.
- There is adequate discussion of differences in toxicokinetics between humans and animals
- There is adequate discussion of the relevance of animal toxicokinetic data to humans
- There does not need to be discussion for different forms of ethylene glycol

Section 3.5

All possible mechanisms of action have been addressed.

Section 3.8

- The biomarkers listed are specific for exposure
- Would move discussion of anion gap and osmolal gap (pages 52 and 53) to Section 3.8.1 as biomarker to aid in diagnosis. This (along with renal function tests) would measure the biomarker of effect.

Section 3.9

- There is adequate discussion of interactive effect
- Few other interactive effects are known

Section 3.10

- Population susceptibility data is scarce. I agree with the data and conclusions presented.

Section 3.11

Update the following references:

Page 93: Lines 35 to 36

Dart RC, ed. 2004. Medical Toxicology. Lippincott, Williams and Wilkins. Philadelphia, PA: 1223-1229
Page 94 Lines 1 to 2
Flomenbaum NE, Goldfrank LR, Hoffman RS, et al. 2006. Goldfrank's Toxicologic Emergencies. 8th ed. New York, NY: McGraw-Hill, 1447-1459

Add these references:
Pellegrino B, Parravani A, Cook L, Mackay K. 2006. Ethylene Glycol Intoxication: Disparate Findings of Immediate versus Delayed Presentation. W.V. Med J 102(4): 32-34

Shannon MW, Borron SW, Burns MJ. 2007. Haddad and Winchester's Clinical Management of Poisoning and Drug Overdose 4th ed. St. Louis, MO: Saunders Elsevier 611-621

Hess R, Bartles MJ, Pottenger LH. 2004. Ethylene Glycol: an estimate of tolerable levels of exposure based on a review of animal and human data. Arch Toxicol 78: 671-680

Section 3.11.2

- Management is specific to the substance. Suggest mentioning that although not formally studied, activated charcoal (at 1 gram per kilogram body weight) given orally may be effective in partially preventing ethylene glycol gastrointestinal absorption. (replace page 94 Lines 20-22)
- The well accepted treatment /antidote is fomepizole which should be emphasized. The American Dosing Regimen is 15 mg/kg loading intravenous dose follwed by ten mg/ kg intravenous every 12 hours for four doses. Subsequent doses (if needed) are 15 mg/kg I.V. until plasma ethylene glycol level falls under 20 mg/dl. Suggest adding that ethanol should only be used if fomepizole is not available.
- The major hazards with ethanol therapy in pediatric patients are the potential development of hypoglycemia and increased risk for sedation.

Page 95 Line 14
Suggest changing level of ethylene glycol from 20 mg/dl to "when initial serum ethylene glycol levels exceed 50 mg/dl..."

- There are no issues of ethylene glycol being stored in adipose tissues so this part is not applicable.

Section 3.12

- I know of no other studies that would fill the data gap
- The data is presented in a neutral, non-judgmental manner.
- I agree with the identified data needs. I would add (on pages 97 and page 107) the fact that inhalation data studies do not take into account the potential increased toxicity of ethylene glycol when it is heated.
- The text adequately addresses and justifies the data needs.

Page 108, Line 31

I would also add that with exposure copious irrigation with water or saline can aid in ocular decontamination.

Pages 95 (line 21) and page 109 (line 21): Suggest elimination of the word magnesium form the text.

Chapter 4

Table 4-1 appears to be primarily complete and accurate. Suggest some additions to Table 4-2

-Suggest adding units to Density (1.1135 grams/ml) and molecular weight (62.07 g/mol)

-Suggest adding pH (neutral)

-Suggest adding that it is soluble in ether

Chapter 5

- I am not aware of any information that is incorrect or missing.

Chapter 6

- The text appropriately traces the substance from its environmental point of release until it reaches the receptor population and it provides technically sound information.
- The text covers pertinent information relative to transport, partitioning, transformation and substance degradation.
- The text provides adequate information on monitored and estimation of ethylene glycol levels in the environment. Proper units are utilized.
- The text provides sources and pathways of exposure for the general and special population. I agree with the selection of special population.

Section 6.8.1 and Section 7.3.1

The data needs are presented in a neutral, non-judgmental fashion-no apparent bias is noted. I agree with the information on identified data needs. The text adequately justifies further data needs development.

Chapter 7

- I am not aware of any other additional methods articulated in Tables 7-1 and 7-2
- The methods (particularly human-related) are mentioned in the text
- Unique aspects have been addressed

Section 7.31-see Section 6.8.1

Chapter 8

- I am not aware of any other regulations or guidelines that may be appropriate for Table 8-1

Chapter 9

Additional references are included in the specific sections and are enclosed.

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GENERAL COMMENTS ON DRAFT

This draft is very thorough and the authors have done an exceptional job of pulling together nearly all of the critical studies that impact on ethylene glycol. I have no comments on the general structure or on the writing. Also, the authors have accomplished the answers to most of the questions posed for the individual questions listed for each chapter in the Guide for Peer Review. As such, I have not directly listed my thoughts on each of those questions. Instead my comments are listed below by page number – in some cases, these comments address the suggested questions

CHAPTER 1. PUBLIC HEALTH STATEMENT

The Public Health Statement is very well written.

p. 6 – how are drinking water levels set?

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

Section 2.2 Summary of Health Effects

p. 8 – a better reference for # of poisonings in US would be one of the annual reports by the AAPCC (such as the elsewhere cited Litovitz et al. 2002 and Watson et al, 2004).

p.11 line 18/19 – Delete sentence because glycolic acid accumulation and acidosis do not contribute to the renal failure, which is solely caused by oxalate crystal accumulation (Green et al., 2005 and Cruzan et al., 2004).

p.11 line 21-23 – Revise sentence – Blood 1965 is incorrect reference. NTP 1993 did show hepatic effect at a dose lower than that producing kidney effect in mice, but the effective dose in mice was much higher than that producing kidney effect in rats, which are more sensitive to renal toxicity. The sentence as written is misleading therefore.

p. 12 – Insert sentence: However in rats dosed on Gd 6-15 with 1000 mg/kg/day in feed, skeletal malformations were not observed (Maronpot et al., 1983), suggesting the importance of dose-rate in producing developmental effects.

p. 13 – revise sentence to read "...; because these changes are not likely to alter ethylene glycol kinetics (Pottenger et al., 2001), this model may be useful in predicting developmental toxicity in humans." As discussed below (Chapter 3), the Pottenger study demonstrates the lack of effect of pregnancy on EG kinetics, indicating that the physiologic changes in pregnancy are not important for EG metabolism or distribution, such that the Corley PBPK model should be useful.

p. 15 – As discussed below under Section 2.3 (MRLs), the Gaunt study is not suitable for determining an intermediate-duration oral dose MRL, so this sentence should be altered to state Cruzan et al.

p. 15 – There now is a chronic oral dose study in Wistar rats (Wilson et al., 2005, copy provided), so the following sentences can be added in place of the deleted phrase. "In the male Wistar rat, which is particularly sensitive to ethylene glycol nephrotoxicity following intermediate duration exposure, renal effects after exposure in the diet for 12 months included crystal-induced nephropathy and renal dysfunction at doses ≥ 300 mg/kg/d. Dose-response data for compound-induced nephropathy in male Wistar rats were used to derive a chronic oral MRL for ethylene glycol (see Section 2.3)." e-pdf

Section 2.3 MRLs

p. 23 line 15-17. The Corley 2005a (liver homogenates) and Booth 2004 (liver slices) studies are not really opposed, as discussed below (Chapter 3), since both indicate that glycolate metabolism in humans in vitro is faster than that in rats. As such, the first suggested change is indicated. Nevertheless, the data may not yet support a less than 10-fold uncertainty factor at this time so no other changes are suggested in the text here.

p. 23 – revise last sentence to read "...; because these changes are not likely to alter ethylene glycol kinetics (Pottenger et al., 2001), this model may be useful in predicting developmental toxicity in humans." As discussed below (Chapter 3), the Pottenger study demonstrates the lack of effect of pregnancy on EG kinetics, indicating that the physiologic changes in pregnancy are not important for EG metabolism or distribution, such that the Corley PBPK model should be useful.

p.24 The paragraph on the uncertainty in acute oral MRL can be stated more strongly since Carney 2001 has shown that bolus doses produce higher glycolate levels than equal doses given over time. Also the two rat studies given equal doses by gavage or in diet show that only gavage (bolus) dosing produces developmental effects.

p. 26 Insert sentence: "Note that these intakes were averaged among rats (housed five per cage) and varied greatly during the 16-week exposure (since the amount of ethylene glycol in the diet was not adjusted for changing food consumption and body weights throughout the study).

p. 27 Insert sentences (at line 13 as indicated): " The Gaunt et al. (1974) study is not suitable for MRL consideration since the animal care in this study was questionable. Nearly all of the rats, possibly from the beginning of the study, showed evidence of respiratory infection (pneumonia) and infectious salivary adenitis, either of which could have confounded the results. Also, rats were fed a constant % ethylene glycol in the diet, such that daily consumption varied throughout the study (for example in the apparent effect group of 180 mg/kg, rats were exposed to ≥ 300 mg/kg for two weeks which is a level above the threshold for renal toxicity based on the chronic

study of Wilson et al., 2005). Furthermore, rats were housed in groups of five such that consumption of individual rats among the groups likely varied greatly. Hence, the dose levels in this study are not reliably consistent, unlike the study by Cruzan et al. (2004), which was conducted in the same strain, by the same route and for the same duration."

A further reason to use the Cruzan study for the intermediate MRL is the recent chronic study of Wilson et al. (2005) in the male Wistar rat which showed the same NOAEL of 150 mg/kg and a LOAEL of 300 mg/kg, thus appearing to substantiate the results of Cruzan et al, not Gaunt et al.

p.29 line 8-10 - I would not consider the slight fatty metamorphosis reported in female F344 rats in the DePass et al (1986a) study to be an adverse effect. At the 200 mg/kg dose, the increase in this parameter was of borderline statistical significance and there was no other evidence of hepatic pathology in these animals or animals receiving the highest dose of ethylene glycol. At no time (6, 12, 18 or 24 months) was there an increase in parameters of liver function (serum chemistry) or in liver weight, even in animals dosed at 1000 mg/kg. Hence there is no dose-dependency to this parameter. I would thus revise the indicated sentence to reflect a NOAEL of 200 mg/kg/day and a LOAEL of 1000 mg/kg/day based on renal pathology.

p.29 after line 25 – this would be the spot to insert a complete paragraph to describe the Wilson et al (2005) study here. Although the authors will want to be more or less thorough, the following is a succinct description.

Male Wistar rats were exposed to ethylene glycol in a low protein diet at 0, 50, 150, 300 and 400 mg/kg/day for 12 months. Endpoints included food and water consumption, body and organ weights, urinalysis, and renal and bladder histopathology. The concentrations of ethylene glycol and its metabolites, glycolate and oxalate, in blood, urine and kidneys were also determined. Benchmark dose (BMD) analyses were conducted using compound-induced nephropathy and kidney birefringent crystal data. No treatment-related effects occurred in the rats at 50 or 150 mg/kg/day. Toxicity was pronounced at 400 mg/kg/day, as shown by increased mortality and excessive weight loss, which led to humane termination of the remaining animals on Day 203. Rats in this group also showed increased kidney weights and major renal pathology (basophilic foci of crystal-related nephropathy, tubule dilatation, birefringent crystal deposition and transitional cell hyperplasia) and urinary bladder pathology (calculi, hemorrhage of bladder wall). Rats given 300 mg/kg were also severely affected, with increased mortality, decreased body weight, increased water consumption, increased urine volume (decreased specific gravity), increased kidney weights, and significant renal and bladder histopathology. In the kidney tissue, there were no differences in the concentrations of glycolate or oxalate at doses $\leq$ 150 mg/kg compared with controls, with clear non-linear increases in both

metabolites at 300 and 400 mg/kg/day. At the latter dose, concentrations of glycolate and oxalate reached an average of 14 μg/kg and 19,000 μg/kg, respectively. Thus, accumulation of calcium oxalate in the kidney correlated with the appearance of renal toxicity. These results indicate a NOAEL of 150 mg/kg/day and an LOAEL of 300 mg/kg/day from this study. BMD analyses showed a BMD05 and BMDL05 for compound-induced nephropathy of 170 and 150 mg/kg/day, respectively, and for compound-induced renal crystal deposition of 170 and 160 mg/kg/day, respectively.

p. 29 line 30. Insert: " kidney lesions (oxalate nephrosis) and mortality at 300 mg/kg/day in male Wistar rats (Wilson et al., 2005)"

p.30 line 4 Insert : "a NOAEL of 150 mg/kg/day and serious LOAEL of 300 mg/kg/day in Wistar males (Wilson et al., 2005).

CHAPTER 3. HEALTH EFFECTS

Section 3.1 INTRODUCTION

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

p.38 – possible typo – as written, states "decreases in live fetuses" as well as "increases in live fetuses".

p.45 – Insert line 26: " There were no effects on parameters of liver function measured in the serum at any dose in either male or female rats."

p. 45 – line 29 insert: "The significance of these minor hepatic lesions is questionable because of the lack of effects on liver weight and on liver function measures, even at the highest dose."

p.45 – last sentence in the paragraph needs to be referenced.

p.47 – Insert a sentence about the paper by Smith et al (1990) (in reference list) – this is a key paper in dogs since it showed that gavage dosing with 3.3 g/kg of ethylene glycol in dogs leads to a progressive development of renal toxicity as noted histopathologically, from 2 – 30 hours after dosing.

p. 48 – move sentence about the MRL from Gaunt paragraph to Cruzan paragraph as discussed for Chapter 2.

p. 49 – alter sentence to indicate that a chronic study has been done in Wistar rats, then describe the renal results from that study here.

p. 51 – add body weight results from Cruzan et al. (2004) – changes the ranges in the sentence as indicated.

p. 54 – add sentence: "In another case of fatal ethylene glycol poisoning, the development of rapid cerebral edema was documented by CT scan and was accompanied by definitive

evidence of calcium oxalate crystals within walls of CNS blood vessels, with associated inflammation and edema (Froberg et al., 2006)." (copy supplied) e-pdf

Toxicity - Quality of Human Studies

No comments needed.

Toxicity - Quality of Animal Studies

As noted above under Section 2.3, the Gaunt study is significantly flawed and a more recent study of the same rat strain and duration (Cruzan) can be substituted in terms of analysis of renal toxicity.

Levels of Significant Exposure (LSE) Tables and Figures

Add data from Wilson et al. (2005) chronic oral study to Table 3-2 and Fig 3-2.

Evaluation of Text

Section 3.3 GENOTOXICITY

Section 3.4 TOXICOKINETICS

p. 72 – the first paragraph needs major revision. The unpublished data of Bartels are published in the report by Corley et al., 2005a. The in vitro rates of metabolism of glycolate would be better assessed by comparing the Vmax (not the Km) determined by the Booth and Corley studies. An even better parameter for assessing relative rates of metabolism would be the Vmax/Km. In the Corley study, the respective Vmax/Km for human and rat liver homogenates are 2.15 and 0.65 L/g/h. In the Booth study, the respective Vmax/Km for human and rat liver slices are 0.43 and 0.28 L/g/h. As such, both studies indicate that the rate of metabolism of glycolate tends to be higher in human liver than in rat liver in vitro.

p. 73 – Insert at end of first paragraph: "In a series of 19 patients, the mean half-life of ethylene glycol during a period without ADH inhibitor treatment and without dialysis was 8.6 hours, while elimination after fomepizole therapy was slower, with a half-life of 19.7 hours (Sivilotti et al., 2000, copy provided)." hard copy

p. 77 last paragraph – I disagree with the assessment by CERHR and the authors on the Pottenger study. First Gd10 is in the middle of the critical window for the developmental effects, so is the most appropriate time to examine the effects of pregnancy on the kinetics of ethylene glycol and glycolate. The fact that pregnancy did not alter these kinetics suggests that thePBPK model of Corley does not need to be calibrated for the physiologic changes that occur during pregnancy such that the model does have significant predictive usefulness. Furthermore, from a mechanistic point of view, the physiologic changes during pregnancy would not be expected to significantly change the kinetics of ethylene glycol or glycolate, which are primarily controlled

by hepatic enzyme activity and renal excretory mechanisms (and not hepatic or renal blood flow). As such, the Pottenger study confirms what one would expect anyway.

Although the Pottenger study did not measure the fetal levels, another report (Corley et al., 2002, copy provided) examined the kinetics of ethylene glycol and glycolate in pregnant Spragu-Dawley rats given ethylene glycol either by gavage (100 or 1000 mg/kg) or by subcutaneous infusion pumps (1000 or 2000 mg/kg/day). By either dosing method, ethylene glycol levels in maternal blood paralleled those in conceptuses (embryos and embryonic fluid, analyzed separately), while glycolate levels in conceptuses also paralleled those in maternal blood, albeit at a consistently higher level (1.4-4 fold). As such, the glycolate levels in maternal blood could be used as a predictor of fetal glycolate levels and thus be useful for extrapolating across species. The continuous infusion dosing led to significantly lower ethylene glycol and glycolate levels than the equivalent dose administered by bolus gavage, confirming the importance of dose-rate in ethylene glycol developmental toxicity. e-pdf

These two studies (Pottenger 2001 and Corley 2002) indicate that pregnancy does not appear to alter ethylene glycol and glycolate kinetics and most importantly help to validate the usefulness of the Corley PBPK model for its predictive value. For these reasons, the last paragraph on p. 77 needs to be substantially revised.

p. 78 the first paragraph needs major revision. The unpublished data of Bartels are published in the report by Corley et al., 2005a. The in vitro rates of metabolism of glycolate would be better assessed by comparing the Vmax (not the Km) determined by the Booth and Corley studies. An even better parameter for assessing relative rates of metabolism would be the Vmax/Km. In the Corley study, the respective Vmax/Km for human and rat liver homogenates are 2.15 and 0.65 L/g/h. In the Booth study, the respective Vmax/Km for human and rat liver slices are 0.43 and 0.28 L/g/h. As such, both studies indicate that the <u>rate</u> of metabolism of glycolate tends to be higher in human liver than in rat liver in vitro.

p. 78 line 30 insert sentence: "Corley et al (2002) have confirmed that the rat embryo and embryonic fluid concentrate glycolic acid, reaching levels roughly twice that in maternal blood."

Section 3.5 MECHANISMS OF ACTION

p. 82 line 32 insert sentence: "Corley et al (2002) have confirmed that the rat embryo and embryonic fluid concentrate glycolic acid, reaching levels roughly twice that in maternal blood."

Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Section 3.7 CHILDREN'S SUSCEPTIBILITY

p. 86 – in the first paragraph, there is a strange dichotomy where ~5000 cases of poisoning are reported in 2001, but only 735 in 2003. The AAPCC reports are sometimes hard to decipher

because they change categories around – sometimes ethylene glycol is a chemical and sometimes it is an automobile product. These two sentences need to be reviewed for accuracy. Note also that Watson 2004 is not in the reference list.

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

p. 90 line 18-30 – The authors are technically correct that glycolate, being an endogenous chemical that can be obtained also from the diet, is not a pure biomarker. However, an increase in glycolate above the general background found in human plasma (which is < 1 mM) is a specific biomarker and probably the best indication of ethylene glycol exposure in humans. It is particularly useful in those situations where there is a lengthy period between exposure and the blood sampling. In those situations, there is often no ethylene glycol in the plasma (due its metabolism and elimination), while there still are elevated levels of glycolate. As such, the second paragraph of Section 3.8.1 needs to be reworded to indicate the usefulness of elevated plasma glycolate levels in diagnosing human exposure to ethylene glycol.

Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

Section 3.12 ADEQUACY OF THE DATABASE

p. 100 – change first paragraph to reflect discussion above for Section 2.3 that Cruzan is better for MRL than Gaunt.

p.100/101 – change the last paragraph of 100 and first of 101 as recommended to reflect the existence of the chronic study in Wistar rats by Wilson et al., 2005. It can probably be stated that the existing data are sufficient to produce a chronic oral MRL and no further studies are needed.

p. 105 – Add sentence where indicated: "However, increased blood glycolate above normal human background levels is strongly indicative of ethylene glycol exposure and is often used for clinical diagnosis or confirmation."

p.108 – insert: ", while Corley et al (2002) have shown in rats that glycolic acid is consistently higher in the conceptus compared to the maternal blood. "

Existing Information on Health Effects

Identification of Data Needs

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

CHAPTER 7. ANALYTICAL METHODS

CHAPTER 8. REGULATIONS AND ADVISORIES

CHAPTER 9. REFERENCES

p.9 Hogue et al 2006 not in list of references (and not on CD).

UNPUBLISHED STUDIES (IF APPLICABLE TO REVIEW)

The Gaunt et al. (1974) study is not suitable for MRL consideration since the animal care in this study was questionable. Nearly all of the rats, possibly from the beginning of the study, showed evidence of respiratory infection (pneumonia) and infectious salivary adenitis, either of which could have confounded the results. Also, rats were fed a constant % ethylene glycol in the diet, such that daily consumption varied throughout the study (for example in the apparent effect group of 180 mg/kg, rats were exposed to $\geq$ 300 mg/kg for two weeks which is a level above the threshold for renal toxicity based on the chronic study of Wilson et al., 2005). Furthermore, rats were housed in groups of five such that consumption of individual rats among the groups likely varied greatly. Hence, the dose levels in this study are not reliably consistent, unlike the study by Cruzan et al. (2004), which was conducted in the same strain, by the same route and for the same duration.

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SECTION II

ADDITIONAL REFERENCES AND DATA SUBMITTED BY THE PEER REVIEWERS

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Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats

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Green, Mike L., Marguerite Hatch, and Robert W. Freel. Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats. *Am J Physiol Renal Physiol* 289: F536–F543, 2005. First published April 26, 2005; doi:10.1152/ajprenal.00025.2005.—Ethylene glycol (EG) consumption is commonly employed as an experimental regimen to induce hyperoxaluria in animal models of calcium oxalate nephrolithiasis. This approach has, however, been criticized because EG overdose induces metabolic acidosis in humans. We tested the hypothesis that EG consumption (0.75% in drinking water for 4 wk) induces metabolic acidosis by comparing arterial blood gases, serum electrolytes, and urinary chemistries in five groups of Sprague-Dawley rats: normal controls (CON), those made hyperoxaluric (HYP) with EG administration, unilaterally nephrectomized controls (UNI), unilaterally nephrectomized rats fed EG (HRF), and a metabolic acidosis (MA) reference group imbibing sweetened drinking water (5% sucrose) containing 0.28 M NH_4Cl. Arterial pH, plasma bicarbonate concentrations, anion gap, urinary pH, and the excretion of titratable acid, ammonium, phosphate, citrate, and calcium in HYP rats were not significantly different from CON rats, indicating that metabolic acidosis did not develop in HYP rats with two kidneys. Unilateral nephrectomy alone (UNI group) did not significantly affect arterial pH, plasma bicarbonate, anion gap, or urinary pH compared with CON rats; however, HRF rats exhibited some signs of a nascent acidosis in having an elevated anion gap, higher phosphate excretion, lower urinary pH, and an increase in titratable acid. Frank metabolic acidosis was observed in the MA rats: decreased arterial pH and plasma HCO_3^- concentration with lower urinary pH and citrate excretion with elevated excretion of ammonium, phosphate and, hence, titratable acid. We conclude that metabolic acidosis does not develop in conventional EG treatments but may ensue with renal insufficiency resulting from an oxalate load.

chronic renal failure; kidney stone; arterial blood gases

ELEVATED URINARY OXALATE EXCRETION (hyperoxaluria) is a clinical condition affecting some 30% of the U.S. stone-forming population (19). Hyperoxaluria can result from both genetic and nonheritable causes. For example, extreme hyperoxaluria may be observed in patients having a relatively rare genetic disorder known as primary hyperoxaluria (types I and 2), where hepatic oxalate production is enhanced by the absence of alanine-glyoxalate transferase or glycolate oxidase, whereas more benign hyperoxaluria may result from enhanced enteric absorption of dietary oxalate or oxalate precursors (22).

While there are several animal models (5, 33, 42) that are used to study hyperoxaluria and its consequences, the most commonly employed and simplest approach to induce hyperoxaluria is to provide ethylene glycol (EG) in an animal's drinking water. EG is readily absorbed along the intestine and is metabolized in the liver to oxalate. Despite being one of the most popular animal models for studies of hyperoxaluria (20, 21), oxalate-induced renal tubular injury (2, 27, 35, 47), and calcium oxalate nephrolithiasis (9, 28, 33, 34), the use of EG as an oxalate precursor in experimental models has been criticized on the grounds that it produces metabolic acidosis (4, 5, 11), which clearly could confound interpretation of studies employing EG-induced hyperoxaluria. The acidosis hypothesis has been generally extrapolated from clinical observations of accidental or intentional overdoses (10, 15, 30–32) of antifreeze formulations, which may contain up to 95% EG. Remarkably, whether the dosage of EG commonly utilized to induce experimental hyperoxaluria (0.75% vol/vol provided in the drinking water) also results in metabolic acidosis has not been established. Given the substantial volume of information and conclusions derived from studies employing EG-induced hyperoxaluria regarding calcium oxalate nephrolithiasis and oxalate-induced renal injury, it is necessary that the issue of metabolic acidosis be experimentally resolved.

Accordingly, we chose to test the hypothesis that the standard EG-induced model of hyperoxaluria produces metabolic acidosis in male Sprague-Dawley rats by arterial blood-gas analyses and serum and urinary chemistries. We also evaluated these acid-base parameters in unilaterally nephrectomized rats (control and EG-treated), which is a more severe hyperoxaluria model (20). A fifth group of rats served as a metabolic acidosis reference group. Our results indicate that two-kidney rats made hyperoxaluric by administering of EG are not acidotic after 4 wk. However, signs of nascent acidosis are evident in rats that manifest compromised renal function as a consequence of chronic hyperoxaluria; whether this acidosis is caused by the EG ingestion or by the renal insufficiency alone remains to be determined. We conclude that at the dosage schedules commonly employed, EG does not produce metabolic acidosis in Sprague-Dawley rats.

MATERIALS AND METHODS

Animals

A total of 80 male Sprague-Dawley rats (275–300 g) were utilized in the current study and were purchased from Harlan (Indianapolis, IN). All rats had free access to Purina Rat Chow 5001 during the entire course of the study. The chemical composition of the diet is provided in Table 1. All experimental protocols were conducted in accordance with the guidelines of the University of Florida Institutional Animal Care and Use Committee and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Experimental Models and Protocols

Two experimental models of EG-induced hyperoxaluria, together with their respective controls, were examined in the current study, which provided varying degrees of hyperoxaluria. A fifth group of rats

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Table 1. *Chemical composition of the diet provided ad libitum to rats*

Nutrients	
Protein, %	23.4
Fat (ether extract), %	4.5
Fat (acid hydrolysis), %	5.5
Fiber (crude), %	5.3
Nitrogen-free extract, %	49.9
Total digestible nutrients, %	76.0
Gross energy, kcal/g	4.0
Metabolizable energy, kcal/g	3.04
Minerals	
Ash, %	6.9
Cations	
Na^+, %	0.40
K^+, %	1.10
Ca^{2+}, %	0.95
Mg^{2+}, %	0.21
Anions	
Cl^-, %	0.65
SO_4^{2-}, %	0.28
HPO_4^{2-}, %	0.67

was made acidotic and served as positive controls for metabolic acidosis.

Control group. For the control group (CON), 17 rats were provided free access to food and normal drinking water served as controls.

Hyperoxaluric group. For the hyperoxaluric group (HYP), 17 rats were given free access to food and drinking water that contained 0.75% (vol/vol) EG (changed daily) for a period of 4 wk (21). The provision of this dose of EG has generated hyperoxaluria in as early as 3 days (28) and as long as 60 days (47) with no discernable effect on renal function as judged by creatinine clearance (21, 27, 29).

Unilateral nephrectomy group. For the unilateral nephrectomy group (UNI), 17 rats were unilaterally nephrectomized (see below) and given 1 wk to recover. These nephrectomized rats were provided free access to food and normal drinking water and served as a nephrectomy control group.

Hyperoxaluria-induced chronic renal failure group. For the hyperoxaluria-induced chronic renal failure group (HRF), 17 unilaterally nephrectomized rats were given 1 wk to recover from surgery before being given free access to food and drinking water that contained 0.75% (vol/vol) EG for a period of 4 wk as previously described (20).

Metabolic acidosis group. For the metabolic acidosis group (MA), acidosis was produced by providing free access to food and drinking water that contained 0.28 M NH_4Cl plus 5% (wt/vol) sucrose for 4 ($n = 6$) or 14 days ($n = 6$). This is a well-established protocol for the induction of metabolic acidosis in the rat (1, 38, 39). An initial analysis indicated no significant differences between the two acidotic groups for any parameter examined, so the rats were combined into a single metabolic acidosis group ($n = 12$).

Unilateral Nephrectomy

To produce oxalate-induced chronic renal failure, unilateral nephrectomies were performed on 34 rats. Briefly, a surgical plane of anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt). A small dorsal incision, ~1.5–2 cm, was made along the upper flank overlying the left kidney. The kidney was exposed through the dorsal incision, decapsulated, and the renal vasculature was ligated before excision of the renal mass. The flanking musculature was sutured, and the skin was closed using Autoclip wound clips. Before treatments were initiated, all rats that underwent surgery were given 1 wk to recover.

Urine Collection

Two weeks after the initiation of treatment and on the penultimate day of the study (4 wk), rats were placed in metabolic cages and 24-h urine collections were made. Urine was collected in 20 μl of 20% sodium azide as a preservative. Particulate matter was sedimented by low-speed centrifugation. A 5-ml aliquot was removed, and the remainder was acidified by the addition of 3 N HCl (~1 ml/5 ml urine volume). The acidified urine was used for the determination of citrate, calcium, and oxalate, whereas osmolality, phosphate, chloride, titratable acid, and ammonium excretion were determined from the nonacidified aliquot.

Blood Collection

At the end of their respective treatment period, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt). Arterial blood samples (~1 ml) were drawn from the abdominal aorta ($n = 11$ rats/treatment group) with blood-gas sampling syringes (PROVENT^{PLUS}, Portex, Keene, NH), and the samples were immediately transported to the STAT lab of Shands Hospital at the University of Florida for blood-gas analysis with an ABL system 500 (Radiometer, Westlake, OH). Arterial blood samples were also drawn from the acidotic reference groups (MA; $n = 12$) and processed as described above. Base excesses were calculated with an algorithm provided by Radiometer and represent the concentration of titratable base when the blood is titrated with strong base or acid to a plasma pH of 7.4 at a P_{CO_2} of 40 Torr and 37°C at the actual oxygen saturation (37). A separate group of rats ($n = 6$ rats/treatment group) was utilized for analysis of serum electrolytes. Serum Na^+, K^+, and Cl^- and CO_2 were measured with a Roche Hitachi Modular P800 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) in venous blood drawn from the anterior vena cava. Anion gaps were calculated from the serum electrolytes as follows: anion gap = serum $[Na^+]$ − (serum $[Cl^-]$ + serum $[CO_2]$) (43). We did not perform serum electrolyte analysis on the MA reference group as it is well established that NH_4Cl causes a hyperchloremic (normal anion gap) acidosis (4, 43). All rats were fully exsanguinated via cardiac puncture, and the serum was collected by centrifugation at 3,000 *g* for 15 min. An aliquot was immediately processed for oxalate determination with all precautions to prevent oxalogenesis (18), and the remainder of the serum was frozen for osmolality and creatinine determination.

Biochemical Determinations

Urinary chloride concentrations were determined with a chloridometer (Labconco, Kansas City, MO). Urine and serum osmolalities were measured with a freezing-point osmometer (Fiske Associates, Norwood, MA). Free orthophosphate (phosphate) was measured with a malachite green phosphate assay kit (BioAssay Systems, Hayward, CA). Creatinine was determined in the urine and serum samples using a modification of the Jaffé reaction as described by Slot (45) and further modified by Heinegard and Tiderstrom (24). Urinary ammonium was measured with an ammonium ion-selective electrode (detect*ION* 3051, World Precision Instruments, Sarasota, FL). Calcium (Pointe Scientific, Lincoln Park, MI), citrate (R-Biopharm, Marshall, MI), and urinary oxalate (Trinity Biotech, St. Louis, MO) were measured with commercially available kits. Serum for oxalate determination was ultrafiltered using an Amicon Ultra-4 device, and oxalate was measured as previously described (18). Titratable acid was quantitated by titrating the urine samples to pH = 7.4 with either 1.0 N NaOH or 1.0 N HCl.

Statistical Analyses

All data were subjected to least-squares ANOVA using the general linear models procedures of the Statistical Analysis System (3). Significant treatment effects were separated by a Student-Newman-Keuls sequential range test (46). Main effects of EG and unilateral

nephrectomy were tested by orthogonal contrasts. When the main effect of unilateral nephrectomy was significant, comparisons were made between the UNI rats and the HRF rats. Differences between the MA group and the CON group were compared by an unpaired Student's t-test. All data were tested for heterogeneity of variance with the Levene median test and for normality with the Kolmogorov-Smirnov test before ANOVA procedures (12). When normality and/or heterogeneity of variance tests failed, data were rank transformed before ANOVA. All data are expressed as means ± SE, and differences between means were considered statistically significant if $P < 0.05$.

RESULTS

Ingestion of excess non-carbonic acid loads or of compounds that are metabolized to such acids (e.g., EG and NH_4Cl) may produce a metabolic acidosis characterized by a fall in arterial pH and HCO_3^- concentration at normal P_{CO_2}. Additionally, the anion gap [serum $[Na^+]$ − (the sum of serum $[Cl^-]$ + serum $[CO_2]$)] produced by non-carbonic acid ingestion may be elevated in metabolic acidosis.

Blood-Gas and Electrolyte Analyses

Comparisons of arterial blood-gas analyses for the five groups of rats are shown in Fig. 1. Metabolic acidosis caused a significant reduction in arterial pH (Fig. 1*A*; 7.43 ± 0.01 vs. 7.33 ± 0.02 in CON vs. MA, respectively) and arterial bicarbonate concentrations (Fig. 1*B*; 29.1 ± 0.6 vs. 21.6 ± 1.1 meq/l in CON vs. MA, respectively). However, arterial pH and bicarbonate concentrations did not statistically differ among CON, HYP, UNI, and HRF, but the lowest numerical values for each were consistently observed in HRF rats. P_{CO_2} (Fig. 1*C*) and P_{O_2} (83.8 ± 4.8 Torr in CON) were unaffected by any of the treatments (data not shown). However, there was a tendency for P_{CO_2} to be lower in the MA group, which may represent a respiratory compensation for metabolic acidosis. Base excess was normal in the HYP group relative to the CON group (Fig. 1*D*). In contrast, base excess tended to be reduced in the HRF rats relative to CON rats, whereas a base deficit of 1.4 ± 1.6 meq/l was observed in the MA rats.

Serum Na^+, K^+, and Cl^- were also measured in all rats except the MA group (Table 2). Anion gaps were calculated from these data and the serum bicarbonates (Fig. 1*B*). As shown in Table 2, only the unilaterally nephrectomized rats treated with EG exhibited a significant elevation in anion gap, indicating the accumulation of anions other than chloride and bicarbonate (perhaps EG metabolites like glycolate, glyoxylate).

Thus these results demonstrate that chronic EG ingestion, at the dosages provided in this study, does not have an impact on the acid-base chemistries of rats with normal renal function (see below). The more severe model, coupling reduced renal mass with EG ingestion, appears to exhibit some, but certainly not all, of the characteristics of metabolic acidosis. In contrast, the conventional model of NH_4Cl-induced metabolic acidosis exhibited the primary hallmarks of this state: decreased arterial pH with a fall in plasma bicarbonate and a base deficit.

Urine Chemistries

Metabolic acidosis may also be manifest in urinary chemistries as a reduction in urinary pH, an increase in the excretion of titratable acid (principally phosphate), and an increase in urinary ammonium ion excretion. Additionally, urinary citrate excretion, a principal organic anion of urine, is reduced in acidosis (16) and calcium excretion may be enhanced (6). Consequently, changes in urinary chemistries in the five experimental groups were evaluated using two 24-h urine collections. Collections for the CON, HYP, UNI, and HRF groups were made at 14 and 28 days and are depicted separately as noted below. In the MA group, there were no significant differences in the parameters measured in 4- and 14-day collections; hence these were combined and are depicted as noted below as a single time point (4–14).

Urinary pH after 2 and 4 wk of treatment followed a similar pattern and was not different among CON, HYP, and UNI rats, as illustrated in Fig. 2*A*. In contrast, urinary pH was significantly lower in the HRF rats than in the CON, HYP, and UNI rats at 2 and 4 wk, but this fall in urinary pH was not nearly as

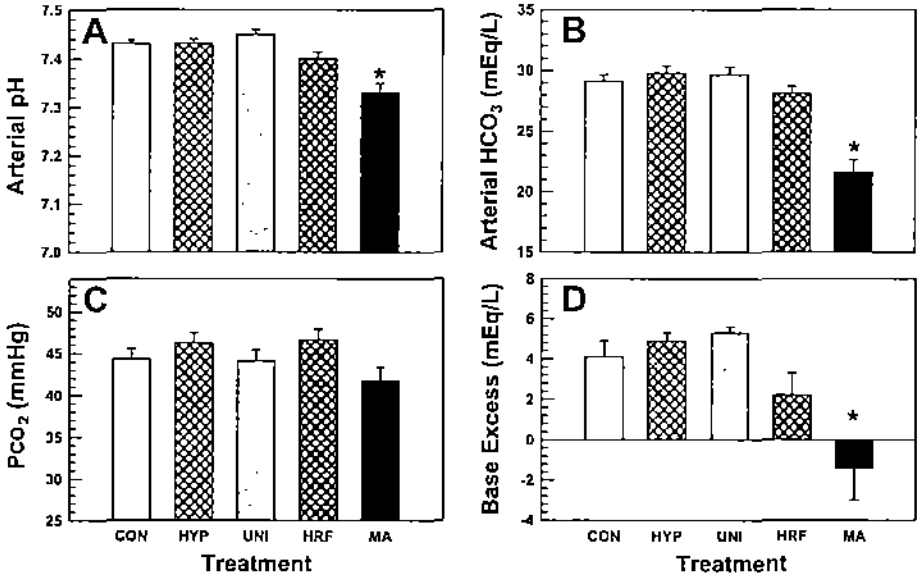

Fig. 1. Arterial blood-gas analysis in normal rats (CON), hyperoxaluric rats (HYP), unilateral nephrectomized rats (UNI), rats in renal failure induced by hyperoxaluria (HRF), and rats with metabolic acidosis (MA) after 4 wk on their respective treatment protocols. See MATERIALS AND METHODS for details on experimental models. Arterial pH (*A*) was significantly reduced in MA rats relative to all other groups. Arterial concentrations of bicarbonate (*B*) were lower in MA rats than in all other groups. P_{CO_2} (*C*) did not differ among any of the treatment groups. Base excess (*D*) was similar among CON, HYP, and UNI groups but tended to be lower in HRF rats relative to CON rats. In contrast, a base deficit was present in MA rats. *$P < 0.05$.

Table 2. *Serum and urinary electrolyte measurements after 4 wk on ethylene glycol*

Parameter	Treatment				
	CON	HYP	UNI	HRF	MA
Body wt, g	389.2±4.8 (n=17)	390.8±6.6 (n=17)	384.0±5.3 (n=17)	381.8±5.5 (n=17)	368.9±5.0 (n=12)
Kidney wt, g/kg	2.63±0.07 (n=11)	2.85±0.03 (n=11)	3.75±0.12* (n=11)	5.15±0.34*† (n=11)	2.77±0.07 (n=12)
Serum					
Osmolality, mosmol/kgH_2O	329.0±6.6 (n=9)	341.6±7.8 (n=9)	320.7±9.5 (n=11)	332.1±9.1 (n=10)	329.0±3.6 (n=12)
Na^+, meq/l	138.7±0.8 (n=6)	140.2±0.9 (n=6)	139.3±0.3 (n=6)	142.3±1.4*† (n=6)	ND
K^-, meq/l	7.5±0.4 (n=6)	7.7±0.4 (n=6)	6.8±0.3 (n=6)	6.5±0.1* (n=6)	ND
Cl^-, meq/l	99.3±0.6 (n=6)	100.0±0.5 (n=6)	99.5±0.6 (n=6)	101.3±1.5 (n=6)	ND
Anion gap, meq/l	15.5±0.6 (n=6)	17.7±1.8 (n=6)	14.7±0.6 (n=6)	21.8±1.7*† (n=6)	ND
Urine					
Volume, ml/24 h	14.0±0.9 (n=17)	21.1±2.9* (n=17)	19.1±0.8 (n=17)	49.0±3.9*† (n=17)	15.7±2.6 (n=12)
Osmolality, mosmol/kgH_2O	1,950±93 (n=11)	1,749±119 (n=11)	1,584±32 (n=11)	746±77*† (n=11)	1,992±51 (n=12)
Na^+, meq/24 h	2.34±0.14 (n=11)	2.62±0.14 (n=11)	2.55±0.08 (n=11)	2.95±0.54 (n=11)	1.97±0.16 (n=12)
K^+, meq/24 h	3.83±0.22 (n=11)	4.38±0.19 (n=11)	4.41±0.15 (n=11)	3.75±0.22 (n=11)	3.98±0.23 (n=12)
Cl^-, meq/24 h	3.09±0.12 (n=17)	3.57±0.11 (n=17)	3.63±0.10 (n=17)	3.38±0.12 (n=17)	10.93±0.49* (n=12)
Unmeasured A^-, meq/24 h	2.96±0.21 (n=10)	3.41±0.22 (n=9)	3.30±0.15 (n=10)	2.97±0.48 (n=9)	0.07±0.24* (n=12)

Values are means ± SE. CON, normal control; HYP, rats made hyperoxaluric by administration of ethylene glycol; UNI, unilaterally nephrectomized control rats; HRF, unilaterally nephrectomized rats fed ethylene glycol; MA, metabolic acidosis reference group; ND, not determined; A^-, anions; Anion gap, serum $[Na^+]$ − (serum $[CO_2]$ + serum $[Cl^-]$). Kidney wet weights are presented as the average kidney weight per kilogram body weight. For the UNI and HRF groups, kidney wet weights are presented as the weight of the single remaining kidney per kilogram body weight. Unmeasured anions in the urine were calculated as the difference between the number of milliequivalents of the measured cations ($Na^+ + K^+ + Ca^{2+} + NH_4^+$) and the number of milliequivalents of measured anions (Cl^- + phosphate) as previously described (8, 41). *$P < 0.05$ vs. CON. †$P < 0.05$ vs. UNI.

striking as that observed in the acidosis reference group (MA), where urinary pH fell below 5.5. Titratable acid was not different among CON, HYP, and UNI rats with the mean titratable acid in all these groups at 2 and 4 wk not significantly deviating from zero. However, titratable acid was increased in the HRF rats relative to the CON rats at 2 and 4 wk, with titratable acid in HRF rats being 0.22 ± 0.09 and 0.13 ± 0.08 meq/24 h at 2 and 4 wk, respectively. Titratable acid was higher in the MA rats than in all other groups, averaging 1.41 ± 0.08 meq/24 h. Total acid excretion, defined as the sum of potentially ionizable H^+ ions (titratable acidity) and bound (nonionizable) H^+ ions in the form of ammonium, was similar among CON, HYP, UNI, and HRF rats, but was higher in MA rats (Fig. 2*B*). The lack of a significant acid load in CON rats from the current study is most likely due to the alkali load provided by the diet (8, 41). Twenty-four hour urinary excretion of ammonium was nearly 30-fold higher in MA rats than in CON rats (Fig. 3*A*). In contrast, urinary excretion of ammonium did not differ between CON and HYP rats at 2 or 4 wk. Unilateral nephrectomy caused a significant decline in urinary ammonium excretion that was not additionally affected by hyperoxaluria-induced renal failure.

Metabolic acidosis induced hyperphosphaturia, with 24-h phosphate excretion being about fourfold higher in the MA group than in the CON group (Fig. 3*B*). The hyperphosphaturia was not evident in the HYP group, again illustrating the dichotomy between the results obtained in the MA and HYP groups. Urinary excretion of phosphate was similar between UNI and HRF groups after 2 wk but was significantly higher in HRF rats than in UNI rats after 4 wk.

Urinary chloride excretion was similar among CON, HYP, UNI, and HRF groups after 2 and 4 wk (Table 2). In contrast, chloride excretion in the MA reference group was about threefold higher than in the CON group.

The MA group showed a significant decrease in urine citrate excretion compared with CON rats (Fig. 4*A*). In contrast, EG-induced hyperoxaluria (HYP group) had no effect on excretion of citrate in the urine after either 2 or 4 wk compared with the CON group. However, unilateral nephrectomy (UNI group) caused a modest rise in urinary citrate excretion, and this increase was attenuated in HRF rats, suggesting some tendency toward acidosis after 4 wk on EG treatment which correlates with the reduction in renal function as judged by a twofold increase in serum creatinine and a 50% reduction in renal creatinine clearance as described in a subsequent section.

Urinary excretion of calcium (Fig. 4*B*) was significantly increased with metabolic acidosis (MA group) and unilateral nephrectomy (UNI group) but tended to be reduced in all hyperoxaluric groups compared with their appropriate controls (HYP vs. CON and HRF vs. UNI). This trend was apparent after both 2 and 4 wk on treatment.

Oxalate Handling

Consistent with our previous studies that utilized these rat models (20, 21), the EG-treated rats (HYP and HRF) exhibited significant hyperoxaluria, hyperoxalemia, and an increased renal clearance of oxalate compared with their respective controls (Fig. 5). By 2 wk, urinary oxalate excretion was increased about four- and sevenfold in HYP and HRF rats, respectively, compared with CON rats. Further significant increases were apparent at 28 days of EG treatment. The significant elevation in serum oxalate and the reduced renal clearance of oxalate in HRF compared with HYP, which we have previously reported (20), is confirmed here and is clearly a direct consequence of reduced renal function in these rats. This study also confirms an earlier report (20) demonstrating no differences in oxalate handling in rats with one kidney compared with healthy controls (both kidneys intact). Interestingly, we find here that metabolic acidosis is not associated with any significant alterations in oxalate homeostasis, as judged by results showing that urinary oxalate excretion, serum

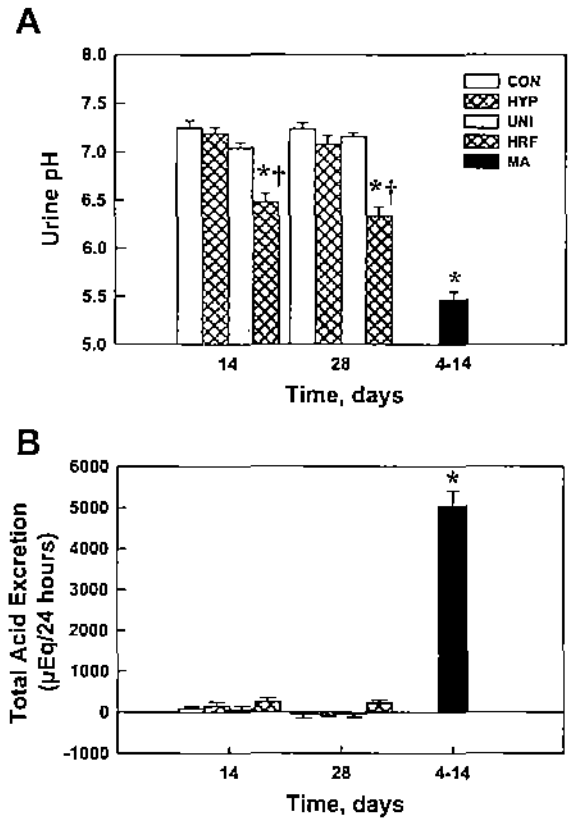

Fig. 2. Effects of hyperoxaluria (HYP group), hyperoxaluria-induced renal failure (HRF group), and metabolic acidosis (MA group) on urine pH and total acid excretion in male Sprague-Dawley rats. After 2 and 4 wk on treatment regimes (see text for details), rats were placed in metabolic cages and 24-h urine collections were performed. Urinary pH (*A*) was significantly lower in HRF rats than in the CON and UNI rats at 2 and 4 wk, but this fall in urinary pH was not nearly as striking as that observed in the MA group. Total acid excretion (*B*) was not different among CON, HYP, UNI, and HRF rats but was higher in MA rats. *$P < 0.05$ vs. CON. †$P < 0.05$ vs. UNI.

oxalate concentrations, and renal oxalate clearances are all within the normal limits for MA rats (20, 21, 23). To our knowledge, this is the first report of oxalate handling in acidotic rats.

Assessment of Renal Function

Serum creatinine (Fig. 6*A*) and creatinine clearance (Fig. 6*B*) were similar among all groups examined with the exception of the HRF group. Consistent with previous investigations of this animal model (20, 23), serum creatinine was over twofold higher in HRF rats than in all other groups and, consequently, creatinine clearance was reduced by ~50% in the HRF compared with all other groups.

Urine volumes were similar among CON, HYP, and UNI rats after 2 wk on treatment (data not shown). In contrast, urine volume in the HRF group after 2 wk was nearly threefold higher than in any other group. After 4 wk on their respective treatments, 24-h urine output was significantly higher in HYP rats than in CON rats (Table 2). Urine output in HRF rats at 4 wk followed a similar trend to that observed at 2 wk, with volumes being ~2.5-fold higher in HRF than in CON, HYP, and UNI rats. Urine volumes in the MA rats were similar to those in CON rats (Table 2).

DISCUSSION

EG-induced hyperoxaluria models have been employed in numerous studies of calcium oxalate nephrolithiasis, and much of our current knowledge base in experimental hyperoxaluria and calcium oxalate kidney stone disease is based on this model (20, 21, 27, 28, 34, 35, 47). Like any experimental model, EG-induced hyperoxaluria has advantages and disadvantages. EG is inexpensive and simple to deliver in drinking water, where it is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase/aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxylic acid, which, in turn, is further oxidized to oxalic acid by glycolate oxidase (13, 31) or lactate dehydrogenase (26), thus promoting hyperoxaluria. There has been some concern, however, that this model also initiates a metabolic acidosis that may confound the interpretation of studies using this oxalate precursor (4, 5, 11). This notion undoubtedly arises from the fact that ingestion of large doses of EG by humans or animals does, indeed, induce metabolic acidosis (25). For example, there are many reports of metabolic acidosis following ingestion of sweet-tasting anti-

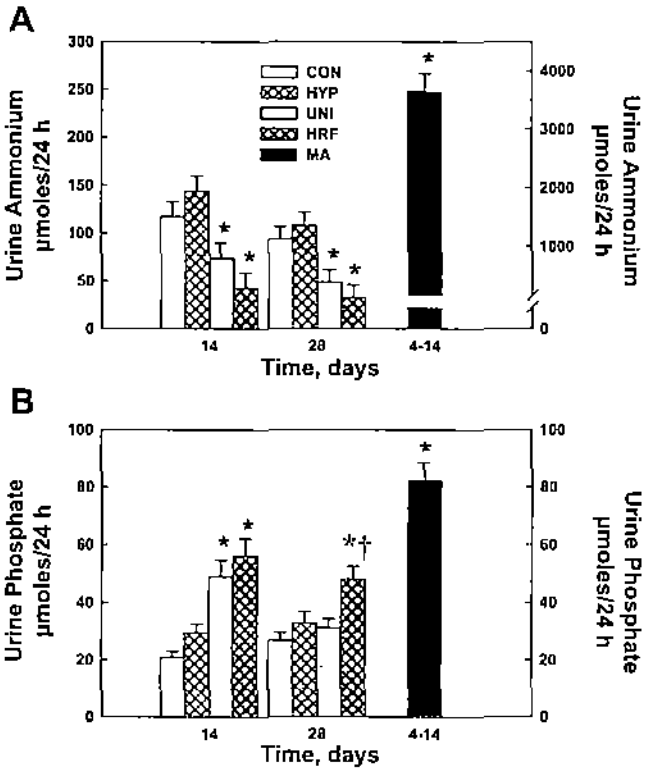

Fig. 3. Comparison of the renal excretion of ammonium (*A*) and phosphate (*B*) among CON, HYP, UNI, HRF, and MA rats. See MATERIALS AND METHODS for details of the various models. Twenty-four-hour urine collections were performed after 2 and 4 wk of treatment. Statistical differences were determined relative to CON for HYP and MA groups and relative to UNI for the HRF group. *A*: urinary excretion of ammonium was similar between CON and HYP rats at 2 and 4 wk, but ammonium excretion was significantly reduced in both UNI and HRF rats at 2 and 4 wk. Note that the MA group is plotted vs. the right ordinate, as ammonium excretion was nearly 30-fold higher in this particular group. *B*: urinary excretion of phosphate was ~4-fold higher in MA than in CON rats. A similar trend was not observed for HYP rats, with phosphate excretion being similar to CON rats at 2 and 4 wk. Urinary excretion of phosphate was higher in HRF rats than in UNI rats after 4 wk of treatment. *$P < 0.05$ vs. CON. †$P < 0.05$ vs. UNI.

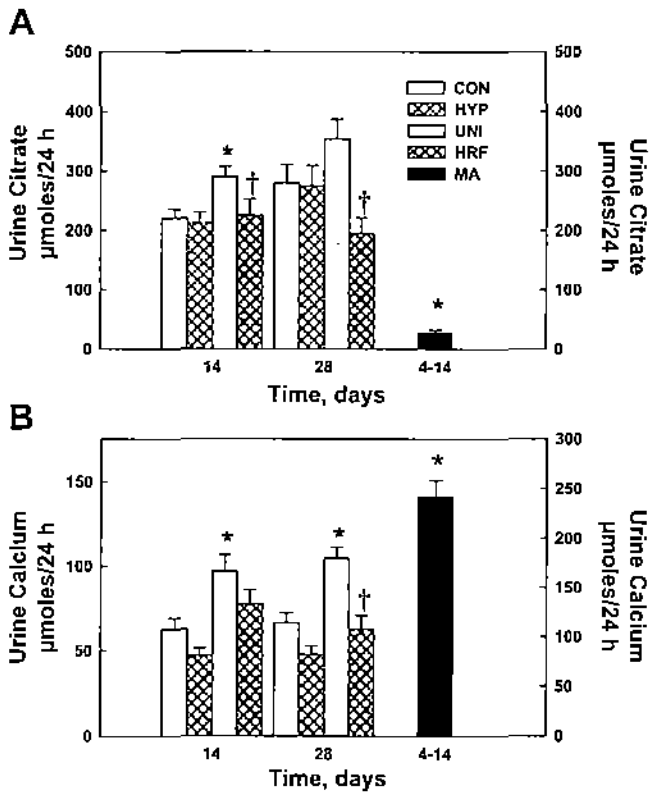

Fig. 4. Comparison of the renal excretions of citrate (*A*) and calcium (*B*) among CON, HYP, UNI, HRF, and MA rats. See MATERIALS AND METHODS for details of the various models. Twenty-four-hour urine collections were performed after 2 and 4 wk of treatment. Statistical differences were determined relative to CON for HYP and MA groups and relative to UNI for the HRF group. *A*: metabolic acidosis caused a significant reduction in urinary citrate excretion. In contrast, hyperoxaluria had no effect on citrate excretion after either 2 or 4 wk. Unilateral nephrectomy caused a modest rise in urinary citrate excretion, and this increase was attenuated in HRF rats, suggesting some tendency toward acidosis. *B*: unilateral nephrectomy and metabolic acidosis caused a significant increase in urinary calcium excretion. This increase was attenuated in the HRF group at 4 wk. Note that the MA group is plotted vs. the right ordinate as urinary excretion of calcium in the MA rats was ~4-fold higher than that of CON rats. $^*P < 0.05$ vs. CON. $\dagger P < 0.05$ vs. UNI.

freeze (primarily EG) by household pets and of humans intentionally imbibing antifreeze (15, 25). Remarkably, the proposal that EG-induced hyperoxaluria models are complicated by the presence of EG-induced metabolic acidosis has not been experimentally evaluated before the present report.

Metabolic acidosis is most simply defined as a decline in systemic pH produced primarily by a reduction in systemic bicarbonate concentrations (4). A metabolic acidosis induced by the ingestion of nonvolatile acids or acid precursors, like EG, is usually associated with an increase in the anion gap due to the presence of organic anions, principally glycolate (15, 32, 44), generated by EG metabolism. Additionally, metabolic acidosis may be associated with alterations in urine chemistry that reflect biochemical/physiological responses to the increased acid load, such as decreases in urinary pH (17, 38) and urinary citrate excretion (1, 16) and increases in urinary calcium excretion (6), urinary ammonium excretion (6, 17), and phosphate excretion (6). We have evaluated these parameters in several models to test the hypothesis that EG consumption produces acidosis at dosages commonly employed to induce hyperoxaluria (20, 21) and nephrolithiasis in rats (27, 28, 35).

Two-Kidney Hyperoxaluria Model

Rats consuming 0.75% EG in their drinking water did not develop any signs of metabolic acidosis after 4 wk. Thus arterial pH, bicarbonate concentrations, and anion gap of these (HYP) rats were not significantly different from two-kidney controls (CON). Urinary pH, titratable acid, and the urinary excretion of citrate, calcium, ammonium, and phosphate were similar in both groups, which further supports the conclusion

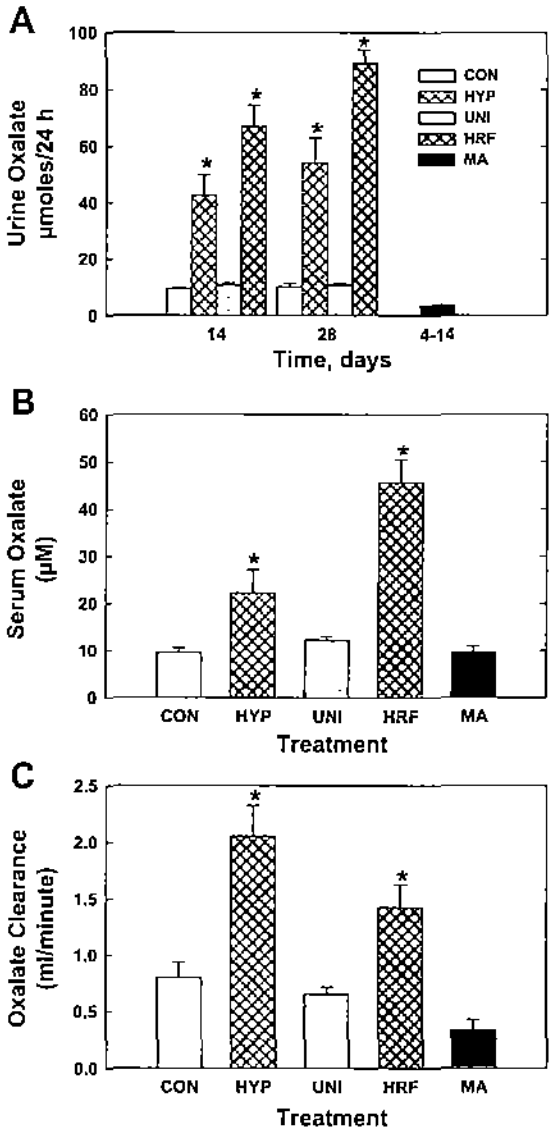

Fig. 5. Serum oxalate and urinary oxalate excretion in CON, HYP, UNI, HRF, and MA rats after 4 wk on their respective treatment protocols. *A*: urinary excretion of oxalate was increased ~4- and 7-fold in the HYP and HRF rats, respectively, by 2 wk with further increases in oxalate excretion apparent after 4 wk of ethylene glycol treatment. *B*: serum concentrations of oxalate were elevated in HYP and HRF rats compared with CON rats but were similar between MA and CON rats. *C*: renal oxalate clearances were increased in both hyperoxaluric groups (HYP and HRF) but were similar between CON and MA groups. $^*P < 0.05$ vs. CON.

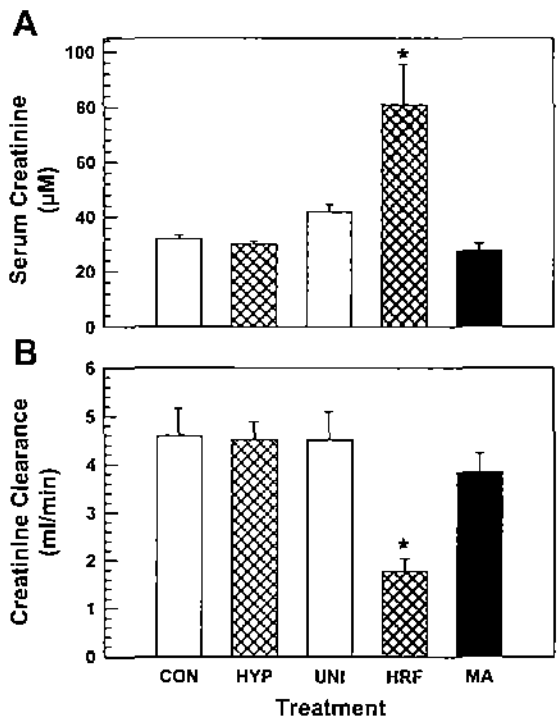

Fig. 6. Assessment of renal function as judged by serum creatinine (*A*) and creatinine clearance (*B*) in CON, HYP, UNI, HRF, and MA rats. See MATERIALS AND METHODS for details of the various models. Renal function was normal in HYP and MA rats but was compromised in HRF rats. Serum creatinine (*A*) in HRF rats was 2-fold higher than in any other group. Consequently, creatinine clearance (*B*) in HRF rats was approximately half that of all other groups after 4 wk of treatment. *$P < 0.05$ vs. all others.

that this frequently employed regimen does not produce metabolic acidosis. In contrast, rats in the commonly employed NH_4Cl ingestion model of metabolic acidosis (MA group) did exhibit all of the hallmarks of acidosis: decreased arterial pH and serum HCO_3^- concentrations, together with lower urinary pH and citrate excretion but elevated urinary ammonium and phosphate excretion, which engenders increases in both titratable acid and total acid excretion.

Most likely, acidosis does not develop in the HYP rat model because the EG is delivered at lower dose over a greater time period compared with situations that arise in a clinical environment where accidental or intentional ingestion of antifreeze results in an acutely high dose. Only EG, glycolate, and oxalate accumulate in appreciable quantities in blood and/or urine (7, 14, 44) following EG ingestion. Because glycolate oxidase (GO) is one of the rate-limiting enzymes in the metabolism of EG (14, 44), high doses of EG (>2,500 mg/kg body wt), particularly when given as an oral bolus, cause the saturation-dependent accumulation of glycolic acid in the plasma (7, 32, 44), with metabolic acidosis ensuing (7, 36). Metabolic acidosis probably never emerges in this model of hyperoxaluria because glycolate oxidase never becomes saturated; hence plasma levels of glycolate in this time frame do not rise significantly. The fact that the anion gap in HYP rats was not significantly different from controls further suggests that glycolate (or other anionic metabolites of EG) does not significantly accumulate in this animal model.

One-Kidney Hyperoxaluria Model

Unilateral nephrectomy (UNI group) did not produce metabolic acidosis as arterial pH, serum HCO_3^- concentration, and the anion gap were not significantly different from CON rats. Furthermore, there was no significant increase in total acid excretion, reduction in urinary pH, or decreased excretion of citrate in the UNI group, as would be anticipated in acidosis, further suggesting that reduced renal mass per se does not lead to metabolic acidosis. (Ammonium excretion in UNI rats was depressed, which is contrary to expectations of enhanced NH_4^+ production in acidosis but consistent with the impaired ammonia excretion that accompanies loss of renal mass.)

In contrast, while nephrectomized rats (HRF) given 0.75% EG in their drinking water for 4 wk did not exhibit frank metabolic acidosis, there were some signs that they may be developing an acidotic state. Thus although arterial pH and HCO_3^- concentrations were not significantly different from either CON or UNI controls, the HRF rats did exhibit a slightly larger anion gap and had a higher urinary phosphate excretion, a lower urinary pH, and an increase in titratable acid. Ammonium excretion in HRF rats was not significantly different from UNI rats, and, as noted above, both were actually lower than in the CON group. It should be noted that the changes in urinary chemistry suggestive of acidosis in HRF rats are quantitatively minor compared with the MA group.

Renal Function and Oxalate Handling in Models

Of the five models examined in this study, only nephrectomized rats ingesting EG (HRF) exhibited renal failure as judged by a significant fall in creatinine clearance and a significant elevation of serum creatinine concentration. This finding is consistent with earlier studies of the HRF model and suggests that oxalate load imposed on nephrectomized rats is a contributing factor in promoting renal failure (20). Indeed, this experimental model was developed to mimic oxalate-related disease states like primary hyperoxaluria with renal insufficiency caused by chronic hyperoxaluria (20). The fact that the HRF model exhibits some characteristics suggestive of a nascent metabolic acidosis is not surprising because renal failure itself causes increased anion gap metabolic acidosis (4) and metabolic acidosis has been observed in patients with primary hyperoxaluria (40).

A novel finding of this study is the observation that metabolic acidosis is not associated with any significant alterations in oxalate homeostasis. In 2001, Bushinsky et al. (6) reported that urinary oxalate excretion was significantly reduced in genetically hypercalciuric stone-forming rats given 0.5–1.5% NH_4Cl for periods of 4–14 wk and, by way of explanation, he suggested that metabolic acidosis alters oxalate metabolism. In our study, which included urine and serum oxalate measurements, as well as an assessment of renal clearance of oxalate in Sprague-Dawley rats, we find that mean urinary excretion of oxalate is somewhat, but not significantly, lower than in controls. Furthermore, all values for each of the parameters examined were within the normal ranges that we have established in our laboratory for Sprague-Dawley rats (20, 21, 23). Thus we conclude from this study that oxalate homeostasis is not influenced by metabolic acidosis. It is, however, quite possible that GHS rats exhibit unusual metabolic patterns because of their extensive inbreeding.

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1: W V Med J. 2006 Jul-Aug;102(4):32-4.

Links

Ethylene glycol intoxication: Disparate findings of immediate versus delayed presentation.

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Ethylene glycol is a common household substance responsible for a large number of ingestions in the U.S. each year. In 2001, nearly 5,000 ethylene glycol exposures were reported with more than 1,600 patients requiring medical treatment. There were 16 deaths attributed to ethylene glycol in 2001, second only to ethanol overdose for lethal ingestions. Diagnosis of ethylene glycol ingestion is relatively straightforward when an individual with a history of exposure is found to have a high anion-gap metabolic acidosis and an elevated osmolar gap. Appropriate treatment can be immediately employed and the diagnosis confirmed by the finding of elevated ethylene glycol levels in the serum. In the absence of exposure history, the differential diagnosis of a high anion-gap metabolic acidosis and an elevated osmolar gap will also lead to consideration of ethylene glycol ingestion. This well-recognized presentation of ethylene glycol toxicity includes findings expected in individuals who present for care soon after their ingestion. A less well-known pattern may be seen in those for whom care is delayed. We present a patient with delayed presentation of ethylene glycol ingestion and review the physiology and biochemistry that underlies this different presentation. Unfortunately, without history or strong laboratory evidence, ethylene glycol ingestion may be easily overlooked in individuals with delayed presentation.

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although quantitative differences have been reported. Comparison between species is difficult, however, because the information on humans is derived mainly from acute poisoning cases whereas the effects of repeated exposures have been investigated in animal experiments. Based on published data the minimum human lethal dose of EG has been estimated at approx. 100 ml for a 70-kg adult or 1.6 g/kg body weight (calculation of dose in ml/kg to mg/kg based in EG density=1.11 g/l). However, human data from case reports are generally insufficient for the determination of a clear dose-response relationship and quantification of threshold doses for systemic toxicity, in particular renal effects, is limited. As toxicity is largely a consequence of metabolism of EG to GA, it is important to note that no signs of renal injury have developed at initial plasma glycolate concentrations of up to 10.1 mM (76.7 mg/dl). Plasma EG levels of 3.2 mM (20 mg/dl) are considered the threshold of toxicity for systemic exposure, if therapeutic strategy is based on the EG concentration alone.

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Ethylene Glycol Intoxication: Disparate Findings of Immediate Versus Delayed Presentation

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Abstract

Ethylene glycol is a common household substance responsible for a large number of ingestions in the U.S. each year. In 2001, nearly 5,000 ethylene glycol exposures were reported with more than 1,600 patients requiring medical treatment. There were 16 deaths attributed to ethylene glycol in 2001, second only to ethanol overdose for lethal ingestions (1). Diagnosis of ethylene glycol ingestion is relatively straight-forward when an individual with a history of exposure is found to have a high anion-gap metabolic acidosis and an elevated osmolar gap. Appropriate treatment can be immediately employed and the diagnosis confirmed by the finding of elevated ethylene glycol levels in the serum. In the absence of exposure history, the differential diagnosis of a high anion-gap metabolic acidosis and an elevated osmolar gap will also lead to consideration of ethylene glycol ingestion. This well-recognized presentation of ethylene glycol toxicity includes findings expected in individuals who present for care soon after their ingestion. A less well-known pattern may be seen in those for whom care is delayed. We present a patient with delayed presentation of ethylene glycol ingestion and review the physiology and biochemistry that underlies this different presentation. Unfortunately, without history or strong laboratory evidence, ethylene glycol ingestion may be easily overlooked in individuals with delayed presentation.

Case Report

A 54-year-old man was brought to the emergency department after being found unresponsive by his wife. He had visited his parents' home the day before to help care for his ailing father, and his wife said he had done several hours' worth of yard work when he returned. Towards the end of the day, she noticed that he was stumbling and appeared intoxicated. He claimed sobriety over the previous four months, but he had a long history of alcohol abuse that primarily involved drinking in private. His wife was dismayed, but not alarmed by his apparent intoxicated state, and had helped him to bed. The next day, she found him lying on the floor and had called for an ambulance.

Upon arrival in the ED, his temperature was 38.8°C, pulse was 109 beats/min, BP 183/77 mm Hg, and respiratory rate 27 breaths/min. On neurological examination, he withdrew all extremities in response to painful stimuli and moaned in response to his name. His pupils were reactive to light. He had no papilledema or facial asymmetry. His lungs, heart, abdomen, extremities were normal, as well as his gag and his deep tendon reflexes. He evidenced no Babinski sign. His white blood cell count was 28,000 cells/μL with 71% neutrophils, 4% bands, and 18% lymphocytes. Other studies revealed: hematocrit 52%; arterial blood gas: pH 7.12, pCO2 21 mmHg, pO2 102 mmHg and bicarbonate 8.8 mEq/L; serum electrolytes: sodium 139 mEq/L, potassium 6.8 mEq/L, chloride 112 mEq/L, glucose 185 mg/dL (10.2 mmol/l), BUN 16 mg/dL (6 mmol/L), creatinine 1.7 mg/dl (150 μmol/L), and lactate 10.4 mmol/L. Measured osmolality was 309 mosm/L with calculated osmolality of 312 mosm/L. Urinalysis dipstick revealed SG 1.015, negative nitrate and leukocyte esterase, protein of 30 mg/dL. Microscopic examination showed 10-20 red blood cells per high power field and no casts. A urine drug screen was positive for benzodiazepines. He underwent a lumbar puncture that revealed 7 white blood cells/μL; 154 red blood cells/μL; glucose of 120 mg/dL (6.6 mmmol/L); and a total protein of 77 mg/dL. Tests for ethanol, methanol, and ethylene glycol were negative.

A CT scan of the brain without contrast and chest X-ray was normal. He was diagnosed as having sepsis and started on broad-spectrum antibiotics and given bicarbonate due to his severe metabolic acidosis. The next day, his white blood count rose to 31,000 cells/μL and the serum creatinine increased to 3.8 mg/dL (336 μmol/L). His mental status was unchanged and he was transferred to our facility for further care of his presumed sepsis and acute non-oliguric renal failure. He was hypoxemic on arrival and required intubation.

Chest radiograph was consistent with pulmonary edema. An MRI of the brain and repeat lumbar puncture were unremarkable. All cultures remained negative. A repeat urinalysis showed 20 to 50 red blood cells, 20 to 50 white blood cells, 15 mg/dL of protein, no casts, no bacteria and calcium oxalate crystaluria with both enveloped and needle-shaped crystals. His creatinine climbed the following day to 5.7 mg/dL. Hemodialysis was initiated and a renal biopsy was performed which revealed normal glomeruli with multiple tubules occluded with birefringent crystals (Figure 1).

During the next few days, he was continued on dialysis, the antibiotics were discontinued, his mental status improved, and he was extubated. His renal function gradually improved after a week of dialysis support. He recalled very few details of the day preceeding his admission, but he remembered he had planned to change the anti-freeze in his mother's car. A family member checked and found that the antifreeze had been changed.

Discussion

Classically, ethylene glycol ingestion is associated with a severe metabolic acidosis. After ingestion, ethylene glycol is oxidized by hepatic

Figure 1. Routine light microscopy showing refractile cystals within tubules. On closer inspection, destruction of tubular epithelium can be appreciated (hematoxylin and eosin; original magnification x 100).

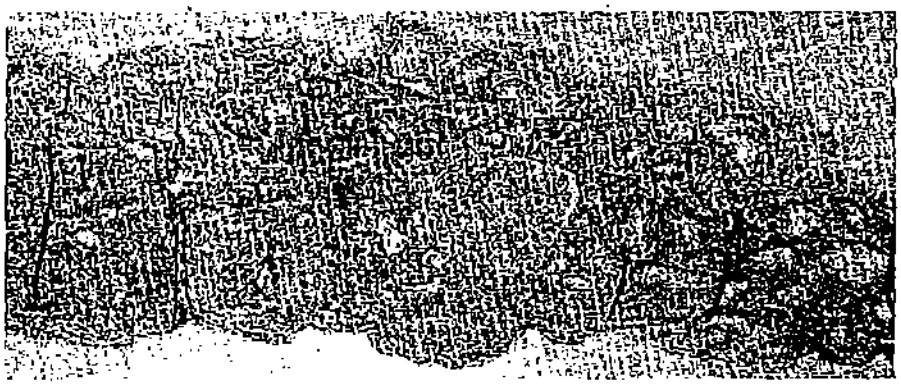
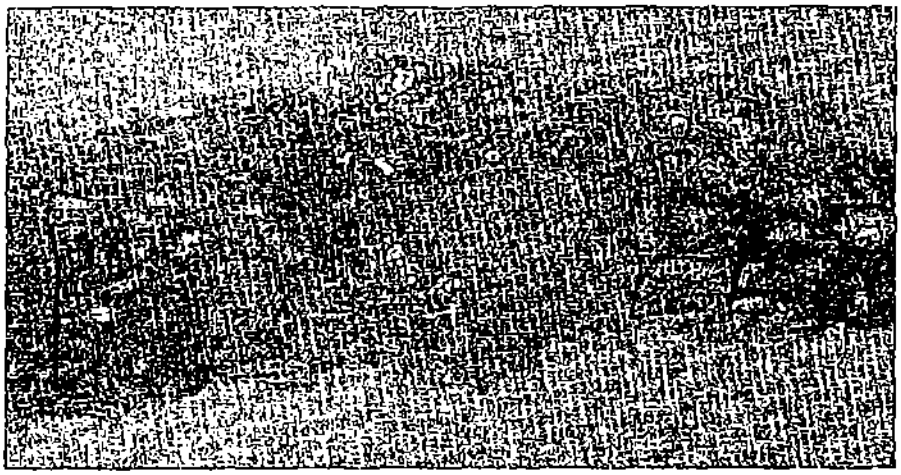


Figure 2. A view of the same biopsy under polarized light shows a moderate number of birefringent calcium oxalate crystals (polarized light microscopy; original mag.)



alcohol dehydrogenase in an NAD-dependant reaction. Breakdown of ethylene glycol (Figure 2), which itself is not toxic to tissues, generates the metabolites responsible for the acidosis and clinical features of toxicity (2,3,4). Glyoxalate is the most damaging to tissues, while glycolate is responsible for up to 96% of the anion gap seen later in the clinical course (3).

Observed clinical sequalae of ethylene glycol ingestion can be seen as early as 30 minutes after exposure because of rapid absorption from the gastrointestinal tract. Initially neurological symptoms predominate, ranging from inebriation and confusion to coma. After 12 to 24 hours, patients may begin to experience more systemic features including pulmonary edema, tachycardia, and hypertension. Renal manifestations occur late, up to 72 hrs. after ingestion if left untreated (4). Severity of the process is dependent on multiple factors including amount of ethylene glycol ingested and the time to presentation and to intervention. The symptoms may appear in any order, and there is poor correlation between ethylene glycol levels and clinical symptoms (3,5).

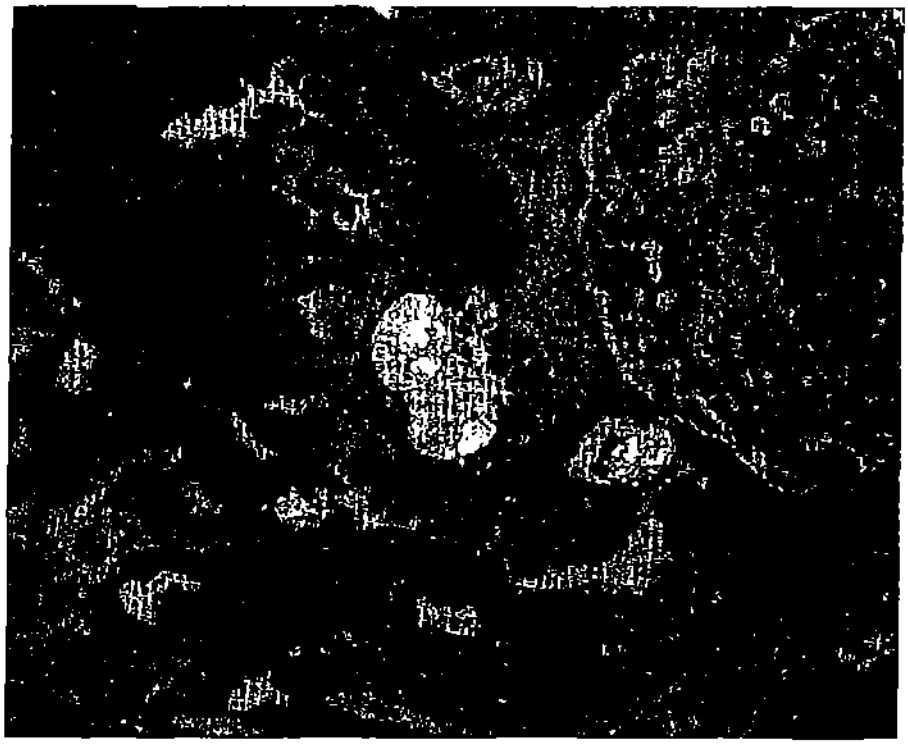
Ethylene glycol toxicity is suggested by several laboratory abnormalities. After ingestion, ethylene glycol molecules are responsible for the commonly seen osmolar gap. Ethylene glycol has a molecular weight of 62.5 gm/mol, which is electrically neutral at physiologic pH. Generally, in ethylene glycol ingestion, the osmolal gap is elevated in the range of 15 to 20 mOsm/L (5). A level of 100 mg/dl (16 mosmol/L) will raise the osmolal gap by 16 (3). Serum levels as low as 20 mg/dL have been associated with severe morbidity and death and are an indication for treatment with dialysis. Such a level would be associated with an osmolar gap of only 3 mosml/L. However, if the patient presents after the ethylene glycol has been metabolized to glycolate and glyoxalate the osmolal gap caused by the ethylene glycol will disappear leaving the clinician without this important diagnostic clue. In addition, assays for ethylene glycol may be also reveal low or non-detectable levels of ethylene glycol if testing is done after time has allowed complete or near-complete metabolism of ethylene glycol to its toxic metabolites. The metabolites of ethylene glycol are rarely measured, but glycolate levels can be determined by gas chromatography. Unfortunately, this is of little clinical utility at the present time because of lack of availability (4,6).

High anion gap metabolic acidosis is a feature of approximately 50% to 86% of patients (3). The toxic metabolites (glycolate, glyoxalate) are unmeasured anions, which are responsible for the elevation in anion gap. There is typically a lag time from the time of ingestion to the development of the anion gap as a result of the time taken to metabolize ethylene glycol. Therefore, depending on the time from initial ethylene glycol exposure, a patient may present with an osmolal gap, an anion gap, or both.

Interpretation of the elevated anion gap may be made more challenging by the fact that lactic acidosis may also be seen with ethylene glycol ingestion. Two sources of the elevated lactate have been described. The oxidation of ethylene glycol increases the ratio of NADH to NAD (4). The increased NADH levels inhibit the metabolism of pyruvate, resulting in the accumulation of lactate (Figure 2) (4,7). Along with a small absolute increase in lactate, the assay for lactic acid may be falsely increased secondary to the NADH levels. This is due to the fact that in some lactate assays, the level of NADH is used to determine lactate levels. Glycolic acid, a metabolite of ethylene glycol, can also interfere with the lactate assay. As a result of similar structures of the two acids (Figure 3), glycolate can be misidentified as lactate in two of the most common lactate assays (4).

Urinalysis may reveal calcium oxalate crystals in association with ethylene glycol ingestion. As oxalate accumulates during the metabolism of ethylene glycol, it begins to precipitate with calcium ions to form calcium oxalate crystals (2). The accumulation of the crystals is the cause of much of the tissue damage seen in these patients, especially the damage to the kidney.

Figure 3. Calcium oxalate crystals are present within tubular lumina and demonstrate characteristic aggregration into fan, sheave, or rosette-shaped structures (hematoxylin and cosin; 400x polarized).



Initially, the crystals are the enveloped-shaped dihydrate form, which are formed by high concentrations of oxalate. These rapidly change into the monohydrate form, which is more commonly seen. They are needle-shaped and can be mistaken for hippurate crystals (8). Massive crystalluria can be seen and should prompt evaluation for ethylene glycol ingestion in the appropriate setting. Other findings on urinalysis may include a low specific gravity, mild proteinuria, and microscopic hematuria.

Our patient's symptoms and laboratory findings can be better understood with an awareness of the metabolism of ethylene glycol described above. His initial symptoms of apparent intoxication probably developed several hours after his ingestion of ethylene glycol. If he had presented for medical care at this point, laboratory studies would likely have demonstrated the high anion gap metabolic acidosis, elevated osmolar gap, and elevated blood levels of ethylene glycol, which together are characteristic of a toxic ingestion of the substance. However, nearly 24 additional hours passed before he was evaluated medically. During this time he appeared to have completely metabolized ethylene glycol to its toxic metabolites, leading to the low osmolar gap and negative ethylene glycol level. His recent ethanol abuse history could have accelerated this metabolism because of the more rapid metabolism of both ethanol and ethylene glycol from up-regulation of alcohol dehydrogenase in individuals with an ethanol abuse history (10). His lactic acidosis was likely a consequence of the increased ratio of NADH to NAD generated during the metabolism of ethylene glycol, which favors the production of lactate from pyruvate. Resulting glycolate production may also interfere with lactic acid assays, causing spurious levels. His elevated white blood cell count, which was initially interpreted as additional evidence of sepsis-related lactic acidosis, was likely a consequence of his ethylene glycol ingestion (9).

Conclusion

Ethylene glycol toxicity is a life-threatening clinical condition. Textbook cases are readily diagnosed and treated with good clinical outcomes. However, as our case demonstrates, there are many laboratory pitfalls that can lead the clinician astray. Invasive measures may be required to reach a definitive diagnosis, but subtle clues often overlooked can help to point the right direction. By paying careful attention to findings on urinalysis, understanding the physiology behind the laboratory findings, and by being persistent, the correct diagnosis of ethylene glycol intoxication can be made and lifesaving treatment provided.

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Update on antidotes for pediatric poisoning.

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Antidotes are playing an increasing role in therapy for pediatric poisonings. Although initial response to all pediatric poisonings begins with basic stabilization, knowledge of specific antidotes, their mechanisms of action, safety profile in pediatrics, and dosing regimens can be life-saving for pediatric victims of nerve gas exposure, acetaminophen toxicity, methanol and ethylene glycol ingestion, and snakebites. This article presents an overview of the pathophysiology, symptoms, antidotes, and emergency management of these toxicological emergencies.

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Update on Antidotes for Pediatric Poisoning

Marjorie Lee White, MD and Erica L. Liebelt, MD

Abstract: Antidotes are playing an increasing role in therapy for pediatric poisonings. Although initial response to all pediatric poisonings begins with basic stabilization, knowledge of specific antidotes, their mechanisms of action, safety profile in pediatrics, and dosing regimens can be life-saving for pediatric victims of nerve gas exposure, acetaminophen toxicity, methanol and ethylene glycol ingestion, and snakebites. This article presents an overview of the pathophysiology, symptoms, antidotes, and emergency management of these toxicological emergencies.

Key Words: antidote, poisoning, nerve gas, acetaminophen, methanol, ethylene glycol, snakebites

TARGET AUDIENCE

This article targets health care providers who care for children and adolescents in acute emergency settings, including physicians, nurses, and prehospital personnel. Emergency physicians, pediatric emergency physicians, pediatricians, medical students, and family practitioners will find the information relevant.

LEARNING OBJECTIVES

1. Identify poisonings for which new antidotes are available.
2. Describe the pathophysiology and clinical presentation of 4 toxicological emergencies.
3. Identify the mechanism of action of these antidotes.
4. Describe the general emergency department management for nerve gas exposure, acetaminophen overdose, crotaline envenomation, and methanol and ethylene glycol poisoning as it relates to antidote administration.

Pediatric emergency response for poisonings always starts with basic supportive measures; however, antidotal therapy for specific poisoning is continuing to play an important role. Antidotes are chemical or physiological antagonists that prevent or reverse the toxic effects of specific poisons. Although the frequency of many of these poisonings may not be high in the pediatric population, the morbidity and mortality rates associated with them can be high, justifying the availability and cost of these antidotes. This article focuses on antidotes available for nerve gases, acetaminophen, snakebites, and methanol (METH) and ethylene glycol (EG) exposures or ingestions.

NERVE GASES

Overview

Nerve gases were first developed in the 1930s initially by the Germans and later by the British military. Examples include Tabun (GA), Sarin (GB), Soman (GD), and VX. As clear, colorless, usually odorless liquids with an evaporation rate similar to water, these agents pose a threat both in their liquid and aerosolized form. They are extremely potent organic phosphorus cholinesterase inhibitors and are the most toxic of current chemical weapons.[1]

Poisoning

Nerve agents alter cholinergic synaptic transmission at neuroeffector, skeletal myoneural and autonomic ganglia, and in the central nervous system, resulting in both muscarinic and nicotinic effects. Initial symptoms depend on the dose and route of exposure. Victims who have inhaled nerve agent vapor present differently from those who contact liquid with their skin or mucous membranes.

The resultant toxidrome includes muscarinic effects (pinpoint pupils, blurred or dim vision, lacrimation, salivation, bronchorrhea, nausea, vomiting, diarrhea, crampy abdominal pain, urinary and fecal incontinence, and bradycardia), nicotinic effects (skeletal muscle twitching, cramping, weakness, and flaccid paralysis), and central effects (loss of consciousness, seizures, and respiratory depression). Nicotinic stimulation can sometimes obscure certain muscarinic effects and produce tachycardia and hypertension (Table 1).

A mild inhaled exposure may only cause miosis, rhinorrhea, and mild dyspnea whereas a moderate exposure may cause bronchoconstriction, excessive bronchial secretions, and more severe dyspnea. Mild to moderate dermal exposure results in sweating and muscular fasciculations at the site of contact, nausea, vomiting, diarrhea, and weakness. The onset of signs and symptoms after a dermal exposure may be delayed for 18 to 24 hours. Higher exposures by any route may result in loss of consciousness, seizures, muscle fasciculations, flaccid paralysis, copious secretions, apnea, and death.

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TABLE 1. Signs and Symptoms of Nerve Agent Exposure

Peripheral
- Miosis
- Bradycardia or tachycardia
- Lacrimation
- Salivation
- Bronchorrhea
- Bronchospasms
- Hypertension
- Urinary incontinence
- Diarrhea

Skeletal muscle
- Weakness
- Fasciculations
- Paralysis

Central nervous system
- Confusion
- Agitation
- Hallucinations
- Seizures
- Coma

In the event of an exposure, children are more likely than adults to be the first to appear with symptoms. They may develop different patterns of clinical toxicity with more severe manifestations and be hospitalized more frequently. As respiratory failure is the primary cause of death in nerve agent–exposed patients, it is important to keep in mind pediatric anatomy and physiology. Children's higher respiratory rates and minute ventilations lead to higher dose effects. The smaller airway, increased nose breathing, larger tongue size, and more compliant chests of children lead to increased respiratory symptoms seen in children exposed to nerve gas.[2]

Antidotes

There are 3 antidotes available for nerve gas toxicity—atropine, pralidoxime (2-PAM), and benzodiazepines. Dosing guidelines are recommended based on severity of symptoms and weight/age of the child (see Table 2). Atropine works at the muscarinic synapses by competitively antagonizing the accumulated acetylcholine and is used to terminate sweating, salivation, rhinorrhea, and lacrimation. Oximes are purported to reactivate acetylcholinesterase at nicotinic synapses. Diazepam is used to control seizures and relax skeletal muscles that are overstimulated.[3] In circumstances with ample supplies, it is both medically prudent and compassionate to treat moderately affected and even mildly affected children. A child who initially appears to be only moderately poisoned can decompensate quickly. Instituting early therapy can arrest progression of symptoms.

Emergency Department Management

Management begins before patients arrive with preparation for decontamination. Therapy starts with ABCs and supportive care and is quickly followed by administration of antidotes to reverse underlying processes via administration of anticholinergics and oximes. The Pediatric Expert Advisory Panel of the National Center for Disaster Preparedness recommends treatment with atropine for marked secretions, bronchospasm, and 2-PAM for persistent weakness or high atropine requirements. They also recommend diazepam, lorazepam, or midazolam for seizures or severe exposures.[3] Table 2 describes the triage and treatment of children with nerve agent exposures based on initial symptoms. Table 3 outlines specific guidelines for antidotal therapy for nerve agent exposure. Note that 2-PAM should

TABLE 2. Triage of Children With Nerve Agent Exposures[2]

Symptoms	Triage Level: Disposition	Anticholinergics	2-PAM	Benzodiazepines
Asymptomatic	Delayed: observe	None	None	None
Miosis and mild rhinorrhea	Delayed: observe	None	None	None
Miosis and any other symptom	Immediate: admit	Atropine Repeat as needed every 5–10 min until pulmonary resistance improves or secretions resolve	2-PAM Repeat every hour as needed	Neurological symptoms? 1. Diazepam or 2. Lorazepam or 3. Midazolam
		Alternatives: peripheral effects only, glycopyrrolate	Watch for: muscle rigidity, laryngospasm, tachycardia, hypertension	
Apnea, seizures, cardiopulmonary arrest	Immediate: pediatric intensive care	Atropine	2-PAM	Diazepam

Adapted with permission from Rotenberg.[4]

TABLE 3. Guidelines for Antidotal Therapy for Nerve Agent Poisoning

Patient (Age)	Antidotes: Mild/Moderate Symptoms*	Antidotes: Severe Symptoms†	Other Treatments
Infant (0–2 y)	Atropine: 0.05 mg/kg IM or 0.05 mg/kg IV (minimum, 0.1 mg) 2-PAM Cl: 25 mg/kg IV‡	Atropine: 0.1 mg/kg IM or 0.05 mg/kg IV (minimum, 0.1 mg) 2-PAM Cl: 25 mg/kg IV‡	• Assist ventilation • Repeat atropine at 5- to 10-min intervals until secretions have decreased and breathing is comfortable and/or airway resistance has returned to near normal
Child (2–10 y)	Atropine: 1 mg IM 2-PAM Cl: 25 mg/kg IV‡	Atropine: 2 mg IM 2-PAM Cl: 25 mg/kg IV‡	• Seizures
Adolescent (>10 y)	Atropine 2 mg IM 2-PAM Cl: 25 mg/kg (maximum, 1 g IV 2 g IM)‡	Atropine: 4 mg IM or 2 mg IV or 2-PAM Cl: 25 mg/kg IV (maximum, 1 g IV, 2 g IM)‡	Diazepam: 0.05–0.3 mg/kg IV/PR (maximum, 10 mg/dose), 5–10 mg/dose IV (adult), repeat every 15–30 min, as needed
Adult	Atropine 2–4 mg IM 2-PAM Cl: 1 g IV,‡ 2 g IM	Atropine: 6 mg IM or 2-PAM Cl: 1 g IV‡ 2 g IM	or Midazolam: 0.15–0.2 mg/kg IV/IM (maximum, 10 mg), repeat as necessary or start continuous intravenous drip
Autoinjectors	AtroPen >10 y: 2 mg for 40 kg§ 5–10 y: 1 mg for 20 kg§ 6 mo to 10 y: 0.5 mg for 10 kg	Mark 1: see text Atropine: 2 mg IM 2-PAM Cl: 600 mg IM	or Lorazepam: 0.1 mg/kg (maximum, 4 mg) IV/IM/PR

*Mild-moderate symptoms include nausea, vomiting, salivation, lacrimation, weakness, and dyspnea.
†Severe symptoms include seizures, apnea, unconsciousness, and flaccid paralysis.
‡2-PAM Cl should be administered slowly for 20 minutes. Rapid intravenous administration can cause laryngospasm and rigidity. 2-PAM Cl may be repeated within 30 to 60 min, as needed, then again every 1 hour for 1 or 2 doses, as needed, for persistent weakness and/or high atropine requirements.

be infused slowly intravenously because of the risk for laryngospasm and muscle rigidity.

Table 4 describes autoinjector usage in children. There are currently no combination autoinjector kits made specifically for pediatric patients, only pediatric atropine autoinjectors. Pediatric AtroPen autoinjectors (Meridian Medical Technologies, Columbia, Md) come in 3 separate strengths -0.5, 1, and 2 mg. Each Mark 1 kit contains 2 autoinjectors (0.8-in needle insertion depth), one each of atropine 2 mL (0.7 mL) and 2-PAM 600 mg (2 mL); although not approved for pediatric use, they should be used as initial treatment in circumstances for children with severe life-threatening nerve agent toxicity for whom intravenous treatment is not possible or available or for whom more precise intramuscular (milligram per kilogram) dosing would be logistically impossible. Suggested dosing guidelines are offered. There is a potential for excess of initial atropine and 2-PAM dosage for age/weight. General guidelines exist for a recommended total during the first 60 to 90 minutes of therapy for severe exposures. This table lists usage of the Mark 1 kit only down to age 3 years based on adherence to recommended dosages for atropine and 2-PAM. However, if an adult Mark 1 kit is the only available source of atropine and 2-PAM after a nerve agent exposure, it should not be withheld from even the youngest child.

TABLE 4. Autoinjector Usage

Approximate Age (y)	Approximate Weight (kg)	Number of Autoinjectors (Each Type)	Atropine Dosage Range (mg/kg)	2-PAM Dosage Range (mg/kg)
3–7	13–25	1	0.08–0.13	24–46
8–14	26–50	2	0.08–0.13	24–46
>14	>51	3	≤0.11	≤35

Adapted with permission from Henretig et al.[6]

ACETAMINOPHEN TOXICITY

Overview

Accidental and intentional ingestions of acetaminophen account for a significant proportion of toxic pediatric ingestions. These ingestions can lead to irreversible liver damage and death. In 2004, the American Association of Poison Control Centers received reports of 150 deaths.[7] Liver damage is related to the metabolism of acetaminophen by the liver and the production of *N*-acetyl-*p*-benzoquinoneimine. In the overdose setting with excessive production of

TABLE 5. Acetylcysteine Dosage Guidelines Adult

Body Weight		Acetylcysteine (mL)		
kg	lb	First Dose	Second Dose	Third Dose
100	220	75	25	50
90	198	67.5	22.5	45
80	176	60	20	40
70	154	52.5	17.5	35
60	132	45	15	30
50	110	37.5	12.5	25
40	88	30	10	20

First dose, 150 mg/kg in 200 mL of 5% dextrose infused for 60 minutes; second dose, 50 mg/kg in 500 mL of 5% dextrose infused for 4 hours; third dose, 100 mg/kg in 1000 mL of 5% dextrose infused for 16 hours.
Source: Package insert, Acetadote, Cumberland Pharmaceuticals.

N-acetyl-*p*-benzoquinoneimine, the usual glutathione deactivation system is overwhelmed, and liver toxicity results in what remains an unclear mechanism. It is clear that outcomes are directly related to the timeliness of antidote administration. The recent release of the intravenous form of *N*-acetylcysteine (NAC), Acetadote (Cumberland Pharmaceuticals, Nashville, Tenn), has changed the management of acute acetaminophen poisoning.

Antidote

The standard antidote for acetaminophen toxicity has been oral NAC with a loading dose of 140 mg/kg followed by 17 doses of 70 mg/kg every 4 hours. A shortened course of oral NAC for 24 to 36 hours can be done safely if there is no measurable parent acetaminophen at these times and no evidence of liver toxicity (no elevation of liver enzymes or prothrombin time).[8]

Intravenous regimens for NAC have been used for more than 30 years in Europe and Canada. These are 20-hour intravenous NAC regimens with a cumulative dose of 300 mg/kg.[9] In 1991, Smilkstein et al demonstrated an effective and shorter alternative to the 72 hour oral regimen using an investigational pyrogen-free form of intravenous NAC for 48 hours.[10] The dosing included a 140-mg/kg loading dose, followed by 12 maintenance doses of 70 mg/kg every 4 hours. All doses were infused for 1 hour, and each subsequent dose was started 4 hours after the previous one (ie, 3 hours "off" per period). The total treatment dose was 980 mg/kg for 48 hours. The incidence of hepatotoxicity in the 48-hour intravenous protocol was comparable to previously noted percentages for treatment groups pre– and post–10 hours of ingestion in the 72-hour oral protocol as well as the 20-hour intravenous protocol.

Perry and Shannon studied intravenous versus oral NAC in an open-label clinical trial in a pediatric population.[11] The intravenous NAC regimen was 140 mg/kg loading dose followed by 12 doses of 70 mg/kg, all for 1 hour, 4 hours apart. The historical control subjects were those treated with oral NAC in the accepted regimen, with the same eligibility requirements as the intravenous NAC group. No patients in the intravenous protocol had hepatotoxicity if treated within 10 hours and 9.8% if treated within 10 to 24 hours.

The Food and Drug Administration (FDA) approved an intravenous formulation of NAC in early 2004 (Acetadote) using a 20-hour, continuous-infusion protocol. The package insert was revised in February 2006, which extended the loading dose infusion time from 15 to 60 minutes, making it a 21-hour infusion.[12] For adult intravenous dosing, the loading dose is 150 mg/kg in 200 mL of 5% dextrose for 60 minutes, followed by 50 mg/kg in 500 mL of 5% dextrose for 4 hours, and 100 mg/kg in 1000 mL of 5% dextrose for 16 hours (Table 5). In regard to pediatric intravenous dosing, it has been shown that standard intravenous dosing can cause hyponatremia and secondary seizures due to the free water load.[13] Therefore, the convention is to dilute 20% NAC to a final concentration of 40 mg/mL (see Table 6 for a depiction of the usual pediatric dosing schedule).[12] The final milligram-per-kilogram dosing (loading dose, 150 mg/kg; 50 mg/kg for 4 hours and 100 mg/kg for 16 hours) is the same; the free water is less than in the adult schedule. Adverse reactions to intravenous NAC include anaphylactoid reactions (rash,

TABLE 6. Acetylcysteine Dosage Guidelines Pediatric (Weight < 40 kg)

Body Weight		Loading Dose		Second Dose		Third Dose	
kg	lb	Acetadote (mL)	5% Dextrose (mL)	Acetadote (mL)	5% Dextrose (L)	Acetadote (mL)	5% Dextrose (mL)
30	66	22.5	100	7.5	250	15	500
25	55	18.75	100	6.25	250	12.5	500
20	44	15	60	5	140	10	280
15	33	11.25	45	3.75	105	7.5	210
10	22	7.5	30	2.5	70	5	140

Loading dose, 150 mg/kg for 60 minutes; second dose, 50 mg/kg for 4 hours; third dose, 100 mg/kg for 16 hours.
Acetadote is hyperosmolar (2600 MOsm/L) and is compatible with 5% dextrose, 0.5 normal saline (0.45% sodium chloride injection), and water for injection.
Source: Package insert, Acetadote, Cumberland Pharmaceuticals.

urticaria, and pruritis), which most commonly occur during the initial loading dose.

Emergency Department Management

Traditional therapy for acetaminophen ingestions aside from the primary survey and supportive care has revolved around serum levels at 4 hours postingestion and administration of oral or nasogastric NAC. The oral regimen has been used with success for more than 20 years in this country. With the FDA approval of the intravenous form, there is now an alternative for selective patients. One suggested guideline for patients is presented in Table 7. Although the cost for Acetadote is significantly higher than oral NAC, shorter hospitalization stays and less laboratory testing should ultimately reduce overall costs.

SNAKEBITES

Overview

In 2004, there were almost 3000 crotaline snakebites reported to poison control centers in the United States, of which 24% were in patients younger than 19 years.[7] Crotaline snakes, including rattlesnakes, water moccasins, and copperheads, account for the large majority of clinically significant envenomations in this country. Since its introduction in 2000, CroFab (Crotalidae polyvalent immune Fab ovine antivenom; Protherics Inc, Brentwood, Tenn) has dramatically improved the management of snakebite victims.

TABLE 7. Clinical Guideline for Intravenous NAC

Patients considered for treatment with intravenous NAC

Patients requiring treatment of acetaminophen toxicity as determined by serum acetaminophen concentration plotted on Rumack-Matthew nomogram and/or other laboratory/clinical parameters and

- Patients who cannot tolerate oral NAC
- Patients with gastrointestinal bleeding or obstruction
- Patients with medical or surgical condition(s) precluding oral NAC administration
- Patients with acetaminophen toxicity presenting as encephalopathy
- Patients with neonatal acetaminophen toxicity from maternal overdose
- Other patients may be considered for treatment with intravenous NAC after consultation with the medical toxicologist on-call

Clinical practice guidelines

1. Draw acetaminophen level and plot on Rumack-Matthew nomogram
2. If APAP level falls above the "possible toxicity" line, begin therapy with NAC.
3. Draw AST/ALT, PT/INR, electrolytes, BUN, Cr, and CBC.
4. At the end of the infusion, draw PT/INR, AST/ALT, BUN/Cr. If any of the laboratory results are abnormal, infusion should be continued at a rate of 6.3 mg/(kg · h)$^{-1}$ until liver function improves.
5. Consult with medical toxicologist regarding duration of therapy.
6. If the patient develops hepatic injury/failure secondary to acetaminophen, NAC therapy should be continued until liver function and/or clinical status improves.

E. Liebelt, MD, personal communication, 2006.

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; INR, international normalized ratio; PT, prothrombin time.

TABLE 8. Grading of Crotaline Envenomations[14,15]

Category	Local	Systemic	Coagulation
Minimal	Swelling, pain, and ecchymosis limited to immediate bite site	None	None
Moderate	Swelling, pain, and ecchymosis involving less than a full extremity or <50 cm	Non–life-threatening systemic signs and symptoms may be present including nausea, vomiting, oral paresthesias, metallic taste, mild hypotension, tachycardia, and tachypnea	Coagulation parameters may be abnormal but no clinical evidence of bleeding
Severe	Swelling, pain, and ecchymosis involving more than 1 extremity or threatening the airway	Systemic signs and symptoms abnormal including altered mental status, severe hypotension, tachycardia, tachypnea	Abnormal coagulation parameters with serious bleeding or severe threat of bleeding

Poisoning

Envenomation by crotaline snakes results in a dynamic clinical disease. Manifestations are related primarily to the site of envenomation; the amount of venom injected and host factors are not well described to date. Classification of envenomations for clinical studies were developed by investigators and Protherics, Inc.[14,15] Table 8 lists this classification system and denotes the 3 categories of clinical toxicity and their severity—local, coagulation, and systemic toxicity.

Antidote

CroFab is manufactured from sheep and is derived from the venom of 4 snake species, including the western diamondback rattlesnake (*Crotalus atrox*), eastern diamondback rattlesnake (*Crotalus adamanteus*), cottonmouth (*Agkistrodon piscivorus*), and Mojave rattlesnake (*Crotalus scutulatus*). Evidence for CroFab's efficacy, safety profile, and dosing regimen have been documented in several prerelease clinical trials, postmarketing case series, and multiple case reports. Although there is minimal evidence for pediatric use, that which exists supports its use.

Initial and postmarketing surveillance have demonstrated a low rate of acute allergic reactions to CroFab administration. Rash, pruritus, and wheezing have been reported. Serum sickness from CroFab is uncommon.

Symptoms include pruritus, rash, low-grade fever, and myalgias. Patients receiving CroFab should be followed for the development of serum sickness for at least 3 weeks.

Emergency Department Management

Indication for use of crotaline snake antivenom is progression of envenomation syndrome.[16] Adult and pediatric dosing is the same regardless of weight—the initial dose is 4 to 6 vials. Table 9 outlines indications for antivenom administration and the dosing schedule. The antidote should be repeated each hour until initial control has been achieved; indicators are cessation of progression in swelling/edema, improving coagulation studies, and no systemic signs or symptoms. Follow-up or maintenance doses every 6 hours after initial control is achieved are recommended on the package insert based on initial clinical trials; however, clinical experience and observation of the patient should dictate whether these doses are necessary. As stated previously, snake envenomation is a dynamic process and depends on many factors including amount of venom injected. If there is no further progression at 12 hours, then no further antivenom is needed. Although many times pain control is usually achieved with antivenom administration alone, adjunctive analgesia may be required. Coagulation studies, including complete blood count, prothrombin time, and fibrinogen, need to be evaluated.

METHANOL AND ETHYLENE GLYCOL POISONING

Overview

Methanol and ethylene glycol are serious causes of poisoning due to nonpharmaceutical substances, especially in young children, because small amounts can cause significant toxicity. In small children, ingestions of as little as 10 to 15 mL can be fatal. Thus, it is imperative to have a safe and efficacious treatment that is readily available and easily administered. In 2004, there were more than 6500 exposures to these toxic alcohols, of which 14% occurred in children less than 6 years.[7]

Methanol (METH) is a component of windshield washer fluid and is toxic in doses of 0.1 mL/kg of 100% solution. Presentation of METH toxicity is often delayed. Toxicity is manifested by a high anion gap metabolic acidosis and visual disturbances including blindness. Ethylene glycol is found in radiator antifreeze, deicers, and engine coolants and is toxic at doses of 0.2 mL/kg of 100% solution. Clinical presentation is more rapid than METH and manifestations include cranial nervous system depression and high anion gap metabolic acidosis. Late effects include renal and cardiac failure. Traditional therapy for these overdoses involves intravenous ethanol that is often complicated by large volume infusions sometimes requiring central line access, metabolic derangements, including hypoglycemia and hyponatremia, and cranial nervous system depression from the ethanol even at therapeutic doses. Other disadvantages of ethanol therapy include difficulty achieving and maintaining adequate serum ethanol levels via continuous infusion and the need for intensive care nursing and monitoring.

TABLE 9. Indications and Dosing for CroFab

Indications

- Crotaline (rattlesnake, copperhead, cottonmouth) envenomation with worsening edema or any systemic symptom including coagulopathy

Contraindications

- Known hypersensitivity to CroFab, papain, papaya

Dosing

- Intravenously infuse 4–6 vials of CroFab diluted in 250 mL normal saline for 1 h; volume may be adjusted for very small children or fluid-sensitive patients
- Initially infuse at 25 to 50 mL/h for 10 min while monitoring closely for signs of acute allergic reaction
- Observe for up to 1 h after infusion, assessing for initial control (halted progression of edema and improvement in all aspects of systemic manifestations)
- Repeat 4 to 6 vials as needed to gain initial control
- Schedule follow-up or maintenance doses of 2 vials every 6 h for 18 h
- Monitor patients for delayed or recurrent toxicity requiring additional antivenom
- Poison control centers or medical toxicologists can assist with management of individual cases

Antidotes

Fomepizole received FDA approval in 1997, with the indication for treatment of EG poisoning. Several years later, the indication for METH poisoning was added. The drug is commonly referred to as 4-MP, or 4-methylpyrazole, its chemical name, and has been used in France for more than 20 years to treat these poisonings. Fomepizole acts as a competitive inhibitor of alcohol dehydrogenase with an affinity for this enzyme 8000 times greater than ethanol, preventing the metabolism of EG and METH to its toxic metabolites. Clinical evidence also suggest in patients with normal renal function and acid-base status that fomepizole is sufficient therapy for severe EG and METH poisoning without adjunctive hemodialysis.[17,18]

Fomepizole has many advantages over ethanol therapy as an antidote for EG and METH poisonings. It is safe and has very few side effects. It may be administered via a peripheral intravenous site, obviating the need for an infusion pump or central line access. Patients receiving fomepizole may be admitted to a general floor instead of intensive care unit if otherwise clinically and metabolically stable. Use of fomepizole may void the need for hemodialysis and all of its accompanying risks and complications. Like many orphan drugs though, it is expensive (~$1000 per 1.5 g). An average course of fomepizole in an adult with EG poisoning is about $4000, compared with an equivalent course of ethanol, which is about $1000. However, its advantages over ethanol therapy or hemodialysis may actually decrease the overall cost of care to the patient.

A loading dose of 15 mg/kg should be administered, followed by doses of 10 mg/kg every 12 hours for 4 doses,

then 15 mg/kg every 12 hours thereafter until the EG or METH levels decreases below 20 mg/dL. For patients requiring hemodialysis, a separate dosing schedule is recommended because the drug is removed through this procedure. Adequate urine output should be maintained throughout therapy to enhance the excretion of unmetabolized EG and METH in the urine. Minimal side effects have been reported and include headache, nausea, and dizziness, as well as minor allergic reactions.

Emergency Department Management

As always, management of suspected ingestions begins with supportive care. Fomepizole is indicated for known EG or METH poisoning defined as a documented serum level greater than 20 mg/dL. Because these blood levels are difficult to obtain, therapy should not be delayed if poisoning is suspected based on the history or other laboratory parameters such as high anion gap metabolic acidosis, increased osmolal gap, or calcium oxalate crystals in the urine. Early administration is important and may prevent the need for hemodialysis. Furthermore, additional dosing is not needed for another 12 hours, allowing further laboratory testing and evaluation. Other therapies to consider include sodium bicarbonate for severe acidosis, correction of symptomatic hypocalcemia, and administration of cofactors, including thiamine and pyridoxine for EG and folate for METH toxicity.

CONCLUSIONS

Management of pediatric toxicological emergencies is a controversial but changing field. This update has reviewed the pathophysiology, clinical symptoms, available antidotes, and emergency management principles for 4 classes of pediatric poisoning. Pediatric patients with exposure to nerve gases, such as Sarin and VX, will be the first to develop symptoms and should be treated early and aggressively. Acetaminophen toxicity may now be treated with a 21-hour regimen of intravenous NAC, which has been demonstrated in pediatric trials to decrease hepatotoxicity. Snakebite therapy has been radically changed by the development of polyvalent immune Fab (ovine) antivenom with low rates of serum sickness. Methanol and ethylene glycol poisoning can be treated with fomepizole, which has a proven record for safety and efficacy. All of these antidotes have already significantly improved the care of patients with these often life-threatening toxicological emergencies.

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CME EXAM
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CME EXAMINATION
November 2006

Please mark your answers on the ANSWER SHEET.

Upate on Antidotes for Pediatric Poisoning, *White and Liebelt*

1. What is an advantage of fomepizole as an antidote over ethanol?
   a. Less expensive
   b. Less nursing care needed
   c. May obviate need for hemodialysis
   d. More efficacious than ethanol
   e. Shorter hospitalization stay
2. A 6-year-old boy presents with an unknown snakebite to his ankle 1 hour ago. After 2 hours of observation in the emergency department, the swelling has progressed to his knee, and he is in severe pain. There is a large blood bullae over the envenomation site. His prothrombin time is 20 seconds. What is the most appropriate next step?
   a. Administration of 6 vials of crotaline polyvalent immune Fab fragments
   b. Consultation with surgical service for possible fasciotomy
   c. Debridement of the bullae and administration of diphenhydramine
   d. Fresh frozen plasma administration
   e. Ice to the ankle and elevation of the leg
3. A 16-year-old presents after ingesting the contents of a whole bottle of acetaminophen. An acetaminophen level drawn 6 hours after ingestion is 275 μg/mL. He has vomited multiple times despite administration of antiemetics. What is the next most appropriate step in this patient's management?
   a. Activated charcoal
   b. Intravenous N-acetylcysteine
   c. Observe
   d. Oral N-acetylcysteine
   e. Whole-bowel irrigation
4. An unknown chemical release occurred in a junior high school. Several adolescents presented to the emergency department complaining of dizziness, blurry vision, eye tearing, and coughing. Physical examination demonstrated tachycardia, miotic pupils, and mild elevation of blood pressure in most of the students. Which antidote is most appropriate for the reversal of these signs and symptoms?
   a. Atropine
   b. Diazepam
   c. Naloxone
   d. Pyridostigmine
   e. Pralidoxime
5. What is a known adverse effect of 2-PAM if infused too rapidly?
   a. Anaphylaxis
   b. Flaccid paralysis
   c. Laryngospasm
   d. Red man syndrome
   e. Skin necrosis

ANSWER SHEET FOR THE *PEDIATRIC EMERGENCY CARE* CME PROGRAM EXAM

November 2006

Please answer the questions on page 747 by filling in the appropriate circles on the answer sheet below. Please mark the one best answer and fill in the circle until the letter is no longer visible. To process your exam, you must also provide the following information:

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1: Am J Forensic Med Pathol. 2006 Jun;27(2):106-12.

Toxicologic findings in suicide: a 10-year retrospective review of Kentucky medical examiner cases.

Shields LB, Hunsaker DM, Hunsaker JC, Ward MK.

Office of the Chief Medical Examiner, Louisville, Kentucky, USA.

Toxicologic analysis is an integral component in the investigation of suicide and requires correlation with a detailed scene inspection, with an extensive exploration into the decedent's medical and social background to uncover suicidal ideation or intent and a postmortem examination of the body. In this review, the authors analyzed 2864 cases classified as suicide upon autopsy and toxicologic examinations between 1993 and 2002 in the Kentucky Division of Medical Examiner's Services. Blood and urine were collected in 95.0% and 72.3% of cases, respectively. A total of 32.5% of the victims had negative blood toxicologic results, and 52.7% of urine toxicology screens yielded no drugs. Analysis of the data indicated that 3 times as many women had taken antidepressants and more than twice as many had consumed opioids. Drug toxicity ("overdose") ranked as the third (9.9%) leading cause of suicide after firearm injury (67.5%) and hanging (13.7%). Women succumbed to drug toxicity more than men (27.5% versus 5.9%). Of the overdose deaths, 66.5% had a negative blood alcohol concentration (BAC), while antidepressants, opioids, and benzodiazepines were detected in blood in 54.4%, 37.4%, and 29.2% of the subjects, respectively. The collection of these data serves the goals of public health and clinicians in devising strategies for suicide prevention.

PMID: 16738426 [PubMed - indexed for MEDLINE]

1: Semin Dial. 2006 Sep-Oct;19(5):402-7.

The role of continuous renal replacement therapy in the treatment of poisoning.

Goodman JW, Goldfarb DS.

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Extracorporeal elimination of drugs and toxins is a critical component in the management of poisonings, though specific techniques and indications remain a matter of debate. Conventional hemodialysis is frequently the treatment of choice because of its widespread availability and proven effectiveness for certain drugs and toxins. With the increased availability of continuous renal replacement therapy (CRRT) modalities, there is yet another therapeutic option, but one that has yet to find a definitive role in this field. The continuous nature of these therapies is attractive for the management of acute renal failure, but the relatively slower clearance rates as compared to conventional hemodialysis is a distinct drawback in patients with acute xenobiotic-induced toxicity. There are abundant case reports as well as a few small case series in the medical literature documenting the use of CRRT, but specific techniques and the clinical outcomes vary considerably. Therefore one cannot draw definitive conclusions regarding benefit. Some patients, particularly those who are hemodynamically unstable and are not candidates for conventional hemodialysis, may warrant a trial of CRRT. However, at the present time, routine use for the treatment of poisoning is not supported. Controlled trials to better clarify its role would be beneficial, though such studies would be extremely difficult to conduct in this field. We believe that the intelligent application of extracorporeal modalities requires a thorough knowledge of drug pharmacokinetics, of the techniques utilized, and a skeptical analysis of the available literature.

PMID: 16970740 [PubMed - indexed for MEDLINE]

1: Arch Toxicol. 2004 Dec;78(12):671-80. Epub 2004 Sep 15.

Ethylene glycol: an estimate of tolerable levels of exposure based on a review of animal and human data.

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Upon ingestion ethylene glycol (EG, monoethylene glycol) is rapidly absorbed from the gastrointestinal tract, and depending on the severity of exposure signs of toxicity may progress through three stages. Neurological effects characterize the first step consisting of central nervous depression (intoxication, lethargy, seizures, and coma). The second stage, usually 12-24 h after ingestion, is characterized by metabolic acidosis due to the accumulation of acidic metabolites of EG, primarily glycolic acid (GA), contributing to the ensuing osmolal and anion gaps. Stage 3, generally 24-72 h after ingestion, is determined mainly by oxalic acid excretion, nephropathy, and eventual renal failure. Because the toxicity of EG is mediated principally through its metabolites, adequate analytical methods are essential to provide the information necessary for diagnosis and therapeutic management. The severe metabolic acidosis and multiple organ failure caused by ingestion of high doses of EG is a medical emergency that usually requires immediate measures to support respiration, correct the electrolyte imbalance, and initiate hemodialysis. Since metabolic acidosis is not specific to EG, whenever EG intoxication is suspected, every effort should be made to determine EG as well as its major metabolite GA in plasma to confirm the diagnosis and to institute special treatment without delay. A number of specific and sensitive analytical methods (GC, GC-MS, or HPLC) are available for this purpose. Due to the rapid metabolism of EG, the plasma concentration of GA may be higher than that of EG already upon admission. As toxicity is largely a consequence of metabolism of EG to GA and oxalic acid, the simultaneous quantification of EG and GA is important. Formation of calcium oxalate monohydrate in the urine may be a useful indicator of developing oxalate nephrosis although urine crystals can result without renal injury. The pathways involved in the metabolism of EG are qualitatively similar in humans and laboratory animals,

1: Crit Rev Toxicol. 2005 Oct-Nov;35(8-9):691-702.

Links

Mode of action: oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis--renal and developmental effects of ethylene glycol.

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Ethylene glycol can cause both renal and developmental toxicity, with metabolism playing a key role in the mode of action (MOA) for each form of toxicity. Renal toxicity is ascribed to the terminal metabolite oxalic acid, which precipitates in the kidney in the form of calcium oxalate crystals and is believed to cause physical damage to the renal tubules. The human relevance of the renal toxicity of ethylene glycol is indicated by the similarity between animals and humans of metabolic pathways, the observation of renal oxalate crystals in toxicity studies in experimental animals and human poisonings, and cases of human kidney and bladder stones related to dietary oxalates and oxalate precursors. High-dose gavage exposures to ethylene glycol also cause axial skeletal defects in rodents (but not rabbits), with the intermediary metabolite, glycolic acid, identified as the causative agent. However, the mechanism by which glycolic acid perturbs development has not been investigated sufficiently to develop a plausible hypothesis of mode of action, nor have any cases of ethylene glycol-induced developmental effects been reported in humans. Given this, and the variations in sensitivity between animal species in response, the relevance to humans of ethylene glycol-induced developmental toxicity in animals is unknown at this time.

PMID: 16417036 [PubMed - indexed for MEDLINE]

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FINAL REPORT

Battelle Project No. 29812 Amendment No. 4
ACC No. EG-29.0-BATT-PBPK

April 16, 2002

Pharmacokinetics of Ethylene Glycol in Pregnant SD Rats Following Bolus Oral Gavage or Continuous Subcutaneous Infusion

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ABSTRACT

This study, along with its companion study (Carney et al., 2001), was designed to test the hypothesis that dose-rate is a critical factor in predicting developmental toxicity in laboratory animals. This report reflects the results from an oral gavage pharmacokinetic study conducted with ethylene glycol in pregnant Sprague-Dawley rats (gestational day 11-12) at Battelle Northwest and the analytical results from a kinetic study conducted at The Dow Chemical Company where pregnant Sprague-Dawley rats were administered ethylene glycol by continuous, subcutaneous infusion over gestational days 6 to 11 or 12 (Carney et al., 2001). Additional data on the levels of ethylene glycol and glycolic acid in maternal blood following bolus subcutaneous injection or continuous infusion are summarized by Carney et al. (2001) along with evaluations of maternal and developmental toxicity.

By all methods of administration, ethylene glycol levels were similar in maternal blood, kidney and conceptuses while glycolic acid levels were consistently higher (1.4-4 fold) in target tissues (maternal kidneys and conceptuses) than maternal blood. Administration of ethylene glycol by continuous infusion resulted in significantly lower maternal blood, tissue and conceptus levels of ethylene glycol (8- to 14-fold lower) and glycolic acid (46- to 83-fold lower) than comparable total daily doses following bolus oral gavage. In fact, continuous infusion of even 2000 mg/kg/day, a dose level that results in significant developmental toxicity when administered as a bolus dose, did not result in glycolic acid maternal blood levels that exceeded the putative threshold for developmental effects (~2 mM) and no developmental toxicity was thus, observed by Carney et al. (2001). Thus, these comparative kinetic studies along with the toxicological evaluations in the companion study of Carney et al. (2001) demonstrate that dose-rate is a critical determinant of ethylene glycol developmental toxicity.

following oral gavage vs. subcutaneous injection (Carney et al., 1999). Additional data were collected by Carney et al. (2001) for comparison to the present study to verify the similarity in kinetics by these two routes of exposure. Thus, these two routes of administration were utilized to efficiently compare the effects of dose and dose-rate on the pharmacokinetics of ethylene glycol and to provide a bridge to existing developmental toxicity studies conducted by oral gavage.

MATERIALS AND METHODS

Study Design. This study was conducted in two laboratories, the Developmental and Reproductive Toxicology Laboratory of The Dow Chemical Company (the "Dow Study") and the Chemical Dosimetry Group of Battelle, Pacific Northwest Division (the "BNW Study"). In the Dow study, 6 time-mated female Sprague-Dawley rats/dose were exposed to 1000 or 2000 mg/kg/day ethylene glycol via continuous, subcutaneous infusion pumps. These rats were implanted with pumps on gestational day (gd) 6 for continuous dosing through gd 11 or 12. On the morning of gd 11, 3 rats/dose level were euthanized for the collection of maternal blood, kidneys, extraembryonic fluid (EEF; pooled by litter) and embryos (pooled by litter). The remaining 3 rats/dose level were transferred to individual metabolism cages for the collection of urine (0-12 and 12-24 hr). These remaining rats were then sacrificed on gd 12 for the collection of the same maternal and conceptus samples. All blood and tissue samples collected from these animals were flash frozen and shipped on dry ice to the Chemical Dosimetry Group, Battelle Northwest Division, where they were stored frozen (-80°C) until analyzed for ethylene glycol, glycolic acid and oxalic acid. Only the results from these analyses are presented in this report. The in-life phase of the Dow study and the results from the toxicological evaluations are reported in Carney et al. (2001).

For the BNW study, ethylene glycol was administered in a water vehicle by oral gavage to two groups of 18 pregnant (gd 11) Sprague-Dawley rats at dose levels

of 100 or 1000 mg/kg. Subgroups of 3 animals/time period were sacrificed at 1, 3, 6, 9, 12 and 24 hr post-dosing. Animals from the 24-hr sacrifice were housed in metabolism cages for the collection of urine (0-12 and 12-24 hr). All animals were sacrificed under CO_2 anesthesia for the collection of maternal blood (cardiac puncture), kidneys, extraembryonic fluid (pooled by litter) and embryos (pooled by litter). Extra animals were dosed at each dose level to replace animals that were found to be either not pregnant at the time of sacrifice or had problems associated with dosing. Each sample was analyzed for ethylene glycol, glycolic acid and oxalic acid.

Test Materials and Chemicals. Ethylene glycol (Lot No. JR00244CR) and glycolic acid (Lot No. 16802LR) were obtained from the Aldrich Chemical Company (Milwaukee, WI). Oxalic acid (Lot No. 123H1122) was obtained from Sigma (St. Louis, MO). Deuterated internal standards D2-glycolic acid (Lot No. I1-5086), D4-ethylene glycol (Lot No. P-6136) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA) while the internal standard, 2-butoxyethanol (Lot No. 07847HN) was obtained from the Aldrich Chemical Company. Derivatizing reagents, pentafluorobenzoyl chloride and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamaide (MTBSTFA) were also obtained from the Aldrich Chemical Company. All other compounds and solvents were reagent grade or better.

Test Animals. Adult, time-mated female Sprague-Dawley rats were purchased from Charles River Laboratories. To obtain the numbers of animals required to conduct the study as designed, animals were received from both the Raleigh, NC and Portage, MI facilities of Charles River Laboratories. Animals were shipped on gestation day 6 and arrived at the laboratory (an AAALAC accredited facility) on gestation day 7. The animals were housed in suspended plastic cages with chipped bedding and acclimated to the laboratory for 4 days prior to dosing with ethylene glycol. The rooms in which the animals were housed were on a 12-hr light cycle (7 am – 7 pm) and designed to maintain adequate temperatures,

relative humidity and airflows for the species under study. Deionized water and Purina Certified Rodent Chow #5002 (Purina Mills, Inc., St. Louis, MO) were provided *ad libitum* during the pre-dosing period except that on the day prior to dosing a uniform amount of chow was fed (~15 g/rat). During the 4-day acclimation, animals were uniquely marked with a tail tattoo, weighed and randomly assigned to subgroups based upon targeted sacrifice times.

On the day prior to dosing, the animals were transferred from the barrier facility to the *in vivo* metabolism room and animals scheduled for urine collection were placed in individual metabolism cages for acclimation and collection of control urine. On the day of dosing (gd 11), feed was withheld ~2 hr prior to dosing and returned ~4 hr post-dosing. Three extra animals/dose group were administered ethylene glycol as potential replacements in the event that a rat was identified as non-pregnant when sacrificed or problems were encountered during dosing.

Dose Solutions and Administration. Aqueous solutions of ethylene glycol were prepared for each dose level on the day prior to dosing. For the 100 mg/kg dose group, a target concentration of 20 mg/ml of ethylene glycol was prepared in deionized (Milli Q) water. For the 1000 mg/kg dose group, ethylene glycol was prepared at a target concentration of 200 mg/ml in deionized water. The dose levels were chosen to be below and above the saturation of glycolic acid metabolism while ethylene glycol metabolism was expected to be linear over this dose range. The highest dose level, 1000 mg/kg, is equivalent to the LOEL for developmental toxicity (Neeper-Bradley et al., 1995) and was a dose level also used in the companion study by Carney et al. (2001).

Samples of each dose solution were taken for analysis on the day of dosing to confirm the targeted concentrations. Each animal was administered dose solutions at a rate of ~5 ml/kg body weight to achieve the targeted dose levels using a glass syringe fitted with a blunted, stainless steel feeding needle. The dosing syringe was weighed before and after dosing to determine the actual dose delivered to each animal. Immediately after dosing, the 24-hr sacrifice

group animals were placed in individual metabolism cages for the collection of urine (0-12 and 12-24 hr). The remaining animals were returned to their home cages until their scheduled time of sacrifice (1, 3, 6, 9, and 12 hr post-dosing).

Specimen Collection. At each scheduled sacrifice time, animals were anesthetized in an 80% CO_2 atmosphere and exsanguinated via cardiac puncture. Blood samples were collected in heparinized Vacutainers® and immediately frozen on dry ice. The time of death was recorded at the end of the blood draw and all animals were rapidly dissected to remove, trim extraneous tissues, and weigh the uterus, and kidneys. The kidneys were flash-frozen and stored along with the blood samples at –80°C until analyzed.

Each uterus was dissected to remove the decidual swellings containing the conceptuses (total recorded). Each conceptus was further dissected to separate extraembryonic fluid and embryos according to the procedure of Cockroft (1990). The time to complete each dissection was recorded. Extraembryonic fluid (pooled by litter) and embryos (pooled by litter) were weighed, flash-frozen and stored at –80°C until analyzed.

All urine voided during the study from the 24-hr sacrifice group animals were collected in dry ice-cooled traps at 12-hr intervals. Each cage was rinsed with a minimal volume of deionized water (~10 ml) and combined with each 0-12 and 12-24 hr urine sample for analysis. Each urine sample was stored at –80°C until analyzed.

Quality Control Samples. Quality control storage spikes of control blood, urine, kidneys and extraembryonic fluid containing 36-69 μg/g (low level) or 475-600 μg/g (high level) ethylene glycol, glycolic acid and oxalic acid were prepared and stored at –80°C along with the samples from both the Battelle and Dow kinetic studies. Due to the small amount of control embryos available, quality control samples were only prepared at a single, mid-level (183-218 μg/g) of ethylene glycol, glycolic acid and oxalic acid. Aliquots of these QC controls were analyzed

along with each preparation of samples from both studies to determine the potential losses of analytes over the period of time it took to complete all analyses.

Specimen Analysis. Samples of heparinized whole blood, urine, extraembryonic fluid and embryos were analyzed for ethylene glycol, glycolic acid and oxalic acid by gas chromatography/mass spectrometry (GC/MS) following the general extraction and derivatization methods of Pottenger et al. (2001). 2-Butoxyethanol and deuterated ethylene glycol and glycolic acid were utilized as internal standards. Kidneys were first homogenized directly (no diluent) then analyzed by the method used for analysis of blood. At the highest dose levels, blood and kidneys were analyzed for ethylene glycol using gas chromatography/flame ionization detection (GC/FID) and 2-butoxyethanol as an internal standard (glycolic acid and oxalic acid were analyzed by GC/MS). For urine samples containing very high concentrations of ethylene glycol, a direct analysis of urine by GC/FID was also conducted using 2-butoxyethanol as an internal standard. Each of these methods is described briefly below.

GC/MS analyses of ethylene glycol, glycolic acid and oxalic acid were performed on a Hewlett Packard 7683 Mass Selective Detector equipped with a Hewlett Packard 6890 Plus gas chromatograph and 7683 autosampler (Hewlett Packard, Avondale, PA). Separations were achieved with a Restec RTX-5MS fused silica capillary column (30 m x 0.25 mmid, 0.25 μm film thickness; Restec, Bellefonte, PA). Injections were either splitless (GA, OX) or pulsed splitless (EG) using an unpacked Restec 4 mmid cyclo double gooseneck liner. Representative chromatography conditions for glycolic acid and oxalic acid were as follows: injector temperature was 210°C, the initial oven temperature was 110°C, which was increased at 15°C/min to 200°C, with a final ramp of 25°C/min to 300°C; initial head pressure was a constant 25 psi with helium as the carrier gas. For ethylene glycol, the injection temperature was 190°C, the initial oven temperature was 130°C, which was increased at 20°C/min to 200°C, with a final

ramp of 50°C/min to 300°C. The initial head pressure was pulsed at 35 psi for 0.5 min followed by a constant head pressure of 25 psi with helium as a carrier gas. The masses used for quantitation of ethylene glycol were 238 or 450 (depending upon column conditions); 241 or 454 for D4-ethylene glycol; 247 for glycolic acid; 249 for D2-glycolic acid; and 261 for oxalic acid.

GC/FID analyses of ethylene glycol were performed on a Hewlett Packard 6890 gas chromatograph equipped with an FID detector and autosampler. Separations were achieved with a J&W DB-Wax fused silica column (15 m x 0.53 mmid x 1.0 df; J&W Scientific, Folsom, CA). For direct injection of urine, injections of 1.0 µl (splitless) of urine spiked with 2-butoxyethanol internal standard (250 µg/g) were injected at 220°C with an initial head pressure of 5 psi (helium) for 2 min, increasing to 10 psi at 20 psi/min. The initial oven temperature was 80°C, increasing to 125°C at 20°C/min with a final ramp of 30°C/min to 230°C. A Restek 4 mmid cyclo-double gooseneck injection liner was also used. For analysis of high dose blood and kidney extracts, the same column and injection liner were used with a constant head pressure of 7 psi (helium), injection temperature of 225°C, initial oven temperature of 70°C for 2 min, increasing at 20°C/min to 130°C with a final ramp to 230°C at 50°C/min.

Statistics and data analysis. Descriptive statistics (i.e. mean ± SD) were used where applicable to present the data. In some instances, only one or two samples within a group had levels of analytes above the limits of reliable quantitation. In these cases, the LOQ/2 was arbitrarily used as a surrogate to calculate the mean ± SD for plotting and pharmacokinetic parameter estimations. Individual data are presented in the Appendix tables. Areas under the concentration vs. time curves were calculated for each analyte from the oral gavage study according to the trapezoidal rule (Gibaldi and Perrier, 1982). Half-lives (ß-elimination phase) were determined by:

$$t_{1/2} = \frac{0.693}{Ke}$$

where Ke is determined from the slope of the line in the ß-elimination phase.

RESULTS AND DISCUSSION

Oral Gavage Study (BNW Study).

Dose Confirmation. Each of the dosing solutions were considered homogenous with actual concentrations of ethylene glycol within 1-2% of target by gravimetric and GC/FID analyses (Table 1). The final GC/FID analyzed concentrations were used to determine the actual doses of ethylene glycol in subsequent analyses. The body weight of each animal was determined just prior to dosing to calculate the target dose volumes. Syringe weights, before and after dosing, were used to calculate the actual dose administered. With the exception of a few animals, the actual dose levels were within 1-2% of the targeted doses (Table 2). Individual animal data are summarized in Appendix Table A-1.

Terminal Body and Tissue Weights. Each animal was anesthetized under 80% CO_2, weighed and exsanguinated by cardiac puncture. Time of death was recorded at the completion of the blood draw. Terminal body weights, numbers of implants and kidney, uterine, and embryo weights are summarized in Table 3. Extraembryonic fluid weights were also recorded. However, these values are more variable reflecting the difficulties in extracting the fluid, especially earlier in gestation. Although no statistical analyses were conducted (no controls were included for comparison), the only biologically significant effects observed in terminal body and tissue weights were associated with the expected growth in the uterus and conceptuses over the 24-hr kinetic study. Individual animal data are summarized in Appendix Tables A-2 and A-3.

Quality Control Samples. Samples of control blood, urine, kidneys, extraembryonic fluid and embryos spiked with ethylene glycol, glycolic acid and oxalic acid and stored frozen at the beginning of the study were analyzed with

each set of samples from the oral gavage study and the Carney et al. (2001) continuous infusion study. There was some variability in the analyzed results, primarily with the tissue spikes, with most samples within ±20% of the targeted concentrations (Figure 2). The variability was attributed to the lack of use of a diluent in the homogenization of the samples and the use of small sub-samples (50-200 mg) for analysis.

On February 7, 2001, after 268 days of storage, the –80°C freezer used to store the samples from this dose-rate study inadvertently shut down due to a tripped circuit breaker (another –80°C freezer had been plugged into the same circuit). Temperatures within the freezer were approximately 0°C when discovered and several of the smaller volume samples appeared to have thawed. Even with the brief, partial thawing of samples, no significant losses of ethylene glycol, glycolic acid or oxalic acid occurred from the quality control samples over the course of the study and up to 542 days. Thus, all samples analyzed over the course of this study were considered reliable and no corrections were necessary to account for degradation during storage.

Kinetics of EG and Metabolites in Maternal Blood, Urine and Kidneys. Maternal blood, urine and kidney levels of ethylene glycol, glycolic acid and oxalic acid are summarized in Tables 4 – 6 and Figures 3 – 5. Individual animal data are summarized in Appendix Tables A-4 to A-6. Pharmacokinetic parameters (AUC's and $t_{1/2}$'s) are presented in Table 7.

Ethylene glycol was well-absorbed orally and achieved peak blood concentrations prior to the collection of the first sample (1 hr). The kinetics of ethylene glycol was, as expected from prior studies, proportional to dose. The clearance of ethylene glycol from blood followed first-order (log-linear) kinetics and, along with the kinetics of glycolic acid, was similar to previously published results of gestation day 10 rat kinetics from Pottenger et al. (2001) as shown in Figure 3. The elimination half-life for EG ranged from 1.1-2.5 hr in blood and tissues

(Table 7) and were similar to the blood elimination half-lives reported by Pottenger et al. (2001).

Maternal blood ethylene glycol and glycolic acid levels following gavage dosing in the present study were also similar to those following subcutaneous bolus injections (Carney et al., 2001), as shown in Figure 4. Although only a single time point after bolus subcutaneous dosing was analyzed (3 hr), the concentrations of ethylene glycol and glycolic acid in maternal blood following oral gavage (also at 3 hr) were comparable.

Ethylene glycol levels in blood were slightly higher than kidneys at 1000 mg/kg but followed a similar kinetic profile (Figure 5). Interestingly, glycolic acid levels in the maternal kidneys were the same as maternal blood levels at the lowest dose level, but were consistently 2.4- to 3.2-fold higher than the corresponding blood levels at 1000 mg/kg (Figure 5 and Table 7).

Unmetabolized ethylene glycol was the major component found in urine at the lowest dose level (21.2% of dose) with the majority excreted in the first 12 hr following dosing (Table 6). Ethylene glycol was excreted in the urine at a similar dose-proportionate rate at 1000 mg/kg (25.8% of dose), consistent with the results of Pottenger et al. (2001), who evaluated the kinetics of ethylene glycol and its metabolites over a bolus oral dose range of 10 – 2500 mg/kg.

Based upon prior studies, the two dose levels used in this kinetics study were chosen to bracket the saturation of glycolic acid metabolism. As such, there was an expected pronounced shift in the kinetics of glycolic acid in maternal blood, urine and kidneys as the ethylene glycol dose level was increased ten-fold from 100 to 1000 mg/kg. At the lowest dose level, glycolic acid accounted for<10% of the metabolites found in urine while at 1000 mg/kg, glycolic acid accounted for greater than 45% of the urinary metabolites (Table 6). The area under the curve for glycolic acid in blood was approximately 74-fold higher after dosing at 1000 mg/kg than at the 10-fold lower dose level. This observation was even more dramatic in the kidneys where the AUC's for glycolic acid were increased

190-fold for a corresponding 10-fold increase in administered dose. In theory, this could play a potential role in high-dose renal toxicity if kidney tissues continue to metabolize glycolic acid to oxalic acid, which, in turn, crystalizes in renal tissues as calcium oxalate. However, as discussed below, the oxalic acid results yielded little information as to dose-related kinetics and the potential relationships to potential renal toxicity.

The concentrations of oxalic acid in blood, urine and kidneys were slightly elevated at the higher dose (Figure 5 and Tables 4-7). For example, the areas under curve for oxalic acid in blood was approximately 2.2-fold higher while the total amounts excreted over 24 hr in urine were 2.7-fold higher at 1000 mg/kg than at 100 mg/kg. However, oxalic acid still only accounted for 0.5-1.33% of the administered dose (Table 6). These results were similar to Pottenger et al. (2001) where oxalic acid accounted for only 0.36-0.66% of the dose over an oral gavage dose range of 10-2500 mg EG/kg.

More importantly for renal toxicity, the areas under the curves for oxalic acid in kidneys were 78-fold higher at 1000 mg/kg than at 100 mg/kg. However, these concentrations were (a) generally near the levels found in a small number of control samples (0-10 μg/g), (b) highly variable, and (c) showed little evidence of clearance. Thus, it was unclear how much of the oxalic acid present in these samples resulted from the administered doses of ethylene glycol or reflected endogenous metabolism or metabolism of constituents in the rodent diet. Since only a small number of control blood, kidney and urine samples were available for analysis and the oxalic acid levels were highly variable, no attempt was made to correct the results for background levels of oxalic acid. Thus, the results for oxalic acid levels in the kidney are intriguing and may reflect a local dose-rate related build-up that may contribute to toxicity.

Kinetics of EG and Metabolites in Conceptuses. The kinetics of ethylene glycol in extraembryonic fluid and embryos were nearly identical to that of the maternal blood (Figure 6, Tables 7-8). The half-life for elimination of ethylene glycol from

conceptuses was slightly longer than blood (Table 7). However, this result was primarily due to the 24-hr time point where no ethylene glycol was detected in maternal blood, thus, no biological significance was placed on the slight differences in terminal, elimination phase half-lives.

Glycolic acid levels, however, were consistently higher in extraembryonic fluid and embryos than their corresponding maternal blood levels. No further compartmentalization was observed within the conceptus given that glycolic acid levels were similar in embryos and extraembryonic fluid at most time points. At the lowest dose level (100 mg/kg), glycolic acid in extraembryonic fluid and embryos ranged from 2- to 4-fold higher than corresponding maternal blood levels for the first few hours after dosing. After 6 hr, the levels of glycolic acid in extraembryonic fluid decreased to the low levels found in maternal blood while the embryo levels were maintained at detectable levels through 24 hr. At 1000 mg/kg, the ratios of conceptus:maternal blood ranged from 1.4-3.1 for the first 12 hr. By 24 hr, glycolic acid levels in the embryo decreased to maternal blood levels while the extraembryonic fluid levels remained slightly elevated (just the opposite of what occurred at 100 mg/kg). These results were also reflected in ratios of the areas under the curves, which ranged from 1.6-2.6 over these two dose levels (Table 7).

These ratios are very similar to those reported in a metabolism probe study by Carney et al. (1998) where extraembryonic fluid levels of glycolic acid ranged from 1.3-1.8 fold higher than corresponding maternal blood levels in the first 3 hr after dosing with either 500 or 2500 mg/kg ethylene glycol by oral gavage. While the exact mechanism behind the finding of higher levels of glycolic acid in conceptuses than the corresponding maternal blood concentrations is unknown, it has been proposed that weak organic acids are trapped in extraembryonic fluid and embryos due to pH-dependent ion-trapping (O'Flaherty et al., 1992; Terry et al., 1995; Pottenger et al., 2001).

The concentrations of glycolic acid in extraembryonic fluid and embryos generally paralleled those of maternal blood, albeit, at consistently higher (1.4-3 fold) levels (Figure 6) for the first 6-12 hr depending upon the dose. If one considers that glycolic acid conceptus levels were almost always within a factor of 3 of maternal blood levels, maternal blood concentrations of glycolic acid could be used as a potential dose surrogate for extrapolating across route of exposure, high-to-low dose and, more importantly, across species. This is particularly important for human health risk assessments where no studies could conceivably be conducted in pregnant women to validate the extrapolations.

As with maternal blood and tissues, oxalic acid levels were generally low, showed very little change with dose or time and were within or near the normal control ranges observed in blood (0-10 μg/g). Individual animal data are summarized in Appendix Tables A-7 to A-8.

Subcutaneous Infusion Study (Dow Study).

To evaluate the impact of dose-rate on developmental toxicity, Carney et al. (2001) utilized an ESOX infusion pump (ESOX, Model V01, Access Technologies, Skokie, IL) implanted subcutaneously in the scapular region of the rat torso to continuously infuse ethylene glycol at a constant rate. The pumps, which were calibrated by the manufacturer to deliver approximately 0.9 ml/day, were implanted on gestation day 6 and re-filled each day (through gestation days 11 or 12) with approximately 1 ml of either 300 or 600 mg/ml of ethylene glycol in distilled water to deliver target dose levels of 1000 or 2000 mg/kg/day, respectively. Maternal blood data from this the subcutaneous infusion study (samples generated at The Dow Chemical Company and analyzed at Battelle Northwest) are plotted in Figure 7 and summarized in Table 9. Individual animal data are summarized in Appendix Table A-9.

In contrast to the blood levels following oral gavage (Figure 3), blood levels of ethylene glycol and glycolic acid following constant infusion of comparable total doses (i.e. 1000 mg/kg) averaged 8- to 14-fold and 46- to 83-fold lower,

respectively (Figure 7). Figure 7 also shows additional data from Carney et al. (2001) in which maternal blood was collected from the tail vein on gestation days 7, 9, 11 and 15 for analysis of ethylene glycol and glycolic acid. Several of the blood samples contained lower levels of ethylene glycol than expected from the tail-vein data reported by Carney et al. (2001). Since ethylene glycol rapidly clears from the blood when intake ceases ($t_{1/2}$ < 2 hr; Table 7), the low levels observed in some animals may have been due to a decrease in the rate of infusion as the pump reservoirs neared depletion before the blood samples were collected. This clearly did not occur in the tail vein blood samples reported in the Carney et al. study. Glycolic acid levels in maternal blood were more consistent with the levels observed by Carney et al. (2001) in the tail-vein bled animals (Table 9 and Figure 7) and, most importantly, never achieved the putative threshold of 2 mM (152 μg/g) suggested by Carney et al. (2001) for developmental toxicity following continuous infusion.

Urine levels of ethylene glycol were consistent with the oral gavage data at comparable dose levels (1000 mg/kg) indicating that the pumps delivered the expected total dose of ethylene glycol (Table 11). Overall, the glycolic acid levels in blood and urine were considerably lower following continuous infusion than following oral gavage (Figures 3 vs. 7 and Tables 6 vs. 11). In fact, for the 1000 mg/kg/day dose level, total urinary glycolic acid collected over 24 hours was 15-fold lower when EG was given via pump vs. gavage.

As observed in the oral gavage study, ethylene glycol levels in the kidneys following continuous infusion were consistent with the corresponding blood levels while glycolic acid levels averaged 1.2- to 1.5-fold higher in the kidneys (Table 10). The impact of dose-rate on glycolic acid levels was also striking in the kidneys where peak glycolic acid levels were approximately 84- to 121-fold lower when 1000 mg/kg of ethylene glycol was administered by continuous subcutaneous infusion than if it was administered by bolus oral gavage (Tables 5 vs. Table 10). Oxalic acid levels in the kidneys following continuous infusion

were near background (0-10 μg/g) and showed no dose-response indicating that renal toxicity may also be attributed to a high dose-rate exposure.

Consistent with results from the oral gavage study, ethylene glycol levels in extraembryonic fluid and embryos following continuous infusion were similar to corresponding maternal blood levels (Tables 9 vs. 12). Glycolic acid levels consistently averaged 1.6- to 2.8-fold higher in extraembryonic fluid and embryos following continuous infusion than the corresponding maternal blood levels. Constant rate pump infusion of 1000 mg/kg/day of ethylene glycol resulted dramatically lower exposure (48- to 57-fold lower than peak blood concentrations) of conceptuses to the proximate toxicant, glycolic acid, as compared to gavage exposure (Tables 8 vs. 12). However, as in the oral gavage study, the kinetics of glycolic acid in the conceptuses mirrored the kinetics in maternal blood. Oxalic acid levels were also variable and near background in the conceptuses following continuous subcutaneous infusion.

CONCLUSIONS

Although numerous kinetic studies have been conducted in male, female and pregnant rats, this study along with its companion (Carney et al., 2001), are the first to include specific analysis of ethylene glycol, glycolic acid and oxalic acid in maternal target organs (kidneys) and the developing embryo (gd 11-12) as a function of dose, route of exposure and dose-rate. The remarkable consistency in results from this study and a number of other studies where similar doses have been used (e.g. Carney et al., 1996, 1998, 1999, 2001; Frantz et al., 1996) and the fact that Pottenger et al. (2001) have shown that pregnancy does not affect the maternal blood kinetics of ethylene glycol and glycolic acid will enable the pooling of a significant amount of pharmacokinetic information across a broad range of doses and routes of exposure to facilitate the interpretation of the toxicity data.

Bolus dosing of ethylene glycol, whether by oral gavage or subcutaneous injection, results in significantly higher maternal blood, tissue and conceptus levels of glycolic acid, the developmentally toxic metabolite, than equivalent daily doses administered by continuous infusion. Interestingly, the dose-rate effect on glycolic acid levels were significant in both the kidneys and the conceptus. Although glycolic acid has not been implicated in renal toxicity, high levels may contribute to renal toxicity if further metabolism of glycolic acid to oxalic acid occurs within the tissue itself. The pharmacokinetics of oxalic acid in renal tissues only showed a significant dose-response in the oral gavage study where the areas under the curves were increased 78-fold for a 10-fold increase in dose suggesting that oxalic acid levels are sensitive to dose-rate. However, the levels were highly variable and were at or near background levels. Further research would be required to differentiate oxalic acid associated with ethylene glycol metabolism from endogenous sources (i.e. through the use of ^{13}C-analogues) to adequately characterize the dose-response relationship of oxalic acid *in vivo*.

As for the conceptus, dose-rate had a profound effect on the levels of the developmentally toxic metabolite, glycolic acid. Glycolic acid was a minor metabolite following oral gavage at 100 mg/kg while at 1000 mg/kg, glycolic acid was a major metabolite with AUC's increasing 44- to 63-fold in the embryos and extraembryonic fluid, respectively. Glycolic acid levels were also significantly elevated (48- to 57-fold) in conceptuses following bolus oral gavage vs. comparable total doses of 1000 mg/kg ethylene glycol administered by continuous subcutanteous infusion. No significant differences were observed in the concentrations of ethylene glycol and glycolic acid in gestational day 11-12 embryos vs. the extraembryonic fluid regardless of dose or dose-rate except at later time periods when most of the ethylene glycol metabolites had been cleared.

Coupled with the results of Carney et al. (2001), these comparative kinetic studies support the hypothesis that dose-rate is a critical determinant of ethylene glycol developmental toxicity. Furthermore, given that the kinetics of the developmentally toxic metabolite, glycolic acid, in rat conceptuses were generally within a factor of 3 of maternal blood levels following either bolus or continuous dose-rates, maternal blood levels may be an effective internal dose surrogate for high-to-low dose, route-to-route and species-to-species extrapolations for developmental risk assessments.

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Figure 1. Metabolism scheme for ethylene glycol.

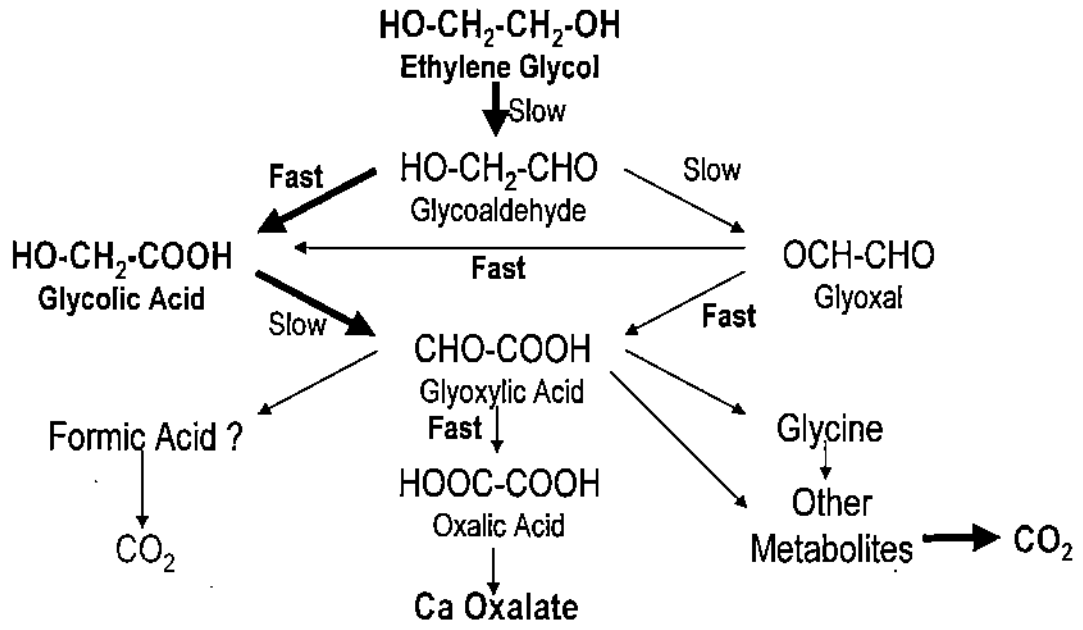


Figure 2. Quality control spikes of blood, urine, kidneys, extraembryonic fluid and embryos. Results expressed as % of target for two concentrations of each analyte. Data from all matrices are combined.

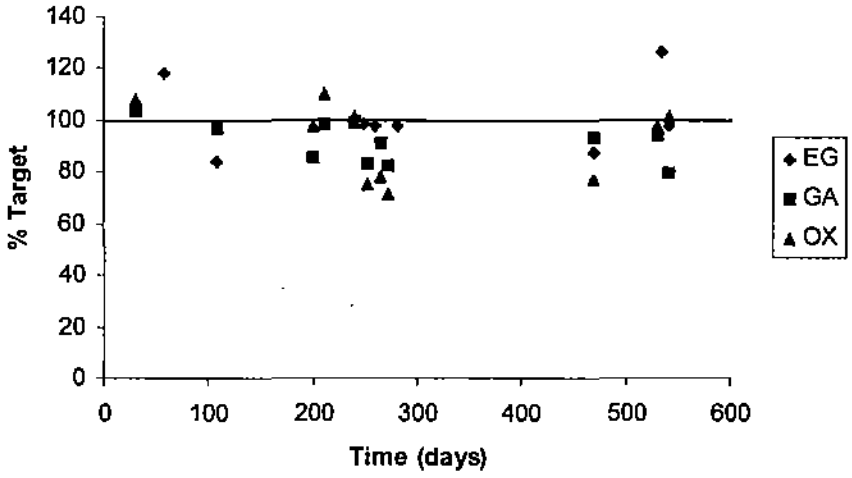


Figure 3. Concentrations of (a) ethylene glycol and (b) glycolic acid in the blood of pregnant Sprague Dawley rats following oral gavage doses of 100 or 1000 mg/kg ethylene glycol. Data from Pottenger et al. (2001) where pregnant Sprague Dawley rats were orally dosed with 150 and 1000 mg/kg ethylene glycol by gavage are included for comparison.

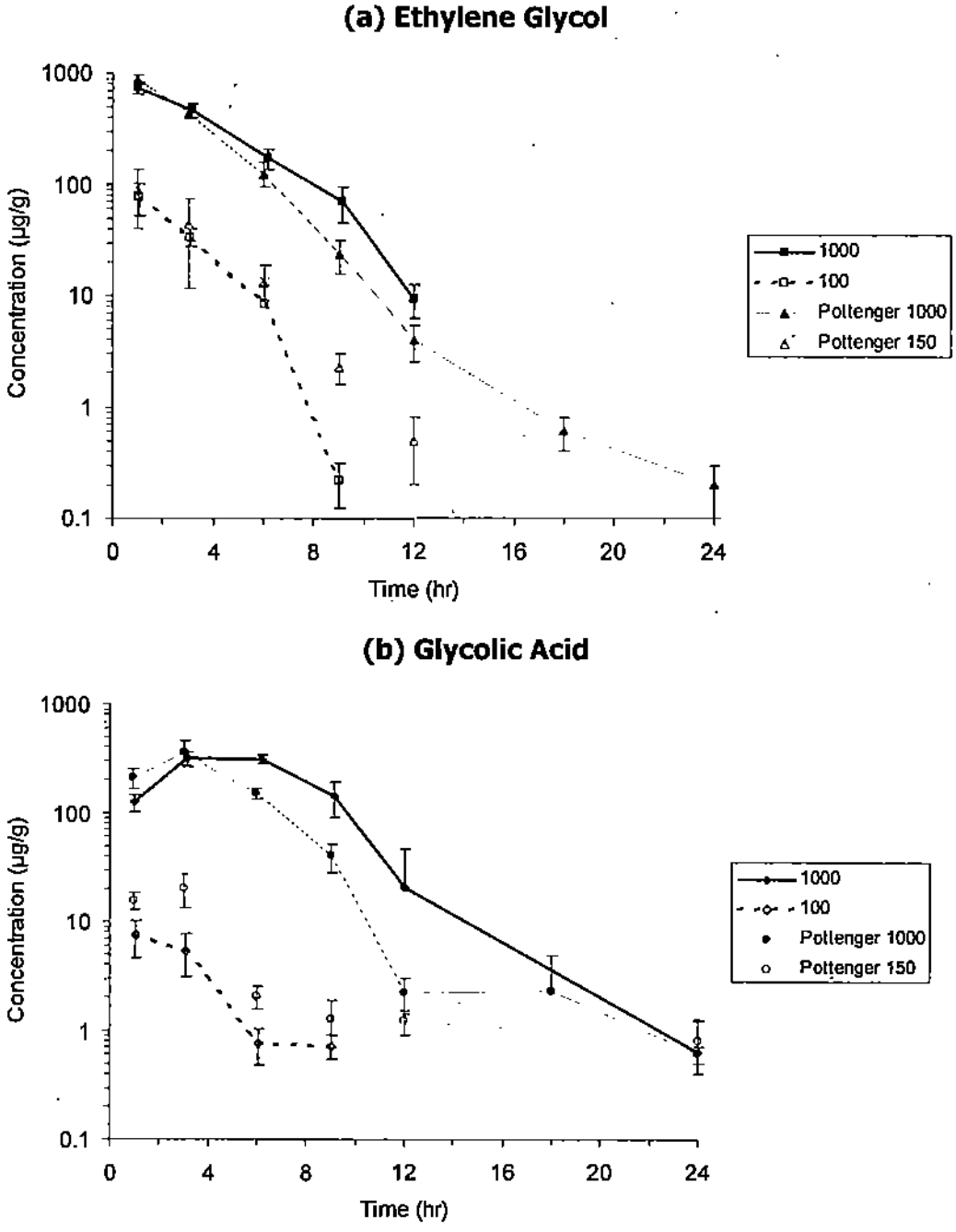
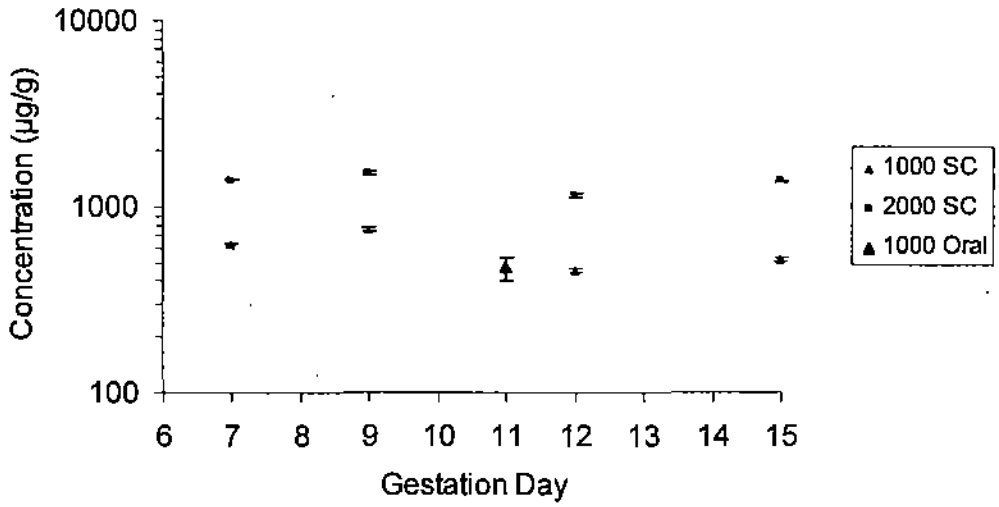


Figure 4. Concentrations of (a) ethylene glycol and (b) glycolic acid in the blood of pregnant Sprague Dawley rats three hours after a bolus dose of ethylene glycol by either the oral route on gestation day 11 (1000 mg/kg; present study) or the subcutaneous route (1000 and 2000 mg/kg/d injections on gestation days 6-15 as reported by Carney et al., 2001).

(a) Ethylene Glycol



(b) Glycolic Acid

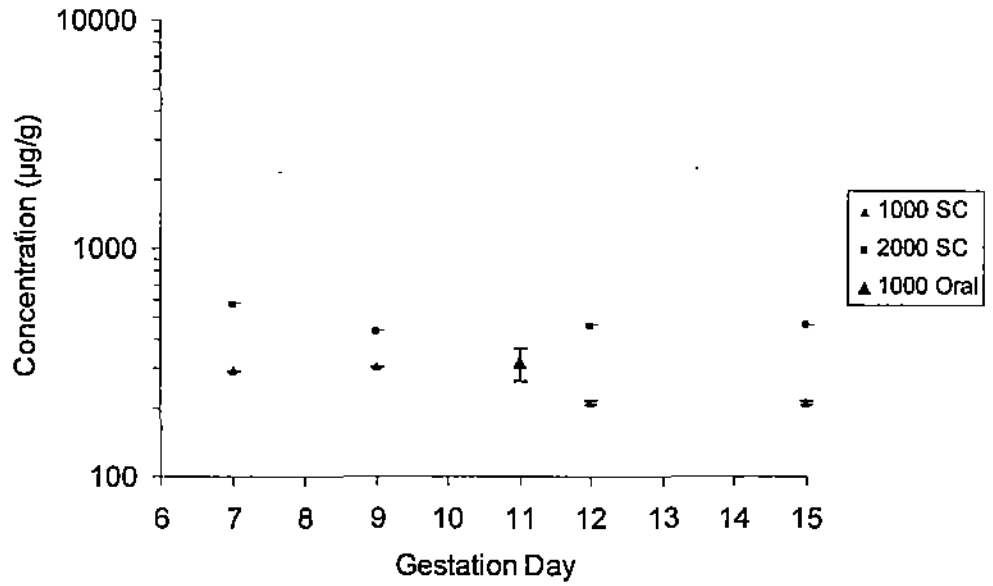


Figure 5. Concentrations of (a) ethylene glycol, (b) glycolic acid and (c) oxalic acid in the kidneys of pregnant Sprague Dawley rats following oral gavage doses of 100 and 1000 mg/kg ethylene glycol. Concentrations of each metabolite in blood are included for comparison.

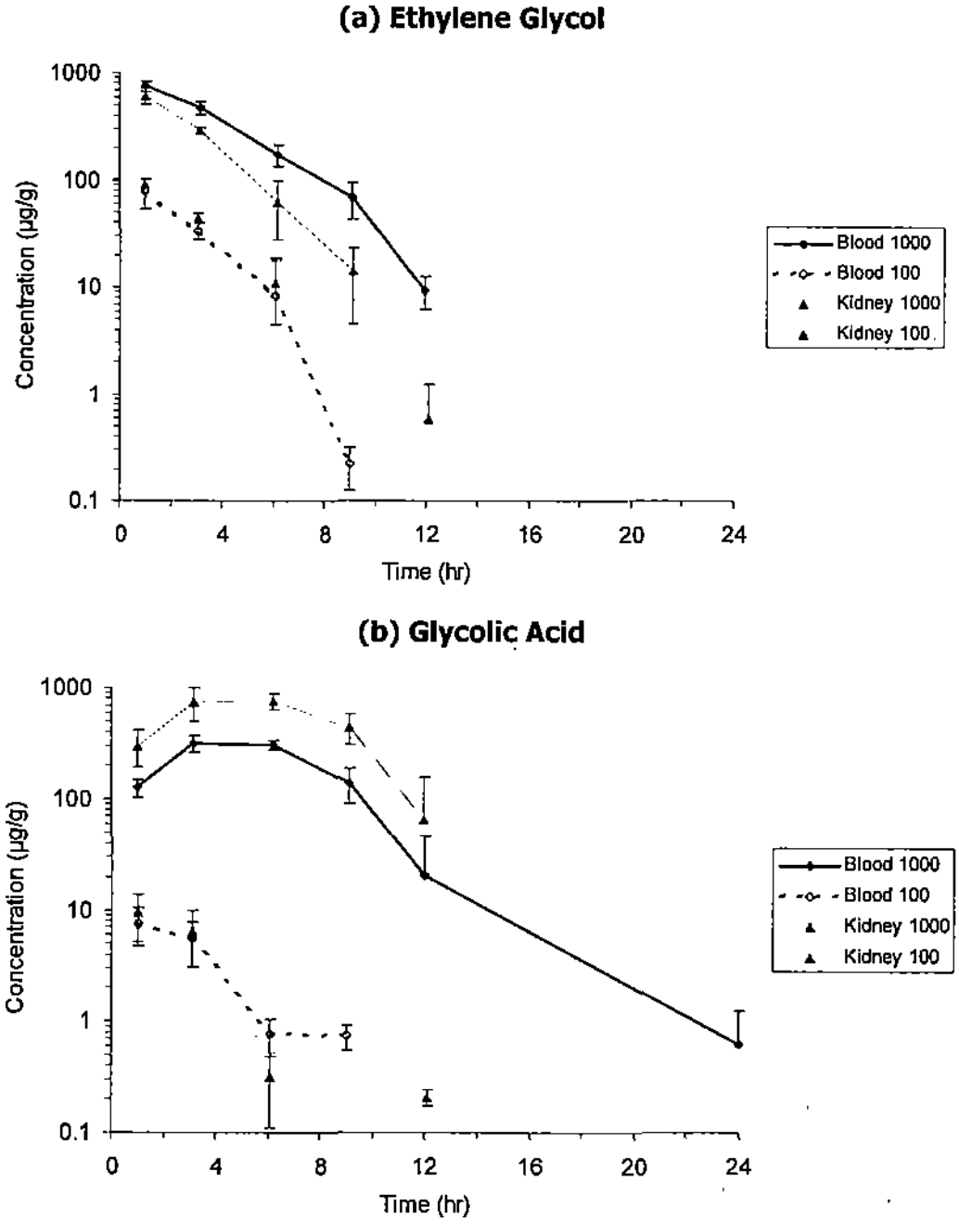


Figure 5 (continued).

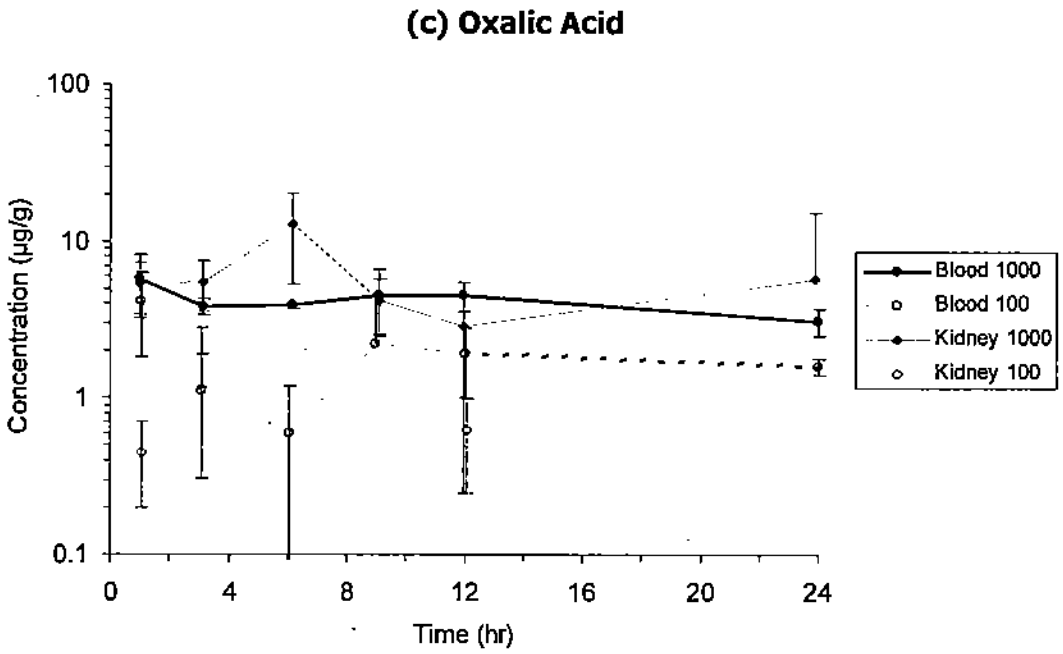
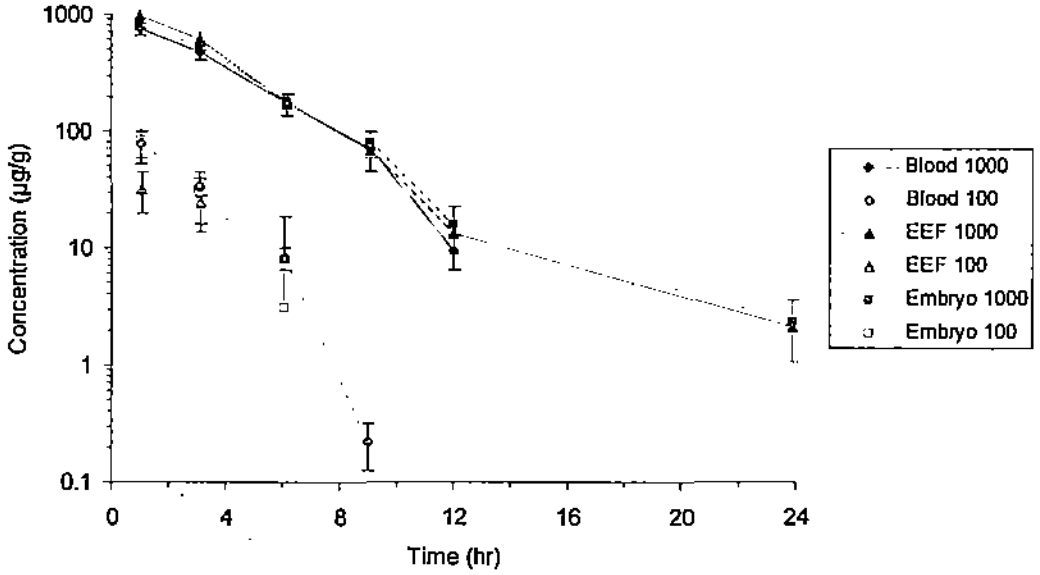


Figure 6. Concentrations of (a) ethylene glycol, (b) glycolic acid and (c) oxalic acid in the embryos and extraembryonic fluid (EEF) of pregnant Sprague Dawley rats following oral gavage doses of 100 and 1000 mg/kg ethylene glycol. Concentrations of each metabolite in blood are included for comparison.

(a) Ethylene Glycol



(b) Glycolic Acid

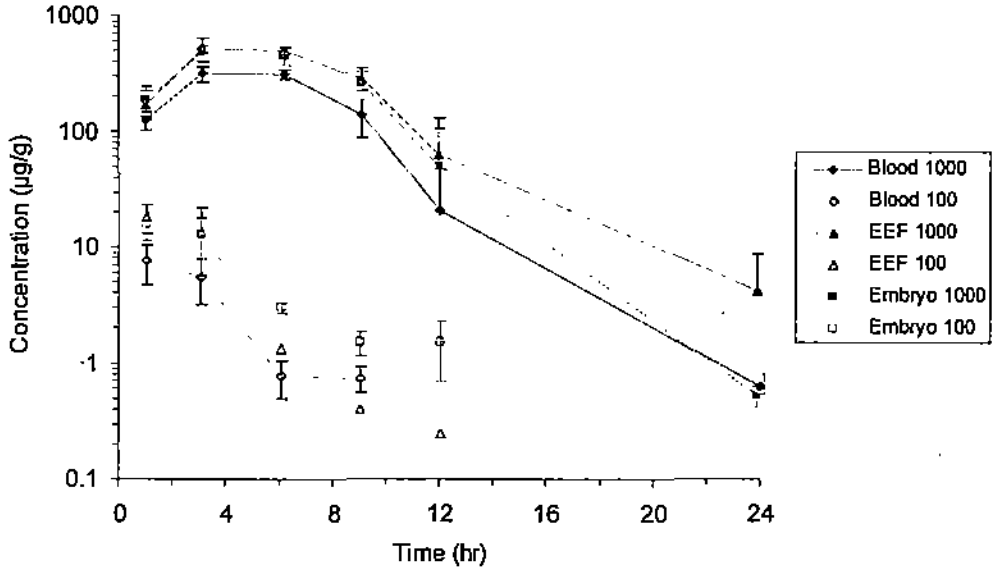


Figure 6 (continued).

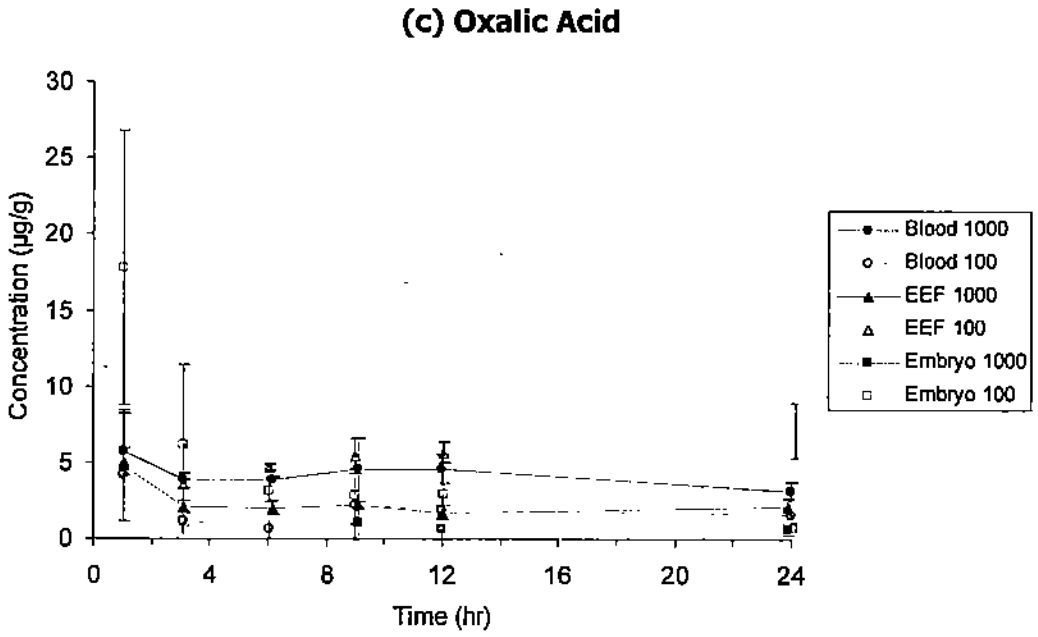


Figure 7. Concentrations of (a) ethylene glycol and (b) glycolic acid in the blood of individual pregnant Sprague Dawley rats following subcutaneous infusion of ethylene glycol at 1000 or 2000 mg/kg/day on gestation days 11 and 12. Mean data from Carney et al. (2001) are included for comparison.

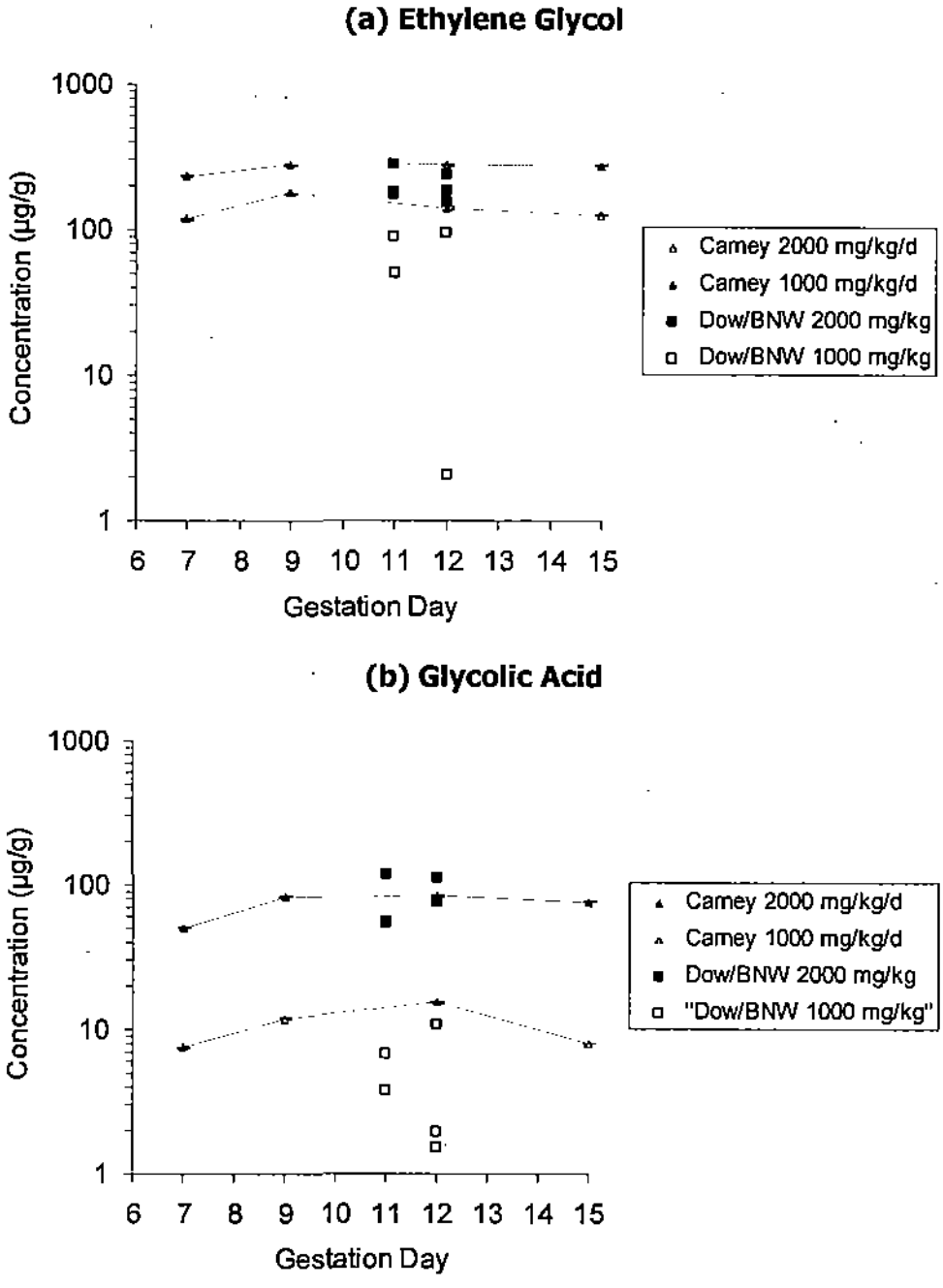


Table 1. Oral Gavage Study: Analysis of EG Dose Solutions.

Target Conc. (mg/ml)	Gravimetric Conc. (mg/ml)	GC/FID Sample Location	GC/FID Conc. (mg/ml)	GC/FID % of Target
20	20.45	Average	20.26	101.3
		Top	*20.215*	
		Middle	*20.281*	
		Bottom	*20.294*	
200	200.1	Average	196.65	98.3
		Top	*193.22*	
		Middle	*197.22*	
		Bottom	*199.50*	

Table 2. Oral Gavage Study: Body Weights, Sacrifice Times and Amounts of Ethylene Glycol Administered.

Scheduled Sacrifice Time (hr)	Actual Sacrifice Time (hr)[a]	Body Wt. At Dosing (kg)	Actual Dose (mg)	Actual Dose (mg/kg)	Actual Dose % Target
		100 mg/kg Dose Group			
1	1.03 ± 0.07	0.272 ± 0.008	27.4 ± 1.2	100.8 ± 1.8	100.8
3	3.10 ± 0.05	0.261 ± 0.007	26.6 ± 1.0	101.9 ± 1.5	101.9
6	6.07 ± 0.07	0.276 ± 0.024	28.1 ± 1.9	101.9 ± 2.3	101.9
9	9.02 ± 0.06	0.264 ± 0.017	27.7 ± 1.0	105.4 ± 4.7	105.4
12	12.06 ± 0.08	0.271 ± 0.014	27.0 ± 1.8	99.6 ± 1.3	99.6
24	24.15 ± 0.14	0.265 ± 0.022	25.9 ± 1.1	98.2 ± 4.0	98.2
		1000 mg/kg Dose Group			
1	1.02 ± 0.12	0.265 ± 0.009	264.3 ± 7.1	997.0 ± 20.5	99.7
3	3.12 ± 0.10	0.256 ± 0.013	257.1 ± 9.0	1005.1 ± 19.8	100.5
6	6.19 ± 0.30	0.250 ± 0.018	248.6 ± 18.0	992.8 ± 2.7	99.3
9	9.13 ± 0.25	0.244 ± 0.037	241.5 ± 41.6	986.5 ± 22.5	98.7
12	11.98 ± 0.07	0.259 ± 0.014	258.4 ± 10.5	999.3 ± 17.8	99.9
24	23.89 ± 0.14	0.245 ± 0.027	244.4 ± 24.1	997.0 ± 18.7	99.7

[a] Actual sacrifice time recorded at completion of exsanguinations.

Table 3. Oral Gavage Study: Terminal Body, Organ and Tissue Weights.

Scheduled Sacrifice (hr)	Terminal Body Wt. (g)	Kidney Wt. (g)	Uterine Wt. (g)	Implants (no.)	Total Embryo Wt. (g)	Total EEF Wt. (g)
			100 mg/kg Dose Group			
1	263.8 ± 7.7	2.159 ± 0.112	3.290 ± 0.396	12.3 ± 1.2	0.050 ± 0.022	0.113 ± 0.083
3	252.9 ± 5.7	1.773 ± 0.109	3.178 ± 0.440	12.7 ± 1.5	0.050 ± 0.024	0.100 ± 0.056
6	266.6 ± 23.0	1.796 ± 0.297	3.381 ± 0.681	13.7 ± 4.5	0.087 ± 0.033	0.126 ± 0.062
9	259.1 ± 11.6	1.949 ± 0.209	3.768 ± 0.515	13.3 ± 0.6	0.124 ± 0.040	0.200 ± 0.044
12	269.0 ± 13.6	2.186 ± 0.189	4.167 ± 1.050	13.3 ± 2.9	0.134 ± 0.082	0.216 ± 0.004
24	283.3 ± 22.9	2.095 ± 0.089	5.218 ± 0.754	13.3 ± 1.5	0.375 ± 0.087	0.260 ± 0.135
			1000 mg/kg Dose Group			
1	256.2 ± 10.2	1.929 ± 0.133	2.998 ± 0.830	12.3 ± 3.8	0.052 ± 0.015	0.084 ± 0.051
3	240.4 ± 12.7	1.717 ± 0.094	3.225 ± 0.168	12.0 ± 1.0	0.080 ± 0.016	0.143 ± 0.078
6	237.2 ± 17.8	1.997 ± 0.163	3.667 ± 0.963	12.7 ± 3.1	0.092 ± 0.010	0.114 ± 0.062
9	234.7 ± 31.5	1.816 ± 0.067	3.795 ± 0.969	12.7 ± 3.2	0.155 ± 0.039	0.118 ± 0.019
12	260.4 ± 14.9	2.213 ± 0.195	4.111 ± 0.478	14.7 ± 1.5	0.154 ± 0.010	0.224 ± 0.098
24	256.3 ± 29.3	2.183 ± 0.195	5.265 ± 1.128	13.7 ± 1.5	0.330 ± 0.060	0.222 ± 0.043

Table 4. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Blood. Data are presented as the means ± standard deviations except when animal numbers reduced from three; individual animal data are presented (see footnotes).

Scheduled Sacrifice Time (hr)	Actual Sacrifice Time (hr)	EG[a] (μg/g)	GA[b] (μg/g)	OX[c] (μg/g)
		100 mg/kg Dose Group		
1	1.03 ± 0.07	76.9 ± 24.1	7.6 ± 2.9	4.1 ± 2.3
3	3.10 ± 0.05	33.8 ± 6.0	5.5 ± 2.4	1.1 ± 0.8
6	6.07 ± 0.07	8.4 ± 10.3	0.8 ± 0.3	0.6 ± 0.6
9	9.02 ± 0.06	0.2 ± 0.10	0.7 ± 0.2	2.2 ± 2.3
12	12.06 ± 0.08	nq	nq	1.9 ± 0.9
24	24.15 ± 0.14	nq	nq	1.6 ± 0.2
		1000 mg/kg Dose Group		
1	1.02 ± 0.12	745.8 ± 84.9	124.8 ± 22.8	5.7 ± 2.5
3	3.12 ± 0.10	473.1 ± 65.9	314.4 ± 48.8	3.8 ± 0.4
6	6.19 ± 0.30	173.1 ± 36.9	310.0 ± 27.4	3.9 ± 0.1
9	9.13 ± 0.25	70.6 ± 25.8	139.9 ± 50.1	4.6 ± 2.1
12	11.98 ± 0.07	9.5 ± 3.1	20.3 ± 26.2	4.5 ± 1.0
24	23.89 ± 0.14	nq	0.6 ± 0.6	3.1 ± 0.6

[a]Not quantifiable at a limit of quantitation of 0.22 μg EG/g of blood.
[b]Not quantifiable at a limit of quantitation of 0.44 μg GA/g of blood.
[c]Not quantifiable at a limit of quantitation of 0.92 μg OX/g of blood.

Table 5. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Kidneys. Data are presented as the means ± standard deviations except when animal numbers reduced from three; individual animal data are presented (see footnotes).

Scheduled Sacrifice Time (hr)	Actual Sacrifice Time (hr)	EG[a] (μg/g)	GA[b] (μg/g)	OX[c] (μg/g)
		100 mg/kg Dose Group		
1	1.03 ± 0.07	88.8 ± 12.6	9.5 ± 4.3	0. 5 ± 0.3
3	3.10 ± 0.05	43.5 ± 5.0	6.4 ± 3.4	1.2 ± 1.6
6	6.07 ± 0.07	10.9 ± 6.4	0.3 ± 0.2	nq
9	9.02 ± 0.06	0.6 ± 0.7	0.2 ± 0.03	1.0 ± 1.3
12	12.06 ± 0.08	nq	nq	0.6 ± 0.4
24	24.15 ± 0.14	nq	nq	nq
		1000 mg/kg Dose Group		
1	1.02 ± 0.12	605.5 ± 98.3	300.6 ± 107.9	5.3 ± 1.9
3	3.12 ± 0.10	290.9 ± 22.3	748.6 ± 257.0	5.5 ± 2.0
6	6.19 ± 0.30	62.9 ± 35.0	760.8 ± 133.5	12.8 ± 7.5
9	9.13 ± 0.25	13.9 ± 9.4	437.5 ± 133.1	4.2 ± 1.6
12	11.98 ± 0.07	nq	65.3 ± 93.0	2.9 ± 2.6
24	23.89 ± 0.14	nq	nq	5.8 ± 9.5

[a]Not quantifiable at a limit of quantitation of 0.24 μg EG/g of kidney.
[b]Not quantifiable at a limit of quantitation of 0.46 μg GA/g of kidney.
[c]Not quantifiable at a limit of quantitation of 0.44 μg OX/g of kidney.

Table 6. Oral Gavage Study: Cumulative Amounts of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) Excreted in the Urine.

Collection Interval (hr)	EG (mg)	EG (% of Dose)	GA (mg)	GA (% of Dose)	OX (mg)	OX (% of Dose)	Total (% of Dose)
			100 mg/kg Dose Group				
0 – 12	5.37 ± 0.62	20.8 ± 2.9	0.36 ± 0.22	1.2 ± 0.7	0.37 ± 0.33	1.0 ± 0.9	22.9 ± 3.7
12 – 24	0.11 ± 0.07	0.4 ± 0.3	0.05 ± 0.06	0.2 ± 0.2	0.14 ± 0.13	0.4 ± 0.4	1.0 ± 0.8
Total	5.48 ± 0.69	21.2 ± 3.1	0.42 ± 0.22	1.3 ± 0.7	0.50 ± 0.26	1.4 ± 0.7	23.9 ± 4.2
			1000 mg/kg Dose Group				
0 – 12	58.97 ± 19.43	24.5 ± 9.6	49.84 ± 7.36	16.7 ± 2.0	0.64 ± 0.08	0.2 ± 0.04	41.3 ± 8.1
12 – 24	3.03 ± 1.62	1.3 ± 0.7	5.06 ± 2.85	1.7 ± 0.8	0.69 ± 0.52	0.2 ± 0.1	3.1 ± 1.5
Total	62.01 ± 20.89	25.8 ± 10.2	54.91 ± 8.12	18.3 ± 1.5	1.33 ± 0.45	0.4 ± 0.1	44.4 ± 8.9

Table 7. Oral Gavage Study: Pharmacokinetic Parameters

Parameter	Dose (mg/kg)	Analyte	Blood	Kidney	EEF	Embryo
AUC_{0-24} (µg/g*hr)	1000	EG	3184	1954	3950	3532
		GA	2499	6444	4437	3963
		OX	272	698	1772	43
	100	EG	234	301	159	212
		GA	34	34	70	90
		OX	124	9	445	97
$t_{1/2}$ (hr)	1000	EG	1.8	1.5	2.5	2.6
		GA	2.0	1.6	2.5	1.8
		OX[a]	nd	nd	nd	nd
	100	EG	1.6	1.5	2.5	1.1
		GA	2.3	1.9	1.6	5.1
		OX[a]	nd	nd	nd	nd

[a]Half life could not be determined.

Table 8. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Extraembryonic Fluid (EEF) and Embryos. Data are presented as the means ± standard deviations except when animal numbers reduced from three; individual animal data are presented (see footnotes).

Scheduled Sacrifice Time (hr)	EG (µg/g) [a]		GA (µg/g) [b]		OX (µg/g) [c]	
	EEF	Embryos	EEF	Embryos	EEF	Embryos
			100 mg/kg Dose Group			
1	31.8 ± 12.1	83.0 ± 24.8	18.3 ± 5.1	15.7 ± 4.4	5.0 ± 0.9	17.7 ± 9.0
3	24.3 ± 8.2	29.1 ± 15.4	13.6 ± 8.0	12.7 ± 4.8	3.6 ± 0.3	9.1 ± 1.7
6	8.2 ± 1.8	3.1 ± 4.9	1.3 ± 1.7	3.0 ± 0.3	4.7 ± 0.2	3.1 ± 0.8
9	nq	nq	0.4 ± 0.1	1.5 ± 0.3	5.4 ± 1.2	2.8 ± 1.8
12	nq	nq	0.2 ± 0.1	1.5 ± 0.8	5.6 ± 0.7	2.9 ± 0.7
24	nq	nq	nq	0.7 ± 0.1	7.1 ± 1.8	0.8 ± 0.3
			1000 mg/kg Dose Group			
1	965.2 ± 170.7	788.6 ± 37.5	171.7 ± 48.1	187.0 ± 53.9	4.8 ± 3.6	4.3 ± 5.6
3	616.6 ± 90.8	539.2 ± 52.8	510.8 ± 117.0	478.6 ± 73.3	2.1 ± 0.4	nq
6	177.4 ± 21.1	179.4 ± 23.9	505.0 ± 31.2	439.0 ± 73.3	2.0 ± 0.5	nq
9	68.1 ± 19.8	79.3 ± 20.6	285.8 ± 63.2	256.6 ± 68.4	2.2 ± 0.2	1.0 ± 0.7
12	13.4 ± 2.4	15.7 ± 6.9	62.7 ± 67.5	49.8 ± 53.6	1.7 ± 0.5	0.7 ± 0.4
24	2.1 ± 1.0	2.3 ± 1.3	4.2 ± 4.4	0.5 ± 0.1	2.1 ± 0.5	0.6 ± 0.3

[a]Not quantifiable at a limit of quantitation of 0.62 µg EG/g of conceptus.
[b]Not quantifiable at a limit of quantitation of 0.22 µg GA/g of conceptus.
[c]Not quantifiable at a limit of quantitation of 0.69 µg OX/g of conceptus.

Table 9. Dow Pump Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Blood. Data are presented as the means ± standard deviations except when animal numbers reduced from three; individual animal data are presented (see footnotes).

Scheduled Sacrifice Time (gestation day)	EG (μg/g)	GA (μg/g)	OX (μg/g)
	1000 mg/kg/day Dose Group		
11	90.2, 50.2	3.8, 6.8	nq[b]
12	2.1, 95.5[b]	4.8 ± 5.3	nq
	2000 mg/kg/day Dose Group		
11	209.7 ± 58.8	75.8 ± 35.3	1.7 ± 2.4
12	192.6 ± 43.2	88.8 ± 20.8	4.4[c]

[a]Pump failed in one animal during dosing between gd 10 and 11 (A2137).
[b] Insufficient blood volume from one animal (A2141) was available for analysis.
[c] Not quantifiable at a limit of quantitation of 0.44 μg OX/g of blood; individual samples reported if above LOQ.

Table 10. Dow Pump Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Kidneys. Data are presented as the means ± standard deviations except when animal numbers reduced from three; individual animal data are presented (see footnotes).

Scheduled Sacrifice Time (gestation day)	EG (μg/g)	GA (μg/g)	OX (μg/g)
	1000 mg/kg/day Dose Group		
11	64.9[a]	6.6, 9.1[a]	6.2, 4.9[a]
12	64.9, 66.5	6.3 ± 7.9	2.3 ± 0.4
	2000 mg/kg/day Dose Group		
11	189.9 ± 44.4	111.5 ± 65.7	3.9 ± 1.1
12	130.4 ± 34.2	108.5 ± 62.1	3.2 ± 0.6

[a]Pump failed in one animal during dosing between gd 10 and 11 (A2137); EG in the kidneys of animal A2136 were below the limit of quantitation (0.22 μg/g).

Table 11. Dow Pump Study: Cumulative Amounts of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) Excreted in the Urine between gestational days 11 and 12.

Collection Interval (hr)	EG (mg)	GA (mg)	OX (mg)
	1000 mg/kg/day Dose Group		
0 – 12	43.46 ± 6.67	1.27 ± 1.25	0.78 ± 0.73
12 – 24	46.18 ± 12.57	2.41 ± 2.60	0.82 ± 0.60
Total	89.64 ± 12.35	3.67 ± 3.83	1.59 ± 1.31
	2000 mg/kg/day Dose Group		
0 – 12	89.10 ± 16.08	8.09 ± 2.94	1.48 ± 1.02
12 – 24	102.00 ± 25.86	18.83 ± 12.43	0.65 ± 0.48
Total	191.10 ± 26.70	26.92 ± 11.73	2.13 ± 1.49

Table 12. Dow Pump Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Extraembryonic Fluid (EEF) and Embryos. Data are presented as the means ± standard deviations except when animal numbers reduced from three; individual animal data are presented (see footnotes).

Scheduled Sacrifice Time (gestation day)	EG (µg/g)		GA (µg/g)		OX (µg/g)	
	EEF	Embryos	EEF	Embryos	EEF	Embryos
			1000 mg/kg/day Dose Group			
11	110, 72.2[a]	139.8, 92.2[a]	5.9, 10.7[a]	5.1, 15.1[a]	11.3, 2.7[a]	21.2, 1.2[a]
12	78.5 ± 45.7	66.6 ± 45.2	9.7 ± 9.2	9.9 ± 10.6	0.81	0.8 ± 0.2
			2000 mg/kg/day Dose Group			
11	309.0 ± 47.2	276.8 ± 101.0	208.9 ± 83.6	178.8 ± 112.5	nq	nq
12	218.4 ± 78.0	180.3 ± 48.9	142.6 ± 9.6	141.7 ± 24.4	nq	0.6, 0.5

[a]Pump failed in one animal during dosing between gd 10 and 11 (A2137).

APPENDIX

INDIVIDUAL ANIMAL DATA

Table A-1. Oral Gavage Study: Body Weights, Sacrifice Times and Amounts of Ethylene Glycol Administered.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. (hr)	Act. Sac. (hr)	Body Wt. (kg)	Total Dose Soln. Admin. (g)	Total Dose of Ethylene Glycol Administered[a] (mg)	(mg/kg)	% of Target
100	401	1	0.97	0.2652	1.3218	26.78	101.0	101.0
	402	1	1.03	0.2809	1.4216	28.81	102.5	102.5
	403	1	1.10	0.2691	1.3138	26.62	98.9	98.9
	420[b]	3	3.07	0.2538	1.2546	25.42	100.2	100.2
	405	3	3.07	0.2616	1.3295	26.94	103.0	103.0
	406	3	3.15	0.2677	1.353	27.42	102.4	102.4
	407	6	6.00	0.2967	1.4538	29.46	99.3	99.3
	408	6	6.07	0.2825	1.4288	28.95	102.5	102.5
	409	6	6.13	0.2493	1.2778	25.89	103.9	103.9
	410	9	8.97	0.2601	1.3238	26.82	103.1	103.1
	419[c]	9	9.00	0.2483	1.357	27.50	110.7	110.7
	412	9	9.08	0.2823	1.4238	28.85	102.2	102.2
	413	12	11.97	0.2863	1.4282	28.94	101.1	101.1
	414	12	12.07	0.2583	1.258	25.49	98.7	98.7
	415	12	12.13	0.269	1.3165	26.68	99.2	99.2
	416	24	24.03	0.2542	1.2595	25.52	100.4	100.4
	417	24	24.13	0.2899	1.339	27.13	93.6	93.6
	418	24	24.30	0.2494	1.2377	25.08	100.6	100.6

[a]Total dose administered based upon GC/FID analysis of dosing solution and total amount of dose solution delivered.
[b] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[c] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-1 (continued). Oral Gavage Study: Body Weights, Sacrifice Times and Amounts of Ethylene Glycol Administered.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. (hr)	Act. Sac. (hr)	Body Wt. (kg)	Total Dose Soln. Admin. (g)	Total Dose of Ethylene Glycol Administered[a] (mg)	(mg/kg)	% of Target
1000	301	1	0.90	0.2554	1.3028	256.19	1003.1	100.3
	302	1	1.02	0.2658	1.3701	269.43	1013.6	101.4
	303	1	1.13	0.2743	1.3588	267.20	974.1	97.4
	304	3	3.02	0.2687	1.3595	267.34	994.9	99.5
	305	3	3.13	0.2555	1.2894	253.56	992.4	99.2
	306	3	3.22	0.2436	1.2733	250.39	1027.9	102.8
	307	6	5.92	0.2626	1.329	261.34	995.2	99.5
	320[d]	6	6.52	0.2295	1.1592	227.95	993.3	99.3
	309	6	6.13	0.259	1.3037	256.37	989.8	99.0
	310	9	8.92	0.2839	1.4616	287.42	1012.4	101.2
	319[e]	9	9.40	0.2112	1.0482	206.13	976.0	97.6
	312	9	9.07	0.2379	1.1749	231.04	971.2	97.1
	313	12	11.92	0.2694	1.3411	263.72	978.9	97.9
	314	12	11.98	0.2633	1.3482	265.12	1006.9	100.7
	315	12	12.05	0.2433	1.2521	246.22	1012.0	101.2
	316	24	23.78	0.2755	1.3836	272.08	987.6	98.8
	317	24	23.85	0.2369	1.1865	233.32	984.9	98.5
	318	24	24.05	0.2237	1.1586	227.84	1018.5	101.9

[d] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[e] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-2. Oral Gavage Study: Terminal Body and Organ Weights.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. (hr)	Terminal BW (g)	Kidney Wt. (g)	Rel. Kidney Wt. (g/100 g BW)	Uterine Wt. (g)	Rel. Uterine Wt. (g/100 g BW)	No. Implants
100	401	1	256.3	2.0309	0.792	3.745	1.461	13
	402	1	271.7	2.2395	0.824	3.1047	1.143	11
	403	1	263.3	2.2053	0.838	3.02	1.147	13
	420[a]	3	246.9	1.87	0.757	3.372	1.366	13
	405	3	253.7	1.6552	0.652	3.4875	1.375	14
	406	3	258.2	1.7933	0.695	2.6745	1.036	11
	407	6	285.2	1.995	0.700	3.8488	1.350	18
	408	6	273.6	1.9385	0.709	3.6945	1.350	14
	409	6	240.9	1.4553	0.604	2.6	1.079	9
	410	9	259.5	1.9916	0.767	4.36	1.680	13
	419[b]	9	247.4	1.7229	0.696	3.5167	1.421	13
	412	9	270.5	2.1336	0.789	3.427	1.267	14
	413	12	283	2.3073	0.815	4.4486	1.572	15
	414	12	255.8	1.968	0.769	3.0058	1.175	10
	415	12	268.2	2.2811	0.851	5.0475	1.882	15
	416	24	270.1	2.0258	0.750	4.6508	1.722	12
	417	24	309.8	2.0636	0.666	6.0735	1.960	15
	418	24	270.1	2.1957	0.813	4.9283	1.825	13

[a] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[b] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-2 (continued). Oral Gavage Study: Terminal Body and Organ Weights.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. (hr)	Terminal BW (g)	Kidney Wt. (g)	Rel. Kidney Wt. (g/100 g BW)	Uterine Wt. (g)	Rel. Uterine Wt. (g/100 g BW)	No. Implants
1000	301	1	245.32	1.8694	0.762	2.1361	0.871	8
	302	1	257.72	2.0815	0.808	3.7913	1.471	14
	303	1	265.47	1.8357	0.691	3.0665	1.155	15
	304	3	253.4	1.7819	0.703	3.356	1.324	12
	305	3	239.73	1.6092	0.671	3.0355	1.266	11
	306	3	228.03	1.7586	0.771	3.282	1.439	13
	307	6	249.16	2.098	0.842	4.5115	1.811	16
	320 [c]	6	216.75	2.0831	0.961	3.8731	1.787	12
	309	6	245.64	1.8092	0.737	2.6178	1.066	10
	310	9	267.6	1.7995	0.672	4.539	1.696	15
	319 [d]	9	204.77	1.7595	0.859	2.6989	1.318	9
	312	9	231.85	1.8896	0.815	4.1482	1.789	14
	313	12	273.25	2.1149	0.774	4.3405	1.588	15
	314	12	263.91	2.4316	0.921	4.4304	1.679	16
	315	12	244.09	2.093	0.857	3.562	1.459	13
	316	24	289	2.4077	0.833	6.4746	2.240	15
	317	24	247.28	2.083	0.842	5.076	2.053	14
	318	24	232.61	2.057	0.884	4.243	1.824	12

[c] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[d] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-3. Oral Gavage Study: Extraembryonic Fluid and Embryo Weights.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. (hr)	No. Implants	Total EEF (g)	Rel. EEF Wt. (g/100 g BW)	Embryo Wt. (g)	Rel. Embryo Wt. (g/100 g BW)
100	401	1	13	0.1978	0.0772	0.0683	0.0266
	402	1	11	0.0324	0.0119	0.0263	0.0097
	403	1	13	0.1101	0.0418	0.0553	0.0210
	420[a]	3	13	0.0818	0.0331	0.0696	0.0282
	405	3	14	0.1624	0.0640	0.0577	0.0227
	406	3	11	0.0554	0.0215	0.0228	0.0088
	407	6	18	0.2011	0.0705	0.1247	0.0437
	408	6	14	0.0708	0.0259	0.0702	0.0257
	409	6	9	0.1072	0.0445	0.065	0.0270
	410	9	13	0.2454	0.0946	0.1662	0.0640
	419[b]	9	13	0.1584	0.0640	0.1194	0.0483
	412	9	14	0.1954	0.0722	0.086	0.0318
	413	12	15	0.2208	0.0780	0.1823	0.0644
	414	12	10	0.2128	0.0832	0.039	0.0152
	415	12	15	0.2147	0.0801	0.1795	0.0669
	416	24	12	0.3102	0.1148	0.2803	0.1038
	417	24	15	0.1074	0.0347	0.4524	0.1460
	418	24	13	0.3636	0.1346	0.392	0.1451

[a] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[b] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-3 (continued). Oral Gavage Study: Extraembryonic Fluid and Embryo Weights.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. (hr)	No. Implants	Total EEF (g)	Rel. EEF Wt. (g/100 g BW)	Embryo Wt. (g)	Rel. Embryo Wt. (g/100 g BW)
1000	301	1	8	0.028	0.0114	0.0467	0.0190
	302	1	14	0.1273	0.0494	0.0693	0.0269
	303	1	15	0.0972	0.0366	0.0404	0.0152
	304	3	12	0.1503	0.0593	0.0951	0.0375
	305	3	11	0.2172	0.0906	0.0812	0.0339
	306	3	13	0.0617	0.0271	0.0638	0.0280
	307	6	16	0.1853	0.0744	0.1031	0.0414
	320[c]	6	12	0.073	0.0337	0.0826	0.0381
	309	6	10	0.0849	0.0346	0.0897	0.0365
	310	9	15	0.1102	0.0412	0.1823	0.0681
	319[d]	9	9	0.1395	0.0681	0.1103	0.0539
	312	9	14	0.1041	0.0449	0.1734	0.0748
	313	12	15	0.2206	0.0807	0.1575	0.0576
	314	12	16	0.3238	0.1227	0.1624	0.0615
	315	12	13	0.1281	0.0525	0.1434	0.0587
	316	24	15	0.1786	0.0618	0.2797	0.0968
	317	24	14	0.2643	0.1069	0.3967	0.1604
	318	24	12	0.2238	0.0962	0.3124	0.1343

[c] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[d] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-4. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Blood.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (µg/g)	EG Mean ± SD	GA (µg/g)	GA Mean ± SD	OX (µg/g)	OX Mean ± SD
100	401	1	103.4	76.9 ± 24.1	4.7	7.6 ± 2.9	6.7	4.1 ± 2.3
	402	1	56.3		10.4		2.5	
	403	1	70.9		7.6		3.1	
	420[a]	3	27.6	33.8 ± 6.0	8.1	5.5 ± 2.4	nq	1.1 ± 0.8[b]
	405	3	34.2		4.9		1.8	
	406	3	39.6		3.5		1.3	
	407	6	20.3	8.4 ± 10.3	1.1	0.8 ± 0.3	1.3	0.6 ± 0.6[b]
	408	6	3.1		0.7		nq	
	409	6	1.9		0.5		0.4	
	410	9	nq	0.2 ± 0.1[b]	0.9	0.7 ± 0.2	1.1	2.2 ± 2.3[b]
	419[c]	9	0.3		0.5		nq	
	412	9	0.3		0.8		4.8	
	413	12	nq		nq		2.9	1.9 ± 0.9
	414	12	nq		nq		1.2	
	415	12	nq		nq		1.5	
	416	24	nq		nq		1.6	1.6 ± 0.2
	417	24	nq		nq		1.4	
	418	24	nq		nq		1.7	

[a] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[b] Not quantifiable data estimated as LOQ/2 in calculation of mean ± sd when other samples within group were above LOQ.
[c] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-4 (continued). Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Blood.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (µg/g)	EG Mean ± SD	GA (µg/g)	GA Mean ± SD	OX (µg/g)	OX Mean ± SD
Control			nq		nq		10.4	
1000	301	1	749.7	745.8 ± 84.9	130.4	124.8 ± 22.8	8.5	5.7 ± 2.5
	302	1	659.0		99.7		4.6	
	303	1	828.6		144.2		3.9	
	304	3	427.5	473.1 ± 65.9	302.9	314.4 ± 48.8	3.8	3.8 ± 0.4
	305	3	548.7		272.3		3.4	
	306	3	443.1		367.9		4.3	
	307	6	132.3	173.1 ± 36.9	340.8	310.0 ± 27.4	3.9	3.9 ± 0.1
	320[c]	6	183.0		288.6		3.8	
	309	6	204.0		300.5		4.0	
	310	9	75.2	70.6 ± 25.8	136.1	139.9 ± 50.1	3.0	4.6 ± 2.1
	319[d]	9	93.8		191.8		3.7	
	312	9	42.8		91.8		6.9	
	313	12	13.1	9.5 ± 3.1	50.6	20.3 ± 26.2	5.5	4.5 ± 1.0
	314	12	7.8		4.1		4.6	
	315	12	7.6		6.3		3.5	
	316	24	nq		nq	0.6 ± 0.6[b]	3.6	3.1 ± 0.6
	317	24	nq		1.4		3.3	
	318	24	nq		nq		2.4	

[c] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[d] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-5. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Kidneys.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (µg/g)	EG Mean ± SD	GA (µg/g)	GA Mean ± SD	OX (µg/g)	OX Mean ± SD
100	401	1	86.7	88.8 ± 12.6	5.1	9.5 ± 4.3	nq	0.5 ± 0.3[a]
	402	1	102.3		13.6		0.7	
	403	1	77.4		9.7		nq	
	420[b]	3	39. 8	43.5 ± 5.0	10.3	6.4 ± 3.4	3.1	1.2 ± 1.6
	405	3	49.1		5.1		nq	
	406	3	41.5		3.9		0.3	
	407	6	18.1	10.9 ± 6.4	0.6	0.3 ± 0.2	nq	
	408	6	8.6		nq		nq	
	409	6	5.9		nq		nq	
	410	9	nq	0.6 ± 0.7[a]	nq		nq	1.0 ± 1.3[a]
	419[c]	9	nq		nq		nq	
	412	9	1.3		nq		2.5	
	413	12	nq		nq		nq	0.6 ± 0.4[a]
	414	12	nq		nq		1.0	
	415	12	nq		nq		0.7	
	416	24	nq		nq		nq	
	417	24	nq		nq		nq	
	418	24	nq		nq		nq	

[a] Not quantifiable data estimated as LOQ/2 in calculation of mean ± sd when other samples within group were >LOQ.
[b] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[c] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-5 (continued). Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Kidneys.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (μg/g)	EG Mean ± SD	GA (μg/g)	GA Mean ± SD	OX (μg/g)	OX Mean ± SD
1000	301	1	704.4	605.5 ± 98.3	326.2	300.6 ± 107.9	7.4	5.3 ± 1.9
	302	1	507.8		182.2		5.1	
	303	1	604.3		393.5		3.5	
	304	3	316.8	290.9 ± 22.3	823.8	748.6 ± 257.0	6.8	5.5 ± 2.0
	305	3	278.4		462.4		3.2	
	306	3	277.7		959.5		6.5	
	307	6	58.4	62.9 ± 35.0	891.7	760.8 ± 133.5	13.9	12.8 ± 7.5
	320[c]	6	30.3		624.7		4.9	
	309	6	99.8		766.1		19.7	
	310	9	24.7	13.9 ± 9.4	510.2	437.5 ± 133.1	5.8	4.2 ± 1.6
	319[d]	9	7.8		518.5		4.3	
	312	9	9.1		283.9		2.5	
	313	12	nd		172.7	65.3 ± 93.0	5.8	2.9 ± 2.6
	314	12	nd		9.1		1.7	
	315	12	nd		14.1		1.0	
	316	24	nd		nd		16.7	5.8 ± 9.5[a]
	317	24	nd		nd		nd	
	318	24	nd		nd		0.1	

[c] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[d] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-6. Oral Gavage Study: Cumulative Amounts of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) Excreted in the Urine.

Dose Level (mg/kg)	Animal ID No.	Collection Interval (hr)	EG (mg)	GA (mg)	OX (mg)	% of Dose
100	416	0 – 12	6.079	0.377	0.288	25.8
		12 – 24	0.190	0.123	0.261	1.8
		Total	6.269	0.500	0.549	27.7
	417	0 – 12	4.914	0.137	0.083	25.8
		12 – 24	0.066	0.034	0.145	1.8
		Total	4.980	0.171	0.228	27.7
	418	0 – 12	5.106	0.578	0.727	24.2
		12 – 24	0.082	ND	ND	0.3
		Total	5.188	0.578	0.727	24.6

Table A-6 (continued). Oral Gavage Study: Cumulative Amounts of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) Excreted in the Urine.

Dose Level (mg/kg)	Animal ID No.	Collection Interval (hr)	EG (mg)	GA (mg)	OX (mg)	% of Dose
1000	316	0 – 12	51.153	54.56	0.567	35.3
		12 – 24	3.225	7.95	1.239	3.9
		Total	54.378	62.51	1.806	39.2
	317	0 – 12	44.68	53.61	0.718	38.1
		12 – 24	1.32	2.25	0.194	1.4
		Total	46.00	55.86	0.912	39.5
	318	0 – 12	81.093	41.364	0.631	50.6
		12 – 24	4.549	4.985	0.645	4.0
		Total	85.642	46.349	1.276	54.6

Table A-7. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Extraembryonic Fluid (EEF).

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (µg/g)	EG Mean ± SD	GA (µg/g)	GA Mean ± SD	OX (µg/g)	OX Mean ± SD
100	401	1	18.9	31.8 ± 12.1	13.3	18.3 ± 5.1	4.6	5.0 ± 0.9
	402	1	42.9		18.3		6.1	
	403	1	33.5		23.5		4.4	
	420[a]	3	33.8	24.3 ± 8.2	21.8	13.6 ± 8.0	3.2	3.6 ± 0.3
	405	3	19.8		13.3		3.8	
	406	3	19.4		5.7		3.7	
	407	6	7.2	8.2 ± 1.8	3.2	1.3 ± 1.7[b]	4.4	4.7 ± 0.2
	408	6	10.3		nq		4.9	
	409	6	7.1		nq		4.7	
	410	9	3.4	3.9 ± 0.5	nq		5.2	5.4 ± 1.2
	419[c]	9	4.3		nq		6.7	
	412	9	4.2		nq		4.4	
	413	12	3.3	3.4 ± 0.3	nq		6.3	5.6 ± 0.7
	414	12	3.7		nq		4.9	
	415	12	3.2		nq		5.8	
	416	24	2.2	3.1 ± 1.7	nq		5.3	7.1 ± 1.8
	417	24	5.2		nq		8.9	
	418	24	2.1		nq		7.3	

[a] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[b] Not quantifiable data estimated as LOQ/2 in calculation of mean ± sd when other samples within group were >LOQ.
[c] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-7 (continued). Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Extraembryonic Fluid (EEF).

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (μg/g)	EG Mean ± SD	GA (μg/g)	GA Mean ± SD	OX (μg/g)	OX Mean ± SD
1000	301	1	1155.2	965.2 ± 170.7	166.5	171.7 ± 48.1	8.9	4.8 ± 3.6
	302	1	915.9		126.4		3.6	
	303	1	824.7		222.1		1.9	
	304	3	647.7	616.6 ± 90.8	501.0	510.8 ± 117.0	1.9	2.1 ± 0.4
	305	3	687.8		399.0		1.8	
	306	3	514.3		632.3		2.6	
	307	6	153.8	177.4 ± 21.1	510.4	505.0 ± 31.2	1.7	2.0 ± 0.5
	320[c]	6	183.9		471.5		1.8	
	309	6	194.6		533.2		2.6	
	310	9	69.1	68.1 ± 19.8	281.3	285.8 ± 63.2	2.2	2.2 ± 0.2
	319[d]	9	87.3		351.2		2.3	
	312	9	47.7		225.0		2.0	
	313	12	14.8	13.4 ± 2.4	140.4	62.7 ± 67.5	1.5	1.7 ± 0.5
	314	12	10.6		18.1		1.4	
	315	12	14.6		29.7		2.3	
	316	24	2.8	2.1 ± 1.0	9.2	4.2 ± 4.4	2.3	2.1 ± 0.5
	317	24	2.6		2.4		2.4	
	318	24	1.0		0.9		1.5	

[c] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[d] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-8. Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Embryos.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (µg/g)	EG Mean ± SD	GA (µg/g)	GA Mean ± SD	OX (µg/g)	OX Mean ± SD
100	401	1	67.8	83.0 ± 24.8	10.7	15.7 ± 4.4	16.9	17.7 ± 9.0
	402	1	111.7		17.6		27.2	
	403	1	69.6		18.8		9.1	
	420[a]	3	27.9	29.1 ± 15.4	18.0	12.7 ± 4.8	nq	6.1 ± 5.3[b]
	405	3	45.0		11.7		10.3	
	406	3	14.3		8.5		7.9	
	407	6	8.8	3.1 ± 4.9[b]	3.2	3.0 ± 0.3	3.0	3.1 ± 0.8
	408	6	nq		3.1		4.0	
	409	6	nq		2.6		2.4	
	410	9	nq		1.1	1.5 ± 0.3	2.3	2.8 ± 1.8
	419[c]	9	nq		1.8		1.2	
	412	9	nq		1.6		4.8	
	413	12	nq		0.9	1.5 ± 0.8	3.6	2.9 ± 0.7
	414	12	nq		2.4		2.1	
	415	12	nq		1.2		3.1	
	416	24	nq		0.8	0.7 ± 0.1	1.1	0.8 ± 0.3
	417	24	nq		0.6		0.9	
	418	24	nq		0.6		0.5	

[a] Animal no. 420 replaced animal no. 404 which was not pregnant at time of sacrifice.
[b] Not quantifiable data estimated as LOQ/2 in calculation of mean ± sd when other samples within group were >LOQ.
[c] Animal no. 419 replaced animal no. 411 which refluxed part of dose.

Table A-8 (continued). Oral Gavage Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Embryos.

Dose Level (mg/kg)	Animal ID No.	Sched. Sac. Time (hr)	EG (µg/g)	EG Mean ± SD	GA (µg/g)	GA Mean ± SD	OX (µg/g)	OX Mean ± SD
1000	301	1	765.1	788.6 ± 37.5	179.3	187.0 ± 53.9	10.7	4.3 ± 5.6[b]
	302	1	768.8		137.3		1.9	
	303	1	831.9		244.3		nq	
	304	3	600.1	539.2 ± 52.8	462.2	478.6 ± 73.3	nq	
	305	3	511.5		414.9		nq	
	306	3	506.0		558.7		nq	
	307	6	153.9	179.4 ± 23.9	486.7	439.0 ± 73.3	nq	
	320[c]	6	182.9		354.6		nq	
	309	6	201.4		475.5		nq	
	310	9	83.8	79.3 ± 20.6	277.0	256.6 ± 68.4	nq	1.0 ± 0.7[b]
	319[d]	9	97.2		312.4		1.8	
	312	9	56.8		180.3		0.9	
	313	12	22.8	15.7 ± 6.9	111.4	49.8 ± 53.6	0.4	0.7 ± 0.4[b]
	314	12	15.2		13.3		1.1	
	315	12	9.1		24.9		0.5	
	316	24	1.1	2.3 ± 1.3	0.5	0.5 ± 0.1	0.8	0.6 ± 0.3[b]
	317	24	2.3		0.6		0.7	
	318	24	3.6		0.5		nq	

[c] Animal no. 320 replaced animal no. 308 which refluxed part of dose.
[d] Animal no. 319 replaced animal no. 311 which refluxed part of dose.

Table A-9. Dow Pump Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Blood.

Dose (mg/kg/day)	Animal ID No.	Sacrifice Time (gestation day)	EG (μg/g)	GA (μg/g)	OX (μg/g)
1000	A2135	11	90.2	3.8	nd
	A2136	11	50.2	6.8	nd
	A2137	11	Pump Failed	Pump Failed	Pump Failed
	Mean ± SD		70.2	5.3	
	A2138	12	2.1	1.5	nd
	A2139	12	95.5	11.0	nd
	A2141	12	NA[a]	1.9	nd
	Mean ± SD		48.8	4.8 ± 5.3	
2000	A2142	11	277.4	116.6	0.3
	A2143	11	171.9	55.8	4.5
	A2144	11	179.7	55.1	0.2
	Mean ± SD		209.7 ± 58.8	75.8 ± 35.3	1.7 ± 2.4
	A2145	12	154.1	77.4	nd
	A2146	12	183.4	112.8	nd
	A2147	12	239.2	76.2	4.4
	Mean ± SD		192.3 ± 43.2	88.8 ± 20.8	

[a]NA=Not Analyzed; insufficient sample.

Table A-10. Dow Pump Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Kidneys.

Dose (mg/kg/day)	Animal ID No.	Sacrifice Time (gestation day)	EG (μg/g)	GA (μg/g)	OX (μg/g)
1000	A2135	11	64.9	6.6	6.2
	A2136	11	nd	9.1	4.9
	A2137	11	Pump Failed	Pump Failed	Pump Failed
	Mean ± SD		64.9	7.9	5.6
	A2138	12	nd	0.4	2.0
	A2139	12	64.9	15.4	2.6
	A2141	12	66.5	3.2	2.2
	Mean ± SD		65.7	6.3 ± 7.9	2.3 ± 0.4
2000	A2142	11	194.7	187.4	5.1
	A2143	11	143.2	72.4	2.8
	A2144	11	231.7	74.8	3.7
	Mean ± SD		189.9 ± 44.4	111.5 ± 65.7	3.9 ± 1.1
	A2145	12	115.1	71.8	3.2
	A2146	12	106.6	180.2	3.8
	A2147	12	169.6	73.4	2.6
	Mean ± SD		130.2 ± 34.2	108.5 ± 62.1	3.2 ± 0.6

Table A-11. Dow Pump Study: Cumulative Amounts of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) Excreted in the Urine Between Gestational Days 11 and 12.

Dose Level (mg/kg)	Animal ID No.	Collection Interval (hr)	EG (mg)	GA (mg)	OX (mg)
1000	A2138	0 – 12	35.758	0.419	0.435
		12 – 24	50.163	1.181	0.75
		Total	85.921	1.600	1.185
	A2139	0 – 12	47.143	2.697	1.617
		12 – 24	56.281	5.399	1.448
		Total	103.424	8.096	3.065
	A2141	0 – 12	47.481	0.681	0.282
		12 – 24	32.106	0.643	0.251
		Total	79.587	1.324	0.533
2000	A2145	0 – 12	90.591	11.478	0.371
		12 – 24	72.627	14.456	0.180
		Total	163.218	25.934	0.551
	A2146	0 – 12	72.327	6.249	2.372
		12 – 24	121.321	32.856	1.148
		Total	193.648	39.105	3.52
	A2147	0 – 12	104.380	6.542	1.688
		12 – 24	112.065	9.174	0.628
		Total	216.445	15.716	2.316

Table A-12. Dow Pump Study: Concentrations of Ethylene Glycol (EG), Glycolic Acid (GA) and Oxalic Acid (OX) in Extraembryonic Fluid (EEF) and Embryos.

Dose (mg/kg/day)	Animal ID No.	Sacrifice Time (gestation day)	EG (µg/g)		GA (µg/g)		OX (µg/g)	
			EEF	Embryos	EEF	Embryos	EEF	Embryos
1000	A2135	11	110.0	139.8	5.9	5.1	11.3	21.2
	A2136	11	72.2	92.2	10.7	15.1	2.7	1.2
	A2137	11	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]
			91.1	116.0	8.3	10.1	7.0	11.2
	A2138	12	29.0	18.5	3.5	2.8	0.8	1.1
	A2139	12	119.2	108.3	20.2	22.1	0.3	0.7
	A2141	12	87.2	73.1	5.3	4.8	0.4	0.7
			78.5 ± 45.7	66.6 ± 45.2	9.7 ± 9.2	9.9 ± 10.6	0.5 ± 0.3	0.8 ± 0.2
2000	A2142	11	296.3	366.6	304.5	307.5	nq	nq
	A2143	11	269.4	167.5	172.4	129.5	nq	nq
	A2144	11	361.2	296.2	149.7	99.2	nq	nq
			309.0 ± 47.2	276.8 ± 101.0	208.9 ± 83.6	178.8 ± 112.5		
	A2145	12	175.1	155.8	136.2	127.3	nq	0.57
	A2146	12	171.6	148.5	153.6	169.8	nq	0.49
	A2147	12	308.4	236.6	137.9	128.0	nq	nq
			218.4 ± 78.0	180.3 ± 48.9	142.6 ± 9.6	141.7 ± 24.4		0.53

[a]NA=Not Analyzed; Pump failed.

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CASE REPORT

The Role of Calcium Oxalate Crystal Deposition in Cerebral Vessels During Ethylene Glycol Poisoning

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Ethylene glycol (EG) poisoning can lead to serious morbidity or death, which occurs following conversion of ethylene glycol to toxic metabolites. These metabolites affect multiple organ/systems leading to metabolic acidosis, cardiopulmonary depression, acute renal failure and central nervous system deficits. Treatment consists of correcting metabolic acidosis with bicarbonate administration, dialysis to remove toxic metabolites and administration of fomepizole or ethanol to prevent conversion of EG to toxic intermediates. Occasionally in the literature, fatal cases of EG poisoning have been described in which calcium oxalate crystal deposition has occurred in the walls of CNS vessels, sometimes with associated neuropathy. We describe a case of fatal EG poisoning in which the development of rapid cerebral edema was documented by CT scan and was accompanied by definitive evidence of birefringent crystals within walls of CNS blood vessels, with associated inflammation and edema. This case and others in the literature suggest that cerebral edema, and perhaps injury to other organs, could result from oxalate crystal deposition in small blood vessels in the brain and other organs.

INTRODUCTION

Ethylene glycol (EG) poisoning may cause significant morbidity or mortality through the toxic effects of its active metabolites (1,2). Although the parent compound is essentially nontoxic, EG is converted through the action of alcohol dehydrogenase to several metabolites that are believed to be responsible for most of the clinical manifestations, which include metabolic acidosis, neurological deficits, cardiopulmonary symptoms, renal failure, hypocalcemia and hyperoxaluria. For this reason, EG poisoning has been treated primarily with attempts to block the formation of these metabolites by alcohol dehydrogenase, either by the administration of the co-substrate ethanol or the competitive inhibitor fomepizole (4-methylpyrazole) (2–5). Hemodialysis is usually performed in cases of EG poisoning in an attempt to remove EG and toxic metabolites from the bloodstream (6). Once substantial amounts of EG are metabolized, however, the efficacy of metabolic blockade as a treatment strategy would likely be greatly diminished. Because deposition of oxalate crystals has been observed in various tissues at post mortem examination of patients who died from EG poisoning, the possible role of oxalate crystal-induced tissue damage in the pathogenesis of EG poisoning has been debated in the literature (2,7). Thus development of alternative treatment strategies aimed at potential oxalate toxicity may be useful in patients where EG metabolism is well underway. We describe a case of fatal ethylene glycol intoxication in which oxalate crystal formation was found within the walls of cerebral vessels at post mortem examination, and discuss the significance of this finding to the pathogenesis and treatment of EG poisoning.

CASE REPORT

A 25-year-old male was found unresponsive at home by his spouse at 17:00. At the scene, the patient was given oxygen (100% by a non-rebreather mask, obtained 96–98% saturation) and intravenous fluids were started. Upon admission to the Emergency Department at 17:45, he appeared comatose with a temperature of 37°C, respiratory rate of 30/min, pulse rate of 116 beats/min, and blood pressure of 148/95 mm Hg. Pupillary light reflexes were sluggish. Measurement of arterial blood

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gases revealed the following: PO_2, 202.7 mm Hg; PCO_2, 10.4 mm Hg; bicarbonate, 2.9 mmol/L and pH, 7.059, with a base excess of −24.5 mmol/L. The total white cell count was 29,000/mm^3 with a normal differential. Hemoglobin level was 143 g/L with a hematocrit of 42.8%. Serum analysis yielded a serum sodium of 139 mmol/L; potassium of 6.9 mmol/L, chloride of 107 mmol/L, BUN of 2.856 mmol/L, creatinine of 159 µmol/L and glucose of 234 mg/L. The anion gap was 36 mmol/L. Serum lactate level was 1.86 mmol/L, while liver transaminases were within normal limits. Bicarbonate (2 ampules at 18:00 and 2 at 19:15) was given to correct the metabolic acidosis. A urine toxicology screen was negative for drugs of abuse and blood ethanol was negative. The patient was intubated, given a slurry of charcoal orally, while naloxone and 50% dextrose were administered intravenously. An initial head CT scan showed a calcified lesion within an atrophic left cerebellum. Blood, urine and tracheal aspirates were cultured for routine pathogens and were negative at 24 hours. Latex agglutination studies and cultures of CSF were negative, although the CSF showed a pleocytosis of polymorphonuclear leukocytes. Shortly after admission to the Intensive Care Unit at 22:15, the patient developed generalized seizures which were refractory to diazepam and phenytoin. A repeat head CT scan, eight hours after the initial head CT, demonstrated generalized cerebral edema. An EEG showed severe depression of electrical activity. Toxicology screens were negative for salicylates and methanol. Further history obtained from the patient's family indicated that one gallon of ethylene glycol-based antifreeze had been obtained by the patient at about 16 hours prior to admission. 225 cc of antifreeze were missing from the antifreeze container. The patient expired within 24 hours of admission, and blood obtained at that time contained 55 mg/dL (8.9 mmol/L) of ethylene glycol with urine levels of 2.6 mg/ml (42 mmol/L). Assays for oxalic acid were performed at a reference laboratory by gas chromatographic techniques and showed serum levels of 0.36 µmol/L and urine levels of 22 µmol/L. An autopsy was performed.

AUTOPSY FINDINGS

Post mortem examination performed within 18 hours of death supported the clinical impression of EG poisoning. Microscopic examination of the kidneys showed changes of acute tubular necrosis with numerous birefringent crystals consistent with oxalate present within the renal tubules. Sections of the brain demonstrated small blood vessels containing birefringent crystals within vessel walls (Fig. 1A). There was prominent perivascular edema and collections of polymorphonuclear leukocytes adjacent to these vessels (Fig. 1B). Sections of the meninges also showed a neutrophilic infiltrate. Sections of the left cerebellum showed an atrophic lobe containing a calcified arteriovenous malformation.

DISCUSSION

The major clinical manifestations of EG poisoning include metabolic acidosis, CNS alterations and acute renal failure. Data from an epidemic of EG poisonings in Sweden found that metabolic acidosis was present in 86% of patients, 75% had CNS manifestations and 67% had acute renal failure (8). Experimental and human studies suggested that these sequelae were due to the action of active metabolites including glycolate, glyoxalate and oxalate (6,7,9). Administration of ethanol prevents the conversion of EG to glycolaldehyde and downstream metabolites, but is in itself intoxicating and requires careful clinical monitoring. Recently, fomepizole has been shown to be a safe and effective treatment for EG, as well as methanol poisoning (3,10). The reported half-life of EG based on serial blood and urine levels of EG from an untreated patient, indicated the t1/2 to be approximately three hours (5),

FIG. 1. A. Section of the cerebrum showing birefringent COM crystals (arrows) within the walls of several small blood vessels. There is prominent perivascular edema and inflammation (hematoxylin & eosin, original magnification × 100). B. CNS blood vessel containing COM crystals. Layers of the vessel wall are separated (arrowheads) by intervening leukocytes and COM crystals (hematoxylin & eosin, original magnification × 200).

suggesting that ethanol or fomepizole therapy needs to be started soon after EG is absorbed in order to be effective.

EG poisoning is often characterized as including three stages(11): an initial CNS depression within the first 6 hours of ingestion; a cardiopulmonary stage from 6–24 hours after ingestion that includes hypertension, tachycardia, pulmonary edema and eventually cardiac failure; and an acute renal failure that becomes manifest about 24–48 hours after ingestion. In many cases, including the present case, these stages are indistinct, with the initial CNS depression progressing to the level of coma by 24–48 hours after ingestion. The late-developing CNS depression is probably caused by the developing brain edema rather than by the ethylene glycol itself (12). Autopsy investigations have shown edema, hemorrhage and perivascular inflammation adjacent to small blood vessels in the brain, lungs and heart, suggesting exudative damage to the endothelial cells in these vessels (11,13–15). The mechanism for this toxic effect on the endothelium has not been determined for EG poisoning.

The historical explanation for the CNS and the cardiopulmonary damage has been the increased levels of the aldehyde metabolites of EG (glycolaldehyde and glyoxylic acid), because of their high reactivity and potential cytotoxicity (16,17). However, plasma glyoxylate accumulation in EG-poisoned humans and animals is minor (<0.2 mmol/L) (6,18), and glycolaldehyde has also not been detected in these situations. Furthermore, these metabolites would be expected to accumulate during the early hours after EG ingestion, since the elimination of EG through its metabolism is rapid (5). Because the brain edema is a late phenomenon, with coma ensuing after most of the EG has been metabolized (24–48 hours), it is more likely to result from another metabolite.

Glycolic acid accumulates to very high levels in most cases of EG intoxication, up to 20–25 mmol/L, and is responsible for the severe metabolic acidosis (6,18). Accumulation of glycolate ion is not likely to be responsible for the tissue damage in the vascular endothelium, because glycolate is not cytotoxic (17,19). In this patient, the initial CT scan, which did not show brain edema, was performed at a time of apparent high accumulation of glycolic acid (evidenced by a severe acidosis with markedly elevated anion gap). The second CT scan showed a subsequent development of brain edema, suggesting involvement of another factor than glycolate accumulation.

Accumulation of oxalic acid as calcium oxalate monohydrate (COM) crystals in the kidney tissue is most likely responsible for the renal cell necrosis that is linked with the acute renal failure. Concentrations of oxalate or COM from 1–5 mmol/L induce cytotoxicity in kidney cell lines and in normal human kidney cells, with an increase in reactive oxygen species (20–22). In 19 patients with EG poisoning, urinary oxalate concentrations were 2–5 mmol/L (3), so the cytotoxic effects of COM occur at toxicologically relevant levels. Studies with low dose-rate exposures of rats to EG have further suggested a major role for COM or oxalate accumulation in the renal toxicity (23). Dietary exposure of rats to EG induces a dose-related accumulation of COM in kidney tissue that correlates strongly with evidence of renal tubular necrosis (24), while plasma glycolate concentrations remain about 1–2 mmol/L. Hence, with no acidosis nor glycolate accumulation, there was massive renal tissue damage, due to the accumulation of COM.

The present study has demonstrated that COM crystals rapidly accumulate in the walls of cerebral blood vessels (within 24 hours), and are associated with perivascular edema and inflammation. The deposition of COM within the endothelium could result in cellular damage to the endothelial cells as seen in Fig. 1B, which would then lead to exudation of capillary fluid into the interstitium; hence, cerebral edema and an inflammatory reaction. This result could occur if oxalate or COM is toxic to endothelial cells like it is to kidney cells. Accumulation of oxalate crystals in the circulation has not been commonly linked with the vascular endothelial damage that occurs in later stages of EG poisoning. The main reason may be because plasma oxalate concentrations, as in the present case, remain very low, due to its low solubility in blood (25). However, in the recent feeding studies with EG in rats, plasma oxalate concentrations were low (<0.2 mmol/L), yet extremely high levels of oxalate, mostly as COM crystals, accumulated in kidney tissue (1 mmol/g tissue, equal to about 1 mol/L concentration) (24). So plasma oxalate concentrations do not indicate its potential for tissue accumulation and damage.

The observation that COM crystal deposition in cerebral blood vessel walls may be linked with the cerebral edema, perivascular hemorrhage and inflammation that occur in EG poisonings has been reported in the past, but has been discounted by more recent reviewers (16,26), who alternatively promote the aldehyde hypothesis. The first major report, in 1946 (13), examined 10 cases in young healthy adult males that died from EG and underwent early autopsy, suggesting a low level of post-mortem or age-related artifacts. All cases had brain edema, perivascular hemorrhage, cell degeneration and inflammation. This damage was accompanied by deposits of crystals that were obvious under polarized light, a strong indication of oxalate crystal accumulation. Crystals were reported as abundant in the vessel walls and perivascular space of damaged areas in 4 cases (but were not shown in vessels in figures), and were present with less frequency in the other 6 cases. Hagemann and Chiffelle in 1948 (14) reported similar findings in the brains of 3 cases (again with no figures), and extended the observations to include endothelial damage to pulmonary and pericardium vessels. Similar autopsy findings have subsequently been reported on multiple occasions (15,27–29). Also, the total amount of oxalate accumulation in the CNS appears to be greater than commonly assumed. In one case, the total oxalate content of the brain and the kidney was determined to be 0.43 and 4.4 mmol/kg tissue, respectively(6), indicating a substantial oxalate deposition in the CNS. Unlike most of the reported cases (13–15,27–29), the unique finding in the present

case was that the onset of generalized seizures correlated with CT documentation of diffuse cerebral edema and with pathologic confirmation of edema and perivascular inflammation being associated with the presence of COM crystals in CNS vessel walls. The documentation of COM crystals in cerebral vessel walls with tissue damage (Fig. 1) is stronger in this case than in the other reports, with one exception (27).

The results in this study reaffirm these repressed observations, and point towards the possibility that the cerebral edema and perhaps injury to other organs could result from COM crystal deposition within small blood vessels in the brain, lungs or heart. These various studies, in conjunction with recent studies on the cytotoxicity of oxalate or COM, suggest that accumulation of oxalate crystals in these vessels may be linked with the endothelial damage, because of the potential for cell damage from the contact with oxalate crystals. However, our results do not exclude the possibility that the cerebral edema resulted from the severe metabolic acidosis, as is known to occur in other poisonings (30). In this case, severe acidosis was present at admission and could have started a series of events that led to the cerebral edema observed in the repeat CT scan 8 hours later. Also, the presence of oxalate crystals in the vessel walls could be an epiphenomenon without a direct causal relation to the edema. Further controlled studies will be needed to differentiate these possibilities. Nevertheless, our results suggest that additional treatment modalities directed at oxalate-induced tissue injury may be important for patients with severe EG poisoning.

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Toxicokinetics of Ethylene Glycol During Fomepizole Therapy: Implications for Management

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Other members of the Methylpyrazole for Toxic Alcohols (META) Study Group are listed in the Appendix.

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Study objective: The elimination kinetics of ethylene glycol (EG) in human subjects treated with fomepizole (4-methylpyrazole) were analyzed to establish the efficacy of alcohol dehydrogenase (ADH) inhibition and to characterize elimination pathways.

Methods: Drug concentration data from patients enrolled in the EG arm of the Methylpyrazole for Toxic Alcohols trial, a prospective, multicenter, open-label trial of fomepizole, were analyzed and compared with published estimates.

Results: In 19 patients analyzed (EG concentrations of 3.5 to 211 mg/dL), elimination was first order during fomepizole monotherapy (half-life of 19.7±1.3 hours) and was not affected by the presence of ethanol. The elimination rate was significantly faster (half-life of <8.6±1.1 hours, $P<.001$) in the absence of fomepizole and ethanol. EG elimination by the kidneys was directly proportional to remaining renal function as estimated by creatinine clearance, with a fractional excretion of 25.5%±9.4%. Renal elimination and hemodialysis were the only significant routes of EG elimination as long as fomepizole concentrations were maintained well above 10 µmol/L (EG/fomepizole molar ratio, <100:1). All patients with normal serum creatinine concentrations at the initiation of fomepizole treatment had rapid rates of renal elimination (half-life of 16.8±0.8 hours).

Conclusion: At doses used, fomepizole effectively inhibits ADH-mediated metabolism of EG. Serum creatinine concentration at presentation and creatinine clearance can be used to predict EG elimination during fomepizole therapy and can help determine which patients will require hemodialysis to expedite EG elimination. An absolute EG concentration above 50 mg/dL should no longer be used as an independent criterion for hemodialysis in patients treated with fomepizole.

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INTRODUCTION

Ethylene glycol (EG) accounted for more than 6,000 poison exposures and 27 deaths reported to US poison centers in 1998.[1] Undoubtedly, the total number of cases and deaths is substantially greater. EG poisoning most commonly occurs after the ingestion of automotive antifreeze and is characterized by central nervous system (CNS) depression, metabolic acidosis, acute renal failure, hypocalcemia, seizures, and severe cardiopulmonary dysfunction.[2-4] The toxicity of EG results largely from its bioactivation to glycolic and oxalic acids. Hepatic alcohol dehydrogenase (ADH) catalyzes the initial step in this metabolic pathway. Although ethanol has traditionally been used to inhibit this enzymatic step, fomepizole (4-methylpyrazole [4-MP]) was approved for this indication by the US Food and Drug Administration in 1997 after a multicenter phase III clinical trial (the Methylpyrazole for Toxic Alcohols [META] study).[5] In this trial, previous human case reports, and animal studies, fomepizole has been shown to both prevent and reverse the development of metabolic acidosis and renal failure by halting the accumulation of toxic metabolites.[5-11]

The availability of fomepizole has the potential to fundamentally change the treatment of EG poisoning.[12] Hemodialysis has been an integral part of the treatment of patients with EG poisoning receiving ethanol therapy. Hemodialysis was recommended in part to expedite removal of EG and thus reduce the duration of ethanol treatment, which is difficult to dose effectively and requires admission to an ICU. Because fomepizole does not share these limitations,[13] it has been suggested that patients with EG poisoning who present before significant ADH-mediated bioactivation (ie, before the development of metabolic acidosis and renal insufficiency) can be treated effectively and safely with fomepizole alone, forgoing hemodialysis.[8-10,12,14,15] Such an approach relies on accurate understanding of the elimination pathways of EG. To date, the only available estimates for EG elimination kinetics in human subjects have been derived from a small number of case reports.[8-10,14-17]

In this study, we sought to characterize the elimination kinetics of EG observed in subjects enrolled in the META study. To demonstrate the efficacy of ADH inhibition caused by fomepizole, the elimination rate during fomepizole treatment was compared with rates observed when ethanol was present, during fomepizole loading, and after fomepizole therapy was halted. These rates were also compared with values previously reported for patients with EG poisoning. By examining elimination rates at various plasma EG and fomepizole concentrations, we also sought to identify a minimal effective inhibitor concentration (both absolute and relative to EG concentrations) necessary to provide good ADH inhibition. Finally, the effects of renal function and hemodialysis on EG elimination were analyzed. Measures of renal function, particularly pretreatment serum creatinine concentration, were compared with the subsequent rate of EG elimination during fomepizole therapy. We hypothesized that a normal pretreatment serum creatinine concentration would predict rapid EG elimination, and an elevated creatinine concentration would predict prolonged EG elimination during fomepizole monotherapy. The ability to predict EG elimination kinetics on the basis of initial serum creatinine concentrations would be clinically useful to rapidly identify patients in need of hemodialysis.

MATERIALS AND METHODS

The META study protocol has been described in detail elsewhere.[5] Patients were enrolled in this prospective, multicenter, open-label trial for suspected or documented toxic ingestions of EG or methanol. Patients were excluded for age younger than 12 years, pregnancy, known sensitivity to pyrazoles, or antidotal treatment with ethanol at the participating study hospital. Patients were not excluded if they had coingested ethanol or if they had received ethanol treatment at a referring hospital before transfer to a study center. Subjects from the EG arm of the trial were retained for this analysis if EG concentrations were greater than 20 mg/dL (3.2 mmol/L)* at enrollment. The study protocol was approved by institutional review committees at each participating center, and informed consent was obtained.

Subjects received a 15-mg/kg intravenous load of fomepizole (Antizol, Orphan Medical, Inc., Minnetonka, MN) over 30 minutes followed by 10 mg/kg administered intravenously every 12 hours for 4 doses, then 15 mg/kg administered intravenously every 12 hours thereafter until EG concentrations decreased below 20 mg/dL.

*To convert EG values from milligrams per deciliter, multiply by 0.161, for ethanol, multiply by 0.217.

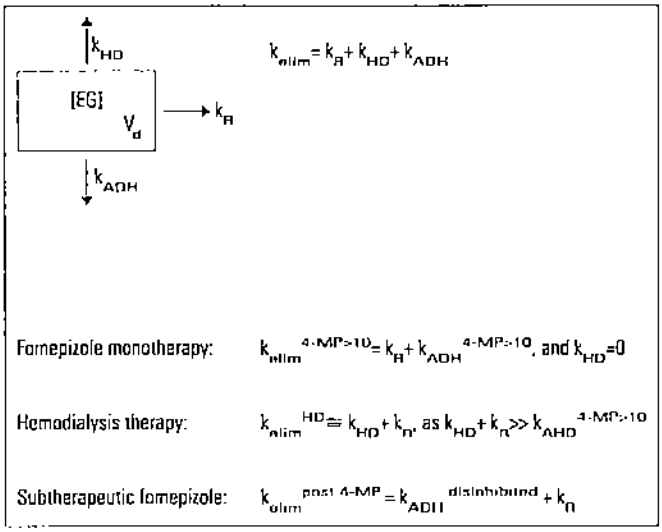
Because fomepizole is dialyzable,[18,19] the dosing interval was shortened to every 4 hours during hemodialysis. This dose schedule was constructed to maintain serum fomepizole concentrations well above 10 µmol/L, which is considered the minimally effective concentration on the basis of previous animal studies.[20,21] Patients underwent hemodialysis for any of the following: EG concentrations greater than 50 mg/dL, arterial pH of less than 7.1, serum creatinine concentration of greater than 3 mg/dL, or any significant deterioration in acid-base status or renal function after enrollment.

Venous blood samples were drawn for pharmacokinetic analysis at 0, 1, 2, 4, 8, and 12 hours after initial fomepizole loading, then every 6 hours until EG concentrations decreased below 20 mg/dL, and then every 12 hours thereafter. During hemodialysis, samples were drawn from the arterial and venous ports every 2 hours, and dialysis flow rates were recorded before, during, and after sampling. Samples were collected in lithium heparin tubes and centrifuged, and the plasma fraction was frozen at −20°C (−4°F) and transferred to a single reference laboratory for analysis. EG and ethanol concentrations were determined in duplicate by using gas chromatography.[22,23] EG concentrations near the sensitivity limit were retested and thus determined in quadruplicate. The validated lower limit of quantitation was 5 mg/dL for EG and ethanol. Fomepizole concentrations were assayed by using high-performance liquid chromatography, with a sensitivity limit of 5 µmol/L.[24]

A one-compartment pharmacokinetic model was used, with 3 main routes of elimination (Figure 1). The activity of these routes was not constant under the varying experimental conditions encountered. The presence of ethanol and fomepizole were assumed to affect the rate of ADH metabolism. By study hypothesis, the rate of renal elimination was assumed to vary between subjects on the basis of the severity of poisoning. Finally, hemodialysis was intermittent. Consequently, kinetic conditions were defined according to prospectively identified criteria. All intervals (ie, time periods between any 2 consecutive EG measurements) during which fomepizole concentrations remained above 10 µmol/L were considered to represent therapeutic fomepizole. Ethanol concentrations greater than 10 mg/dL were termed "ethanol present." Thus, all intervals with therapeutic fomepizole, ethanol absent, and hemodialysis not in progress represented fomepizole monotherapy ($k_{elim}^{4\text{-MP}>10}$). Intervals of therapeutic fomepizole or ethanol present were termed "ADH inhibition." Fomepizole loading denotes the (nonhemodialysis) time interval spanning initial fomepizole administration at trial enrollment. Subtherapeutic fomepizole ($k_{elim}^{post\ 4\text{-MP}}$) was any nonhemodialysis ethanol-absent interval during which fomepizole concentrations decreased below 10 µmol/L after trial initiation. Hemodialysis therapy (k_{elim}^{HD}) is self-explanatory.

For each condition, the effect of renal function was considered. Two mutually exclusive subgroups, namely patients with normal renal function and those with renal insufficiency, were derived for dichotomous comparisons. Renal insufficiency was defined as an abnormal serum creatinine concentration (≥1.5 mg/dL [130 µmol/L]) at presentation. For continuous comparisons between subjects, the independent variable of creatinine clearance was calculated from the patient's age, sex, mass, and serial serum creatinine values using the method of Lott and Hayton.[25] Hemodialysis clearance was calculated by using the Fick principle (clearance=hemodialysis flow×([conc]$_{inlet}$−[conc]$_{outlet}$)/[conc]$_{inlet}$). Volume of distribution was derived from the clearance/k_{elim}^{HD} ratio on the basis of the assumption $k_{HD}>k_R$.

Figure 1.
Pharmacokinetic model and definitions. [EG] represents the plasma concentration of ethylene glycol in the central compartment of volume V_d. The 3 main routes of EG elimination contributing to overall elimination are denoted symbolically by k_R (renal elimination [a function of creatinine clearance]), k_{HD} (intermittently active hemodialysis), and k_{ADH} (ADH-mediated metabolism blocked by fomepizole or ethanol). Three operative conditions described in the text (see "Materials and Methods" section) are formally defined by using superscripts to denote the condition.



Concentration-time data were analyzed by using a pooled population approach.[26-28] Briefly, rather than calculating kinetic parameters for each subject individually and then using the unweighted average as the best estimate of the parameter in the population (standard 2-step technique), data from all patients were analyzed simultaneously to derive the overall estimate. Such population pharmacokinetic techniques have been developed to overcome limitations of the standard 2-step technique when applied to data obtained outside the pharmacokinetic laboratory[26,27] and have recently gained the endorsement of regulatory agencies.[29,30] To illustrate, our patients had wide variations in the number and range of EG measurements, the time interval over which they were obtained, and the timing of hemodialysis. As a result, under certain kinetic conditions, some patients had relatively few observations obtained over a narrow time interval. Clearly, the uncertainty associated with the kinetic parameter calculated for such subjects was much greater than for subjects with more complete concentration-time profiles. Merely averaging the k_{elim} observed in each patient, without considering the precision of the individual estimates, could lead to a biased estimate of overall k_{elim} and is known to systematically inflate the variance of this estimate.[31] Pooling incorporates a more balanced measure of uncertainty in the overall estimate and provides a less-biased estimate of overall kinetics.[32]

Accordingly, we pooled concentration-time data from each subject after transformation along the time and concentration axes to maximize overlap. Mathematically, this transformation was performed by performing simple linear regression on an individual subject's log concentration-time data for the entire duration of a given kinetic condition and then mapping the predicted midinterval value to the origin of the log-linear graph and translating (shifting) all points accordingly. Overall population estimates for kinetic parameters were derived from performing linear regression on the pooled set of data.[31,32]

To identify the operative kinetics during ADH inhibition and the range of EG concentrations across which these kinetics held, 2 additional plots were constructed. First, original EG concentrations were retained, and the time axis for each subject was translated to optimize overlaps with other subjects. Next, original time data were held constant, and the log EG concentrations were translated to determine whether EG elimination accelerated appreciably over the course of the treatment. For fomepizole monotherapy, intervals from an individual subject separated by hemodialysis were joined end-to-end. This convention was adopted on the basis of the assumption of less intrasubject than intersubject variability in elimination during intervals of fomepizole monotherapy.

After fomepizole therapy was stopped and concentrations of inhibitor were allowed to decrease below 1.0 μmol/L, subsequent EG concentration measurements (called terminal segments) were examined for evidence of accelerated clearance of EG because of ADH disinhibition. Furthermore, because fomepizole is a competitive inhibitor,[33-35] further analysis was attempted to define a maximal effective ratio of substrate/inhibitor rather than merely an absolute concentration of inhibitor. The observed first-order slopes between any 2 concentration-time points during fomepizole monotherapy were plotted against the molar ratio of EG/fomepizole to search for evidence of a breakpoint ratio at which EG metabolism accelerates.

Elimination rates were derived by linear regression and compared by using an unpaired, 2-tailed, Student's *t* test with unequal variances. An α level of .05 was considered statistically significant. Goodness of fit between zero- and first-order models was tested by comparison of Pearson correlation coefficients. Population estimates are expressed as means±SE.

Table 1.
Subjects at presentation.

Initial Serum Concentration (mg/dL)	All Subjects	Subjects With Normal Renal Function	Subjects With Renal Insufficiency	*P* Value
EG	122.8±23.5	144.2±41.6	99.1±21.5	.34
Creatinine	1.55±0.19	0.92±0.08	2.24±0.21	<.001
Ethanol	62.8±15.7	62.4±24.6	63.3±22.0	.98
Glycolic acid	89.7±18.2	28.1±11.4	158.9±17.4	<.001
Bicarbonate (mEq/L)	12.9±1.7	18.3±2.1	7.0±0.8	<.001

RESULTS

Of 23 subjects enrolled in the EG arm of the META study, 4 were excluded from this analysis because of EG concentrations less than 20 mg/dL at enrollment, leaving data from 206 phlebotomy samples obtained from 19 subjects. At presentation, EG concentrations averaged 123±24 mg/dL (range 24 to 446 mg/dL). At enrollment, 12 (63%) had measurable ethanol concentrations, but only 4 had ethanol concentrations greater than 100 mg/dL. Of 8 patients to whom the referring physician had administered ethanol, 2 had ethanol concentrations greater than 100 mg/dL. The median time from estimated ingestion to fomepizole treatment was 11.4 hours (range 6.6 to 20.8 hours; unknown in 4 cases). In each patient, EG concentrations were declining (postpeak) at the time of enrollment. Nine (47%) patients had renal insufficiency. These patients had significantly greater delays to presentation, elevated plasma glycolate levels, and acidemia but similar EG and ethanol concentrations at enrollment compared with patients with normal renal function (Table 1). Seventeen (89%) patients underwent a total of 21 rounds of hemodialysis (mean duration, 5.5 hours per round). One patient died.

Seventeen of 19 patients had ADH inhibition data available for analysis, with a total of 120 observations. Most of these occurred during conditions of fomepizole monotherapy, comprising 99 observations (48% of total). First-order EG elimination constants for individual subjects (ie, unpooled) during ADH inhibition ranged from 0.0049 to 0.1129 $hour^{-1}$, with an unweighted mean of 0.0439±0.0079 $hour^{-1}$.

When EG concentration-time data were translated along the time axis, EG elimination fit first-order kinetics across concentrations ranging from 3.5 to 211 mg/dL (r^2=0.95, Figure 2). The zero-order model was much less satisfactory (r^2=0.74). The transformation of EG concentrations, while retaining original time values, demonstrated no evidence of change in elimination rates during up to 61.4 hours of treatment.

The overall pooled first-order model for fomepizole monotherapy yielded an elimination constant of $k_{elim}^{4\text{-MP}>10}$ = (0.0351±0.0023 $hour^{-1}$, r^2=0.72), which represents an elimination half-life of 19.7±1.3 hours

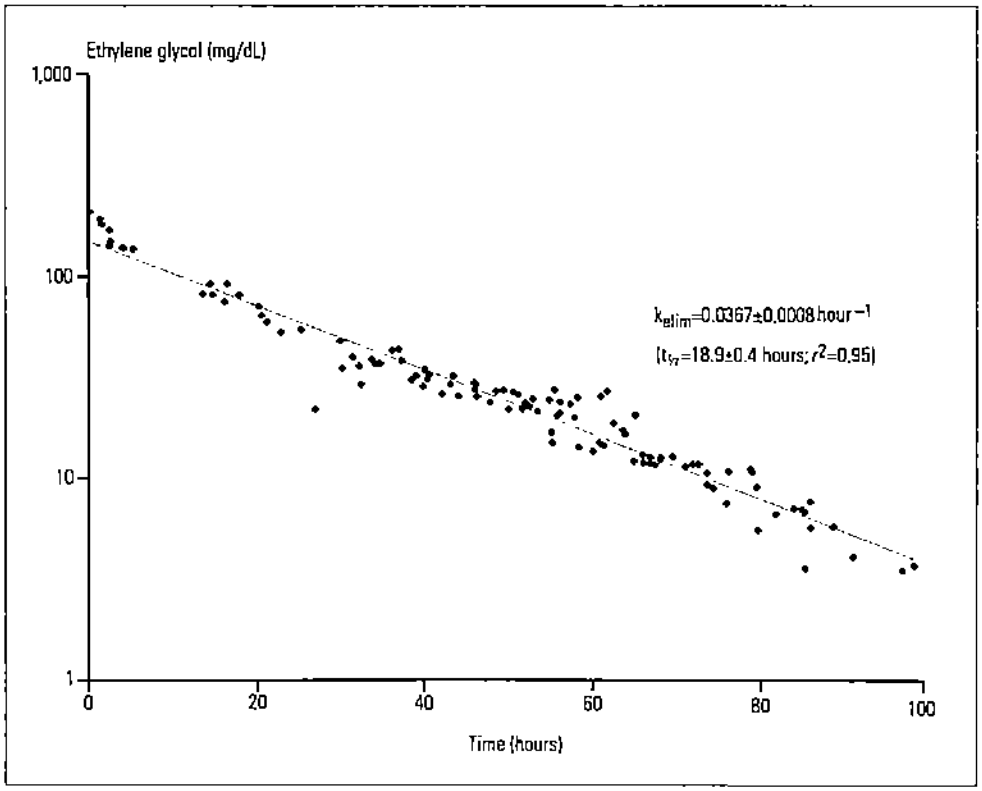


Figure 2.
EG elimination during ADH inhibition. Data have been linearly translated along the time axis to optimize overlap. First-order kinetics are operative across the entire range of EG concentrations, with an average elimination rate as indicated.

(Figure 3).* The zero-order model was again less satisfactory (0.516 mg/dL per hour, r^2=0.61). The presence of ethanol did not appreciably affect the rate of EG elimination at therapeutic fomepizole concentrations. On the other hand, significantly faster elimination rates were observed during fomepizole loading in the absence of ethanol (Table 2). Together, these findings support the pharmacokinetic efficacy of fomepizole in halting EG metabolism.

Eight patients had a total of 10 EG concentrations in samples obtained after fomepizole concentrations decreased below the target minimum of 10 µmol/L. All had EG concentrations in a low range (≤18.1 mg/dL). Nevertheless, EG elimination accelerated dramatically (indicated by short lines in Figure 4). To permit quantitative analysis, given that 4 of 8 available intervals ended with EG concentrations below the sensitivity limit of the assay, the first undetectable EG concentration was considered to be equal to this sensitivity limit (indicated by open circles in Figure 4). This conservative strategy yielded an overall elimination constant of $k_{elim}^{post\ 4\text{-}MP}$ greater than 0.0809±0.0092 hour^{-1} (P<.001 versus fomepizole monotherapy) or a half-life shorter than 8.6±1.1 hours.

During fomepizole monotherapy, there was no evidence of accelerated EG metabolism at higher EG/fomepizole ratios (r^2=0.0003, P value not significant). The median molar ratio attained was 15.9 (95% percentile range 4.46 to 95.9).

During fomepizole monotherapy, EG elimination was significantly slower for patients who had elevated serum creatinine concentrations at presentation compared with patients with normal serum creatinine concentrations (P<.001; Table 3 and open versus solid diamonds in Figure 3). All 7 patients with an elimination half-life greater than 24 hours had a serum creatinine concentration greater than or equal to 1.5 mg/dL at presentation. Among patients with normal serum creatinine concentrations at enrollment, none had elevations of creati-

*To convert elimination constant to half-life, use half-life of $0.693/k_{elim}$.

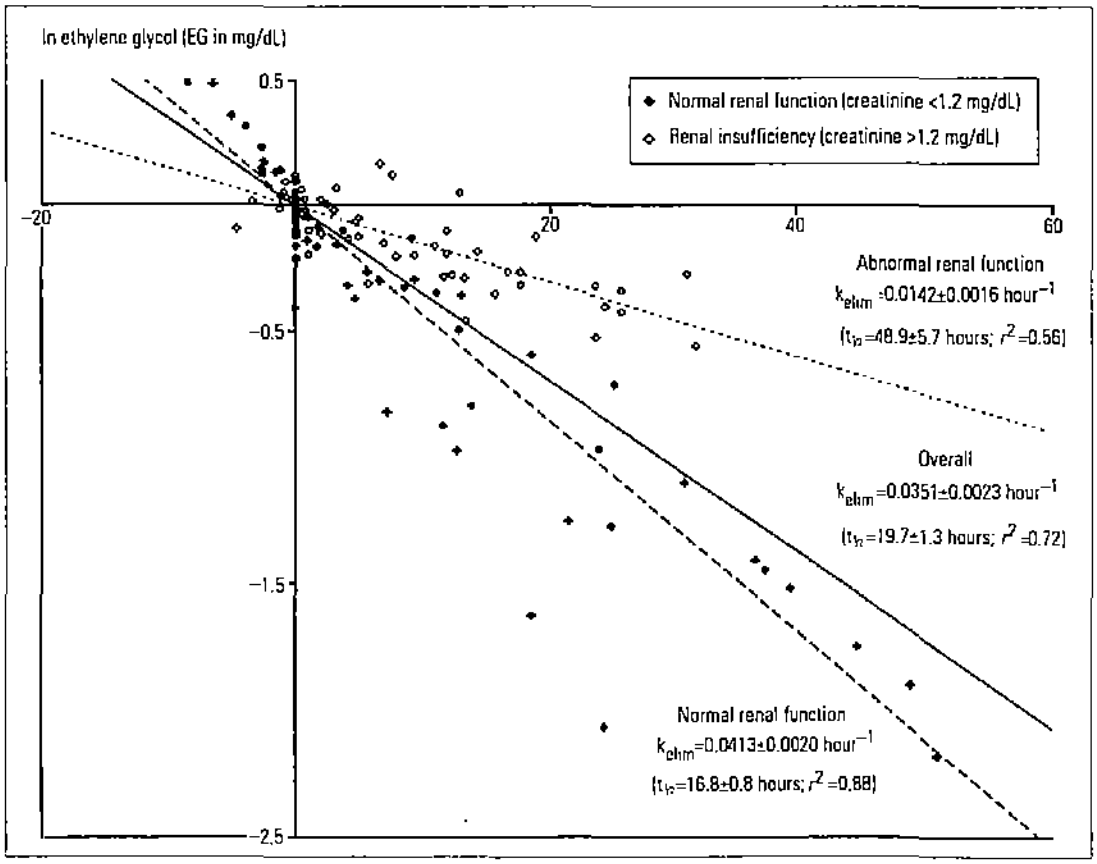


Figure 3.
Pooled EG elimination data during fomepizole monotherapy. Data have been linearly translated along both time and concentration (natural logarithm) axes. Patients with normal serum creatinine concentrations at presentation are indicated by solid diamonds, and all others are indicated by open diamonds. The solid line indicates the best fit for all patients, the long dashes for patients with normal renal function, and the short dashes for patients with renal insufficiency. Elimination constants and corresponding half-lives are indicated adjacent to each line.

nine into the abnormal range during the trial. Thus, initial creatinine concentrations were an excellent predictor of prolonged elimination and subsequent renal function.

Creatinine clearance correlated well with EG elimination during fomepizole monotherapy (r^2=0.53). Postulating a linear relationship between these 2 variables, the *y*-intercept for the model ($k_{elim}=\beta_0+\beta_1\times$creatinine clearance)

Table 2.
EG elimination rate categorized by ADH inhibition therapy.

k_{elim} (hour^{-1})	Ethanol Present (>10 mg/dL)		Ethanol Absent (<10 mg/dL)	Fomepizole±Ethanol
Fomepizole loading (at enrollment)	−0.0067±0.0175	P<.001	0.1013±0.0083	NA
	P<.01*		P<.001*	
Fomepizole therapeutic (>10 µmol/L)	0.0548±0.0153	P>.05	0.0351±0.0023†	0.0355±0.0018
			P<.001	
Subtherapeutic fomepizole (<10 µmol/L)	NA		>0.0809±0.00922‡	NA
Ethanol±fomepizole	0.0416±0.0105		NA	0.0353±0.0019§

See the "Materials and Methods" section for definitions of kinetic conditions.
NA, Not applicable.
*Where comparisons between conditions are appropriate, the significance level is indicated by symbol between those cells. Fomepizole±ethanol denotes fomepizole concentrations >10 µmol/L without restriction on the presence or absence of ethanol. Conversely, ethanol±fomepizole represents ethanol concentrations ≥10 mg/dL without restriction on the concentration of fomepizole.
†Fomepizole monotherapy.
‡Subtherapeutic fomepizole.
§ADH inhibition.

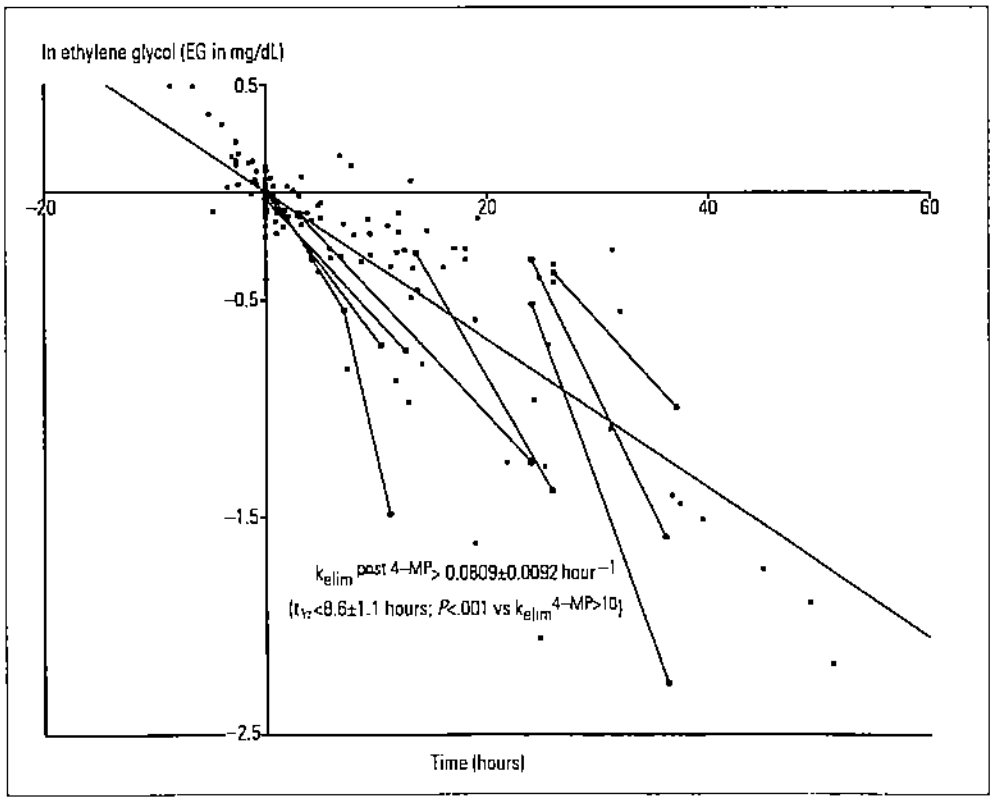


Figure 4.
Acceleration of EG elimination when fomepizole concentrations were subtherapeutic. Terminal segments (time interval during which fomepizole concentrations decreased below 10 µmol/L) are superimposed on the pooled fomepizole monotherapy data. EG concentrations below the sensitivity limit of the assay are represented by open circles located at this sensitivity limit and thus represent a conservative estimate of the rate of elimination. The overall elimination rate for fomepizole monotherapy is represented by the long diagonal line and is significantly slower (slope is less steep).

was 0.0204±0.0070 hour^{-1} (half-life, 33.9±13.3 hours). This parameter represents the k_{elim} in the absence of (or independent of) renal function, namely a non-ADH and nonrenal elimination pathway. The slope of the model was 0.375±0.092 kg/L, which yields an estimate of the fractional excretion of EG by the kidneys of 25.5%±9.4% after multiplication by the volume of distribution. This value corresponds to a renal clearance of 0.46±0.17 mL/kg per minute among the patients with preserved renal function.

During hemodialysis, clearance of EG was excellent (Table 3). The fractional excretion during hemodialysis was 68.4%±3.0% (range 42.9% to 91.8%), with negligible deterioration over several hours. In subjects undergoing hemodialysis the first-order elimination constant k_{elim}^{HD} averaged 0.259±0.021 hour^{-1} (half-life of 2.68±0.22 hours). During hemodialysis, overall elimination was significantly slower in the subgroup of patients with renal insufficiency (P=.028, Table 3). This difference, attributable to the difference in native renal function, is partly accounted for by the 0.027 hour^{-1} difference between groups in k_R (from the fomepizole monotherapy analysis). Plotting k_{elim}^{HD} against $k_{elim}^{4\text{-MP}>10}$ for each patient yielded a line with a slope of 1.06 and y-intercept of 0.206±0.036 hour^{-1}, which is the best estimate of k_{HD}, and represents a half-life of 3.37±0.61 hours.

DISCUSSION

An understanding of EG toxicokinetics allows the physician to select optimal therapy for a patient with EG poisoning. Decisions regarding both antidotal therapy and extracorporeal removal depend on accurate prediction of the kinetics of elimination. The two major endogenous routes of EG elimination are metabolic bioactivation by

Table 3.
EG pharmacokinetics during fomepizole monotherapy and during hemodialysis.

Kinetic Parameter	All Subjects	Subjects With Normal Renal Function	Subjects With Renal Insufficiency	*P* Value
Fomepizole monotherapy				
$k_{elim}^{4\text{-MP}>10}$ (hour^{-1})	0.0351±0.0023	0.0413±0.0020	0.0142±0.0016	<.001
Half-life (hour)	19.7±1.3	16.8±0.8	49.9±5.7	<.001
Hemodialysis				
k_{elim}^{HD} (hour^{-1})	0.259±0.021	0.302±0.029	0.215±0.025	.028
Half-life (h)	2.68±0.22	2.29±0.22	3.23±0.38	.028
Clearance (mL/min)	209±15	226±31	177±22	NS
V_d (L/kg)	0.680±0.084	0.774±0.124	0.585±0.134	NS

NS, Not significant.

Table 4.
Summary of EG elimination kinetics under various conditions and component pathway activities.

First-Order Rate Constant	Mean±SE (hour^{-1})
Overall elimination rates	
$k_{elim}^{4\text{-MP}>10}=k_R+k_{ADH}^{inhib}$	0.0351±0.0023
$k_{elim}^{post\ 4\text{-MP}}$	>0.0809±0.0092
$k_{elim}^{HD}=k_{HD}+k_R$	0.259±0.021
Component pathway activities	
$k_{elim}^{4\text{-MP}>10}$ as function of creatinine clearance	(0.375±0.092 kg/L)xCl_{creat}+0.0204±0.0070
$k_{ADH}^{uninhibited}$	>0.131±0.126
$k_{HD}=k_{elim}^{HD}$ as creatinine clearance → 0	0.206±0.036
$k_{non\text{-}ADH}=k_{elim}^{4\text{-MP}>10}$ as creatinine clearance → 0	0.0204±0.0070

ADH and excretion by the kidneys. Untreated EG poisoning results in renal insufficiency caused by the accumulation of toxic metabolites. Renal insufficiency in turn delays EG elimination and promotes further toxic metabolite accumulation.[2-4] Thus, it is useful to model the elimination kinetics of EG, especially in terms of renal function remaining at the time of initiation of antidotal therapy. The data presented here are from the largest and best-characterized series of patients ever reported and are derived from the only prospective trial of any treatment for EG poisoning in human subjects.

A mainstay in the treatment of EG poisoning is the inhibition of ADH to prevent metabolism to toxic organic acids. Traditionally, ethanol at concentrations of at least 100 mg/dL has been recommended for ADH inhibition.[2] The utility of ethanol as an antidote is limited by the unpredictable elimination kinetics of ethanol itself, restricted availability, and, by its CNS, gastrointestinal, and metabolic toxicity.[3,4,9,12,15] Although ethanol appears clinically effective, kinetic data to support its efficacy for human EG poisoning are limited. EG elimination rate constants during ethanol monotherapy were 0.039 and 0.041 $hour^{-1}$ in 2 patients with preserved renal function[16,17] and only 0.007 $hour^{-1}$ in 1 patient with renal failure.[14] Fomepizole is a more potent inhibitor of ADH, with a wider therapeutic index, longer duration of action, easier dosing, and more predictable kinetics.[12,13]

In this study, the rates of EG elimination under different therapeutic conditions through available pathways were characterized (Table 4). During fomepizole therapy, first-order noninducible kinetics are operative. Our results demonstrate that ADH-mediated oxidation of EG is effectively inhibited by fomepizole. The small elimination constants observed are comparable with rates previously reported for patients treated with ethanol alone. These rates are also consistent with the 6 reported cases of patients treated with fomepizole, in whom elimination constants ranged from 0.043 to 0.063 $hour^{-1}$, calculated from a relatively small number of data points.[8-10,15] It is interesting to note that the fastest elimination rates were obtained in patients given smaller doses of fomepizole (10.6 to 16.5 mg/kg in the first 24 hours and 1.8 to 4.8 mg/kg in the next 24 hours) than those used in the META trial.[9,15] This observation may represent partial disinhibition of ADH, occurring toward the end of each dosing interval and without any apparent clinical consequence.

Estimates of the rate of ADH-mediated metabolism of EG in the absence of inhibitor appear to be much faster on the basis of even sparser data (total elimination rate of 0.23 $hour^{-1}$ in a patient with normal renal function,[16] 0.12 $hour^{-1}$ in a patient with renal failure,[14] and 0.10 to 0.23 $hour^{-1}$ in animals[36-38]. The rate of ADH metabolism of EG in the absence of inhibitors must at least be greater than the rate of glycolic acid elimination because this metabolite is known to accumulate in EG poisoning.[37] Moreau et al[11] estimate a first-order rate constant for glycolic acid elimination of 0.131 ± 0.126 $hour^{-1}$ on the basis of 4 patients in the META study. In less-advanced cases with preserved renal function, glycolic acid elimination may be even faster. Because this elimination rate constant is nearly an order of magnitude larger than that of EG during fomepizole monotherapy, our analysis establishes the efficacy of ADH inhibition by fomepizole in the doses used. Further evidence of the efficacy of fomepizole is provided in our analysis by the observed deceleration of EG elimination with fomepizole loading, and subsequent acceleration after fomepizole therapy was discontinued. Moreover, the lack of correlation between elimination rate and the EG/fomepizole ratio demonstrates effective ADH blockade across the range of substrate/inhibitor ratios encountered. Finally, the presence of ethanol in addition to fomepizole had no appreciable effect on EG elimination, confirming a very high degree of metabolic inhibition by fomepizole alone.

This report not only demonstrates the inhibitory efficacy of fomepizole but also characterizes the primary routes of EG elimination. Thus, it provides the pharmacokinetic underpinnings for selecting rational and cost-effective management strategies for EG poisoning. Specifically, our kinetic data support the recent American Academy of Clinical Toxicology practice guidelines, which recommend modification of the traditional criteria for hemodialysis.[12] Although a plasma EG concentration of 50 mg/dL or greater has historically been used as an independent indication for hemodialysis in patients treated with ethanol, this concentration alone does not denote toxicity, predict prolonged elimination, or correlate with patient outcome. If EG metabolism were effectively blocked and the renal elimination pathway was intact, unmetabolized EG should have little potential for toxicity. Rather than EG concentration, the primary indications for hemodialysis in patients treated with fomepizole should be significant end-organ toxicity manifested by metabolic acidosis, elevated serum creatinine concentration, or clinical deterioration despite treatment.

Previous human case reports have yielded conflicting results on the importance of renal clearance on EG elimination, reflecting the variable degree of renal injury by the time of presentation and treatment.[9,14,17] In patients with normal renal function, renal excretion is believed

to account for a significant proportion of EG elimination.[8-10,15,17,39] Our data indicate that patients with normal serum creatinine concentrations and acid-base status at presentation can be expected to eliminate EG by means of renal excretion with a half-life of approximately 17 hours, obviating the need for hemodialysis. However, patients with metabolic acidosis or renal insufficiency appear to need hemodialysis, both to remove toxic organic acid metabolites and to remove EG itself, which has a mean half-life of over 48 hours when renal function is compromised.

Given the difficulty obtaining EG concentrations at most hospitals within a clinically useful turnaround time, reducing the dependence of treatment decisions on quantifying the absolute EG concentration would be of significant benefit to streamlining care in the emergency department. Moreover, the common dilemma of identifying which patients should be exposed to the risks of ethanol loading and hemodialysis before laboratory confirmation of EG poisoning could be resolved by initiating fomepizole therapy on the basis of the clinical suspicion of EG poisoning, reserving hemodialysis for renal insufficiency and metabolic acidosis. The enrollment criteria used in the META trial demonstrated high specificity for identifying patients with EG poisoning,[5] but it is unknown whether this specificity can be reproduced outside the setting of a clinical trial. Although the drug cost of fomepizole is greater than that of ethanol, a reduction in hemodialysis, ICU admission, and monitoring ethanol concentrations may make fomepizole cost-effective. A formal cost-benefit analysis should be undertaken, incorporating our pharmacokinetic data.

Using pooled data is a powerful technique, particularly when analyzing data obtained from critically ill patients.[26,27] The wide range in EG concentrations, delays to presentation, and presence of ethanol resulted in a heterogeneous (albeit representative) group of subjects at enrollment. Subsequent variability in initiation and duration of hemodialysis further complicated direct comparison of EG concentrations. By pooling the data, several objectives were accomplished. First, graphical methods could be used to explore and present the entire data set. Second, all data collected were used, an important concern given the difficulty obtaining high-quality EG concentration data in severely poisoned patients. Third, subjects in whom more data points were obtained over a longer interval were ascribed a greater weight in deriving overall kinetic estimates. Although the standard 2-step approach would provide an approximate estimate, the use of pooled data permitted a more precise quantification of these kinetics. This increased precision allowed exploration of the effect of renal function on elimination, even during hemodialysis, and the quantification of a nonrenal elimination pathway during ADH blockade.

There are limitations to this analysis. The assumptions implicit in the pooling of transformed data are that the kinetics are constant across the broad range of time and concentrations involved, as well as between subjects. The transformations along either time or concentration axes and the absence of significant curvilinear or higher order trends in the pooled data support these assumptions of stability for the first-order model. However, because patients with high EG concentrations underwent hemodialysis by study protocol, there are comparatively few data at concentrations of 100 to 200 mg/dL. Future studies of patients treated with fomepizole alone will be necessary to establish whether the kinetics described here continue to hold at higher EG concentrations when hemodialysis is withheld.

Pooling, by its nature, cannot differentiate between the variability in the kinetic parameter resulting from intersubject variation, and variability resulting from all other sources (eg, intrasubject, measurement error, and model misspecification).[28,31] A more complex nonlinear mixed effects model could be used to better characterize population variance. This technique, however, is less intuitive and more difficult to represent graphically, and the resultant model is more difficult to retain for infrequent clinical use. Moreover, current software implementations have difficulty accommodating our model with intermittent hemodialysis and ADH inhibition. The experimental design of the META trial with predetermined and frequent sampling times resulted in a data set intermediate in complexity between the classical data-rich pharmacokinetic drug-dosing study in healthy volunteers (standard 2-step technique acceptable), and the typical data-poor clinical observational study in which the number of observations per subject often is less than the number of kinetic parameters being estimated (nonlinear mixed effects model necessary).[29,30] With our linear single-compartment model, a single kinetic parameter (k_{elim}) independent of time and concentration, and at least several observations per subject, the population estimate of k_{elim} by using a mixed-effects model should be of comparable accuracy to our methodology.[31,32] Moreover, satisfactory r^2 values corroborate the adequacy of our model choice. The estimates of variance using our methodology are likely to be slightly inflated (although less so than using standard 2-step techniques), but this is expected to give only a minor reduction in power given that the num-

ber of observations per subject was usually much greater than the number of parameters being estimated.[32,40]

A one-compartment model was selected, ignoring the partitions of total body water across which EG distributes. There was, however, minimal posthemodialysis rebound in plasma EG concentrations, justifying this model. Equilibration between the intravascular and extravascular compartments therefore occurs with kinetics at least as rapid as hemodialysis elimination.

Although EG metabolism clearly accelerates if fomepizole concentrations decrease below 10 μmol/L, a precise minimum effective fomepizole concentration could not be identified, nor could a subtherapeutic ratio of substrate to inhibitor. The power of this analysis was limited by the relatively high serum concentrations of fomepizole. By design, a deliberate excess of fomepizole was administered to maintain a large margin of safety, and fomepizole therapy was only halted when EG concentrations were less than 20 mg/dL. Because of the relatively slow elimination of fomepizole,[41] substantial concentrations of fomepizole persisted after inhibitor therapy was stopped. Thus, there were few observations involving large excesses of substrate over inhibitor. The dosing schedule was constructed to maintain absolute fomepizole concentrations at least an order of magnitude greater than the minimum effective concentration of 10 μmol/L suggested by animal studies.[20,21] These studies involved methanol-poisoned monkeys and identified substrate/inhibitor molar ratios of roughly 3,000:1 to 6,000:1 (trough) as the threshold for inadequate ADH blockade. EG is believed to have an affinity for ADH comparable with that of methanol.[42] Animals successfully treated with fomepizole for EG toxicity may have attained ratios of up to 700:1 at mid-dosing interval.[6,7,43] The META patients were exposed to ratios of no greater than 100:1. On the basis of the relative affinities for human ADH in vitro, such ratios could be expected to inhibit more than 99% of the ADH-mediated metabolism of EG.[34,35,42,44] Given the minimal toxicity of fomepizole and the convenience of intermittent dosing, finding such a minimum effective concentration in human subjects is more of scientific curiosity than of clinical utility. With zero-order kinetics for fomepizole,[3,41] the cumulative dose needed for a course of therapy and hence the drug cost is unaffected by identifying a lower effective trough concentration.

The lack of control groups is a limitation of the META study design. Withholding or delaying antidotal therapy for EG poisoning would be unethical. There were no data on ethanol monotherapy available for comparison because patients were effectively loaded with fomepizole at enrollment and because ethanol was eliminated much more rapidly than EG (presumably through non–ADH-mediated metabolism,[45] as well as hemodialysis). In the absence of prospective data on patients treated with ethanol alone, historic controls from the literature were used for comparison. Our analysis cannot address the pharmacokinetic merits of withholding hemodialysis despite high EG concentrations for patients treated with ethanol. However, clinicians are unlikely to favor such a strategy because of difficulties maintaining ethanol concentrations at or above 100 mg/dL for prolonged periods and the metabolic and CNS consequences of prolonged ethanol therapy,[12] even if clinical data on the effective inhibitory ratio of EG/ethanol were to be available.

In summary, pharmacokinetic evidence is presented that demonstrates that fomepizole effectively inhibits ADH in human subjects with EG poisoning. An abnormal pretreatment creatinine concentration (≥1.5 mg/dL) predicts significantly prolonged EG elimination during fomepizole therapy. In the absence of metabolic acidosis, patients who present with normal renal function would not be expected to require hemodialysis, regardless of the EG concentration. Because EG elimination is exponential, even patients with very high concentrations but no other signs of end-organ toxicity should eliminate EG rapidly, despite ADH inhibition. Criteria for hemodialysis for the patient treated with fomepizole should be modified accordingly.

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APPENDIX.

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STUDY TITLE

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

Data Requirement

OECD Guideline 407
EEC Part B.7
USEPA - OPPTS 870.3050

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Study Completion Date

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Laboratory Project Study ID

031079

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: ETHYLENE GLYCOL

Title: ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

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

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

US Environmental Protection Agency - TSCA GLPs
Title 40 CFR, Part 792 - Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards, Final Rule

Organisation for Economic Co-Operation and Development (OECD)
OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997)
ENV/MC/CHEM(98)17

European Community (EC)
EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999)

Exceptions:

1. Concurrent with the study, the test material was characterized, checked for structural conformation and assayed for purity in accordance with GLPs.
2. The work conducted at Battelle, Pacific Northwest Division, Richland, WA, The Sapphire Group, Arlington, VA and by Dr. Gordon Hard were conducted in the spirit of GLPs, however, there were no formal QAU audits.
3. In compliance with the protocol, the work conducted at WIL Research Laboratories LLC was audited under the ordinance of their facility.

M. D. Dryzga, B.S. 6/8/05
M. D. Dryzga, B.S. (Date)
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R-R-ALBEE 6/8/2005
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QUALITY ASSURANCE STATEMENT

Compound: ETHYLENE GLYCOL

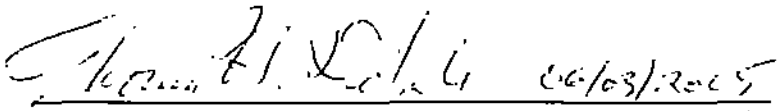
Title: ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

This study was examined for conformance with Good Laboratory Practices as published by the USEPA (TSCA), MITI, OECD, and EC. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 02 September 2003

TYPE OF AUDIT:	DATE OF AUDIT:	DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Final protocol	08 September 2003	08 September 2003
Study conduct #1	24-25 September 2003	26 September 2003
Study conduct #2	25 March 2004	25 March 2004
Study conduct #3	15 June 2004	09 July 2004
Study conduct #4	27-28 July 2004	28 July 2004
Study conduct #5	20 August 2004	20 August 2004
Protocol, data, and draft report	22 February 2005 - 02 June 2005	02 June 2005
Final report	The date of the signature below is the date of the final report audit.	

The final report accurately reflects the raw data of the study.



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Compound: ETHYLENE GLYCOL

Title: ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

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SUMMARY

The objective of this study was to assess the renal toxicity potential of ethylene glycol (EG) in male Wistar Han rats after 12 months dietary administration. The concentrations of EG and its metabolites, glycolic acid (GA) and oxalic acid (OA), in the blood, kidneys and urine, and the clearance kinetics of OA were also assessed. Additionally, the strain and age-dependence of clearance was evaluated in satellite groups of naïve male F-344 and Wistar rats. Benchmark dose (BMD) analyses were conducted for human health risk assessment purposes using compound-induced nephropathy and birefringent crystal data.

EG was given in NTP 2000 (lower protein) diet at 0, 50, 150, 300, and 400-mg/kg body weight/day (mkd) to groups of twenty male Wistar rats for 12 months. Ten rats per group (main group) were used to evaluate renal toxicity, five rats per group were used to evaluate metabolites, and five animals per group were used to determine renal clearance. Parameters assessed in the main group included cage-side and clinical observations, body weights, feed and water consumption, urinalysis, organ weights, gross necropsy, and histopathologic examination of kidneys and bladders. Strain and age dependence of OA clearance were assessed using four naïve Fischer-344 rats approximately 1-yr old and groups of five 9-12-wk old naïve Wistar and F344 rats. Ten sentinel animals were maintained in the study room for the 12-month duration of the study.

In-life treatment, necropsy, and clearance analyses were conducted at The Dow Chemical Company, Midland, Michigan. Histological staining of the urinary bladder and kidney slides was done by WIL Research. Microscopic histopathology evaluation was conducted by Gordon C. Hard, BVSc., Ph.D., D.Sc. Metabolic analyses were conducted by Richard Corley, Ph.D., Battelle Northwest. BMD analyses were conducted by the Sapphire Group.

One control rat died (day 307), no rats given 50-mkd died, one rat given 150-mkd died of a spontaneous rat lymphoma (day 267), and four rats given 300-mkd died (on day 111, 207, 213, or 221) with a fifth rat at that dose level declared moribund on day 138. At 400-mkd, 4 rats died spontaneously or were humanely euthanized in a moribund state (on day 43, 154, 187, or 193). On day 203, the sixteen remaining animals given 400-mkd were humanely euthanized because of excessive body weight loss. The mortality at 300 and 400-mkd was considered treatment-related.

All rats given 300-mkd that died or were declared moribund prior to study termination had gross findings on the bladder and four of them had gross findings on the kidney,

with the cause of death attributed to sequelae of urinary obstruction. The underlying cause of death/moribundity determined following gross and histopathologic examination was related to effects on the urinary bladder or kidney as described below.

During the study, animals given 300 or 400-mkd had occasional treatment-related absent/decreased feces, blood in the cage, red urine, red perioral and perinasal soiling, and/or perineal soiling. There were no treatment-related clinical signs at 50 or 150-mkd.

Rats given 300 or 400-mkd had treatment-related decrements in body weight and body weight gain. The differences from controls occurred within the first few months in animals given 400-mkd and were first statistically identified on day 141, when body weights and body weight gains were 12.7% and 21.4% less than controls, respectively. On study day 197 at 400-mkd, body weights were 20.1% less than controls and body weight gains were 31.3% less; therefore, the remaining rats at this dose were humanely euthanized on study day 203 because of excessive body weight loss. Body weights for rats given 300-mkd were typically lower than controls by mid-study, with all but one animal usually having body weight less than the control mean. These effects were considered related to treatment but were not statistically identified because of the large standard deviations. The body weight effects for rats given 300-mkd occurred gradually, and on study day 141, body weights and body weight gains were 5.2% and 8.4% less than controls, respectively. After day 141, differences from controls in body weights and body weight gains leveled off. No body weight effects occurred at 50 or 150 mkd.

Feed aversion/scratching occurred at ≥ 150-mkd, which was reflected in the smaller sample size as these feed consumption data were not collected. Rats given 400-mkd had treatment-related decreases in feed consumption at every time point through termination on day 203, which were typically statistically identified from study day 106. There were no treatment-related effects on feed consumption for rats given 50, 150, or 300-mkd.

Water consumption was analyzed near the 12-month end of the study. Rats given 300-mkd had a treatment-related increase in water consumption of 151% of controls. There were no treatment-related effects on water consumption for animals given 50 or 150-mkd.

After 12 months, decreased urinary pH occurred in all treatment groups but was not considered adverse but rather likely due to the presence of metabolic products of EG.

Animals given 300-mkd had increased urine volume and concomitantly decreased urine specific gravity compared to controls, which correlated with the increase in water consumption. The more dilute urine in the 300-mkd group might also explain the finding that less animals in this group had decreased urinary pH than in the 150-mkd group. Analysis of urinary crystals demonstrated treatment-related effects at all EG doses, with the proportion of crystals that were composed of calcium oxalate increasing with increasing EG dose, and those composed of phosphate decreasing with increasing EG dose. This compositional effect was considered a metabolic consequence of EG exposure as no adverse effects were seen from the crystals observed in the 50 or 150-mkd groups.

Increases in absolute and relative kidney weights occurred in animals given 300 or 400-mkd. These were not statistically identified at 300-mkd and were not statistically analyzed at 400-mkd, but were considered treatment-related. There were no contemporaneous controls for the animals given 400-mkd since they were sacrificed early, but remarkable increases occurred in their absolute and relative kidney weights versus all other groups that went to term, although rats at 400-mkd weighed much less.

Treatment-related gross pathological observations occurred in animals given 300 or 400-mkd and were primarily confined to the kidney and urinary bladder, with secondary treatment-related observations occurring in the lung. For rats given 300-mkd, of 15 rats examined, 7 had findings on the kidney and 8 had findings on the urinary bladder. For rats given 400 mkd, of 20 rats examined, 17 had findings on the kidney and 10 had findings on the urinary bladder. The most relevant observation in the 300-mkd group was the presence of calculi in the bladder (and sometimes the renal pelvis or ureter) in 8 of the total 15 rats examined. This also occurred in 8 of 20 rats at 400-mkd. Calculus formation in the urinary bladder was usually accompanied by dilatation of the bladder and, for the 5 unscheduled deaths at 300-mkd, hemorrhage of the bladder wall, usually with ascites or other edematous change. Three animals given 300-mkd had calculi in the renal pelvis. Almost all rats at 400-mkd showed signs of kidney and/or urinary bladder involvement, including a roughened kidney surface, renal pelvic dilatation, thickened bladder wall, and calculi in the renal pelvis, ureter, or bladder. Of the four unscheduled deaths occurring before early termination of this group, three were observed to have hemorrhage of the bladder wall. Some animals given 400-mkd also had decreased body fat, increased size of the renal lymph nodes, and calculus in the ureter or a dilated ureter. Treatment-related gross pathological effects on the lung, which were less frequent and considered secondary sequelae to effects on the kidney,

consisted of a mottled appearance in four rats given 400-mkd. Gross pathological findings of congestion and edema that occurred in the lungs of several animals given either 300 or 400-mkd may have been associated with agonal effects as these animals were found dead. The decrease in body fat observed for five animals given 400-mkd was considered reflective of the general decrease in body weight demonstrated by animals at this dose level. Ureter dilatation and calculi observed in two animals given 400-mkd are considered secondary to effects on the kidney and bladder. The increased size of the renal lymph nodes was considered a secondary consequence of the renal findings observed in eight animals given 400-mkd.

Histopathological examination showed that a compound-induced nephropathy associated with crystalluria affected the majority of the animals at 300-mkd, and all of those given 400-mkd. None of the renal alterations associated with EG exposure (basophilic foci of crystalluria-related nephropathy, tubule dilatation, birefringent crystals particularly in the pelvic fornix, renal pelvic dilatation, or transitional cell hyperplasia) were observed in the rats given 50 or 150-mkd, establishing the latter dose-level as a NOAEL.

Calculi, up to 2-mm diameter, were found in the bladder, and sometimes in the renal pelvis, at the two highest doses. Since the cause of early death for 3 animals at 300-mkd was unlikely to be related to the extent of the compound-associated kidney changes, which were less than end-stage, bladder tissue from some animals in each group was examined. Histological findings in the bladder and ureter correlated well with the observations of calculi. The basic change was simple transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. In animals dying before scheduled termination in groups given 300 or 400-mkd, the acute inflammation and hemorrhage of the bladder wall was a consistent finding in all but one case, and considered to be related to the cause of death. Such severe bladder pathology was often accompanied by a necropsy record of ascites or other edematous change, suggesting that infection via the bladder wall and septicemia may have been the terminal event.

The renal clearance rates of ^{3}H-inulin and ^{14}C-oxalate were evaluated in the control, 50, 150, and 300-mkd groups as well as naïve, male, young Wistar and F344 rats (9-12 weeks of age) and naïve, male, old F344 rats (47-56 weeks of age) to obtain information on renal clearance capability in rats of different strains and ages.

There were no treatment-related changes in oxalate or inulin clearance in the male Wistar rats after 12-months. Clearance ratios were 0.82 for controls and 0.73-0.87 for the 50, 150, and 300-mkd groups. Oxalate clearance rates ranged from 3.91-4.79 ml/min/kg bw.

Clearance ratios were not significantly different for the young versus old Wistar rats and varied from 0.59 to 0.82, respectively. While these results suggest an age-dependent increase in oxalate clearance, the actual clearance of oxalate was found to be quite constant with age (3.80-3.91). This variation in oxalate/inulin clearance ratios was most probably due to an age-dependent decrease in inulin clearance. In contrast, the ratio of oxalate to inulin clearance was lower in the young versus old F344 rats (0.70 vs. 0.81; not statistically significant), while the oxalate clearance rate was higher for the young versus old F344 rats (6.06 versus 4.56 ml/min/kg, respectively; not statistically significant), suggesting a higher rate of oxalate and inulin clearance in the young versus old F344 rats.

The only statistically identified difference in the rate of oxalate clearance was between the naïve young Wistar and F344 rats, which was significantly higher in the F344 rat. The clearance of oxalate was slightly higher in the old F344 versus Wistar rat (4.56 vs. 3.91 ml/min/kg, respectively; not statistically significant). Although old male F344 rats also have a reduced capacity for clearance of OX, similar to that of young and old male Wistar rats, the strain differences in sensitivity are maintained even through one year of exposure.

Blood, urine, and kidney samples collected from the metabolite satellite group of Wistar rats exposed for 12 months at 0, 50, 150, or 300-mkd EG were analyzed for EG, glycolic acid (GA), and oxalic acid (OX). A section of kidney from each animal in the 400-mkd group that was sacrificed on study day 203, and a section of kidney from all main study animals at 12 months, were also analyzed for EG, GA, and OX. There was a contaminant in the derivatization agent used for the analysis of EG in all samples except urine, which was analyzed directly. Thus for EG, only the urine data are reported.

The clearance of EG in urine followed a linear dose-response relationship across all dose levels. A linear increase in urinary clearance of GA was observed at 50 and 150 mg/kg-day while a disproportionate (non-linear) increase was observed at 300-mkd. Urinary clearance of OX was similar to controls across all dose levels. In the kidneys, there were no differences in the concentrations of GA and OX at dose levels up to

150-mkd, compared with controls. However, there were clear non-linear increases in the concentrations of GA and OX at dose levels of 300 and 400-mkd. Concentrations at 400-mkd reached an average of 14 µg/g and 18,800 µg/g for GA and OX, respectively, with some animals having considerably higher concentrations of each metabolite than average. In fact, OX concentrations, when expressed as calcium oxalate, accounted for an average of 2.9% of the total kidney weight (with one animal approaching 11.2%) in the animals exposed to 400 mg/kg-day and sacrificed early in the study. As with the results from the kidneys, the concentrations of GA in blood were not significantly different from controls up to 150-mkd. At 300-mkd, the concentrations in blood were approximately 3.3-fold higher than controls although the concentrations were all <10 µg/g regardless of dose level. The concentrations of OA in blood were also similar across all dose levels, averaging 3.7-5.1 µg/g. These results were expected from the low solubility of OA at physiological pH in aqueous media.

BMD analyses were conducted using compound-induced nephropathy and birefringent crystal data from Wistar rats chronically exposed to EG for the purposes of defining a dose corresponding to an extra risk of 5% (BMD05) and its lower confidence limit (BMDL05). The respective BMD05 and BMDL05 values using incidence and severity were
170 mg/kg-day and 150 mg/kg-day for compound-induced nephropathy, and 170 mg/kg-day and 160 mg/kg-day for compound-induced birefringent crystals.

In conclusion, chronic dietary administration of EG to male Wistar Han rats for 12 months resulted in:

- The maximum tolerated dose (MTD) was exceeded at 400 mkd as excessive body weight loss at this level necessitated early termination and there were histopathologic manifestations of marked renal toxicity.
- The no-observed-adverse-effect level (NOAEL) was 150 mkd based on the absence of manifestations of systemic or renal toxicity at this dose.
- A no-observed-effect level (NOEL) was not established as decreased urinary pH and increased urinary oxalate crystals occurred at all treatment levels (≥ 50 mkd), however, these were not considered adverse but rather normal metabolic/physiological consequences of chronic EG exposure.
- There were no treatment-related effects on oxalate or inulin clearance.

- Urinary clearance of OX was similar to controls across all doses, that of EG followed a linear dose-response relationship, and that of GA was linear between 50 and 150-mkd, with a disproportionate non-linear increase at 300-mkd.
- Kidney concentrations of GA and OX were similar to controls at doses up to 150-mkd. However, there were clear non-linear increases in the kidney concentrations of GA and OX at dose levels of 300 and 400-mkd.
- The respective BMD05 and BMDL05 values using incidence and severity data were170 mg/kg-day and 150 mg/kg-day for compound-induced nephropathy, and 170 mg/kg-day and 160 mg/kg-day for compound-induced birefringent crystals.

INTRODUCTION

Previous Toxicity Information

In subchronic and chronic EG toxicity studies, no-observed-adverse-effect levels (NOAELs) have been established based upon renal toxicity, with male Wistar Han rats being more sensitive than male F344 rats (Mertens, 2002; Hard, 2002; DePass, 1986). This strain-dependence might be attributable to differences in metabolism or disposition. Developmental effects have been associated with the intermediate metabolite, glycolic acid (GA). Renal toxicity has been associated with the metabolite, oxalic acid (OA), which can bind to calcium and precipitate as calcium oxalate crystals. Calcium oxalate-induced crystal or stone formation (and nephrotoxicity) has been shown to occur to a greater extent in males than females, possibly due to rate of metabolism (Richardson, 1965).

The male Wistar Han rat has a reduced ability to clear both GA and OA relative to other strains of rats and species, which may explain the enhanced sensitivity to renal toxicity (Corley, personal communication). Given the apparent differences between male Wistar Han rats and other rat strains, a comparison of the renal toxicity of EG and the pharmacokinetics of EG and its metabolites, GA and OA, following 16-weeks of dietary EG administration was recently conducted in male F344 rats and Wistar Han rats (Mertens, 2002; Hard, 2002; Corley, personal communication; Cruzan *et al.*, 2004). The kidneys of male Wistar Han rats were approximately two-fold more sensitive than those of male F344 rats to OA-induced crystal nephropathy. This toxicity difference correlates with a 3- to 4-fold decreased capacity to clear GA and OA metabolites in male Wistar Han rats versus other rat strains (Corley, personal communication). Following one week of dietary EG administration, male Wistar Han rats had significantly higher levels of OA in the kidneys at 500 and 1000 mkd than similarly dosed male F344 rats. By 16 weeks of exposure, the kidney concentrations of OA in male Wistar Han rats and, to a lesser extent, male F344 rats were greater than after one week of exposure. Relative to control, kidney OA levels following 16 weeks of dietary exposure to EG were elevated 4- or 2,530-fold in male F344 rats given 500 and 1000 mkd, respectively, versus 6-, 6153- and 18,740-fold in male Wistar Han rats given 150, 500, and 1000 mkd, respectively.

Purpose

The toxicity of ethylene glycol (EG) in rats has exhibited strain-dependence that might be attributable to strain-dependent differences in metabolism or disposition. Therefore, this study had five purposes: (1) to evaluate the renal toxicity potential of EG when

administered to male Wistar Han rats for 12 months via the diet; (2) to investigate the pharmacokinetics and disposition of EG in male Wistar Han rats by determining the levels of EG and its metabolites, glycolic acid (GA) and oxalic acid (OA), in the blood, kidneys, and urine from a satellite group of rats exposed to EG for 12 months via the diet; (3) to compare the strain and age-dependence of OA clearance in male F-344 versus male Wistar Han rats; (4) to investigate the impact of chronic (12-months) dietary administration of EG on the clearance kinetics of OA in male Wistar Han rats; and (5) to conduct benchmark dose (BMD) analyses on the results of the chronic study for EG in Wistar rats using the histopathology data for compound-induced nephropathy and birefringent crystals.

Test Guidelines

There were no guidelines relevant to this study design.

Quality Assurance

The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

Archiving

The data, protocol, protocol changes/revisions, and final report are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

TEST MATERIAL INFORMATION

Test Material Name

Ethylene glycol

Chemical Name

Ethylene Glycol Polyester Grade

Supplier, City, State (lot, reference number)

The Dow Chemical Company, Midland, Michigan (Lot# RG2355U1DC)

Purity/Characterization (method of analysis and reference)

>99.9% (Certificate of analysis, Jamerson, 2003). Characterization of ethylene glycol resulted in a purity of 99.4% ± 0.07% (corrected for water) by gas chromatography with thermal conductivity detection and the structure of ethylene glycol was confirmed by

infrared spectroscopy and gas chromatographic mass spectroscopy (Megregian *et al.*, 2003). In addition, the water content was determined to be 0.30% by Karl Fischer coulometric titration.

Characteristics

Appearance (physical state, color)

Clear Liquid

Molecular Formula

$C_2H_6O_2$

Molecular Weight

62.07

Chemical Structure

Vehicle

NTP 2000 Rodent Feed (Zeigler Brothers, Inc., Gardners, Pennsylvania)

Storage Conditions of Test Material

The test material was stored in amber-colored glass bottles at approximately 20°C. The headspace was purged with nitrogen prior to sealing the containers. The test material was allowed to warm to room temperature prior to use.

TEST SPECIES AND HUSBANDRY

Species and Sex

Rats–males

Strain and Justification

Strain-specific differences in EG toxicity have previously been identified that might have been attributable to strain-dependent differences in metabolism or disposition. Therefore, two strains of rats were used in different parts of the current study:

- Wistar Han (Crl:WI(Gix/BRL/Han)IGSBR rats
- Fischer 344 (CDF(F-344)/CrlBR) rats

These strains were selected based on toxicity studies previously conducted on EG (Mertens, 2002). Wistar Han rats were used in the chronic dosing portion of the study to assess renal toxicity, metabolism, and disposition, as these data have not previously been generated in this strain of rat subsequent to chronic (1-year) dosing via the feed.

Additionally, naïve, young Wistar Han and F344 male rats and old F344 rats were used in a satellite group designed to compare the strain-dependent clearance of OA.

Supplier and Location

Charles River Laboratories, Inc. (Raleigh, North Carolina)

This supplier and specific breeding facility were selected to mimic that used in a previous toxicity study conducted on this compound (Mertens, 2002).

Age at Study Start

Animals were approximately six weeks old at the start of the study.

Physical and Acclimation

Each animal was evaluated by a laboratory veterinarian, or a trained animal/toxicology technician under the direct supervision of a lab veterinarian, to determine the general health status and acceptability for study purposes upon arrival at the laboratory[1]. The animals were housed 1-2 per cage in stainless steel cages, in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle), and acclimated to the laboratory for one week prior to the start of the study.

Housing

Animals were housed one per cage in stainless steel cages in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle). A 12-hour light/dark photocycle was maintained for all animal room(s) with lights on at 6:00 a.m. and off at 6:00 p.m. Room air was exchanged approximately 12-15 times/hour. Cages had wire-mesh floors and were suspended above catch pans. Cages contained feed containers and pressure activated, nipple-type watering systems. Room temperature was recorded daily. The relative humidity was maintained within a range of 48.6-63.0%. The room temperature was maintained within a range of 21.5-22.3°C. These values were within the laboratory recommended range for rats.

[1] Fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Randomization and Identification

Animals were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers.

Text Table 1. Animal Identification Numbers

Dose Level (mkd)	Main Toxicity Group	Metabolism Group	Oxalate Clearance Group
Sentinel	Not Applicable		
0	03A4401-03A4410	03A4411-03A4415	03A4416-03A4420
50	03A4421-03A4430	03A4431-03A4435	03A4436-03A4440
150	03A4441-03A4450	03A4451-03A4455	03A4456-03A4460
300	03A4461-03A4470	03A4471-03A4475	03A4476-03A4480
400	03A4481-03A4490	03A4491-03A4495	03A4496-03A4500

Note: This table does not include the nine naive F344 or five naive Wistar Han rats used for the clearance study.

Feed and Water

Animals assigned to the study (03A4401-03A4500), including sentinel animals, were provided with NTP 2000 (Zeigler Brothers, Inc., Gardners, Pennsylvania) in meal form. The feed was treated with gamma irradiation to minimize the potential for mold growth in relation to the high moisture content of the feed (Neutron Products, Inc., Dickerson, Maryland). NTP 2000 diet has lower protein content than other rodent feeds and was used to minimize potential confounding of increased incidence and severity of an age-related spontaneous disease of most rat strains (chronic progressive nephritis), increase by elevated levels of protein in the diet (Rao, *et al.*, 1993). The naïve animals in the clearance group were fed LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form and housed in the stock animal room until selected for surgery – an exception to this was several sentinel animals were used as aged naïve Wistar controls and these had been maintained on NTP 2000 for the study duration. Analysis of the LabDiet® feed was performed by PMI Nutrition International to confirm the diet provided adequate nutrition and to quantify the levels of selected contaminates. Feed and municipal water were provided *ad libitum*. Analyses of the NTP 2000 feed was performed by Covance Laboratories, Inc., Madison, Wisconsin and received from Zeigler Bros., Inc. to confirm the diet provided adequate nutrition and to quantify the levels of selected contaminants. Copies of feed analyses and gamma

irradiation records are in the study file. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department (Appendix Table 17). In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent testing facility (Appendix Table 18). Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. There were no contaminates detected in the feed or water at levels that adversely affected this study.

Animal Welfare

In response to the Final Rules amending the U.S. Animal Welfare Act promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activities (ACUA) required for the conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC determined that the proposed Activities were in full accordance with these Final Rules. The IACUC assigned File No. Subchronic/Chronic Tox 01, Metabolism 01, Metabolism 03, and Animal ID 01 to these Animal Care and Use Activities.

STUDY DESIGN

Experimental Design and Critical Dates

Groups of 20 male Wistar Han rats were fed diets formulated to contain 0, 50, 150, 300, or 400 mg ethylene glycol/kg/day (mg/kg/day, mkd, mg/kg/d) for up to 12 months to evaluate the potential for systemic toxicity. Ten animals per group were considered as main group animals and were used to evaluate the potential for renal toxicity. Five animals per group were pre-selected as a satellite group for evaluation of metabolic parameters, with samples shipped to Battelle, Pacific Northwest Division, Richland, Washington. The remaining five animals per group were pre-selected as a satellite group for determination of oxalate clearance. A group of 10 male Wistar Han rats was included on study to serve as a naïve sentinel population for possible pathogen or viral analyses. These animals were maintained with the main group animals.

Prior to the completion of the dietary study, four to six age-matched, older (approximately 1 year), naïve Wistar Han and Fischer-344 (F344) rats were used to compare oxalate clearance between strains. In addition, five Wistar Han and five

Fischer-344 rats of a younger age (approximately 7-12 weeks) were used to measure oxalate clearance for comparison to oxalate clearance in older animals.

The following parameters were measured in the main, metabolism, and oxalate clearance groups (Table 1): Cage-side clinical observations, pre-exposure ophthalmologic exams, body weights, feed consumption, urinalysis (main group only), organ weights, gross necropsy, and histopathologic examination of tissues. Test material administration for all animals began on September 5, 2003. As a result of excessive body weight loss, the 400 mkd dose group was euthanized on March 25, 2004 (test day 203). Rats from the main and metabolism groups were necropsied on September 7 and 8, 2004, respectively (test days 369 and 370).

Route, Method of Administration, Frequency, Duration and Justification

The route of administration was via the diet since it was used in previous toxicity studies (Mertens, 2002), and is a potential route of exposure for humans. Thus, administration of the test material to rats via the diet represented an appropriate means of exposure.

Dose Levels and Justification

The high-dose (400 mkd) was chosen based on results of a 16-week toxicity study (Mertens, 2002). The remaining dose levels were expected to provide dose-response data for any treatment-related effects observed in the high-dose group and identify a NOEL.

Dose Preparation and Analysis

Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed periodically throughout the study based on stability data. Diets were prepared based upon the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from pre-exposure body weights and feed consumption data. Subsequently, the concentrations of the test material in the feed were adjusted weekly for the first 12 weeks of the study and at 2-week intervals thereafter, based upon the most recent body weight and feed consumption data.

Analysis

Concentration and Homogeneity

Concentration analyses of all dose levels, plus control and premix were conducted pre-exposure and again at weeks approximately 1, 4, 8, 12, 26, 43, and 52. The

homogeneity of the low-dose and the high-dose diets were determined once prior to the start of dosing. The method used for analyzing the test material in feed was a solvent extraction method followed by analysis using gas chromatography-mass spectrometry (GC-MS) and solvent standards incorporating an internal standard.

Stability

Ethylene glycol was determined to be stable in the NTP diet for up to 12 days in the range of concentrations used in this study (Mertens, 2002). The stability of the test material was further determined out to 21 days.

Retainer Samples

Reference samples (one/ dose/mix and premix) were retained and stored frozen (approximately -20°C) in sealed amber vials. Reference samples were discarded upon issuance of the final report.

Cage-Side Observations

A cage-side (general) clinical examination was conducted on all animals at least once a day, usually in the morning, taking into consideration the peak period of anticipated effects. This examination was performed with the animals in their cages and was designed to 1) detect significant clinical abnormalities that were clearly visible upon a limited examination, and 2) to monitor the general health of the animals. Significant clinical abnormalities that would have been observed included, but were not limited to: activity, repetitive behavior, vocalization, incoordination/lameness, injury, neuromuscular function (convulsion, fasciculation, tremor, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), fecal consistency, and fecal/urinary quantity. At least twice daily, usually at the beginning and end of each day, all animals were also observed for morbidity and mortality, and the availability of feed/water.

Clinical Observations

Clinical observations were conducted on all animals pre-exposure. Main group animals were observed weekly during the first 12 weeks of the study and at approximately 2-week intervals thereafter. Animals in the metabolism and oxalate clearance groups were observed at approximately 4-week intervals throughout the study period for health surveillance only. The examination includes cage-side, hand-held, and open-field observations that are recorded categorically as outlined in Table 2.

Ophthalmology

The eyes of all animals were examined by a veterinarian pre-exposure using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examinations.

Body Weights/Body Weight Gains

All rats were weighed during the pre-exposure period. Main group animals were weighed weekly during the first 12 weeks of the study and at approximately two-week intervals thereafter until March 12, 2004 (test day 190) when body weights were recorded weekly through study termination. Animals in the metabolism and oxalate clearance groups were weighed prior to study start, at approximately four-week intervals throughout the study period for health surveillance until March 12, 2004 (test day 190) when body weights were recorded weekly through study termination. Body weight gains were calculated throughout the duration of the study.

Feed Consumption

Feed consumption data on the main group animals was collected during the pre-exposure period, weekly during the first 12 weeks of the study and at approximately two-week intervals thereafter by weighing feed containers at the start and end of a measurement cycle. Starting on March 12, 2004 (test day 190), bi-monthly feed consumption was collected on animals from the metabolism and oxalate clearance groups through study termination. Feed consumption was calculated using the following equation:

$$\text{Feed consumption (g/day)} = \frac{\text{(initial weight of feed container – final weight of feed container)}}{\text{(\# of days in measurement cycle) (\# of animals per cage)}}$$

Water Consumption

Animals in the control, 50, 150, and 300 mkd main groups were acclimated to water bottles for approximately three days, eight hours per day, prior to collecting water parameters. Water consumption data were collected for a 24-hour period (main group animals only) during the urine collection period. Animals had access to water bottles instead of the automatic watering system. The water consumption was determined by weighing each water container at the start and end of the measurement cycle and calculated using the following equation:

$$\text{Water consumption (g/day)} = \frac{\text{(initial weight of water container – final weight of water container)}}{\text{(\# of days in measurement cycle) (\# of animals per cage)}}$$

Feed Efficiency

Feed efficiency on the main group animals was calculated using mean body weight gain and mean feed consumption data from the first 13 weeks of the study using the following equation:

$$\text{Feed efficiency} = \frac{\text{(g feed consumed/day)}}{\text{(g body weight gain/day)}}$$

Test Material Intake

The actual test material intake (TMI) for the main group animals was calculated using test material feed concentrations, body weights, and feed consumption using the following equation:

$$TMI = \frac{\left(\text{feed consumption}\left(\frac{\text{g}}{\text{day}}\right)\right) * (1000\ \text{mg/g}) * \frac{\text{(\% of test material in feed)}}{100}}{\frac{\left(\text{current BW [g]} + \text{previous BW [g]}\right)}{2}}$$

$$1000\ \text{g/kg}$$

Urinalysis

Main Group

Urine was obtained from all surviving fasted rats (main group) the week prior to necropsy. Animals were housed in metabolism cages and the urine collected for an approximately 24-hour period.

Assay: Color, appearance, specific gravity (refractometer), volume

Semiquantitative analyses (Multistix® Reagent Strips, Bayer Corporation, Elkhardt, Indiana on the Clinitek 200+) of the following were conducted:

pH
Bilirubin
Glucose
Proteins
Ketones
Blood
Urobilinogen

Microscopic evaluation for crystal types via micro-sediment analysis on individual animals.

Metabolite Group

Urine was obtained from all surviving non-fasted rats (metabolite group) the day prior to necropsy. Animals were housed in metabolism cages and the urine collected for an approximately 24-hour period. Urine samples were collected on dry ice. At the end of the collection period, the metabolism cages were rinsed with a minimal amount (<100 ml) of deionized water to adequately collect residual urine. Urine and rinse samples were weighed, stored frozen at approximately –80°C, and subsequently shipped to Battelle for analyses.

Oxalic Acid Clearance

A satellite group of male Wistar Han rats on the 12-month dietary EG study were used to quantitate the dose/exposure duration-dependent ability of the male Wistar Han rat to clear oxalic acid in the urine. At least 4 animals/group were used for this evaluation. Two additional rats were randomly selected from the sentinel group and data from these animals included with the control group. Prior to sacrifice, each animal (under anesthesia) was infused via a jugular cannula with a mixture of oxalic acid and inulin (to determine glomerular filtration rates). Blood and urine samples were collected via an abdominal aorta cannula or cannulated bladder, respectively. The methodology used was based on methods of Hautmann and Osswald (1978) and Sugimoto *et al.* (1993). Four age-matched, older (approximately one year) naïve Fischer 344 rats were used to compare oxalate clearance in Wistar Han and Fischer 344 rats. In addition, five naïve Wistar Han and five naïve Fischer 344 rats of a younger age (approximately 9-12 weeks) were used to measure oxalate clearance for comparison to that of older animals.

Animals were deprived of food for approximately 14 hours prior to the clearance study, while having free access to water. After anesthesia, the rats were placed on a heated pad to maintain their body temperature. The jugular vein was cannulated for infusion of solutions and the abdominal aorta was cannulated for withdrawal of blood samples. Urine was collected from the bladder via a catheter. The oxalate clearance infusion procedure began with lactated Ringers solution infused through the jugular vein cannula at a rate of 0.1 ml/min for 0.5 hour. Next, a radioactive solution (0.5-1.0 µCi of ^{14}C-oxalate and 0.25-4.5 µCi ^{3}H-inulin/ml isotonic saline) was infused at a rate of 0.2 ml/min for 5 minutes and then at 0.06 ml/min for 0.5 hr. After 0.5 hr of infusion of the radioactive solution, blood and urine sampling (urine at 10 min intervals and blood at midpoint) began while the radioactive solution continued to be infused at a rate of

0.06 ml/min for up to 90 minute. In some situations, sampling of the blood and urine were delayed until the urine flow had started. Inulin and oxalate concentrations in plasma and urine were determined from ^{3}H and ^{14}C-radioactivity, respectively.

Anatomic Pathology

Necropsy

Main Group

Fasted rats submitted for necropsy were anesthetized by the inhalation of CO_2 and then weighed. Their tracheas were exposed and clamped, and the animals were euthanized by decapitation.

A complete necropsy was conducted on all main group animals by a veterinary pathologist assisted by a team of trained individuals. The necropsy included an examination of the external tissues and all orifices. The head was removed, the cranial cavity opened and the brain, pituitary and adjacent cervical tissues examined. The eyes were examined *in situ* by application of a moistened microscope slide to each cornea. The nasal cavity was flushed via the nasopharyngeal duct. The skin was reflected from the carcass, the thoracic and abdominal cavities were opened and the viscera examined. All visceral tissues were dissected from the carcass, re-examined, and selected tissues incised. The lungs were distended to an approximately normal inspiratory volume with neutral, phosphate-buffered 10% formalin using a hand-held syringe and blunt needle. The liver (excluding the 400 mkd group) and kidneys were trimmed and weighed immediately. One kidney was cut longitudinally and the other kidney was cut transversely. One-half of each kidney was fixed in formalin for kidney pathology, and the remaining half was weighed and frozen in liquid nitrogen for evaluation of metabolic parameters. In addition, the prosector recorded any gross observations at necropsy; and saved any gross lesions in formalin. The ratios of organ weight to terminal body weight were calculated. The frozen kidneys were wrapped in foil, flash frozen in liquid nitrogen, kept at -80°C, and subsequently shipped frozen to Battelle for evaluation. The kidneys and urinary bladders fixed in formalin were sent to WIL Research Laboratories, LLC for processing by standard histologic procedures and the slides were shipped to Dr. Gordon Hard for histologic evaluation.

Representative samples of tissues listed in Table 3 were collected and preserved in neutral, phosphate-buffered 10% formalin. Transponders were removed and placed in jars with the tissues. Similar necropsy procedures were followed for all animals (main, metabolite, and oxalate clearance) found dead or moribund, except that body weights, organ weights, and urine samples were not obtained.

Metabolite Satellite Group

Animals were anesthetized by the inhalation of CO_2 and weighed. Whole blood was collected via the vena cava into heparinized tubes and immediately placed on dry ice then stored frozen at -80°C and subsequently sent to Battelle for evaluation. The tracheas of each animal were exposed and clamped, and the animals euthanized by decapitation.

The skin was reflected from the carcass, the abdominal cavity opened, and the kidneys excised. Kidneys were weighed immediately and the ratio of organ weight to terminal body weight was calculated. One kidney was cut longitudinally and the other kidney was cut transversely. One-half of each kidney was fixed in formalin for kidney pathology, and the remaining half was weighed and frozen in liquid nitrogen for evaluation of metabolic parameters. In addition, the prosector recorded any gross observations at necropsy, and saved any gross lesions in formalin. The remaining carcass with the transponder was placed in appropriate containers and stored frozen until the final report was issued. The frozen kidneys were wrapped in foil, flash frozen in liquid nitrogen, kept at -80°C, and subsequently shipped frozen to Battelle for evaluation. The kidneys and urinary bladders fixed in formalin were sent to WIL Research Laboratories, LLC for processing by standard histologic procedures and the slides were shipped to Dr. Gordon Hard for histologic evaluation.

Oxalate Clearance Group

There was no further histopathologic evaluation of tissues from animals in the oxalate clearance group. Animals were humanely euthanized at the completion of the clearance study with no further samples collected.

Early Termination

As the study progressed, several animals given 400 mkd died and the remaining animals at this dose level generally had excessive body weight loss, therefore, the remaining animals (16) were euthanized on March 25, 2004 (study day 203).

Animals were weighed the day prior to euthanasia, fasted overnight, and terminal fasted body weights were collected prior to necropsy. Kidneys from all animals were processed for histologic and metabolite evaluation as described below. Standard tissues (main group, Table 3) and any gross lesions (all groups) were preserved in formalin.

Sentinel Group

Two animals from the Wistar sentinel group were used for oxalate clearance and the remaining animals were humanely euthanized at the completion of the oxalate clearance study with no further samples collected.

Histopathology

Main and Metabolite Groups

One section from each kidney was processed by standard histologic procedures from all animals in the main and metabolite groups. One section from urinary bladder was processed by standard histologic procedures from all animals in the main group, all animals that had gross findings on the urinary bladder at necropsy, or from animals that died early or were declared moribund. The remaining tissues (described above) from the main group were maintained in formalin fixative for potential future evaluation at the discretion of the Sponsor. The frozen kidneys were wrapped in foil, flash frozen in liquid nitrogen, kept at -80°C, and subsequently shipped frozen to Battelle for evaluation. The kidneys and urinary bladders fixed in formalin were sent to WIL Research Laboratories, LLC for processing by standard histologic procedures and the slides were shipped to Dr. Gordon Hard, New Zealand, for histopathologic evaluation.

Oxalate Clearance Group.

One section from urinary bladder was processed by standard histologic procedures from animals in the oxalate clearance group that died spontaneously or had gross findings at necropsy. There was no further histopathologic evaluation of tissues from animals in the oxalate clearance group.

STATISTICS

All Animals

Means and standard deviations were calculated for all continuous data.

Main Group

Body weights, feed consumption, organ weights, urine volume, and urine specific gravity were evaluated by Bartlett's test for equality of variances (alpha = 0.01; Winer, 1971). Based on the outcome of Bartlett's test, exploratory data analyses were performed by a parametric (Steel and Torrie, 1960) or nonparametric analysis of variance (ANOVA) (Hollander and Wolfe, 1973). If the ANOVA was significant at alpha = 0.05, it was followed respectively by Dunnett's test (Winer, 1971) or the Wilcoxon Rank-Sum test (Hollander and Wolfe, 1973) with a Bonferroni correction for multiple comparisons to the control (Miller, 1966). The experiment-wise alpha level of 0.05 was reported for Dunnett's test and Wilcoxon Rank-Sum test. Descriptive statistics only (means and standard deviations) were reported for body weight gains and feed efficiency. Statistical outliers were identified by a sequential test (alpha = 0.02; Grubbs, 1969), but routinely excluded only from feed consumption statistics. Outliers were excluded from other analyses only for documented, scientifically sound reasons.

Gross pathologic observations were tabulated and considered in the interpretation of final histopathologic data, but were not evaluated statistically.

Oxalate Clearance Group

Inulin and oxalate concentrations in plasma and urine were determined from ^{3}H and ^{14}C-radioactivity, respectively. For all clearance group animals, the clearance of oxalate and inulin were calculated as the product of the urine to plasma concentration ratio and urinary flow rate, respectively. Oxalate clearance rates and ratios of oxalate/inulin clearance rates were evaluated by analysis of variance for the Wistar Han rats in the control and dosed groups. T-tests were used for the following comparisons: young Fischer 344 rats versus old Fischer 344 rats, young Fischer 344 rats versus young Wistar Han rats, control Wistar Han rats versus old Fischer 344 rats, and control Wistar Han rats versus young Wistar Han rats.

Metabolism Group

Statistical analyses for the work conducted at Battelle were included in a separate report that is appended to this report.

Benchmark Dose Calculations

Benchmark Dose (BMD) modeling was conducted by the Sapphire Group, Inc. Their report in appended to this report as Appendix C. BMD modeling was performed using the

histopathology data described by Dr. Gordon Hard for kidney effects in Wistar rats chronically exposed to EG using data for compound-induced nephropathy, birefringent crystals, and spontaneous nephropathy. However, the data for spontaneous nephropathy are not considered relevant for use in human health risk assessment for the following reasons: (1) the study author concluded there was no effect of EG treatment on the severity of spontaneous nephropathy; (2) the incidence for this endpoint in control male rats is very high and variable; (3) the dose-response data are nonmonotonic (i.e., decreasing at the lowest dose) which is often difficult for simple dose-response models to provide an acceptable fit; (4) measurement of this endpoint is confounded by compound-induced nephropathy, in that data from the 400 mkd dose group could not be used, and it is likely that the data from the 300 mkd dose group were impacted as well (data from only 8 animals); and (5) this form of spontaneous nephropathy is specific to rodents, and therefore this endpoint is not relevant to renal toxicity or to human health. For these reasons, the BMD analyses for spontaneous nephropathy are not discussed herein, but are provided for the sake of completeness in an Appendix to the BMD report (which is included with this report as Appendix C). Incidence data and combined incidence X severity data were used for the purposes of defining a dose corresponding to an extra risk of 5% (BMD05) and its lower confidence limit (BMDL05). Statistical tests were done to assess the significance of any treatment-related effects, and the goodness-of-fit for the dose-response model. The multistage model was used for fitting to the dose-response data. All BMD modeling and statistical tests were performed using USEPA's Benchmark Dose Software (BMDS, version 1.3.2).

RESULTS AND DISCUSSION

Analytical Chemistry

The homogeneity of EG in rodent feed was determined from eight separate mixing batches for the 50 mkd, 2 mixing batches at 300 mkd, and six mixing batches at 400 mkd, the lowest and highest concentrations used in the study at the specified time period (Table 4). The homogeneity was considered acceptable, with relative standard deviations for all diets sampled between 1.02 and 6.75%, with the exception of one analysis (mixed 23 September 2003) where the relative standard deviation was 36.6 and 16.1% for the 50 and 400 mkd groups, respectively. Visual inspection of the diet mix indicated the presence of some small clumps that was assumed to be composed of more highly concentrated EG with ground feed. A new premix and diet were prepared, with the

premix passed through a Comil (Quadro Engineering Incorporated, Waterloo, Ontario) prior to further use. An analysis conducted on this diet mix (mixed 02 October 2003) resulted in relative standard deviations of 2.32 and 4.78% for the 50 and 400 mkd groups, respectively. The Comil step was implemented for the remainder of the study.

Stability of EG was determined at concentrations of 0.005, 0.05, 0.5, and 5% in unsealed feed crocks that were exposed to an indirect light/dark cycle at ambient temperature, and also in feed stored in sealed containers with no direct light at ambient temperature (Table 5-A and B). EG was determined to be stable in unsealed feed crocks for at least eight days at concentrations from 0.005 to 5%, for which concentrations were 95.9-101% of the initial concentration. EG was also determined to be stable in stored sealed containers at concentrations from 0.005 to 5% for 22 days, for which concentrations were 93.5-104% of the initial concentration.

The concentrations of EG were determined for the control and test diets from eight time points and were found to be acceptable (Table 6). The mean concentration for each dose level over the course of the study ranged from 95.8 to 104% of the targeted concentration. No EG was found in the control diet. GC-MS analysis of samples of fed diets indicated 82.2-117% of the target concentration of EG.

Mortality

By the end of the study across all of the study groups, one control animal died (cause of death-probable lymphoid tumor), no animals given 50 mkd died, one animal given 150 mkd died of a spontaneous rat leukemia, and four rats given 300 mkd died with a fifth rat at that dose level declared moribund on day 138. At the high dose of 400 mkd, 4 rats died spontaneously or were humanely euthanized in a moribund state prior to study day 203. On study day 203, the sixteen remaining animals from the high dose were humanely euthanized because of excessive body weight loss. The mortality observed in the 300 and 400 mkd dose groups was considered treatment-related. The underlying cause of death or moribund condition was determined following gross and histopathologic (urinary bladder and kidney) examination and is presented in Table 27 and Appendix Table 19 and further described for some rats in the pathology section below. At necropsy, all rats given 300 mkd that died or were declared moribund had gross findings on the bladder and four of them had gross findings on the kidney, with the cause of death attributed to sequelae of urinary obstruction. There were no treatment-

related differences in the overall moribundity/mortality in rats given 50 or 150 mkd when compared to the control animals.

Clinical and Cage-Side Observations

Clinical and cage-side observations are summarized in Tables 7-9, and individual data are reported in Appendix Tables 1-3. Examinations performed on all animals given 0, 50, or 150 mkd revealed no treatment-related findings as cited observations were found in at most one animal/group and there was no pattern suggesting an increased incidence with an increased dose of EG. Overall, the incidence of clinical or cage-side observations was minimal. Animals given 300 or 400 mkd were observed with absent/decreased feces, blood in the cage, red urine, red perioral and perinasal soiling, and/or perineal soiling. These findings were considered related to treatment and are presented in Text Table 2. One animal given 150 mkd was observed with decreased feces, red perinasal soiling, and pale skin; however, these findings were associated with size increases in the lymph node, spleen (probable lymphoid tumor) and thymus indicative of rat leukemia and were not considered related to treatment.

Text Table 2. Salient Clinical Observations of Wistar Han Rats Given EG (All Groups)

	Dose Level (mkd)				
Observation	0	50	150	300	400
Feces- Absent/Decreased	0	0	**1**	**2**	**1**
Blood in the Cage	0	0	0	0	**1**
Soiling- Perioral, Red	0	0	0	**3**	0
Soiling- Perineal, Urine	0	0	0	**1**	0
Soiling- Perinasal, Red	0	0	**1**	**3**	0
Moribund/Spontaneous Death	**1**	0	**1**	**5**	**3**

Boldtype indicates effects considered treatment related.

Ophthalmology

Ophthalmology results are summarized in Table 10, and individual data are reported in Appendix Table 4. Examinations performed on all animals prior to the study revealed some animals with cloudy lens. These findings were considered incidental and a remnant opacity from the hyaloid artery; therefore, these animals were considered healthy and suitable for study purposes.

Body Weights/Body Weight Gains

Mean body weight and body weight gain data for rats are presented in Figures 1-6, summarized in Tables 11-13, and individual data are reported in Appendix Tables 5-7. There were no statistically identified or treatment-related effects in body weights for animals given 50 or 150 mkd, which had considerable variability. Rats given 300 or 400 mkd had treatment-related decrements in body weight (Text Table 3). The differences from controls were observed within the first few months in animals given 400 mkd. On study day 43, body weights and body weight gains for the 400 mkd group were decreased 5.9% and 12.5%, respectively. The differences from controls and the 400 mkd group continued to develop and were first statistically identified on study day 141, when body weights and body weight gains were 12.7% and 21.4% less than controls, respectively. Body weight and body weight gains continued to decrease and, on study day 197, body weights were 20.1% less than controls and body weight gains were 31.3% less. On study day 203, the 400 mkd dose group was humanely euthanized because of excessive body weight loss. Body weights for rats given 300 mkd were typically lower than controls by mid-study on with all but one animal having body weights less than the mean of the control group. These body weight decreases from controls were considered related to treatment but were not statistically identified because of the large standard deviations. The differences from controls for the 300 mkd dose group developed gradually and on study day 141, body weights were 5.2% less than controls and body weight gains were 8.4% less than controls. After day 141, differences from controls in body weights and body weight gains leveled off and on study day 358 body weight decreases were 5.4% of controls and body weight gains were 8.5% of controls. At study termination, body weights were 2.3% less than controls and body weight gains were only 3.8% less than controls for the 300 mkd group. The reported changes in body weights and body weight gains were calculated from the main study animals. Similar trends in body weight and body weight gains were observed in the oxalate clearance group but not in the metabolism group. The absence of these trends in the metabolism group may be a reflection of the smaller sample size and individual animal variability.

Text Table 3. Mean Body Weights of Wistar Han Rats Given EG (Main Group) – Selected Intervals

	Dose Level (mkd)				
Test Day	0	50	150	300	400
	Males (g)				
1	182.0	183.3	183.3	181.1	182.0
43	341.7	343.7	329.8	334.2	**321.7**
141	447.7	441.6	429.3	**424.2**	**390.8***
197	461.3	464.3	444.3	**433.5**	**368.7***
281	482.2	492.8	465.2	**456.3**	–
323	484.8	505.4	471.8	**458.4**	–
358	501.0	521.8	488.0	**473.8**	–
365	504.4	523.8	490.1	493.0	–

*Statistically Different from Control Mean by Dunnett's Test, alpha = 0.05.
Boldtype indicates effects considered treatment related.
-- No data

Feed Consumption

Mean feed consumption data for rats are summarized in Tables 14-16, and individual data are reported in Appendix Tables 8-10. The reported effects in feed consumption were summarized from the main study animals. Similar trends in feed consumption were observed in the metabolite and oxalate clearance groups. Feed aversion/scratching occurred at ≥ 150-mkd, which was reflected in the smaller sample size as these feed consumption data were not collected. There was no noticeable treatment or dose-related trend in feed consumption for rats given 50, 150, or 300 mkd compared to controls, and differences were never statistically identified (Text Table 4). From day 1 through termination on study day 203, feed consumption for rats given 400 mkd was decreased at every time point when compared to controls, typically statistically identified from study day 106 to termination and was considered related to treatment (Text Table 4).

Text Table 4. Mean Feed Consumption of Wistar Han Rats Given EG (Main Group) – Selected Intervals

	Dose Level (mkd)				
Test Day	0	50	150	300	400
	Males (g/day)				
1-8	23.4	23.4	23.9	22.9	22.7
85-92	23.9	23.5	24.8	23.8	22.1
106-113	24.4	22.7	23.1	22.8	**20.4$**
190-197	22.9	22.7	22.0	22.8	**17.4***
288-295	21.7	21.9	21.6	22.5	–
358-365	23.8	22.6	21.8	23.1	–

*Statistically Different from Control Mean by Dunnett's Test, alpha = 0.05.
$Statistically Different from Control Mean by Wilcoxon's Test, alpha = 0.05.
Boldtype indicates effects considered treatment related.
-- No data

Water Consumption

Mean water consumption data for rats are summarized in Table 17, and individual data are reported in Appendix Table 11. Water consumption data were collected for main group animals in the control, 50, 150, and 300 mkd groups for a 24-hour period during the urine collection period. Water consumption for rats given 300 mkd was increased 151% of controls and considered to be treatment related. There were no differences in water consumption for animals given 50 or 150 mkd when compared to controls.

Feed Efficiency

Feed efficiency data for the first 13-weeks of the study are summarized in Table 18, and individual data are reported in Appendix Table 12. Rats given 400 mkd were observed with decreases in feed efficiency toward the end of the 13-week period. These decreases were considered related to treatment. There was no consistent pattern of altered feed efficiency at doses of 50, 150, or 300 mkd.

Test Material Intake

Test material intake data are summarized in Table 19. Group test material intakes for 0-12 months were calculated on the parts per million of test material in the feed (% test material in the feed), feed consumption, and body weight data as reported above. The test material intake was consistent with the targeted concentrations for all dose levels over the course of the study. Mean test material intake data (mg/kg/day) are presented in Text Table 5.

Text Table 5. Targeted (mg/kg/day in the diet) and Mean Calculated Dose (mg/kg/day) of EG

Targeted dose (mg/kg/day)	0	50	150	300	400[1]
	Actual dose (mg/kg/day)*				
0-12 months					
Males	0	51 (± 4)	152 (± 14)	303 (± 24)	390 (± 36)

* Mean (± Standard Deviation) of calculated dosage levels.
[1] Study duration was 203 days for the 400 mg/kg/day dose group.

Clinical Pathology

Urinalysis

Urinalysis data for rats are summarized in Tables 20 and 21 and Text Table 6, and individual data are reported in Appendix Table 13. Decreases in urinary pH occurred in all treatment groups but were not considered adverse but rather likely resulted from the presence of urinary metabolic products of EG, which were anticipated. Animals given 300 mkd had increased urine volume and concomitantly decreased urine specific gravity compared to controls. These findings correlated with the increased water consumption observed at this dose level. The more dilute urine in the 300 mkd group might also explain the finding that there were less animals in this group with decreased urinary pH than in the 150 mkd group.

Urinary crystal data are summarized in Text Table 7. Treatment-related effects occurred in all treatment groups, with the proportion of crystals that were composed of calcium oxalate increasing with increasing dose of EG, and those composed of phosphate decreasing with increasing dose of EG. The change in the composition of urinary crystals was considered a normal metabolic consequence of EG exposure and not an adverse effect.

Text Table 6. Salient Urinalysis Findings

Dose (mg/kg/day)	0	50	150	300
Urine pH	7.0 (1) 7.5 (1) 8.0 (2) 8.5 (2) >9 (2)	**6.5 (3)** 7.5 (1) 8.0 (2) 8.5 (2) >9 (2)	**5.0 (2)** **5.5 (1)** **6.0 (2)** 7.5 (2) >9 (2)	**6.5 (1)** 7.0 (1) 8.5 (1) >9 (2)
Urine volume (ml)	10.6	8.8	7.9	**16.3**
Urine specific gravity (mOsmol/l)	1.031	1.034	1.038	**1.025**

Urine pH data tabulated as number of animals (N) with the stated value.
Bold type indicates the effects judged to be treatment-related.

Text Table 7. Urine Crystals

Dose (mg/kg/day)	0	50	150	300
N	8	10	9	5
Triple Phosphate (+++)	7	7	3	3
Triple Phosphate (++)	1	2	1	1
Triple Phosphate (+)		1	3	1
Triple Phosphate (rare)			**1**	
Calcium Oxalate (rare)		**1**		
Calcium Oxalate (+)		**2**	**1**	
Calcium Oxalate (++)			**2**	**3**
Calcium Oxalate (+++)			**6**	**2**

Numbers represent incidence and are not mutually exclusive (i.e., some animals had multiple crystal types).
Bold type indicates the effects judged to be treatment-related.

Anatomic Pathology

Organ Weights

Terminal body and organ weight data for rats are summarized in Tables 24-26, and individual data are reported in Appendix Tables 14-16. Salient organ weight effects are presented in Text Table 8. At scheduled sacrifice, liver and kidneys were weighed for the main group animals and kidneys were weighed for the metabolism group animals given 0, 50, 150, or 300 mkd. Kidneys were weighed at early sacrifice for animals given 400 mkd.

There were no statistically identified differences in any of the measured organs for any treated groups when compared to their respective controls. Statistical analyses were not conducted on organ weights of animals sacrificed early. Treatment-related increases in absolute and relative kidney weights occurred in animals given 300 or 400 mkd. Although there were no contemporaneous controls for the animals given 400 mkd that were sacrificed early, there were remarkable increases in their absolute and relative kidney weights compared to all other groups that went to term, even though the body weights of the 400 mkd group were considerably less. The treatment-related changes in kidney weight were consistent with histopathological evidence of mineralization that occurred at the corresponding doses.

Text Table 8. Treatment-Related Organ Weight Effects

GROUP	Mean Weight	DOSE(mg/kg/day)				
		0	50	150	300	400
Main group	Terminal Body (g)	483.5	498.7	467.6	466	
	Kidney (g)	2.551	2.692	2.417	**2.806**	-
	Kidney (g/100)	0.530	0.539	0.517	**0.612**	-
Metabolism group	Terminal Body (g)	488.4	484.1	485.2	457.9	-
	Kidney (g)	2.455	2.593	2.649	**3.242**	-
	Kidney (g/100)	0.505	0.539	0.548	**0.713**	-
Early termination group	Terminal Body (g)	-	-	-	-	367.7
	Kidney (g)	-	-	-	-	**4.021**
	Kidney (g/100)	-	-	-	-	**1.122**

- No data.
Bold type indicates the effects judged to be treatment related.

Gross Pathology

The gross pathologic observations are summarized in Table 27, and individual data are reported in Appendix Table 19. Salient gross pathological observations are presented in Text Table 9. Treatment-related gross pathological observations were primarily confined to the kidney and urinary bladder in animals given 300 or 400 mkd, with secondary treatment-related observations occurring at a lesser incidence in the lung at these dose levels. For rats given 300 mkd, of 15 rats examined grossly at necropsy, 7 had findings on the kidney and 8 had findings on the urinary bladder. For rats given 400 mkd, of 20 rats examined grossly at necropsy, 17 had findings on the kidney and 10 had findings on the urinary bladder. Some animals given 400 mkd also had decreased body fat, increased size of the renal lymph nodes, and calculus in the ureter or a dilated ureter. Gross pathological effects on the kidney consisted of calculi, dilated renal pelvis,

or a mottled, pale or roughened surface. Gross pathological effects on the urinary bladder consisted of calculi, dilatation, bloody urine, or a thickened or hemorrhagic wall. Treatment-related gross pathological effects on the lung, which were less frequent and considered secondary sequelae to effects on the kidney, consisted of a mottled appearance in four rats given 400 mkd. Effects in the lung secondary to chronic renal disease are well established in the literature (Boorman and Eustis. 1990). Gross pathological findings of congestion and edema that occurred in the lungs of several animals given either 300 or 400 mkd may have been associated with agonal changes as these animals were found dead. The decrease in body fat observed for five animals given 400 mkd was considered reflective of the general decrease in body weight demonstrated by animals at this dose level. The increased size of the renal lymph nodes was considered a secondary consequence of the renal findings observed in eight animals given 400 mkd.

Text Table 9. Salient Gross Pathological Observations

Dose (mg/kg/day)	0	50	150	300	400
Kidneys (# examined)	(14)	(15)	(15)	(15)	(20)
No visible lesions	14	15	15	8	3
Calculus, unilateral	0	0	0	**2**	0
Calculus, bilateral	0	0	0	**1**	**3**
Dilated renal pelvis, unilateral	0	0	0	**3**	**3**
Dilated renal pelvis, bilateral	0	0	0	**3**	**6**
Mottled	0	0	0	0	**1**
Pale, bilateral	0	0	0	**3**	**14**
Roughened surface, bilateral	0	0	0	**2**	**14**
Urinary bladder (# examined)	(14)	(15)	(15)	(15)	(20)
No visible lesions	14	15	15	7	10
Calculus	0	0	0	**7**	**4**
Calculus, multifocal	0	0	0	**1**	**1**
Dilatation	0	0	0	**8**	**3**
Hemorrhage of the wall	0	0	0	**5**	**3**
Thickened wall	0	0	0	**1**	**7**
Bloody urine	0	0	0	0	**2**
Lung (# examined)	(14)	(15)	(15)	(15)	(20)
No visible lesions	14	15	14	14	14
Congestion	0	0	0	1	2
Edema	0	0	0	1	**1**
Focus, dark, multifocal	0	0	1	0	0
Mottled	0	0	0	0	**4**
Decreased body fat	0	0	0	0	**5**
Increased size of renal lymph nodes	0	0	0	0	**8**
Ureter with calculus present	0	0	0	0	**2**
Ureter with unilateral dilatation	0	0	0	0	**2**

Bold type indicates the effects judged to be treatment related.

Histopathology

Histopathologic observations are summarized in the Pathology Report submitted by Dr. Gordon Hard and attached to this report as Appendix A.

A compound-induced nephropathy associated with crystalluria affected the majority of the animals at 300-mkd, and all of those given the highest dose of 400-mkd with a severity that led to early termination of this group. In contrast, none of the renal alterations associated with EG exposure (basophilic foci of crystalluria-related nephropathy, renal tubule dilatation, birefringent crystals representative of calcium oxalate, dilatation of the renal pelvis, or transitional cell hyperplasia in the renal pelvis) were observed in the group of rats administered 50 or 150-mkd, establishing the latter dose-level as a NOAEL.

Calculi, up to 2 mm in diameter, were found in the bladder, and sometimes in the renal pelvis, at the two highest doses. Since the cause of death of the 3 animals dying in Group 4 (300-mkd) was unlikely to be related to the extent of the compound-associated kidney changes, which was less than end-stage in each case, bladder tissue from most animals in each group was examined. Histological findings in the bladder and/or ureter correlated well with the necropsy observations of calculi. The basic change was simple transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. In animals dying before scheduled termination in groups given 300 or 400-mkd, the acute inflammation and hemorrhage of the bladder wall was a consistent finding in all but one case, and considered to be related to the cause of death. Such severe bladder pathology was often accompanied by a necropsy record of ascites or other edematous change, suggesting that infection via the bladder wall and septicemia may have been the terminal event in these cases. Although the cause of death may have been related to the consequences of calculi in the bladder, the most sensitive marker of the adverse effects of ethylene glycol was in the kidneys.

Calculus formation as a consequence of EG administration is a predictable finding given the chronic duration of exposure. DePass *et al.* (1986), in their 2-year bioassay of EG in Fischer 344 rats, reported the presence of oxalate crystals in the urinary bladder by 12 months, and sometimes calculi in the pelvic space, ureters, and bladder, often in association with hydronephrosis, by 18 months. The greater sensitivity of the Wistar rat may explain the more rapid development of calculi by 12 months in the present study. In a subchronic study of calcium oxalate crystalluria induced by EG in the Sprague-Dawley rat, Khan (1995) described the formation of "ministones" on the surface of the renal papilla after 8 weeks, and referred to the potential for this to lead to stone development. On the basis of the crystalline structures observed in some of the bladders in the current 12-month study with ethylene glycol, it seems likely that

the calculi diagnosed at necropsy were not true concretions, which are usually solid, but merely organization of crystal clumps into larger aggregates.

Oxalic Acid Clearance

Oxalic acid clearance data are summarized in Table 22 and Text Table 10.

Text Table 10. Summary of Oxalic Acid Clearance

GROUP	Mean Clearance (ml/min/kg BW)	EG DOSE (mg/kg/day)			
		0	50	150	300
Main Study Rats (age range 55-60 wk)	Mean	3.91	4.50	4.70	4.79
	Std Dev	1.03	0.59	0.77	1.53
Naïve Younger Wistar Rats (age range 9-12 wk)	Mean	3.80	-	-	-
	Std Dev	0.70	-	-	-
Naïve Older F-344 Rats (age range 47-56 wk)	Mean	4.56	-	-	-
	Std Dev	1.26	-	-	-
Naïve Younger F-344 Rats (age range 9-12 wk)	Mean	6.06	-	-	-
	Std Dev	0.68	-	-	-

(-) No data

The renal clearance rates of ^{3}H-inulin and ^{14}C-oxalate were evaluated in all surviving dose groups of the study (control, 10, 150, and 300 mkd) to evaluate dose-dependent effects on renal function. These parameters were also determined for control, male, young Wistar Han and F344 rats (9-12 weeks of age) and control, male, old F344 rats (47-56 weeks of age) to obtain reference level information on renal function in rats of different strains and ages. Naïve animals were purchased from the vendor shortly prior to clearance assessment, and therefore, were not on the lower-protein NTP diet that the main study animals were fed. No assessment of the potential impact of this variable was done as part of this study.

There were no test material-related changes in oxalate or inulin clearance in the male Wistar Han rat at the end of the 12-month study. As shown in Table 23, the ratio of oxalate/inulin clearance was 0.82 for control male rats and 0.73-0.87 for the three surviving dose groups. No statistically significant, dose-dependent trends were observed in the clearance ratio between these groups. Evaluation of the oxalate clearance alone (Figure 7, Table 22) also showed no statistically significant changes in renal elimination of oxalate between the control and dosed groups, with clearance rates of 3.91-4.79 ml/min/kg bw.

The only statistically significant difference observed in the rate of oxalate clearance was between the non-dosed young Wistar Han and F344 rats. As shown in Figure 8C, the renal elimination of oxalate was significantly higher in the F344 rat. These results are consistent with the higher kidney oxalate levels and increased renal toxicity reported previously in the Wistar rat vs. the F344 (Cruzan *et al.* 2004), following dietary administration of ethylene glycol for 16 weeks.

The ratio of oxalate to inulin clearance appeared lower in the young vs. the old F344 rats (0.70 vs. 0.81; not statistically significant), while the oxalate clearance rate appeared higher for the young F344 rats (6.06 vs. 4.56 ml/min/kg, respectively; not statistically significant) (Tables 22 and 23, Figure 8B). These results are consistent with both a higher rate of oxalate and inulin clearance in the young vs. the old F344 rat, as reported for inulin in Wister rats (Corman *et al.* 1988).

The clearance of oxalate appeared slightly higher in the old F344 vs. Wistar Han rats (4.56 vs. 3.91 ml/min/kg, respectively; not statistically significant). Although old male F344 rats also have a reduced capacity for clearance of OX, similar to that of young and old male Wistar rats, the strain differences in sensitivity are maintained even through one year of exposure.

Metabolism Satellite Group

EG metabolism analysis was conducted by Richard Corley, Ph.D., Battelle Northwest. The report is appended to this report as Appendix B. Blood, urine, and kidney samples collected from the metabolite satellite group of male Wistar rats exposed for 12 months at 0, 50, 150, or 300-mkd EG were analyzed for EG, glycolic acid (GA), and oxalic acid (OX) (Text Tables 11a-c). In addition to these samples, a section of kidney from male rats in the 400-mkd group that were sacrificed on study day 203 due to excessive body weight loss, and a section of kidney from all main study animals necropsied at 12 months, were also analyzed for EG, GA, and OX. The presence of a contaminant in the derivatization agent used for the analysis of EG in all samples except urine, which was analyzed directly, prevented the accurate quantitation of EG. Thus, for EG, only the urine data are reported.

The elimination of EG in urine followed a linear, dose-related relationship. The results represented a slight under-estimate of the total amounts of EG cleared in urine because of the inability to quantitate EG in cage wash samples due to a contaminant in the derivatization reagent, PFBCl. A linear increase in urinary excretion of GA was

observed at 50 and 150 mg/kg/d while a disproportionate (non-linear) increase was observed at 300-mkd. Urinary excretion of OX was similar to controls across all dose levels.

In the kidneys, there were no differences in the concentrations of GA and OX at dose levels up to 150-mkd, compared with controls. However, there were clear non-linear increases in the concentrations of GA and OX at dose levels of 300 and 400-mkd. Concentrations at 400-mkd reached an average of 14 µg/g and 18,800 µg/g for GA and OX, respectively, with some animals having considerably higher concentrations of each metabolite than average. In fact, OX concentrations, when expressed as calcium oxalate, accounted for an average of 2.9% of the total kidney weight (with one animal approaching 11.2%) in the animals exposed to 400 mg/kg/d and sacrificed early in the study.

The concentrations of GA in blood were not significantly different from controls up to 150-mkd. At 300-mkd, the concentrations in blood were approximately 3.3-fold higher than controls although the concentrations were all <10 µg/g regardless of dose level. The concentrations of OA in blood were also similar across all dose levels, averaging 3.7-5.1 µg/g. These results were expected from the low solubility of OA at physiological pH in aqueous media.

Text Table 11a. Total Amounts (mean ± s.d.) of EG, GA, and OX Eliminated in the Urine + Cage Wash Collected 24 hours Prior to Sacrifice of Male Wistar Han Rats Administered Ethylene Glycol in the Diets For Up to 12 Months

Dose Group (mg/kg/day)	EG (µg)	GA (µg)	OX (µg)
Control	nd(1)	52.0 ±40.9	3,015 ±1486
50	2,576 ±1,375	231.5 ±112.0	1,519 ± 989
150	6,469 ± 1892	358.9 ± 105.9	4,211 ± 2,964
300	13,945 ± 8,021	2,100 ± 1,160	4,274 ± 3,111

(1)One control urine had detectable amounts of EG, while no EG was detected in all other samples.

Text Table 11b. Concentrations (mean ± s.d.) of GA and OX in the Kidneys of Male Wistar Han Rats Administered Ethylene Glycol in the Diets For Up to 12 Months

Dose Group (mg/kg/day)	GA (µg/g)	OX (µg/g)
Control (n=13)	1.72 ± 0.85	5.31 ± 4.22
50 (n=15)	1.79 ± 0.97	16.07 ± 35.03
150 (n=14)	1.67 ± 0.95	8.72 ± 7.33
300 (n=10)	8.64 ± 14.11	6,561 ± 18,644
400 (n=15)[1]	13.97 ± 9.54	18,789 ± 23,446

[1]Early sacrifice (day 203).

Text Table 11c. Concentrations (mean ± s.d., n=5) of GA and OX in the Blood of Male Wistar Han Rats Administered Ethylene Glycol in the Diets For Up to 12 Months

Dose Group (mg/kg/day)	GA (µg/g)	OX (µg/g)
Control	2.06 ± 1.38	3.87 ± 2.35
50	3.42 ± 0.87	3.74 ± 2.80
150	2.67 ± 1.89	3.83 ± 0.65
300	6.78 ± 1.75	5.10 ± 2.18

Benchmark Dose Analysis

BMD modeling was conducted by the Sapphire Group, Inc., using the histopathology data described by Dr. Gordon Hard for kidney effects in Wistar rats chronically exposed to EG using data for compound-induced nephropathy, and birefringent crystals. Incidence data and combined incidence X severity data were used for the purposes of defining a dose corresponding to an extra risk of 5% (BMD05) and its lower confidence limit (BMDL05). The BMD05 and BMDL05 values calculated for compound-induced nephropathy using incidence data were120 mg/kg-day and 82 mg/kg-day, respectively. The BMD05 and BMDL05 values calculated for compound-induced nephropathy using incidence X severity data were170 mg/kg-day and 150 mg/kg-day, respectively. The BMD05 and BMDL05 values calculated for compound-induced birefringent crystals using incidence data were140 mg/kg-day and 94 mg/kg-day, respectively. The BMD05 and BMDL05 values calculated for compound-induced birefringent crystals using incidence X severity data were

170 mg/kg-day and 160 mg/kg-day, respectively. The Sapphire BMD report is appended to this report as Appendix C.

DISCUSSION AND CONCLUSIONS

As a result of excessive body weight loss in the 400-mkd group, remaining animals at this dose level were humanely euthanized on test day 203. For the 300-mkd group, due to deaths that occurred prior to the end of the one-year dosing period, some rats previously dedicated to the main group were shifted between groups at the end of the in-life phase of the study to ensure that at least five animals/dose level were in each the metabolism and clearance groups. Since this shifting of animals sometimes resulted in less than 10 animals/dose level remaining in the main toxicity group, a portion of the kidneys from all animals finally dedicated to either the main or metabolism groups were collected for both histopathology as well as metabolite identification, thus resulting in a variable "N" greater than the original design of ten or five animals for general toxicity or kidney metabolites, respectively. Due to deaths that occurred during surgery or due to some non-successful surgeries, some rats previously dedicated to either the main or sentinel control groups were shifted to the clearance control group at the end of the in-life phase of the study to ensure that at least five animals/dose level were in the clearance group.

One control rat died (day 307), no rats given 50-mkd died, one rat given 150-mkd died of a spontaneous rat lymphoma (day 267), and four rats given 300-mkd died (on day 111, 207, 213, or 221) with a fifth rat at that dose level declared moribund on day 138. At 400-mkd, 4 rats died spontaneously or were humanely euthanized in a moribund state (on day 43, 154, 187, or 193). On day 203, the sixteen remaining animals given 400-mkd were humanely euthanized because of excessive body weight loss. The mortality at 300 and 400-mkd was considered treatment-related.

All rats given 300-mkd that died or were declared moribund prior to study termination had gross findings on the bladder and four of them had gross findings on the kidney, with the cause of death attributed to sequelae of urinary obstruction. The underlying cause of death/moribundity determined following gross and histopathologic examination was related to effects on the urinary bladder or kidney as described below.

During the study, animals given 300 or 400-mkd had occasional treatment-related absent/decreased feces, blood in the cage, red urine, red perioral, and perinasal soiling, and/or perineal soiling. There were no treatment-related clinical signs at 50 or 150-mkd.

Rats given 300 or 400-mkd had treatment-related decrements in body weight and body weight gain. The differences from controls occurred within the first few months in animals given 400-mkd and were first statistically identified on day 141, when body weights and body weight gains were 12.7% and 21.4% less than controls, respectively. On study day 197 at 400-mkd, body weights were 20.1% less than controls and body weight gains were 31.3% less; therefore, the remaining rats at this dose were humanely euthanized on study day 203 because of excessive body weight loss. Body weights for rats given 300-mkd were typically lower than controls by mid-study, with all but one animal usually having body weight less than the control mean. These effects were considered related to treatment but were not statistically identified because of the large standard deviations. The body weight effects for rats given 300-mkd occurred gradually, and on study day 141, body weights and body weight gains were 5.2% and 8.4% less than controls, respectively. After day 141, differences from controls in body weights and body weight gains leveled off. No body weight effects occurred at 50 or 150 mkd.

Feed aversion/scratching occurred at ≥ 150-mkd. This was reflected in the smaller sample data set at these doses as feed consumption data were not collected for rats documented as having scratched feed out of their feed crock. Rats given 400-mkd had treatment-related decreases in feed consumption at every time point through termination on day 203, which were typically statistically identified from study day 106. There were no treatment-related decreases in feed consumption for rats given 50, 150, or 300-mkd.

Water consumption was analyzed near the end of the study. Rats given 300-mkd had a treatment-related increase in water consumption of 151% of controls. There were no treatment-related effects on water consumption for animals given 50 or 150-mkd.

After 12 months, decreased urinary pH occurred in all treatment groups but was not considered adverse but rather likely due to the presence of acidic metabolic products of EG. Animals given 300-mkd had increased urine volume and concomitantly decreased urine specific gravity compared to controls, which correlated with the increase in water consumption. The more dilute urine in the 300 mkd group might also explain the finding that less animals in this group had decreased urinary pH than in the 150-mkd group. Analysis of urinary crystals demonstrated treatment-related effects at all EG doses, with the proportion of crystals that were composed of calcium oxalate increasing with increasing EG dose, and those composed of phosphate decreasing with increasing EG dose. This

compositional effect was considered a metabolic consequence of EG exposure as no adverse effects were seen from the crystals observed in the 50 or 150-mkd groups.

Increases in absolute and relative kidney weights occurred in animals given 300 or 400-mkd. These were not statistically identified at 300-mkd and were not statistically analyzed at 400-mkd, but were considered treatment-related. There were no contemporaneous controls for the animals given 400-mkd since they were sacrificed early, but remarkable increases occurred in their absolute and relative kidney weights versus all other groups that went to term, although rats at 400-mkd weighed much less.

Treatment-related gross pathological observations occurred in animals given 300 or 400-mkd and were primarily confined to the kidney and urinary bladder, with secondary treatment-related observations occurring in the lung. For rats given 300-mkd, of 15 rats examined, 7 had findings on the kidney and 8 had findings on the urinary bladder. For rats given 400 mkd, of 20 rats examined, 17 had findings on the kidney and 10 had findings on the urinary bladder. The most relevant observation in the 300-mkd group was the presence of calculi in the bladder (and sometimes the renal pelvis or ureter) in 8 of the total 15 rats examined. This also occurred in 8 of 20 rats at 400-mkd. Calculus formation in the urinary bladder was usually accompanied by dilatation of the bladder and, for the 5 unscheduled deaths at 300-mkd, hemorrhage of the bladder wall, usually with ascites or other edematous change. Three animals given 300-mkd had calculi in the renal pelvis. Almost all rats at 400-mkd showed signs of kidney and/or urinary bladder involvement, including a roughened kidney surface, renal pelvic dilatation, thickened bladder wall, and calculi in the renal pelvis, ureter, or bladder. Of the four unscheduled deaths occurring before early termination of this group, three were observed to have hemorrhage of the bladder wall. Some animals given 400-mkd also had decreased body fat, increased size of the renal lymph nodes, and calculus in the ureter or a dilated ureter. Treatment-related gross pathological effects on the lung, which were less frequent and considered secondary sequelae to effects on the kidney, consisted of a mottled appearance in four rats given 400-mkd. Gross pathological findings of congestion and edema that occurred in the lungs of several animals given either 300 or 400-mkd may have been associated with agonal effects as these animals were found dead. The decrease in body fat observed for five animals given 400-mkd was considered reflective of the general decrease in body weight demonstrated by animals at this dose level. The increased size of the renal lymph nodes was considered a secondary consequence of the renal findings observed in eight animals given 400-mkd.

Histopathological examination showed that a compound-induced nephropathy associated with crystalluria affected the majority of the animals at 300-mkd, and all of those given 400-mkd. None of the renal alterations associated with EG exposure (basophilic foci of crystalluria-related nephropathy, tubule dilatation, birefringent crystals particularly in the pelvic fornix, renal pelvic dilatation, or transitional cell hyperplasia) were observed in the rats given 50 or 150-mkd, establishing the latter dose-level as a NOAEL.

Calculi, up to 2-mm diameter, were found in the bladder, and sometimes in the renal pelvis, at the two highest doses. Since the cause of early death for 3 animals at 300-mkd was unlikely to be related to the extent of the compound-associated kidney changes, which were less than end-stage, bladder tissue from some animals in each group was examined. Histological findings in the bladder and ureter correlated well with the observations of calculi. There were no treatment-related findings in the bladder or ureters of the rats in the 50 and 150 mkd groups. The basic change was simple transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. In animals dying before scheduled termination in groups given 300 or 400-mkd, the acute inflammation and hemorrhage of the bladder wall was a consistent finding in all but one case, and considered to be related to the cause of death. Such severe bladder pathology was often accompanied by a necropsy record of ascites or other edematous change, suggesting that infection via the bladder wall and septicemia may have been the terminal event. Although the cause of death may have been related to the consequences of calculi in the bladder, the most sensitive marker of the adverse effects of ethylene glycol was in the kidneys.

Near the end of the 12-month study, the renal clearance rates of ^{3}H-inulin and ^{14}C-oxalate were evaluated in the control, 50, 150, and 300-mkd groups as well as naïve, male, young Wistar and F344 rats (9-12 weeks of age) and naïve, male, old F344 rats (47-56 wk of age) to obtain information on renal clearance capability in rats of different strains and ages.

There were no treatment-related changes in oxalate or inulin clearance in the male Wistar rats after 12-months. Oxalate/inulin clearance ratios were 0.82 for controls and 0.73-0.87 for the 50, 150, and 300-mkd groups. Oxalate clearance rates ranged from 3.91-4.79 ml/min/kg bw.

Clearance ratios were not significantly different for the young versus old Wistar rats and varied from 0.59 to 0.82, respectively. While these results suggest an age-dependent

increase in oxalate clearance, the actual clearance of oxalate was found to be quite constant with age (3.80-3.91). This variation in oxalate/inulin clearance ratios was most probably due to an age-dependent decrease in inulin clearance. In contrast, the ratio of oxalate to inulin clearance was lower in the young versus old F344 rats (0.70 vs. 0.81; not statistically significant), while the oxalate clearance rate was higher for the young versus old F344 rats (6.06 versus 4.56 ml/min/kg, respectively; not statistically significant), suggesting a higher rate of oxalate and inulin clearance in the young versus old F344 rats.

The only statistically identified difference in the rate of oxalate clearance was between the naïve young Wistar and F344 rats, which was significantly higher in the F344 rat. The clearance of oxalate was slightly higher in the old F344 versus Wistar rat (4.56 vs. 3.91 ml/min/kg, respectively; not statistically significant). Although old male F344 rats also have a reduced capacity for clearance of OX, similar to that of young and old male Wistar rats, the strain differences in sensitivity are maintained even through one year of exposure.

Blood, urine, and kidney samples collected from the metabolite satellite group of Wistar rats exposed for 12 months at 0, 50, 150, or 300-mkd EG were analyzed for EG, glycolic acid (GA), and oxalic acid (OX). A portion of kidney from each animal in the 400-mkd group that was sacrificed on study day 203, and a portion of kidney from all main study animals at 12 months, were also analyzed for EG, GA, and OX. There was a contaminant in the derivatization agent used for the analysis of EG in all samples except urine, which was analyzed directly. Thus for EG, only the urine data are reported.

The excretion of EG in urine followed a linear dose-response relationship across all dose levels. A linear increase in urinary excretion of GA was observed at 50 and 150 mg/kg-day while a disproportionate (non-linear) increase was observed at 300-mkd. Urinary concentrations of OX were similar to controls across all dose levels. In the kidneys, there were no differences in the concentrations of GA and OX at dose levels up to 150-mkd, compared with controls. However, there were clear non-linear increases in the concentrations of GA and OX at dose levels of 300 and 400-mkd. Concentrations at 400-mkd reached an average of 14 µg/g and 18,800 µg/g for GA and OX, respectively, with some animals having considerably higher concentrations of each metabolite than average. In fact, OX concentrations, when expressed as calcium oxalate, accounted for an average of 2.9% of the total kidney weight (with one animal approaching 11.2%) in the animals exposed to 400 mg/kg/d and sacrificed early in the study. As with the results

from the kidneys, the concentrations of GA in blood were not significantly different from controls up to 150-mkd. At 300-mkd, the concentrations in blood were approximately 3.3-fold higher than controls although the concentrations were all <10 µg/g regardless of dose level. The concentrations of OA in blood were also similar across all dose levels, averaging 3.7-5.1 µg/g. These results were expected from the low solubility of OA at physiological pH in aqueous media.

BMD analyses were conducted using compound-induced nephropathy and birefringent crystal data from Wistar rats chronically exposed to EG for the purposes of defining a dose corresponding to an extra risk of 5% (BMD05) and its lower confidence limit (BMDL05). The respective BMD05 and BMDL05 values using incidence and severity were170 mg/kg-day and 150 mg/kg-day for compound-induced nephropathy, and 170 mg/kg-day and 160 mg/kg-day for compound-induced birefringent crystals.

In conclusion, chronic dietary administration of EG to male Wistar Han rats for 12 months resulted in:

- The maximum tolerated dose (MTD) was exceeded at 400-mkd as excessive body weight loss at this level necessitated early termination and there were histopathologic manifestations of marked renal toxicity.
- The lowest-observed-adverse-effect level (LOAEL) was 300-mkd based on gross and microscopic observations of compound-induced nephropathy and urinary bladder changes associated with crystalluria (representative of calcium oxalate), increased absolute and relative kidney weights, decrements in body weight and body weight gain, increased mortality, increased water consumption, a non-linear increase in urinary and blood concentrations of GA, and non-linear increases in the kidney concentrations of GA and OX.
- The no-observed-adverse-effect level (NOAEL) was 150-mkd based on the absence of manifestations of systemic, bladder or renal toxicity at this dose. The most sensitive marker of the adverse effects of EG was in the kidneys at levels greater than 150-mkd.
- A no-observed-effect level (NOEL) was not established as decreased urinary pH and increased urinary oxalate crystals occurred at all treatment levels (≥ 50-mkd), however, these were not considered adverse but rather normal metabolic/physiological consequences of chronic EG exposure.
- There were no treatment-related effects on renal clearance of oxalate or inulin.

- Urinary excretion of OX was similar to controls across all doses, that of EG followed a linear dose-response relationship, and that of GA was linear between 50 and 150-mkd, with a disproportionate non-linear increase at 300-mkd.
- Kidney concentrations of GA and OX were similar to controls at doses up to 150-mkd. However, there were clear non-linear increases in the kidney concentrations of GA and OX at dose levels of 300 and 400-mkd.
- The respective BMD05 and BMDL05 values using incidence and severity data were 170 mg/kg-day and 150 mg/kg-day for compound-induced nephropathy, and 170 mg/kg-day and 160 mg/kg-day for compound-induced birefringent crystals.

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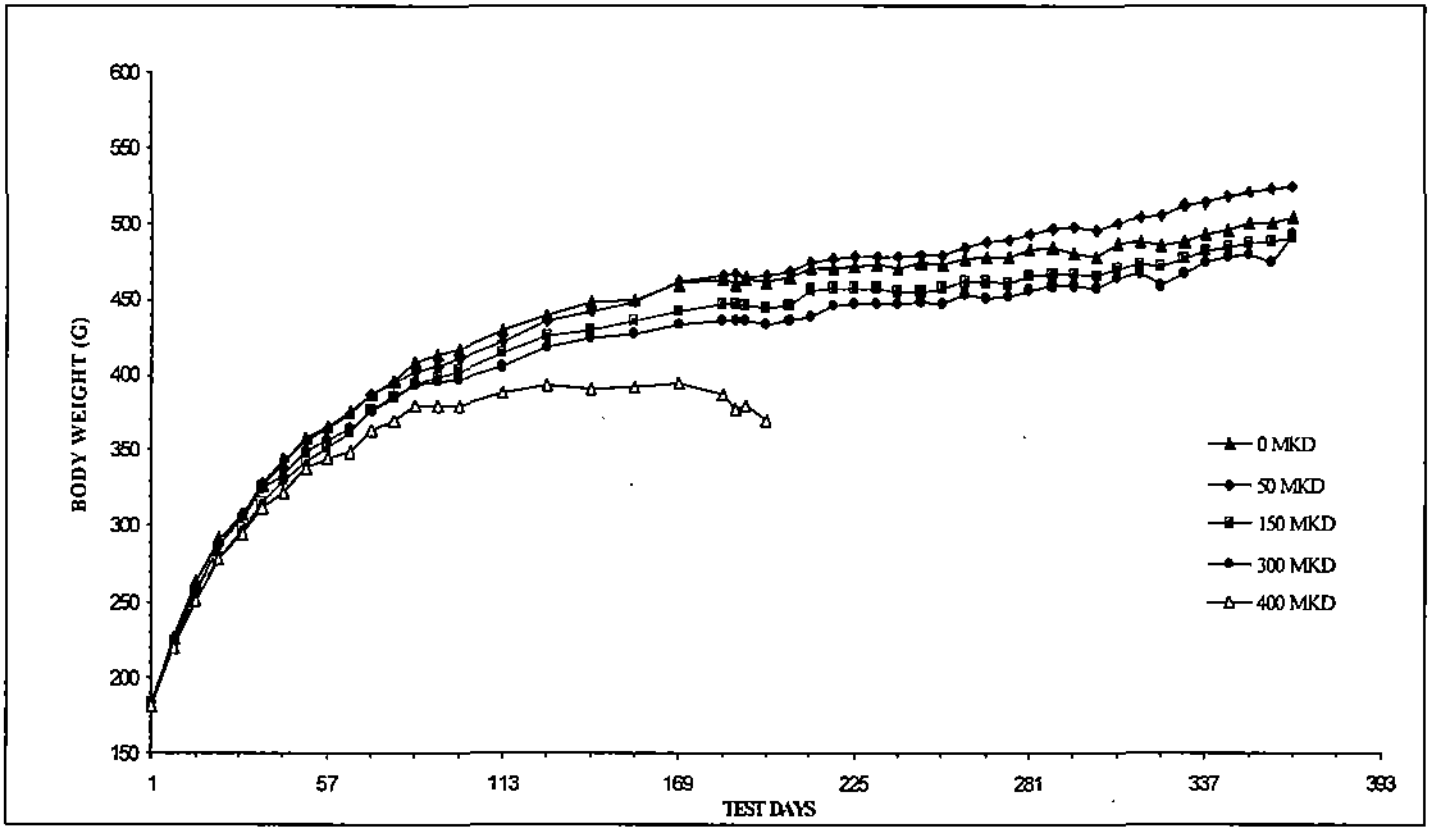
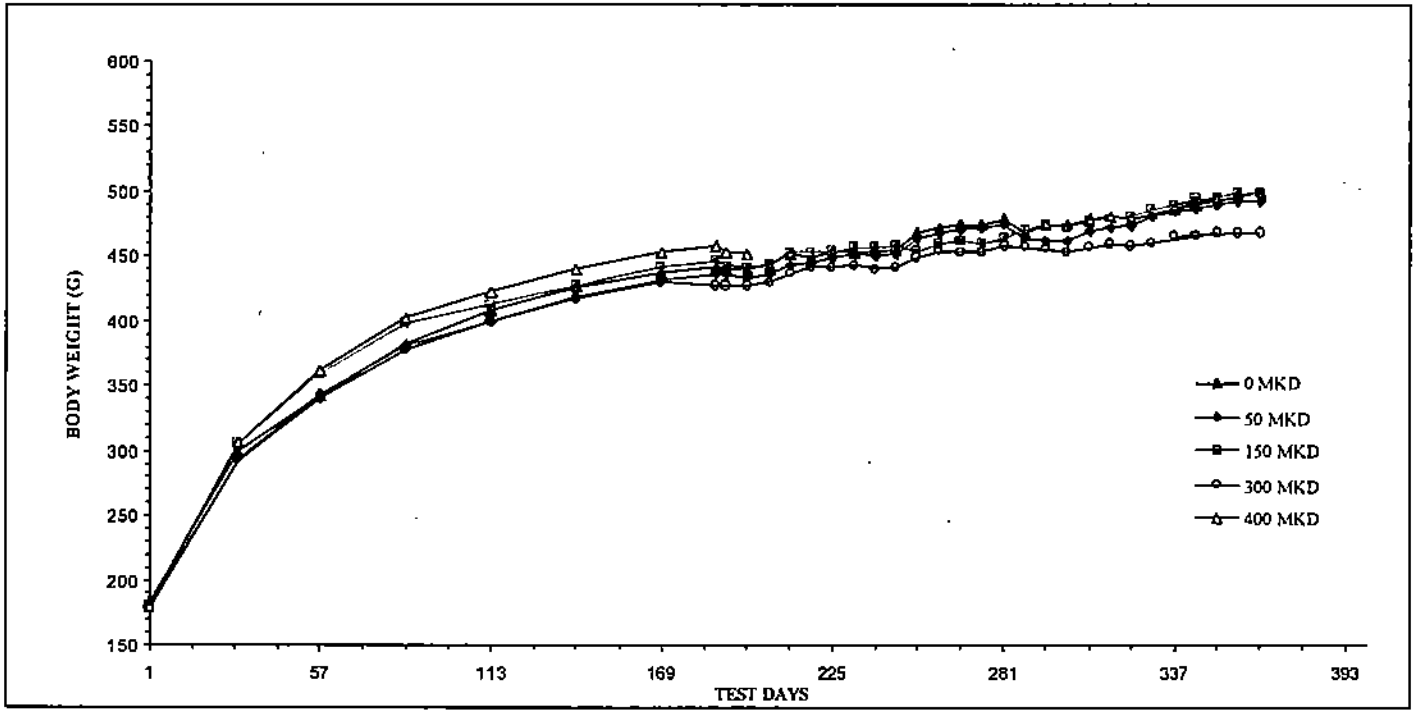
ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

FIGURE 1. Body Weights – Main Group



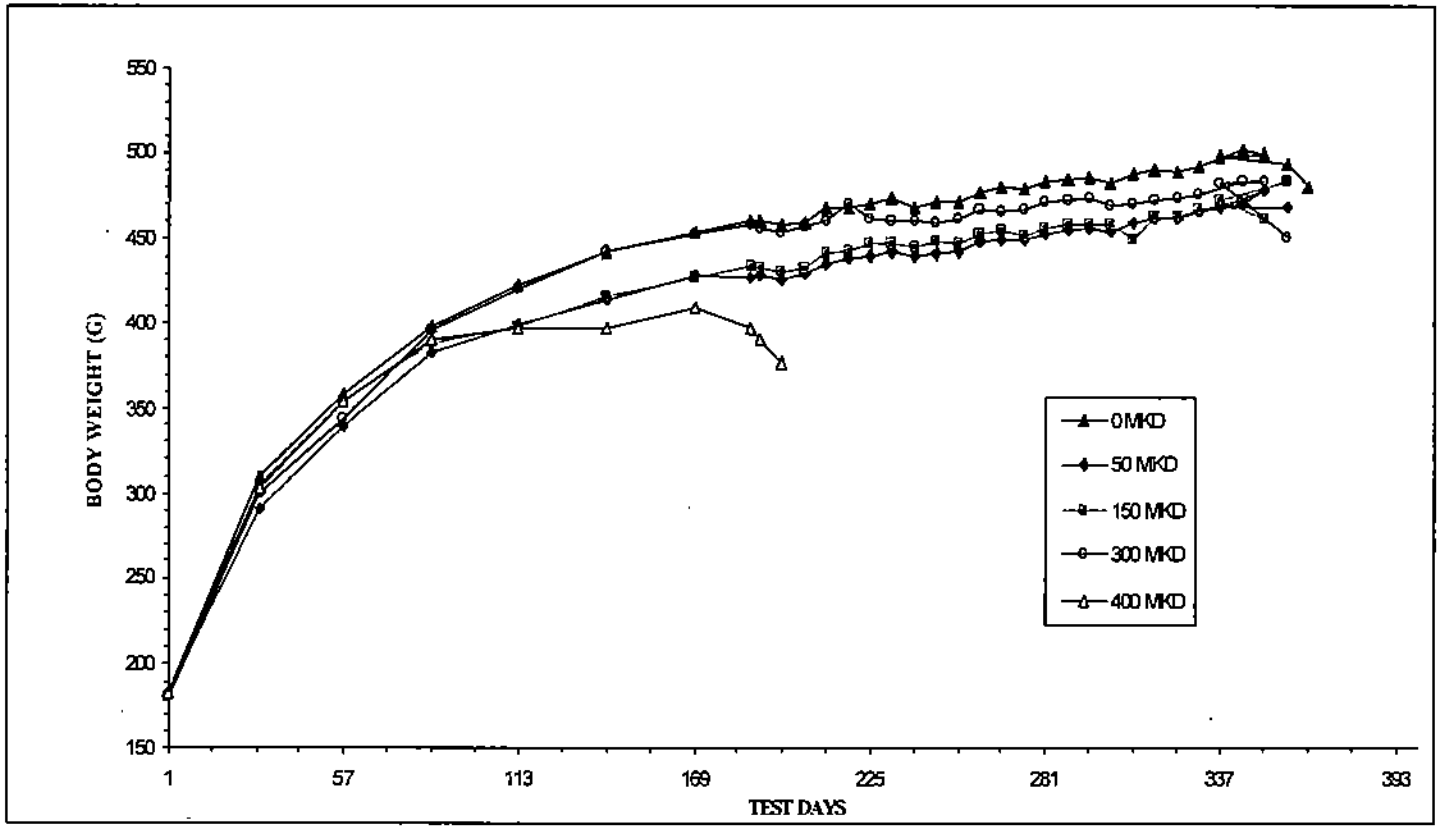
ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

FIGURE 2. Body Weights – Metabolism Group



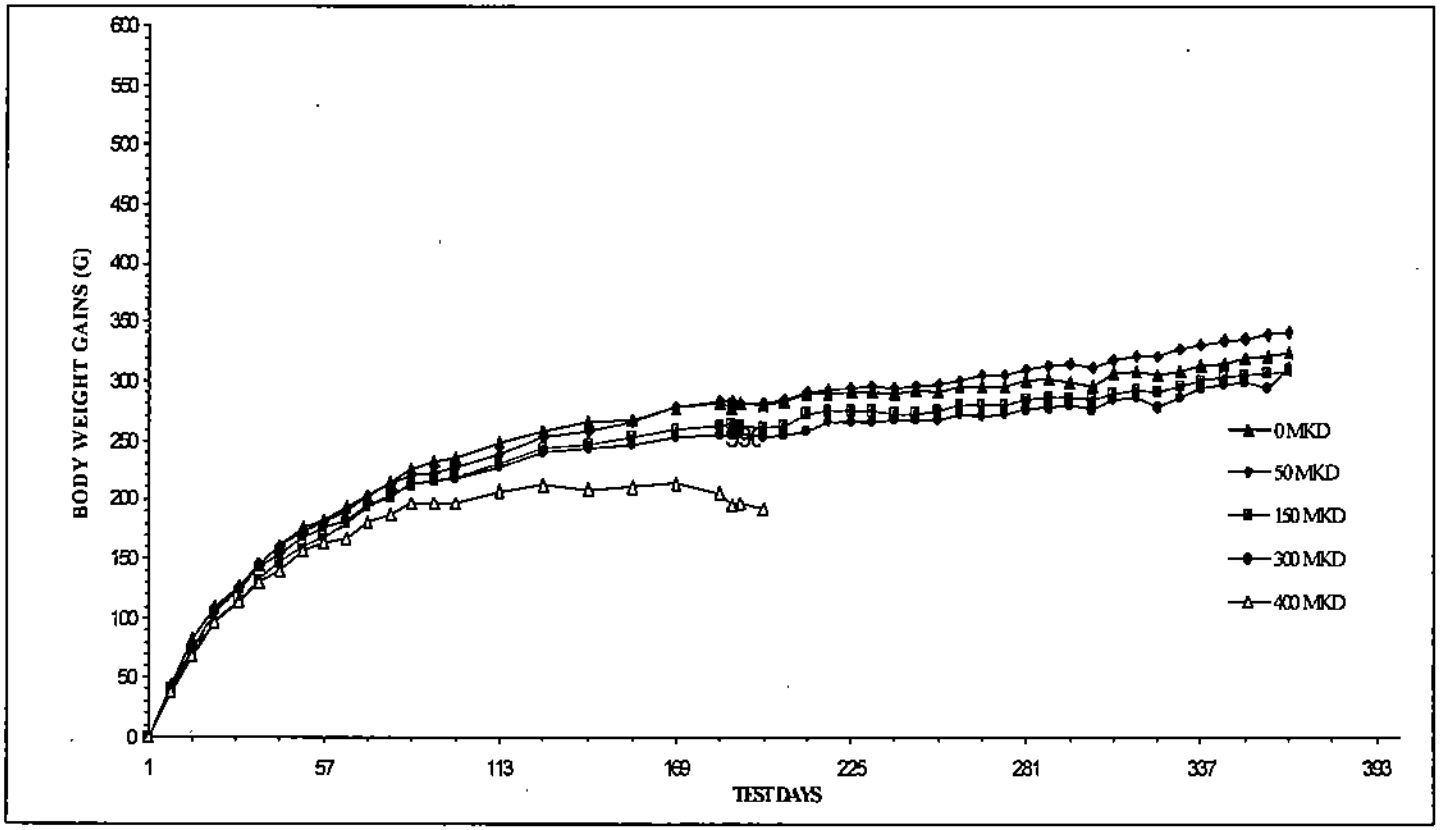
ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

FIGURE 3. Body Weights – Oxalate Clearance Group



ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

FIGURE 4. Body Weights Gains – Main Group



ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

FIGURE 5. Body Weights Gains – Metabolism Group

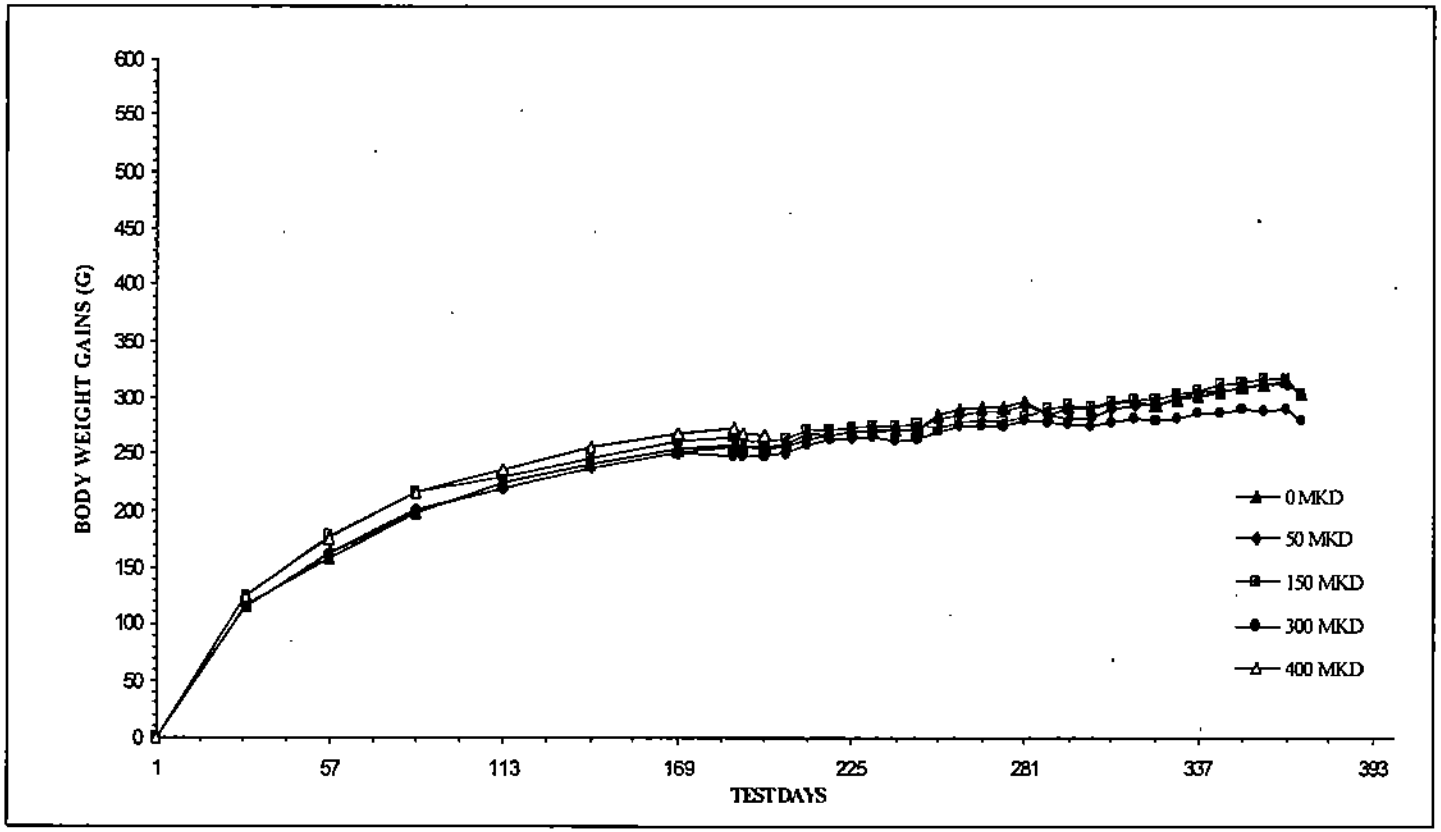


FIGURE 6. Body Weights Gains – Oxalate Clearance Group

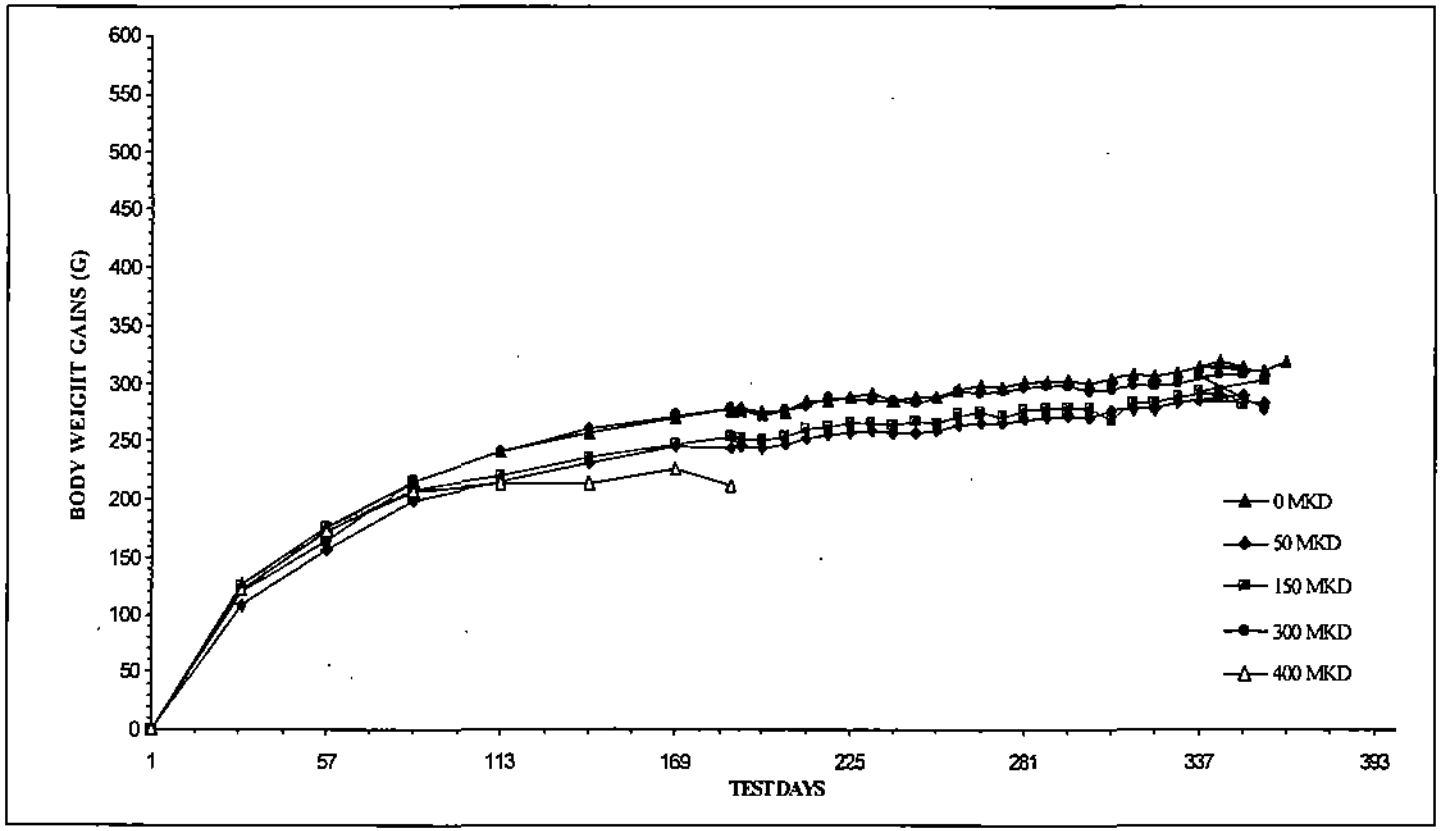


FIGURE 7. Renal Clearance of Oxalate in the Wistar Rat Administered EG for 12 Months

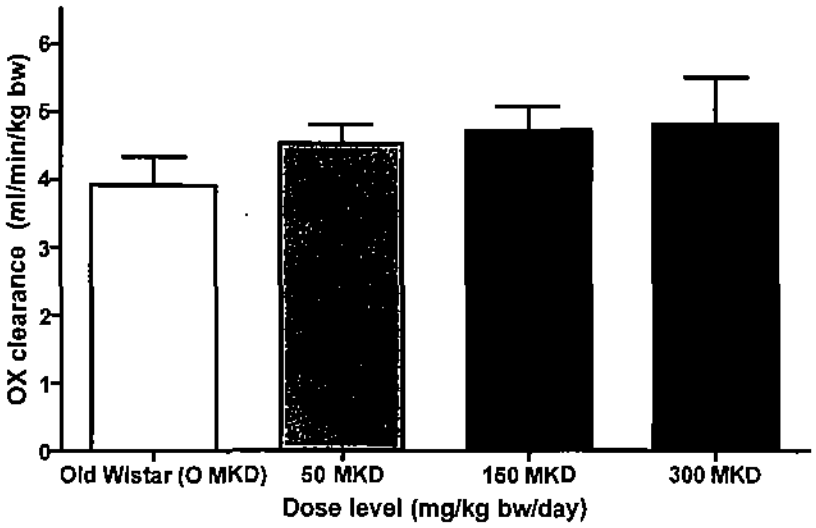


FIGURE 8. Renal Clearance of Oxalate in Control Rats

A) 3-month Wistar vs. 12-month Wistar; B) 3-month F344 vs. 12-month F344; C) 3-month F344 vs. 3-month Wistar (* statistically different, $p < 0.001$); D) 12-month F344 vs. 12-month Wistar.

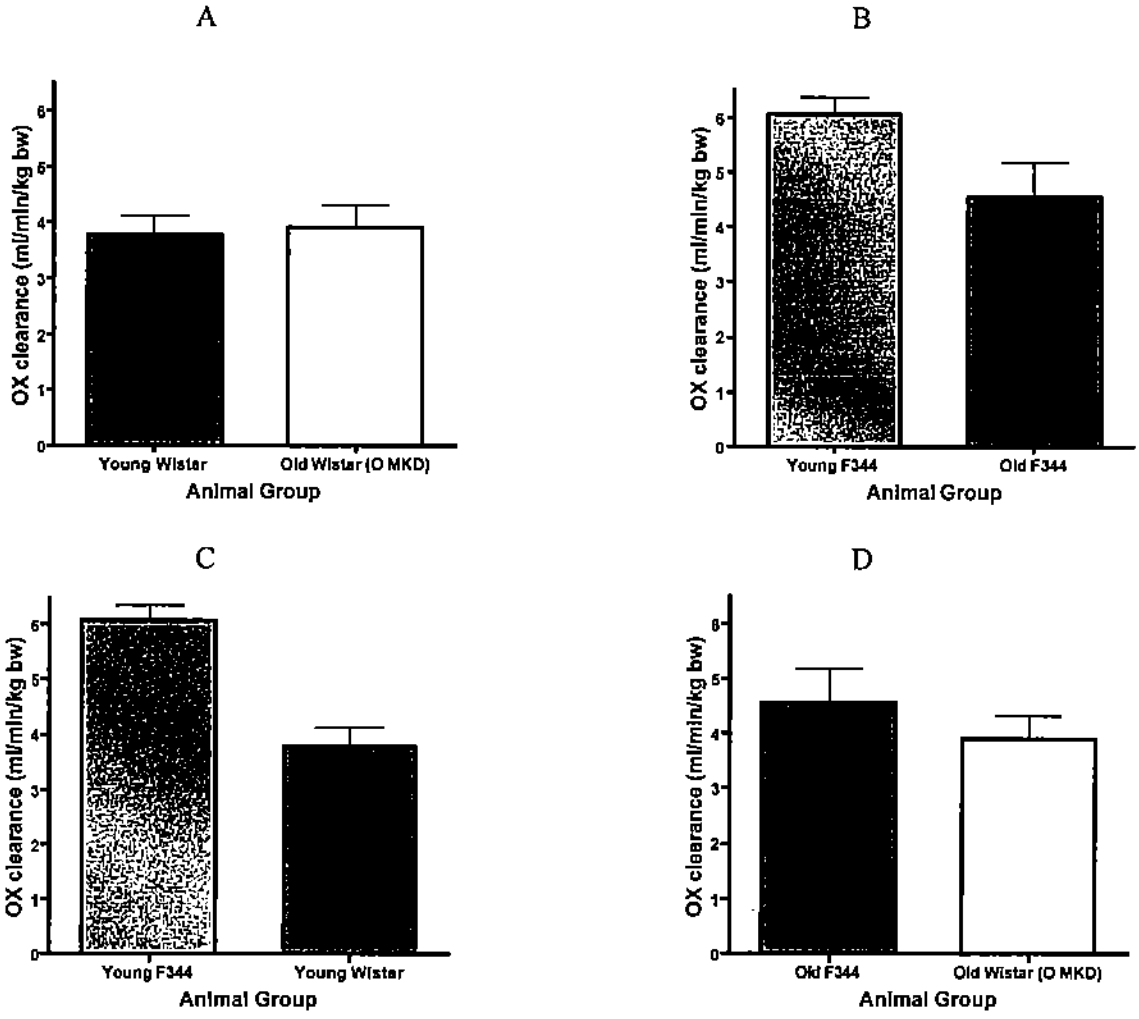


TABLE 1. Study Specific Parameters

Study Parameters	Main Group # of Animals/Dose	Metabolite Satellite Group # of Animals/Dose	Oxalate Clearance Group # of Animals/Dose
Cage-side Observations	10	5	5
Body Weights	10	5	5
Feed Consumption	10	-	-
Test Material Intake	10	-	-
Feed Efficiency	10	-	-
Clinical Observations	10	5	5
Urinalysis	10	5	-
Necropsy	10	5	5
Organ Weights	10	5	-
Histopathology	10	-	-

TABLE 2. DCO Parameters, Functional Tests and Mode of Recording

Clinical Observations

For the categories listed below, the observer directly records the positive observation.

1.	Abnormal behavior	Description
2.	Abnormalities of the eye	Description
3.	Abnormal urine or feces	Description
4.	Abnormalities of the gastrointestinal tract	Description
5.	Injury	Description
6.	Missing extremity	Description
7.	Abnormal muscle movements	Description
8.	Palpable mass/swellings	Description
9.	Abnormal posture	Description
10.	Abnormalities of the reproductive system	Description
11.	Abnormal respiration	Description
12.	Abnormal skin or haircoat/mucous membranes	Description
13.	Excessive soiling	Description
14.	General abnormalities	Description

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TABLE 3. Tissues Collected and Preserved at Necropsy

ADRENALS
AORTA
AUDITORY SEBACEOUS GLANDS
BONE (INCLUDING JOINT)
BONE MARROW
BRAIN (CEREBRUM, BRAINSTEM, CEREBELLUM)
CECUM

COAGULATING GLANDS
COLON
CRANIAL NERVE - OPTIC
DUODENUM
EPIDIDYMIDES
ESOPHAGUS
EYES
GROSS LESIONS
HEART
ILEUM
JEJUNUM
KIDNEYS
LACRIMAL/HARDERIAN GLANDS
LARYNX
LIVER
LUNGS

MEDIASTINAL LYMPH NODE
MEDIASTINAL TISSUES
MESENTERIC LYMPH NODE
MESENTERIC TISSUES
NASAL TISSUES/PHARYNX
ORAL TISSUES

PANCREAS
PARATHYROID GLANDS
PERIPHERAL NERVE - TIBIAL
PITUITARY
PROSTATE
RECTUM
SALIVARY GLANDS
SEMINAL VESICLES
SKELETAL MUSCLE
SKIN AND SUBCUTIS
SPINAL CORD (CERVICAL, THORACIC, LUMBAR)
SPLEEN
STOMACH
TESTES
THYMUS
THYROID GLAND
TONGUE
TRACHEA
URINARY BLADDER

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TABLE 4. Homogeneity of Test Material in Diet

ACL Report # :	2003-143		2003-154		2003-167		2003-176		2003-205		2004-27		2004-100		2004-128	
Date Mixed:	2-Sep-03		23-Sep-03		2-Oct-03		20-Oct-03		30 Nov-03		22-Feb-04		27-Jun-04		22-Aug-04	
Target Conc. (%w/w)	0.0553	0.443	0.0644	0.498	0.0693	0.549	0.076	0.593	0.0892	0.689	0.104	0.858	0.114	0.612	0.116	0.62
Dose Level:	50 MKD	400 MKD	50 MKD	400 MKD	50 MKD	400 MKD	50 MKD	400 MKD	50 MKD	400 MKD	50 MKD	400 MKD	50 MKD	300 MKD	50 MKD	300 MKD
Location																
Top - A	0.0522	0.420	0.109	0.400	0.0648	0.510	0.0686	0.536	0.0837	0.677	0.108	0.914	0.106	0.554	0.111	0.619
Bottom - A	0.0602	0.411	0.0624	0.412	0.0661	0.542	0.0691	0.547	0.0808	0.687	0.108	0.929	0.103	0.567	0.112	0.608
Top - B	0.0570	0.411	0.0553	0.493	0.0620	0.538	0.0664	0.560	0.0802	0.698	0.105	0.965	0.109	0.58	0.114	0.611
Bottom - B	0.0558	0.398	0.0431	0.343	0.0660	0.553	0.0717	0.592	0.0855	0.709	0.107	0.802	0.107	0.564	0.114	0.602
Top - C	0.0543	0.413	0.0640	0.474	0.0647	0.558	0.0684	0.555	0.0843	0.699	0.103	0.849	0.103	0.556	0.111	0.617
Botom - C	0.0600	0.405	0.0493	0.539	0.0643	0.589	0.0723	0.555	0.0778	0.763	0.109	0.861	0.101	0.602	0.112	0.609
Mean	0.0566	0.410	0.0638	0.444	0.0647	0.548	0.0694	0.558	0.0821	0.706	0.107	0.887	0.105	0.571	0.112	0.611
% RSD	5.56	1.83	36.6	16.1	2.32	4.78	3.19	3.38	3.56	4.29	2.11	6.75	2.86	3.15	1.22	1.02

TABLE 5. Stability of Test Material in Diet

A. Stability in feed crocks (unsealed) and exposed to indirect light/dark cycle at ambient temperature

Dose Level	Date of Analysis	Observed Conc. % (w/w)	Percent of Initial Day	ACL Report #
0.005%	8/11/2003	0.00500	NA	2003-151
	8/19/2003	0.00504	101%	2003-151
0.05%	8/11/2003	0.0485	NA	2003-151
	8/19/2003	0.0473	97.7%	2003-151
0.5%	8/11/2003	0.481	NA	2003-151
	8/19/2003	0.461	95.9%	2003-151
5%	8/11/2003	5.07	NA	2003-151
	8/19/2003	4.96	97.9%	2003-151

B. Stability in sealed containers at ambient temperature and no direct light

Dose Level	Date of Analysis	Observed Conc. % (w/w)	Percent of Initial Day	ACL Report #
0.005%	8/11/2003	0.00500	NA	2003-151
	9/2/2003	0.00518	104%	2003-151
0.05%	8/11/2003	0.0485	NA	2003-151
	9/2/2003	0.0471	97.3%	2003-151
0.5%	8/11/2003	0.481	NA	2003-151
	9/2/2003	0.449	93.5%	2003-151
5%	8/11/2003	5.07	NA	2003-151
	9/2/2003	4.96	98.0%	2003-151

NA = Not Applicable
Data obtained from ACL Reports which are Dow Internal Reports

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TABLE 6. Concentration of Test Material in Diet

Mean Percent of Target Concentration

Dose Level	ACL Report #: Mix Date:	2003-143 2-Sep-03	2003-154 23-Sep-03	2003-167 2-Oct-03	2003-176 20-Oct-03	2003-205 30-Nov-03	2004-27 22-Feb-04	2004-100 27-Jun-04	2004-128 22-Aug-04	Overall Mean	Number of Analyses
Control		<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	8
M-50 MKD		102%	99.0%	93.3%	91.3%	92.0%	103%	92.0%	96.8%	96.2%	8
M-150 MKD		96.2%	110%	98.9%	93.4%	96.1%	106%	94.1%	99.0%	99.2%	8
M-300 MKD		96.0%	82.2%	97.8%	93.5%	98.0%	107%	93.2%	98.5%	95.8%	8
M-400 MKD		92.5%	89.1%	99.9%	94.0%	102%	103%	N/A	N/A	96.8%	8
5% Premix		102%	111%	104%	98.5%	117%	98.4%	98.3%	105%	104%	8

<LLQ = less than lower limit of quantitation ranging from: 0.000410% (w/w) to 0.0209% (w/w) in feed
Data obtained from ACL Reports which are Dow Internal Reports

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 7. Summary of Clinical Observations – Main Group

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Number of Animals Examined						
DAY	1	10	10	10	10	10
DAY	8	10	10	10	10	10
DAY	15	10	10	10	10	10
DAY	22	10	10	10	10	10
DAY	30	10	10	10	10	10
DAY	36	10	10	10	10	10
DAY	43	10	10	10	10	10
DAY	50	10	10	10	10	10
DAY	57	10	10	10	10	10
DAY	64	10	10	10	10	10
DAY	71	10	10	10	10	10
DAY	78	10	10	10	10	10
DAY	85	10	10	10	10	10
DAY	92	10	10	10	10	10
DAY	99	10	10	10	10	10
DAY	113	10	10	10	9	10
DAY	127	10	10	10	9	10
DAY	141	10	10	10	8	10
DAY	155	10	10	10	8	10
DAY	169	10	10	10	8	10
DAY	183	10	10	10	8	10
DAY	187	-	-	-	-	1
DAY	197	10	10	10	8	8
DAY	211	10	10	10	7	-
DAY	225	10	10	10	7	-
DAY	239	10	10	10	7	-
DAY	253	10	10	10	7	-
DAY	266	-	-	1	-	-
DAY	267	10	10	10	7	-
DAY	281	10	10	9	7	-
DAY	295	10	10	9	7	-
DAY	302	1	-	-	-	-
DAY	307	1	-	-	-	-
DAY	309	9	10	9	7	-
DAY	323	9	10	9	7	-
DAY	337	9	10	9	7	-
DAY	351	9	10	9	7	-
DAY	365	9	10	9	5	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 7. Summary of Clinical Observations – Main Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
-						
Number of Animals Examined						
All Categories, Within Normal Limits						
DAY	1	10	10	9	10	10
DAY	8	10	10	9	10	10
DAY	15	10	10	9	10	10
DAY	22	10	10	9	10	10
DAY	30	9	10	9	10	9
DAY	36	9	10	9	10	10
DAY	43	9	10	9	10	10
DAY	50	9	10	9	10	10
DAY	57	9	10	9	10	10
DAY	64	9	10	9	10	9
DAY	71	9	10	9	10	9
DAY	78	9	10	9	10	10
DAY	85	9	10	9	10	10
DAY	92	9	10	9	10	10
DAY	99	9	10	9	10	10
DAY	113	9	10	9	9	10
DAY	127	9	10	9	9	10
DAY	141	8	10	9	8	10
DAY	155	8	10	9	8	10
DAY	169	8	10	9	8	10
DAY	183	8	10	9	8	10
DAY	197	8	10	9	8	8
DAY	211	8	10	9	7	-
DAY	225	8	10	9	7	-
DAY	239	8	10	9	7	-
DAY	253	8	10	9	7	-
DAY	267	8	10	8	7	-
DAY	281	8	10	8	7	-
DAY	295	8	10	8	7	-
DAY	309	8	10	8	7	-
DAY	323	8	10	8	7	-
DAY	337	8	10	8	7	-
DAY	351	8	10	7	7	-
DAY	365	8	10	7	5	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 7. Summary of Clinical Observations – Main Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
-						
Eyes, Cloudy						
DAY	141	1	0	0	0	0
DAY	155	1	0	0	0	0
DAY	169	1	0	0	0	0
DAY	183	1	0	0	0	0
DAY	197	1	0	0	0	0
DAY	211	1	0	0	0	-
DAY	225	1	0	0	0	-
DAY	239	1	0	0	0	-
DAY	253	1	0	0	0	-
DAY	267	1	0	0	0	-
DAY	281	1	0	0	0	-
DAY	295	1	0	0	0	-
DAY	309	1	0	0	0	-
DAY	323	1	0	0	0	-
DAY	337	1	0	0	0	-
DAY	351	1	0	0	0	-
DAY	365	1	0	0	0	-
Eyes, Enlarged or Protruding						
DAY	253	1	0	0	0	-
DAY	267	1	0	0	0	-
DAY	281	1	0	0	0	-
DAY	295	1	0	0	0	-
DAY	309	1	0	0	0	-
DAY	323	1	0	0	0	-
DAY	337	1	0	0	0	-
DAY	351	1	0	0	0	-
DAY	365	1	0	0	0	-
Feces, Abnormal Quantity, Absent						
DAY	187	-	-	-	-	1
Feces, Abnormal Quantity, Decreased						
DAY	266	-	-	1	-	-
DAY	267	0	0	1	0	-
Gait, Dragging Hindquarters, Limbs Flacid						
DAY	307	1	-	-	-	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 7. Summary of Clinical Observations – Main Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Gait, Dragging Hindquarters, Limbs Flacid						
Gastrointestinal Tract, Malocclusion Incisors						
DAY	30	1	0	0	0	0
DAY	36	1	0	0	0	0
DAY	43	1	0	0	0	0
DAY	50	1	0	0	0	0
DAY	57	1	0	0	0	0
DAY	64	1	0	0	0	1
DAY	71	1	0	0	0	1
DAY	78	1	0	0	0	0
DAY	85	1	0	0	0	0
DAY	92	1	0	0	0	0
DAY	99	1	0	0	0	0
DAY	113	1	0	0	0	0
DAY	127	1	0	0	0	0
DAY	141	1	0	0	0	0
DAY	155	1	0	0	0	0
DAY	169	1	0	0	0	0
DAY	183	1	0	0	0	0
DAY	197	1	0	0	0	0
DAY	211	1	0	0	0	-
DAY	225	1	0	0	0	-
DAY	239	1	0	0	0	-
DAY	253	1	0	0	0	-
DAY	267	1	0	0	0	-
DAY	281	1	0	0	0	-
DAY	295	1	0	0	0	-
DAY	302	1	-	-	-	-
DAY	307	1	-	-	-	-
Injury, Apparent Mechanical, Trauma						
DAY	1	0	0	1	0	0
DAY	8	0	0	1	0	0
DAY	15	0	0	1	0	0
DAY	22	0	0	1	0	0
DAY	30	0	0	1	0	0
DAY	36	0	0	1	0	0
DAY	43	0	0	1	0	0

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 7. Summary of Clinical Observations – Main Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
-						
Injury, Apparent Mechanical, Trauma						
DAY	50	0	0	1	0	0
DAY	57	0	0	1	0	0
DAY	64	0	0	1	0	0
DAY	71	0	0	1	0	0
DAY	78	0	0	1	0	0
DAY	85	0	0	1	0	0
DAY	92	0	0	1	0	0
DAY	99	0	0	1	0	0
DAY	113	0	0	1	0	0
DAY	127	0	0	1	0	0
DAY	141	0	0	1	0	0
DAY	155	0	0	1	0	0
DAY	169	0	0	1	0	0
DAY	183	0	0	1	0	0
DAY	197	0	0	1	0	0
DAY	211	0	0	1	0	-
DAY	225	0	0	1	0	-
DAY	239	0	0	1	0	-
DAY	253	0	0	1	0	-
DAY	267	0	0	1	0	-
DAY	281	0	0	1	0	-
DAY	295	0	0	1	0	-
DAY	309	0	0	1	0	-
DAY	323	0	0	1	0	-
DAY	337	0	0	1	0	-
DAY	351	0	0	1	0	-
DAY	365	0	0	1	0	-
Miscellaneous, Blood in Cage						
DAY	187	-	-	-	-	1
Skin/Fur/Mucous Membranes, Flaking/Scaling, Focal						
DAY	30	0	0	0	0	1
Skin/Fur/Mucous Membranes, Skin, Scab, Focal						
DAY	351	0	0	1	0	-
DAY	365	0	0	1	0	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 7. Summary of Clinical Observations – Main Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
-						
Skin/Fur/Mucous Membranes, Skin/Mucous Membranes Pale						
DAY	266	-	-	1	-	-
DAY	267	0	0	1	0	-
DAY	302	1	-	-	-	-
DAY	307	1	-	-	-	-
Soiling, Perinasal, Red						
DAY	266	-	-	1	-	-
DAY	267	0	0	1	0	-
Soiling, Periocular, Red						
DAY	30	1	0	0	0	0
DAY	36	1	0	0	0	0
DAY	43	1	0	0	0	0
DAY	50	1	0	0	0	0
DAY	57	1	0	0	0	0
DAY	64	1	0	0	0	0
DAY	71	1	0	0	0	0
DAY	78	1	0	0	0	0
DAY	85	1	0	0	0	0
DAY	92	1	0	0	0	0
DAY	99	1	0	0	0	0
DAY	113	1	0	0	0	0
DAY	127	1	0	0	0	0
DAY	141	1	0	0	0	0
DAY	155	1	0	0	0	0
DAY	169	1	0	0	0	0
DAY	183	1	0	0	0	0
DAY	197	1	0	0	0	0
DAY	211	1	0	0	0	-
DAY	225	1	0	0	0	-
DAY	239	1	0	0	0	-
DAY	253	1	0	0	0	-
DAY	267	1	0	0	0	-
DAY	281	1	0	0	0	-
DAY	295	1	0	0	0	-
DAY	302	1	-	-	-	-
DAY	307	1	-	-	-	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 8. Summary of Clinical Observations – Metabolism Group

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Number of Animals Examined						
DAY	1	5	5	5	5	5
DAY	30	5	5	5	5	5
DAY	57	5	5	5	5	4
DAY	85	5	5	5	5	4
DAY	113	5	5	5	5	4
DAY	141	5	5	5	5	4
DAY	169	5	5	5	5	4
DAY	197	5	5	5	5	4
DAY	211	5	5	5	5	-
DAY	225	5	5	5	5	-
DAY	239	5	5	5	5	-
DAY	253	5	5	5	5	-
DAY	267	5	5	5	5	-
DAY	281	5	5	5	5	-
DAY	295	5	5	5	5	-
DAY	309	5	5	5	5	-
DAY	323	5	5	5	5	-
DAY	337	5	5	5	5	-
DAY	351	5	5	5	5	-
DAY	365	5	5	5	5	-
All Categories, Within Normal Limits						
DAY	1	5	5	5	4	5
DAY	30	5	4	5	4	5
DAY	57	5	4	5	4	4
DAY	85	5	4	5	4	4
DAY	113	5	4	5	4	4
DAY	141	5	3	5	4	4
DAY	169	5	3	5	4	4
DAY	197	5	3	5	4	4
DAY	211	5	4	5	4	-
DAY	225	5	4	5	4	-
DAY	239	5	4	5	4	-
DAY	253	5	4	5	4	-
DAY	267	5	4	5	4	-
DAY	281	5	4	5	4	-
DAY	295	5	4	5	4	-
DAY	309	5	4	5	4	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 8. Summary of Clinical Observations – Metabolism Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
All Categories, Within Normal Limits						
DAY	323	5	4	5	4	-
DAY	337	5	4	5	4	-
DAY	351	5	4	5	4	-
DAY	365	5	4	5	4	-
Gastrointestinal Tract, Malocclusion Incisors						
DAY	30	0	1	0	0	0
DAY	57	0	1	0	0	0
DAY	85	0	1	0	0	0
DAY	113	0	1	0	0	0
DAY	141	0	1	0	0	0
DAY	169	0	1	0	0	0
DAY	197	0	1	0	0	0
DAY	211	0	1	0	0	-
DAY	225	0	1	0	0	-
DAY	239	0	1	0	0	-
DAY	253	0	1	0	0	-
DAY	267	0	1	0	0	-
DAY	281	0	1	0	0	-
DAY	295	0	1	0	0	-
DAY	309	0	1	0	0	-
DAY	323	0	1	0	0	-
DAY	337	0	1	0	0	-
DAY	351	0	1	0	0	-
DAY	365	0	1	0	0	-
Injury, Apparent Mechanical, Other						
DAY	1	0	0	0	1	0
DAY	30	0	0	0	1	0
DAY	57	0	0	0	1	0
DAY	85	0	0	0	1	0
DAY	113	0	0	0	1	0
DAY	141	0	0	0	1	0
DAY	169	0	0	0	1	0
DAY	197	0	0	0	1	0
DAY	211	0	0	0	1	-
DAY	225	0	0	0	1	-
DAY	239	0	0	0	1	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 8. Summary of Clinical Observations – Metabolism Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
-						
Injury, Apparent Mechanical, Other						
DAY	253	0	0	0	1	-
DAY	267	0	0	0	1	-
DAY	281	0	0	0	1	-
DAY	295	0	0	0	1	-
DAY	309	0	0	0	1	-
DAY	323	0	0	0	1	-
DAY	337	0	0	0	1	-
DAY	351	0	0	0	1	-
DAY	365	0	0	0	1	-
Skin/Fur/Mucous Membranes, Excessive Hairloss						
DAY	141	0	1	0	0	0
DAY	169	0	1	0	0	0
DAY	197	0	1	0	0	0
Soiling, Periocular, Red						
DAY	30	0	1	0	0	0
DAY	57	0	1	0	0	0
DAY	85	0	1	0	0	0
DAY	113	0	1	0	0	0
DAY	141	0	1	0	0	0
DAY	169	0	1	0	0	0
DAY	197	0	1	0	0	0
DAY	211	0	1	0	0	-
DAY	225	0	1	0	0	-
DAY	239	0	1	0	0	-
DAY	253	0	1	0	0	-
DAY	267	0	1	0	0	-
DAY	281	0	1	0	0	-
DAY	295	0	1	0	0	-
DAY	309	0	1	0	0	-
DAY	323	0	1	0	0	-
DAY	337	0	1	0	0	-
DAY	351	0	1	0	0	-
DAY	365	0	1	0	0	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 9. Summary of Clinical Observations Oxalate Clearance Group

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Number of Animals Examined						
DAY	1	5	5	5	5	5
DAY	30	5	5	5	5	5
DAY	53	-	-	-	1	-
DAY	57	5	5	5	5	5
DAY	85	5	5	5	5	5
DAY	92	-	-	1	-	-
DAY	113	5	5	5	5	5
DAY	141	5	5	5	5	5
DAY	169	5	5	5	5	4
DAY	197	5	5	5	5	4
DAY	211	5	5	5	5	-
DAY	212	-	-	-	1	-
DAY	218	-	-	-	1	-
DAY	219	-	-	-	1	-
DAY	225	5	5	5	3	-
DAY	239	5	5	5	3	-
DAY	246	-	-	1	-	-
DAY	253	5	5	5	3	-
DAY	267	5	5	5	3	-
DAY	281	5	5	5	3	-
DAY	295	5	5	5	3	-
DAY	309	5	5	5	3	-
DAY	323	5	5	5	3	-
DAY	330	-	-	-	1	-
DAY	337	5	5	5	3	-
DAY	351	5	3	5	3	-
DAY	365	1	-	-	-	-
All Categories, Within Normal Limits						
DAY	1	5	5	5	5	5
DAY	30	5	5	5	5	5
DAY	57	5	5	5	4	5
DAY	85	5	5	5	4	5
DAY	113	5	5	4	4	5
DAY	141	5	5	4	4	5
DAY	169	5	5	3	4	4
DAY	197	5	5	3	4	4
DAY	211	5	5	3	3	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 9. Summary of Clinical Observations – Oxalate Clearance Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
-						
All Categories, Within Normal Limits						
DAY	225	5	5	3	2	-
DAY	239	5	5	3	2	-
DAY	253	5	5	3	2	-
DAY	267	5	5	3	2	-
DAY	281	5	4	3	2	-
DAY	295	5	5	3	2	-
DAY	309	5	5	3	2	-
DAY	323	5	5	3	2	-
DAY	337	5	5	3	2	-
DAY	351	5	3	3	2	-
DAY	365	1	-	-	-	-
Feces, Abnormal Quantity, Decreased						
DAY	212	-	-	-	1	-
DAY	219	-	-	-	1	-
Gastrointestinal Tract, Malocclusion Incisors						
DAY	53	-	-	-	1	-
DAY	57	0	0	0	1	0
DAY	85	0	0	0	1	0
DAY	92	-	-	1	-	-
DAY	113	0	0	1	1	0
DAY	141	0	0	1	1	0
DAY	169	0	0	1	1	0
DAY	197	0	0	1	1	0
DAY	211	0	0	1	1	-
DAY	225	0	0	1	1	-
DAY	239	0	0	1	1	-
DAY	246	-	-	1	-	-
DAY	253	0	0	1	1	-
DAY	267	0	0	1	1	-
DAY	281	0	0	1	1	-
DAY	295	0	0	1	1	-
DAY	309	0	0	1	1	-
DAY	323	0	0	1	1	-
DAY	337	0	0	1	1	-
DAY	351	0	0	1	1	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 9. Summary of Clinical Observations – Oxalate Clearance Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Injury, Apparent Mechanical, Trauma						
DAY	281	0	1	0	0	-
Swellings/Masses, Palpable Mass 1, 0.3 to 1.0 cm, Ulcerated						
DAY	246	-	-	1	-	-
Swellings/Masses, Swelling						
DAY	197	0	0	1	0	0
DAY	211	0	0	1	0	-
DAY	225	0	0	1	0	-
DAY	239	0	0	1	0	-
DAY	253	0	0	1	0	-
DAY	267	0	0	1	0	-
DAY	281	0	0	1	0	-
DAY	295	0	0	1	0	-
DAY	309	0	0	1	0	-
DAY	323	0	0	1	0	-
DAY	337	0	0	1	0	-
DAY	351	0	0	1	0	-
Skin/Fur/Mucous Membranes, Excessive Hairloss						
DAY	169	0	0	1	0	0
DAY	197	0	0	1	0	0
DAY	211	0	0	1	0	-
DAY	225	0	0	1	0	-
DAY	239	0	0	1	0	-
DAY	253	0	0	1	0	-
DAY	267	0	0	1	0	-
DAY	281	0	0	1	0	-
DAY	295	0	0	1	0	-
DAY	309	0	0	1	0	-
DAY	323	0	0	1	0	-
DAY	337	0	0	1	0	-
DAY	351	0	0	1	0	-
Skin/Fur/Mucous Membranes, Thin Hair Coat						
DAY	295	0	0	1	0	-
DAY	309	0	0	1	0	-
DAY	323	0	0	1	0	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 9. Summary of Clinical Observations – Oxalate Clearance Group (continued)

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Skin/Fur/Mucous Membranes, Thin Hair Coat						
DAY	337	0	0	1	0	-
DAY	351	0	0	1	0	-
Soiling, Perinasal, Red						
DAY	53	-	-	-	1	-
DAY	57	0	0	0	1	0
DAY	211	0	0	0	1	-
DAY	212	-	-	-	1	-
DAY	218	-	-	-	1	-
DAY	219	-	-	-	1	-
Soiling, Perineal, Urine						
DAY	218	-	-	-	1	-
DAY	219	-	-	-	1	-
Soiling, Periocular, Red						
DAY	53	-	-	-	1	-
DAY	57	0	0	0	1	0
DAY	92	-	-	1	-	-
Soiling, Perioral, Red						
DAY	211	0	0	0	1	-
DAY	212	-	-	-	1	-
DAY	218	-	-	-	1	-
DAY	219	-	-	-	1	-
Urine, Abnormal Color , Red						
DAY	211	0	0	0	1	-
DAY	212	-	-	-	1	-
DAY	218	-	-	-	1	-
DAY	219	-	-	-	1	-
DAY	330	-	-	-	1	-

- No Data

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 10. Ophthalmic Observations Summary

SEX		MALES				
DOSE (mkd)		0	50	150	300	400
Number of Animals Examined						
DAY	-2	20	20	20	20	20
Eyes, Cloudy Lens						
DAY	-2	0	2	1	1	0

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group

DOSE MKD		DAYS ON TEST 1	8	GAIN	15	GAIN	22	GAIN	30	GAIN	36	GAIN	43	GAIN
0	MEAN	182.0	226.2	44.2&	263.0	81.0&	291.6	109.6&	308.5	126.5&	327.3	145.3&	341.7	159.7&
	S.D.	11.9	14.6	3.3	18.3	7.7	21.4	11.7	22.8	18.4	24.7	18.4	25.3	17.4
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	MEAN	183.3	225.2	41.9&	258.3	75.0&	288.1	104.7&	308.2	124.9&	328.6	145.2&	343.7	160.3&
	S.D.	11.3	12.1	4.5	16.4	14.0	15.0	12.4	16.3	14.0	15.9	12.0	18.3	14.3
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	224.0	40.6&	256.9	73.6&	280.5	97.2&	298.5	115.1&	316.0	132.7&	329.8	146.5&
	S.D.	14.7	19.7	7.6	24.4	15.1	29.6	22.3	33.7	27.9	36.6	31.4	39.1	34.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	MEAN	181.1	220.4	39.3&	256.5	75.4&	287.1	106.1&	305.8	124.7&	324.6	143.5&	334.2	153.1&
	S.D.	17.0	18.5	4.8	22.3	11.5	26.6	16.7	29.0	19.5	32.4	23.7	34.0	25.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
400	MEAN	182.0	219.7	37.7&	250.8	68.8&	278.5	96.6&	295.9	113.9&	311.7	129.8&	321.7	139.7&
	S.D.	17.2	18.1	5.9	21.8	17.1	26.8	24.6	26.1	25.7	27.1	27.4	29.2	30.6
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE		DAYS ON TEST												
MKD		1	50	GAIN	57	GAIN	64	GAIN	71	GAIN	78	GAIN	85	GAIN
0	MEAN	182.0	357.4	175.4&	365.4	183.4&	376.0	194.1&	386.6	204.6&	396.1	214.1&	407.8	225.8&
	S.D.	11.9	28.2	19.6	32.0	23.6	34.2	25.8	35.1	26.7	33.5	25.2	34.5	25.6
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	MEAN	183.3	355.8	172.5&	364.3	180.9&	374.7	191.3&	386.7	203.4&	395.3	212.0&	402.7	219.3&
	S.D.	11.3	19.8	16.3	19.1	15.7	21.7	18.7	21.5	19.1	22.3	19.3	24.3	20.9
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	343.0	159.7&	351.7	168.4&	361.9	178.6&	377.0	193.7&	385.4	202.1&	395.1	211.8&
	S.D.	14.7	38.6	34.3	38.2	33.8	38.6	34.0	40.8	36.1	40.7	35.8	41.7	36.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	MEAN	181.1	349.1	168.1&	356.4	175.3&	364.0	183.0&	375.9	194.9&	384.1	203.0&	393.2	212.1&
	S.D.	17.0	36.1	27.5	38.7	31.0	41.1	33.3	43.8	35.7	44.1	35.9	45.2	37.3
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
400	MEAN	182.0	338.5	156.5&	344.6	162.6&	348.4	166.4&	363.4	181.5&	369.4	187.4&	379.5	197.5&
	S.D.	17.2	33.4	32.8	36.2	35.5	45.7	44.9	36.5	36.9	36.6	36.2	35.9	35.7
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE MKD		DAYS ON TEST 1	92	GAIN	99	GAIN	113	GAIN	127	GAIN	141	GAIN	155	GAIN
0	MEAN	182.0	412.9	230.9&	416.7	234.7&	429.7	247.7&	439.6	257.6&	447.7	265.7&	449.3	267.4&
	S.D.	11.9	35.8	26.9	37.1	28.2	37.9	29.0	36.2	27.5	38.8	30.0	39.6	31.0
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	MEAN	183.3	405.4	222.1&	409.7	226.4&	422.0	238.7&	435.4	252.1&	441.6	258.3&	448.7	265.3&
	S.D.	11.3	23.8	20.4	23.1	20.4	22.6	20.2	23.7	21.2	25.3	21.7	27.5	25.1
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	398.9	215.5&	402.1	218.7&	414.0	230.6&	425.6	242.2&	429.3	246.0&	436.0	252.6&
	S.D.	14.7	42.5	36.9	42.0	36.0	43.6	38.2	43.6	37.8	43.4	37.1	43.8	37.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	MEAN	181.1	395.7	214.6&	397.4	216.4&	404.9	226.6&	417.9	239.6&	424.2	243.4&	426.5	245.6&
	S.D.	17.0	45.5	37.8	46.5	38.9	47.8	42.1	49.3	43.9	56.6	51.3	56.9	51.2
	N=	10	10	10	10	10	9	9	9	9	8	8	8	8
400	MEAN	182.0	379.0	197.0&	379.9	197.9&	388.6	206.6&	393.2	211.2&	390.8*	208.8&	392.8*	210.9&
	S.D.	17.2	37.0	37.2	34.7	35.7	35.2	36.1	34.9	37.0	36.6	37.8	32.8	33.3
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE		DAYS ON TEST										
MKD		1	169	GAIN	183	GAIN	187	GAIN	190	GAIN	197	GAIN
0	MEAN	182.0	459.9	277.9&	462.9	280.9&	459.6	277.7&	461.7	279.8&	461.3	279.3&
	S.D.	11.9	39.7	31.3	40.2	31.9	39.9	31.4	38.7	30.7	37.7	29.6
	N=	10	10	10	10	10	10	10	10	10	10	10
50	MEAN	183.3	460.6	277.2&	465.0	281.7&	465.6	282.3&	463.6	280.3&	464.3	281.0&
	S.D.	11.3	28.7	26.2	30.4	28.1	29.2	26.9	29.0	26.1	29.8	27.0
	N=	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	442.4	259.0&	446.7	263.3&	446.9	263.6&	445.9	262.6&	444.3	261.0&
	S.D.	14.7	44.4	38.7	43.9	38.4	44.5	38.7	44.2	38.4	43.6	37.9
	N=	10	10	10	10	10	10	10	10	10	10	10
300	MEAN	181.1	432.9	252.0&	436.0	255.1&	435.5	254.7&	436.1	255.2&	433.5	252.7&
	S.D.	17.0	60.6	54.4	61.1	54.3	63.8	57.0	61.5	55.1	61.4	56.0
	N=	10	8	8	8	8	8	8	8	8	8	8
400	MEAN	182.0	394.9*	212.9&	387.1*	205.1&	377.4*	195.4&	379.1*	197.8&	368.7*	192.0&
	S.D.	17.2	32.3	33.2	25.4	26.3	32.8	34.9	26.5	27.4	27.8	26.1
	N=	10	10	10	10	10	10	10	9	9	8	8

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE		DAYS ON TEST												
MKD		1	204	GAIN	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN
0	MEAN	182.0	464.0	282.0&	470.6	288.7&	470.6	288.6&	471.8	289.8&	472.2	290.3&	470.2	288.2&
	S.D.	11.9	37.0	29.2	37.7	29.3	37.4	29.4	36.3	28.1	36.3	28.1	34.4	26.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	MEAN	183.3	467.0	283.7&	473.2	289.8&	476.0	292.6&	477.3	294.0&	477.7	294.3&	477.1	293.7&
	S.D.	11.3	31.6	28.9	32.2	29.9	31.2	29.3	31.7	29.6	31.2	29.2	32.6	29.9
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	446.3	262.9&	455.4	272.1&	456.7	273.4&	457.5	274.2&	457.3	273.9&	455.0	271.7&
	S.D.	14.7	43.2	37.2	44.2	37.7	43.2	36.3	44.1	37.4	43.1	36.5	43.4	37.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	MEAN	181.1	435.8	254.9&	438.7	258.3&	446.0	265.6&	447.0	266.5&	446.9	266.5&	447.0	266.6&
	S.D.	17.0	61.7	56.4	61.3	55.7	65.1	59.7	64.9	59.3	67.4	62.6	69.0	64.0
	N=	10	8	8	7	7	7	7	7	7	7	7	7	7

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE		DAYS ON TEST												
MKD		1	246	GAIN	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN
0	MEAN	182.0	473.5	291.6&	472.0	290.1&	476.3	294.3&	477.2	295.2&	477.2	295.3&	482.2	300.2&
	S.D.	11.9	35.4	27.3	34.4	26.4	34.3	25.5	34.3	26.0	33.0	25.0	33.7	25.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	MEAN	183.3	478.8	295.5&	479.4	296.0&	484.2	300.8&	487.7	304.4&	489.0	305.6&	492.8	309.5&
	S.D.	11.3	33.9	31.4	33.3	31.0	35.2	33.1	36.5	34.5	37.5	35.6	36.5	34.8
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	454.9	271.5&	456.8	273.5&	461.4	278.1&	461.6	278.3&	459.8	278.4&	465.2	283.8&
	S.D.	14.7	43.6	37.8	43.5	37.7	43.9	38.0	42.8	38.2	44.5	39.4	43.8	38.4
	N=	10	10	10	10	10	10	10	10	10	9	9	9	9
300	MEAN	181.1	448.3	267.9&	447.4	267.0&	452.8	272.4&	451.3	270.9&	452.2	271.8&	456.3	275.8&
	S.D.	17.0	69.1	64.3	68.7	64.4	71.0	66.4	70.2	66.1	71.4	66.7	73.0	68.8
	N=	10	7	7	7	7	7	7	7	7	7	7	7	7

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE		DAYS ON TEST												
MKD		1	288	GAIN	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN
0	MEAN	182.0	483.7	301.7&	480.5	298.5&	477.4	295.4&	486.5	306.3&	488.4	308.2&	484.8	304.6&
	S.D.	11.9	33.8	26.5	32.1	27.2	34.7	31.9	34.5	27.8	35.2	28.3	34.5	27.8
	N=	10	10	10	10	10	10	10	9	9	9	9	9	9
50	MEAN	183.3	496.6	313.3&	497.5	314.2&	495.1	311.7&	500.9	317.5&	504.3	320.9&	505.4	322.0&
	S.D.	11.3	37.5	36.2	37.8	35.9	36.4	35.0	37.3	35.8	38.0	36.0	38.3	36.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	466.3	284.9&	466.6	285.2&	465.4	284.0&	470.1	288.7&	472.6	291.2&	471.8	290.3&
	S.D.	14.7	42.7	37.5	43.3	38.2	43.0	38.0	44.1	38.9	44.6	39.0	44.4	38.5
	N=	10	9	9	9	9	9	9	9	9	9	9	9	9
300	MEAN	181.1	458.1	277.7&	458.5	278.1&	456.6	276.2&	463.4	283.0&	466.1	285.6&	458.4	277.9&
	S.D.	17.0	74.2	70.1	72.5	68.0	72.5	68.0	74.8	70.1	74.5	69.8	76.3	73.5
	N=	10	7	7	7	7	7	7	7	7	7	7	7	7

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 11. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE		DAYS ON TEST												
MKD		1	330	GAIN	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
0	MEAN	182.0	488.4	308.2&	492.5	312.3&	495.3	315.1&	500.1	319.8&	501.0	320.8&	504.4	324.2&
	S.D.	11.9	36.3	29.2	36.4	29.5	36.6	29.8	36.8	29.9	36.6	29.8	38.5	31.7
	N=	10	9	9	9	9	9	9	9	9	9	9	9	9
50	MEAN	183.3	511.8	328.4&	513.9	330.6&	517.6	334.3&	519.8	336.5&	521.8	338.4&	523.8	340.4&
	S.D.	11.3	39.8	37.9	39.3	37.7	41.9	40.7	43.7	42.4	42.5	41.0	43.3	42.1
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
150	MEAN	183.3	476.8	295.3&	481.9	300.5&	484.1	302.6&	486.5	305.0&	488.0	306.5&	490.1	308.7&
	S.D.	14.7	44.8	39.2	44.5	38.5	45.0	39.2	45.7	39.2	46.6	40.1	46.2	39.6
	N=	10	9	9	9	9	9	9	9	9	9	9	9	9
300	MEAN	181.1	466.1	285.6&	473.4	293.0&	478.0	297.5&	478.6	298.2&	473.8	293.4&	493.0	311.8&
	S.D.	17.0	75.4	71.9	77.3	73.1	79.8	75.5	81.2	77.4	83.5	80.4	95.4	92.5
	N=	10	7	7	7	7	7	7	7	7	7	7	5	5

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 12. Body Weight/Body Weight Gains Summary (G) – Metabolism Group

DOSE		DAYS ON TEST												
MKD		1	30	GAIN	57	GAIN	85	GAIN	113	GAIN	141	GAIN	204	GAIN
0	MEAN	184.3	299.5	115.2&	342.9	158.6&	382.5	198.2&	408.8	224.6&	426.5	242.2&	442.5	258.2&
	S.D.	14.5	21.6	16.6	26.9	21.4	27.7	20.2	36.0	26.9	40.7	32.4	45.9	38.0
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	179.9	294.5	114.6&	343.5	163.6&	380.3	200.4&	399.5	219.6&	419.3	239.3&	436.3	256.4&
	S.D.	14.3	27.8	20.4	32.1	22.9	40.7	33.0	43.4	36.0	44.3	36.6	51.1	42.9
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	181.6	305.7	124.1&	359.6	178.0&	398.7	217.1&	412.5	230.9&	428.1	246.5&	444.4	262.8&
	S.D.	15.2	23.8	10.9	28.1	18.7	30.1	20.2	29.9	19.5	32.2	21.6	30.7	22.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	179.0	293.2	114.1&	340.7	161.7&	378.8	199.8&	399.5	220.4&	417.3	238.3&	429.7	250.7&
	S.D.	16.3	21.2	18.7	28.2	28.8	30.7	33.2	34.2	35.5	34.0	36.4	34.4	36.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
400	MEAN	180.6	305.8	125.1&	361.8	176.4&	402.7	217.3&	423.1	237.7&	440.3	254.9&	===	===&
	S.D.	14.8	26.2	23.9	38.7	34.6	41.3	37.9	47.5	44.4	55.2	53.5	===	===
	N=	5	5	5	4	4	4	4	4	4	4	4	0	0

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
=== NO DATA AVAILABLE FOR MEAN AND S.D.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 12. Body Weight/Body Weight Gains Summary (G) – Metabolism Group (continued)

DOSE		DAYS ON TEST												
MKD		1	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN	246	GAIN
0	MEAN	184.3	451.6	267.4&	449.7	265.4&	453.5	269.3&	453.0	268.7&	454.8	270.5&	454.9	270.7&
	S.D.	14.5	47.6	39.3	49.1	41.2	50.6	43.4	51.1	44.1	52.0	45.0	52.6	45.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	179.9	442.3	262.4&	445.9	266.0&	449.3	269.4&	451.5	271.5&	450.6	270.7&	451.7	271.8&
	S.D.	14.3	56.9	49.5	52.8	45.1	54.5	46.7	54.2	45.9	51.9	43.8	50.8	42.2
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	181.6	452.4	270.8&	452.9	271.3&	454.7	273.1&	456.2	274.6&	456.6	275.0&	457.7	276.1&
	S.D.	15.2	31.6	23.1	29.2	19.3	30.9	22.4	30.7	22.3	30.2	22.3	29.7	22.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	179.0	436.7	257.7&	441.1	262.1&	441.2	262.2&	443.2	264.2&	440.4	261.4&	441.4	262.4&
	S.D.	16.3	34.2	37.4	34.4	37.2	36.0	39.0	37.0	40.1	35.3	39.2	36.8	40.9
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 12. Body Weight/Body Weight Gains Summary (G) – Metabolism Group (continued)

DOSE		DAYS ON TEST												
MKD		1	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN	288	GAIN
0	MEAN	184.3	456.5	272.2&	460.4	276.2&	462.2	277.9&	462.5	278.2&	467.4	283.1&	468.7	284.4&
	S.D.	14.5	54.2	46.9	53.2	46.2	55.0	48.1	54.7	47.8	54.6	48.0	57.5	50.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	179.9	451.4	271.4&	456.1	276.1&	459.7	279.8&	458.6	278.7&	463.0	283.0&	464.1	284.1&
	S.D.	14.3	49.6	41.0	51.6	42.7	53.7	44.9	53.3	44.1	53.7	44.8	59.4	51.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	181.6	459.4	277.8&	464.1	282.5&	466.9	285.3&	465.2	283.6&	470.3	288.7&	471.1	289.5&
	S.D.	15.2	31.0	23.5	30.4	22.9	31.8	24.7	31.3	24.1	32.6	25.7	33.3	26.0
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	179.0	443.3	264.3&	448.3	269.3&	447.8	268.7&	447.0	268.0&	452.7	273.7&	456.2	277.2&
	S.D.	16.3	36.2	40.1	38.4	41.9	36.8	40.0	37.2	41.1	38.0	41.4	37.5	40.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 12. Body Weight/Body Weight Gains Summary (G) – Metabolism Group (continued)

DOSE		DAYS ON TEST												
MKD		1	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN	330	GAIN
0	MEAN	184.3	475.1	290.9&	473.9	289.6&	478.8	294.6&	480.7	296.5&	478.2	294.0&	483.3	299.1&
	S.D.	14.5	55.9	48.9	59.7	52.7	61.9	54.8	61.4	54.7	59.8	52.8	61.1	54.5
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	179.9	461.7	281.8&	461.9	282.0&	469.3	289.3&	472.6	292.6&	473.4	293.5&	480.6	300.6&
	S.D.	14.3	65.1	58.3	69.1	61.5	64.3	55.5	63.1	54.4	61.5	52.5	65.0	55.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	181.6	474.1	292.5&	472.4	290.8&	477.5	295.9&	480.2	298.6&	480.7	299.1&	485.8	304.2&
	S.D.	15.2	33.3	26.4	34.5	27.4	34.4	27.8	33.2	27.0	33.1	27.1	31.8	24.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	179.0	455.5	276.4&	452.9	273.9&	456.6	277.6&	459.8	280.8&	458.5	279.4&	460.9	281.9&
	S.D.	16.3	34.5	37.3	38.2	41.4	36.5	39.8	37.8	40.6	39.8	42.5	41.0	44.9
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 12. Body Weight/Body Weight Gains Summary (G) – Metabolism Group (continued)

DOSE		DAYS ON TEST										
MKD		1	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
0	MEAN	184.3	486.7	302.5&	490.0	305.7&	494.2	310.0&	496.5	312.2&	499.6	315.3&
	S.D.	14.5	62.3	55.6	61.9	55.2	64.9	58.5	64.4	58.0	65.8	58.9
	N=	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	179.9	484.9	304.9&	486.7	306.8&	489.1	309.2&	492.0	312.0&	492.0	312.1&
	S.D.	14.3	63.1	53.9	65.0	55.7	71.4	61.9	68.5	58.9	67.2	57.4
	N=	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	181.6	489.1	307.5&	494.2	312.6&	495.3	313.7&	498.7	317.1&	499.2	317.6&
	S.D.	15.2	37.3	29.7	35.0	27.2	36.0	27.8	37.4	29.1	37.0	28.6
	N=	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	179.0	465.4	286.3&	465.7	286.7&	468.3	289.3&	467.7	288.7&	468.1	289.0&
	S.D.	16.3	41.2	44.4	43.5	47.8	40.7	44.1	42.9	47.6	42.7	47.1
	N=	5	5	5	5	5	5	5	5	5	5	5

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 13. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group

DOSE MKD		DAYS ON TEST 1	30	GAIN	57	GAIN	85	GAIN	113	GAIN	141	GAIN	204	GAIN
0	MEAN	183.5	310.4	126.8&	358.2	174.7&	398.1	214.6&	423.2	239.6&	441.5	257.9&	459.1	275.6&
	S.D.	13.3	16.5	17.3	17.6	18.7	15.2	16.1	18.5	17.4	22.1	21.5	25.6	25.1
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	183.2	290.9	107.7&	339.5	156.3&	381.8	198.6&	399.0	215.8&	413.8	230.6&	429.8	246.6&
	S.D.	15.3	22.8	28.3	23.9	32.5	19.0	27.7	19.4	24.9	18.2	26.4	26.3	32.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	179.9	305.1	125.2&	354.2	174.3&	388.0	208.1&	398.0	218.1&	415.6	235.7&	433.1	253.2&
	S.D.	15.7	23.1	9.8	32.5	17.9	32.0	17.8	31.7	16.9	35.8	22.9	42.4	28.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	180.7	299.7	118.9&	343.7	163.0&	395.7	214.9&	420.3	239.5&	442.0	261.3&	456.1	275.3&
	S.D.	16.6	25.8	11.0	38.1	22.5	26.3	14.7	30.0	15.4	34.1	19.8	36.0	21.0
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
400	MEAN	183.1	303.3	120.2&	353.9	170.8&	390.5	207.3&	397.0	213.9&	396.2	213.1&	===	===&
	S.D.	15.8	24.6	14.8	32.6	22.5	31.7	20.7	24.5	13.3	28.9	15.5	===	===
	N=	5	5	5	5	5	5	5	5	5	5	5	0	0

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
=== NO DATA AVAILABLE FOR MEAN AND S.D.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 13. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE		DAYS ON TEST												
MKD		1	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN	246	GAIN
0	MEAN	183.5	467.2	283.7&	468.0	284.4&	470.5	286.9&	473.5	290.0&	468.3	284.8&	470.9	287.4&
	S.D.	13.3	26.7	27.0	25.0	24.8	25.2	25.8	26.9	27.0	28.8	28.2	27.6	27.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	183.2	435.1	251.9&	438.4	255.2&	439.8	256.6&	442.1	258.9&	439.8	256.6&	440.8	257.6&
	S.D.	15.3	24.9	31.1	22.3	29.5	23.6	29.6	24.9	32.1	26.2	33.3	24.1	32.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	179.9	440.9	261.0&	441.4	261.5&	445.8	265.9&	446.1	266.2&	444.4	264.5&	447.4	267.5&
	S.D.	15.7	43.9	30.3	45.4	31.3	46.2	32.3	46.7	33.1	45.1	31.3	46.5	32.5
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	180.7	459.3	278.5&	470.1	287.4&	461.3	285.9&	459.5	284.1&	459.3	283.9&	458.5	283.1&
	S.D.	16.6	38.0	23.7	35.2	17.8	25.6	14.1	23.0	11.8	23.3	12.1	23.5	12.5
	N=	5	5	5	4	4	3	3	3	3	3	3	3	3

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 13. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE		DAYS ON TEST												
MKD		1	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN	288	GAIN
0	MEAN	183.5	471.1	287.6&	477.1	293.5&	480.4	296.8&	478.8	295.2&	483.7	300.1&	484.1	300.6&
	S.D.	13.3	28.0	28.2	27.4	26.6	28.3	27.6	28.0	27.1	29.4	30.1	29.7	30.6
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	183.2	441.3	258.1&	447.0	263.8&	449.0	265.8&	449.0	265.8&	451.7	268.5&	454.5	271.3&
	S.D.	15.3	24.9	32.9	24.2	31.9	24.9	32.8	26.7	34.0	25.3	32.2	25.8	33.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	179.9	445.9	266.0&	451.6	271.7&	454.5	274.6&	451.0	271.1&	455.2	275.3&	457.1	277.2&
	S.D.	15.7	44.7	30.7	45.0	31.2	46.9	33.0	45.2	31.3	45.4	31.5	45.8	32.1
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	180.7	461.0	285.6&	467.1	291.7&	465.4	290.0&	467.0	291.6&	471.6	296.2&	472.5	297.1&
	S.D.	16.6	24.8	13.5	25.3	13.7	25.1	13.5	26.9	14.4	27.3	15.4	25.9	13.7
	N=	5	3	3	3	3	3	3	3	3	3	3	3	3

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 13. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE		DAYS ON TEST											
MKD		1	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN	330	GAIN
0	MEAN	183.5	485.8	302.3&	482.7	299.2&	487.8	304.3&	490.5	307.0&	488.9	305.4&	492.8	309.3&
	S.D.	13.3	30.2	30.1	30.2	30.1	30.1	30.4	28.8	28.7	29.7	29.2	29.4	29.7
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	MEAN	183.2	455.1	271.9&	453.2	270.0&	458.5	275.3&	460.9	277.7&	461.0	277.8&	465.7	282.5&
	S.D.	15.3	23.9	31.4	23.9	31.1	24.3	31.0	24.1	30.5	25.7	32.2	25.8	31.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	MEAN	179.9	458.0	278.1&	457.4	277.5&	448.7	268.8&	461.8	281.9&	462.4	282.5&	467.1	287.2&
	S.D.	15.7	46.2	32.2	47.7	33.8	40.8	28.8	43.7	29.7	43.0	28.7	45.4	31.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
300	MEAN	180.7	473.5	298.1&	468.6	293.2&	469.7	294.3&	472.6	297.2&	473.7	298.3&	475.1	299.7&
	S.D.	16.6	27.4	15.0	29.9	16.7	26.6	13.5	26.8	13.8	33.1	20.3	19.0	5.5
	N=	5	3	3	3	3	3	3	3	3	3	3	3	3

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 13. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE		DAYS ON TEST										
MKD		1	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
0	MEAN	183.5	498.4	314.9&	502.1	318.5&	498.8	314.3&	493.6	311.7&	480.7	318.5&
	S.D.	13.3	28.8	29.6	30.6	30.9	31.9	31.6	23.4	28.0	.	.
	N=	5	5	5	5	5	4	4	4	4	1	1
50	MEAN	183.2	467.8	284.6&	469.9	286.7&	477.5	289.5&	467.6	281.9&	===	===&
	S.D.	15.3	24.8	30.0	23.1	28.1	19.1	8.3	.	.	===	===
	N=	5	5	5	5	5	3	3	1	1	0	0
150	MEAN	179.9	472.0	292.1&	472.8	292.9&	461.1	281.2&	483.7	302.6&	===	===&
	S.D.	15.7	45.9	31.9	48.4	34.5	43.7	31.9	.	.	===	===
	N=	5	5	5	5	5	5	5	1	1	0	0
300	MEAN	180.7	482.0	306.6&	483.8	308.4&	483.5	308.1&	449.9	277.6&	===	===&
	S.D.	16.6	21.4	7.8	24.3	10.8	24.1	10.5	33.7	16.1	===	===
	N=	5	3	3	3	3	3	3	2	2	0	0

& INDICATES NO STATISTICAL COMPARISON OF MEANS.
=== NO DATA AVAILABLE FOR MEAN AND S.D.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 14. Feed Consumption (G/Day) Summary – Main Group

DOSE MKD		DAYS ON TEST 1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
0	MEAN	23.4	25.0	24.8	24.0	23.8	24.0	23.8	22.5	22.8	23.8	25.3	24.6	23.9
	S.D.	1.3	1.6	1.8	2.2	2.1	1.7	1.8	1.8	2.5	2.5	2.0	1.9	2.1
	N=	10	10	10	10	10	9	9	8	7	10	8	9	8
50	MEAN	23.4	23.9	24.0	23.7	24.1	23.8	23.3	22.6	23.2	24.5	24.5	23.4	23.5
	S.D.	1.1	1.5	1.4	1.0	1.0	1.1	1.2	1.3	1.7	1.3	1.2	1.7	1.3
	N=	10	10	10	10	10	10	10	10	8	10	9	9	10
150	MEAN	23.9	24.9	23.7	22.4	24.3	23.1	22.9	22.2	23.2	26.2	24.7	24.1	24.8
	S.D.	2.3	2.6	2.8	1.8	2.4	2.5	2.4	2.3	2.5	2.6	3.0	2.6	2.8
	N=	9	10	8	7	10	7	7	6	8	8	6	7	9
300	MEAN	22.9	24.3	23.7	22.7	23.8	23.3	22.9	21.9	22.0	24.4	24.0	24.4	23.8
	S.D.	1.9	2.4	1.6	1.8	1.4	1.7	2.7	1.6	2.6	2.6	3.0	2.4	2.4
	N=	10	10	9	7	9	8	9	7	7	8	6	9	9
400	MEAN	22.7	23.9	23.3	22.1	22.7	22.3	22.5	21.8	21.3	23.0	23.0	23.2	22.1
	S.D.	1.6	2.3	2.2	1.6	2.1	1.9	2.7	2.1	2.4	1.5	1.9	2.0	1.7
	N=	9	10	9	9	10	9	6	9	7	8	7	9	7

THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 14. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD		DAYS ON TEST 92-99	106-113	120-127	134-141	148-155	162-169	176-183	190-197
0	MEAN	24.1	24.4	23.6	23.3	23.3	23.6	22.5	22.9
	S.D.	2.1	0.8	1.8	2.9	1.2	0.4	1.9	1.9
	N=	9	8	9	9	8	6	9	9
50	MEAN	22.7	22.7	22.6	22.6	22.4	22.7	22.4	22.7
	S.D.	1.5	1.1	1.2	1.6	1.5	1.8	1.7	1.7
	N=	10	9	9	10	9	10	9	9
150	MEAN	22.7	23.1	22.5	22.7	22.0	21.2	21.8	22.0
	S.D.	3.0	3.1	2.3	3.0	2.3	2.8	2.2	2.0
	N=	5	6	7	8	6	5	7	6
300	MEAN	22.8	22.8	22.7	22.7	21.5	22.0	22.3	22.8
	S.D.	2.3	3.1	2.3	2.7	3.4	3.2	3.0	3.0
	N=	8	4	5	7	4	4	4	4
400	MEAN	21.5	20.4$	20.1*	20.0	19.6*	18.5$	17.8*	17.4*
	S.D.	1.2	3.0	2.2	2.5	1.2	1.7	2.3	4.4
	N=	7	6	7	6	6	6	6	5

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA=0.05.
$ STATISTICALLY DIFFERENT FROM CONTROL MEAN BY WILCOXON'S TEST, ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 14. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD		DAYS ON TEST 204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
0	MEAN	23.4	23.2	22.9	22.5	22.7	23.2	21.7	21.2	22.8	23.2	23.6	23.8
	S.D.	1.6	0.8	1.7	1.4	1.8	1.8	2.2	1.6	0.8	3.4	0.5	0.6
	N=	9	7	9	9	9	9	8	6	6	9	5	6
50	MEAN	22.9	22.9	22.3	22.4	23.0	23.2	21.9	21.4	24.2	22.5	22.6	22.6
	S.D.	1.6	1.6	2.0	1.5	1.9	1.5	1.7	1.7	2.1	1.5	1.8	1.6
	N=	9	9	9	10	10	10	10	8	10	9	9	10
150	MEAN	22.0	21.7	21.1	22.1	20.5	23.0	21.6	20.1	22.1	22.4	22.1	21.8
	S.D.	2.0	2.4	1.8	2.6	4.6	3.2	2.3	1.9	2.5	2.4	2.5	2.1
	N=	6	6	6	8	7	6	6	5	5	6	6	6
300	MEAN	23.5	22.8	22.7	22.4	22.8	23.2	22.5	21.4	23.4	22.3	23.2	23.1
	S.D.	1.9	4.2	3.7	4.0	2.7	4.5	3.2	3.3	4.1	3.4	4.1	5.8
	N=	3	2	3	2	5	2	4	3	2	3	3	2

THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 15. Feed Consumption (G/Day) Summary – Metabolism Group

DOSE MKD		DAYS ON TEST 190-197	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
0	MEAN	22.3	23.0	22.6	22.2	22.1	22.3	22.6	22.6	21.2	21.8	21.8	22.7	22.5
	S.D.	2.3	2.6	2.9	3.5	2.9	2.6	2.4	2.7	4.0	2.4	2.8	2.9	2.9
	N=	4	4	4	3	4	4	4	4	3	4	4	4	4
50	MEAN	21.5	21.8	22.5	21.9	21.5	21.9	22.5	19.9	20.6	22.7	21.8	22.2	22.1
	S.D.	1.8	2.5	2.0	1.6	1.9	1.9	1.7	4.3	2.8	1.9	1.8	2.8	1.4
	N=	5	4	3	4	4	4	5	5	3	3	3	3	4
150	MEAN	23.1	23.5	23.3	23.1	23.1	23.1	24.1	23.0	22.0	23.8	23.1	23.4	22.4
	S.D.	1.4	1.6	1.2	1.0	1.2	1.4	1.3	1.4	1.3	1.3	1.7	1.6	1.7
	N=	5	5	4	5	4	4	5	5	4	4	5	4	4
300	MEAN	21.2	22.2	22.1	19.6	20.6	21.5	23.4	20.8	20.2	21.5	20.6	20.7	20.0
	S.D.	1.3	1.8	1.1	2.9	0.7	2.0	2.2	1.1	1.1	1.9	1.9	1.4	1.6
	N=	3	3	3	3	3	3	4	3	3	3	3	3	3
400	MEAN	24.0	---	---	---	---	---	---	---	---	---	---	---	---
	S.D.	2.0	---	---	---	---	---	---	---	---	---	---	---	---
	N=	3	0	0	0	0	0	0	0	0	0	0	0	0

--- NO DATA AVAILABLE FOR MEAN AND S.D.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 16. Feed Consumption (G/Day) Summary – Oxalate Clearance Group

DOSE MKD		DAYS ON TEST 190-197	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
0	MEAN	22.7	22.7	22.9	21.7	20.9	21.6	23.4	21.6	20.3	20.6	21.8	21.2	24.6
	S.D.	1.0	1.2	2.0	0.2	0.1	0.1	3.7	0.9	0.6	0.8	2.3	0.8	.
	N=	5	4	4	4	2	2	3	2	2	2	3	2	1
50	MEAN	21.1	21.5	21.9	21.6	21.0	22.1	22.0	20.8	20.1	22.3	21.7	20.8	---
	S.D.	0.9	0.7	0.5	1.0	1.0	1.7	1.0	1.1	0.8	0.7	0.6	2.0	---
	N=	5	5	5	5	5	5	5	5	5	5	4	3	0
150	MEAN	21.6	22.2	22.4	21.9	21.6	22.2	22.1	21.6	20.5	22.4	21.8	20.7	---
	S.D.	1.5	2.0	2.0	1.6	1.2	2.0	1.7	1.7	1.4	2.1	1.9	2.0	---
	N=	5	5	5	5	5	5	5	5	5	5	5	5	0
300	MEAN	21.5	20.6	22.1	21.0	23.0	21.1	21.4	20.3	21.1	23.6	20.8	21.1	---
	S.D.	2.4	5.3	1.9	1.3	3.5	2.0	1.5	1.6	2.8	4.2	1.8	2.6	---
	N=	4	4	2	2	3	2	2	2	3	3	2	2	0
400	MEAN	16.4	---*	---	---	---	---	---	---	---	---	---	---	---
	S.D.	0.9	---	---	---	---	---	---	---	---	---	---	---	---
	N=	3	0	0	0	0	0	0	0	0	0	0	0	0

* STATISTICALLY DIFFERENT FROM CONTROL MEAN BY DUNNETT'S TEST, ALPHA=0.05.
--- NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 17. Water Consumption (G/Day) Summary – Main Group

DOSE MKD		DAYS ON 368-369
0	MEAN	10.9
	S.D.	3.8
	N=	8
50	MEAN	9.7
	S.D.	3.0
	N=	10
150	MEAN	8.9
	S.D.	4.0
	N=	9
300	MEAN	16.5
	S.D.	8.7
	N=	5

THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 18. Feed Efficiency (G/Day) Summary– Main Group

		DAYS ON TEST												
DOSE		1,8	8,15	15,22	22,30	30,36	36,43	43,50	50,57	57,64	64,71	71,78	78,85	85,92
MKD		1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
0	MEAN	3.7	4.8	6.2	9.4	8.5	13.1	11.7	25.0	18.1	14.5	17.6	16.6	33.4
	S.D.	0.1	0.5	0.7	1.7	2.6	4.4	3.3	10.3	8.5	2.9	6.5	6.5	7.0
	N=	10	10	10	9	10	9	9	7	7	8	7	9	7
50	MEAN	4.0	4.6	5.9	9.6	7.5	11.0	14.2	18.7	17.8	14.7	19.3	22.7	76.1
	S.D.	0.4	0.5	1.1	1.4	1.8	3.1	3.6	5.4	6.7	2.6	5.0	9.4	41.8
	N=	10	9	10	10	10	9	10	9	8	10	8	8	9
150	MEAN	4.2	5.5	6.3	13.4	8.6	13.0	13.0	23.4	15.8	12.3	22.3	21.7	48.6
	S.D.	0.7	1.4	0.7	6.7	1.6	3.6	3.0	11.9	3.5	1.5	4.5	1.4	24.1
	N=	9	10	6	7	10	7	7	6	7	8	5	6	7
300	MEAN	4.1	4.9	5.7	10.6	7.7	20.0	11.2	22.2	24.8	12.3	25.0	16.0	14.6
	S.D.	0.4	0.9	1.2	2.4	1.3	9.1	2.2	12.2	10.4	2.4	6.3	2.4	132.5
	N=	10	10	9	7	8	8	9	6	6	6	6	7	9
400	MEAN	4.3	5.5	6.3	10.7	8.8	16.1	8.8	29.4	18.3	17.9	-54.8	15.5	-106.8
	S.D.	0.7	1.4	1.4	2.9	1.1	4.2	1.4	15.4	3.9	9.9	136.9	2.1	96.6
	N=	9	9	9	9	10	8	6	8	6	8	7	8	7

DAYS ON TEST GIVEN AS BODY WEIGHT INTERVAL OVER FEED CONSUMPTION INTERVAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 19. Test Material Intake Summary – Main Group

DOSE		0			50 MKD				150 MKD				300 MKD				600 MKD				
Feed Period (in Days)	Factor[1]	PPM	MKD	% Test Material in Diet	PPM[2]	Adjusted PPM[3]	MKD[4]	Adjusted MKD[5]	% Test Material in Diet	PPM[2]	Adjusted PPM[3]	MKD[4]	Adjusted MKD[5]	% Test Material in Diet	PPM[2]	Adjusted PPM[3]	MKD[4]	Adjusted MKD[5]	% Test Material in Diet	PPM[2]	Adjusted PPM[3]	MKD[4]	Adjusted MKD[5]
1-8	1	0	0	0.0553	553	553	64.9	65	0.1660	1660	1660	195	195	0.3321	3321	3321	378.8	379	0.4427	4427	4427	500	500.0
8-15	1	0	0	0.0636	636	636	65.9	66	0.1930	1930	1930	200	200	0.3783	3783	3783	385.5	386	0.5073	5073	5073	516	516.0
15-22	1	0	0	0.0618	618	618	54.5	55	0.1722	1722	1722	152	152	0.3671	3671	3671	320.1	320	0.4871	4870	4870	429	429.0
22-29	1	0	0	0.0644	644	644	49.8	50	0.1845	1845	1845	143	143	0.3835	3835	3835	293.7	294	0.4978	4978	4978	383	383.0
29-36	1	0	0	0.0693	693	693	54.8	55	0.1999	1999	1999	158	158	0.4215	4215	4215	318.3	318	0.5494	5494	5494	411	411.0
36-43	1	0	0	0.0701	701	701	50.1	50	0.2142	2142	2142	153	153	0.4335	4335	4335	306.6	307	0.5730	5730	5730	403	403.0
43-50	1	0	0	0.0756	756	756	51.5	52	0.2140	2140	2140	146	146	0.4506	4506	4506	302.0	302	0.5980	5980	5980	408	408.0
50-57	1	0	0	0.0770	770	770	49.2	49	0.2276	2276	2276	145	145	0.4488	4488	4488	278.6	279	0.6040	6039	6039	385	385.0
57-64	1	0	0	0.0803	802	802	52.1	52	0.2376	2376	2376	154	154	0.4866	4866	4866	297.2	297	0.6466	6466	6466	397	397.0
64-71	1	0	0	0.0834	834	834	59.1	59	0.2465	2465	2465	175	175	0.5032	5032	5032	331.9	332	0.6491	6491	6491	419	419.0
71-78	1	0	0	0.0841	841	841	54.5	55	0.2439	2439	2439	158	158	0.5119	5119	5119	323.3	323	0.6650	6650	6650	417	417.0
78-85	1	0	0	0.0826	826	826	51.0	51	0.2288	2288	2288	141	141	0.4841	4841	4841	303.9	304	0.6711	6711	6711	416	416.0
85-92	1	0	0	0.0833	833	833	52.0	52	0.2417	2417	2417	151	151	0.4960	4955	4955	299.0	299	0.6581	6581	6581	383	383.0
92-99	1	0	0	0.0892	892	892	50.6	51	0.2580	2580	2580	146	146	0.5058	5058	5058	290.8	291	0.6891	6891	6891	390	390.0
106-120	2	0	0	0.0921	921	1842	52.1	104	0.2699	2699	5398	153	306	0.5274	5274	10548	299.8	600	0.7101	7101	14202	377	754.0
120-134	2	0	0	0.0957	957	1914	51.3	103	0.2766	2766	5532	148	296	0.5426	5426	10852	299.4	599	0.7790	7790	15580	401	802.0
134-148	2	0	0	0.0993	993	1986	52.7	105	0.2915	2915	5830	155	310	0.5695	5695	11390	307.0	614	0.7916	7916	15832	404	808.0
148-162	2	0	0	0.0991	991	1982	50.4	101	0.2861	2861	5722	145	290	0.5689	5689	11378	287.6	575	0.7768	7768	15536	389	778.0
162-176	2	0	0	0.1041	1041	2082	50.2	100	0.3176	3175	6350	153	306	0.5991	5990	11980	306.7	613	0.8584	8584	17168	403	806.0
176-190	2	0	0	0.1049	1048	2096	51.4	103	0.3105	3105	6210	152	304	0.5910	5910	11820	303.4	607	0.8511	8511	17022	387	774.0
190-204	2	0	0	0.1048	1048	2096	51.6	103	0.3103	3103	6206	153	306	0.5907	5907	11814	309.1	618	0.8524	8524	17048	384	776.0
204-218	2	0	0	0.1026	1026	2052	50.6	101	0.3008	3008	6016	148	296	0.5636	5636	11272	303.9	608	0.7998	7998	15996	368	736.0
218-232	2	0	0	0.1060	1060	2120	51.7	103	0.3229	3229	6458	157	314	0.5675	5674	11348	297.5	595					
232-246	2	0	0	0.1048	1048	2096	49.7	99	0.3174	3174	6348	150	300	0.5908	5908	11816	308.6	617					
246-260	2	0	0	0.1067	1067	2134	52.3	105	0.3085	3085	6170	151	302	0.5910	5910	11820	302.8	606					
260-274	2	0	0	0.1073	1073	2146	48.2	96	0.3126	3126	6252	141	282	0.5968	5968	11936	307.6	615					
274-288	2	0	0	0.1075	1075	2150	54.1	108	0.3381	3380	6760	170	340	0.5899	5899	11798	306.5	613					
288-302	2	0	0	0.1079	1078	2156	50.9	102	0.3104	3104	6208	147	294	0.6007	6006	12012	302.3	605					
302-316	2	0	0	0.1140	1140	2280	50.2	100	0.3244	3244	6488	143	286	0.6124	6124	12248	293.2	586					
316-330	2	0	0	0.1197	1197	2394	58.1	116	0.3578	3578	7156	174	348	0.6687	6687	13374	349.5	699					
330-344	2	0	0	0.1049	1049	2098	51.5	103	0.3191	3191	6382	157	314	0.5680	5679	11358	282.9	566					
344-358	2	0	0	0.1151	1151	2302	55.4	111	0.3295	3295	6590	159	318	0.6565	6565	13130	338.4	677					
358-365	1	0	0	0.1160	1160	1160	54.8	55	0.3335	3335	3335	158	158	0.6204	6204	6204	317.0	317					
Time Weighed Avg.[7]		0	0			948		51			2857		152			5371		303			6799		390
SD					177		4.06			539		14			865		24			30	1261		36	

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 20. Urinalysis Summary – Main Group

DOSE MKD		URINE VOL (ML)	SPECIFIC GRAVITY	Color	Appear	pH	PROTEIN (MG/DL)	GLUCOSE (MG/DL)	KETONES (MG/DL)	BILI-RUBIN	BLOOD	UROBIL-INOGEN
0	MEAN	10.6	1.031	YELLOW (8)	SL. CL (8)	7.0 (1)	+ (5)	NEG (8)	NEG (4)	NEG (8)	NEG (8)	<=1 (8)
	S.D.	3.6	0.007			7.5 (1)	++ (3)		TRC (4)			
	N=	8	8			8.0 (2)						
						8.5 (2)						
						>=9 (2)						
50	MEAN	8.8	1.034	YELLOW (10)	SL. CL (10)	6.5 (3)	+ (3)	NEG (10)	NEG (4)	NEG (7)	NEG (7)	<=1 (10)
	S.D.	3.7	0.009			7.5 (1)	++ (7)		TRC (6)	+ (3)	+ (2)	
	N=	10	10			8.0 (2)					++ (1)	
						8.5 (2)						
						>=9 (2)						
150	MEAN	7.9	1.038	YELLOW (9)	SL. CL (9)	5.0 (2)	+ (4)	NEG (9)	NEG (3)	NEG (7)	NEG (8)	<=1 (9)
	S.D.	3.2	0.013			5.5 (1)	++ (5)		TRC (5)	+ (2)	++ (1)	
	N=	9	9			6.0 (2)			+ (1)			
						7.5 (2)						
						>=9 (2)						
300	MEAN	16.3	1.025	YELLOW (4)	SL. CL (5)	6.5 (1)	+ (3)	NEG (5)	NEG (3)	NEG (4)	NEG (4)	<=1 (5)
	S.D.	12.2	0.012	BROWN (1)		7.0 (1)	++ (1)		TRC (2)	+ (1)	++++ (1)	
	N=	5	5			8.5 (1)	+++ (1)					
						>=9 (2)						

THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.
URINE VOLUME AND SPECIFIC GRAVITY VALUES ARE MEAN AND S.D. FOR THE SPECIFIED NUMBER (N) OF ANIMALS.
ALL OTHER DATA TABULATED AS NUMBER OF ANIMALS (N) WITH THE STATED VALUE.
THE TOTAL NUMBER OF ANIMALS FOR SOME PARAMETERS MAY NOT EQUAL THE NUMBER OF
ANIMALS IN THE DOSE GROUP DUE TO INSUFFICIENT QUANTITY OF SAMPLE OR EXCLUSION OF ANIMAL(S) FROM ANALYSIS
NEG=NEGATIVE TRC=TRACE SL. CL=SLIGHTLY CLOUDY UROBILINOGEN IS MEASURED IN EU/DL=EHRLICH UNITS/DECILITER
N = NORMAL; + = SLIGHT; ++ = MODERATE; +++ = SEVERE

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 21. Urine Microscopic – Main Group

DOSE UNIT.	ANIMAL NUMBER	WBC /HPF	RBC /HPF	CASTS /LPF	CRYSTALS /LPF	EPI /LPF	BACTERIA /HPF	YEAST /HPF	MUCUS /LPF	MICROSCOPIC COMMENTS
0	4402				TR PHOS +++ CA OXAL RARE		PRESENT			SPERM PRESENT
	4403				TR PHOS +++		PRESENT			SPERM PRESENT
	4405				TR PHOS ++		PRESENT			SPERM PRESENT
	4406				TR PHOS +++		PRESENT			SPERM PRESENT
	4407				TR PHOS +++		PRESENT			SPERM PRESENT
	4408				TR PHOS +++		PRESENT			SPERM PRESENT
	4409				TR PHOS +++		PRESENT			SPERM PRESENT
	4410				TR PHOS +++		PRESENT			SPERM PRESENT

DATA ARE COLLECTED FROM INDIVIDUAL ANIMALS (NOT POOLED)

AM PHOS=AMORPHOUS PHOSPHATES
CA CARB=CALCIUM CARBONATE
CA OXAL=CALCIUM OXALATE
TR PHOS=TRIPLE PHOSPHATES
+=FEW
++=MODERATE
+++=MANY
EPI=EPITHELIAL
GRAN=GRANULAR
HYLN=HYALINE
SULF=SULFONAMIDE
URIC=URIC ACID
OCC=OCCASIONAL
TNTC=TOO NUMEROUS TO COUNT
CRS=COARSE GRANULAR CASTS
FINE=FINE GRANULAR CASTS
/HPF=PER HIGH POWER FIELD
/LPF=PER LOW POWER FIELD

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 21. Urine Microscopic – Main Group (continued)

DOSE UNIT.	ANIMAL NUMBER	WBC /HPF	RBC /HPF	CASTS /LPF	CRYSTALS /LPF	EPI /LPF	BACTERIA /HPF	YEAST /HPF	MUCUS /LPF	MICROSCOPIC COMMENTS
50	4421				TR PHOS +++		PRESENT			SPERM PRESENT
	4422				TR PHOS ++ CA OXAL +		PRESENT			SPERM PRESENT
	4423				TR PHOS +++		PRESENT			SPERM PRESENT
	4424				TR PHOS +++		PRESENT			SPERM PRESENT
	4425				TR PHOS +++		PRESENT			SPERM PRESENT
	4426				TR PHOS +++ CA OXAL +		PRESENT			SPERM PRESENT
	4427				TR PHOS +		PRESENT			SPERM PRESENT
	4428				TR PHOS +++ CA OXAL RARE		PRESENT			SPERM PRESENT
	4429				TR PHOS +++		PRESENT			SPERM PRESENT
	4430				TR PHOS ++		PRESENT			SPERM PRESENT

DATA ARE COLLECTED FROM INDIVIDUAL ANIMALS (NOT POOLED)
AM PHOS=AMORPHOUS PHOSPHATES EPI=EPITHELIAL URIC=URIC ACID CRS=COARSE GRANULAR CASTS
CA CARB=CALCIUM CARBONATE GRAN=GRANULAR FINE=FINE GRANULAR CASTS
CA OXAL=CALCIUM OXALATE HYLN=HYALINE OCC=OCCASIONAL /HPF=PER HIGH POWER FIELD
TR PHOS=TRIPLE PHOSPHATES SULF=SULFONAMIDE TNTC=TOO NUMEROUS TO COUNT /LPF=PER LOW POWER FIELD
+=FEW ++=MODERATE +++=MANY

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 21. Urine Microscopic – Main Group (continued)

DOSE UNIT.	ANIMAL NUMBER	WBC /HPF	RBC /HPF	CASTS /LPF	CRYSTALS /LPF	EPI /LPF	BACTERIA /HPF	YEAST /HPF	MUCUS /LPF	MICROSCOPIC COMMENTS
150	4441				TR PHOS + CA OXAL +++		PRESENT			SPERM PRESENT
	4442				TR PHOS +++ CA OXAL +		PRESENT			SPERM PRESENT
	4443				TR PHOS + CA OXAL +++		PRESENT			SPERM PRESENT
	4445				TR PHOS RARE CA OXAL +++		PRESENT			SPERM PRESENT
	4446				TR PHOS ++ CA OXAL +++		PRESENT			SPERM PRESENT
	4447				TR PHOS +++ CA OXAL ++		PRESENT			SPERM PRESENT
	4448				TR PHOS +++ CA OXAL ++		PRESENT			SPERM PRESENT
	4449				TR PHOS + CA OXAL +++		PRESENT			SPERM PRESENT
	4450				CA OXAL +++		PRESENT			SPERM PRESENT

DATA ARE COLLECTED FROM INDIVIDUAL ANIMALS (NOT POOLED)

AM PHOS=AMORPHOUS PHOSPHATES EPI=EPITHELIAL URIC=URIC ACID CRS=COARSE GRANULAR CASTS
CA CARB=CALCIUM CARBONATE GRAN=GRANULAR FINE=FINE GRANULAR CASTS
CA OXAL=CALCIUM OXALATE HYLN=HYALINE OCC=OCCASIONAL /HPF=PER HIGH POWER FIELD
TR PHOS=TRIPLE PHOSPHATES SULF=SULFONAMIDE TNTC=TOO NUMEROUS TO COUNT /LPF=PER LOW POWER FIELD
+=FEW ++=MODERATE +++=MANY

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 21. Urine Microscopic – Main Group (continued)

DOSE UNIT.	ANIMAL NUMBER	WBC /HPF	RBC /HPF	CASTS /LPF	CRYSTALS /LPF	EPI /LPF	BACTERIA /HPF	YEAST /HPF	MUCUS /LPF	MICROSCOPIC COMMENTS
300	4464				TR PHOS +++ CA OXAL +++		PRESENT			SPERM PRESENT
	4465				TR PHOS +++ CA OXAL +++		PRESENT			SPERM PRESENT
	4467				TR PHOS +++ CA OXAL ++		PRESENT			SPERM PRESENT
	4468				TR PHOS + CA OXAL ++		PRESENT			SPERM PRESENT
	4469				TR PHOS ++ CA OXAL ++		PRESENT			SPERM PRESENT

DATA ARE COLLECTED FROM INDIVIDUAL ANIMALS (NOT POOLED)

AM PHOS=AMORPHOUS PHOSPHATES
CA CARB=CALCIUM CARBONATE
CA OXAL=CALCIUM OXALATE
TR PHOS=TRIPLE PHOSPHATES
+=FEW
++=MODERATE
+++=MANY
EPI=EPITHELIAL
GRAN=GRANULAR
HYLN=HYALINE
SULF=SULFONAMIDE
URIC=URIC ACID
OCC=OCCASIONAL
TNTC=TOO NUMEROUS TO COUNT
CRS=COARSE GRANULAR CASTS
FINE=FINE GRANULAR CASTS
/HPF=PER HIGH POWER FIELD
/LPF=PER LOW POWER FIELD

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 22. Oxalic Acid Clearance

Renal clearance of oxalate in the Wistar rat administered EG for 12 months, and control young (9-12 weeks of age) Wistar and F344 rats and old (approximately 12 months of age) F344 rats

Study Animals								Control Animals					
0 MKD		50 MKD		150 MKD		300 MKD		Young Wistar		Old F344		Young F344	
Animal number	Clearance (ml/min/kg bw)	Animal number	Clearance (ml/min/kg bw)	Animal number	Clearance (ml/min/kg bw)	Animal number	Clearance (ml/min/kg bw)	Animal number	Clearance (ml/min/kg bw)	Animal number	Clearance (ml/min/kg bw)	Animal number	Clearance (ml/min/kg bw)
04A4996 **	2.74	03A4436	4.46	03A4456	3.92	03A4476	2.61	NA *	4.41	NA *	5.81	NA *	5.22
04A4998 **	3.42	03A4438	5.23	03A4458	5.71	03A4477	5.51	NA	2.93	NA	5.15	NA	6.08
03A4416	4.47	03A4439	4.54	03A4459	4.83	03A4480	6.08	NA	3.46	NA	2.89	NA	6.27
03A4417	3.27	03A4440	3.79	03A4460	4.34	03A4461	3.80	NA	4.61	NA	4.38	NA	7.04
03A4419	5.62					03A4466	5.97	NA	3.56			NA	5.67
03A4420	3.95												
Mean	3.91		4.50		4.70		4.79		3.80		4.56		6.06
SD	1.03		0.59		0.77		1.53		0.70		1.26		0.68

* No animal number assigned to control animals used for clearance determinations only.
** Sentinel animals.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 23. Ratios of Renal Clearance of Oxalate versus Inulin

Ratios of renal clearance of oxalate vs. inulin in the Wistar rat administered EG for 12 months, and control young (9-12 weeks of age) Wistar and F344 rats and old (approximately 12 months of age) F344 rats

Study Animals								Control Animals					
0 MKD		50 MKD		150 MKD		300 MKD		Young Wistar		Old F344		Young F344	
Animal number	Clearance ratio (OX/IN)	Animal number	Clearance ratio (OX/IN)	Animal number	Clearance ratio (OX/IN)	Animal number	Clearance ratio (OX/IN)	Animal number	Clearance ratio (OX/IN)	Animal number	Clearance ratio (OX/IN)	Animal number	Clearance ratio (OX/IN)
04A4996 **	0.54	03A4436	0.72	03A4456	0.74	03A4476	0.83	NA *	0.50	NA *	.0.90	NA *	0.68
04A4998 **	0.73	03A4438	1.03	03A4458	0.70	03A4477	0.67	NA	0.90	NA	0.81	NA	0.76
03A4416	0.70	03A4439	1.04	03A4459	0.65	03A4480	1.15	NA	0.38	NA	0.66	NA	0.70
03A4417	1.40	03A4440	0.70	03A4460	0.85	03A4461	0.82	NA	0.58	NA	0.86	NA	0.72
03A4419	0.92					03A4466	0.41	NA	0.61			NA	0.65
03A4420	0.64												
Mean	0.82		0.87		0.73		0.78		0.59		0.81		0.70
SD	0.31		0.19		0.08		0.27		0.19		0.10		0.04

* No animal number assigned to control animals used for clearance determinations only.
** Sentinel animals.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 24. Organ and Organ/Body Weights Summary – Main Group

DOSE MKD		FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)	LIVER (G)	LIVER (G/100)
0	MEAN	483.5	2.551	0.530	11.717	2.427
	S.D.	39.4	0.140	0.036	0.897	0.109
	N=	8	8	8	8	8
50	MEAN	498.7	2.692	0.539	11.995	2.400
	S.D.	40.3	0.297	0.034	1.431	0.127
	N=	10	10	10	10	10
150	MEAN	467.6	2.417	0.517	11.129	2.373
	S.D.	45.5	0.248	0.028	1.539	0.143
	N=	9	9	9	9	9
300	MEAN	466.0	2.806	0.612	11.284	2.426
	S.D.	93.1	0.515	0.122	2.203	0.161
	N=	5	5	5	5	5

THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 25. Organ and Organ/Body Weights Summary – Metabolism Group

DOSE MKD		FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)
0	MEAN	488.4	2.455	0.505
	S.D.	68.5	0.270	0.028
	N=	5	5	5
50	MEAN	484.1	2.593	0.539
	S.D.	71.3	0.275	0.044
	N=	5	5	5
150	MEAN	485.2	2.649	0.548
	S.D.	38.8	0.182	0.047
	N=	5	5	5
300	MEAN	457.9	3.242	0.713
	S.D.	39.2	1.286	0.304
	N=	5	5	5

THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROL AT ALPHA=0.05.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 26. Organ and Organ/Body Weights Summary – Early Termination

DOSE MKD		FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)
400	MEAN	367.7	4.021	1.122
	S.D.	54.5	0.746	0.278
	N=	16	16	16

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations

Removal Reasons: All of those SELECTED

	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
ADRENAL GLANDS;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
AORTA;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
AUDITORY SEBACEOUS GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
BONE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
BONE - JOINT;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
BONE MARROW;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
BRAIN;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
CECUM;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	19
Hemorrhage; wall; multifocal	0	0	0	0	1

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
COAGULATING GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	14	20
Dark; bilateral	0	0	0	1	0
COLON;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
CRANIAL NERVE - OPTIC;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
KIDNEYS;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	8	3
Calculus; unilateral; pelvis	0	0	0	2	0
Calculus; bilateral; pelvis	0	0	0	1	3
Dilatation; left; pelvis	0	0	0	0	2
Dilatation; right; pelvis	0	0	0	3	1
Dilatation; bilateral; pelvis	0	0	0	3	6
Mottled	0	0	0	0	1
Pale; bilateral	0	0	0	3	14
Roughened Surface; bilateral	0	0	0	2	14
DUODENUM;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
EPIDIDYMIDES;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
ESOPHAGUS;.					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
EYE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	13	15	15	15	20
Cloudy; left; cornea	1	0	0	0	0
GENERAL;					
Submitted	(0)	(0)	(0)	(3)	(6)
No Visible Lesions	0	0	0	0	0
Ascites	0	0	0	3	0
Congestion; viscera	0	0	0	0	1
Decreased Amount Of Fat	0	0	0	0	5
Hemolyzed Blood; gastrointestinal tract	0	0	0	1	0
Hydrothorax; clear	0	0	0	0	1
Hydrothorax; serosanguineous	0	0	0	1	0
HEART;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	14	20
Mottled; bilateral; ventricle	0	0	0	1	0
ILEUM;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
JEJUNUM;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
LACRIMAL/HARDERIAN GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
LACRIMAL/HARDERIAN GLAND; (continued)					
No Visible Lesions	14	15	15	15	19
Increased Size; bilateral	0	0	0	0	1
LARYNX;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
LIVER;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	14	14	20
Hernia; hiatal	0	0	0	1	0
Pale	0	0	1	0	0
LUNG;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	14	14	14
Congestion; generalized	0	0	0	1	2
Edema	0	0	0	1	1
Focus; dark; multifocal	0	0	1	0	0
Mottled	0	0	0	0	4
LYMPH NODE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	14	14	15	12
Dark; mesenteric	0	1	0	0	0
Increased Size; generalized	0	0	1	0	0
Increased Size; renal	0	0	0	0	8
MEDIASTINAL TISSUE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
MESENTERIC TISSUE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
NASAL TISSUE - PHARYNX;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
ORAL TISSUE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
PANCREAS;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
PARATHYROID GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
PERIPHERAL NERVE - TIBIAL;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
PITUITARY GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
PROSTATE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
RECTUM;					
Submitted	(14)	(15)	(15)	(15)	(20)

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
RECTUM; (continued)					
No Visible Lesions	14	15	15	15	20
SALIVARY GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
SEMINAL VESICLES;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	13	19
Dark; bilateral	0	0	0	1	1
Inflammation; bilateral	0	0	0	1	0
SKELETAL MUSCLE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
SKIN AND SUBCUTIS;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	13	15	14	14	20
Perineal Soiling	0	0	0	1	0
Scab; right; muzzle	0	0	1	0	0
Soiling; right; periocular	1	0	0	0	0
SPINAL CORD;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
SPLEEN;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	13	15	14	15	20
Increased Size; probable lymphoid tumor	1	0	1	0	0

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
STOMACH;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	13	19
Erosion - Ulcer; glandular mucosa; multifocal	0	0	0	2	0
Hemolyzed Blood	0	0	0	0	1
Mineralization; glandular mucosa	0	0	0	0	1
TESTES;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	13	15	15	14	20
Flaccid; unilateral	1	0	0	1	0
THYMUS;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	14	15	20
Increased Size	0	0	1	0	0
THYROID GLAND;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
TONGUE;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	20
TRACHEA;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	15	19
Froth	0	0	0	0	1
URETER;					
Submitted	(0)	(0)	(0)	(0)	(2)
No Visible Lesions	0	0	0	0	0
Calculus	0	0	0	0	2

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 27. Gross Pathological Observations (continued)

Removal Reasons: All of those SELECTED	MALES				
	0 mkd	50 mkd	150 mkd	300 mkd	400 mkd
Number of Animals on Study :	14	15	15	15	20
Number of Animals Completed:	(14)	(15)	(15)	(15)	(20)
URETER; (continued)					
Dilatation; left	0	0	0	0	1
Dilatation; right	0	0	0	0	1
URINARY BLADDER;					
Submitted	(14)	(15)	(15)	(15)	(20)
No Visible Lesions	14	15	15	7	10
Calculus	0	0	0	7	4
Calculus; multifocal	0	0	0	1	1
Dilatation	0	0	0	8	3
Hemorrhage; wall	0	0	0	5	3
Thickened; wall	0	0	0	1	7
Urine - Bloody	0	0	0	0	2

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 1. Clinical Observations – Main Group

Dose	Animal Number	Day Observed First	Last	Observation/Comment
0 mkd				
	4401	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy
	4402	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4403	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4404	-8	22	No Remarkable Observations
		30	307	Soiling, Periocular, Red, Right
		30	307	GI, Maloccluded Incisors
		302	307	Skin/Mucous Membranes Pale
		307	307	Gait, Dragging Hindquarters, Limbs Flacid
		307	307	Disposition, Moribund - Unscheduled
	4405	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4406	-8	127	No Remarkable Observations
		141	365	Eye, Cloudy, Left
		253	365	Eye, Enlarged or Protruding, Left
		369	369	Disposition, Scheduled Necropsy
	4407	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4408	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4409	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4410	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 1. Clinical Observations – Main Group (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
50 mkd				
	4421	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4422	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4423	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4424	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4425	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4426	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4427	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4428	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4429	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4430	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
150 mkd				
	4441	-8	337	No Remarkable Observations
		351	365	Skin, Scab, Focal, Muzzle, Right
		369	369	Disposition, Scheduled Necropsy
	4442	-8	365	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 1. Clinical Observations – Main Group (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
150 mkd				
	4442	369	369	Disposition, Scheduled Necropsy
	4443	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4444	-8	253	No Remarkable Observations
		266	267	Soiling, Perinasal, Red
		266	267	Skin/Mucous Membranes Pale
		266	267	Feces, Abnormal Quantity, Decreased
		267	267	Disposition, Moribund - Unscheduled
	4445	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4446	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4447	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4448	-8	-8	No Remarkable Observations
		1	365	Injury, Apparent Mechanical, Trauma, Tail, Tip
		369	369	Disposition, Scheduled Necropsy
	4449	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4450	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
300 mkd				
	4461	-8	351	No Remarkable Observations
		363	363	Disposition, Scheduled Necropsy
	4462	-8	127	No Remarkable Observations
		138	138	Disposition, Moribund - Unscheduled

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 1. Clinical Observations – Main Group (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
300 mkd				
	4463	-8	99	No Remarkable Observations
		111	111	Disposition, Spontaneous - Unscheduled
	4464	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4465	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4466	-8	351	No Remarkable Observations
		363	363	Disposition, Scheduled Necropsy
	4467	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4468	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4469	-8	365	No Remarkable Observations
		369	369	Disposition, Scheduled Necropsy
	4470	-8	197	No Remarkable Observations
		207	207	Disposition, Spontaneous - Unscheduled
400 mkd				
	4481	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4482	-8	57	No Remarkable Observations
		64	71	GI, Maloccluded Incisors
		78	183	No Remarkable Observations
		187	187	Misc, Blood in Cage
		187	187	Feces, Abnormal Quantity, Absent
		187	187	Disposition, Moribund - Unscheduled
	4483	-8	22	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 1. Clinical Observations – Main Group (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
400 mkd				
	4483	30	30	Skin, Flaking/Scaling, Focal, Neck, Left
		36	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4484	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4485	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4486	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4487	-8	183	No Remarkable Observations
		193	193	Disposition, Moribund - Unscheduled
	4488	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4489	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4490	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 2. Clinical Observations – Metabolism Group

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
0 mkd				
	4411	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4412	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4413	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4414	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4415	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
50 mkd				
	4431	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4432	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4433	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4434	-8	113	No Remarkable Observations
		141	197	Skin, Excessive Hairloss, Axillary, Right
		211	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4435	-8	1	No Remarkable Observations
		30	365	Soiling, Periocular, Red, Right
		30	365	GI, Maloccluded Incisors
		370	370	Disposition, Scheduled Necropsy - Metabolism

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 2. Clinical Observations – Metabolism Group (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
150 mkd				
	4451	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4452	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4453	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4454	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4455	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
300 mkd				
	4471	-8	-8	Injury, Apparent Mechanical, Other, Tail, Tip
		-8	-8	tail is kinked.
		1	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4472	-8	-8	Tail is kinked.
		-8	365	Injury, Apparent Mechanical, Other, Tail, Tip
		1	1	Tail is kinked.
		30	30	Tail is kinked.
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4473	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4474	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism
	4475	-8	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy - Metabolism

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 2. Clinical Observations – Metabolism Group (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
400 mkd				
	4491	-8	30	No Remarkable Observations
		43	43	Disposition, Spontaneous - Unscheduled
	4492	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4493	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4494	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4495	-8	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 3. Clinical Observations – Oxalate Clearance Group

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
0 mkd				
	4416	1	351	No Remarkable Observations
	4417	1	351	No Remarkable Observations
		357	357	Disposition, Scheduled Necropsy
	4418	1	351	No Remarkable Observations
		362	362	Disposition, Scheduled Necropsy
	4419	1	351	No Remarkable Observations
		362	362	Disposition, Scheduled Necropsy
	4420	1	365	No Remarkable Observations
		370	370	Disposition, Scheduled Necropsy
50 mkd				
	4436	1	337	No Remarkable Observations
		350	350	Disposition, Scheduled Necropsy
	4437	1	337	No Remarkable Observations
		350	350	Disposition, Scheduled Necropsy
	4438	1	351	No Remarkable Observations
		355	355	Disposition, Scheduled Necropsy
	4439	1	351	No Remarkable Observations
		355	355	Disposition, Scheduled Necropsy
	4440	1	267	No Remarkable Observations
		281	281	Injury, Apparent Mechanical, Trauma, Hinddigit, Left
		295	351	No Remarkable Observations
		361	361	Disposition, Scheduled Necropsy
150 mkd				
	4456	1	85	No Remarkable Observations
		92	92	Soiling, Periocular, Red, Right

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 3. Clinical Observations – Oxalate Clearance Group (continued)

Dose	Animal Number	Day Observed First	Last	Observation/Comment
150 mkd				
	4456	92	351	GI, Maloccluded Incisors
		246	246	Palpable Mass 1, 0.3 to 1.0 cm, Ulcerated, Hock (Tarsus), Right
		351	351	Disposition, Scheduled Necropsy
	4457	1	141	No Remarkable Observations
		169	351	Skin, Excessive Hairloss, Muzzle, General
		169	351	Skin, Excessive Hairloss, Chin
		197	351	Swelling , Muzzle, General
		197	351	Swelling , Chin
		295	351	Skin, Thin Hair Coat
		351	351	Disposition, Scheduled Necropsy
	4458	1	351	No Remarkable Observations
		356	356	Disposition, Scheduled Necropsy
	4459	1	351	No Remarkable Observations
		356	356	Disposition, Scheduled Necropsy
	4460	1	351	No Remarkable Observations
		361	361	Disposition, Scheduled Necropsy
300 mkd				
	4476	1	351	No Remarkable Observations
		354	354	Disposition, Scheduled Necropsy
	4477	1	30	No Remarkable Observations
		53	57	Soiling, Periocular, Red, Bilateral
		53	57	Soiling, Perinasal, Red
		53	351	GI, Maloccluded Incisors
		358	358	Disposition, Scheduled Necropsy
	4478	1	197	No Remarkable Observations
		211	212	Urine, Abnormal Color, Red
		211	212	Soiling, Perioral, Red
		211	212	Soiling, Perinasal, Red
		212	212	Feces, Abnormal Quantity, Decreased

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 3. Clinical Observations – Oxalate Clearance Group (continued)

Dose	Animal Number	Day Observed First	Last	Observation/Comment
300 mkd				
	4478	213	213	Disposition, Spontaneous - Unscheduled
	4479	1	211	No Remarkable Observations
		218	219	Urine, Abnormal Color, Red
		218	219	Soiling, Perioral, Red
		218	219	Soiling, Perineal, Urine
		218	219	Soiling, Perinasal, Red
		219	219	Feces, Abnormal Quantity, Decreased
		221	221	Disposition, Spontaneous - Unscheduled
	4480	1	323	No Remarkable Observations
		330	330	Urine, Abnormal Color, Red
		337	351	No Remarkable Observations
		358	358	Disposition, Scheduled Necropsy
400 mkd				
	4496	1	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4497	1	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4498	1	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4499	1	197	No Remarkable Observations
		203	203	Disposition, Scheduled Necropsy - Early Termination
	4500	1	141	No Remarkable Observations
		154	154	Disposition, Spontaneous - Unscheduled

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 4. Ophthalmic Observations

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
0 mkd				
	4401	-2	-2	No Remarkable Observations
	4402	-2	-2	No Remarkable Observations
	4403	-2	-2	No Remarkable Observations
	4404	-2	-2	No Remarkable Observations
	4405	-2	-2	No Remarkable Observations
	4406	-2	-2	No Remarkable Observations
	4407	-2	-2	No Remarkable Observations
	4408	-2	-2	No Remarkable Observations
	4409	-2	-2	No Remarkable Observations
	4410	-2	-2	No Remarkable Observations
	4411	-2	-2	No Remarkable Observations
	4412	-2	-2	No Remarkable Observations
	4413	-2	-2	No Remarkable Observations
	4414	-2	-2	No Remarkable Observations
	4415	-2	-2	No Remarkable Observations
	4416	-2	-2	No Remarkable Observations
	4417	-2	-2	No Remarkable Observations
	4418	-2	-2	No Remarkable Observations
	4419	-2	-2	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 4. Ophthalmic Observations (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
0 mkd				
	4420	-2	-2	No Remarkable Observations
50 mkd				
	4421	-2	-2	No Remarkable Observations
	4422	-2	-2	No Remarkable Observations
	4423	-2	-2	No Remarkable Observations
	4424	-2	-2	No Remarkable Observations
	4425	-2	-2	No Remarkable Observations
	4426	-2	-2	No Remarkable Observations
	4427	-2	-2	No Remarkable Observations
	4428	-2	-2	No Remarkable Observations
	4429	-2	-2	No Remarkable Observations
	4430	-2	-2	Eye, Cloudy Lens, Right
	4431	-2	-2	No Remarkable Observations
	4432	-2	-2	Eye, Cloudy Lens, Right
	4433	-2	-2	No Remarkable Observations
	4434	-2	-2	No Remarkable Observations
	4435	-2	-2	No Remarkable Observations
	4436	-2	-2	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 4. Ophthalmic Observations (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
50 mkd				
	4437	-2	-2	No Remarkable Observations
	4438	-2	-2	No Remarkable Observations
	4439	-2	-2	No Remarkable Observations
	4440	-2	-2	No Remarkable Observations
150 mkd				
	4441	-2	-2	No Remarkable Observations
	4442	-2	-2	No Remarkable Observations
	4443	-2	-2	No Remarkable Observations
	4444	-2	-2	No Remarkable Observations
	4445	-2	-2	No Remarkable Observations
	4446	-2	-2	No Remarkable Observations
	4447	-2	-2	Eye, Cloudy Lens, Right
	4448	-2	-2	No Remarkable Observations
	4449	-2	-2	No Remarkable Observations
	4450	-2	-2	No Remarkable Observations
	4451	-2	-2	No Remarkable Observations
	4452	-2	-2	No Remarkable Observations
	4453	-2	-2	No Remarkable Observations
	4454	-2	-2	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 4. Ophthalmic Observations (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
150 mkd				
	4455	-2	-2	No Remarkable Observations
	4456	-2	-2	No Remarkable Observations
	4457	-2	-2	No Remarkable Observations
	4458	-2	-2	No Remarkable Observations
	4459	-2	-2	No Remarkable Observations
	4460	-2	-2	No Remarkable Observations
300 mkd				
	4461	-2	-2	No Remarkable Observations
	4462	-2	-2	No Remarkable Observations
	4463	-2	-2	No Remarkable Observations
	4464	-2	-2	No Remarkable Observations
	4465	-2	-2	No Remarkable Observations
	4466	-2	-2	No Remarkable Observations
	4467	-2	-2	No Remarkable Observations
	4468	-2	-2	No Remarkable Observations
	4469	-2	-2	No Remarkable Observations
	4470	-2	-2	No Remarkable Observations
	4471	-2	-2	No Remarkable Observations
	4472	-2	-2	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 4. Ophthalmic Observations (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
300 mkd				
	4473	-2	-2	No Remarkable Observations
	4474	-2	-2	No Remarkable Observations
	4475	-2	-2	Eye, Cloudy Lens, Left
	4476	-2	-2	No Remarkable Observations
	4477	-2	-2	No Remarkable Observations
	4478	-2	-2	No Remarkable Observations
	4479	-2	-2	No Remarkable Observations
	4480	-2	-2	No Remarkable Observations
400 mkd				
	4481	-2	-2	No Remarkable Observations
	4482	-2	-2	No Remarkable Observations
	4483	-2	-2	No Remarkable Observations
	4484	-2	-2	No Remarkable Observations
	4485	-2	-2	No Remarkable Observations
	4486	-2	-2	No Remarkable Observations
	4487	-2	-2	No Remarkable Observations
	4488	-2	-2	No Remarkable Observations
	4489	-2	-2	No Remarkable Observations
	4490	-2	-2	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 4. Ophthalmic Observations (continued)

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
400 mkd				
	4491	-2	-2	No Remarkable Observations
	4492	-2	-2	No Remarkable Observations
	4493	-2	-2	No Remarkable Observations
	4494	-2	-2	No Remarkable Observations
	4495	-2	-2	No Remarkable Observations
	4496	-2	-2	No Remarkable Observations
	4497	-2	-2	No Remarkable Observations
	4498	-2	-2	No Remarkable Observations
	4499	-2	-2	No Remarkable Observations
	4500	-2	-2	No Remarkable Observations

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	8	GAIN	15	GAIN	22	GAIN	30	GAIN	36	GAIN	43	GAIN
0	4401	173.3	216.2	42.9	253.7	80.4	283.4	110.1	306.0	132.7	323.2	149.9	333.5	160.2
	4402	183.3	228.9	45.6	262.0	78.7	285.4	102.1	308.2	124.9	322.6	139.3	340.0	156.7
	4403	179.9	225.8	45.9	258.6	78.7	286.1	106.2	302.2	122.3	318.4	138.5	326.3	146.4
	4404	197.8	244.1	46.3	282.2	84.4	317.6	119.8	291.7	93.9	328.2	130.4	361.0	163.2
	4405	165.2	201.7	36.5#	230.3	65.1	250.8	85.6	269.3	104.1	280.1	114.9	293.0	127.8
	4406	185.7	230.8	45.1	262.9	77.2	288.3	102.6	303.5	117.8	313.8	128.1	327.9	142.2
	4407	192.6	240.9	48.3	287.2	94.6	320.7	128.1	350.5	157.9	369.0	176.4	381.1	188.5
	4408	165.2	206.7	41.5	244.0	78.8	274.1	108.9	298.9	133.7	320.9	155.7	334.8	169.6
	4409	179.0	224.2	45.2	263.6	84.6	296.6	117.6	316.0	137.0	335.4	156.4	348.3	169.3
	4410	197.8	242.7	44.9	285.6	87.8	313.0	115.2	338.2	140.4	360.9	163.1	371.3	173.5
	MEAN	182.0	226.2	44.2	263.0	81.0	291.6	109.6	308.5	126.5	327.3	145.3	341.7	159.7
	S.D.	11.9	14.6	3.3	18.3	7.7	21.4	11.7	22.8	18.4	24.7	18.4	25.3	17.4
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	4421	182.9	231.9	49.0	276.0	93.1	310.1	127.2	332.6	149.7	353.3	170.4	373.8	190.9
	4422	171.2	214.7	43.5	256.4	85.2	285.1	113.9	308.4	137.2	327.9	156.7	339.0	167.8
	4423	177.8	215.2	37.4	244.0	66.2	265.5	87.7	284.7	106.9	308.5	130.7	324.0	146.2
	4424	186.5	230.4	43.9	228.5	42.0	276.7	90.2	293.7	107.2	324.0	137.5	340.1	153.6
	4425	191.9	225.9	34.0	260.6	68.7	285.9	94.0	309.9	118.0	334.0	142.1	339.8	147.9
	4426	187.9	235.6	47.7	271.8	83.9	305.2	117.3	327.5	139.6	342.9	155.0	364.9	177.0
	4427	183.3	224.8	41.5	260.9	77.6	289.3	106.0	312.0	128.7	325.9	142.6	343.8	160.5
	4428	175.1	215.4	40.3	250.5	75.4	281.0	105.9	298.9	123.8	317.7	142.6	327.2	152.1
	4429	169.0	209.1	40.1	249.8	80.8	274.5	105.5	290.7	121.7	304.8	135.8	320.1	151.1
	4430	207.8	249.2	41.4	284.4	76.6	307.3	99.5	323.6	115.8	346.6	138.8	363.8	156.0
	MEAN	183.3	225.2	41.9	258.3	75.0	288.1	104.7	308.2	124.9	328.6	145.2	343.7	160.3
	S.D.	11.3	12.1	4.5	16.4	14.0	15.0	12.4	16.3	14.0	15.9	12.0	18.3	14.3
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	8	GAIN	15	GAIN	22	GAIN	30	GAIN	36	GAIN	43	GAIN
150	4441	184.8	229.1	44.3	272.0	87.2	300.3	115.5	325.8	141.0	347.0	162.2	371.0	186.2
	4442	164.4	206.5	42.1	246.7	82.3	275.7	111.3	297.4	133.0	321.4	157.0	335.5	171.1
	4443	178.4	219.1	40.7	256.1	77.7	285.3	106.9	308.0	129.6	326.4	148.0	340.1	161.7
	4444	200.4	248.3	47.9	276.0	75.6	303.4	103.0	320.6	120.2	334.5	134.1	344.4	144.0
	4445	192.3	231.0	38.7	266.4	74.1	291.9	99.6	312.7	120.4	331.1	138.8	348.0	155.7
	4446	186.0	216.3	30.3	236.0	50.0	244.1	58.1	250.9	64.9	266.4	80.4	275.8	89.8
	4447	195.0	249.0	54.0	292.9	97.9	327.1	132.1	349.9	154.9	372.0	177.0	388.1	193.1
	4448	172.3	212.5	40.2	243.3	71.0	264.4	92.1	284.9	112.6	303.6	131.3	316.1	143.8
	4449	201.0	241.1	40.1	272.0	71.0	287.1	86.1	295.8	94.8	309.4	108.4	318.7	117.7
	4450	158.7	186.6	27.9	207.8	49.1	226.0	67.3	238.5	79.8	248.4	89.7	260.7	102.0
	MEAN	183.3	224.0	40.6	256.9	73.6	280.5	97.2	298.5	115.1	316.0	132.7	329.8	146.5
	S.D.	14.7	19.7	7.6	24.4	15.1	29.6	22.3	33.7	27.9	36.6	31.4	39.1	34.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	4461	183.1	228.9	45.8	268.7	85.6	307.5	124.4	326.7	143.6	345.2	162.1	353.8	170.7
	4462	157.7	197.6	39.9	235.0	77.3	264.2	106.5	279.4	121.7	294.5	136.8	301.6	143.9
	4463	206.0	242.6	36.6	277.3	71.3	311.4	105.4	334.7	128.7	354.4	148.4	370.1	164.1
	4464	176.2	207.8	31.6	232.6	56.4	255.8	79.6	275.5	99.3	284.5	108.3	293.3	117.1
	4465	187.5	235.5	48.0	283.5	96.0	318.2	130.7	345.5	158.0	371.8	184.3	382.1	194.6
	4466	174.1	211.3	37.2	234.5	60.4	252.5	78.4	264.4	90.3	279.5	105.4	286.1	112.0
	4467	153.3	189.9	36.6	227.6	74.3	258.8	105.5	282.0	128.7	304.9	151.6	319.0	165.7
	4468	203.7	244.7	41.0	284.1	80.4	314.4	110.7	333.8	130.1	351.7	148.0	364.2	160.5
	4469	185.1	224.1	39.0	257.9	72.8	289.6	104.5	303.5	118.4	320.6	135.5	324.5	139.4
	4470	183.9	221.3	37.4	263.3	79.4	298.7	114.8	312.4	128.5	338.7	154.8	347.2	163.3
	MEAN	181.1	220.4	39.3	256.5	75.4	287.1	106.1	305.8	124.7	324.6	143.5	334.2	153.1
	S.D.	17.0	18.5	4.8	22.3	11.5	26.6	16.7	29.0	19.5	32.4	23.7	34.0	25.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	8	GAIN	15	GAIN	22	GAIN	30	GAIN	36	GAIN	43	GAIN
400	4481	194.6	222.0	27.4	235.8	41.2	252.0	57.4	269.8	75.2	287.7	93.1	298.9	104.3
	4482	188.9	226.5	37.6	250.5	61.6	275.6	86.7	291.1	102.2	304.7	115.8	312.1	123.2
	4483	185.1	225.2	40.1	264.1	79.0	296.6	111.5	313.9	128.8	334.7	149.6	345.2	160.1
	4484	172.5	214.2	41.7	248.4	75.9	275.7	103.2	296.1	123.6	310.0	137.5	324.0	151.5
	4485	164.8	204.4	39.6	237.2	72.4	266.2	101.4	280.4	115.6	295.0	130.2	302.6	137.8
	4486	174.5	207.0	32.5	235.3	60.8	261.2	86.7	276.4	101.9	290.9	116.4	298.9	124.4
	4487	217.3	264.2#	46.9	307.1#	89.8	342.1#	124.8	353.2	135.9	366.9	149.6	376.2	158.9
	4488	184.2	217.7	33.5	236.1	51.9	253.8	69.6	274.2	90.0	287.3	103.1	296.4	112.2
	4489	183.1	216.6	33.5	242.3	59.2	268.4	85.3	283.9	100.8	298.4	115.3	301.0	117.9
	4490	154.8	198.7	43.9	251.2	96.4	293.7	138.9	319.7	164.9	341.7	186.9	361.6	206.8
	MEAN	182.0	219.7	37.7	250.8	68.8	278.5	96.6	295.9	113.9	311.7	129.8	321.7	139.7
	S.D.	17.2	18.1	5.9	21.8	17.1	26.8	24.6	26.1	25.7	27.1	27.4	29.2	30.6
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	50	GAIN	57	GAIN	64	GAIN	71	GAIN	78	GAIN	85	GAIN
0	4401	173.3	348.6	175.3	352.1	178.8	358.2	184.9	371.5	198.2	381.1	207.8	392.8	219.5
	4402	183.3	350.1	166.8	357.0	173.7	369.0	185.7	378.3	195.0	395.6	212.3	408.8	225.5
	4403	179.9	338.1	158.2	343.7	163.8	348.0	168.1	356.3	176.4	368.0	188.1	384.6	204.7
	4404	197.8	394.4	196.6	411.1	213.3	429.2	231.4	433.0	235.2	435.5	237.7	453.0	255.2
	4405	165.2	302.8	137.6	303.0	137.8	313.1	147.9	316.3	151.1	323.9	158.7	332.9	167.7
	4406	185.7	346.9	161.2	354.9	169.2	366.4	180.7	384.9	199.2	399.6	213.9	411.6	225.9
	4407	192.6	394.6	202.0	405.2	212.6	417.8	225.2	429.8	237.2	435.9	243.3	448.7	256.1
	4408	165.2	351.4	186.2	366.2	201.0	377.5	212.3	388.4	223.2	397.5	232.3	403.1	237.9
	4409	179.0	361.7	182.7	366.9	187.9	377.6	198.6	390.7	211.7	401.5	222.5	410.3	231.3
	4410	197.8	385.0	187.2	394.1	196.3	403.6	205.8	416.4	218.6	422.5	224.7	432.4	234.6
	MEAN	182.0	357.4	175.4	365.4	183.4	376.0	194.1	386.6	204.6	396.1	214.1	407.8	225.8
	S.D.	11.9	28.2	19.6	32.0	23.6	34.2	25.8	35.1	26.7	33.5	25.2	34.5	25.6
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	4421	182.9	392.3	209.4#	401.2	218.3#	416.0	233.1#	428.9	246.0#	438.1	255.2	449.3	266.4
	4422	171.2	348.0	176.8	357.5	186.3#	367.1	195.9	379.8	208.6	389.1	217.9	398.3	227.1
	4423	177.8	338.7	160.9	347.9	170.1	357.8	180.0	376.0	198.2	385.1	207.3	396.7	218.9
	4424	186.5	350.0	163.5	357.6	171.1	371.1	184.6	383.0	196.5	393.7	207.2	389.0	202.5
	4425	191.9	351.7	159.8	364.0	172.1	369.6	177.7	378.7	186.8	393.1	201.2	403.8	211.9
	4426	187.9	380.9	193.0#	384.8	196.9#	403.4	215.5#	415.2	227.3#	422.2	234.3	429.4	241.5
	4427	183.3	352.5	169.2	361.5	178.2	371.0	187.7	382.4	199.1	388.7	205.4	392.8	209.5
	4428	175.1	339.9	164.8	344.8	169.7	353.1	178.0	364.9	189.8	365.9	190.8	373.2	198.1
	4429	169.0	331.3	162.3	342.5	173.5	349.0	180.0	360.0	191.0	369.1	200.1	373.1	204.1
	4430	207.8	373.1	165.3	381.0	173.2	388.5	180.7	398.0	190.2	407.9	200.1	421.2	213.4
	MEAN	183.3	355.8	172.5	364.3	180.9	374.7	191.3	386.7	203.4	395.3	212.0	402.7	219.3
	S.D.	11.3	19.8	16.3	19.1	15.7	21.7	18.7	21.5	19.1	22.3	19.3	24.3	20.9
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	50	GAIN	57	GAIN	64	GAIN	71	GAIN	78	GAIN	85	GAIN
150	4441	184.8	382.5	197.7	386.3	201.5	391.2	206.4	406.3	221.5	409.4	224.6	418.1	233.3
	4442	164.4	349.7	185.3	358.9	194.5	369.7	205.3	384.9	220.5	393.9	229.5	404.4	240.0
	4443	178.4	355.6	177.2	363.5	185.1	379.7	201.3	394.9	216.5	403.5	225.1	410.7	232.3
	4444	200.4	357.2	156.8	363.7	163.3	373.0	172.6	389.2	188.8	396.2	195.8	413.1	212.7
	4445	192.3	364.1	171.8	372.0	179.7	382.5	190.2	400.1	207.8	408.8	216.5	419.8	227.5
	4446	186.0	288.6	102.6	305.9	119.9	316.7	130.7	330.5	144.5	342.2	156.2	350.9	164.9
	4447	195.0	399.1	204.1	414.2	219.2	424.3	229.3	444.2	249.2	453.6	258.6	462.4	267.4
	4448	172.3	328.7	156.4	334.3	162.0	345.5	173.2	360.5	188.2	371.9	199.6	383.1	210.8
	4449	201.0	328.9	127.9	334.8	133.8	346.3	145.3	358.2	157.2	368.8	167.8	376.2	175.2
	4450	158.7	276.0	117.3	283.7	125.0	289.9	131.2	301.3	142.6	306.0	147.3	312.1	153.4
	MEAN	183.3	343.0	159.7	351.7	168.4	361.9	178.6	377.0	193.7	385.4	202.1	395.1	211.8
	S.D.	14.7	38.6	34.3	38.2	33.8	38.6	34.0	40.8	36.1	40.7	35.8	41.7	36.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	4461	183.1	366.6	183.5	376.2	193.1	381.0	197.9	396.8	213.7	402.7	219.6	406.4	223.3
	4462	157.7	314.5	156.8	323.1	165.4	333.0	175.3	345.1	187.4	355.8	198.1	367.4	209.7
	4463	206.0	386.0	180.0	398.5	192.5	407.2	201.2	428.3	222.3	434.9	228.9	445.9	239.9
	4464	176.2	308.4	132.2	312.5	136.3	312.3	136.1	320.9	144.7	328.2	152.0	334.3	158.1
	4465	187.5	402.2	214.7	414.1	226.6	428.7	241.2	442.4	254.9	453.5	266.0	468.1	280.6
	4466	174.1	297.8	123.7	301.1	127.0	305.0	130.9	315.2	141.1	322.5	148.4	331.8	157.7
	4467	153.3	334.6	181.3	347.8	194.5	351.4	198.1	365.4	212.1	369.7	216.4	379.1	225.8
	4468	203.7	380.1	176.4	386.8	183.1	393.7	190.0	410.3	206.6	419.9	216.2	425.9	222.2
	4469	185.1	333.9	148.8	331.5	146.4	345.0	159.9	350.8	165.7	362.0	176.9	370.0	184.9
	4470	183.9	367.3	183.4	372.3	188.4	382.9	199.0	384.0	200.1	391.6	207.7	402.6	218.7
	MEAN	181.1	349.1	168.1	356.4	175.3	364.0	183.0	375.9	194.9	384.1	203.0	393.2	212.1
	S.D.	17.0	36.1	27.5	38.7	31.0	41.1	33.3	43.8	35.7	44.1	35.9	45.2	37.3
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	50	GAIN	57	GAIN	64	GAIN	71	GAIN	78	GAIN	85	GAIN
400	4481	194.6	313.9	119.3	320.0	125.4	327.9	133.3	341.6	147.0	347.2	152.6	356.6	162.0
	4482	188.9	330.3	141.4	336.4	147.5	343.3	154.4	352.5	163.6	351.8	162.9	363.7	174.8
	4483	185.1	365.6	180.5	374.6	189.5	382.3	197.2	393.7	208.6	399.6	214.5	412.0	226.9
	4484	172.5	338.6	166.1	348.2	175.7	361.8	189.3	372.7	200.2	379.4	206.9	389.4	216.9
	4485	164.8	312.0	147.2	317.0	152.2	324.9	160.1	331.6	166.8	340.5	175.7	349.4	184.6
	4486	174.5	311.3	136.8	311.5	137.0	267.2	92.7	333.2	158.7	337.7	163.2	347.9	173.4
	4487	217.3	403.9	186.6	413.6	196.3	422.2	204.9	426.8	209.5	438.9	221.6	445.2	227.9
	4488	184.2	311.2	127.0	313.4	129.2	321.5	137.3	329.4	145.2	334.1	149.9	344.4	160.2
	4489	183.1	317.8	134.7	321.1	138.0	327.0	143.9	338.2	155.1	350.8	167.7	362.5	179.4
	4490	154.8	379.9	225.1	389.8	235.0	405.4	250.6	414.7	259.9	414.1	259.3	423.5	268.7
	MEAN	182.0	338.5	156.5	344.6	162.6	348.4	166.4	363.4	181.5	369.4	187.4	379.5	197.5
	S.D.	17.2	33.4	32.8	36.2	35.5	45.7	44.9	36.5	36.9	36.6	36.2	35.9	35.7
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	92	GAIN	99	GAIN	113	GAIN	127	GAIN	141	GAIN	155	GAIN
0	4401	173.3	398.5	225.2	395.8	222.5	408.3	235.0	418.9	245.6	425.3	252.0	427.9	254.6
	4402	183.3	412.5	229.2	414.7	231.4	434.1	250.8	441.0	257.7	450.5	267.2	444.3	261.0
	4403	179.9	385.5	205.6	393.8	213.9	409.2	229.3	422.1	242.2	439.8	259.9	438.6	258.7
	4404	197.8	455.9	258.1	463.7	265.9	472.1	274.3	475.9	278.1	491.2	293.4	488.8	291.0
	4405	165.2	336.5	171.3	337.8	172.6	346.3	181.1	358.4	193.2	356.5	191.3#	356.1	190.9#
	4406	185.7	417.4	231.7	421.3	235.6	435.0	249.3	448.6	262.9	453.0	267.3	460.9	275.2
	4407	192.6	458.9	266.3	464.9	272.3	481.2	288.6	489.8	297.2	493.9	301.3	498.9	306.3
	4408	165.2	407.8	242.6	412.8	247.6	423.2	258.0	435.3	270.1	445.3	280.1	447.6	282.4
	4409	179.0	416.8	237.8	420.7	241.7	434.5	255.5	442.8	263.8	448.9	269.9	456.4	277.4
	4410	197.8	439.2	241.4	441.6	243.8	452.8	255.0	462.9	265.1	472.2	274.4	473.8	276.0
	MEAN	182.0	412.9	230.9	416.7	234.7	429.7	247.7	439.6	257.6	447.7	265.7	449.3	267.4
	S.D.	11.9	35.8	26.9	37.1	28.2	37.9	29.0	36.2	27.5	38.8	30.0	39.6	31.0
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	4421	182.9	450.6	267.7	451.2	268.3	461.2	278.3	476.7	293.8	479.0	296.1	493.4	310.5
	4422	171.2	402.3	231.1	410.9	239.7	422.9	251.7	438.2	267.0	437.5	266.3	450.8	279.6
	4423	177.8	399.6	221.8	408.0	230.2	425.7	247.9	443.4	265.6	454.4	276.6	464.1	286.3
	4424	186.5	396.5	210.0	399.0	212.5	416.6	230.1	431.9	245.4	442.9	256.4	447.5	261.0
	4425	191.9	407.4	215.5	411.1	219.2	425.2	233.3	438.3	246.4	443.7	251.8	447.8	255.9
	4426	187.9	430.9	243.0	436.6	248.7	447.7	259.8	457.2	269.3	470.4	282.5	478.2	290.3
	4427	183.3	393.0	209.7	398.0	214.7	406.0	222.7	419.2	235.9	423.2	239.9	427.2	243.9
	4428	175.1	374.3	199.2	376.5	201.4	388.1	213.0	399.3	224.2	405.3	230.2	409.6	234.5
	4429	169.0	376.2	207.2	381.8	212.8	393.3	224.3	402.9	233.9	403.5	234.5	408.8	239.8
	4430	207.8	423.2	215.4	424.3	216.5	433.5	225.7	447.2	239.4	456.3	248.5	459.3	251.5
	MEAN	183.3	405.4	222.1	409.7	226.4	422.0	238.7	435.4	252.1	441.6	258.3	448.7	265.3
	S.D.	11.3	23.8	20.4	23.1	20.4	22.6	20.2	23.7	21.2	25.3	21.7	27.5	25.1
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	92	GAIN	99	GAIN	113	GAIN	127	GAIN	141	GAIN	155	GAIN
150	4441	184.8	416.2	231.4	421.1	236.3	432.7	247.9	439.5	254.7	442.4	257.6	451.5	266.7
	4442	164.4	408.7	244.3	406.5	242.1	424.6	260.2	435.6	271.2	438.4	274.0	442.6	278.2
	4443	178.4	412.5	234.1	412.7	234.3	429.1	250.7	439.3	260.9	441.6	263.2	448.7	270.3
	4444	200.4	418.1	217.7	423.6	223.2	434.7	234.3	446.5	246.1	454.3	253.9	461.7	261.3
	4445	192.3	423.4	231.1	424.0	231.7	430.6	238.3	443.9	251.6	448.6	256.3	448.5	256.2
	4446	186.0	353.7	167.7	360.0	174.0	373.9	187.9	386.7	200.7	390.1	204.1	395.0	209.0
	4447	195.0	470.7	275.7	475.3	280.3	488.6	293.6	497.7	302.7	500.4	305.4	509.4	314.4
	4448	172.3	387.3	215.0	393.0	220.7	407.2	234.9	425.1	252.8	425.0	252.7	431.4	259.1
	4449	201.0	383.6	182.6	386.0	185.0	394.1	193.1	409.7	208.7	416.6	215.6	429.0	228.0
	4450	158.7	314.5	155.8	318.5	159.8	324.1	165.4	331.5	172.8	335.8	177.1	341.9	183.2
	MEAN	183.3	398.9	215.5	402.1	218.7	414.0	230.6	425.6	242.2	429.3	246.0	436.0	252.6
	S.D.	14.7	42.5	36.9	42.0	36.0	43.6	38.2	43.6	37.8	43.4	37.1	43.8	37.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	4461	183.1	405.3	222.2	405.9	222.8	418.9	235.8	427.9	244.8	434.6	251.5	438.0	254.9
	4462	157.7	375.1	217.4	374.9	217.2	386.3	228.6	400.3	242.6	***	***	***	***
	4463	206.0	451.0	245.0	450.6	244.6	***	***	***	***	***	***	***	***
	4464	176.2	330.5	154.3	331.4	155.2	344.0	167.8	357.9	181.7	359.3	183.1	361.0	184.8
	4465	187.5	468.8	281.3	476.2	288.7	497.3	309.8	514.0	326.5	531.1	343.6	535.3	347.8
	4466	174.1	334.9	160.8	338.7	164.6	350.7	176.6	358.8	184.7	363.9	189.8	364.4	190.3
	4467	153.3	382.6	229.3	384.0	230.7	393.6	240.3	410.4	257.1	412.0	258.7	408.4	255.1
	4468	203.7	430.5	226.8	434.2	230.5	448.7	245.0	463.1	259.4	469.1	265.4	467.7	264.0
	4469	185.1	376.3	191.2	375.3	190.2	387.1	202.0	397.6	212.5	395.0	209.9	405.1	220.0
	4470	183.9	401.8	217.9	403.0	219.1	417.7	233.8	431.2	247.3	428.9	245.0	431.9	248.0
	MEAN	181.1	395.7	214.6	397.4	216.4	404.9	226.6	417.9	239.6	424.2	243.4	426.5	245.6
	S.D.	17.0	45.5	37.8	46.5	38.9	47.8	42.1	49.3	43.9	56.6	51.3	56.9	51.2
	N=	10	10	10	10	10	9	9	9	9	8	8	8	8

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	92	GAIN	99	GAIN	113	GAIN	127	GAIN	141	GAIN	155	GAIN
400	4481	194.6	355.9	161.3	357.2	162.6	376.9	182.3	389.3	194.7	395.0	200.4	394.3	199.7
	4482	188.9	360.8	171.9	367.4	178.5	374.2	185.3	373.3	184.4	354.2	165.3	365.7	176.8
	4483	185.1	413.9	228.8	416.8	231.7	427.3	242.2	435.4	250.3	430.9	245.8	439.0	253.9
	4484	172.5	389.2	216.7	391.5	219.0	400.4	227.9	404.7	232.2	403.7	231.2	402.0	229.5
	4485	164.8	348.8	184.0	350.6	185.8	365.3	200.5	371.9	207.1	364.9	200.1	372.3	207.5
	4486	174.5	347.2	172.7	350.0	175.5	357.1	182.6	365.8	191.3	365.5	191.0	363.0	188.5
	4487	217.3	443.7	226.4	435.6	218.3	444.8	227.5	440.9	223.6	444.0	226.7	441.4	224.1
	4488	184.2	342.0	157.8	340.7	156.5	350.1	165.9	348.8	164.6	347.6	163.4	354.7	170.5
	4489	183.1	361.4	178.3	364.3	181.2	356.6	173.5	361.6	178.5	364.2	181.1	370.2	187.1
	4490	154.8	427.1	272.3	424.8	270.0	433.3	278.5	440.3	285.5	437.6	282.8	425.7	270.9
	MEAN	182.0	379.0	197.0	379.9	197.9	388.6	206.6	393.2	211.2	390.8	208.8	392.8	210.9
	S.D.	17.2	37.0	37.2	34.7	35.7	35.2	36.1	34.9	37.0	36.6	37.8	32.8	33.3
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST										
MKD	NUMBER	1	169	GAIN	183	GAIN	187	GAIN	190	GAIN	197	GAIN
0	4401	173.3	433.3	260.0	435.2	261.9	434.2	260.9	432.6	259.3	442.0	268.7
	4402	183.3	463.5	280.2	466.4	283.1	466.3	283.0	464.6	281.3	461.6	278.3
	4403	179.9	455.1	275.2	459.9	280.0	459.7	279.8	460.6	280.7	460.8	280.9
	4404	197.8	498.3	300.5	503.4	305.6	507.1	309.3	503.7	305.9	504.1	306.3
	4405	165.2	365.0	199.8#	367.3	202.1#	368.2	203.0	370.1	204.9#	369.5#	204.3#
	4406	185.7	468.6	282.9	468.8	283.1	466.1	280.4	463.5	277.8	465.8	280.1
	4407	192.6	508.6	316.0	513.0	320.4	505.6	313.0	506.0	313.4	504.0	311.4
	4408	165.2	459.3	294.1	463.1	297.9	463.4	298.2	468.5	303.3	464.2	299.0
	4409	179.0	465.0	286.0	469.1	290.1	442.1	263.1	465.6	286.6	462.0	283.0
	4410	197.8	482.2	284.4	483.0	285.2	483.6	285.8	482.2	284.4	479.2	281.4
	MEAN	182.0	459.9	277.9	462.9	280.9	459.6	277.7	461.7	279.8	461.3	279.3
	S.D.	11.9	39.7	31.3	40.2	31.9	39.9	31.4	38.7	30.7	37.7	29.6
	N=	10	10	10	10	10	10	10	10	10	10	10
50	4421	182.9	505.5	322.6	515.5	332.6	513.8	330.9	505.7	322.8	508.4	325.5
	4422	171.2	466.3	295.1	472.0	300.8	473.8	302.6	469.5	298.3	469.0	297.8
	4423	177.8	475.7	297.9	481.9	304.1	481.2	303.4	484.6	306.8	488.1	310.3
	4424	186.5	464.6	278.1	473.1	286.6	464.2	277.7	464.8	278.3	470.2	283.7
	4425	191.9	463.4	271.5	467.3	275.4	472.1	280.2	465.4	273.5	467.4	275.5
	4426	187.9	487.3	299.4	487.0	299.1	488.6	300.7	486.6	298.7	484.2	296.3
	4427	183.3	433.5	250.2	435.8	252.5	437.6	254.3	439.0	255.7	437.1	253.8
	4428	175.1	415.0	239.9	417.6	242.5	418.8	243.7	414.9	239.8	415.6	240.5
	4429	169.0	422.4	253.4	424.2	255.2	428.6	259.6	424.9	255.9	423.3	254.3
	4430	207.8	472.0	264.2	475.8	268.0	477.3	269.5	480.9	273.1	479.7	271.9
	MEAN	183.3	460.6	277.2	465.0	281.7	465.6	282.3	463.6	280.3	464.3	281.0
	S.D.	11.3	28.7	26.2	30.4	28.1	29.2	26.9	29.0	26.1	29.8	27.0
	N=	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST										
MKD	NUMBER	1	169	GAIN	183	GAIN	187	GAIN	190	GAIN	197	GAIN
150	4441	184.8	448.4	263.6	459.7	274.9	462.5	277.7	458.7	273.9	456.2	271.4
	4442	164.4	457.9	293.5	463.0	298.6	459.2	294.8	458.5	294.1	457.6	293.2
	4443	178.4	458.3	279.9	460.1	281.7	460.0	281.6	460.8	282.4	459.9	281.5
	4444	200.4	472.6	272.2	476.6	276.2	475.3	274.9	474.9	274.5	475.0	274.6
	4445	192.3	457.2	264.9	458.9	266.6	461.5	269.2	461.6	269.3	455.0	262.7
	4446	186.0	399.5	213.5	405.4	219.4	404.5	218.5	403.8	217.8	403.4	217.4
	4447	195.0	513.7	318.7	516.8	321.8	520.0	325.0	518.0	323.0	516.8	321.8
	4448	172.3	440.2	267.9	443.8	271.5	442.5	270.2	440.4	268.1	437.5	265.2
	4449	201.0	427.9	226.9	428.8	227.8	430.3	229.3	429.7	228.7	428.7	227.7
	4450	158.7	347.8	189.1	353.4	194.7	353.0	194.3	352.5	193.8	353.2	194.5
	MEAN	183.3	442.4	259.0	446.7	263.3	446.9	263.6	445.9	262.6	444.3	261.0
	S.D.	14.7	44.4	38.7	43.9	38.4	44.5	38.7	44.2	38.4	43.6	37.9
	N=	10	10	10	10	10	10	10	10	10	10	10
300	4461	183.1	441.7	258.6	438.3	255.2	437.9	254.8	440.6	257.5	440.4	257.3
	4462	157.7	***	***	***	***	***	***	***	***	***	***
	4463	206.0	***	***	***	***	***	***	***	***	***	***
	4464	176.2	362.7	186.5	374.0	197.8	371.0	194.8	374.4	198.2	371.0	194.8
	4465	187.5	548.1	360.6	555.6	368.1	562.3	374.8	559.0	371.5	561.3	373.8
	4466	174.1	370.8	196.7	372.3	198.2	371.8	197.7	370.4	196.3	370.0	195.9
	4467	153.3	408.4	255.1	401.4	248.1	398.6	245.3	403.2	249.9	405.0	251.7
	4468	203.7	480.6	276.9	482.2	278.5	482.6	278.9	474.8	271.1	458.9	255.2
	4469	185.1	408.4	223.3	414.7	229.6	413.2	228.1	415.6	230.5	413.8	228.7
	4470	183.9	442.3	258.4	449.3	265.4	446.7	262.8	450.4	266.5	447.9	264.0
	MEAN	181.1	432.9	252.0	436.0	255.1	435.5	254.7	436.1	255.2	433.5	252.7
	S.D.	17.0	60.6	54.4	61.1	54.3	63.8	57.0	61.5	55.1	61.4	56.0
	N=	10	8	8	8	8	8	8	8	8	8	8

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST										
MKD	NUMBER	1	169	GAIN	183	GAIN	187	GAIN	190	GAIN	197	GAIN
400	4481	194.6	401.2	206.6	399.1	204.5	393.8	199.2	392.3	197.7	390.7	196.1
	4482	188.9	356.5	167.6	358.0	169.1	317.7	128.8	***	***	***	***
	4483	185.1	447.6	262.5	436.2	251.1	439.7	254.6	437.6	252.5	423.6	238.5
	4484	172.5	412.3	239.8	397.9	225.4	388.8	216.3	382.6	210.1	371.7	199.2
	4485	164.8	378.0	213.2	377.7	212.9	374.2	209.4	374.5	209.7	363.3	198.5
	4486	174.5	366.5	192.0	356.8	182.3	344.4	169.9	347.6	173.1	340.6	166.1
	4487	217.3	435.9	218.6	413.1	195.8	398.1	180.8	388.6	171.3	***	***
	4488	184.2	366.2	182.0	369.4	185.2	370.3	186.1	361.0	176.8	361.6	177.4
	4489	183.1	367.6	184.5	370.8	187.7	362.1	179.0	356.1	173.0	337.5	154.4
	4490	154.8	416.7	261.9	392.0	237.2	384.5	229.7	371.2	216.4	360.4	205.6
	MEAN	182.0	394.9	212.9	387.1	205.1	377.4	195.4	379.1	197.8	368.7	192.0
	S.D.	17.2	32.3	33.2	25.4	26.3	32.8	34.9	26.5	27.4	27.8	26.1
	N=	10	10	10	10	10	10	10	9	9	8	8

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	204	GAIN	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN
0	4401	173.3	441.8	268.5	451.1	277.8	452.7	279.4	453.6	280.3	449.0	275.7	448.3	275.0
	4402	183.3	463.2	279.9	470.9	287.6	466.2	282.9	473.8	290.5	470.4	287.1	470.3	287.0
	4403	179.9	464.9	285.0	468.5	288.6	475.4	295.5	475.3	295.4	477.8	297.9	480.2	300.3
	4404	197.8	503.5	305.7	516.1	318.3	510.3	312.5	509.2	311.4	510.3	312.5	500.4	302.6
	4405	165.2	374.0#	208.8#	380.6	215.4#	378.6#	213.4#	381.9#	216.7#	385.0#	219.8#	384.8#	219.6#
	4406	185.7	467.0	281.3	473.5	287.8	475.1	289.4	478.8	293.1	476.9	291.2	476.3	290.6
	4407	192.6	506.8	314.2	512.9	320.3	512.8	320.2	512.8	320.2	514.5	321.9	508.1	315.5
	4408	165.2	467.8	302.6	472.1	306.9	472.7	307.5	470.4	305.2	473.4	308.2	470.4	305.2
	4409	179.0	469.8	290.8	469.8	290.8	472.3	293.3	472.6	293.6	472.6	293.6	473.9	294.9
	4410	197.8	481.4	283.6	490.8	293.0	490.1	292.3	489.5	291.7	492.5	294.7	489.1	291.3
	MEAN	182.0	464.0	282.0	470.6	288.7	470.6	288.6	471.8	289.8	472.2	290.3	470.2	288.2
	S.D.	11.9	37.0	29.2	37.7	29.3	37.4	29.4	36.3	28.1	36.3	28.1	34.4	26.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	4421	182.9	513.0	330.1	521.6	338.7	520.4	337.5	521.3	338.4	519.2	336.3	516.1	333.2
	4422	171.2	475.1	303.9	479.4	308.2	485.9	314.7	485.7	314.5	489.3	318.1	486.6	315.4
	4423	177.8	493.5	315.7	502.8	325.0	507.0	329.2	510.2	332.4	507.3	329.5	506.4	328.6
	4424	186.5	470.4	283.9	474.7	288.2	478.8	292.3	482.3	295.8	483.8	297.3	485.7	299.2
	4425	191.9	467.7	275.8	471.0	279.1	469.6	277.7	472.9	281.0	473.0	281.1	472.5	280.6
	4426	187.9	488.8	300.9	496.7	308.8	499.1	311.2	498.8	310.9	500.2	312.3	503.6	315.7
	4427	183.3	436.3	253.0	442.1	258.8	445.4	262.1	445.1	261.8	443.9	260.6	445.0	261.7
	4428	175.1	417.3	242.2	422.0	246.9	428.5	253.4	428.1	253.0	426.4	251.3	419.0	243.9
	4429	169.0	423.4	254.4	432.3	263.3	434.1	265.1	435.4	266.4	439.8	270.8	438.5	269.5
	4430	207.8	484.4	276.6	489.2	281.4	490.8	283.0	493.5	285.7	493.9	286.1	497.4	289.6
	MEAN	183.3	467.0	283.7	473.2	289.8	476.0	292.6	477.3	294.0	477.7	294.3	477.1	293.7
	S.D.	11.3	31.6	28.9	32.2	29.9	31.2	29.3	31.7	29.6	31.2	29.2	32.6	29.9
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	204	GAIN	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN
150	4441	184.8	456.8	272.0	469.4	284.6	466.8	282.0	465.1	280.3	466.0	281.2	464.0	279.2
	4442	164.4	457.9	293.5	463.8	299.4	463.1	298.7	470.2	305.8	466.1	301.7	465.0	300.6
	4443	178.4	463.8	285.4	472.2	293.8	472.7	294.3	472.5	294.1	472.5	294.1	474.3	295.9
	4444	200.4	476.3	275.9	483.7	283.3	485.6	285.2	492.2	291.8	492.7	292.3	491.5	291.1
	4445	192.3	458.5	266.2	468.6	276.3	473.0	280.7	476.0	283.7	470.6	278.3	466.5	274.2
	4446	186.0	405.9	219.9	415.7	229.7	418.7	232.7	419.0	233.0	417.5	231.5	410.7	224.7
	4447	195.0	517.3	322.3	526.3	331.3	524.7	329.7	523.6	328.6	522.1	327.1	521.6	326.6
	4448	172.3	438.0	265.7	450.2	277.9	452.1	279.8	449.2	276.9	454.8	282.5	450.0	277.7
	4449	201.0	432.8	231.8	445.1	244.1	449.4	248.4	447.4	246.4	448.2	247.2	443.5	242.5
	4450	158.7	355.3	196.6	358.9	200.2	361.0	202.3	360.1	201.4	362.2	203.5	363.3	204.6
	MEAN	183.3	446.3	262.9	455.4	272.1	456.7	273.4	457.5	274.2	457.3	273.9	455.0	271.7
	S.D.	14.7	43.2	37.2	44.2	37.7	43.2	36.3	44.1	37.4	43.1	36.5	43.4	37.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
300	4461	183.1	440.1	257.0	443.4	260.3	449.1	266.0	448.5	265.4	453.9	270.8	453.1	270.0
	4462	157.7	***	***	***	***	***	***	***	***	***	***	***	***
	4463	206.0	***	***	***	***	***	***	***	***	***	***	***	***
	4464	176.2	372.5	196.3	375.8	199.6	381.0	204.8	387.0	210.8	382.1	205.9	381.0	204.8
	4465	187.5	562.5	375.0	556.4	368.9	570.4	382.9	572.2	384.7	578.6	391.1	582.4	394.9
	4466	174.1	371.4	197.3	384.5	210.4	386.6	212.5	385.3	211.2	384.6	210.5	381.9	207.8
	4467	153.3	407.3	254.0	419.0	265.7	429.7	276.4	428.3	275.0	432.0	278.7	429.5	276.2
	4468	203.7	460.5	256.8	471.4	267.7	482.8	279.1	483.2	279.5	476.2	272.5	475.8	272.1
	4469	185.1	413.5	228.4	420.7	235.6	422.3	237.2	424.2	239.1	420.9	235.8	425.4	240.3
	4470	183.9	458.5	274.6	***	***	***	***	***	***	***	***	***	***
	MEAN	181.1	435.8	254.9	438.7	258.3	446.0	265.6	447.0	266.5	446.9	266.5	447.0	266.6
	S.D.	17.0	61.7	56.4	61.3	55.7	65.1	59.7	64.9	59.3	67.4	62.6	69.0	64.0
	N=	10	8	8	7	7	7	7	7	7	7	7	7	7

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	246	GAIN	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN
0	4401	173.3	448.6	275.3	449.2	275.9	456.5	283.2	459.8	286.5	460.5	287.2	464.7	291.4
	4402	183.3	472.8	289.5	471.5	288.2	481.7	298.4	479.8	296.5	482.5	299.2	486.3	303.0
	4403	179.9	480.8	300.9	478.7	298.8	482.6	302.7	478.0	298.1	483.4	303.5	489.9	310.0
	4404	197.8	511.1	313.3	509.2	311.4	513.0	315.2	514.4	316.6	505.4	307.6	513.8	316.0
	4405	165.2	387.8#	222.6#	390.0	224.8#	394.1	228.9#	393.4#	228.2#	395.1#	229.9#	398.8#	233.6#
	4406	185.7	474.6	288.9	469.7	284.0	475.3	289.6	478.7	293.0	475.8	290.1	479.3	293.6
	4407	192.6	510.1	317.5	512.0	319.4	515.1	322.5	515.7	323.1	515.3	322.7	518.9	326.3
	4408	165.2	475.9	310.7	473.2	308.0	467.8	302.6	475.0	309.8	475.4	310.2	479.3	314.1
	4409	179.0	477.7	298.7	476.4	297.4	480.0	301.0	479.8	300.8	481.0	302.0	486.2	307.2
	4410	197.8	495.9	298.1	490.4	292.6	496.4	298.6	497.1	299.3	497.9	300.1	504.4	306.6
	MEAN	182.0	473.5	291.6	472.0	290.1	476.3	294.3	477.2	295.2	477.2	295.3	482.2	300.2
	S.D.	11.9	35.4	27.3	34.4	26.4	34.3	25.5	34.3	26.0	33.0	25.0	33.7	25.5
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10
50	4421	182.9	523.8	340.9	521.1	338.2	529.1	346.2	537.8	354.9	535.9	353.0	543.0	360.1
	4422	171.2	488.7	317.5	491.3	320.1	496.3	325.1	497.4	326.2	501.5	330.3	502.5	331.3
	4423	177.8	508.3	330.5	511.1	333.3	518.4	340.6	524.4	346.6	531.5	353.7	533.1	355.3
	4424	186.5	492.1	305.6	486.0	299.5	495.4	308.9	500.5	314.0	497.8	311.3	501.1	314.6
	4425	191.9	474.3	282.4	475.8	283.9	477.8	285.9	483.9	292.0	485.7	293.8	488.7	296.8
	4426	187.9	501.2	313.3	504.3	316.4	508.7	320.8	511.5	323.6	514.2	326.3	517.6	329.7
	4427	183.3	446.0	262.7	445.2	261.9	448.5	265.2	448.0	264.7	450.3	267.0	451.6	268.3
	4428	175.1	418.4	243.3	420.5	245.4	420.6	245.5	426.9	251.8	424.0	248.9	432.8	257.7
	4429	169.0	438.8	269.8	441.3	272.3	446.0	277.0	444.8	275.8	446.0	277.0	452.9	283.9
	4430	207.8	496.5	288.7	496.9	289.1	500.8	293.0	502.1	294.3	502.9	295.1	505.1	297.3
	MEAN	183.3	478.8	295.5	479.4	296.0	484.2	300.8	487.7	304.4	489.0	305.6	492.8	309.5
	S.D.	11.3	33.9	31.4	33.3	31.0	35.2	33.1	36.5	34.5	37.5	35.6	36.5	34.8
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	246	GAIN	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN
150	4441	184.8	470.1	285.3	467.8	283.0	475.7	290.9	480.0	295.2	479.2	294.4	485.3	300.5
	4442	164.4	468.1	303.7	471.9	307.5	473.8	309.4	480.9	316.5	476.8	312.4	481.3	316.9
	4443	178.4	471.4	293.0	470.4	292.0	479.0	300.6	480.9	302.5	476.2	297.8	478.8	300.4
	4444	200.4	490.9	290.5	498.0	297.6	500.4	300.0	473.7	273.3	***	***	***	***
	4445	192.3	462.9	270.6	470.8	278.5	473.5	281.2	477.4	285.1	478.9	286.6	485.3	293.0
	4446	186.0	408.0	222.0	408.7	222.7	414.5	228.5	416.1	230.1	420.3	234.3	432.4	246.4
	4447	195.0	523.2	328.2	519.2	324.2	526.4	331.4	529.3	334.3	529.4	334.4	531.6	336.6
	4448	172.3	447.6	275.3	453.1	280.8	454.5	282.2	457.7	285.4	458.4	286.1	465.9	293.6
	4449	201.0	440.5	239.5	441.8	240.8	446.1	245.1	449.3	248.3	449.3	248.3	452.9	251.9
	4450	158.7	365.9	207.2	366.7	208.0	370.2	211.5	371.0	212.3	370.1	211.4	373.3	214.6
	MEAN	183.3	454.9	271.5	456.8	273.5	461.4	278.1	461.6	278.3	459.8	278.4	465.2	283.8
	S.D.	14.7	43.6	37.8	43.5	37.7	43.9	38.0	42.8	38.2	44.5	39.4	43.8	38.4
	N=	10	10	10	10	10	10	10	10	10	9	9	9	9
300	4461	183.1	450.9	267.8	454.8	271.7	460.7	277.6	457.3	274.2	456.8	273.7	462.7	279.6
	4462	157.7	***	***	***	***	***	***	***	***	***	***	***	***
	4463	206.0	***	***	***	***	***	***	***	***	***	***	***	***
	4464	176.2	388.5	212.3	388.9	212.7	393.3	217.1	396.2	220.0	391.8	215.6	396.1	219.9
	4465	187.5	587.1	399.6	585.7	398.2	596.6	409.1	598.2#	410.7#	598.0	410.5#	605.7	418.2#
	4466	174.1	381.6	207.5	378.1	204.0	384.8	210.7	389.5	215.4	387.2	213.1	387.1	213.0
	4467	153.3	430.1	276.8	431.8	278.5	432.4	279.1	429.1	275.8	430.5	277.2	439.0	285.7
	4468	203.7	472.8	269.1	464.9	261.2	472.4	268.7	460.4	256.7	472.8	269.1	472.3	268.6
	4469	185.1	427.4	242.3	427.6	242.5	429.4	244.3	428.3	243.2	428.4	243.3	430.9	245.8
	4470	183.9	***	***	***	***	***	***	***	***	***	***	***	***
	MEAN	181.1	448.3	267.9	447.4	267.0	452.8	272.4	451.3	270.9	452.2	271.8	456.3	275.8
	S.D.	17.0	69.1	64.3	68.7	64.4	71.0	66.4	70.2	66.1	71.4	66.7	73.0	68.8
	N=	10	7	7	7	7	7	7	7	7	7	7	7	7

STATISTICAL OUTLIERS INCLUDED.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST											
MKD	NUMBER	1	288	GAIN	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN
0	4401	173.3	464.1	290.8	468.8	295.5	466.3	293.0	473.4	300.1	471.7	298.4	468.3	295.0
	4402	183.3	489.9	306.6	487.3	304.0	486.6	303.3	490.8	307.5	492.2	308.9	489.3	306.0
	4403	179.9	496.8	316.9	494.4	314.5	497.9	318.0	507.5	327.6	510.6	330.7	507.0	327.1
	4404	197.8	503.6	305.8	469.4	271.6	440.1	242.3	***	***	***	***	***	***
	4405	165.2	398.9#	233.7#	402.0#	236.8	400.7	235.5	406.3#	241.1#	406.9#	241.7#	403.8#	238.6#
	4406	185.7	483.0	297.3	479.6	293.9	479.9	294.2	482.7	297.0	488.6	302.9	486.5	300.8
	4407	192.6	523.4	330.8	522.7	330.1	521.9	329.3	529.5	336.9	532.1	339.5	526.8	334.2
	4408	165.2	486.0	320.8	488.8	323.6	487.2	322.0	491.0	325.8	491.4	326.2	485.9	320.7
	4409	179.0	484.7	305.7	483.6	304.6	488.4	309.4	486.7	307.7	489.9	310.9	491.5	312.5
	4410	197.8	506.7	308.9	508.1	310.3	504.9	307.1	511.0	313.2	512.6	314.8	503.9	306.1
	MEAN	182.0	483.7	301.7	480.5	298.5	477.4	295.4	486.5	306.3	488.4	308.2	484.8	304.6
	S.D.	11.9	33.8	26.5	32.1	27.2	34.7	31.9	34.5	27.8	35.2	28.3	34.5	27.8
	N=	10	10	10	10	10	10	10	9	9	9	9	9	9
50	4421	182.9	548.7	365.8	546.6	363.7	546.3	363.4	553.8	370.9	557.6	374.7	560.2	377.3
	4422	171.2	511.5	340.3	507.7	336.5	503.2	332.0	509.8	338.6	513.0	341.8	508.2	337.0
	4423	177.8	538.0	360.2	540.6	362.8	538.7	360.9	542.4	364.6	544.0	366.2	550.2	372.4
	4424	186.5	509.4	322.9	510.8	324.3	503.4	316.9	511.3	324.8	515.0	328.5	509.7	323.2
	4425	191.9	488.5	296.6	496.5	304.6	493.9	302.0	497.4	305.5	503.9	312.0	505.7	313.8
	4426	187.9	517.9	330.0	518.0	330.1	516.2	328.3	525.5	337.6	528.6	340.7	531.6	343.7
	4427	183.3	453.2	269.9	453.4	270.1	455.5	272.2	463.1	279.8	462.2	278.9	464.1	280.8
	4428	175.1	438.6	263.5	434.3	259.2	435.3	260.2	438.2	263.1	441.8	266.7	445.9	270.8
	4429	169.0	452.5	283.5	455.4	286.4	455.2	286.2	458.5	289.5	460.2	291.2	459.8	290.8
	4430	207.8	507.7	299.9	511.6	303.8	502.9	295.1	508.6	300.8	516.4	308.6	518.2	310.4
	MEAN	183.3	496.6	313.3	497.5	314.2	495.1	311.7	500.9	317.5	504.3	320.9	505.4	322.0
	S.D.	11.3	37.5	36.2	37.8	35.9	36.4	35.0	37.3	35.8	38.0	36.0	38.3	36.2
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	288	GAIN	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN
150	4441	184.8	485.1	300.3	487.1	302.3	481.9	297.1	485.5	300.7	492.9	308.1	490.4	305.6
	4442	164.4	483.7	319.3	482.6	318.2	482.5	318.1	486.5	322.1	484.5	320.1	481.7	317.3
	4443	178.4	483.0	304.6	483.4	305.0	482.8	304.4	488.2	309.8	494.7	316.3	488.2	309.8
	4444	200.4	***	***	***	***	***	***	***	***	***	***	***	***
	4445	192.3	484.5	292.2	484.9	292.6	482.2	289.9	489.9	297.6	495.8	303.5	497.3	305.0
	4446	186.0	434.2	248.2	433.5	247.5	432.6	246.6	435.6	249.6	437.0	251.0	436.3	250.3
	4447	195.0	530.1	335.1	531.9	336.9	530.6	335.6	537.1	342.1	535.0	340.0	536.6	341.6
	4448	172.3	464.3	292.0	466.7	294.4	467.4	295.1	471.8	299.5	473.6	301.3	475.2	302.9
	4449	201.0	455.5	254.5	453.6	252.6	453.4	252.4	458.7	257.7	462.8	261.8	462.5	261.5
	4450	158.7	376.3	217.6	376.0	217.3	375.2	216.5	377.7	219.0	377.2	218.5	377.8	219.1
	MEAN	183.3	466.3	284.9	466.6	285.2	465.4	284.0	470.1	288.7	472.6	291.2	471.8	290.3
	S.D.	14.7	42.7	37.5	43.3	38.2	43.0	38.0	44.1	38.9	44.6	39.0	44.4	38.5
	N=	10	9	9	9	9	9	9	9	9	9	9	9	9
300	4461	183.1	468.5	285.4	464.3	281.2	462.1	279.0	469.1	286.0	468.1	285.0	470.7	287.6
	4462	157.7	***	***	***	***	***	***	***	***	***	***	***	***
	4463	206.0	***	***	***	***	***	***	***	***	***	***	***	***
	4464	176.2	397.8	221.6	403.1	226.9	401.5	225.3	408.7	232.5	409.0	232.8	402.3	226.1
	4465	187.5	608.6	421.1	607.1	419.6#	606.6	419.1#	616.8	429.3#	619.2	431.7#	616.9#	429.4#
	4466	174.1	386.9	212.8	389.6	215.5	388.5	214.4	393.8	219.7	397.3	223.2	384.1	210.0
	4467	153.3	442.7	289.4	438.6	285.3	435.8	282.5	440.2	286.9	444.0	290.7	447.9	294.6
	4468	203.7	476.1	272.4	477.3	273.6	475.5	271.8	485.7	282.0	488.8	285.1	458.6	254.9
	4469	185.1	426.1	241.0	429.5	244.4	427.3	242.2	429.5	244.4	436.1	251.0	428.0	242.9
	4470	183.9	***	***	***	***	***	***	***	***	***	***	***	***
	MEAN	181.1	458.1	277.7	458.5	278.1	456.6	276.2	463.4	283.0	466.1	285.6	458.4	277.9
	S.D.	17.0	74.2	70.1	72.5	68.0	72.5	68.0	74.8	70.1	74.5	69.8	76.3	73.5
	N=	10	7	7	7	7	7	7	7	7	7	7	7	7

STATISTICAL OUTLIERS INCLUDED.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G)– Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	330	GAIN	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
0	4401	173.3	459.9	286.6	466.0	292.7	469.5	296.2	479.9	306.6	482.2	308.9	483.9	310.6
	4402	183.3	493.9	310.6	502.3	319.0	505.0	321.7	509.2	325.9	512.2	328.9	516.9	333.6
	4403	179.9	513.6	333.7	515.0	335.1	513.4	333.5	525.9	346.0	528.9	349.0	530.8	350.9
	4404	197.8	***	***	***	***	***	***	***	***	***	***	***	***
	4405	165.2	406.7	241.5#	408.4#	243.2#	410.1#	244.9#	413.0#	247.8#	415.3#	250.1#	413.9#	248.7#
	4406	185.7	492.6	306.9	498.5	312.8	504.2	318.5	507.4	321.7	502.8	317.1	511.0	325.3
	4407	192.6	528.3	335.7	533.1	340.5	536.1	345.5	540.0	347.4	541.9	349.3	549.5	356.9
	4408	165.2	492.7	327.5	497.5	332.3	501.0	335.8	502.0	336.8	502.4	337.2	505.0	339.8
	4409	179.0	492.8	313.8	497.5	318.5	500.7	321.7	501.5	322.5	502.4	323.4	506.9	327.9
	4410	197.8	515.5	317.7	514.6	316.8	515.6	317.8	521.6	323.8	521.3	323.5	522.0	324.2
	MEAN	182.0	488.4	308.2	492.5	312.3	495.3	315.1	500.1	319.8	501.0	320.8	504.4	324.2
	S.D.	11.9	36.3	29.2	36.4	29.5	36.6	29.8	36.8	29.9	36.6	29.8	38.5	31.7
	N=	10	9	9	9	9	9	9	9	9	9	9	9	9
50	4421	182.9	568.8	385.9	569.0	386.1	580.1	397.2	582.2	399.3	580.5	397.6	583.3	400.4
	4422	171.2	512.9	341.7	520.9	349.7	521.0	349.8	524.6	353.4	529.8	358.6	531.8	360.6
	4423	177.8	561.1	383.3	563.0	385.2	574.4	396.6	581.4	403.6	582.3	404.5	586.7	408.9
	4424	186.5	519.7	333.2	526.7	340.2	527.4	340.9	532.7	346.2	525.7	339.2	526.5	340.0
	4425	191.9	511.7	319.8	509.9	318.0	512.7	320.8	512.5	320.6	516.4	324.5	515.7	323.8
	4426	187.9	536.9	349.0	534.2	346.3	537.6	349.7	538.6	350.7	541.7	353.8	547.2	359.3
	4427	183.3	469.1	285.8	469.3	286.0	470.9	287.6	472.8	289.5	475.6	292.3	475.0	291.7
	4428	175.1	449.1	274.0	455.0	279.9	454.8	279.7	454.7	279.6	460.5	285.4	461.6	286.5
	4429	169.0	467.0	298.0	466.6	297.6	473.0	304.0	470.7	301.7	471.1	302.1	475.3	306.3
	4430	207.8	521.2	313.4	524.6	316.8	524.2	316.4	527.9	320.1	533.9	326.1	534.5	326.7
	MEAN	183.3	511.8	328.4	513.9	330.6	517.6	334.3	519.8	336.5	521.8	338.4	523.8	340.4
	S.D.	11.3	39.8	37.9	39.3	37.7	41.9	40.7	43.7	42.4	42.5	41.0	43.3	42.1
	N=	10	10	10	10	10	10	10	10	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 5. Body Weight/Body Weight Gains Summary (G) – Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	330	GAIN	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
150	4441	184.8	490.4	305.6	497.1	312.3	500.6	315.8	499.7	314.9	501.3	316.5	503.6	318.8
	4442	164.4	488.4	324.0	491.1	326.7	494.9	330.5	494.6	330.2	496.0	331.6	496.8	332.4
	4443	178.4	499.5	321.1	505.1	326.7	507.5	329.1	508.2	329.8	510.0	331.6	512.9	334.5
	4444	200.4	***	***	***	***	***	***	***	***	***	***	***	***
	4445	192.3	502.6	310.3	505.3	313.0	510.2	317.9	515.8	323.5	519.4	327.1	523.7	331.4
	4446	186.0	440.1	254.1	451.9	265.9	452.2	266.2	455.2	269.2	453.7	267.7	458.8	272.8
	4447	195.0	538.0	343.0	541.0	346.0	543.0	348.0	547.8	352.8	549.1	354.1	549.2	354.2
	4448	172.3	483.2	310.9	488.7	316.4	489.8	317.5	491.4	319.1	493.7	321.4	495.4	323.1
	4449	201.0	468.6	267.6	474.7	273.7	474.3	273.3	480.7	279.7	484.2	283.2	483.4	282.4
	4450	158.7	380.0	221.3	382.1	223.4	384.2	225.5	384.8	226.1	384.4	225.7	387.0	228.3
	MEAN	183.3	476.8	295.3	481.9	300.5	484.1	302.6	486.5	305.0	488.0	306.5	490.1	308.7
	S.D.	14.7	44.8	39.2	44.5	38.5	45.0	39.2	45.7	39.2	46.6	40.1	46.2	39.6
	N=	10	9	9	9	9	9	9	9	9	9	9	9	9
300	4461	183.1	474.5	291.4	482.0	298.9	485.1	302.0	489.6	306.5	465.6	282.5	***	***
	4462	157.7	***	***	***	***	***	***	***	***	***	***	***	***
	4463	206.0	***	***	***	***	***	***	***	***	***	***	***	***
	4464	176.2	414.4	238.2	419.4	243.2	424.3	248.1	422.9	246.7	424.6	248.4	414.6	238.4
	4465	187.5	622.9#	435.4#	632.0	444.5#	641.8	454.3#	647.6#	460.1#	651.4#	463.9#	656.1	468.6
	4466	174.1	393.9	219.8	396.7	222.6	399.0	224.9	403.2	229.1	395.1	221.0	***	***
	4467	153.3	450.9	297.6	455.3	302.0	458.9	305.6	459.1	305.8	463.3	310.0	468.2	314.9
	4468	203.7	475.3	271.6	489.5	285.8	497.0	293.3	489.2	285.5	480.8	277.1	488.0	284.3
	4469	185.1	430.6	245.5	438.8	253.7	439.7	254.6	438.5	253.4	435.7	250.6	437.9	252.8
	4470	183.9	***	***	***	***	***	***	***	***	***	***	***	***
	MEAN	181.1	466.1	285.6	473.4	293.0	478.0	297.5	478.6	298.2	473.8	293.4	493.0	311.8
	S.D.	17.0	75.4	71.9	77.3	73.1	79.8	75.5	81.2	77.4	83.5	80.4	95.4	92.5
	N=	10	7	7	7	7	7	7	7	7	7	7	5	5

STATISTICAL OUTLIERS INCLUDED.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G) – Metabolism Group

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	30	GAIN	57	GAIN	85	GAIN	113	GAIN	141	GAIN	204	GAIN
0	4411	203.4	314.6	111.2	360.5	157.1	403.5	200.1	428.9	225.5	444.0	240.6	459.4	256.0
	4412	178.5	266.4	87.9	303.2	124.7	350.0	171.5	376.4	197.9	396.0	217.5	402.3	223.8
	4413	185.4	308.5	123.1	345.4	160.0	376.8	191.4	406.9	221.5	416.5	231.1	434.7	249.3
	4414	190.0	318.5	128.5	372.8	182.8	417.5	227.5	458.6	268.6	468.1	298.1	512.7	322.7
	4415	164.0	289.5	125.5	332.5	168.5	364.6	200.6	373.4	209.4	387.9	223.9	403.2	239.2
	MEAN	184.3	299.5	115.2	342.9	158.6	382.5	198.2	408.8	224.6	426.5	242.2	442.5	258.2
	S.D.	14.5	21.6	16.6	26.9	21.4	27.7	20.2	36.0	26.9	40.7	32.4	45.9	38.0
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4431	184.7	309.0	124.3	359.9	175.2	401.0	216.3	416.3	231.6	436.1	251.4	461.7	277.0
	4432	174.8	279.8	105.0	327.9	153.1	358.1	183.3	386.8	212.0	407.4	232.6	404.2	229.4
	4433	158.6	277.0	118.4	318.2	159.6	358.4	199.8	375.4	216.8	392.5	233.9	410.6	252.0
	4434	197.0	336.6	139.6	392.3	195.3	441.8	244.8	465.7	268.7	487.1	290.1	514.1	317.1
	4435	184.6	270.2	85.6	319.2	134.6	342.2	157.6	353.5	168.9	373.3	188.7	391.0	206.4
	MEAN	179.9	294.5	114.6	343.5	163.6	380.3	200.4	399.5	219.6	419.3	239.3	436.3	256.4
	S.D.	14.3	27.8	20.4	32.1	22.9	40.7	33.0	43.4	36.0	44.3	36.6	51.1	42.9
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4451	191.6	333.4	141.8	402.1	210.5	443.6	252.0	454.7	263.1	473.0	281.4	493.3	301.7
	4452	188.9	308.2	119.3	358.0	169.1	405.6	216.7	422.6	233.7	439.1	250.2	447.8	258.9
	4453	161.2	279.0	117.8	336.3	175.1	369.6	208.4	385.1	223.9	399.6	238.4	418.0	256.8
	4454	169.9	284.4	114.5	332.8	162.9	372.3	202.4	382.3	212.4	394.2	224.3	418.7	248.8
	4455	196.4	323.7	127.3	368.8	172.4	402.4	206.0	417.9	221.5	434.6	238.2	444.2	247.8
	MEAN	181.6	305.7	124.1	359.6	178.0	398.7	217.1	412.5	230.9	428.1	246.5	444.4	262.8
	S.D.	15.2	23.8	10.9	28.1	18.7	30.1	20.2	29.9	19.5	32.2	21.6	30.7	22.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	30	GAIN	57	GAIN	85	GAIN	113	GAIN	141	GAIN	204	GAIN
300	4471	195.1	299.3	104.2	350.9	155.8	391.1	196.0	414.2	219.1	435.3	240.2	455.0	259.9
	4472	171.1	299.0	127.9	350.0	178.9	394.9	223.8	419.6	248.5	441.8	270.7	457.4	286.3
	4473	181.7	267.7	86.0	295.4	113.7	325.9	144.2	341.6	159.9	359.0	177.3	372.5	190.8
	4474	192.0	322.1	130.1	371.0	179.0	402.3	210.3	426.0	234.0	434.5	242.5	436.7	244.7
	4475	155.3	277.8	122.5	336.3	181.0	379.8	224.5	396.0	240.7	416.0	260.7	427.0	271.7
	MEAN	179.0	293.2	114.1	340.7	161.7	378.8	199.8	399.5	220.4	417.3	238.3	429.7	250.7
	S.D.	16.3	21.2	18.7	28.2	28.8	30.7	33.2	34.2	35.5	34.0	36.4	34.4	36.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
400	4491	161.8	303.2	141.4	***	***	***	***	***	***	***	***	***	***
	4492	172.4	307.2	134.8	371.0	198.6	415.3	242.9	443.6	271.2	471.9	299.5	***	***
	4493	196.5	346.2	149.7	407.9	211.4	452.0	255.5	474.5	278.0	497.8	301.3	***	***
	4494	177.9	273.2	95.3	314.9	137.0	354.2	176.3	363.6	185.7	375.1	197.2	***	***
	4495	194.6	299.0	104.4	353.2	158.6	389.2	194.6	410.5	215.9	416.3	221.7	***	***
	MEAN	180.6	305.8	125.1	361.8	176.4	402.7	217.3	423.1	237.7	440.3	254.9	===	===
	S.D.	14.8	26.2	23.9	38.7	34.6	41.3	37.9	47.5	44.4	55.2	53.5	===	===
	N=	5	5	5	4	4	4	4	4	4	4	4	0	0

*** DEAD ANIMAL
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G) – Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN	246	GAIN
0	4411	203.4	470.7	267.3	464.0	260.6	464.4	261.0	460.8	257.4	462.6	259.2	461.7	258.3
	4412	178.5	415.9	237.4	412.1	233.6	418.3	239.8	417.1	238.6	419.7	241.2	418.5	240.0
	4413	185.4	439.2	253.8	441.7	256.3	441.2	255.8	443.9	258.5	444.1	258.7	444.8	259.4
	4414	190.0	524.7	334.7	526.4	336.4	535.3	345.3#	536.1	346.1#	539.7	349.7#	541.2	351.2#
	4415	164.0	407.6	243.6	404.3	240.3	408.4	244.4	407.0	243.0	407.9	243.9	408.5	244.5
	MEAN	184.3	451.6	267.4	449.7	265.4	453.5	269.3	453.0	268.7	454.8	270.5	454.9	270.7
	S.D.	14.5	47.6	39.3	49.1	41.2	50.6	43.4	51.1	44.1	52.0	45.0	52.6	45.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4431	184.7	471.5	286.8	472.6	287.9	472.8	288.1	473.5	288.8	472.1	287.4	476.0	291.3
	4432	174.8	415.8	241.0	417.2	242.4	425.4	250.6	428.3	253.5	425.8	251.0	423.2	248.4
	4433	158.6	416.0	257.4	420.8	262.2	421.8	263.2	421.0	262.4	423.1	264.5	422.5	263.9
	4434	197.0	526.3	329.3	525.2	328.2	532.5	335.5	535.4	338.4	531.1	334.1	529.6	332.6
	4435	184.6	381.9	197.3	393.8	209.2	394.0	209.4	399.1	214.5	400.9	216.3	407.2	222.6
	MEAN	179.9	442.3	262.4	445.9	266.0	449.3	269.4	451.5	271.5	450.6	270.7	451.7	271.8
	S.D.	14.3	56.9	49.5	52.8	45.1	54.5	46.7	54.2	45.9	51.9	43.8	50.8	42.2
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4451	191.6	501.6	310.0	496.4	304.8	503.4	311.8	504.1	312.5	505.1	313.5	506.3	314.7
	4452	188.9	460.5	271.6	459.4	270.5	459.7	270.8	464.0	275.1	461.4	272.5	460.0	271.1
	4453	161.2	424.8	263.6	424.8	263.6	428.9	267.7	428.6	267.4	430.9	269.7	432.8	271.6
	4454	169.9	425.4	255.5	427.2	257.3	427.4	257.5	431.0	261.1	432.1	262.2	434.9	265.0
	4455	196.4	449.8	253.4	456.8	260.4	454.1	257.7	453.3	256.9	453.6	257.2	454.5	258.1
	MEAN	181.6	452.4	270.8	452.9	271.3	454.7	273.1	456.2	274.6	456.6	275.0	457.7	276.1
	S.D.	15.2	31.6	23.1	29.2	19.3	30.9	22.4	30.7	22.3	30.2	22.3	29.7	22.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G) – Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST											
MKD	NUMBER	1	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN	246	GAIN
300	4471	195.1	460.0	264.9	467.0	271.9	467.4	272.3	469.1	274.0	466.7	271.6	470.5	275.4
	4472	171.1	465.5	294.4	465.0	293.9	466.6	295.5	465.5	294.4	467.3	296.2	471.1	300.0
	4473	181.7	379.5	197.8	382.4	200.7	379.7	198.0	378.7	197.0	380.8	199.1	381.1	199.4
	4474	192.0	442.4	250.4	448.6	256.6	448.2	256.2	453.6	261.6	441.9	249.9	437.6	245.6
	4475	155.3	436.3	281.0	442.5	287.2	444.1	288.8	449.3	294.0	445.5	290.2	446.7	291.4
	MEAN	179.0	436.7	257.7	441.1	262.1	441.2	262.2	443.2	264.2	440.4	261.4	441.4	262.4
	S.D.	16.3	34.2	37.4	34.4	37.2	36.0	39.0	37.0	40.1	35.3	39.2	36.8	40.9
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN	288	GAIN
0	4411	203.4	466.5	263.1	469.5	266.1	471.0	267.6	470.4	267.0	474.7	271.3	479.3	275.9
	4412	178.5	417.3	238.8	419.7	241.2	421.5	243.0	423.9	245.4	429.2	250.7	432.5	254.0
	4413	185.4	446.2	260.8	451.3	265.9	450.6	265.2	451.2	265.8	452.9	267.5	445.3	259.9
	4414	190.0	544.1	354.1	546.6	356.6	552.1	362.1	552.1	362.1#	557.7	367.7#	564.0	374.0#
	4415	164.0	408.4	244.4	415.1	251.1	415.7	251.7	414.9	250.9	422.3	258.3	422.3	258.3
	MEAN	184.3	456.5	272.2	460.4	276.2	462.2	277.9	462.5	278.2	467.4	283.1	468.7	284.4
	S.D.	14.5	54.2	46.9	53.2	46.2	55.0	48.1	54.7	47.8	54.6	48.0	57.5	50.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4431	184.7	475.2	290.5	484.0	299.3	488.5	303.8	485.7	301.0	488.7	304.0	490.0	305.3
	4432	174.8	421.2	246.4	420.9	246.1	425.7	250.9	424.4	249.6	431.7	256.9	433.7	258.9
	4433	158.6	424.0	265.4	426.8	268.2	428.5	269.9	425.9	267.3	431.3	272.7	434.8	276.2
	4434	197.0	527.5	330.5	533.9	336.9	540.9	343.9	540.2	343.2	545.6	348.6	555.4	358.4
	4435	184.6	408.9	224.3	414.8	230.2	415.1	230.5	417.0	232.4	417.6	233.0	406.4	221.8
	MEAN	179.9	451.4	271.4	456.1	276.1	459.7	279.8	458.6	278.7	463.0	283.0	464.1	284.1
	S.D.	14.3	49.6	41.0	51.6	42.7	53.7	44.9	53.3	44.1	53.7	44.8	59.4	51.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4451	191.6	509.8	318.2	513.3	321.7	520.1	328.5#	517.2	325.6	525.3	333.7	526.9	335.3#
	4452	186.9	463.8	274.9	469.0	280.1	465.6	276.7	466.0	277.1	468.2	279.3	467.0	278.1
	4453	161.2	432.9	271.7	438.2	277.0	442.0	280.8	440.4	279.2	446.7	285.5	446.2	285.0
	4454	169.9	435.9	266.0	440.5	270.6	442.9	273.0	441.3	271.4	444.5	274.6	445.2	275.3
	4455	196.4	454.4	258.0	459.4	263.0	463.7	267.3	461.1	264.7	467.0	270.6	470.0	273.6
	MEAN	181.6	459.4	277.8	464.1	282.5	466.9	285.3	465.2	283.6	470.3	288.7	471.1	289.5
	S.D.	15.2	31.0	23.5	30.4	22.9	31.8	24.7	31.3	24.1	32.6	25.7	33.3	26.0
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN	288	GAIN
300	4471	195.1	469.9	274.8	476.1	281.0	480.6	285.5	477.4	282.3	487.2	292.1	489.2	294.1
	4472	171.1	470.0	298.9	477.0	305.9	468.7	297.6	473.9	302.8	478.3	307.2	477.4	306.3
	4473	181.7	381.7	200.0	383.0	201.3	385.7	204.0	385.1	203.4	390.4	208.7	392.7	211.0
	4474	192.0	445.7	253.7	451.8	259.8	448.9	256.9	444.7	252.7	448.7	256.7	459.3	267.3
	4475	155.3	449.2	293.9	453.6	298.3	455.0	299.7	454.0	298.7	458.9	303.6	462.5	307.2
	MEAN	179.0	443.3	264.3	448.3	269.3	447.8	268.7	447.0	268.0	452.7	273.7	456.2	277.2
	S.D.	16.3	36.2	40.1	38.4	41.9	36.8	40.0	37.2	41.1	38.0	41.4	37.5	40.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN	330	GAIN
0	4411	203.4	485.0	281.6	482.3	278.9	488.1	284.7	485.8	282.4	485.2	281.8	488.3	284.9
	4412	178.5	436.2	257.7	426.7	248.2	432.5	254.0	434.5	256.0	433.5	255.0	438.3	259.8
	4413	185.4	460.0	274.6	466.0	280.6	467.7	282.3	472.0	286.6	469.9	284.5	472.8	287.4
	4414	190.0	566.8	376.8#	570.7	380.7	579.7	389.7	581.8	391.8	575.8	385.8	584.5	394.5
	4415	164.0	427.7	263.7	423.6	259.6	426.1	262.1	429.5	265.5	426.8	262.8	432.8	268.8
	MEAN	184.3	475.1	290.9	473.9	289.6	478.8	294.6	480.7	296.5	478.2	294.0	483.3	299.1
	S.D.	14.5	55.9	48.9	59.7	52.7	61.9	54.8	61.4	54.7	59.8	52.8	61.1	54.5
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4431	184.7	492.1	307.4	495.8	311.1	497.0	312.3	502.6	317.9	502.3	317.6	504.8	320.1
	4432	174.8	432.6	257.8	428.7	253.9	431.8	257.0	437.8	263.0	438.2	263.4	444.0	269.2
	4433	158.6	438.0	279.4	431.9	273.3	434.2	275.6	437.1	278.5	437.3	278.7	442.7	284.1
	4434	197.0	557.7	360.7	564.6	367.6	569.6	372.6	569.4	372.4	568.3	371.3	584.1	387.1
	4435	184.6	388.1	203.5	388.5	203.9	413.7	229.1	416.0	231.4	420.9	236.3	427.3	242.7
	MEAN	179.9	461.7	281.8	461.9	282.0	469.3	289.3	472.6	292.6	473.4	293.5	480.6	300.6
	S.D.	14.3	65.1	58.3	69.1	61.5	64.3	55.5	63.1	54.4	61.5	52.5	65.0	55.8
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4451	191.6	530.2	338.6#	530.4	338.8#	535.5	343.9	536.6	345.0	536.9	345.3	538.4	346.8#
	4452	188.9	471.1	282.2	469.9	281.0	478.8	289.9	479.5	290.6	481.9	293.0	485.9	297.0
	4453	161.2	450.6	289.4	448.0	286.8	452.1	290.9	457.5	296.3	456.8	295.6	460.4	299.2
	4454	169.9	447.3	277.4	444.4	274.5	452.0	282.1	454.8	284.9	456.9	287.0	460.6	290.7
	4455	196.4	471.2	274.8	469.4	273.0	469.1	272.7	472.7	276.3	470.9	274.5	483.6	287.2
	MEAN	181.6	474.1	292.5	472.4	290.8	477.5	295.9	480.2	298.6	480.7	299.1	485.8	304.2
	S.D.	15.2	33.3	26.4	34.5	27.4	34.4	27.8	33.2	27.0	33.1	27.1	31.8	24.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN	330	GAIN
300	4471	195.1	486.1	291.0	484.5	289.4	489.3	294.2	494.0	298.9	492.9	297.8	499.0	303.9
	4472	171.1	475.9	304.8	480.2	309.1	481.2	310.1	482.7	311.6	484.9	313.8	491.8	320.7
	4473	181.7	397.4	215.7	389.1	207.4	396.3	214.6	396.5	214.8	391.8	210.1	396.7	215.0
	4474	192.0	458.6	266.6	453.2	261.2	454.8	262.8	461.2	269.2	460.1	268.1	448.9	256.9
	4475	155.3	459.4	304.1	457.5	302.2	461.4	306.1	464.8	309.5	462.7	307.4	468.3	313.0
	MEAN	179.0	455.5	276.4	452.9	273.9	456.6	277.6	459.8	280.8	458.5	279.4	460.9	281.9
	S.D.	16.3	34.5	37.3	38.2	41.4	36.5	39.8	37.8	40.6	39.8	42.5	41.0	44.9
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
0	4411	203.4	492.7	289.3	495.7	292.3	498.4	295.0	502.3	298.9	507.9	304.5
	4412	178.5	438.7	260.2	445.4	266.9	446.0	267.5	448.0	269.5	448.3	269.8
	4413	185.4	477.0	291.6	477.9	292.5	480.8	295.4	481.6	296.2	486.9	301.5
	4414	190.0	589.2	399.2	592.4	402.4#	602.6	412.6#	603.8	413.8#	607.7	417.7
	4415	164.0	436.0	272.0	438.5	274.5	443.4	279.4	446.6	282.6	447.1	283.1
	MEAN	184.3	486.7	302.5	490.0	305.7	494.2	310.0	496.5	312.2	499.6	315.3
	S.D.	14.5	62.3	55.6	61.9	55.2	64.9	58.5	64.4	58.0	65.8	58.9
	N=	5	5	5	5	5	5	5	5	5	5	5
50	4431	184.7	509.8	325.1	515.1	330.4	514.3	329.6	513.9	329.2	516.3	331.6
	4432	174.8	451.1	276.3	452.3	277.5	452.5	277.7	455.6	280.8	456.5	281.7
	4433	158.6	446.4	287.8	446.6	288.0	444.6	286.0	448.8	290.2	447.2	288.6
	4434	197.0	584.6	387.6	588.0	391.0	603.3	406.3	602.8	405.8	599.5	402.5
	4435	184.6	432.4	247.8	431.7	247.1	430.9	246.3	438.7	254.1	440.5	255.9
	MEAN	179.9	484.9	304.9	486.7	306.8	489.1	309.2	492.0	312.0	492.0	312.1
	S.D.	14.3	63.1	53.9	65.0	55.7	71.4	61.9	68.5	58.9	67.2	57.4
	N=	5	5	5	5	5	5	5	5	5	5	5
150	4451	191.6	551.4	359.8#	551.7	360.1#	554.3	362.7#	560.5	368.9#	557.4	365.8
	4452	188.9	489.4	300.5	496.9	308.0	496.6	307.7	497.0	308.1	509.5	320.6
	4453	161.2	460.6	299.4	465.0	303.8	465.0	303.8	467.9	306.7	467.2	306.0
	4454	169.9	459.8	289.9	467.6	297.7	467.3	297.4	470.0	300.1	469.2	299.3
	4455	196.4	484.5	288.1	489.7	293.3	493.2	296.8	498.1	301.7	492.5	296.1
	MEAN	181.6	489.1	307.5	494.2	312.6	495.3	313.7	498.7	317.1	499.2	317.6
	S.D.	15.2	37.3	29.7	35.0	27.2	36.0	27.8	37.4	29.1	37.0	28.6
	N=	5	5	5	5	5	5	5	5	5	5	5

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 6. Body Weight/Body Weight Gains Summary (G)– Metabolism Group (continued)

DOSE	ANIMAL	DAYS ON TEST										
MKD	NUMBER	1	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
300	4471	195.1	504.0	308.9	507.4	312.3	508.9	313.8	509.3	314.2	509.8	314.7
	4472	171.1	495.0	323.9	494.9	323.8	497.8	326.7	498.8	327.7	500.6	329.5
	4473	181.7	399.4	217.7	398.4	216.7	405.9	224.2	404.4	222.7	405.6	223.9
	4474	192.0	458.1	266.1	449.2	257.2	455.3	263.3	445.7	253.7	446.3	254.3
	4475	155.3	470.4	315.1	478.6	323.3	473.8	318.5	480.4	325.1	478.1	322.8
	MEAN	179.0	465.4	286.3	465.7	286.7	468.3	289.3	467.7	288.7	468.1	289.0
	S.D.	16.3	41.2	44.4	43.5	47.8	40.7	44.1	42.9	47.6	42.7	47.1
	N=	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	30	GAIN	57	GAIN	85	GAIN	113	GAIN	141	GAIN	204	GAIN
0	4416	196.0	326.5	130.5	377.6	181.6	416.7	220.7	436.1	240.1	448.5	252.5	464.5	268.5
	4417	189.9	323.1	133.2	373.0	183.1	406.1	216.2	437.4	247.5	456.6	266.7	487.7	297.8
	4418	179.6	314.3	134.7	352.2	172.6	400.2	220.6	436.3	256.7	465.8	286.2	477.6	298.0
	4419	190.0	286.4	96.4#	333.7	143.7	376.8	186.8	400.5	210.5	417.1	227.1	427.2	237.2
	4420	162.2	301.5	139.3	354.7	192.5	390.7	228.5	405.5	243.3	419.4	257.2	438.5	276.3
	MEAN	183.5	310.4	126.8	358.2	174.7	398.1	214.6	423.2	239.6	441.5	257.9	459.1	275.6
	S.D.	13.3	16.5	17.3	17.6	18.7	15.2	16.1	18.5	17.4	22.1	21.5	25.6	25.1
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4436	191.8	254.8	63.0	305.6	113.8	353.9	162.1	374.1	182.3	389.1	197.3	390.4	198.6
	4437	160.2	293.8	133.6	355.3	195.1	393.9	233.7	409.7	249.5	429.0	268.8	448.5	288.3
	4438	200.5	302.4	101.9	340.7	140.2	386.2	185.7	417.4	216.9	424.8	224.3	455.8	255.3
	4439	177.8	287.6	109.8	328.5	150.7	372.8	195.0	382.3	204.5	399.6	221.8	418.0	240.2
	4440	185.7	315.9	130.2	367.2	181.5	402.3	216.6	411.4	225.7	426.6	240.9	436.3	250.6
	MEAN	183.2	290.9	107.7	339.5	156.3	381.8	198.6	399.0	215.8	413.8	230.6	429.8	246.6
	S.D.	15.3	22.8	28.3	23.9	32.5	19.0	27.7	19.4	24.9	18.2	26.4	26.3	32.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4456	189.0	321.7	132.7	379.7	190.7	419.7	230.7	420.6	231.6	452.4	263.4	479.2	290.2
	4457	169.5	278.6	109.1	325.2	155.7	361.5	192.0	373.1	203.6	379.2	209.7	397.7	228.2
	4458	199.8	329.7	129.9	385.3	185.5	414.9	215.1	429.3	229.5	439.3	239.5	466.8	267.0
	4459	160.1	283.0	122.9	314.2	154.1	348.0	187.9	356.2	196.1	375.4	215.3	381.7	221.6
	4460	181.1	312.7	131.6	366.8	185.7	396.0	214.9	410.8	229.7	431.6	250.5	440.1	259.0
	MEAN	179.9	305.1	125.2	354.2	174.3	388.0	208.1	398.0	218.1	415.6	235.7	433.1	253.2
	S.D.	15.7	23.1	9.8	32.5	17.9	32.0	17.8	31.7	16.9	35.8	22.9	42.4	28.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	30	GAIN	57	GAIN	85	GAIN	113	GAIN	141	GAIN	204	GAIN
300	4476	181.6	302.0	120.4	358.3	176.7	395.8	214.2	410.5	228.9	428.1	246.5	443.9	262.3
	4477	159.8	275.9	116.1	290.8	131.0	378.9	219.1	394.7	234.9	412.5	252.7	425.4	265.6
	4478	172.6	275.3	102.7	320.3	147.7	362.7	190.1	395.9	223.3	414.9	242.3	429.2	256.6
	4479	204.8	337.7	132.9	387.7	182.9	429.7	224.9	465.5	260.7	490.4	285.6	512.8	308.0
	4480	184.8	307.4	122.6	361.3	176.5	411.2	226.4	434.7	249.9	464.3	279.5	469.0	284.2
	MEAN	180.7	299.7	118.9	343.7	163.0	395.7	214.9	420.3	239.5	442.0	261.3	456.1	275.3
	S.D.	16.6	25.8	11.0	38.1	22.5	26.3	14.7	30.0	15.4	34.1	19.8	36.0	21.0
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
400	4496	161.2	287.0	125.8	340.1	178.9	374.4	213.2	378.7	217.5	366.1	204.9	***	***
	4497	205.3	342.1	136.8	409.3	204.0	443.9	238.6	434.5	229.2	440.3	235.0	***	***
	4498	185.4	287.1	101.7	338.6	153.2	388.3	202.9	406.8	221.4	408.1	222.7	***	***
	4499	184.9	313.7	128.8	355.4	170.5	384.6	199.7	391.1	206.2	381.6	196.7	***	***
	4500	178.9	286.8	107.9	326.2	147.3	361.2	182.3	374.1	195.2	384.9	206.0	***	***
	MEAN	183.1	303.3	120.2	353.9	170.8	390.5	207.3	397.0	213.9	396.2	213.1	===	===
	S.D.	15.8	24.6	14.8	32.6	22.5	31.7	20.7	24.5	13.3	28.9	15.5	===	===
	N=	5	5	5	5	5	5	5	5	5	5	5	0	0

*** DEAD ANIMAL
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G)– Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN	246	GAIN
0	4416	196.0	468.4	272.4	467.8	271.8	471.4	275.4	478.6	282.6	475.1	279.1	481.5	285.5
	4417	189.9	497.0	307.1	496.6	306.7	499.6	309.7	500.6	310.7	499.3	309.4	498.9	309.0
	4418	179.6	488.9	309.3	488.8	309.2	489.9	310.3	496.3	316.7	489.7	310.1	489.3	309.7
	4419	190.0	433.8	243.8	439.8	249.8	438.9	248.9	438.5	248.5	431.2	241.2	432.5	242.5
	4420	162.2	447.9	285.7	446.8	284.6	452.5	290.3	453.6	291.4	446.3	284.1	452.3	290.1
	MEAN	183.5	467.2	283.7	468.0	284.4	470.5	286.9	473.5	290.0	468.3	284.8	470.9	287.4
	S.D.	13.3	26.7	27.0	25.0	24.8	25.2	25.8	26.9	27.0	28.8	28.2	27.6	27.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4436	191.8	402.3	210.5	408.1	216.3	408.1	216.3	406.9	215.1	402.0	210.2	403.4	211.6
	4437	160.2	456.0	295.8	458.7	298.5	458.3	298.1	464.9	304.7	463.4	303.2	463.4	303.2
	4438	200.5	459.8	259.3	460.2	259.7	465.5	265.0	465.7	265.2	463.3	262.8	459.8	259.3
	4439	177.8	416.9	239.1	424.7	246.9	425.4	247.6	429.4	251.6	426.0	248.2	434.2	256.4
	4440	185.7	440.5	254.8	440.1	254.4	441.9	256.2	443.5	257.8	444.5	258.8	443.2	257.5
	MEAN	183.2	435.1	251.9	438.4	255.2	439.8	256.6	442.1	258.9	439.8	256.6	440.8	257.6
	S.D.	15.3	24.9	31.1	22.3	29.5	23.6	29.6	24.9	32.1	26.2	33.3	24.1	32.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4456	189.0	487.9	298.9	484.7	295.7	493.4	304.4	494.7	305.7	487.0	298.0	493.1	304.1
	4457	169.5	402.4	232.9	399.6	230.1	403.3	233.8	401.7	232.2	400.9	231.4	399.6	230.1
	4458	199.8	471.3	271.5	476.7	276.9	480.9	281.1	479.1	279.3	478.3	278.5	485.5	285.7
	4459	160.1	387.2	227.1	386.3	226.2	390.8	230.7	391.4	231.3	391.0	230.9	396.4	236.3
	4460	181.1	455.7	274.6	459.5	278.4	460.8	279.7	463.6	282.5	465.0	283.9	462.4	281.3
	MEAN	179.9	440.9	261.0	441.4	261.5	445.8	265.9	446.1	266.2	444.4	264.5	447.4	267.5
	S.D.	15.7	43.9	30.3	45.4	31.3	46.2	32.3	46.7	33.1	45.1	31.3	46.5	32.5
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G)– Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	211	GAIN	218	GAIN	225	GAIN	232	GAIN	239	GAIN	246	GAIN
300	4476	181.6	449.3	267.7	456.6	275.0	461.6	280.0	459.3	277.7	459.2	277.6	457.9	276.3
	4477	159.8	432.5	272.7	429.8	270.0	435.5	275.7	436.6	276.8	436.0	276.2	435.3	275.5
	4478	172.6	422.7	250.1	***	***	***	***	***	***	***	***	***	***
	4479	204.8	517.6	312.8	512.2	307.4	***	***	***	***	***	***	***	***
	4480	184.8	474.2	289.4	481.9	297.1	486.7	301.9	482.5	297.7	482.6	297.8	482.3	297.5
	MEAN	180.7	459.3	278.5	470.1	287.4	461.3	285.9	459.5	284.1	459.3	283.9	458.5	283.1
	S.D.	16.6	38.0	23.7	35.2	17.8	25.6	14.1	23.0	11.8	23.3	12.1	23.5	12.5
	N=	5	5	5	4	4	3	3	3	3	3	3	3	3

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN	288	GAIN
0	4416	196.0	475.5	279.5	490.1	294.1	490.5	294.5	486.3	290.3	484.2	288.2	482.5	286.5
	4417	189.9	502.1	312.2	502.7	312.8	508.2	318.3	508.7	318.8	517.4	327.5	518.2	328.3
	4418	179.6	491.7	312.1	496.2	316.6	501.4	321.8	499.3	319.7	506.3	326.7	508.0	328.4
	4419	190.0	434.0	244.0	439.7	249.7	442.6	252.6	443.3	253.3	445.3	255.3	445.9	255.9
	4420	162.2	452.2	290.0	456.7	294.5	459.1	296.9	456.2	294.0	465.1	302.9	465.9	303.7
	MEAN	183.5	471.1	287.6	477.1	293.5	480.4	296.8	478.8	295.2	483.7	300.1	484.1	300.6
	S.D.	13.3	28.0	28.2	27.4	26.6	28.3	27.6	28.0	27.1	29.4	30.1	29.7	30.6
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4436	191.8	404.4	212.6	409.1	217.3	409.3	217.5	408.5	216.7	413.4	221.6	414.2	222.4
	4437	160.2	465.3	305.1	467.4	307.2	470.1	309.9	472.5	312.3	472.1	311.9	476.7	316.5
	4438	200.5	463.1	262.6	468.2	267.7	469.9	269.4	472.1	271.6	475.9	275.4	477.1	276.6
	4439	177.8	432.8	255.0	441.0	263.2	444.2	266.4	438.6	260.8	442.9	265.1	448.4	270.6
	4440	185.7	440.9	255.2	449.4	263.7	451.7	266.0	453.2	267.5	454.3	268.6	455.9	270.2
	MEAN	183.2	441.3	258.1	447.0	263.8	449.0	265.8	449.0	265.8	451.7	268.5	454.5	271.3
	S.D.	15.3	24.9	32.9	24.2	31.9	24.9	32.8	26.7	34.0	25.3	32.2	25.8	33.4
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4456	189.0	486.5	297.5	497.6	308.6	503.1	314.1	495.5	306.5	499.2	310.2	504.0	315.0
	4457	169.5	399.0	229.5	407.5	238.0	407.3	237.8	402.6	233.1	408.0	238.5	409.6	240.1
	4458	199.8	484.2	284.4	485.7	285.9	491.2	291.4	489.1	289.3	492.7	292.9	492.3	292.5
	4459	160.1	396.9	236.8	400.1	240.0	402.9	242.8	403.8	243.7	405.7	245.6	407.4	247.3
	4460	181.1	462.9	281.8	466.9	285.8	468.2	287.1	463.9	282.8	470.5	289.4	472.3	291.2
	MEAN	179.9	445.9	266.0	451.6	271.7	454.5	274.6	451.0	271.1	455.2	275.3	457.1	277.2
	S.D.	15.7	44.7	30.7	45.0	31.2	46.9	33.0	45.2	31.3	45.4	31.5	45.8	32.1
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	253	GAIN	260	GAIN	267	GAIN	274	GAIN	281	GAIN	288	GAIN
300	4476	181.6	460.8	279.2	467.7	286.1	465.8	284.2	470.4	288.8	472.6	291.0	474.7	293.1
	4477	159.8	436.3	276.5	441.5	281.7	440.2	280.4	438.6	278.8	443.8	284.0	445.6	285.8
	4478	172.6	***	***	***	***	***	***	***	***	***	***	***	***
	4479	204.8	***	***	***	***	***	***	***	***	***	***	***	***
	4480	184.8	485.9	301.1	492.1	307.3	490.3	305.5	492.0	307.2	498.3	313.5	497.2	312.4
	MEAN	180.7	461.0	285.6	467.1	291.7	465.4	290.0	467.0	291.6	471.6	296.2	472.5	297.1
	S.D.	16.6	24.8	13.5	25.3	13.7	25.1	13.5	26.9	14.4	27.3	15.4	25.9	13.7
	N=	5	3	3	3	3	3	3	3	3	3	3	3	3

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN	330	GAIN
0	4416	196.0	486.0	290.0	480.1	284.1	485.1	289.1	488.6	292.6	484.6	288.6	487.8	291.8
	4417	189.9	520.9	331.0	518.1	328.2	525.1	335.2	527.2	337.3	527.5	337.6	530.3	340.4
	4418	179.6	509.5	329.9	508.1	328.5	509.9	330.3	510.3	330.7	510.3	330.7	514.6	335.0
	4419	190.0	448.8	258.8	448.0	258.0	451.5	261.5	456.9	266.9	457.4	267.4	459.5	269.5
	4420	162.2	463.9	301.7	459.4	297.2	467.4	305.2	469.5	307.3	464.9	302.7	471.8	309.6
	MEAN	183.5	485.8	302.3	482.7	299.2	487.8	304.3	490.5	307.0	488.9	305.4	492.8	309.3
	S.D.	13.3	30.2	30.1	30.2	30.1	30.1	30.4	28.8	28.7	29.7	29.2	29.4	29.7
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
50	4436	191.8	416.5	224.7	416.3	224.5	421.0	229.2	422.4	230.6	421.1	229.3	426.6	234.8
	4437	160.2	473.2	313.0	472.1	311.9	476.3	316.1	475.7	315.5	479.2	319.0	481.5	321.3
	4438	200.5	476.8	276.3	476.4	275.9	482.9	282.4	485.8	285.3	487.5	287.0	494.3	293.8
	4439	177.8	453.6	275.8	449.1	271.3	452.1	274.3	459.0	281.2	456.8	279.0	458.1	280.3
	4440	185.7	455.6	269.9	451.9	266.2	460.0	274.3	461.8	276.1	460.6	274.9	467.8	282.1
	MEAN	183.2	455.1	271.9	453.2	270.0	458.5	275.3	460.9	277.7	461.0	277.8	465.7	282.5
	S.D.	15.3	23.9	31.4	23.9	31.1	24.3	31.0	24.1	30.5	25.7	32.2	25.8	31.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5
150	4456	189.0	503.4	314.4	502.9	313.9	442.4	253.4	503.8	314.8	505.5	316.5	512.6	323.6
	4457	169.5	409.3	239.8	406.4	236.9	411.4	241.9	415.6	246.1	418.2	248.7	420.4	250.9
	4458	199.8	497.0	297.2	496.9	297.1	499.3	299.5	498.8	299.0	502.8	303.0	506.2	306.4
	4459	160.1	408.9	248.8	406.6	246.5	408.7	248.6	415.1	255.0	418.6	258.5	418.7	258.6
	4460	181.1	471.4	290.3	474.3	293.2	481.5	300.4	475.6	294.5	466.7	285.6	477.5	296.4
	MEAN	179.9	458.0	278.1	457.4	277.5	448.7	268.8	461.8	291.9	462.4	282.5	467.1	287.2
	S.D.	15.7	46.2	32.2	47.7	33.8	40.8	28.8	43.7	29.7	43.0	28.7	45.4	31.3
	N=	5	5	5	5	5	5	5	5	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G)– Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1	295	GAIN	302	GAIN	309	GAIN	316	GAIN	323	GAIN	330	GAIN
300	4476	181.6	476.6	295.0	476.2	294.6	476.0	294.4	478.3	296.7	478.8	297.2	484.9	303.3
	4477	159.8	444.7	284.9	435.7	275.9	440.6	280.8	443.4	283.6	438.3	278.5	453.2	293.4
	4478	172.6	***	***	***	***	***	***	***	***	***	***	***	***
	4479	204.8	***	***	***	***	***	***	***	***	***	***	***	***
	4480	184.8	499.2	314.4	494.0	309.2	492.6	307.8	496.0	311.2	503.9	319.1	487.3	302.5
	MEAN	180.7	473.5	298.1	468.6	293.2	469.7	294.3	472.6	297.2	473.7	298.3	475.1	299.7
	S.D.	16.6	27.4	15.0	29.9	16.7	26.6	13.5	26.8	13.8	33.1	20.3	19.0	5.5
	N=	5	3	3	3	3	3	3	3	3	3	3	3	3

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST										
MKD	NUMBER	1	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
0	4416	196.0	490.3	294.3	497.3	301.3	495.1	299.1	491.1	295.1	---	---
	4417	189.9	537.6	347.7	542.5	352.6	544.4	354.5	***	***	***	***
	4418	179.6	517.7	338.1	522.1	342.5	527.7@	348.1@	527.3	347.7	***	***
	4419	190.0	466.7	276.7	466.2	276.2	471.4	281.4	474.7	284.7	***	***
	4420	162.2	479.9	317.7	482.2	320.0	484.3	322.1	481.4	319.2	480.7	318.5
	MEAN	183.5	498.4	314.9	502.1	318.5	498.8	314.3	493.6	311.7	480.7	318.5
	S.D.	13.3	28.8	29.6	30.6	30.9	31.9	31.6	23.4	28.0	.	.
	N=	5	5	5	5	5	4	4	4	4	1	1
50	4436	191.8	430.7	238.9	438.7	246.9	***	***	***	***	***	***
	4437	160.2	481.8	321.6	484.2	324.0	***	***	***	***	***	***
	4438	200.5	496.8	296.3	498.7	298.2	499.4	298.9	***	***	***	***
	4439	177.8	461.2	283.4	458.5	280.7	464.5	286.7	***	***	***	***
	4440	185.7	468.3	282.6	469.5	283.8	468.7	283.0	467.6	281.9	***	***
	MEAN	183.2	467.8	284.6	469.9	286.7	477.5	289.5	467.6	281.9	===	===
	S.D.	15.3	24.8	30.0	23.1	28.1	19.1	8.3	.	.	===	===
	N=	5	5	5	5	5	3	3	1	1	0	0
150	4456	189.0	517.4	328.4	522.6	333.6	496.0	307.0	***	***	***	***
	4457	169.5	424.8	255.3	423.4	253.9	402.2	232.7	***	***	***	***
	4458	199.8	510.7	310.9	510.5	310.7	492.6	292.8	***	***	***	***
	4459	160.1	422.2	262.1	419.9	259.8	426.2	266.1	***	***	***	***
	4460	181.1	485.0	303.9	487.5	306.4	488.4	307.3	483.7	302.6	***	***
	MEAN	179.9	472.0	292.1	472.8	292.9	461.1	281.2	483.7	302.6	===	===
	S.D.	15.7	45.9	31.9	48.4	34.5	43.7	31.9	.	.	===	===
	N=	5	5	5	5	5	5	5	1	1	0	0

@ VALUES EXCLUDED FROM ANALYSIS.
--- NO DATA
*** DEAD ANIMAL
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 7. Body Weight/Body Weight Gains Summary (G) – Oxalate Clearance Group (continued)

DOSE	ANIMAL	DAYS ON TEST										
MKD	NUMBER	1	337	GAIN	344	GAIN	351	GAIN	358	GAIN	365	GAIN
300	4476	181.6	490.4	308.8	496.4	314.8	495.9	314.3	***	***	***	***
	4477	159.8	457.7	297.9	455.8	296.0	455.7	295.9	426.0	266.2	***	***
	4478	172.6	***	***	***	***	***	***	***	***	***	***
	4479	204.8	***	***	***	***	***	***	***	***	***	***
	4480	184.8	497.9	313.1	499.3	314.5	498.8	314.0	473.7	288.9	***	***
	MEAN	180.7	482.0	306.6	483.8	308.4	483.5	308.1	449.9	277.6	===	===
	S.D.	16.6	21.4	7.8	24.3	10.8	24.1	10.5	33.7	16.1	===	===
	N=	5	3	3	3	3	3	3	2	2	0	0

*** DEAD ANIMAL
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
0	4401	22.4	24.4	24.8	24.9	23.7	23.2	22.3	21.7	21.3	22.3	24.6	23.4	22.9
	4402	23.5	24.7	23.4	23.9	23.1	23.3	23.2	22.7	23.0	23.0	26.6	24.5	24.2
	4403	23.5	25.2	24.2	23.9	22.9	23.3	22.4	21.6	21.2	21.4	24.1	23.5	22.6
	4404	24.0	25.1	25.9	19.5	21.7	26.6	26.9	26.6@	26.6	23.9	25.7	26.8	26.7@
	4405	21.0	22.4	22.4	21.9	20.8	21.2	21.3	19.0	19.2	19.0	21.1	20.7	19.7
	4406	23.7	24.4	23.2	22.7	22.0	22.4	23.1	22.7	22.8@	24.0	26.7	25.3	25.4
	4407	25.5	28.2	28.2	27.7	27.8	27.5@	27.3@	27.2@	26.9@	26.6	30.2@	29.5@	29.5@
	4408	22.1	23.6	23.9	24.6	25.1	24.6	24.7	24.3	24.8	24.3	27.3@	24.8	24.4
	4409	23.4	25.4	26.6	25.2	25.2	25.3	24.4	23.4	23.6	27.1	27.0	26.8	25.8
	4410	24.7	26.7	25.8	25.6	25.7	25.8	25.5	24.7	23.8@	26.5	26.7	25.9	26.0
	MEAN	23.4	25.0	24.8	24.0	23.8	24.0	23.8	22.5	22.8	23.8	25.3	24.6	23.9
	S.D.	1.3	1.6	1.8	2.2	2.1	1.7	1.8	1.8	2.5	2.5	2.0	1.9	2.1
	N=	10	10	10	10	10	9	9	8	7	10	8	9	8
50	4421	23.9	25.3	24.1	24.4	23.8	24.8	24.4	23.7	23.6	24.8	24.9	23.8	24.0
	4422	23.9	25.0	24.8	24.3	24.9	24.5	23.5	22.6	23.4@	24.0	24.4	23.9	23.2
	4423	22.8	22.0	21.4	22.2	23.9	23.8	22.4	21.7	22.6@	26.3	24.9@	24.4@	24.8
	4424	24.4	21.4	25.6	24.3	24.0	23.0	23.4	21.9	23.6	25.2	25.1	23.8	23.8
	4425	22.8	23.4	23.0	23.7	24.3	23.4	23.2	22.8	23.2	24.2	24.6	24.6	24.5
	4426	25.0	25.4	25.8	24.4	24.4	24.3	24.5	23.6	25.6	25.6	25.6	24.3	23.8
	4427	22.8	24.1	24.5	24.6	24.8	24.3	23.0	22.4	22.5	23.7	24.7	22.2	22.7
	4428	22.8	23.1	23.6	23.2	23.6	22.6	22.0	21.4	22.0	23.8	22.7	21.6	21.6
	4429	21.3	23.4	22.4	21.8	21.7	21.7	21.3	20.6	20.3	21.7	22.6	20.6	21.3
	4430	24.7	25.5	24.7	24.4	25.4	25.2	25.1	24.9	25.1	25.8	26.0	26.0	25.3
	MEAN	23.4	23.9	24.0	23.7	24.1	23.8	23.3	22.6	23.2	24.5	24.5	23.4	23.5
	S.D.	1.1	1.5	1.4	1.0	1.0	1.1	1.2	1.3	1.7	1.3	1.2	1.7	1.3
	N=	10	10	10	10	10	10	10	10	8	10	9	9	10

@ VALUES EXCLUDED FROM ANALYSIS.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
150	4441	23.7	25.4	25.3	25.1	26.5	26.0	25.1	24.2	24.2	27.2	25.9	24.7	23.9
	4442	23.8	25.5	26.5@	26.3@	27.1	28.8@	28.8@	27.8@	28.5@	30.2@	29.6@	28.6@	28.6
	4443	21.8	22.8	23.1	22.3	23.2	23.2	22.7	21.7	22.4	26.2	24.9	24.1	23.3
	4444	24.8	24.5	23.7	22.4	22.8	22.3	22.2	21.9	22.0	25.1	23.6	24.1	24.1
	4445	25.5	26.2	24.9	25.5@	25.3	25.2@	25.2@	24.8@	24.5	27.6	27.9@	27.4@	26.2
	4446	24.8	24.2	20.9	20.5	22.6	22.2	22.4	24.6	24.2@	26.6	26.4@	25.2	25.0
	4447	26.5	28.5	27.8	27.2@	26.9	26.2	26.4	26.1@	26.7	30.4	28.6	27.6	27.7
	4448	25.8@	25.8	24.8@	23.6	25.4	25.9@	25.5@	24.4@	24.5	27.5@	26.9@	26.3@	26.2@
	4449	25.7	26.7	25.2	23.3	23.3	22.8	22.9	22.5	22.6	25.5	25.7	24.4	25.1
	4450	18.9	18.9	19.0	19.7	19.6	18.7	18.9	18.2	18.3	21.2	19.6	18.9	18.9
	MEAN	23.9	24.9	23.7	22.4	24.3	23.1	22.9	22.2	23.2	26.2	24.7	24.1	24.8
	S.D.	2.3	2.6	2.8	1.8	2.4	2.5	2.4	2.3	2.5	2.6	3.0	2.6	2.8
	N=	9	10	8	7	10	7	7	6	8	8	6	7	9
300	4461	22.9	24.4	25.1	24.8	24.2	24.3	23.1	22.8	21.0	24.2	24.2	23.7	22.4
	4462	21.9	24.2	24.1	22.6	23.6	22.4	20.6	21.4	21.6	24.1	24.1@	24.0	24.8
	4463	24.0	24.0	24.2	24.6	24.8	25.1	24.5	23.9	24.5	27.1	26.7	26.2	26.1
	4464	21.3	22.1	22.5	22.8	24.4	24.8@	25.8@	24.8@	23.4	25.4@	24.5@	22.9@	22.9@
	4465	25.9	29.6	28.6@	28.1@	29.2@	27.6@	27.8	26.6@	25.7	29.0	28.2	27.4	26.8
	4466	22.4	22.0	21.2	19.7	21.4	21.0	19.2	19.6	18.7	21.1	21.2	21.4	20.6
	4467	19.6	21.2	21.6	21.3	22.4	22.0	20.8	21.2	19.2	21.9	20.9	21.5	20.3
	4468	25.4	26.6	26.1	26.3@	26.1	25.8	25.5	25.1@	25.0@	28.2@	29.0@	28.0	26.5
	4469	22.4	24.1	23.3	24.3@	22.9	22.0	21.3	20.7	21.9@	23.2	24.7@	23.8	23.4
	4470	23.4	25.1	24.8	23.1	24.3	24.1	23.5	23.6	23.1@	24.2	22.8	23.5	23.5
	MEAN	22.9	24.3	23.7	22.7	23.8	23.3	22.9	21.9	22.0	24.4	24.0	24.4	23.8
	S.D.	1.9	2.4	1.6	1.8	1.4	1.7	2.7	1.6	2.6	2.6	3.0	2.4	2.4
	N=	10	10	9	7	9	8	9	7	7	8	6	9	9

@ VALUES EXCLUDED FROM ANALYSIS.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST												
MKD	NUMBER	1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
400	4481	22.4	22.5	20.7	21.5	22.7	23.0	22.4	22.0	22.2	24.0	24.3	22.5	22.7
	4482	22.9	22.9	22.7	21.8	21.6	22.1	21.9	22.2	20.9	23.3	22.3	22.8	21.5
	4483	24.8	27.8	28.3@	27.3@	27.1	26.9@	26.8@	27.2@	26.3@	30.8@	27.3@	27.2	26.4@
	4484	22.4	24.4	23.9	22.5	23.0	23.7	22.7@	23.3	24.1@	26.8@	26.8@	26.0@	25.3@
	4485	23.3@	23.6	24.6	22.2	21.7	22.8	21.1@	20.3	19.8@	22.0	23.6@	22.5	21.6@
	4486	21.2	21.8	21.6	20.8	21.1	21.4	21.8@	20.2	20.2	22.7	21.8	21.8	21.4
	4487	25.1	26.8	26.8	23.9	23.5	21.6	26.3	25.1	24.1	24.3	26.1	24.9	23.8
	4488	23.5	21.9	21.3	20.7	20.8	20.9	20.0	19.5	19.6	21.3	21.1	20.9	20.0
	4489	20.3	21.3	21.9	20.1	20.4	19.3	19.3	19.5	17.9	21.0	21.3	21.3	20.9
	4490	21.7	26.1	26.2	25.0	25.5	26.1	24.8	24.4	24.5	25.4	24.3	24.8	24.7
	MEAN	22.7	23.9	23.3	22.1	22.7	22.3	22.5	21.8	21.3	23.0	23.0	23.2	22.1
	S.D.	1.6	2.3	2.2	1.6	2.1	1.9	2.7	2.1	2.4	1.5	1.9	2.0	1.7
	N=	9	10	9	9	10	9	6	9	7	8	7	9	7

@ VALUES EXCLUDED FROM ANALYSIS.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 92-99	106-113	120-127	134-141	148-155	162-169	176-183	190-197
0	4401	21.8	23.0	21.8	21.1	21.2	21.1!	20.3	21.7
	4402	24.7	24.4	23.7	22.1	22.2	23.2	22.2	22.0
	4403	23.5	23.7	24.4	24.0	23.4	23.4	22.4	23.3
	4404	26.3	25.0	23.3	24.3	23.6	23.8	25.1	24.5
	4405	19.8	19.3!	19.7	18.2	17.9!	18.4!	19.1	18.8
	4406	24.7	24.0	24.3	23.4	23.7	23.4	23.4	23.8
	4407	30.0@	29.9@	29.2@	28.8	29.2@	31.0@	30.5@	29.1@
	4408	24.3	24.0	24.1	22.8	23.3	23.3	22.4	23.4
	4409	25.8	25.6	25.7	24.6	24.7	24.2	23.9	24.8
	4410	25.8	25.1	25.0	25.1@	24.5	25.0@	24.0	24.1
	MEAN	24.1	24.4	23.6	23.3	23.3	23.6	22.5	22.9
	S.D.	2.1	0.8	1.8	2.9	1.2	0.4	1.9	1.9
	N=	9	8	9	9	8	6	9	9
50	4421	22.1	23.3	23.0	22.8	22.8	23.1	24.0	22.9
	4422	23.2	23.1	22.9	22.5	23.6	24.5	23.1	23.7
	4423	24.4	25.2@	25.0@	24.7	24.2	24.0	24.9@	24.4@
	4424	22.8	23.3	23.3	23.8	23.5	22.6	22.9	23.8
	4425	23.1	23.4	23.2	22.8	22.1	23.9	22.6	22.2
	4426	23.5	23.2	22.6	23.0	23.4	22.3	22.2	22.2
	4427	21.9	21.8	22.1	21.5	21.5	20.9	21.0	21.4
	4428	20.9	21.8	21.5	21.4	21.3	20.4	20.8	21.2
	4429	20.0	20.6	20.5	19.3	19.4	20.2	19.7	20.5
	4430	24.8	24.1	24.6	24.4	24.9@	25.3	25.0	26.0
	MEAN	22.7	22.7	22.6	22.6	22.4	22.7	22.4	22.7
	S.D.	1.5	1.1	1.2	1.6	1.5	1.8	1.7	1.7
	N=	10	9	9	10	9	10	9	9

@ VALUES EXCLUDED FROM ANALYSIS.
! STATISTICAL OUTLIERS EXCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 92-99	106-113	120-127	134-141	148-155	162-169	176-183	190-197
150	4441	23.8	23.2	22.4	22.1	22.4	20.6	21.6	22.2
	4442	25.6@	28.4@	27.9@	27.1	27.8@	28.0@	28.4@	28.0@
	4443	21.7	22.8	22.0	21.2	21.5	21.8	21.2	21.3
	4444	22.9	22.4	21.7	21.7	20.9	21.1	21.1	21.9
	4445	25.0@	25.6	25.1@	24.7	24.0	24.2@	23.9	24.2@
	4446	24.0@	24.0@	23.5	22.2	23.0@	21.2@	22.0	23.0
	4447	26.6	26.8	25.4	25.4	24.7	25.0	24.7	24.8
	4448	26.2@	25.6@	26.3@	26.4@	26.9@	27.1@	25.5@	24.8@
	4449	25.2@	24.2@	24.2	24.9@	25.4@	24.2@	23.2@	24.2@
	4450	18.4	17.9	18.2	17.4	18.2	17.3	17.8	18.6
	MEAN	22.7	23.1	22.5	22.7	22.0	21.2	21.8	22.0
	S.D.	3.0	3.1	2.3	3.0	2.3	2.8	2.2	2.0
	N=	5	6	7	8	6	5	7	6
300	4461	21.5	22.4	22.3	21.9	20.7	21.3	20.2@	23.0
	4462	23.7	24.7@	25.5@	***	***	***	***	***
	4463	24.8	***	***	***	***	***	***	***
	4464	21.5@	22.2@	21.5	21.8	21.1@	21.5@	22.1	21.7@
	4465	26.5	26.8	26.3	26.2	25.6	26.1	25.5	26.2
	4466	20.5	20.5@	20.6@	20.3	20.3@	20.2@	19.3@	20.3@
	4467	19.5	19.2	20.2	19.7	17.5	18.4	18.3	18.8
	4468	26.2@	26.5@	26.7@	26.7	26.0@	27.1@	26.2@	21.3@
	4469	22.5	23.3@	23.9@	22.1@	23.5@	23.2@	23.6@	22.9@
	4470	23.1	22.7	23.1	22.0	22.3	22.0	23.3	23.0
	MEAN	22.8	22.8	22.7	22.7	21.5	22.0	22.3	22.8
	S.D.	2.3	3.1	2.3	2.7	3.4	3.2	3.0	3.0
	N=	8	4	5	7	4	4	4	4

@ VALUES EXCLUDED FROM ANALYSIS.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 92-99	106-113	120-127	134-141	148-155	162-169	176-183	190-197
400	4481	21.3	22.3	22.2	23.2	21.7	21.4	20.9	22.4
	4482	22.2	21.8	19.7	17.4	18.9	17.9	19.0	***
	4483	25.3@	26.8@	23.5@	23.9@	24.4@	24.7@	23.0@	22.2@
	4484	24.5@	23.2@	22.7@	23.4@	24.5@	22.9@	21.1@	18.2@
	4485	22.1@	22.7@	22.9@	22.2@	21.2@	21.5@	21.4@	20.9@
	4486	20.8	21.4@	21.2	21.4@	20.1@	20.2@	19.2@	18.7
	4487	22.7	22.5	21.2	21.6	19.8	18.2	15.5	***
	4488	20.4	18.3	16.8	18.4	19.6	19.5	19.5	19.9
	4489	20.1	15.1	17.7	17.4	18.2	16.8	15.7	11.6
	4490	23.3	22.4	22.2	21.8	19.5	17.4	16.3	14.2
	MEAN	21.5	20.4	20.1	20.0	19.6	18.5	17.8	17.4
	S.D.	1.2	3.0	2.2	2.5	1.2	1.7	2.3	4.4
	N=	7	6	7	6	6	6	6	5

@ VALUES EXCLUDED FROM ANALYSIS.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary -- Main Group (continued)

DOSE	ANIMAL	DAYS ON TEST											
MKD	NUMBER	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
0	4401	23.1	22.4	21.1	21.0	21.9	21.8	22.6	21.2	22.0	21.1	23.9	24.0
	4402	23.4	23.1	23.1	22.4	22.7	22.9	22.8	21.0@	22.2	23.0	24.2	23.5
	4403	23.0	22.7	23.3	23.2	21.4	23.3	21.8	22.7	22.9	22.0	23.1	23.7
	4404	25.7	23.8	24.0	23.5	23.8	22.4	17.6	***	***	***	***	***
	4405	19.8	19.2!	19.4	19.7	19.1	20.0	19.1	18.4	18.2!	17.9	19.4!	19.1!
	4406	23.6	24.8	24.3	23.9	24.1	25.1	23.4	22.4	24.1	25.5	25.8@	24.6
	4407	30.2@	31.2@	30.6@	30.8@	30.4@	32.0@	30.1@	29.8@	31.3@	30.0	31.6@	32.4@
	4408	23.8	22.7	23.0	22.2	22.5	23.1	23.9	22.0	22.6	22.2	23.1	22.9
	4409	23.6	23.2	23.7	23.7	23.3	24.2	22.5	20.7	23.2	21.9	23.9	23.9
	4410	24.9	24.5@	24.6	23.1	25.1	26.0	25.2@	23.4@	23.7@	24.8	26.3@	25.1@
	MEAN	23.4	23.2	22.9	22.5	22.7	23.2	21.7	21.2	22.8	23.2	23.6	23.8
	S.D.	1.6	0.8	1.7	1.4	1.8	1.8	2.2	1.6	0.8	3.4	0.5	0.6
	N=	9	7	9	9	9	9	8	6	6	9	5	6
50	4421	24.0	23.5	23.3	23.0	24.0	25.5	23.3	23.2	24.9	24.0	24.7	23.5
	4422	23.6	24.6@	23.8@	23.6	23.5	24.2	22.5	22.6@	24.3	22.8	23.7	23.3
	4423	25.6@	24.9	23.6	24.2	26.3	24.2	24.0	23.2@	28.8	25.9@	27.0@	25.1
	4424	23.2	23.6	22.6	22.9	23.1	23.6	22.3	22.2	23.6	23.1	23.4	21.6
	4425	22.5	22.8	21.9	22.7	23.4	23.4	22.5	21.8	24.4	22.4	22.2	22.0
	4426	24.4	23.0	23.6	22.0	22.9	22.5	21.9	22.4	24.8	22.5	22.9	23.6
	4427	20.7	21.3	20.9	21.0	21.7	21.1	20.1	20.5	21.9	21.1	20.7	20.6
	4428	21.7	22.2	19.2	20.6	21.1	22.2	19.6	19.4	23.5	22.1	21.6	21.7
	4429	20.7	19.9	19.9	19.8	19.4	21.0	19.4	18.6	20.7	19.7	19.4	20.3
	4430	24.9	25.0	25.3	24.1	24.1	24.6	23.7	22.7	25.2	24.6	24.8	23.9
	MEAN	22.9	22.9	22.3	22.4	23.0	23.2	21.9	21.4	24.2	22.5	22.6	22.6
	S.D.	1.6	1.6	2.0	1.5	1.9	1.5	1.7	1.7	2.1	1.5	1.8	1.6
	N=	9	9	9	10	10	10	10	8	10	9	9	10

@ VALUES EXCLUDED FROM ANALYSIS.
*** DEAD ANIMAL
! STATISTICAL OUTLIERS EXCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 8. Feed Consumption (G/Day) Summary – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
150	4441	22.5	22.0	21.4	21.5	22.3	22.1	21.7	20.0	22.3	21.6	21.6	21.9
	4442	27.8@	28.0@	26.8@	27.4@	27.5@	27.5@	26.5@	25.2@	26.0@	26.2@	25.7@	25.4@
	4443	22.4	21.9	21.3	21.3	21.7	22.2	21.7	20.3	22.9	23.0	21.5	21.0
	4444	21.1	21.4	21.3	19.5	11.1	***	***	***	***	***	***	***
	4445	25.6@	26.8@	24.8@	26.0	26.4@	25.3@	24.2	24.8@	27.3@	25.4	25.0	24.3
	4446	23.4	23.0	21.2	21.9	22.5	23.6	21.4	21.2	22.2	23.2	22.1	21.4
	4447	24.1	24.5	23.6	23.6	24.3	23.7	23.1	22.1	25.1	23.3	24.5	23.9
	4448	27.3@	27.3@	26.9@	27.7@	27.0@	28.2	27.0@	26.5@	29.1@	28.2@	26.6@	26.8@
	4449	26.3@	25.0@	24.8@	24.8	23.7	25.5@	23.2@	23.3@	27.5@	26.9@	25.3@	25.9@
	4450	18.6	17.2	18.0	18.1	17.7	18.4	17.3	17.0	18.1	18.1	17.9	18.4
	MEAN	22.0	21.7	21.1	22.1	20.5	23.0	21.6	20.1	22.1	22.4	22.1	21.8
	S.D.	2.0	2.4	1.8	2.6	4.6	3.2	2.3	1.9	2.5	2.4	2.5	2.1
	N=	6	6	6	8	7	6	6	5	5	6	6	6
300	4461	23.8@	23.1@	22.4	21.7@	22.7	24.5@	22.8	23.9@	27.0@	25.7@	24.8@	***
	4462	***	***	***	***	***	***	***	***	***	***	***	***
	4463	***	***	***	***	***	***	***	***	***	***	***	***
	4464	23.1@	23.7@	23.0@	23.5@	23.9@	26.2@	24.7	23.9@	25.0@	26.6@	25.7@	23.3@
	4465	23.9	25.7	26.5	25.2	26.4	26.4	24.6	25.0	26.3	26.1	27.9	27.2
	4466	22.1@	21.9@	21.0@	19.4@	22.0	19.5@	21.8@	20.8	20.8@	21.4	21.8	***
	4467	21.4	19.8	19.1	19.6	19.0	20.0	17.8	18.5	20.5	19.4	20.0	19.0
	4468	25.2	28.2@	26.5@	22.6@	23.8	25.0@	26.8@	26.1@	22.5@	30.8@	27.1@	27.9@
	4469	24.3@	24.6@	25.8@	25.2@	23.3@	25.4@	23.6@	23.2@	24.8@	26.7@	23.8@	23.2@
	4470	***	***	***	***	***	***	***	***	***	***	***	***
	MEAN	23.5	22.8	22.7	22.4	22.8	23.2	22.5	21.4	23.4	22.3	23.2	23.1
	S.D.	1.9	4.2	3.7	4.0	2.7	4.5	3.2	3.3	4.1	3.4	4.1	5.8
	N=	3	2	3	2	5	2	4	3	2	3	3	2

@ VALUES EXCLUDED FROM ANALYSIS.
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 9. Feed Consumption (G/Day) Summary – Metabolism Group

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 190-197	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
0	4411	23.0	24.1	22.3	23.4@	23.5	23.0	23.0	23.4	21.6@	22.8	21.5	21.9	22.9
	4412	20.3	21.7	21.0	20.5	19.8	20.4	21.1	20.7	18.7	19.7	20.3	20.6	19.9
	4413	23.3@	25.0@	26.6@	26.9@	26.5@	25.8@	27.4@	29.3@	27.5@	27.4@	26.8@	28.1@	28.8@
	4414	25.3	26.0	26.8	26.2	25.6	25.6	25.9	26.0	25.9	24.7	25.8	27.0	26.5
	4415	20.7	20.2	20.2	19.9	19.6	20.1	20.5	20.1	19.1	19.8	19.6	21.2	20.8
	MEAN	22.3	23.0	22.6	22.2	22.1	22.3	22.6	22.6	21.2	21.8	21.8	22.7	22.5
	S.D.	2.3	2.6	2.9	3.5	2.9	2.6	2.4	2.7	4.0	2.4	2.8	2.9	2.9
	N=	4	4	4	3	4	4	4	4	3	4	4	4	4
50	4431	20.8	20.7	21.0	20.7	20.7	21.1	20.9	20.7	19.5	21.7	21.1	21.0	20.9
	4432	18.9	20.9	21.7	20.3	19.1	19.8	20.8	19.0	18.5	21.4	20.5	20.1	20.9
	4433	22.1	23.2@	22.7@	22.7	22.9	22.4	24.5	23.1	21.4@	24.2@	23.9@	22.6@	23.8@
	4434	23.8	25.6	24.8	23.7	23.2	24.2	24.1	23.6	23.7	24.9	23.9	25.4	23.0
	4435	21.9	20.1	22.9@	23.4@	24.7@	22.2@	22.4	13.0	26.4@	23.7@	23.1@	23.4@	23.5
	MEAN	21.5	21.8	22.5	21.9	21.5	21.9	22.5	19.9	20.6	22.7	21.8	22.2	22.1
	S.D.	1.8	2.5	2.0	1.6	1.9	1.9	1.7	4.3	2.8	1.9	1.8	2.8	1.4
	N=	5	4	3	4	4	4	5	5	3	3	3	3	4
150	4451	25.4	24.7	24.7	24.5	24.7	24.4	25.3	24.4	23.4	24.8	24.7	24.9	24.1
	4452	22.7	24.7	24.6@	22.6	23.1@	23.1@	24.6	23.4	22.7@	25.4@	23.7	24.3@	25.8@
	4453	21.9	22.5	22.7	22.9	22.4	23.1	23.6	22.1	22.0	23.3	22.1	22.4	22.5
	4454	22.2	21.2	21.9	22.0	22.0	21.1	22.0	21.1	20.2	22.2	20.6	21.7	20.0
	4455	23.2	24.5	23.9	23.5	23.2	23.8	24.9	23.9	22.5	25.0	24.3	24.7	23.0
	MEAN	23.1	23.5	23.3	23.1	23.1	23.1	24.1	23.0	22.0	23.8	23.1	23.4	22.4
	S.D.	1.4	1.6	1.2	1.0	1.2	1.4	1.3	1.4	1.3	1.3	1.7	1.6	1.7
	N=	5	5	4	5	4	4	5	5	4	4	5	4	4

@ VALUES EXCLUDED FROM ANALYSIS.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

TABLE 9. Feed Consumption (G/Day) Summary – Metabolism Clearance Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 190-197	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
300	4471	22.7	24.0	23.0	22.3	21.3	23.8	24.1	21.9	21.3	23.7	22.6	22.2	21.8
	4472	25.0@	25.4@	25.1@	25.6@	26.6@	26.4@	26.2	26.4@	25.9@	31.0@	27.1@	26.5@	26.0@
	4473	20.4	20.4	20.8	19.9	19.9	20.5	21.3	19.8	19.2	20.1	18.8	20.3	19.7
	4474	20.5	22.1	22.4	16.6	20.6	20.3	22.1	20.8	20.0	20.8	20.4	19.5	18.6
	4475	26.7@	26.3@	26.2@	26.2@	25.9@	26.4@	26.8@	26.1@	26.0@	27.9@	27.4@	28.0@	27.3@
	MEAN	21.2	22.2	22.1	19.6	20.6	21.5	23.4	20.8	20.2	21.5	20.6	20.7	20.0
	S.D.	1.3	1.8	1.1	2.9	0.7	2.0	2.2	1.1	1.1	1.9	1.9	1.4	1.6
	N=	3	3	3	3	3	3	4	3	3	3	3	3	3
400	4491	***	***	***	***	***	***	***	***	***	***	***	***	***
	4492	29.4@	***	***	***	***	***	***	***	***	***	***	***	***
	4493	25.9	***	***	***	***	***	***	***	***	***	***	***	***
	4494	22.0	***	***	***	***	***	***	***	***	***	***	***	***
	4495	24.1	***	***	***	***	***	***	***	***	***	***	***	***
	MEAN	24.0	===	===	===	===	===	===	===	===	===	===	===	===
	S.D.	2.0	===	===	===	===	===	===	===	===	===	===	===	===
	N=	3	0	0	0	0	0	0	0	0	0	0	0	0

@ VALUES EXCLUDED FROM ANALYSIS.
*** DEAD ANIMAL
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 10. Feed Consumption (G/Day) Summary – Oxalate Clearance Group

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 190-197	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
0	4416	21.1	21.5	21.1	21.5	21.0	21.6	20.4	20.9	19.9	20.0	19.7	20.6	---
	4417	22.3	21.9	21.9	21.7	20.8	21.5	22.4	22.2	20.7	21.1	21.5	21.7	***
	4418	23.3	23.1@	26.7@	21.8	24.7@	27.1@	27.5	26.7@	25.4@	26.1@	26.6@	28.4@	***
	4419	23.8	22.9	22.9	21.9	22.8@	24.3@	25.3@	25.5@	25.3@	27.6@	26.6@	28.7@	***
	4420	22.9	24.3	25.7	24.3!	24.8@	25.1@	24.9@	26.0@	23.9@	24.3@	24.3	27.5@	24.6
	MEAN	22.7	22.7	22.9	21.7	20.9	21.6	23.4	21.6	20.3	20.6	21.8	21.2	24.6
	S.D.	1.0	1.2	2.0	0.2	0.1	0.1	3.7	0.9	0.6	0.8	2.3	0.8	.
	N=	5	4	4	4	2	2	3	2	2	2	3	2	1
50	4436	21.2	22.3	21.7	20.5	20.3	20.9	21.5	20.5	19.5	21.1	21.5	***	***
	4437	21.7	21.5	22.3	21.9	22.1	22.4	22.2	21.1	19.9	22.6	22.2	***	***
	4438	19.9	21.2	21.4	20.8	20.0	19.9	20.6	19.1	19.1	22.1	20.9	18.8	***
	4439	20.8	20.6	21.7	21.8	22.1	23.4	22.9	22.1	20.9	23.1	22.7@	22.7	***
	4440	22.1	22.1	22.5	23.0	20.7	24.1	23.0	21.3	21.0	22.5	22.2	20.9	***
	MEAN	21.1	21.5	21.9	21.6	21.0	22.1	22.0	20.8	20.1	22.3	21.7	20.8	===
	S.D.	0.9	0.7	0.5	1.0	1.0	1.7	1.0	1.1	0.8	0.7	0.6	2.0	===
	N=	5	5	5	5	5	5	5	5	5	5	4	3	0
150	4456	24.1	24.6	25.0	23.8	22.8	25.4	24.9	24.4	21.9	25.8	24.5	22.2	***
	4457	20.7	20.0	20.1	20.1	20.0	20.4	21.1	20.1	18.8	21.5	20.0	17.5	***
	4458	21.7	21.9	22.3	21.9	22.1	22.3	21.4	21.3	20.5	22.7	21.8	20.1	***
	4459	20.2	20.8	21.0	20.4	20.8	20.7	20.6	20.5	19.4	22.0	19.9	20.9	***
	4460	21.3	23.9	23.7	23.1	22.4	22.0	22.5	21.6	21.7	20.0	22.7	22.6	***
	MEAN	21.6	22.2	22.4	21.9	21.6	22.2	22.1	21.6	20.5	22.4	21.8	20.7	===
	S.D.	1.5	2.0	2.0	1.6	1.2	2.0	1.7	1.7	1.4	2.1	1.9	2.0	===
	N=	5	5	5	5	5	5	5	5	5	5	5	5	0

@ VALUES EXCLUDED FROM ANALYSIS.
--- NO DATA
*** DEAD ANIMAL
! STATISTICAL OUTLIERS EXCLUDED.
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 10. Feed Consumption (G/Day) Summary – Oxalate Clearance Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON TEST 190-197	204-211	218-225	232-239	246-253	260-267	274-281	288-295	302-309	316-323	330-337	344-351	358-365
300	4476	22.4	23.1	23.4	21.9	22.6	22.5	22.4	21.4	19.7	22.9	22.0	22.9	***
	4477	18.8	20.6	20.7	20.1	19.7	19.7	20.3	19.2	19.2	19.8	19.5	19.2	***
	4478	20.5	13.2	***	***	***	***	***	***	***	***	***	***	***
	4479	24.4	25.3	***	***	***	***	***	***	***	***	***	***	***
	4480	24.8@	29.1@	28.3@	27.6@	26.7	25.8@	28.2@	26.1@	24.3	28.1	24.5@	24.8@	***
	MEAN	21.5	20.6	22.1	21.0	23.0	21.1	21.4	20.3	21.1	23.6	20.8	21.1	===
	S.D.	2.4	5.3	1.9	1.3	3.5	2.0	1.5	1.6	2.8	4.2	1.8	2.6	===
	N=	4	4	2	2	3	2	2	2	3	3	2	2	0
400	4496	17.4	***	***	***	***	***	***	***	***	***	***	***	***
	4497	16.0	***	***	***	***	***	***	***	***	***	***	***	***
	4498	19.3@	***	***	***	***	***	***	***	***	***	***	***	***
	4499	15.7	***	***	***	***	***	***	***	***	***	***	***	***
	4500	***	***	***	***	***	***	***	***	***	***	***	***	***
	MEAN	16.4	===	===	===	===	===	===	===	===	===	===	===	===
	S.D.	0.9	===	===	===	===	===	===	===	===	===	===	===	===
	N=	3	0	0	0	0	0	0	0	0	0	0	0	0

@ VALUES EXCLUDED FROM ANALYSIS.
*** DEAD ANIMAL
=== NO DATA AVAILABLE FOR MEAN AND S.D.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 11. Water Consumption (G/Day) – Main Group

DOSE MKD	ANIMAL NUMBER	DAYS ON 368-369
0	4401	---
	4402	5.1
	4403	17.0
	4404	***
	4405	9.9
	4406	7.4
	4407	10.1
	4408	10.5
	4409	11.7
	4410	15.1
	MEAN	10.9
	S.D.	3.8
	N=	8
50	4421	13.9
	4422	9.1
	4423	9.2
	4424	14.4
	4425	11.3
	4426	5.8
	4427	10.7
	4428	10.1
	4429	7.1
	4430	5.5
	MEAN	9.7
	S.D.	3.0
	N=	10

--- NO DATA
*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 11. Water Consumption (G/Day) – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	DAYS ON 368-369
150	4441	4.6
	4442	13.8
	4443	7.8
	4444	***
	4445	10.8
	4446	16.4
	4447	8.5
	4448	7.0
	4449	5.4
	4450	6.1
	MEAN	8.9
	S.D.	4.0
	N=	9
300	4461	***
	4462	***
	4463	***
	4464	22.8
	4465	15.6
	4466	***
	4467	10.3
	4468	27.6
	4469	6.4
	4470	***
	MEAN	16.5
	S.D.	8.7
	N=	5

*** DEAD ANIMAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 12. Feed Efficiency (G/Day) – Main Group

DOSE	ANIMAL	DAYS ON TEST 1,8	8,15	15,22	22,30	30,36	36,43	43,50	50,57	57,64	64,71	71,78	78,85	85,92
MKD	NUMBER	1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
0	4401	3.7	4.6	5.8	8.8	8.3	15.8	10.3	43.4	24.4	11.7	17.9	14.0	28.1
	4402	3.6	5.2	7.0	8.4	9.6	9.4	16.1	23.0	13.4	17.3	10.8	13.0	45.8
	4403	3.6	5.4	6.2	11.9	8.5	20.6	13.3	27.0	34.5	18.0	14.4	9.9	175.8!
	4404	3.6	4.6	5.1	-6.0!	3.6	5.7	5.6	---	10.3	44.0!	72.0!	10.7	---
	4405	4.0	5.5	7.6	9.5	11.6	11.5	15.2	665.0!	13.3	41.6!	19.4	16.1	38.3
	4406	3.7	5.3	6.4	11.9	12.8	11.1	8.5	19.9	---	9.1	12.7	14.8	30.7
	4407	3.7	4.3	5.9	7.4	9.0	---	---	---	---	15.5	---	---	---
	4408	3.7	4.4	5.6	7.9	6.8	12.4	10.4	11.5	15.4	15.6	---	31.0	36.3
	4409	3.6	4.5	5.6	10.4	7.8	13.7	12.7	31.5	15.4	14.5	17.5	21.3	27.8
	4410	3.9	4.4	6.6	8.1	6.8	17.4	13.0	19.0	---	14.5	30.6	18.3	26.8
	MEAN	3.7	4.8	6.2	9.4	8.5	13.1	11.7	25.0	18.1	14.5	17.6	16.6	33.4
	S.D.	0.1	0.5	0.7	1.7	2.6	4.4	3.3	10.3	8.5	2.9	6.5	6.5	7.0
	N=	10	10	10	9	10	9	9	7	7	8	7	9	7
50	4421	3.4	4.0	4.9	8.7	6.9	8.5	9.2	18.6	11.2	13.5	18.9	14.9	129.2
	4422	3.8	4.2	6.0	8.3	7.7	15.5	18.3	16.7	---	13.2	18.4	18.2	40.6
	4423	4.3	5.3	7.0	9.3	6.0	10.7	10.7	16.5	---	10.1	---	---	59.9
	4424	3.9	-78.8!	3.7	11.4	4.8	10.0	16.5	20.2	12.2	14.8	16.4	-35.4!	22.2
	4425	4.7	4.7	6.4	7.9	6.0	28.2!	13.6	13.0	29.0	18.6	12.0	16.1	47.6
	4426	3.7	4.9	5.4	8.8	9.5	7.7	10.7	42.4!	9.6	15.2	25.6	23.6	111.1
	4427	3.8	4.7	6.0	8.7	10.7	9.5	18.5	17.4	16.6	14.6	27.4	37.9	794.5!
	4428	4.0	4.6	5.4	10.4	7.5	16.7	12.1	30.6	18.6	14.1	158.9!	20.7	137.5
	4429	3.7	4.0	6.3	10.8	9.2	9.9	13.3	12.9	21.9	13.8	17.4	36.1	48.1
	4430	4.2	5.1	7.6	12.0	6.6	10.3	18.9	22.1	23.4	19.0	18.4	13.7	88.6
	MEAN	4.0	4.6	5.9	9.6	7.5	11.0	14.2	18.7	17.8	14.7	19.3	22.7	76.1
	S.D.	0.4	0.5	1.1	1.4	1.8	3.1	3.6	5.4	6.7	2.6	5.0	9.4	41.8
	N=	10	9	10	10	10	9	10	9	8	10	8	8	9

--- NO DATA
! STATISTICAL OUTLIERS EXCLUDED.
DAYS ON TEST GIVEN AS BODY WEIGHT INTERVAL OVER FEED CONSUMPTION INTERVAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 12. Feed Efficiency (G/Day) – Main Group (continued)

		DAYS ON TEST												
DOSE MKD	ANIMAL NUMBER	1,8 1-8	8,15 8-15	15,22 15-22	22,30 22-30	30,36 30-36	36,43 36-43	43,50 43-50	50,57 50-57	57,64 57-64	64,71 64-71	71,78 71-78	78,85 78-85	85,92 85-92
150	4441	3.7	4.1	6.3	7.9	7.5	7.6	15.3	44.6	34.6!	12.6	58.5!	19.9	-88.1!
	4442	4.0	4.4	---	---	6.8	---	---	---	---	---	---	---	---
	4443	3.7	4.3	5.5	7.9	7.6	11.9	10.3	19.2	9.7	12.1	20.3	23.4	90.6
	4444	3.6	6.2	6.1	10.4	9.8	15.8	12.1	23.6	16.6	10.8	23.6	10.0!	33.7
	4445	4.6	5.2	6.8	---	8.2	---	---	---	16.3	11.0	---	---	50.9
	4446	5.7	8.6	18.1!	24.1	8.7	16.5	12.2	10.0	---	13.5	---	20.3	62.5
	4447	3.4	4.5	5.7	---	7.3	11.4	16.8	---	18.5	10.7	21.3	22.0	23.4
	4448	---	5.9	---	9.2	8.1	---	---	---	15.3	---	---	---	---
	4449	4.5	6.0	11.7!	21.4	10.3	17.2	15.7	26.7	13.8	15.0	17.0	23.1	23.7
	4450	4.7	6.2	7.3	12.6	11.9	10.6	8.6	16.5	20.7	13.0	29.2	21.7	55.1
	MEAN	4.2	5.5	6.3	13.4	8.6	13.0	13.0	23.4	15.8	12.3	22.3	21.7	48.6
	S.D.	0.7	1.4	0.7	6.7	1.6	3.6	3.0	11.9	3.5	1.5	4.5	1.4	24.1
	N=	9	10	6	7	10	7	7	6	7	8	5	6	7
300	4461	3.5	4.3	4.5	10.3	7.8	19.8	12.6	16.6	30.6	10.7	28.7	44.8!	-142.5
	4462	3.8	4.5	5.8	11.9	9.4	22.1	11.2	17.4	15.3	13.9	---	14.5	22.5
	4463	4.6	4.8	5.0	8.4	7.6	11.2	10.8	13.4	19.7	9.0	28.3	16.7	35.8
	4464	4.7	6.2	6.8	9.3	16.3!	---	---	---	-819.0!	---	---	---	---
	4465	3.8	4.3	---	---	---	---	9.7	---	12.3	14.8	17.8	13.1	268.0
	4466	4.2	6.6	8.2	13.2	8.5	22.3	11.5	41.6	33.6	14.5	20.3	16.1	46.5
	4467	3.7	3.9	4.8	7.3	5.9	10.9	9.3	11.2	37.3	11.0	34.0	16.0	40.6
	4468	4.3	4.7	6.0	---	8.7	14.4	11.2	---	---	---	---	32.7!	40.3
	4469	4.0	5.0	5.1	---	8.0	39.5	15.9	-60.4!	---	28.0!	---	20.8	26.0
	4470	4.4	4.2	4.9	13.5	5.5	19.8	8.2	33.0	---	154.0!	21.0	15.0	-205.6
	MEAN	4.1	4.9	5.7	10.6	7.7	20.0	11.2	22.2	24.8	12.3	25.0	16.0	14.6
	S.D.	0.4	0.9	1.2	2.4	1.3	9.1	2.2	12.2	10.4	2.4	6.3	2.4	132.5
	N=	10	10	9	7	8	8	9	6	6	6	6	7	9

--- NO DATA
! STATISTICAL OUTLIERS EXCLUDED.
DAYS ON TEST GIVEN AS BODY WEIGHT INTERVAL OVER FEED CONSUMPTION INTERVAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 12. Feed Efficiency (G/Day) – Main Group (continued)

		DAYS ON TEST											
DOSE	ANIMAL	1,8	8,15	15,22	22,30	30,36	36,43	43,50	50,57	57,64	64,71	71,78	78,85	85,92
MKD	NUMBER	1-8	8-15	15-22	22-30	30-36	36-43	43-50	50-57	57-64	64-71	71-78	78-85	85-92
400	4481	5.7	11.4!	8.9	9.7	7.6	14.4	10.5	25.2	19.7	12.3	30.4	16.8	-227.0
	4482	4.3	6.7	6.3	11.3	9.5	20.9	8.4	25.5	21.2	17.7	-223.0	13.4	-51.9
	4483	4.3	5.0	---	---	7.8	---	---	---	---	---	---	15.4	---
	4484	3.8	5.0	6.1	8.8	9.9	11.9	---	17.0	---	---	---	---	---
	4485	---	5.0	5.9	12.5	8.9	21.0	---	28.4	---	23.0	---	17.7	---
	4486	4.6	5.4	5.8	10.9	8.7	18.7	---	707.0!	-3.2!	2.4	33.9	15.0	-214.0
	4487	3.7	4.4	5.4	17.2	10.3	16.3	6.6	18.1	19.6	37.0	15.1	27.7!	-111.1
	4488	4.9	8.3	8.4	8.1	9.5	16.1	9.5	62.0	16.9	18.9	31.4	14.2	-58.3
	4489	4.2	5.8	5.9	10.4	8.4	52.0!	8.0	41.4	21.2	13.1	11.8	12.7	-133.0
	4490	3.5	3.5	4.3	7.7	7.0	9.2	9.5	17.3	11.0	19.1	-283.5	18.5	48.0
	MEAN	4.3	5.5	6.3	10.7	8.8	16.1	8.8	29.4	18.3	17.9	-54.8	15.5	-106.8
	S.D.	0.7	1.4	1.4	2.9	1.1	4.2	1.4	15.4	3.9	9.9	136.9	2.1	96.6
	N=	9	9	9	9	10	8	6	8	6	8	7	8	7

--- NO DATA
! STATISTICAL OUTLIERS EXCLUDED.
DAYS ON TEST GIVEN AS BODY WEIGHT INTERVAL OVER FEED CONSUMPTION INTERVAL

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 13. Urinalysis

DOSE MKD	ANIMAL NUMBER	URINE VOL (ML)	SPECIFIC GRAVITY	COLOR	APPEAR	pH	PROTEIN (MG/DL)	GLUCOSE (MG/DL)	KETONES (MG/DL)	BILI-RUBIN	BLOOD	UROBIL-INOGEN
0	4402	8.7	1.037	YELLOW	SL. CL	8.5	++	NEG	TRC	NEG	NEG	<=1
	4403	14.5	1.026	YELLOW	SL. CL	>=9	+	NEG	TRC	NEG	NEG	<=1
	4405	7.1	1.033	YELLOW	SL. CL	8.0	+	NEG	NEG	NEG	NEG	<=1
	4406	5.1	1.045	YELLOW	SL. CL	7.0	++	NEG	TRC	NEG	NEG	<=1
	4407	13.1	1.028	YELLOW	SL. CL	8.5	++	NEG	NEG	NEG	NEG	<=1
	4408	9.1	1.027	YELLOW	SL. CL	>=9	+	NEG	TRC	NEG	NEG	<=1
	4409	14.2	1.024	YELLOW	SL. CL	8.0	+	NEG	NEG	NEG	NEG	<=1
	4410	13.3	1.024	YELLOW	SL. CL	7.5	+	NEG	NEG	NEG	NEG	<=1
	MEAN	10.6	1.031									
	S.D.	3.6	0.007									
	N=	8	8									
50	4421	15.2	1.024	YELLOW	SL. CL	7.5	+	NEG	NEG	NEG	NEG	<=1
	4422	4.6	1.047	YELLOW	SL. CL	6.5	++	NEG	TRC	+	+	<=1
	4423	6.8	1.036	YELLOW	SL. CL	>=9	++	NEG	TRC	NEG	NEG	<=1
	4424	12.4	1.024	YELLOW	SL. CL	>=9	++	NEG	NEG	NEG	NEG	<=1
	4425	13.0	1.023	YELLOW	SL. CL	8.5	+	NEG	NEG	NEG	NEG	<=1
	4426	8.6	1.039	YELLOW	SL. CL	6.5	++	NEG	TRC	NEG	NEG	<=1
	4427	3.5	1.044	YELLOW	SL. CL	8.0	++	NEG	TRC	+	++	<=1
	4428	8.1	1.032	YELLOW	SL. CL	8.5	+	NEG	TRC	NEG	NEG	<=1
	4429	8.3	1.031	YELLOW	SL. CL	8.0	++	NEG	NEG	NEG	NEG	<=1
	4430	7.1	1.041	YELLOW	SL. CL	6.5	++	NEG	TRC	+	+	<=1
	MEAN	8.8	1.034									
	S.D.	3.7	0.009									
	N=	10	10									

NEG=NEGATIVE TRC=TRACE SL. CL=SLIGHTLY CLOUDY UROBILINOGEN IS MEASURED IN EU/DL=EHRLICH UNITS/DECILITER
@SPECIFIC GRAVITY VALUES ARE MEAN AND S.D. FOR THE SPECIFIED NUMBER (N) OF ANIMALS. ALL OTHER DATA TABULATED AS NUMBER OF ANIMALS (N) WITH THE STATED VALUE. THE TOTAL NUMBER OF ANIMALS FOR SOME PARAMETERS MAY NOT EQUAL THE NUMBER OF ANIMALS IN THE DOSE GROUP
N = NORMAL; + = SLIGHT; ++ = MODERATE; +++ = SEVERE

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 13. Urinalysis (continued)

DOSE MKD	ANIMAL NUMBER	URINE VOL (ML)	SPECIFIC GRAVITY	COLOR	APPEAR	pH	PROTEIN (MG/DL)	GLUCOSE (MG/DL)	KETONES (MG/DL)	BILI-RUBIN	BLOOD	UROBIL-INOGEN
150	4441	6.6	1.047	YELLOW	SL. CL	6.0	++	NEG	TRC	NEG	++	<=1
	4442	12.5	1.025	YELLOW	SL. CL	7.5	+	NEG	NEG	NEG	NEG	<=1
	4443	7.3	1.041	YELLOW	SL. CL	5.0	++	NEG	TRC	NEG	NEG	<=1
	4445	6.4	1.037	YELLOW	SL. CL	5.5	+	NEG	TRC	NEG	NEG	<=1
	4446	11.6	1.023	YELLOW	SL. CL	7.5	+	NEG	NEG	NEG	NEG	<=1
	4447	11.3	1.025	YELLOW	SL. CL	>=9	+	NEG	NEG	NEG	NEG	<=1
	4448	7.1	1.035	YELLOW	SL. CL	>=9	++	NEG	TRC	NEG	NEG	<=1
	4449	5.2	1.047	YELLOW	SL. CL	6.0	++	NEG	TRC	+	NEG	<=1
	4450	3.2	1.062	YELLOW	SL. CL	5.0	++	NEG	+	+	NEG	<=1
	MEAN	7.9	1.038									
	S.D.	3.2	0.013									
	N=	9	9									
300	4464	19.8	1.017	YELLOW	SL. CL	>=9	+	NEG	NEG	NEG	NEG	<=1
	4465	13.9	1.026	YELLOW	SL. CL	8.5	++	NEG	NEG	NEG	NEG	<=1
	4467	7.8	1.029	YELLOW	SL. CL	7.0	+	NEG	TRC	NEG	NEG	<=1
	4468	35.3	1.012	YELLOW	SL. CL	6.5	+	NEG	NEG	NEG	NEG	<=1
	4469	4.5	1.042	BROWN	SL. CL	>=9	+++	NEG	TRC	+	++++	<=1
	MEAN	16.3	1.025									
	S.D.	12.2	0.012									
	N=	5	5									

NEG=NEGATIVE TRC=TRACE SL. CL=SLIGHTLY CLOUDY UROBILINOGEN IN MEASURED IN EU/DL=EHRLICH UNITS/DECILITER
@SPECIFIC GRAVITY VALUES ARE MEAN AND S.D. FOR THE SPECIFIED NUMBER (N) OF ANIMALS. ALL OTHER DATA TABULATED AS NUMBER OF ANIMALS (N) WITH THE STATED VALUE. THE TOTAL NUMBER OF ANIMALS FOR SOME PARAMETERS MAY NOT EQUAL THE NUMBER OF ANIMALS IN THE DOSE GROUP
N = NORMAL; + = SLIGHT; ++ = MODERATE; +++ = SEVERE

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 14. Organ and Organ/Body Weights – Main Group

DOSE MKD	ANIMAL NUMBER	FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)	LIVER (G)	LIVER (G/100)
0	4402	492.7	2.556	0.519	12.709	2.579
	4403	514.0	2.795	0.544	12.649	2.461
	4405	394.7#	2.388	0.605	9.901	2.508
	4406	487.2	2.596	0.533	11.743	2.410
	4407	526.0	2.654	0.505	11.748	2.233
	4408	476.0	2.584	0.543	11.470	2.410
	4409	486.1	2.397	0.493	11.302	2.325
	4410	491.6	2.438	0.496	12.215	2.485
	MEAN	483.5	2.551	0.530	11.717	2.427
	S.D.	39.4	0.140	0.036	0.897	0.109
	N=	8	8	8	8	8
50	4421	554.6	3.115	0.562	14.785	2.666
	4422	503.1	2.610	0.519	12.121	2.409
	4423	560.0	2.841	0.507	13.093	2.338
	4424	495.3	2.490	0.503	12.114	2.446
	4425	487.3	2.749	0.564	12.063	2.475
	4426	525.2	3.038	0.578	12.965	2.469
	4427	455.1	2.566	0.564	10.332	2.270
	4428	441.9	2.321	0.525	10.556	2.389
	4429	457.5	2.241	0.490	10.098	2.207
	4430	507.1	2.945	0.581	11.821	2.331
	MEAN	498.7	2.692	0.539	11.995	2.400
	S.D.	40.3	0.297	0.034	1.431	0.127
	N=	10	10	10	10	10

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 14. Organ and Organ/Body Weights – Main Group (continued)

DOSE MKD	ANIMAL NUMBER	FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)	LIVER (G)	LIVER (G/100)
150	4441	483.5	2.521	0.521	12.286	2.541
	4442	475.0	2.364	0.498	10.430	2.196
	4443	487.1	2.387	0.490	11.386	2.338
	4445	503.2	2.648	0.526	11.619	2.309
	4446	434.3	2.103	0.484	10.662	2.455
	4447	529.7	2.653	0.501	13.752	2.596
	4448	466.5	2.682	0.575	11.077	2.374
	4449	459.8	2.427	0.528	10.933	2.378
	4450	369.6	1.969	0.533	8.019	2.170
	MEAN	467.6	2.417	0.517	11.129	2.373
	S.D.	45.5	0.248	0.028	1.539	0.143
	N=	9	9	9	9	9
300	4464	389.5	2.330	0.598	9.832	2.524
	4465	625.6	3.029	0.484	14.823	2.369
	4467	442.0	2.233	0.505	9.764	2.209
	4468	458.7	3.457	0.754	12.075	2.632
	4469	414.2	2.982	0.720	9.926	2.396
	MEAN	466.0	2.806	0.612	11.284	2.426
	S.D.	93.1	0.515	0.122	2.203	0.161
	N=	5	5	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 15. Organ and Organ/Body Weights – Metabolism Group

DOSE MKD	ANIMAL NUMBER	FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)
0	4411	498.8	2.349	0.471
	4412	436.8	2.312	0.529
	4413	469.8	2.506	0.533
	4414	601.6	2.900	0.482
	4415	435.0	2.210	0.508
	MEAN	488.4	2.455	0.505
	S.D.	68.5	0.270	0.028
	N=	5	5	5
50	4431	501.0	2.458	0.491
	4432	449.4	2.662	0.592
	4433	441.1	2.339	0.530
	4434	601.6	3.040	0.505
	4435	427.5	2.467	0.577
	MEAN	484.1	2.593	0.539
	S.D.	71.3	0.275	0.044
	N=	5	5	5
150	4451	548.9	2.689	0.490
	4452	492.2	2.846	0.578
	4453	455.7	2.779	0.610
	4454	454.8	2.402	0.528
	4455	474.4	2.530	0.533
	MEAN	485.2	2.649	0.548
	S.D.	38.8	0.182	0.047
	N=	5	5	5

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 15. Organ and Organ/Body Weights – Metabolism Group (continued)

DOSE MKD	ANIMAL NUMBER	FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)
300	4471	501.2	2.590	0.517
	4472	485.1	3.136	0.646
	4473	402.7	2.160	0.536
	4474	436.5	5.450	1.249#
	4475	464.1	2.872	0.619
	MEAN	457.9	3.242	0.713
	S.D.	39.2	1.286	0.304
	N=	5	5	5

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 16. Organ and Organ/Body Weights – Early Termination

DOSE MKD	ANIMAL NUMBER	FINAL BODY WT. (G)	KIDNEYS (G)	KIDNEYS (G/100)
400	4481	375.6	2.981	0.794
	4483	400.5	4.342	1.084
	4484	334.3	4.546	1.360
	4485	335.0	3.668	1.095
	4486	327.0	3.150	0.963
	4488	324.0	4.047	1.249
	4489	301.9	4.159	1.378
	4490	338.0	4.027	1.191
	4492	482.9#	3.160	0.654
	4493	495.2#	3.026	0.611
	4494	362.7	3.347	0.923
	4495	399.6	4.002	1.002
	4496	336.9	4.916	1.459
	4497	371.5	5.331	1.435
	4498	358.5	4.870	1.358
	4499	340.1	4.764	1.401
	MEAN	367.7	4.021	1.122
	S.D.	54.5	0.746	0.278
	N=	16	16	16

STATISTICAL OUTLIERS INCLUDED.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: AUGUST 2003

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	14	73	0
TEMPERATURE - °C	20	22	18
pH	8.2	8.7	7.9
CONDUCTIVITY, uS/CM	216	222	206
TURBIDITY, NTU	0.11	0.19	0.08
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	97	101	93
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	67	69	63
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	3	8	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	64	68	60
CALCIUM, as $CaCO_3$	66	70	62
CALCIUM, (mg/L)	27	28	25
MAGNESIUM, (mg/L)	7.5	8.0	6.6
FLUORIDE, (mg/L)	0.73	1.18	0.08
Free Chlorine, (mg/L)	0.72	0.95	0.25
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.0	0.4	-0.3

* STANDARD METHODS FOR EXAMINATION OF WATER AND WASTE WATER, 16TH ED. (1985), AMERICAN PUBLIC HEALTH ASSOCIATION AND AMERICAN WATER WORKS; WASHINGTON, D.C.
NTU - NEPHELOMETRIC TURBIDITY UNITS
INTENSITY LEVEL RECORDED AS I THROUGH IV
M - MUSTY
** NO DATA GIVEN
@ INDICATES WATER CORROSIVITY (A POSITIVE VALUE INDICATES WATER WILL DEPOSIT MINERALS, A NEGATIVE VALUE INDICATES WATER WILL TAKE UP MINERALS)

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: September 2003

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	17	170	0
TEMPERATURE - °C	20	22	18
pH	8.1	8.6	7.9
CONDUCTIVITY, uS/CM	218	226	205
TURBIDITY, NTU	0.11	0.20	0.08
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	99	103	94
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	68	72	63
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	2	6	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	66	69	61
CALCIUM, as $CaCO_3$	68	72	62
CALCIUM, (mg/L)	27	29	25
MAGNESIUM, (mg/L)	7.6	8.0	7.3
FLUORIDE, (mg/L)	0.98	1.13	0.77
Free Chlorine, (mg/L)	0.78	1.05	0.45
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.1	0.3	-0.4

* STANDARD METHODS FOR EXAMINATION OF WATER AND WASTE WATER, 16TH ED. (1985), AMERICAN PUBLIC HEALTH ASSOCIATION AND AMERICAN WATER WORKS; WASHINGTON, D.C.
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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: October 2003

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	4	45	0
TEMPERATURE - °C	16	20	13
pH	8.1	8.6	7.9
CONDUCTIVITY, uS/CM	217	221	207
TURBIDITY, NTU	0.10	0.18	0.07
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	100	102	93
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	69	72	63
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	1	8	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	68	71	61
CALCIUM, as $CaCO_3$	69	72	63
CALCIUM, (mg/L)	28	29	25
MAGNESIUM, (mg/L)	7.6	8.3	7.0
FLUORIDE, (mg/L)	1.06	1.20	0.90
Free Chlorine, (mg/L)	0.75	1.00	0.35
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.2	0.3	-0.4

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: November 2003

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	3	15	0
TEMPERATURE - °C	13	15	11
pH	8.2	8.6	8.0
CONDUCTIVITY, uS/CM	219	227	212
TURBIDITY, NTU	0.09	0.13	0.06
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	100	103	97
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	70	72	68
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	2	4	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	68	70	65
CALCIUM, as $CaCO_3$	70	74	67
CALCIUM, (mg/L)	28	30	27
MAGNESIUM, (mg/L)	7.5	8.0	6.8
FLUORIDE, (mg/L)	1.10	1.19	1.03
Free Chlorine, (mg/L)	0.76	0.90	0.40
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.2	0.2	-0.4

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: December 2003

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	3	42	0
TEMPERATURE - °C	10	15	8
pH	8.3	8.6	8.1
CONDUCTIVITY, uS/CM	218	230	213
TURBIDITY, NTU	0.09	0.22	0.06
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	101	107	98
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	71	77	69
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	3	6	2
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	68	71	66
CALCIUM, as $CaCO_3$	70	77	67
CALCIUM, (mg/L)	28	31	27
MAGNESIUM, (mg/L)	7.5	8.0	7.0
FLUORIDE, (mg/L)	1.11	1.28	0.95
Free Chlorine, (mg/L)	0.81	1.00	0.50
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.2	0.3	-0.3

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: JANUARY 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	0	3	0
TEMPERATURE - °C	8	11	6
pH	8.7	8.9	8.6
CONDUCTIVITY, uS/CM	220	230	215
TURBIDITY, NTU	0.10	0.15	0.07
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	102	106	100
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	72	74	70
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	6	10	4
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	66	69	63
CALCIUM, as $CaCO_3$	71	74	68
CALCIUM, (mg/L)	28	30	27
MAGNESIUM, (mg/L)	7.6	8.3	7.3
FLUORIDE, (mg/L)	1.06	1.16	0.91
Free Chlorine, (mg/L)	0.82	0.95	0.55
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.2	0.4	0.1

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: FEBRUARY 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	0	2	0
TEMPERATURE - °C	6	9	4
pH	8.7	8.9	8.1
CONDUCTIVITY, uS/CM	223	239	213
TURBIDITY, NTU	0.09	0.12	0.07
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	103	109	98
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	71	76	67
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	6	8	4
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	65	70	61
CALCIUM, as $CaCO_3$	71	75	65
CALCIUM, (mg/L)	28	30	26
MAGNESIUM, (mg/L)	7.9	8.7	7.3
FLUORIDE, (mg/L)	1.05	1.11	0.97
Free Chlorine, (mg/L)	0.87	1.17	0.40
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.1	0.4	-0.5

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INTENSITY LEVEL RECORDED AS I THROUGH IV
M - MUSTY
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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: MARCH 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	0	2	0
TEMPERATURE - °C	6	9	4
pH	8.6	8.8	8.5
CONDUCTIVITY, uS/CM	237	268	220
TURBIDITY, NTU	0.09	0.30	0.06
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	109	120	102
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	76	82	69
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	5	8	4
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	71	78	61
CALCIUM, as $CaCO_3$	76	86	70
CALCIUM, (mg/L)	30	34	28
MAGNESIUM, (mg/L)	8.2	10.0	7.5
FLUORIDE, (mg/L)	1.10	1.18	1.02
Free Chlorine, (mg/L)	0.85	1.05	0.55
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.2	0.4	0.0

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\# INTENSITY LEVEL RECORDED AS I THROUGH IV
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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: APRIL 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	0	1	0
TEMPERATURE - °C	9	12	6
pH	8.6	8.8	8.4
CONDUCTIVITY, uS/CM	244	280	222
TURBIDITY, NTU	0.09	0.14	0.07
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	112	125	103
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	77	84	71
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	5	8	2
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	72	80	65
CALCIUM, as $CaCO_3$	78	87	72
CALCIUM, (mg/L)	31	35	29
MAGNESIUM, (mg/L)	8.2	9.2	6.8
FLUORIDE, (mg/L)	1.06	1.14	0.99
Free Chlorine, (mg/L)	0.82	1.08	0.54
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.2	0.4	0.0

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: MAY 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	0	0	0
TEMPERATURE - °C	12	15	9
pH	8.6	8.8	8.2
CONDUCTIVITY, uS/CM	234	265	199
TURBIDITY, NTU	0.11	0.90	0.06
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	105	117	91
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	69	83	60
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	5	10	2
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	64	77	55
CALCIUM, as $CaCO_3$	71	85	60
CALCIUM, (mg/L)	28	34	24
MAGNESIUM, (mg/L)	8.2	9.2	7.3
FLUORIDE, (mg/L)	1.10	1.14	1.04
Free Chlorine, (mg/L)	0.79	1.08	0.40
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.1	0.4	-0.2

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: JUNE 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	0	3	0
TEMPERATURE - °C	16	18	11
pH	8.3	8.6	8.1
CONDUCTIVITY, uS/CM	256	285	225
TURBIDITY, NTU	0.10	0.18	0.07
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	111	119	100
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	72	77	67
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	2	6	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	70	75	64
CALCIUM, as $CaCO_3$	75	79	68
CALCIUM, (mg/L)	30	32	27
MAGNESIUM, (mg/L)	8.7	10.0	7.8
FLUORIDE, (mg/L)	1.02	1.17	0.96
Free Chlorine, (mg/L)	0.77	1.11	0.46
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	0.0	0.3	-0.2

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: JULY 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	1	5	0
TEMPERATURE - °C	19	21	16
pH	8.1	8.4	8.0
CONDUCTIVITY, uS/CM	234	251	223
TURBIDITY, NTU	0.11	0.19	0.07
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	104	110	96
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	70	74	66
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	2	4	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	68	73	64
CALCIUM, as $CaCO_3$	71	76	67
CALCIUM, (mg/L)	28	30	27
MAGNESIUM, (mg/L)	8.0	8.7	7.0
FLUORIDE, (mg/L)	0.98	1.03	0.93
Free Chlorine, (mg/L)	0.77	1.08	0.42
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.1	0.1	-0.3

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: August 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	2	10	0
TEMPERATURE - °C	20	21	17
pH	8.1	8.4	8.0
CONDUCTIVITY, uS/CM	222	244	214
TURBIDITY, NTU	0.13	0.44	0.06
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	98	107	68
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	68	73	63
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	2	4	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	66	70	61
CALCIUM, as $CaCO_3$	68	76	64
CALCIUM, (mg/L)	27	30	26
MAGNESIUM, (mg/L)	7.4	8.7	0.2
FLUORIDE, (mg/L)	1.00	1.07	0.93
Free Chlorine, (mg/L)	0.76	1.08	0.37
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.1	0.2	-0.3

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 17. Municipal Water Analysis (continued)

MONTHLY DISTRIBUTION WATER ANALYSIS REPORT*
MIDLAND WATER TREATMENT PLANT

MONTH: September 2004

PARAMETER	RESULTS		
	Average	Maximum	Minimum
TOTAL COLIFORMS	ND	ND	ND
HETEROTROPHIC PLATE COUNT	2	20	0
TEMPERATURE - °C	20	22	17
pH	8.0	8.4	7.9
CONDUCTIVITY, uS/CM	235	257	220
TURBIDITY, NTU	0.13	0.24	0.08
ODOR# AT 60°C	**	**	**
TOTAL HARDNESS, (mg/L) as $CaCO_3$	103	113	97
TOTAL ALKALINITY, (mg/L) as $CaCO_3$	69	76	65
CARBONATE ALKALINITY, (mg/L) as $CaCO_3$	1	4	0
BICARBONATE ALKALINITY, (mg/L) as $CaCO_3$	69	76	61
CALCIUM, as $CaCO_3$	70	78	65
CALCIUM, (mg/L)	28	31	26
MAGNESIUM, (mg/L)	8.1	9.0	7.3
FLUORIDE, (mg/L)	1.01	1.08	0.96
Free Chlorine, (mg/L)	0.64	1.01	0.22
TOTAL IRON, (mg/L)	**	**	**
LANGELIER SATURATION INDEX@	-0.2	0.1	-0.4

* STANDARD METHODS FOR EXAMINATION OF WATER AND WASTE WATER, 16TH ED. (1985), AMERICAN PUBLIC HEALTH ASSOCIATION AND AMERICAN WATER WORKS; WASHINGTON, D.C.
NTU NEPHELOMETRIC TURBIDITY UNITS
INTENSITY LEVEL RECORDED AS I THROUGH IV
M MUSTY
** NO DATA GIVEN
@ INDICATES WATER CORROSIVITY (A POSITIVE VALUE INDICATES WATER WILL DEPOSIT MINERALS, A NEGATIVE VALUE INDICATES WATER WILL TAKE UP MINERALS)

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 18. Analytical Water Analysis

Date Collected 04/01/03 — *Date Reported* 04/22/03

Parameter	Level Detected (ng/mL*)	MCL (mg/L)
Semi-Volatile Compounds		
Pentachlorophenol	<DL2	0.001
Phenol	<DL2	
Volatile Compounds		
Bromodichloromethane	8	
Bromoform	<DL1	
Carbon Tetrachloride	<DL1	0.005
Chloroform	18	
Total Trihalomethanes	26	0.08
Methyl Ethyl Ketone	<DL1	
Toluene	<DL1	1
Tetrachloroethene	<DL1	0.005
Dibromochloromethane	<DL1	
Trichloroethene	<DL1	0.005
Polychlorinated Biphenyls		
Aroclor 1016	<DL5	0.0005
Aroclor 1221	<DL5	0.0005
Aroclor 1232	<DL5	0.0005
Aroclor 1242	<DL5	0.0005
Aroclor 1248	<DL5	0.0005
Aroclor 1254	<DL5	0.0005
Aroclor 1260	<DL5	0.0005
Organophosphate Insecticides		
Diazinon	<DL5	
Disulfoton (Di-Syston)	<DL5	
Ethyl Parathion	<DL5	
Malathion	<DL5	
Methyl Parathion	<DL5	
Chlorophenoxy Herbicides		
Dicamba	<DL4	
2,4-D	<DL2	0.07
2,4,5-T	<DL4	
2,4,5-TP (Silvex)	<DL4	0.05

<DL1 = Below detection limit of 5 ug/Liter
<DL2 = Below detection limit of 1 ug/Liter
<DL3 = Below detection limit of 2 ug/Liter
<DL4 = Below detection limit of 0.25 ug/Liter
<DL5 = Below detection limit of 0.5 ug/Liter
<DL6 = Below detection limit of 2.5 ug/Liter
<DL7 = Below detection limit of 10 ug/Liter

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 18. Analytical Water Analysis (continued)

Date Collected 04/01/03	*Date Reported*	04/22/03
Parameter	**Municipal Water** Cage Drain Valve **Room 290**	**MCL (mg/L)**
Heavy Metals		
Aluminum	0.04 mg/L.	0.2*
Arsenic	<DL2	0.01
Barium	0.02 mg/L.	2
Cadmium	<DL8	0.005
Chromium	<DL9	0.1
Copper	0.04 mg/L.	1.3
Iron	0.13 mg/L.	0.3*
Lead	<DL9	0.015
Managanese	<DL9	0.05
Mercury	<DL2	0.002
Selenium	<DL2	0.05
Silver	<DL9	0.010*
Zinc	0.02 mg/L.	5*
Analyte		
Fluoride	0.10 mg/L.	4
Nitrate *measured as nitrogen*	0.14 mg/L.	10
Sulfate	18 mg/L.	250*

<DL2 = Below detection limit of 1 ug/Liter	<DL11 = Below detection limit of 0.04 mg/Liter
<DL8 = Below detection limit of 0.005 mg/Liter	<DL12 = Below detection limit of 0.1 mg/Liter
<DL9 = Below detection limit of 0.01 mg/Liter	<DL13 = Below detection limit of 0.5 mg/Liter
<DL10 = Below detection limit of 0.02 mg/Liter	<DL14 = Below detection limit of 1mg/Liter

A&L Great Lakes Laboratories, Inc.

Reported by: Keith L. Henley Jr.

Date: 04/22/03

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 18. Analytical Water Analysis (continued)

Date Collected 10/21/03	*Date Reported*	21-Nov-03
Parameter	**Level Detected (ng/mL*)**	**MCL (mg/L)**
Semi-Volatile Compounds		
Pentachlorophenol	<DL2	0.001
Phenol	<DL2	
Volatile Compounds		
Bromodichloromethane	6 ug/L	
Bromoform	<DL1	
Carbon Tetrachloride	<DL1	0.005
Chloroform	12 ug/L	
Total Trihalomethanes	18 ug/L	0.08
Methyl Ethyl Ketone	<DL1	
Toluene	<DL1	1
Tetrachloroethene	<DL1	0.005
Dibromochloromethane	<DL1	
Trichloroethene	<DL1	0.005
Polychlorinated Biphenyls		
Aroclor 1016	<DL5	0.0005
Aroclor 1221	<DL5	0.0005
Aroclor 1232	<DL5	0.0005
Aroclor 1242	<DL5	0.0005
Aroclor 1248	<DL5	0.0005
Aroclor 1254	<DL5	0.0005
Aroclor 1260	<DL5	0.0005
Organophosphate Insecticides		
Diazinon	<DL5	
Disulfoton (Di-Syston)	<DL2	
Ethyl Parathion	<DL5	
Malathion	<DL5	
Methyl Parathion	<DL5	
Chlorophenoxy Herbicides		
Dicamba	<DL4	
2,4-D	<DL2	0.07
2,4,5-T	<DL4	
2,4,5-TP (Silvex)	<DL4	0.05

<DL1 = Below detection limit of 5 ug/Liter	<DL5 = Below detection limit of 0.5 ug/Liter
<DL2 = Below detection limit of 1 ug/Liter	<DL6 = Below detection limit of 2.5 ug/Liter
<DL3 = Below detection limit of 2 ug/Liter	<DL7 = Below detection limit of 10 ug/Liter
<DL4 = Below detection limit of 0.25 ug/Liter	

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 18. Analytical Water Analysis (continued)

Date Collected 10/21/03	*Date Reported*	21-Nov.-2003
Parameter	**Municipal Water Run Lixit Valve Room 163**	**MCL (mg/L)**
Heavy Metals		
Aluminum	<DL9	0.2*
Arsenic	<DL9	0.01
Barium	0.01 mg/L	2
Cadmium	<DL9	0.005
Chromium	<DL9	0.1
Copper	0.130 mg/L	1.3
Iron	0.04 mg/L	0.3*
Lead	<DL9	0.015
Managanese	<DL9	0.05
Mercury	<DL9	0.002
Selenium	<DL9	0.05
Silver	<DL9	0.010*
Zinc	0.020 mg/L	5*
Analyte		
Fluoride	1.1 mg/L	4
Nitrate *measured as nitrogen*	0.33 mg/L	10
Sulfate	<DL14	250*

<DL2 = Below detection limit of 1 ug/Liter	<DL11 = Below detection limit of 0.04 mg/Liter
<DL8 = Below detection limit of 0.005 mg/Liter	<DL12 = Below detection limit of 0.1 mg/Liter
<DL9 = Below detection limit of 0.001 mg/Liter	<DL13 = Below detection limit of 0.5 mg/Liter
<DL10 = Below detection limit of 0.02 mg/Liter	<DL14 = Below detection limit of 1mg/Liter

A&L Great Lakes Laboratories, Inc.

Reported by: Keith L. Henley Jr.

Date: 21-November, 2003

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 18. Analytical Water Analysis (continued)

5/11/2004	*Date Reported*	6/7/2004
Parameter	**Level Detected (ng/mL*)**	**MCL (mg/L)**
Semi-Volatile Compounds		
Pentachlorophenol	<DL2	0.001
Phenol	<DL2	
Volatile Compounds		
Bromodichloromethane	9 ug/L	
Bromoform	<DL1	
Carbon Tetrachloride	<DL1	0.005
Chloroform	44 ug/L	
Total Trihalomethanes	53 ug/L	0.08
Methyl Ethyl Ketone	<DL1	
Toluene	<DL1	1
Tetrachloroethene	<DL1	0.005
Dibromochloromethane	<DL1	
Trichloroethene	<DL1	0.005
Polychlorinated Biphenyls		
Aroclor 1016	<DL5	0.0005
Aroclor 1221	<DL5	0.0005
Aroclor 1232	<DL5	0.0005
Aroclor 1242	<DL5	0.0005
Aroclor 1248	<DL5	0.0005
Aroclor 1254	<DL5	0.0005
Aroclor 1260	<DL5	0.0005
Organophosphate Insecticides		
Diazinon	<DL5	
Disulfoton (Di-Syston)	<DL2	
Ethyl Parathion	<DL5	
Malathion	<DL5	
Methyl Parathion	<DL5	
Chlorophenoxy Herbicides		
Dicamba	<DL4	
2,4-D	<DL2	0.07
2,4,5-T	<DL4	
2,4,5-TP (Silvex)	<DL4	0.05

<DL1 = Below detection limit of 5 ug/Liter	<DL5 = Below detection limit of 0.5 ug/Liter
<DL2 = Below detection limit of 1 ug/Liter	<DL6 = Below detection limit of 2.5 ug/Liter
<DL3 = Below detection limit of 2 ug/Liter	<DL7 = Below detection limit of 10 ug/Liter
<DL4 = Below detection limit of 0.25 ug/Liter	

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 18. Analytical Water Analysis (continued)

5/11/2004	*Date Reported*	6/7/2004
Parameter	**Municipal Water Water Drain Valve Room 155 Chamber H**	**MCL (mg/L)**
Heavy Metals		
Aluminum	0.018mg/L	0.2*
Arsenic	<DL9	0.01
Barium	0.011 mg/L	2
Cadmium	<DL9	0.005
Chromium	0.002 mg/L	0.1
Copper	0.058 mg/L	1.3
Iron	0.545 mg/L	0.3*
Lead	0.001 mg/L	0.015
Managanese	0.002 mg/L	0.05
Mercury	<DL9	0.002
Selenium	<DL9	0.05
Silver	<DL9	0.010*
Zinc	0.037 mg/L	5*
Analyte		
Fluoride	1.2 mg/L	4
Nitrate *measured as nitrogen*	0.40 mg/L	10
Sulfate	18.6 mg/L	250*

<DL2 = Below detection limit of 1 ug/Liter	<DL11 = Below detection limit of 0.04 mg/Liter
<DL8 = Below detection limit of 0.005 mg/Liter	<DL12 = Below detection limit of 0.1 mg/Liter
<DL9 = Below detection limit of 0.01 mg/Liter	<DL13 = Below detection limit of 0.5 mg/Liter
<DL10 = Below detection limit of 0.02 mg/Liter	<DL14 = Below detection limit of 1mg/Liter

A&L Great Lakes Laboratories, Inc.

Reported by: Keith L. Henley Jr.

Date: 2004 June 7

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report

Animal No.: 4402 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 492.7g

Organ Weights:

KIDNEYS	: 2.556g	LIVER	: 12.709g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4403 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 514g

Organ Weights:

KIDNEYS	: 2.785g	LIVER	: 12.649g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4404 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 07/07/2004 Study Day No. (Week): 307 (44) Mode of Death: Moribund - Unscheduled
Date of Necropsy: 07/07/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Moribund

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

SKIN AND SUBCUTIS;
Soiling; periocular; right

SPLEEN;
Increased Size; probable lymphoid tumor

Any remaining protocol required tissues, which have been examined, have no visible lesions

Probable cause of death:

SPLEEN; Increased Size; probable lymphoid tumor

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4405 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 304.7g

Organ Weights:

KIDNEYS	:	2.388g	LIVER	:	9.901g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4406 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 487.2g

Organ Weights:

KIDNEYS : 2.596g LIVER : 11.743g

Gross Pathology Observations:

EYE;
Cloudy; cornea; left

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4407 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 526g

Organ Weights:

KIDNEYS	: 2.654g	LIVER	: 11.748g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4408 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 476g

Organ Weights:

KIDNEYS	: 2.584g	LIVER	: 11.470g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4409 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 486.1g

Organ Weights:

KIDNEYS	: 2.397g	LIVER	: 11.302g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4410 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 491.6g

Organ Weights:

KIDNEYS	: 2.438g	LIVER	: 12.215g

Gross Pathology Observations:

TESTES;
Flaccid; unilateral

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4411 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 498.8g

Organ Weights:

KIDNEYS : 2.349g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4412 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 436.8g

Organ Weights:

KIDNEYS : 2.312g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4413 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 488.8g

Organ Weights:

KIDNEYS : 2.506g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4414 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 601.6g

Organ Weights:

KIDNEYS : 2.900g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4415 Group: 1 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 0 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 435g

Organ Weights:

KIDNEYS : 2.210g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4421 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 554.6g

Organ Weights:

KIDNEYS	: 3.115g	LIVER	: 14.785g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4422 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 503.1g

Organ Weights:

KIDNEYS	: 2.610g	LIVER	: 12.121g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4423 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 560g

Organ Weights:

KIDNEYS	:	2.841g	LIVER	:	13.093g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:,

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4424 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 495.3g

Organ Weights:.

KIDNEYS	: 2.490g	LIVER	: 12.114g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4425 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 487.3g

Organ Weights:

KIDNEYS	: 2.749g	LIVER	: 12.063g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4426 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 525.2g

Organ Weights:

KIDNEYS	: 3.038g	LIVER	: 12.965g

Gross Pathology Observations:

LYMPH NODE;
Dark; mesenteric

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4427 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 455.1g

Organ Weights:

KIDNEYS	: 2.566g	LIVER	: 10.332g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 442B Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 441.9g

Organ Weights:

KIDNEYS	: 2.321g	LIVER	: 10.556g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4429 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 457.5g

Organ Weights:

KIDNEYS	: 2.241g	LIVER	: 10.098g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4430 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 389 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 507.1g

Organ Weights:

KIDNEYS : 2.945g LIVER : 11.821g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4431 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 501g

Organ Weights:

KIDNEYS : 2.458g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4432 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 449.4g

Organ Weights:

KIDNEYS : 2.662g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4433 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 441.1g

Organ Weights:

KIDNEYS : 2.339g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4434 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 601.6g

Organ Weights:

KIDNEYS : 3.040g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4435 Group: 2 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 50 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

GI, Malocculded Incisors
Soiling, Periocular; Red

Palpable Mass Details:

None

Terminal Body Weight: 427.5g

Organ Weights:

KIDNEYS : 2.467g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4441 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 483.5g

Organ Weights:

KIDNEYS : 2.521g LIVER : 12.286g

Gross Pathology Observations:

SKIN AND SUBCUTIS;
Scab; muzzle; right

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4442 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 08/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 475g

Organ Weights:

KIDNEYS	:	2.364g	LIVER	:	10.430g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4443 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 487.1g

Organ Weights:

KIDNEYS	: 2.387g	LIVER	: 11.386g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4444 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 05/28/2004 Study Day No. (Week): 267 (39) Mode of Death: Moribund - Unscheduled
Date of Necropsy: 05/28/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Feces, Abnormal Quantity; Decreased
Skin/Mucous Membranes Pale
Soiling, Perineal; Red

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

LIVER;
Pale

LUNG;
Focus; dark; multifocal

LYMPH NODE;
Increased Size; generalized

SPLEEN;
Increased Size; probable lymphoid tumor

THYMUS;
Increased Size

Any remaining protocol required tissues, which have been examined, have no visible lesions

Probable cause of death:

SPLEEN; Increased Size; probable lymphoid tumor

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4445 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 503.2g

Organ Weights:

KIDNEYS	: 2.648g	LIVER	: 11.819g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4446 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 434.3g

Organ Weights:

KIDNEYS	: 2.103g	LIVER	: 10.662g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4447 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 529.7g

Organ Weights:

KIDNEYS : 2.653g LIVER : 13.752g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4448 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 466.5g

Organ Weights:

KIDNEYS : 2.682g LIVER : 11.077g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4449 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 469.8g

Organ Weights:

KIDNEYS	: 2.427g	LIVER	: 10.933g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4450 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 369.6g

Organ Weights:

KIDNEYS	: 1.969g	LIVER	: 8.019g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4451 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 548.9g

Organ Weights:

KIDNEYS : 2.689g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4452 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 492.2g

Organ Weights:

KIDNEYS : 2.846g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4453 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 455.7g

Organ Weights:

KIDNEYS : 2.779g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4454 Group: 3 Sex: Male Species: Rat Strain: IGS Wister Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 454.8g

Organ Weights:

KIDNEYS : 2.402g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4455 Group: 3 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 150 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 474.4g

Organ Weights:

KIDNEYS : 2.530g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4402 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 01/20/2004 Study Day No. (Week): 138 (20) Mode of Death: Moribund - Unscheduled
Date of Necropsy: 01/20/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Moribund

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; right: Comments: Both kidneys appear slightly swollen, slightly pale and moist on cut surface.

GENERAL;
Ascites: Comments: The abdominal cavity is distended with clear fluid. There is also a moderate perirenal edema of the fat surrounding the kidneys.
Hemolyzed Blood; gastrointestinal tract
Hydrothorax; serosanguineous

URINARY BLADDER;
Calculus: Comments: Small amounts of fine tan-white calculi are seen in the bladder.
Dilatation: Comments: The bladder is distended with about 2--3 ml of a dark red urine.
Hemorrhage; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions
Kidney disease, and probable obstruction of urinary outflow are suspected to be the cause of death.

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4463 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechaniatic
Date of Death : 12/24/2003 Study Day No. (Week): 111 (16) Mode of Death: Spontaneous - Unscheduled
Date of Necropsy: 12/24/2003 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

GENERAL;
Ascites: Comments: The abdomen was filled with approximately 20 mls of clear fluid.

LIVER;
Hernia; hiatal

URINARY BLADDER;
Calculus
Dilatation: Comments; The bladder is markedly distended with cloudy urine. The bladder wall is hemorrhagic. Small amounts of fine to slightly coarse (about 1-2mm) size calculi are seen in the bladder.
Hemorrhage; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions
OBSTRUCTION OF THE URINARY TRACT

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4464 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 08/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 389.5g

Organ Weights:

KIDNEYS	:	2.330g	LIVER	:	9.832g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4465 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 625.6g

Organ Weights:

KIDNEYS	: 3.029g	LIVER	: 14.823g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4467 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 442g

Organ Weights:

KIDNEYS : 2.233g LIVER : 8.764g

Gross Pathology Observations:

TESTES;
Flaccid; unilateral

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4468 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 458.7g

Organ Weights:

KIDNEYS	: 3.457g	LIVER	: 12.075g

Gross Pathology Observations:

KIDNEYS;
Calculus; pelvis; unilateral
Dilatation; pelvis; bilateral
Roughened Surface; bilateral

URINARY BLADDER;
Calculus
Dilatation

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4469 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/07/2004 Study Day No. (Week): 369 (53) Mode of Death: Scheduled Necropsy
Date of Necropsy: 09/07/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 414.2g

Organ Weights:

KIDNEYS	: 2.982g	LIVER	: 9.926g

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; right

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4470 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/29/2004 Study Day No. (Week): 207 (30) Mode of Death: Spontaneous - Unscheduled
Date of Necropsy: 03/29/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

COAGULATING GLAND;
Dark; bilateral

KIDNEYS;
Calculus; pelvis; unilateral
Dilatation; pelvis; bilateral
Pale; bilateral

LUNG;
Congestion; generalized
Edema

SEMINAL VESICLES;
Dark; bilateral

STOMACH;
Erosion - Ulcer; glandular mucosa; multifocal

URINARY BLADDER;
Calculus; multifocal
Dilatation
Hemorrhage; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4470 Group: 4 Sex: Male (continued)

Probable cause of death:

URINARY BLADDER; Hemorrhage; wall

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4471 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 501.2g

Organ Weights:

KIDNEYS : 2.590g

Grose Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4472 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Injury, Apparent Mechanical; Other

Palpable Mass Details:

None

Terminal Body Weight: 485.1g

Organ Weights:

KIDNEYS : 3.136g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4473 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 08/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 402.7g

Organ Weights:

KIDNEYS : 2.160g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4474 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 436.5g

Organ Weights:

KIDNEYS : 5.450g>

Gross Pathology Observations:

KIDNEYS;
Calculus; pelvis; bilateral
Pale; bilateral
Roughened Surface; bilateral

URINARY BLADDER;
Calculus
Dilatation

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4475 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 09/08/2004 Study Day No. (Week): 370 (53) Mode of Death: Scheduled Necropsy - Metabolism
Date of Necropsy: 09/08/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 464.1g

Organ Weights:

KIDNEYS : 2.872g

Gross Pathology Observations:

URINARY BLADDER;
Calculus
Dilatation
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:,

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4478 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 04/04/2004 Study Day No. (Week): 213 (31) Mode of Death: Spontaneous - Unscheduled
Date of Necropsy: 04/05/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; right: Comments: There is moderate perirenal edema, particularly on the left side.

GENERAL;
Ascites

URINARY BLADDER;
Calculus
Dilatation: Comments; The bladder is distended with about 3 ml of a cloudy urine containing green-grey flaky material. A small yellow-tan calculus (about 2mm in diameter) was seen at the neck of the bladder.
Hemorrhage; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions
OBSTRUCTION OF THE URINARY TRACT

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4479 Group: 4 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 300 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 04/12/2004 Study Day No. (Week): 221 (32) Mode of Death: Spontaneous - Unscheduled
Date of Necropsy: 04/12/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; bilateral
Pale; bilatoral

HEART;
Mottled; ventricle; bilateral

SEMINAL VESICLES;
Inflammation; bilateral

SKIN AND SUBCUTIS;
Perineal Soiling

STOMACH;
Erosion - Ulcer; glandular mucosa; multifocal

URINARY BLADDER;
Calculus; COMMENT: Several irregularly shaped calculi, 1 to 2 mm in diameter, were present in the urinary bladder.
Dilatation
Hemorrhage; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4479 Group: 4 Sex: Male (continued)

Obstruction of the urinary tract contributed to the death of this rat.

Codes Used:,

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4481 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 375.6g

Organ Weights:

KIDNEYS : 2.081g

Gross Pathology Observations:

KIDNEYS;
Mottled

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4482 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/09/2004 Study Day No. (Week): 187 (27) Mode of Death: Moribund - Unscheduled
Date of Necropsy: 03/09/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Moribund

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; bilateral: Comments: Both pelves are markedly dilated with urine and contain small amounts of fine, yellow sand-like material particularly, in the left kidney.

URINARY BLADDER;
Dilatation: Comments: The bladder was distended with about 3 ml of cloudy urine with flecks of green material.

Any remaining protocol required tissues, which have been examined, have no visible lesions
OBSTRUCTION OF THE URINARY TRACT

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4483 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

No Abnormalities Detected

Palpable Mass Details:

None

Terminal Body Weight: 400.5g

Organ Weights:

KIDNEYS : 4.342g

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; bilateral
Pale; bilateral
Roughened Surface; bilateral

URINARY BLADDER;
Calculus
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4484 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 334.3g

Organ Weights:

KIDNEYS : 4.540g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

GENERAL;
Decreased Amount Of Fat

LUNG;
Mottled

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4485 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 335g

Organ Weights:

KIDNEYS : 3.668g

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; left
Pale; bilateral
Roughened Surface; bilateral

LYMPH NODE;
Increased Size; renal

URINARY BLADDER;
Calculus
Dilatation
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4486 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (28) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 327g

Organ Weights:

KIDNEYS : 3.150g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4487 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/15/2004 Study Day No. (Week): 193 (28) Mode of Death: Moribund - Unscheduled
Date of Necropsy: 03/15/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

CECUM;
Hemorrhage; wall; multifocal

KIDNEYS;
Calculus; pelvis; bilateral
Dilatation; pelvis; bilateral
Pale; bilateral
Roughened Surface; bilateral

GENERAL;
Decreased Amount Of Fat

STOMACH;
Hemolyzed Blood
Mineralization; glandular mucosa

URINARY BLADDER;
Dilatation
Hemorrhage; wall
Urine - Bloody

Any remaining protocol required tissues, which have been examined, have no visible lesions
The cause of the moribund condition was chronic renal disease and obstruction of the urinary bladder.

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4488 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 324g

Organ Weights:

KIDNEYS : 4.047g

Gross Pathology Observations:

KIDNEYS;
Calculus; pelvis; bilateral
Dilatation; pelvis; bilateral
Pale; bilateral
Roughened Surface; bilateral

LYMPH NODE;
Increased Size; renal

URETER;
Calculus
Dilatation; left

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4489 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 301.9g

Organ Weights:

KIDNEYS : 4.159g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

GENERAL;
Decreased Amount Of Fat

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4490 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 338g

Organ Weights:

KIDNEYS : 4.027g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

GENERAL;
Decreased Amount Of Fat

LUNG;
Mottled

LYMPH NODE;
Increased Size; renal

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4491 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 10/17/2003 Study Day No. (Week): 43 (7) Mode of Death: Spontaneous - Unscheduled
Date of Necropsy: 10/17/2003 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

LUNG;
Congestion; generalized

URINARY BLADDER;
Hemorrhage; wall
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions
Probable cystitis contributed to the death of this rat.

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4492 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 482.9g

Organ Weights:

KIDNEYS : 3.160g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4493 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 495.2g

Organ Weights:

KIDNEYS : 3.026g

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4494 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 362.7g

Organ Weights:

KIDNEYS : 3.347g

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; left
Pale; bilateral
Roughened Surface; bilateral

URINARY BLADDER;
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4495 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 399.6g

Organ Weights:

KIDNEYS : 4.002g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

LYMPH NODE;
Increased Size; renal

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4496 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 336.0g

Organ Weights:

KIDNEYS : 4.916g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

LYMPH NODE;
Increased Size; renal

URINARY BLADDER;
Calculus
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4497 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 371.5g

Organ Weights:

KIDNEYS : 5.331g>

Gross Pathology Observations:

KIDNEYS;
Calculus; pelvis; bilateral
Dilatation; pelvis; bilateral
Pale; bilateral
Roughened Surface; bilateral

LYMPH NODE;
Increased Size; renal

URETER;
Calculus
Dilatation; right

URINARY BLADDER;
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4498 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 358.5g

Organ Weights:

KIDNEYS : 4.870g

Gross Pathology Observations:

KIDNEYS;
Pale; bilateral
Roughened Surface; bilateral

GENERAL;
Decreased Amount Of Fat

LUNG;
Mottled

LYMPH NODE;
Increased Size; renal

URINARY BLADDER;
Calculus
Thickened; wall

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4499 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 03/25/2004 Study Day No. (Week): 203 (29) Mode of Death: Scheduled Necropsy - Early Termination
Date of Necropsy: 03/25/2004 ** NECROPSY COMPLETE **

Terminal Body Weight: 340.1g

Organ Weights:

KIDNEYS : 4.764g

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; right
Pale; bilateral
Roughened Surface; bilateral

LUNG;
Mottled

LYMPH NODE;
Increased Size; renal

Any remaining protocol required tissues, which have been examined, have no visible lesions

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4500 Group: 5 Sex: Male Species: Rat Strain: IGS Wistar Han

Test Material: Ethylene Glycol Dose: 400 mkd Route: Dietary Study Type: Mechanistic
Date of Death : 02/05/2004 Study Day No. (Week): 154 (22) Mode of Death: Spontaneous - Unscheduled
Date of Necropsy: 02/05/2004 ** NECROPSY COMPLETE **

Last Clinical Observations:

Spontaneous Death

Palpable Mass Details:

None

Terminal Body Weight: None

Gross Pathology Observations:

KIDNEYS;
Dilatation; pelvis; bilateral

GENERAL;
Congestion; viscera
Hydrothorax; clear

LACRIMAL/HARDERIAN GLAND;
Increased Size; bilateral

LUNG;
Congestion; generalized
Edema

SEMINAL VESICLES;
Dark; bilateral

TRACHEA;
Froth

URINARY BLADDER;
Calculus; multifocal
Hemorrhage; wall
Urine - Bloody

Any remaining protocol required tissues, which have been examined, have no visible lesions
Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX TABLE 19. Individual Animal Pathology Report (continued)

Animal No.: 4500 Group: 5 Sex: Male (continued)

Probable cause of death:

URINARY BLADDER; Hemorrhage; wall

Codes Used:.

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX A. Pathology Report – Gordon Hard

EXPERT REPORT ON URINARY SYSTEM HISTOPATHOLOGIC CHANGES IN A 12-MONTH DIETARY TOXICITY STUDY OF ETHYLENE GLYCOL IN MALE WISTAR HAN RATS (STUDY NUMBERS: DOW 031079; WIL-186027)

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX A. Pathology Report – Gordon Hard (continued)

AUTHOR STATEMENT CONCERNING AUTHENTICATION

I, the undersigned, hereby declare that the findings of this point-by-point evaluation, conducted by me, of glass histology slides of kidney and urinary bladder from a 12-month toxicity study with Wistar Han rats receiving ethylene glycol in the diet are accurately reflected in the body of this report, which was compiled by me, and which comprises 20 pages, including seven (7) tables and one (1) appendix.

Gordon C Hard
BVSc, PhD, DSc
FRCPath, FRCVS, FAToxSci

Date

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX A. Pathology Report – Gordon Hard (continued)

SUMMARY

Groups of 10 to 15 male Wistar Han rats were administered ethylene glycol in NTP 2000 diet at doses of 0, 50, 150, 300, and 400 mg/kg/d over 12 months duration for histopathological evaluation of the kidneys. Three rats in the group receiving 300 mg/kg/d were unscheduled deaths caused by the test compound, while all rats remaining in the group administered 400 mg/kg/d were terminated before the scheduled date because of the adverse effects of treatment. None of the rats in the two lower dose groups, 50 and 150 mg/kg/d, showed any evidence of the histopathology associated with ethylene glycol administration, namely, basophilic foci of crystalluria-related nephropathy, renal tubule dilatation, birefringent crystals morphologically representative of calcium oxalate, dilatation of the renal pelvis, or transitional cell hyperplasia (the latter in either renal pelvis or bladder). Accordingly, this study confirmed 150 mg/kg/d to be a NOAEL for the chronic administration of ethylene glycol in the Wistar Han rat.

INTRODUCTION

The main objective of the histopathology phase (WIL-186027) of this toxicity study (Dow Study Number 031079) was to evaluate the renal toxicity potential of ethylene glycol when administered in the diet to male Wistar Han rats over a 12-month period, and to determine if possible a no-observable-adverse-effect-level (NOAEL).

MATERIALS AND METHODS

A total of 70 glass histology slides containing kidney sections from 67 rats were shipped from WIL Research Laboratories, Ashland, Ohio, USA and collected by me from the FedEx repository at Auckland Airport on December 5, 2004. The sections of right and left kidneys were mounted on single slides for 64 rats, but on separate slides for 3 rats. The groups and animal numbers received and evaluated are listed in Appendix 1. Both kidneys from each rat had been sectioned and stained with hematoxylin and eosin (H&E), one kidney in the sagittal plane, and the other transversely.

On January 29, 2005, 56 glass histology slides containing H&E-stained urinary bladder tissue from 56 rats, which had also been shipped from WIL Research Laboratories, Ashland, Ohio, were collected by me from the FedEx repository at Auckland Airport.

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APPENDIX A. Pathology Report – Gordon Hard (continued)

The groups and animal numbers for which kidneys and urinary bladders were received and evaluated are listed in Appendix 1. For groups 1 through 4 (0 to 300 mg/kg/d), kidneys from all of the animals in the Main Study designated for histopathology and those for metabolism were histologically examined, including those that were sacrificed moribund, those that died, and those sacrificed at scheduled termination (12 months). The exceptions were animal nos. 4401 (0 mg/kg/d), 4461 and 4466 (300 mg/kg/d), which were transferred to the clearance group to ensure that there was an adequate number of animals. The bladders from the Main study animals designated for histopathology from groups 1 through 4 (0 to 300 mg/kg/d) were examined, as well as those collected because they had gross changes at necropsy. The exceptions were the three animals (nos. 4401, 4461, and 4466) that were transferred to the clearance group as described above. In group 5 (400 mg/kg/d), kidneys and bladders from all Main study animals (designated for histopathology) were examined, the exception being the bladder in animal no. 4489 as this organ was inadvertently not taken at necropsy.

All rat kidney and bladder slides were examined at 203 Paku Drive, Tairua, New Zealand, by conventional brightfield microscopy and polarized light using an Olympus BX 41 microscope with objectives ranging from 4 to 40x magnification. For evaluation of compound-induced lesions by brightfield microscopy, the entire tissue of each H&E-stained kidney or bladder was examined systematically in a precise turret pattern with the aid of a mechanical stage. The same systematic procedure was followed for examination under polarized light to determine the presence or absence of birefringent crystals. A representative sample from the kidney of each rat was also examined with ultraviolet illumination (fluorescence microscopy) at a wavelength of 420–490 nm in order to assess the presence of lysosomes (Maunsbach, 1966) or increased hyaline droplets (Hard and Snowden, 1990) by autofluorescence.

Where applicable, the distribution of histopathologic change in the kidney was categorized by zone according to the description of Young and Wissig (1964) for rat kidney. In this schema, the zones of kidney comprise the cortex or zone 1 (convoluted segments of proximal and distal tubules and glomeruli), outer stripe of outer medulla (OSOM) or zone 2 (predominantly straight segments of proximal tubule), inner stripe of outer medulla (ISOM) or zone 3 (Henle limbs, collecting ducts and vasa recta), inner medulla or zone 4, and papilla or zone 5 (Henle limbs and collecting ducts).

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Criteria established in the previous 16-week study of ethylene glycol for diagnosing and grading the severity of the nephropathy caused by compound administration associated with crystalluria were used as the basis for grading systems in this study (Hard, 2002). Accordingly, crystal nephropathy was graded on a scale of 0 to 5 as follows: 0, no basophilic foci of the type signifying crystal nephropathy; 1, minimal (one to no more than 4 foci of nephropathy in both kidney sections together); 2, mild, (sparsely scattered foci of nephropathy); 3, moderate (frequent foci or early coalescence of foci into areas of nephropathy, but with at least half of the cortex remaining unaffected); 4, marked (diffuse distribution of nephropathy to involve the majority of the parenchyma); 5, end-stage (nephropathy involving all of the kidney indicative of impending renal failure).

Under polarized light, kidneys were graded for the presence of oxalate-like crystals as follows: 0, no crystals present in any location in either kidney; 1, minimal (solitary, small crystals evident, usually only in the fornix of the renal pelvis or in the adjacent urothelial lining); 2, mild (usually no crystals in the cortex but scattered in the papilla and renal pelvis); 3, moderate (occasional crystals in the cortex, but more frequent in the medulla); 4, marked (frequent crystals in all zones of the kidney, including cortex).

Chronic progressive nephropathy (CPN) is a spontaneous, age-related disease of laboratory rats. It is characterized in the early stages by single tubule profiles or focal lesions of cortical tubule basophilia associated with prominent basement membrane thickening, and with hyaline cast formation involving the medullary segments of the same tubule. With progression of the disease, the foci of tubule alteration enlarge and coalesce into areas of affected tubules, ultimately involving all of the kidney bilaterally (Hard et al, 1999; Hard and Khan, 2004). CPN can be graded based on lesion pathogenesis on a scale of 0 to 8 (Hard and Khan, 2004): 0, no lesions; 1 minimal (fewer than 6 basophilic tubule foci and/or hyaline casts in right and left kidney sections together), 2, mild (6 to 15 CPN lesions); 3 to 8, low-moderate to end-stage (progressive development from focal to diffuse distribution to ultimately involve all kidney parenchyma).

Other lesions, including those of an incidental nature, that were encountered in the kidneys or bladder were also graded on a scale of 1 to 4, representing minimal, mild, moderate, and marked grades of severity.

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Bladder lesions were categorized according to Arden and Frith (1991) and Hard et al (1999).

RESULTS

1. Survival.

Considering all rats on study, including those to be set aside for metabolism and clearance assays (a total of 14, 15 or 20 rats in each group), one rat (animal no. 4404) was sacrificed in a moribund state at day 307 of the study in group 1 (0 mg/kg/d). In group 3 (150 mg/kg/d), one rat (animal no. 4444) was sacrificed in a moribund state at study day 267. In group 4 (300 mg/kg/d), there were 5 unscheduled deaths, one a moribund sacrifice (animal no. 4462) at day 138, and 4 natural deaths (animal nos. 4463, 4470, 4478, 4479) at 111, 257, 213, and 213 days on study, respectively. All other rats in these 4 groups were sacrificed at the scheduled termination date of 369 or 370 days.

Because of excessive body weight loss and 4 unscheduled deaths at 43, 154, 187 and 193 days (animal nos. 4491, 4500, 4482, and 4487, respectively), the remaining rats in group 5 (400 mg/kg/d) were subject to early termination at 203 days (29 weeks).

2. Necropsy findings.

The necropsy findings related to ethylene glycol exposure occurred only in the 300 and 400 mg/kg/d dose groups. The most relevant observation was the presence of calculi in the bladder (but also sometimes in renal pelvis or ureter) in 8 of the total 15 rats on study at the 300 mg/kg/d dose, and in 8 of 20 rats at the 400 mg/kg/d dose. Calculus formation in the bladder was usually accompanied by dilatation of the bladder and in the 5 cases of unscheduled death in group 4 (animal nos. 4462, 4463, 4470, 4478, 4479), hemorrhage of the bladder wall, usually with ascites or other systemic edematous change. Animal nos. 4468, 4470 and 4474 from group 4 had calculi in the renal pelvis.

In group 5 exposed to the highest dose of 400 mg/kg/d, almost all rats showed signs of kidney and/or urinary bladder involvement, usually including roughened kidney surface, renal pelvic dilatation, thickened bladder wall, and sometimes calculi in the renal pelvis, ureter, or bladder. Of the 4 unscheduled deaths occurring before early termination of this group, 3 were observed at necropsy to have hemorrhage of the bladder wall.

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3. Kidney histopathology

a. Conventional brightfield microscopy.

Compound-related (crystal) nephropathy. Nephropathy induced by ethylene glycol exposure was observed as foci, radial tracts, or diffuse areas of basophilic tubules in the cortex, and outer and inner medulla. The cytoplasm of basophilic proximal tubules was foamy, finely vacuolated, or rarefied, with an occasional apoptotic cell or mitotic figure, and mild basement membrane thickening. There was minimal to mild mononuclear inflammatory infiltration and fibrosis accompanying the basophilic alteration. Increasing severity of the nephropathy was manifest by coalescence of foci into areas of diffuse change and an association with tubule dilatation, increasing fibrosis, increasing extracellular matrix, minor tubulitis associated with intralumenal neutrophils, dilatation of the renal pelvis, and some transitional cell hyperplasia of the renal pelvis lining. Proximal tubule mineralization was seen in a few advanced cases, but this was not a constant feature. In many kidneys with compound-induced nephropathy, the outlines of crystals could be observed within tubule lumens, or in the renal pelvis, but these were better visualized and scored for severity under polarized light optics. A few rats at the highest dose had either minimal degeneration of the papilla tip, or some pyelitis, both associated with crystal deposition.

The group incidence and severity of compound-induced nephropathy is presented in Tables 1A and 1B, representing all rats assessed for histopathology (1A), or only those surviving to scheduled termination at 12 months (1B). Considering all rats evaluated, in the group receiving 300 mg/kg/d, compound-related nephropathy was observed in 12 of 13 rats in grades of severity ranging from 1 (minimal) to 4 (marked), with the mode occurring at 1 (minimal). In the 400 mg/kg/d group, all 10 rats had compound-related nephropathy ranging from 3 (moderate) to 5 (end-stage), with the mode at 4 (marked). Four rats in this group had end-stage crystal nephropathy.

Additional changes were present in the kidneys of the unscheduled deaths in group 4 (300 mg/kg/d). In animal nos. 4462 and 4470, mild to moderate tubule dilatation, beyond that associated with the degree of crystalluria-related nephropathy present, was prominent throughout the kidneys, and there was also some perivascular or interstitial edema. An increase in mitotic figures in proximal tubules of the OSOM was apparent in all three, this being minimal in 4463 and 4470, but marked in 4462. Minimal tubule mineralization involving proximal tubules was evident in

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b. Polarized light microscopy.

The group incidence and severity of crystal deposition in the kidney is presented in Tables 3A and 3B, representing all rats assessed regardless of time of death (3A), or only those rats surviving to scheduled termination at 12 months (3B). Under polarized light, birefringent, polycrystalline particles arranged in rosette, fan-shaped or sheaf-like patterns, or individually as near-rectangular plates, were observed in 8 of 13 rats receiving 300 mg/kg/d, and in 10 of 10 rats receiving 400 mg/kg/d. Depending on the severity, crystal deposition occurred in the lumens of tubules from the cortex to the papilla, in outpouchings of the pelvis lining, and in the renal pelvis, particularly in the fornices. In severe cases the cortex showed frequent crystal deposition. In less severe cases there was only an occasional crystal in the cortex, but more of a concentration in the papilla. In the least affected cases, small crystals were usually observed only in the fornix of the renal pelvis, or in the adjacent urothelial lining. The polycrystalline rosettes and plates had the typical morphology and multicolored birefringence of oxalate crystals (Khan et al, 1982; Rushton et al, 1981). In group 3, the mode of severity in rats with crystal deposition was 1 (minimal), and in group 4, the mode was 4 (marked).

No birefringent oxalate-like crystals were observed in rats of the control group, or in groups receiving 50 or 150 mg/kg/d.

c. Fluorescence microscopy.

Under ultraviolet illumination, lysosomes could be visualized in some rats as a sprinkling of small autofluorescing droplets in the cytoplasm of proximal convoluted tubules scattered sparsely through the cortex. On a scale of 0 to 4, the grade of frequency of autofluorescing droplets varied from 0 to 1 within each group, with grade 1 being within the upper range of normality. Exposure to ethylene glycol was therefore not associated with an increase in, or accumulation of, hyaline droplets.

4. Urinary bladder histopathology

a. Conventional brightfield microscopy

Alterations related to ethylene glycol exposure in the bladder and/or ureters were observed only in groups receiving 300 and 400 mg/kg/d doses. These lesions included transitional cell hyperplasia, acute inflammation involving infiltration of the bladder wall with polymorphonuclear neutrophil leucocytes, subepithelial and intramuscular hemorrhage, and ulceration or denudation of the epithelial lining. In some cases, denudation may have resulted as an autolytic change. Transitional

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cell hyperplasia was the primary and consistent lesion indicative of ethylene glycol exposure. It was mostly of the diffuse, simple type, but in more severe cases there could also be focal papillary and/or nodular hyperplasia. An accompanying, subtle change was an increase in mast cells in the submucosa.

The severity of the changes associated with ethylene glycol exposure was graded in a system devised by the author according to lesion progression. Thus, grade 1 (minimal) was represented by minimal to mild, usually diffuse, simple hyperplasia, with no other lesions except an increase in mast cells. Grade 2 (mild) involved mild, diffuse, simple hyperplasia with either focal papillary or nodular hyperplasia or with focal acute inflammation. Grade 3 (moderate) showed diffuse simple hyperplasia accompanied by diffuse acute inflammation. Grade 4 (marked) showed simple hyperplasia together with acute inflammation and either multifocal to diffuse submucosal hemorrhage, or ulceration. The group incidence and results of this grading for rats in the Main study designated for histopathology are presented in Table 4A. All of the unscheduled deaths examined in groups 4 (animal nos. 4462, 4463, 4470, 4478, 4479) and 5 (4482, 4487, 4491, 4500), except animal no. 4482, showed bladder changes of severity grade 4, which were considered to be related to the cause of death. The combination of the kidney and bladder changes in animal no. 4482 was considered consistent with the moribund condition of this rat. The histology observations in the bladders of rats that had bladder necropsy findings from the satellite groups not designated for histopathology are summarized in Table 4B. These animals were nos. 4474, 4475, 4478, and 4479 from group 4 (300 mg/kg/d), and nos. 4491, 4494, 4496, 4497, 4498, and 4500 from group5 (400 mg/kg/d).

None of the above lesions were observed in animals in groups 1 (0 mg/kg/d), 2 (50 mg/kg/d), or 3 (150 mg/kg/d), including an absence of any increase in submucosal mast cell numbers. In the one unscheduled death occurring at the 150 mg/kg/d dose, the bladder wall showed marked infiltration with lymphoma cells.

b. Polarized light microscopy

The group incidence and severity grading for the presence of treatment-related birefringent crystals in bladder and/or ureter for rats in the Main study designated for histopathology are presented in Table 5A. Birefringent crystals compatible with calcium oxalate were observed only in groups 4 (300 mg/kg/d) and 5 (400 mg/kg/d). These were intraluminal

and usually situated close to the epithelial lining. In a few cases, the crystals were organized into concentric profiles of peripherally disposed individual aggregates and an empty or sparsely crystalline interior, with the suggestion of a very thin membrane enveloping the structure. When seen, these structures appeared to be related to the calculi diagnosed at necropsy. The polarized light observations in the bladders of rats that had bladder necropsy findings from the satellite groups not designated for histopathology are summarized in Table 56 (see above for the animal nos.).

DISCUSSION

This study achieved its goal of defining a dose response for the histopathological manifestation of renal toxicity for ethylene glycol administered in the diet to Wistar rats over a chronic period of 12 months. A compound-induced nephropathy associated with crystalluria affected the majority of the animals at 300 mg/kg/d, and all of those given the highest dose of 400 mg/kg/d with a severity that led to early termination of this group. In contrast, none of the renal alterations associated with ethylene glycol exposure (basophilic foci of crystalluria-related nephropathy, tubule dilatation, birefringent crystals particularly in the pelvic fornix as a minimal finding, renal pelvic dilatation, or transitional cell hyperplasia) were observed in the group of rats administered 50 or 150 mg/kg/d, establishing the latter dose-level as a NOAEL. In this regard, the 12-month study recapitulated the results from the 16-week study conducted in both Wistar and Fischer 344 male rats, where the dose of 150 mg/kg/d was also an unequivocal NOAEL for both strains (Hard, 2002; Cruzan et al, 2004). Comparison of these two studies also confirms that there is no progressive or cumulative effect of ethylene glycol with increased duration of exposure at this level of exposure. The 16-week study demonstrated that the Wistar rat was more sensitive to the effects of ethylene glycol administration than the Fischer by approximately a factor of 2 (Hard, 2002; Cruzan et al, 2004).

One difference between the subchronic (16-week) and chronic (12-month) studies was the finding at necropsy of calculi, up to 2 mm in diameter, in the bladder, and sometimes in the renal pelvis, at the two highest doses in the 12-month study. Bladder tissue from animals designated for pathology in each group from the latter study was therefore examined, because the cause of death of the 3 animals dying in group 4 (300 mg/kg/d) was unlikely to be related to the extent of the compound-

associated kidney changes, which were less than end-stage in each case. Histological findings in the bladder and/or ureter correlated well with the necropsy observations of calculi. The basic change was simple transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. In animals dying before scheduled termination in groups 4 and 5, the acute inflammation and hemorrhage of bladder wall was a consistent finding in all but one case, and considered to be related to the cause of death. Such severe bladder pathology was often accompanied by a necropsy record of ascites or other edematous change, suggesting that infection via the damaged bladder wall and septicemia may have been the terminal event in these cases. Although cause of death may have been related to the consequences of calculi in the bladder, the most sensitive markers of the adverse effects of ethylene glycol were in the kidney.

Calculus formation as a consequence of ethylene glycol administration is a predictable finding given the chronic duration of exposure. DePass et al (1986) in their 2-year bioassay of ethylene glycol in Fischer 344 rats, reported the presence of oxalate crystals in the urinary bladder by 12 months, and sometimes calculi in the pelvic space, ureters and bladder, often in association with hydronephrosis, by 18 months. The greater sensitivity of the Wistar rat may explain the more rapid development of calculi by 12 months in the present study. In a subchronic study of calcium oxalate crystalluria induced by ethylene glycol in the Sprague-Dawley strain, Khan (1995) described the formation of "ministones" on the surface of the renal papilla after 8 weeks, and referred to the potential for this to lead to stone development. On the basis of the crystalline structures observed in some of the bladders in the current 12-month study with ethylene glycol, it seems likely that the calculi diagnosed at necropsy are not true concretions, which are usually solid, but merely loose organization of crystal clumps into larger aggregates.

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Table 1A. Group Incidence and Severity of Compound-Induced Nephropathy in All Male Wistar Han Rats Assessed for Histopathology

Dose Group (mg/kg/d)	Number of rats assessed	Rats with severity grade:					
		0	1	2	3	4	5
0	14	14	0	0	0	0	0
50	15	15	0	0	0	0	0
150	15	15	0	0	0	0	0
300	13	1	5	2	2	3	0
400	10	0	0	0	1	5	4

Table 1B. Group Incidence and Severity of Compound-Induced Nephropathy in Male Wistar Han Rats Surviving to Scheduled Termination at 12 Months

Dose Group (mg/kg/d)	Number of rats assessed	Rats with severity grade:					
		0	1	2	3	4	5
0	13	13	0	0	0	0	0
50	15	15	0	0	0	0	0
150	14	14	0	0	0	0	0
300	10	1	5	2	0	2	0
400	0	-	-	-	-	-	-

Severity grades:
0 no lesion
1 minimal
2 mild
3 moderate
4 marked
5 end-stage

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Table 2. Group Incidence and Severity of Spontaneous Nephropathy (CPN) in Male Wistar Han Rats

Dose groups (mg/kg/d)	Number of rats assessed	Rats with severity grade: 0	1	2	3	4 - 8
0	14	4	9	1	0	0
50	15	7	8	0	0	0
150	15	3	12	0	0	0
300	8	0	7	1	0	0
400	*	*	*	*	*	*

Severity grades: 0 no lesions
1 minimal
2 mild
3 low-moderate
4-8 mid-moderate to end-stage

* Because of severe compound-induced nephropathy, CPN could not be assessed in 5 rats at the 300 mg/kg/d dose-level, or on a group basis at the 400 mg/kg/d

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Table 3A. Group Incidence and Severity of Birefringent Crystals in Kidneys of All Male Wistar Han Rats Assessed for Histopathology

Dose group (mg/kg/d)	Number of rats assessed	Rats with severity grade: 0	1	2	3	4
0	14	14	0	0	0	0
50	15	15	0	0	0	0
150	15	15	0	0	0	0
300	13	5	4	1	2	1
400	10	0	0	1	4	5

Table 3B. Group Incidence and Severity of Birefringent Crystals in Kidneys of Male Wistar Han Rats Surviving to Scheduled Termination at 12 Months

Dose group (mg/kg/d)	Number of rats assessed	Rats with severity grade: 0	1	2	3	4
0	13	13	0	0	0	0
50	15	15	0	0	0	0
150	14	14	0	0	0	0
300	10	4	3	1	1	1
400	0	-	-	-	-	-

Severity grades: 0 no crystals
1 minimal
2 mild
3 moderate
4 marked

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Table 4A. Incidence and Severity of Compound-Related Changes* in Bladder Tissue of Male Wistar Han Rats from the Main Group Designated for Histopathology

Dose group (mg/kg/d)	No. of rats assessed	Rats with severity grade: 0	1	2	3	4
0	9	0	0	0	0	0
50	10	0	0	0	0	0
150	10	0	0	0	0	0
300	8	2	3	0	0	3
400	9	2	3	2	0	2

Table 4B. Incidence and Severity of Compound-Related Changes* in Bladder Tissue of Male Wistar Han Rats from Satellite Groups, that had Necropsy Findings in Bladder

Dose group (mg/kg/d)	No. of rats affected	Rats with severity grade: 0	1	2	3	4
0	0					
50	0					
150	0					
300	4	0	1	0	1	2
400	6	1	0	2	1	2

* Includes transitional cell hyperplasia, hemorrhage of bladder wall, acute inflammation, and/or ulceration

Severity grades:
- 0 none of the above lesions
- 1 minimal to mild, focal to diffuse, simple hyperplasia only
- 2 mild, diffuse, simple hyperplasia with focal papillary or nodular hyperplasia, and/or with focal acute inflammation
- 3 simple hyperplasia with diffuse acute inflammation
- 4 simple hyperplasia with acute inflammation and multifocal/diffuse hemorrhage or ulceration

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APPENDIX A. Pathology Report – Gordon Hard (continued)

Table 5A. Incidence and Severity of Birefringent Crystals in Bladder Tissue* of Male Wistar Han Rats from the Main Group Designated for Histopathology

Dose group (mg/kg/d)	No. of rats assessed	Rats with severity grade: 0	1	2	3	4
0	9	9	0	0	0	0
50	10	10	0	0	0	0
150	10	10	0	0	0	0
300	8	6	0	0	2	0
400	9	5	0	2	2	0

Table 5B. Incidence and Severity of Birefringent Crystals in Bladder Tissue* of Male Wistar Han Rats from the Satellite Groups, that had Necropsy Findings in Bladder

Dose group (mg/kg/d)	No. of rats affected	Rats with severity grade: 0	1	2	3	4
0	0					
50	0					
150	0					
300	4	0	1	2	0	1
400	6	1	1	2	1	1

* Could include ureter

Severity grades:
0 no crystals
1 minimal
2 mild
3 moderate
4 marked

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX A. Pathology Report – Gordon Hard (continued)

Appendix a. Dose Groups and Animal Numbers for which Kidneys were Received and Examined

Group 1	0 mg/kg/day	4402, 4403, 4404, 4405, 4406, 4407, 4408, 4409, 4410, 4411, 4412, 4413, 4414, 4415.
Group 2	50 mg/kg/day	4421, 4422, 4423, 4424, 4425, 4426, 4427, 4428, 4429, 4430, 4431, 4432, 4433, 4434, 4435.
Group 3	150 mg/kg/day	4441, 4442, 4443, 4444, 4445, 4446, 4447, 4448, 4449, 4450, 4451, 4452, 4453, 4454, 4455.
Group 4	300 mg/kg/day	4462, 4463, 4464, 4465, 4467, 4468, 4469, 4470, 4471, 4472, 4473, 4474, 4475.
Group 5	400 mg/kg/day	4481, 4482, 4483, 4484, 4485, 4486, 4487, 4488, 4489, 4490.

b. Dose Groups and Animal Numbers for which Urinary Bladder Tissues were Received and Examined

Group 1	0 mg/kg/day	4402, 4403, 4404, 4405, 4406, 4407, 4408, 4409, 4410.
Group 2	50 mg/kg/day	4421, 4422, 4423, 4424, 4425, 4426, 4427, 4428, 4429, 4430.
Group 3	150 mg/kg/day	4441, 4442, 4443, 4444, 4445, 4446, 4447, 4448, 4449, 4450.
Group 4	300 mg/kg/day	4462, 4463, 4464, 4465, 4467, 4468, 4469, 4470, 4474, 4475, 4478, 4479.
Group 5	400 mg/kg/day	4481, 4482, 4483, 4484, 4485, 4486, 4487, 4488, 4490, 4491, 4494, 4495, 4497, 4498, 4500.

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ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

APPENDIX A. Pathology Report – Gordon Hard (continued)

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN MALE WISTAR HAN RATS

Histopathological Observations – Summary Table

		MALES				
Kidney	**Dose**	**0**	**50**	**150**	**300**	**400**
Submitted		14	15	15	13	10
No recordable lesions		4	5	3	0	0
Crystal nephropathy		0	0	0	12	10
	Minimal	0	0	0	5	0
	Mild	0	0	0	2	0
	Moderate	0	0	0	2	1
	Marked	0	0	0	3	5
	End-stage	0	0	0	0	4
Birefringent crystals		0	0	0	8	10
	Minimal	0	0	0	4	0
	Mild	0	0	0	1	1
	Moderate	0	0	0	2	4
	Marked	0	0	0	1	5
Spontaneous nephropathy		10	8	12	8	1
	Not assessable	0	0	0	5	9
	Minimal	9	8	12	7	1
	Mild	1	0	0	1	0
	Moderate to end-stage	0	0	0	0	0
Tubule dilatation		0	0	0	8	10
	Minimal	0	0	0	2	0
	Mild	0	0	0	3	1
	Moderate	0	0	0	3	5
	Marked	0	0	0	0	4
Edema, interstitium/perivascular, mild		0	0	0	2	0
Mitoses increased, tubules		0	0	0	4	0
Papillary degeneration, minimal-mild		0	0	0	1	2
Transitional cell hyperplasia		0	0	0	5	9
	Minimal	0	0	0	3	3
	Mild	0	0	0	1	4
	Moderate	0	0	0	0	2
	Marked	0	0	0	1	0
	Marked	0	0	0	0	1
Pelvic dilatation		0	0	0	6	9
	Minimal	0	0	0	0	4
	Mild	0	0	0	3	2
	Moderate	0	0	0	3	2
	Marked	0	0	0	0	1
Pyelitis		0	0	0	0	2
Mononuclear cells, perivascular, minimal		1	0	0	0	0

Mineralization	1	1	0	2	1
Cortical scar, minimal	0	0	1	0	0
Pigment, tubular, minimal	0	1	0	0	0
Lymphoma, secondary infiltration	0	0	1	0	0
Bladder					
Submitted	9	10	10	12	15
No recordable lesions	7	8	8	2	2
Compound-related lesions (excluding crystals)	0	0	0	10	12
Minimal	0	0	0	4	3
Mild	0	0	0	0	4
Moderate	0	0	0	1	1
Marked	0	0	0	5	4
Transitional cell hyperplasia	0	0	0	10	11
Minimal	0	0	0	1	3
Mild	0	0	0	5	4
Moderate	0	0	0	2	3
Marked	0	0	0	2	1
Hemorrhage, focal to diffuse	0	0	0	5	3
Inflammation, acute or mixed, focal to diffuse	0	0	0	6	7
Minimal	0	0	0	3	2
Mild	0	0	0	2	0
Moderate	0	0	0	1	3
Marked	0	0	0	0	2
chronic, focal to multifocal	2	2	1	1	1
Minimal	2	2	1	1	1
Mild-marked	0	0	0	0	0
Ulceration, focal to multifocal	0	0	0	2	5
Birefringent crystals	0	0	0	6	9*
Minimal	0	0	0	1	1
Mild	0	0	0	2	4
Moderate	0	0	0	2	3
Marked	0	0	0	1	1
Lymphoma, secondary infiltration	0	0	1	0	0

* One animal had crystals in the bladder but no bladder wall lesions, and is not included under Compound-related lesions above

Pathologist: Dr Gordon C Hard

ETHYLENE GLYCOL: 12-MONTH DIETARY TOXICITY STUDY IN WISTAR HAN RATS

Study Numbers: Dow 031079
WIL-186027K

INDIVIDUAL ANIMAL DATA PAGES FOR KIDNEY AND BLADDER HISTOPATHOLOGY

Kidney

Grades of severity:

Compound (crystalluria) related nephropathy

1, minimal	(one to no more than 4 basophilic tubule foci or radial tracts in both kidney sections together)
2, mild	(sparsely scattered basophilic tubule foci or tracts)
3, moderate	(frequent foci, or coalescence into areas of nephropathy, but with at least half of the cortex remaining unaffected)
4, marked	(diffuse distribution of nephropathy to involve the greater proportion of parenchyma)
5, end-stage	(nephropathy involving all of the parenchyma)

Spontaneous nephropathy (chronic progressive nephropathy, CPN)

1, minimal	(fewer than 6 basophilic tubule foci and/or hyaline casts in both kidney sections together)
2, mild	(6 to 15 CPN lesions in both kidney sections together)
3, low moderate to 8, end-stage	(progressive development from focal to diffuse distribution of nephropathy to ultimately involve the entire kidney parenchyma)

Crystal deposition (under polarized light)

1, minimal	(solitary, small crystals, usually only in the fornix of the renal pelvis or in the adjacent urothelial lining)
2, mild	(usually no crystals in the cortex but scattered in the papilla and renal pelvis)
3, moderate	(occasional crystals in the cortex, but more frequent in the medulla)
4, marked	(frequent crystals in all zones of the kidney, including cortex)

Incidental lesions
1, minimal
2, mild
3, moderate
4, marked

Bladder
Grades of severity:
Compound-related and incidental lesions
1, minimal
2, mild
3, moderate
4, marked

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4402, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4403, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Bladder Brightfield:
Within normal limits
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4404, Group 1, Dose 0 mg/kg/d, sex male
Moribund (unscheduled) sacrifice, Days on study 307

Kidneys Brightfield:
CPN, minimal
Mononuclear cell infiltration, perivascular, minimal
Mineralization, minimal, medulla
Polarized light:
Negative
Fluorescence:
Normal

Bladder Brightfield:
Inflammation, chronic, focal, minimal
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4405, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4406, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4407, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4408, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4409, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Inflammation, chronic, focal, minimal
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4410, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4411, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 370
Kidneys Brightfield:
Within normal limits
Polarized light:
Negative
Fluorescence:
Normal
Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4412, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 370
Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal
Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4413, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 370
Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal
Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4414, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, mild
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4415, Group 1, Dose 0 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4421, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys Brightfield:
Pigment, proximal tubules, minimal increase
Polarized light:
Negative
Fluorescence:
Normal

Bladder Brightfield:
Within normal limits
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4422, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Bladder Brightfield:
Within normal limits
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4423, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Mineralization, pelvis, mild
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4424, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4425, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4426, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4427, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Inflammation, chronic, focal, minimal
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4428, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Inflammation, chronic, focal, minimal
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4429, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4430, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4431, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4432, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4433, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4434, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4435, Group 2, Dose 50 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4441, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4442, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4443, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Inflammation, chronic, focal, minimal
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4444, Group 3, Dose 150 mg/kg/d, sex male
Moribund (unscheduled) sacrifice, days on study 267

Kidneys	Brightfield:	
		CPN, minimal
		Lymphoma, secondary infiltration, marked
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Lymphoma, secondary infiltration, marked
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4445, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4446, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4447, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4448, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4449, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4450, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		CPN, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4451, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
Cortical scar, minimal
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4452, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4453, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
Within normal limits
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4454, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
Within normal limits
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4455, Group 3, Dose 150 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4462, Group 4, Dose 300 mg/kg/d, sex male
Moribund (unscheduled) sacrifice, days on study 138

Kidneys Brightfield:
- Compound-related nephropathy, moderate
- CPN, not assessable
- Edema, perivascular, mild
- Mineralization, tubular, minimal
- Mitotic figures, proximal tubules, marked increase
- Tubule dilatation, diffuse, mild
- Degeneration, papilla tip, minimal
- Pelvic dilatation, bilateral, mild

Polarized light:
- Negative

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, marked
- Hemorrhage, diffuse
- Inflammation, acute, focal, minimal

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4463, Group 4, Dose 300 mg/kg/d, sex male
Spontaneous (unscheduled) death, days on study 111

Kidneys Brightfield:
- Compound-related nephropathy, moderate
- CPN, not assessable
- Mineralization, tubular, cortex, minimal
- Mitotic figures, proximal tubules, minimal increase
- Pelvic dilatation, unilateral, mild
- Transitional cell hyperplasia, unilateral, mild

Polarized light:
- Crystals, fornix, minimal

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, moderate
- Hemorrhage, diffuse
- Inflammation, acute, diffuse, minimal
- Ulceration, focal

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4464, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys Brightfield:
Compound-related nephropathy, mild
CPN, minimal
Tubule dilatation, inner medulla, minimal
Transitional cell hyperplasia, unilateral, minimal
Polarized light:
Crystals, mainly medulla, mild
Fluorescence:
Normal for cytoplasmic droplets

Bladder Brightfield:
Within normal limits
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4465, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys Brightfield:
Compound-related nephropathy, minimal
CPN, mild
Transitional cell hyperplasia, unilateral, minimal
Polarized light:
Crystals, fornix, minimal
Fluorescence:
Normal for cytoplasmic droplets

Bladder Brightfield:
Transitional cell hyperplasia, focal, mild
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4467, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Compound-related nephropathy, minimal
		CPN, minimal
		Tubule dilatation, cortex medulla, minimal
	Polarized light:	
		Negative
	Fluorescence:	
		Normal for cytoplasmic droplets
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4468, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys	Brightfield:	
		Compound-related nephropathy, marked
		CPN, not assessable
		Tubule dilatation, diffuse, moderate
		Pelvic dilatation, unilateral, moderate
		Transitional cell hyperplasia, unilateral, minimal
	Polarized light:	
		Crystals, cortex to papilla and pelvis, moderate
	Fluorescence:	
		Normal for cytoplasmic droplets
Bladder	Brightfield:	
		Transitional cell hyperplasia, diffuse, mild
	Polarized light:	
		Crystals, moderate

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4469, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 369

Kidneys Brightfield:
- Compound-related nephropathy, mild
- CPN, minimal
- Tubule dilatation, diffuse, mild
- Pelvic dilatation, unilateral, moderate

Polarized light:
- Crystals, urothelial lining of pelvis, minimal

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, mild
- Inflammation, chronic, multifocal, minimal

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4470, Group 4, Dose 300 mg/kg/d, sex male
Spontaneous (unscheduled) death, days on study 207

Kidneys Brightfield:
- Compound-related nephropathy, marked
- CPN, not assessable
- Edema, interstitium, mild
- Mitotic figures, proximal tubules, minimal increase
- Tubule dilatation, diffuse, moderate
- Pelvic dilatation, bilateral, moderate
- Transitional cell hyperplasia, unilateral, marked

Polarized light:
- Crystals, cortex, medulla, pelvis, moderate

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, minimal
- Hemorrhage, diffuse
- Inflammation, acute, multifocal, mild
- Ulceration, multifocal

Polarized light:
- Crystals, moderate

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4471, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
Compound-related nephropathy, minimal
CPN, minimal
Tubule dilatation, inner medulla, mild
Polarized light:
Negative
Fluorescence:
Normal for cytoplasmic droplets

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4472, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
CPN, minimal
Polarized light:
Negative
Fluorescence:
Normal for cytoplasmic droplets

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4473, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
- Compound-related nephropathy, minimal
- CPN, minimal

Polarized light:
- Negative

Fluorescence:
- Normal for cytoplasmic droplets

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4474, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
- Compound-related nephropathy, marked
- CPN, not assessable
- Mitotic figures, proximal tubules, minimal increase
- Tubule dilatation, diffuse, moderate
- Pelvic dilatation, bilateral, mild

Polarized light:
- Crystals, cortex, medulla, pelvis, marked

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, mild

Polarized light:
- Crystals, mild

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4475, Group 4, Dose 300 mg/kg/d, sex male
Scheduled sacrifice, days on study 370

Kidneys Brightfield:
Compound-related nephropathy, minimal
CPN, minimal
Polarized light:
Crystals, fornix, minimal
Fluorescence:
Normal for cytoplasmic droplets

Bladder Brightfield:
Transitional cell hyperplasia, diffuse, marked
Inflammation, acute, multifocal, mild
Polarized light:
Crystals, marked

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4478, Group 4, Dose 300 mg/kg/d, sex male
Spontaneous (unscheduled) death, days on study 213

Bladder Brightfield:
Transitional cell hyperplasia, diffuse, mild
Hemorrhage, multifocal
Inflammation, acute, diffuse, moderate
Polarized light:
Crystals, minimal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4479, Group 4, Dose 300 mg/kg/d, sex male
Spontaneous (unscheduled) death, days on study 221

Bladder Brightfield:
Transitional cell hyperplasia, diffuse, moderate
Hemorrhage, diffuse
Inflammation, acute, multifocal, minimal
Polarized light:
Crystals, mild

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4481, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys Brightfield:
- Compound-related nephropathy, moderate
- CPN, minimal
- Tubule dilatation, diffuse, mild
- Pelvic dilatation, unilateral minimal
- Transitional cell hyperplasia, bilateral, mild

Polarized light:
- Crystals, medulla, urothelial lining, fornix, mild

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Within normal limits

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4482, Group 5, Dose 400 mg/kg/d, sex male
Moribund (unscheduled) sacrifice, days on study 187

Kidneys — Brightfield:
- Compound-related nephropathy, marked
- CPN, not assessable
- Tubule dilatation, diffuse, moderate
- Pelvic dilatation, bilateral, moderate
- Transitional cell hyperplasia, unilateral, mild

Polarized light:
- Crystals, cortex to papilla, moderate

Fluorescence:
- Normal for cytoplasmic droplets

Bladder — Brightfield:
- Transitional cell hyperplasia, diffuse, mild
- Inflammation, acute/chronic, focal, moderate

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4483, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys	Brightfield:	
		Compound-related nephropathy, marked
		CPN, not assessable
		Mineralization, proximal tubules, unilateral, moderate
		Tubule dilatation, diffuse, moderate
		Pelvic dilatation, bilateral, moderate
	Polarized light:	
		Crystals, cortex to papilla and pelvis, moderate
	Fluorescence:	
		Normal for cytoplasmic droplets
Bladder	Brightfield:	
		Transitional cell hyperplasia, diffuse, mild
	Polarized light:	
		Crystals, moderate

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4484, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys	Brightfield:	
		Compound-related nephropathy, end-stage
		CPN, not assessable
		Tubule dilatation, diffuse, marked
		Transitional cell hyperplasia, bilateral, mild
	Polarized light:	
		Crystals, cortex, medulla, pelvis, marked
	Fluorescence:	
		Normal for cytoplasmic droplets
Bladder	Brightfield:	
		Transitional cell hyperplasia, focal, minimal
	Polarized light:	
		Crystals, mild

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4485, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys Brightfield:
- Compound-related nephropathy, marked
- CPN, not assessable
- Tubule dilatation, diffuse, moderate
- Pelvic dilatation, bilateral, mild
- Pyelitis, bilateral, marked
- Transitional cell hyperplasia, bilateral, moderate

Polarized light:
- Crystals, cortex to papilla, moderate

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, marked
- Inflammation, acute/chronic, multifocal, moderate
- Ulceration, focal

Polarized light:
- Crystals, moderate

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4486, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys	Brightfield:	
		Compound-related nephropathy, marked
		CPN, not assessable
		Tubule dilatation, diffuse, moderate
		Pelvic dilatation, unilateral, minimal
		Transitional cell hyperplasia, bilateral, minimal
	Polarized light:	
		Crystals, cortex to papilla, fornix, moderate
	Fluorescence:	
		Normal for cytoplasmic droplets
Bladder	Brightfield:	
		Within normal limits
	Polarized light:	
		Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4487, Group 5, Dose 400 mg/kg/d, sex male
Moribund (unscheduled) sacrifice, days on study 193

Kidneys — Brightfield:
- Compound-related nephropathy, end-stage
- CPN, not assessable
- Tubule dilatation, diffuse, marked
- Pelvic dilatation, bilateral, marked
- Degeneration, papilla tip, mild
- Transitional cell hyperplasia, unilateral, mild

Polarized light:
- Crystals, cortex, medulla, pelvis, marked

Fluorescence:
- Normal for cytoplasmic droplets

Bladder — Brightfield:
- Hemorrhage, multifocal
- Inflammation, acute, diffuse, marked
- Ulceration, focal

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4488, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys Brightfield:
Compound-related nephropathy, marked
CPN, not assessable
Tubule dilatation, diffuse, moderate
Pelvic dilatation, bilateral, mild,
Pyelitis, unilateral, mild
Transitional cell hyperplasia, unilateral, moderate
Polarized light:
Crystals, cortex, medulla, pelvis, marked
Fluorescence:
Normal for cytoplasmic droplets

Bladder Brightfield:
Transitional cell hyperplasia, multifocal, mild
Polarized light:
Negative

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4489, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys Brightfield:
- Compound-related nephropathy, end-stage
- CPN, not assessable
- Tubule dilatation, diffuse, marked
- Pelvic dilatation, unilateral, minimal
- Degeneration, papilla tip, minimal
- Transitional cell hyperplasia, bilateral, minimal

Polarized light:
- Crystals, cortex, medulla, pelvis, marked

Fluorescence:
- Normal for cytoplasmic droplets

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4490, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203

Kidneys Brightfield:
- Compound-related nephropathy, end-stage
- CPN, not assessable
- Tubule dilatation, diffuse, marked
- Pelvic dilatation, unilateral, minimal
- Transitional cell hyperplasia, bilateral, minimal

Polarized light:
- Crystals, cortex, medulla, pelvis, marked

Fluorescence:
- Normal for cytoplasmic droplets

Bladder Brightfield:
- Transitional cell hyperplasia, focal, minimal

Polarized light:
- Crystals, mild

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4491, Group 5, Dose 400 mg/kg/d, sex male
Spontaneous (unscheduled) death, days on study 43
Bladder Brightfield:
Transitional cell hyperplasia, multifocal, minimal
Hemorrhage, diffuse
Inflammation, acute, diffuse, minimal
Ulceration, focal
Polarized light:
Crystals, minimal

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4494, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203
Bladder Brightfield:
Transitional cell hyperplasia, diffuse, moderate
Inflammation, acute, multifocal, minimal
Polarized light:
Crystals, moderate

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4496, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203
Bladder Brightfield:
Within normal limits (tissue sampling limited)
Polarized light:
Crystals, mild
Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4497, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203
Bladder Brightfield:
Transitional cell hyperplasia, multifocal, mild
Inflammation, acute/chronic, focal, marked
Ulceration, focal
Polarized light:
Crystals, marked

Pathologist: Dr Gordon C Hard

Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4498, Group 5, Dose 400 mg/kg/d, sex male
Early termination sacrifice, days on study 203
Bladder Brightfield:
Transitional cell hyperplasia, diffuse, moderate
Inflammation, chronic, focal, minimal
Polarized light:
Crystals, mild

Pathologist: Dr Gordon C Hard

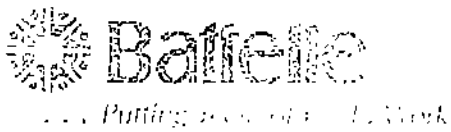
Ethylene glycol: 12-month dietary toxicity study in Wistar Han rats
Study numbers: Dow 031079, WIL-186027K
Histopathology individual animal data record
Animal no. 4500, Group 5, Dose 400 mg/kg/d, sex male
Spontaneous (unscheduled) death, days on study 154

Bladder Brightfield:
- Transitional cell hyperplasia, diffuse, moderate
- Hemorrhage, diffuse
- Inflammation, acute, multifocal, moderate
- Ulceration, multifocal

Polarized light:
- Negative

Pathologist: Dr Gordon C Hard

APPENDIX B. Metabolism Report – Rick Corley



Pacific Northwest National Laboratory
Operated by Battelle for the U.S. Department of Energy

Biological Monitoring and Modeling

AMENDED FINAL REPORT

Battelle Project No. 29812
ACC No. EG-50.0-Battelle

May 20, 2005

Concentrations of Ethylene Glycol, Glycolic Acid and Oxalic Acid in the Blood, Urine and Kidneys of Male Wistar Han Rats Following Dietary Administration of Ethylene Glycol for up to Twelve Months (Dow Study ID 031079)

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PURPOSE OF AMENDMENT

This amendment clarifies a statement made in the abstract of the original final report that previously stated:

"The clearance of EG into urine followed a linear dose-response relationship between 50 and 150 mg/kg/d..."

with the following statement:

"The clearance of EG into urine followed a linear dose-response relationship across all dose levels,..."

which more completely reflects the data and the discussion of the results. This amended report replaces the previous final report dated January 28, 2005.

The amended report was further revised on May 20, 2005 to reference the pathology report of Hard (2005) as a final, rather than a draft report.

Richard A. Corley, May 20, 2005
Richard A. Corley, Ph.D.
Principal Investigator

ABSTRACT

This report summarizes the results from the analysis of blood, urine and kidney samples collected from a toxicokinetic satellite group of male Wistar rats exposed for up to 12 months to 0, 50, 150, 300 or 400 mg/kg/day ethylene glycol via the diet at The Dow Chemical Company (Dow Study ID 031079). The animals from the 400 mg/kg/d group were sacrificed early due to excessive toxicity. Each sample was analyzed at Battelle Northwest (BNW) for ethylene glycol (EG), glycolic acid (GA), and oxalic acid (OX). In addition to the samples from the toxicokinetic satellite group, a section of kidney from each animal from the 400 mg/kg/d group that was sacrificed early (day 203) and all main study animals necropsied after 12 months were also submitted for analysis of EG, GA and OX. The presence of a contaminant in the derivatization agent used for the analysis of EG in all samples except urine, which was analyzed directly, prevented the accurate quantitation of EG. Thus, for EG, only the urine data are reported. The clearance of EG into urine followed a linear dose-response relationship across all dose levels, while non-linearities were observed in the clearance of glycolic acid between 150 and 300 mg/kg/d. In the kidneys, there were also clear non-linear increases in the concentrations of GA and OX at dose levels above 150 mg/kg/d. In fact, OX concentrations, when expressed as calcium oxalate, accounted for an average of 2.9% of the total kidney weight (with one animal approaching 11.2%) in the animals exposed to 400 mg/kg/d and sacrificed early in the study. The dose-response relationships for EG, GA and OX in blood, urine and kidneys of animals exposed for up to 12 months to EG in the diets and the resulting NOEL for renal toxicity were consistent with the previous data and the NOEL for renal toxicity observed in the subchronic toxicity study of Cruzan et al. (2004) and the accepted mode of action that renal toxicity is due to a buildup in calcium oxalate crystals.

INTRODUCTION

Purpose. The objectives of the ethylene glycol (EG) chronic toxicity study conducted at The Dow Chemical Company (Dow Study ID 031079; Midland, MI) were to: (1) evaluate the renal toxicity potential of EG when administered to male Wistar Han rats for 12 months via the diet; (2) investigate the toxicokinetics and disposition of EG in male Wistar Han rats by determining the levels of EG and its metabolites, glycolic acid (GA) and oxalic acid (OA), in the blood, kidneys and urine from a satellite group of rats exposed to EG for 12 months via the diet; (3) compare the strain and age-dependence of OA clearance in male F-344 versus male Wistar Han rats; and (4) investigate the impact of chronic (12-months) dietary administration of EG on the clearance kinetics of OA in male Wistar Han rats. This report presents the results from the analyses of EG, GA and OX in blood, urine and kidneys from the toxicokinetic satellite group of rats (Objective 2). In addition, sections of kidneys from all main study animals (Objective 1) were also analyzed for the metabolites, GA and OX.

Study Design. Groups of 20 male Wistar Han rats were fed diets supplying 0, 50, 150, 300, or 400 mg ethylene glycol/kg body weight/day for up to 12 months. Ten animals per group were considered as main group animals and were used to evaluate the potential for renal toxicity (Objective 1). Five animals per group were pre-selected as a satellite group for analysis of EG, GA and OX in blood, kidneys and urine, with samples shipped to Battelle, Pacific Northwest Division (BNW), Richland, Washington (Objective 2). The remaining five animals per group were pre-selected as a satellite group for determination of oxalate clearance following 12 months of dietary administration of EG (Objective 4).

For the satellite pharmacokinetic group of animals, urine was collected from each animal for 24 hr prior to sacrifice after 12 months of dietary administration of EG. Following the collection of urine, each metabolism cage was rinsed with a minimal amount of water for analysis of EG, GA and OX with the results to be included with urine as total amounts excreted by this route. In addition to the samples collected from the pharmacokinetic satellite group (Objective 2), sections of kidneys from all main study animals that survived until the 12-month necropsy (Objective 1) and all animals from the top dose group, 400 mg/kg/day, that were sacrificed early (day 203) were collected for analysis of EG, GA, and OX. All samples were flash-frozen in liquid nitrogen and shipped on dry ice from The Dow Chemical Company to BNW. Samples were received at BNW on September 21, 2004 and stored frozen (-80°C) until analyzed. Previous studies have shown that samples prepared and stored in this manner remain viable for analysis of EG, GA and OX for up to 542 days (Corley et al., 2002). The samples submitted to BNW are summarized in Appendix Table A-1.

MATERIALS AND METHODS

Test Materials and Chemicals. Ethylene glycol (Lot No. JR00244CR) and glycolic acid (Lot No. 16802LR) were obtained from the Aldrich Chemical Company (Milwaukee, WI). Oxalic acid (Lot No. 123H1122) was obtained from Sigma (St. Louis, MO). Deuterated internal standards D2-glycolic acid (Lot No. I1-5086), D4-ethylene glycol (Lot No. P-6136) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA) while the internal standard, 2-butoxyethanol (Lot No. 07847HN) was obtained from the Aldrich Chemical Company. Derivatizing reagents, pentafluorobenzoyl chloride (PFBCl) and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamaide

(MTBSTFA) were also obtained from the Aldrich Chemical Company. All other compounds and solvents were reagent grade or better.

Specimen Analysis. Samples of heparinized whole blood, kidneys, urine and cage wash were analyzed for EG, GA and OX by gas chromatography/mass spectrometry (GC/MS) following the general extraction and derivatization methods of Pottenger et al. (2001). 2-Butoxyethanol and deuterated ethylene glycol and glycolic acid were utilized as internal standards. Kidneys were first homogenized directly (no diluent) then analyzed by the method used for analysis of blood. For urine samples containing very high concentrations of ethylene glycol, a direct analysis of urine by GC/FID was also conducted using 2-butoxyethanol as an internal standard (Corley et al., 2002). Each of these methods is described briefly below.

GC/MS analyses of ethylene glycol, glycolic acid and oxalic acid were performed on a Hewlett Packard 7683 Mass Selective Detector equipped with a Hewlett Packard 6890 Plus gas chromatograph and 7673 autosampler (Hewlett Packard, Avondale, PA). Separations were achieved with a Restek RTX-5MS fused silica capillary column (30 m x 0.25 mmid, 0.25 μm film thickness; Restek, Bellefonte, PA). Injections were splitless using an unpacked Restek 4 mmid cyclo double gooseneck liner. Representative chromatography conditions for glycolic acid and oxalic acid were as follows: injector temperature was 210°C, the initial oven temperature was 110°C, which was increased at 15°C/min to 200°C, with a final ramp of 25°C/min to 300°C; initial head pressure was a constant 25 psi with helium as the carrier gas. For ethylene glycol, the injection temperature was 210°C, the initial oven temperature was 130°C, which was increased at 15°C/min to 200°C, with a final ramp of 25°C/min to 300°C. Head pressure was constant at 25 psi with helium as a carrier gas. The masses used for quantitation of the pentafluorobenzoyl ester derivatives of ethylene glycol were 238 or 450

(depending upon column conditions); 241 or 454 for D4-ethylene glycol. The masses used for the quantitation of the t-butyldimethylsilyl derivatives of glycolic acid, D2-glycolic acid and oxalic acid were 247, 249 and 261, respectively.

GC/FID analyses of ethylene glycol were performed on a Hewlett Packard 6890 gas chromatograph equipped with an FID detector and 7673 autosampler. Separations were achieved with a J&W DB-Wax fused silica column (30 m x 0.53 mmid x 1.0 df; J&W Scientific, Folsom, CA). For direct injection of urine, injections of 1.0 µl (splitless) of urine spiked with 2-butoxyethanol internal standard (9090 µg/g) were injected at 275°C with a head pressure of 10 psi (helium). The initial oven temperature was 100°C, increasing to 230°C at 20°C/min. A Restek 4 mmid cyclo-double gooseneck injection liner was also used.

Statistics and data analysis. Descriptive statistics (i.e. means ± SD) were used where applicable to present the data. In some instances, only one or two samples within a group had levels of analytes above the limits of reliable quantitation (LOQ). In these cases, the LOQ/2 was arbitrarily used as a surrogate to calculate the mean ± SD for plotting. Individual data are presented in the Appendix tables.

RESULTS AND DISCUSSION

Kidneys. Terminal body weights, kidney weights and the samples submitted to BNW for analysis of EG, GA and OX are summarized in Appendix Table A-1. In Figure 1, the absolute and relative (%body weight) kidney weights from all animals from the main study and the PK satellite group submitted to BNW show a clear relationship of increasing kidney weight with dose, especially relative to body weight, at dose levels above 150 mg/kg/d. No statistical analyses were conducted on these data as the chronic toxicity study (Dow

Study ID 031079), provides more definitive conclusions regarding treatment-related target tissue effects.

The levels of GA (Figure 2a; Table 1) and OX (Figure 3a; Table 1) in the kidneys of these animals also demonstrated similar dose-response relationships. At dose levels up to 150 mg/kg/d, there were no differences in the concentrations of GA and OX, compared with controls. Concentrations generally averaged <2 µg/g and <20 µg/g for GA and OX, respectively at these lower dose levels. However, at dose levels of 300 and 400 mg/kg/d, both GA and OX were increased in a dose-related manner. Concentrations at 400 mg/kg/d reached an average of 14 µg/g and 18,800 µg/g for GA and OX, respectively, with some animals having considerably higher concentrations of each metabolite than average. These results were consistent with previous dose-response relationships observed in male Wistar rats administered EG in the diets for 1 or 16 weeks (Cruzan et al., 2004) which are presented in Figures 2b and 3b. In those studies, there were also no differences from control in GA and OX levels in the kidneys of male Wistar rats at 150 mg/kg/d following 1 or 16 weeks of exposure to EG. The individual animal results from the analysis of GA and OX in kidney samples are presented in Appendix Table A-2.

Interference in EG Analysis. Due to the presence of a contaminant in the derivatization reagent, PFBCl, BNW was unable to complete the analysis of EG in kidneys, blood and cage wash samples. Analysis of EG in urine was successful because this method did not involve derivatization. Alternative sources for PFBCl were obtained and evaluated. Unfortunately, the contaminant, which could not be differentiated by either electron-impact or negative chemical ionization mass spectrometry from the authentic pentafluorobenzoyl-derivative of EG, was also present in the alternative sources at high enough levels to interfere with the analysis of EG at the

concentrations expected in this study (i.e. concentrations were expected to be <50 µg/g in blood and kidneys based upon results from the subchronic study of Cruzan et al., 2004).

Blood. As with the results from the kidneys, the concentrations of GA in blood were not significantly different from controls up to 150 mg/kg/d (Figure 4a; Table 2). At 300 mg/kg/d, the concentrations in blood were approximately 3.3-fold higher than controls although the concentrations were all <10 µg/g regardless of dose level. Again, these results were consistent with those from the subchronic study of Cruzan et al. (2004) which are presented in Figure 4b for comparison.

The concentrations of oxalic acid in blood (Figure 5a; Table 2) were also similar across all dose levels, averaging 3.7-5.1 µg/g. These results were expected from the low solubility of oxalic acid at physiological pH's in aqueous media (~4.2-7.4 µg/g in deionized water or neutral urine; Burgess and Drasdo, 1993; Hodgkinson, 1981) and data from the subchronic study of Cruzan et al. (2004) which are presented in Figure 5b for comparison. Individual animal results from the analysis of GA and OX in blood of rats from the chronic study are presented in Appendix Table A-3.

Urine. The elimination of EG in urine followed a linear, dose-related relationship (Figure 6a; Table 3). These results represent a slight under-estimate of the total amounts of EG cleared in urine because of the inability to quantitate EG in cage wash samples due to a contaminant in the derivatization reagent, PFBCl. A linear increase in urinary clearance of GA was observed at 50 and 150 mg/kg/d while a disproportionate (non-linear) increase was observed at the 300 mg/kg/d dose level (Figure 6b; Table 3). Oxalic acid clearances were similar to controls across all dose levels (Figure 6c; Table 3). As with blood and kidney data, these results were consistent

with those observed in the subchronic study of Cruzan et al. (2004). Individual animal results are presented in Appendix Table A-4.

CONCLUSIONS

All of the results reported in this study are consistent with the generally accepted mode of action of EG-induced renal toxicity (deposition of calcium oxalate crystals in the kidneys) and the determination by Hard (2005) that 150 mg/kg/d represents a NOEL for chronic toxicity of EG administered orally via the diet to male Wistar rats. In the kidneys, there were clear non-linearities in the concentrations of GA and, more importantly, OX, as concentrations of these metabolites significantly increased over control levels at 300 and 400 mg/kg/d. In fact, OX concentrations, when expressed as calcium oxalate, accounted for an average of 2.9% of the total kidney weight (with one animal approaching 11.2%) in the animals exposed to 400 mg/kg/d and sacrificed early (day 203) in the study. The dose-response relationships for EG, GA and OX in blood, urine and kidneys of animals exposed for up to 12 months to EG in the diets and the resulting NOEL for renal toxicity were consistent with the previous data and the NOEL for renal toxicity observed in the subchronic toxicity study of Cruzan et al. (2004).

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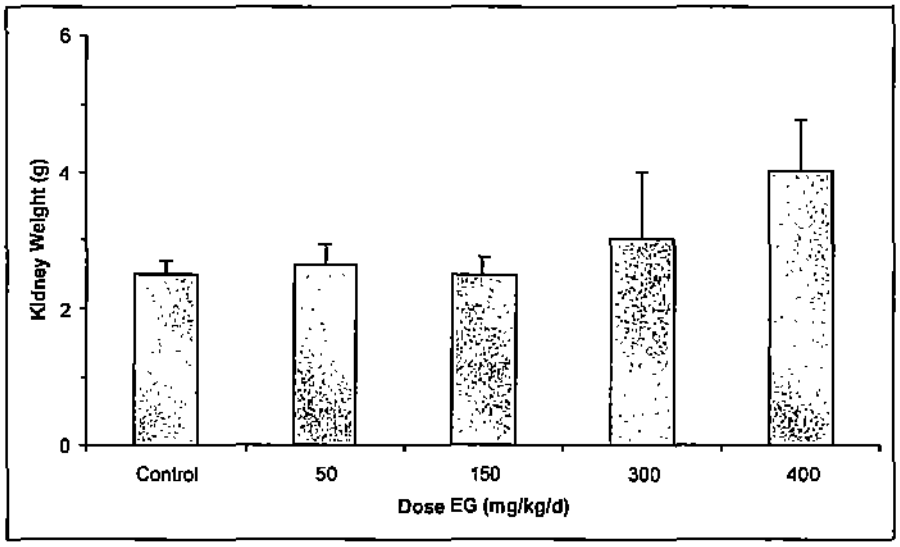
Pottenger, L.H., Carney, E.W. and Bartels, M.J. (2001). Dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites in pregnant (GD10) and nonpregnant Sprague-Dawley rats following oral administration of ethylene glycol. Toxicol. Sci. **62,** 10-19.

Figure 1. Absolute and relative kidney weights (%body weight) of all main study and PK satellite group animals where kidneys were submitted to BNW for analysis of EG, GA and OX (data presented in Appendix Table A-1).

(a) Absolute Kidney Weights



(b) Relative Kidney Weights

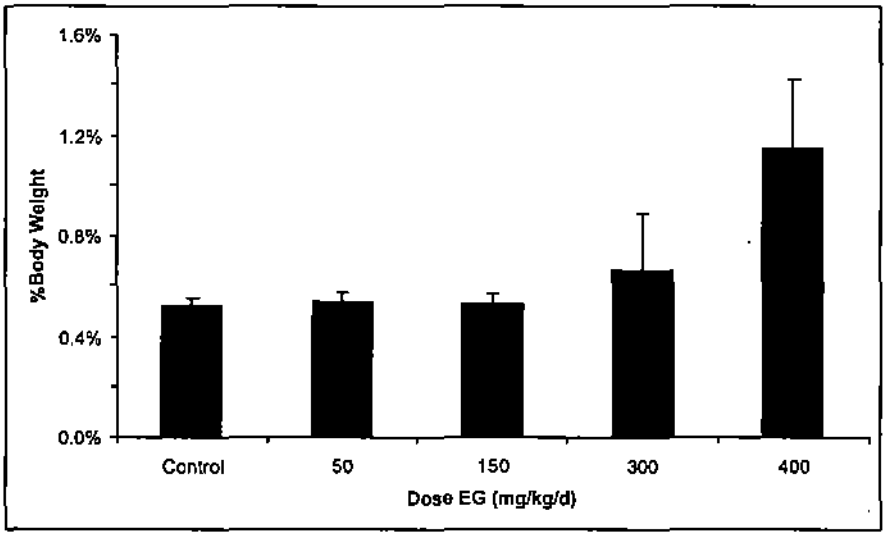
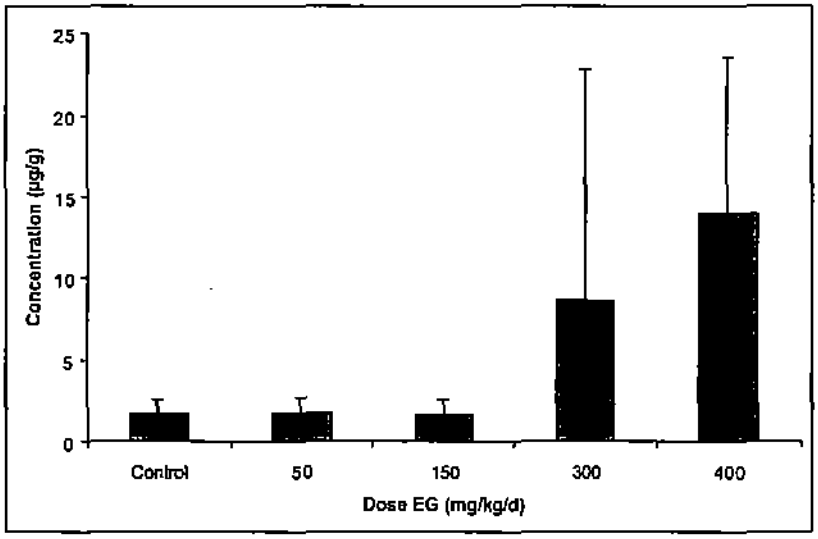


Figure 2. Concentrations of glycolic acid in the kidneys of (a) male Wistar rats administered ethylene glycol at 0, 50, 150 or 300 mg/kg/day for up to 12 months (this study) and (b) male Wistar rats administered 0, 150, 500 or 1000 mg/kg/day for 1 or 16 weeks (from Cruzan et al., 2004). Data are expressed as the means ± standard deviations of up to 5 rats/group.

(a) 12-Month Study



(b) Subchronic Study of Cruzan et al. (2004) — Note Different Scales

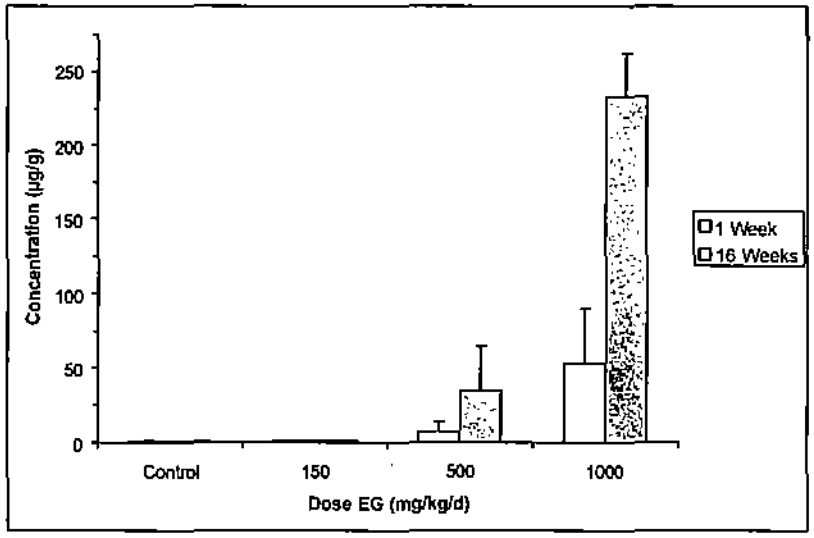
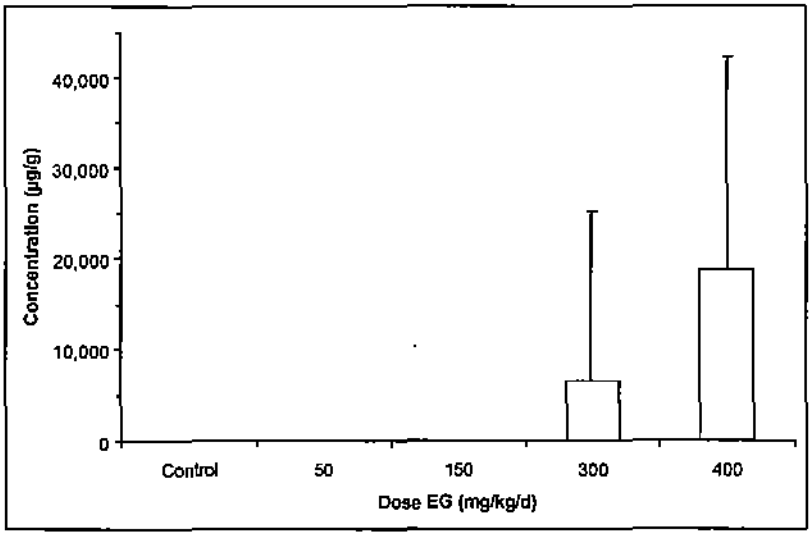


Figure 3. Concentrations of oxalic acid in the kidneys of (a) male Wstar rats administered ethylene glycol at 0, 50, 150 or 300 mg/kg/day for up to 12 months (this study) and (b) male Wistar rats administered 0, 150, 500 or 1000 mg/kg/day for 1 or 16 weeks (from Cruzan et al., 2004). Data are expressed as the means ± standard deviations of up to 5 rats/group.

(a) 12-Month Study



(b) Subchronic Study of Cruzan et al. (2004) — Note Different Scales

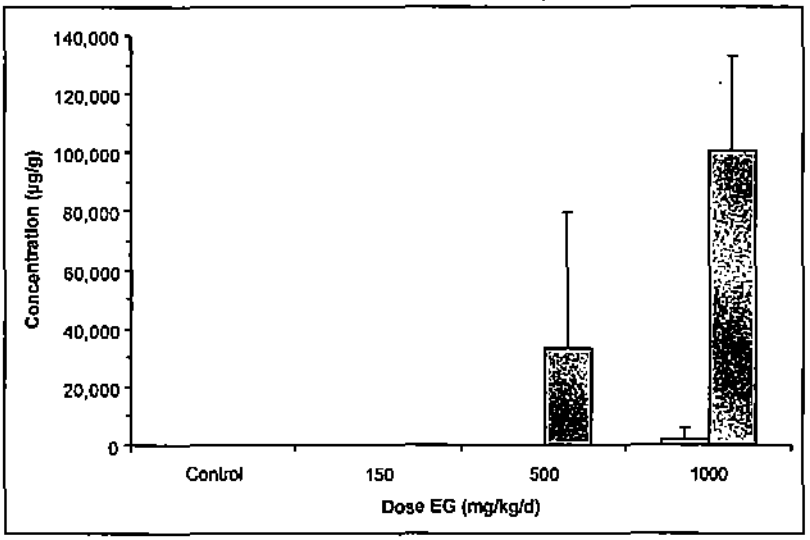
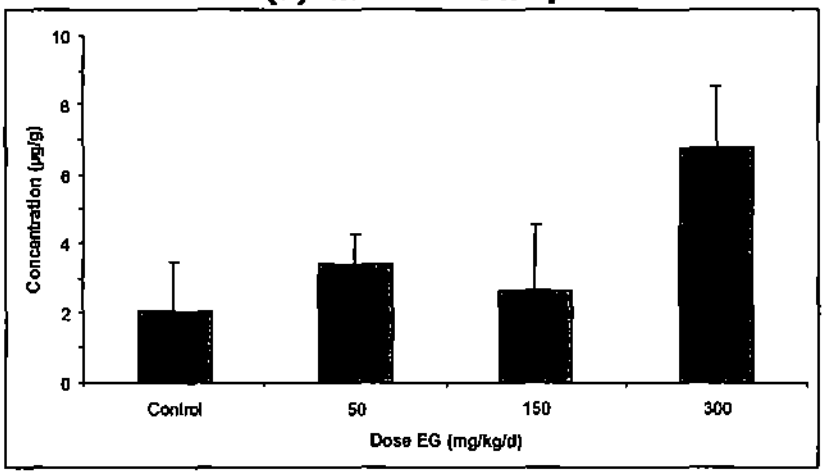


Figure 4. Concentrations of glycolic acid in the blood of (a) male Wistar rats administered ethylene glycol at 0, 50, 150 or 300 mg/kg/day for up to 12 months (this study) and (b) male Wistar rats administered 0, 150, 500 or 1000 mg/kg/day for 1 or 16 weeks (from Cruzan et al., 2004). Data are expressed as the means ± standard deviations of up to 5 rats/group.

(a) 12-Month Study



(b) Subchronic Study of Cruzan et al. (2004) — Note Different Scales

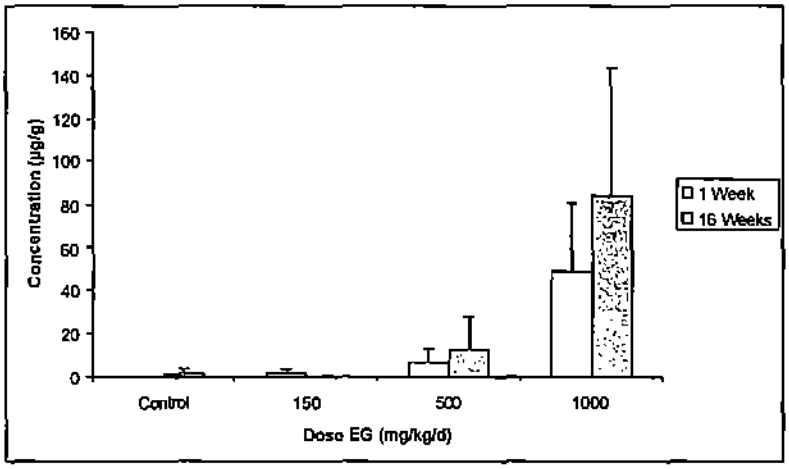
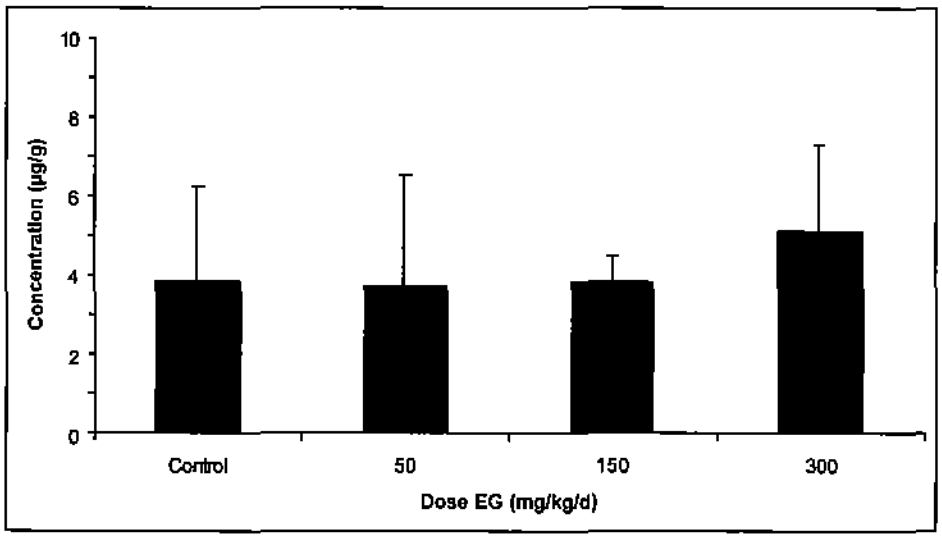


Figure 5. Concentrations of oxalic acid in the blood of (a) male Wistar rats administered ethylene glycol at 0, 50, 150 or 300 mg/kg/day for up to 12 months (this study) and (b) male Wistar rats administered 0, 150, 500 or 1000 mg/kg/day for 1 or 16 weeks (from Cruzan et al., 2004). Data are expressed as the means ± standard deviations of up to 5 rats/group.

(a) 12-Month Study



(b) Subchronic Study of Cruzan et al. (2004) — Note Different Scales

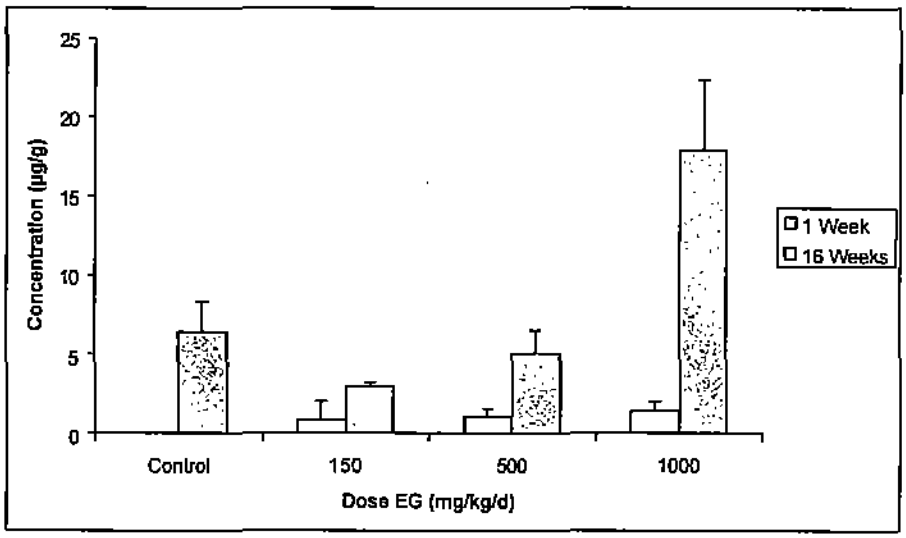


Figure 6. Total amounts of (a) ethylene glycol, (b) glycolic acid, and (c) oxalic acid excreted in the urine and cage wash samples from male Wistar rats collected for 24 hr prior to necropsy following 12 months of dietary exposure to ethylene glycol at 0, 50, 150 or 300 mg/kg/d. Data are expressed as the means ± standard deviations of 5 rats/group. Note that due to a contaminant in the derivatization reagent, the concentrations of ethylene glycol were not determined in the cage wash samples.

(a) Ethylene Glycol

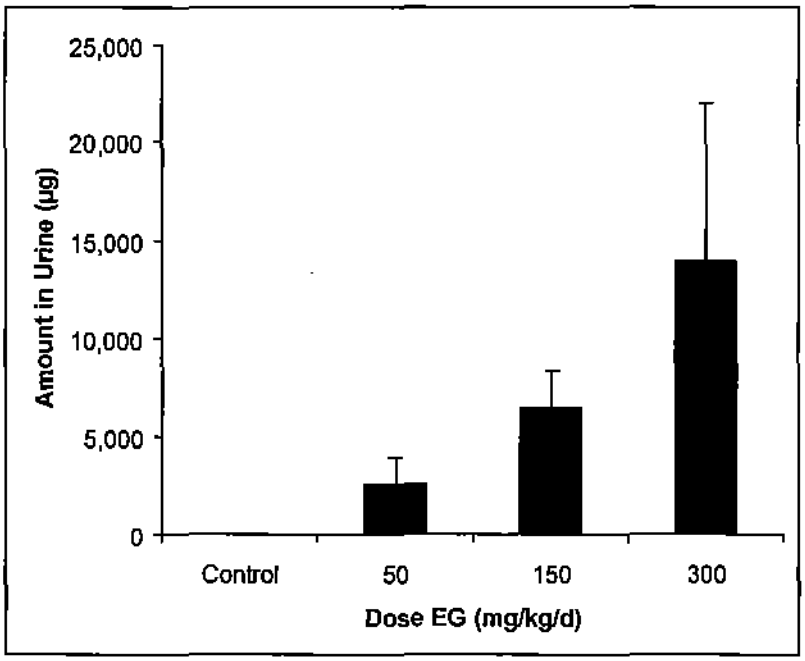
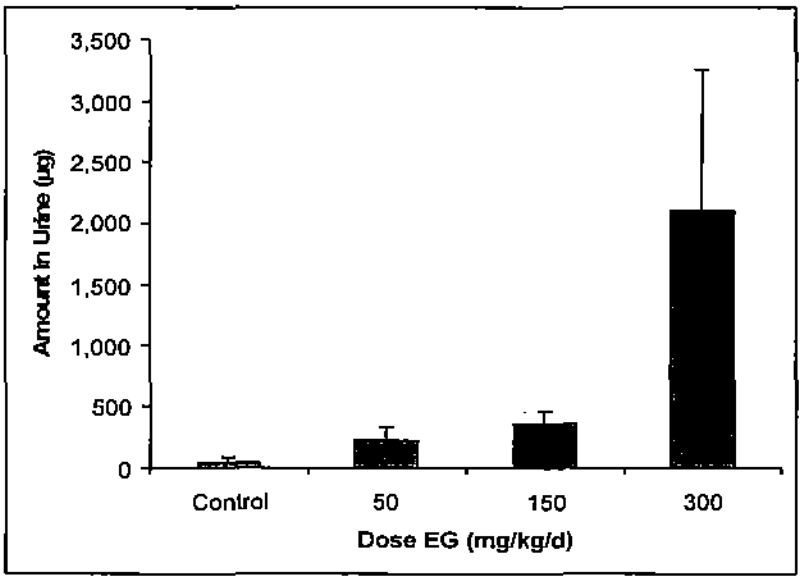


Figure 6 (continued).

(b) Glycolic Acid



(c) Oxalic Acid

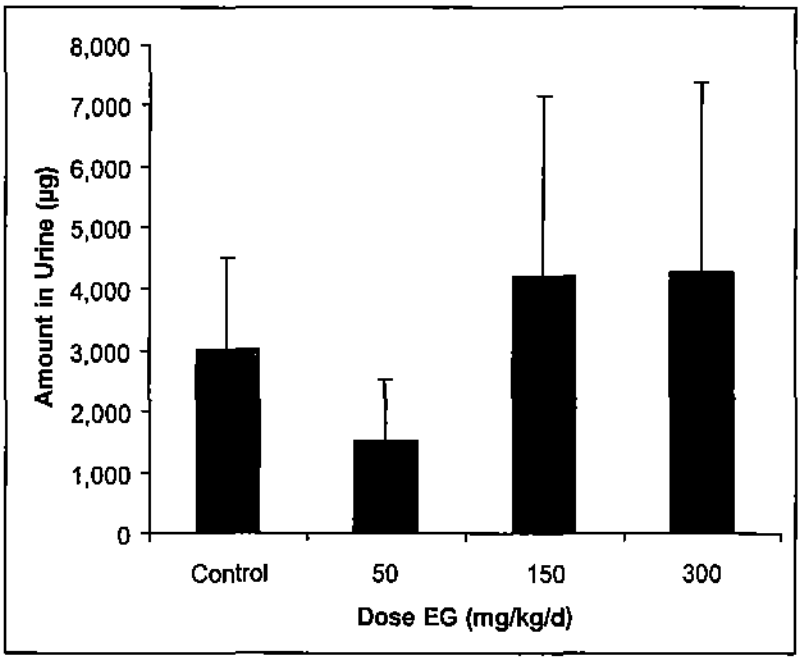


Table 1. Concentrations (mean ± s.d.) of GA and OX in the kidneys of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	GA (µg/g)	OX (µg/g)
Control (n=13)	1.72 ± 0.85	5.31 ± 4.22
50 (n=15)	1.79 ± 0.97	16.07 ± 35.03
150 (n=14)	1.67 ± 0.95	8.72 ± 7.33
300 (n=10)	8.64 ± 14.11	6,561 ± 18,644
400 (n=15)[1]	13.97 ± 9.54	18,789 ± 23,446

[1] Early sacrifice (day 203).

Table 2. Concentrations (mean ± s.d., n=5) of GA and OX in the blood of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	GA (µg/g)	OX (µg/g)
Control	2.06 ± 1.38	3.87 ± 2.35
50	3.42 ± 0.87	3.74 ± 2.80
150	2.67 ± 1.89	3.83 ± 0.65
300	6.78 ± 1.75	5.10 ± 2.18

Table 3. Total amounts (mean ± s.d.) of EG, GA and OX eliminated in the urine + cage wash collected 24 hr prior to sacrifice of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	EG (µg)	GA (µg)	OX (µg)
Control	nd[1]	52.0 ±40.9	3,015 ±1486
50	2,576 ±1,375	231.5 ±112.0	1,519 ± 989
150	6,469 ± 1892	358.9 ± 105.9	4,211 ± 2,964
300	13,945 ± 8,021	2,100 ± 1,160	4,274 ± 3,111

[1] One control urine had detectable amounts of EG, while no EG was detected in all other samples.

APPENDIX

INDIVIDUAL ANIMAL DATA

Table A-1. Terminal body weights, kidney weights, and samples submitted to Battelle Northwest (BNW) from male Wistar Han rats administered ethylene glycol in the diets for up to 12 months for analysis of EG, GA and OX.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	Terminal BW (g)	Kidney Wt. (g)	Samples Submitted to BNW		
					Blood	Kidney	Urine + Cage Wash
Control	Main Study	4402	492.7	2.556		X	
Control	Main Study	4403	514.0	2.795		X	
Control	Main Study	4405	394.7	2.388		X	
Control	Main Study	4406	487.2	2.596		X	
Control	Main Study	4407	526.0	2.654		X	
Control	Main Study	4408	476.0	2.584		X	
Control	Main Study	4409	486.1	2.397		X	
Control	Main Study	4410	491.6	2.438		X	
Control	PK	4411	498.8	2.349	X	X	X
Control	PK	4412	436.8	2.312	X	X	X
Control	PK	4413	469.8	2.506	X	X	X
Control	PK	4414	601.6	2.900	X	X	X
Control	PK	4415	435.0	2.210	X	X	X
Mean			485.4	2.514			
SD			49.8	0.195			
50	Main Study	4421	554.6	3.115		X	
50	Main Study	4422	503.1	2.610		X	
50	Main Study	4423	560.0	2.841		X	
50	Main Study	4424	495.3	2.490		X	
50	Main Study	4425	487.3	2.749		X	
50	Main Study	4426	525.2	3.038		X	
50	Main Study	4427	455.1	2.566		X	

Table A-1 (continued). Terminal body weights, kidney weights, and samples submitted to Battelle Northwest (BNW) from male Wistar Han rats administered ethylene glycol in the diets for up to 12 months for analysis of EG, GA and OX.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	Terminal BW (g)	Kidney Wt. (g)	Samples Submitted to BNW		
					Blood	Kidney	Urine + Cage Wash
50	Main Study	4428	441.9	2.321		X	
50	Main Study	4429	457.5	2.241		X	
50	Main Study	4430	507.1	2.945		X	
50	PK	4431	501.0	2.458	X	X	X
50	PK	4432	449.4	2.662	X	X	X
50	PK	4433	441.1	2.339	X	X	X
50	PK	4434	601.6	3.040	X	X	X
50	PK	4435	427.5	2.467	X	X	X
Mean			493.8	2.659			
SD			50.5	0.284			
150	Main Study	4441	483.5	2.521		X	
150	Main Study	4442	475.0	2.364		X	
150	Main Study	4443	487.1	2.387		X	
150	Main Study	4445	503.2	2.648		X	
150	Main Study	4446	434.3	2.103		X	
150	Main Study	4447	529.7	2.653		X	
150	Main Study	4448	466.5	2.682		X	
150	Main Study	4449	459.8	2.427		X	
150	Main Study	4450	369.6	1.969		X	
150	PK	4451	548.9	2.689	X	X	X
150	PK	4452	492.2	2.846	X	X	X
150	PK	4453	455.7	2.779	X	X	X

Table A-1 (continued). Terminal body weights, kidney weights, and samples submitted to Battelle Northwest (BNW) from male Wistar Han rats administered ethylene glycol in the diets for up to 12 months for analysis of EG, GA and OX.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	Terminal BW (g)	Kidney Wt. (g)	Samples Submitted to BNW		
					Blood	Kidney	Urine + Cage Wash
150	PK	4454	454.8	2.402	X	X	X
150	PK	4455	474.4	2.530	X	X	X
Mean			473.9	2.500			
SD			42.6	0.248			
300	Main Study	4464	389.5	2.330		X	
300	Main Study	4465	625.6	3.029		X	
300	Main Study	4467	442.0	2.233		X	
300	Main Study	4468	458.7	3.457		X	
300	Main Study	4469	414.2	2.982		X	
300	PK	4471	501.2	2.590	X	X	X
300	PK	4472	485.1	3.136	X	X	X
300	PK	4473	402.7	2.160	X	X	X
300	PK	4474	436.5	5.450	X	X	X
300	PK	4475	464.1	2.872	X	X	X
Mean			462.0	3.024			
SD			67.5	0.952			
400	Early Sac[1]	4481	375.6	2.981		X	
400	Early Sac	4483	400.5	4.342		X	
400	Early Sac	4484	334.3	4.546		X	

[1]Early sacrifice (day 203).

Table A-1 (continued). Terminal body weights, kidney weights, and samples submitted to Battelle Northwest (BNW) from male Wistar Han rats administered ethylene glycol in the diets for up to 12 months for analysis of EG, GA and OX.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	Terminal BW (g)	Kidney Wt. (g)	Samples Submitted to BNW		
					Blood	Kidney	Urine + Cage Wash
400	Early Sac	4485	335.0	3.668		X	
400	Early Sac	4486	327.0	3.150		X	
400	Early Sac	4488	324.0	4.047		X	
400	Early Sac	4489	301.9	4.159		X	
400	Early Sac	4490	338.0	4.027		X	
400	Early Sac	4492	482.9	3.160		X	
400	Early Sac	4493	495.2	3.026		X	
400	Early Sac	4494	362.7	3.347		X	
400	Early Sac	4495	399.6	4.002		X	
400	Early Sac	4496	336.9	4.916		X	
400	Early Sac	4497	371.5	5.331		X	
400	Early Sac	4498	358.5	4.870		X	
400	Early Sac	4499	340.1	4.764		X	
Mean			367.7	4.021			
SD			54.5	0.746			

(1)Early sacrifice (day 203).

Table A-2. Concentrations of EG, GA and OX in the kidneys of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	EG[1] (µg/g)	GA (µg/g)	OX (µg/g)
Control	Main Study	4402	14570-11-01	-	2.46	17.72
Control	Main Study	4403	14570-11-02	-	2.11	6.84
Control	Main Study	4405	14570-11-04	-	2.62	5.87
Control	Main Study	4406	14570-11-05	-	2.38	6.10
Control	Main Study	4407	14570-11-06	-	3.71	4.61
Control	Main Study	4408	14570-11-07	-	1.13[2]	5.71
Control	Main Study	4409	14570-11-08	-	1.13[2]	5.53
Control	Main Study	4410	14570-11-09	-	1.13[2]	3.23
Control	PK	4411	14570-11-10	-	1.13[2]	3.37
Control	PK	4412	14570-11-11	-	1.13[2]	0.94
Control	PK	4413	14570-11-12	-	1.13[2]	1.69
Control	PK	4414	14570-11-13	-	1.13[2]	1.47
Control	PK	4415	14570-11-14	-	1.13[2]	6.01
Mean				-	1.72	5.31
SD				-	0.85	4.22
50	Main Study	4421	14570-11-15	-	2.96	5.88
50	Main Study	4422	14570-11-16	-	2.54	5.24
50	Main Study	4423	14570-11-17	-	4.33	4.71
50	Main Study	4424	14570-11-18	-	2.33	6.10

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG.
[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for GA = 2.26 µg/g; LOQ for OX = 2.21 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-2 (continued). Concentrations of EG, GA and OX in the kidneys of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	EG[1] (µg/g)	GA (µg/g)	OX (µg/g)
50	Main Study	4425	14570-11-19	-	1.28	28.46
50	Main Study	4426	14570-11-20	-	1.73	12.74
50	Main Study	4427	14570-11-21	-	2.69	7.82
50	Main Study	4428	14570-11-22	-	1.15	7.84
50	Main Study	4429	14570-11-23	-	1.13[2]	2.22
50	Main Study	4430	14570-11-24	-	1.13[2]	2.40
50	PK	4431	14570-11-25	-	1.13[2]	2.33
50	PK	4432	14570-11-26	-	1.13[2]	2.64
50	PK	4433	14570-11-27	-	1.13[2]	3.16
50	PK	4434	14570-11-28	-	1.13[2]	140.43
50	PK	4435	14570-11-29	-	1.13[2]	9.14
Mean				-	1.79	16.07
SD				-	0.97	35.03
150	Main Study	4441	14570-11-30	-	1.38	9.64
150	Main Study	4442	14570-11-31	-	4.40	19.35
150	Main Study	4443	14570-11-32	-	2.86	8.04
150	Main Study	4445	14570-11-34	-	1.81	8.44
150	Main Study	4446	14570-11-35	-	2.27	6.39
150	Main Study	4447	14570-11-36	-	1.57	2.77
150	Main Study	4448	14570-11-37	-	1.13[2]	3.62
150	Main Study	4449	14570-11-38	-	1.13[2]	4.75

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for GA = 2.26 µg/g; LOQ for OX = 2.21 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-2 (continued). Concentrations of EG, GA and OX in the kidneys of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	EG[1] (µg/g)	GA (µg/g)	OX (µg/g)
150	Main Study	4450	14570-11-39	-	1.13[2]	3.94
150	PK	4451	14570-11-40	-	1.13[2]	5.66
150	PK	4452	14570-11-41	-	1.13[2]	3.17
150	PK	4453	14570-11-42	-	1.13[2]	6.84
150	PK	4454	14570-11-43	-	1.13[2]	9.97
150	PK	4455	14570-11-44	-	1.13[2]	29.55
Mean				-	1.67	8.72
SD				-	0.95	7.33
300	Main Study	4464	14570-11-45	-	2.42	48.07
300	Main Study	4465	14570-11-46	-	1.62	16.68
300	Main Study	4467	14570-11-47	-	1.81	29.88
300	Main Study	4468	14570-11-48	-	8.95	3,377.08
300	Main Study	4469	14570-11-49	-	5.54	1,525.35
300	PK	4471	14570-11-51	-	2.70	74.05
300	PK	4472	14570-11-52	-	2.26	21.64
300	PK	4473	14570-11-53	-	12.04	47.32
300	PK	4474	14570-11-54	-	47.49	59,532.20
300	PK	4475	14570-11-55	-	1.58	937.23
Mean				-	8.64	6,560.95
SD				-	14.11	18,643.57

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for GA = 2.26 µg/g; LOQ for OX = 2.21 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-2 (continued). Concentrations of EG, GA and OX in the kidneys of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	EG[1] (µg/g)	GA (µg/g)	OX (µg/g)
Mean (excluding Animal 4474)				-	4.32	675.26
SD (excluding Animal 4474)				-	3.78	1146.61
400	Early Sac	4483	14570-11-58	-	12.09	2,436.28
400	Early Sac	4484	14570-11-59	-	14.89	11,369.28
400	Early Sac	4485	14570-11-60	-	7.65	2,532.01
400	Early Sac	4486	14570-11-61	-	6.71	3,777.47
400	Early Sac	4488	14570-11-63	-	36.14	5,407.48
400	Early Sac	4489	14570-11-64	-	16.95	9,365.73
400	Early Sac	4490	14570-11-65	-	28.16	16,585.72
400	Early Sac	4492	14570-11-66	-	1.13	1,064.50
400	Early Sac	4493	14570-11-67	-	1.13	234.98
400	Early Sac	4494	14570-11-68	-	9.75	5,661.70
400	Early Sac	4495	14570-11-69	-	11.46	11,770.30
400	Early Sac	4496	14570-11-70	-	8.52	27,400.78
400	Early Sac	4497	14570-11-71	-	21.81	73,168.23
400	Early Sac	4498	14570-11-72	-	13.70	55,192.84
400	Early Sac	4499	14570-11-73	-	19.44	55,863.04
Mean				-	13.97	18,788.69
SD				-	9.54	23,445.69

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for GA = 2.26 µg/g; LOQ for OX = 2.21 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-3. Concentrations of EG, GA and OX in the blood of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	EG[1] (µg/g)	GA (µg/g)	OX (µg/g)
Control	PK	4411	14570-10-1	-	1.29[2]	7.80
Control	PK	4412	14570-10-2	-	1.96	3.99
Control	PK	4413	14570-10-3	-	1.29[2]	1.70
Control	PK	4414	14570-10-4	-	1.29[2]	2.64
Control	PK	4415	14570-10-5	-	4.48	3.23
Mean				-	2.06	3.87
SD				-	1.38	2.35
50	PK	4431	14570-10-6	-	2.08	3.24
50	PK	4432	14570-10-7	-	3.01	1.04
50	PK	4433	14570-10-8	-	4.08	8.48
50	PK	4434	14570-10-9	-	3.80	3.16
50	PK	4435	14570-10-10	-	4.12	2.78
Mean				-	3.42	3.74
SD				-	0.87	2.80

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for GA = 2.57 µg/g; LOQ for OX = 2.21 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-3 (continued). Concentrations of EG, GA and OX in the blood of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	EG[1] (µg/g)	GA (µg/g)	OX (µg/g)
150	PK	4451	14570-10-11	-	4.83	3.18
150	PK	4452	14570-10-12	-	1.29[2]	4.59
150	PK	4453	14570-10-13	-	1.31	4.46
150	PK	4454	14570-10-14	-	4.64	3.46
150	PK	4455	14570-10-15	-	1.29[2]	3.45
Mean				-	2.67	3.83
SD				-	1.89	0.65
300	PK	4471	14570-10-16	-	6.69	4.20
300	PK	4472	14570-10-17	-	7.54	3.80
300	PK	4473	14570-10-18	-	8.71	4.05
300	PK	4474	14570-10-19	-	3.96	8.97
300	PK	4475	14570-10-20	-	7.00	4.48
Mean				-	6.78	5.10
SD				-	1.75	2.18

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for GA = 2.57 µg/g; LOQ for OX = 2.21 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-4. Total amounts of EG, GA, and OX eliminated in the urine + cage wash collected 24 hr prior to sacrifice of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	Amt. Urine (g)	EG[1] (µg)	GA (µg)	OX (µg)	%Target Dose Accounted For
Control	PK	4411	14570-10-1	12.569	39.84	34.99	4,870.93	-
Control	PK	4412	14570-10-2	11.500	nd	3.07	2,535.27	-
Control	PK	4413	14570-10-3	10.372	nd	46.31	3,081.99	-
Control	PK	4414	14570-10-4	9.524	nd	114.20	3,727.99	-
Control	PK	4415	14570-10-5	6.743	nd	61.63	859.25	-
Mean				10.142		52.04	3,015.08	-
SD				2.221		40.86	1,486.16	-
50	PK	4431	14570-10-6	9.320	1,261.7	104.32	791.81	7.56
50	PK	4432	14570-10-7	27.933	2,858.9	276.41	361.15	14.84
50	PK	4433	14570-10-8	12.936	3,797.0	390.04	2,808.09	27.44
50	PK	4434	14570-10-9	16.476	3,938.6	237.72	2,137.89	18.64
50	PK	4435	14570-10-10	11.367	1,025.4	149.06	1,494.13	10.19
Mean				15.606	2,576.3	231.51	1,518.61	15.73
SD				7.371	1,374.7	111.98	989.30	7.81

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG in cage wash samples; total amounts thus reflect only the analysis of EG in urine.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for EG= 1.54 µg/g; GA = 0.61 µg/g; LOQ for OX = 3.89 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

Table A-4 (continued). Total amounts of EG, GA, and OX eliminated in the urine + cage wash collected 24 hr prior to sacrifice of male Wistar Han rats administered ethylene glycol in the diets for up to 12 months.

Dose Group (mg/kg/day)	Study Group	Dow Animal ID	BNW Sample ID	Amt. Urine (g)	EG[1] (µg)	GA (µg)	OX (µg)	%Target Dose Accounted For
150	PK	4451	14570-10-11	19.024	7,534.1	387.86	2,125.03	11.31
150	PK	4452	14570-10-12	14.466	6,709.8	286.25	6,665.49	15.63
150	PK	4453	14570-10-13	11.946	7,917.1	509.06	3,467.30	15.69
150	PK	4454	14570-10-14	22.612	6,995.7	376.88	7,872.28	18.66
150	PK	4455	14570-10-15	11.764	3,190.0	234.26	923.84	5.65
Mean				15.962	6,469.3	358.86	4,210.79	13.39
SD				4.733	1,892.0	105.86	2,963.92	5.06
300	PK	4471	14570-10-16	11.535	16,871.7	2,082.86	8,760.22	16.37
300	PK	4472	14570-10-17	10.802	8,140.6	424.11	2,822.85	7.17
300	PK	4473	14570-10-18	12.655	26,302.3	2,307.30	5,229.05	26.31
300	PK	4474	14570-10-19	50.360	6,253.2	3,686.35	328.56	7.25
300	PK	4475	14570-10-20	14.440	12,155.3	1,997.05	4,229.62	12.00
Mean				19.958	13,944.6	2,099.54	4,274.06	13.82
SD				17.050	8,020.7	1,159.63	3,110.97	7.96

[1]A contaminant in the derivatization agent, PFBCl, prevented accurate quantitation of EG in cage wash samples; total amounts thus reflect only the analysis of EG in urine.

[2]For samples containing analytes at concentrations below the limits of quantitation (LOQ)/2 (LOQ for EG= 1.54 µg/g; GA = 0.61 µg/g; LOQ for OX = 3.89 µg/g), the LOQ/2 was used in the calculation of group statistics (Mean ± SD).

APPENDIX C. Benchmark Dose Analyses – The Sapphire Group

The Sapphire Group[TM]

22 February 2005

Via email: William_Gulledge@AmericanChemistry.com
William Gulledge
American Chemistry Council
1300 Wilson Boulevard
Arlington, VA 22209

RE: Benchmark Dose Analyses of the Chronic Study for Ethylene Glycol (EG) in Wistar Rats in Terms of External Dose

Dear Mr. Gulledge:

The *Sapphire Group, Inc.* is pleased to present the Ethylene Glycols Panel with a report for our benchmark dose (BMD) analyses using the results of the chronic study for EG in Wistar rats (Hard, 2005). As described in our proposal, this work was performed according to the following tasks: (1) BMD analysis of compound-induced nephropathy; and (2) BMD analysis of birefringent crystals. Consideration was also given to conducting BMD analysis for spontaneous nephropathy data from Hard (2005). However, these data are not considered useful for use in human health risk assessment for the following reasons: (1) the study authors concluded that there was no effect of EG on the severity of spontaneous nephropathy; (2) the incidence for this endpoint in control animals is very high (71%) and variable; (3) the dose-response data are nonmonotonic (*i.e.*, decreasing at the lowest dose), which is often difficult for simple dose-response models to provide an acceptable fit; (4) measurement of this endpoint is confounded by compound-induced nephropathy, in that data from the 400 mg/kg-day dose group could not be used, and it is likely that the data from the 300 mg/kg-day dose group were impacted as well (data for only 8 animals); and (5) spontaneous nephropathy is specific to rodents (Hard and Khan, 2004), and therefore this endpoint is not relevant to renal toxicity or to human health. For these reasons, BMD analyses were not conducted on the data for spontaneous nephropathy.

BMD modeling was performed using the data for compound-induced nephropathy an birefringent crystals in Wistar rats exposed to EG for one year as described in Hard (2005) (Table 1). Incidence data and combined incidence×severity data were used for the purposes of defining a dose corresponding to an extra risk of 5% (BMD05) and its lower confidence limit (BMDL05). Statistical tests were done to assess the significance of any treatment related effect, and the goodness-of-fit for the dose-response model. Consistent with our

previous analyses for EG (The Sapphire Group, 2003), the multistage model was selected for fitting to the dose-response data. All BMD modeling and statistical tests were performed using USEPA's Benchmark Dose Software (BMDS, version 1.3.2). The methods and results for both tasks are summarized below.

Task 1: BMD Values for Compound-Induced Nephropathy

BMD05 and BMDL05 values were derived from the dose-response data for compound-induced nephropathy assessed in terms of incidence and incidence×severity (Table 2), as summarized below:

- Incidence - The effect of EG exposure on the incidence of compound-induced nephropathy was highly significant ($p<0.0001$). The multistage model provided an acceptable fit to the incidence data for compound-induced nephropathy ($p=0.66$). Based upon these data, the BMD05 and BMDL05 for this endpoint were calculated to be 120 and 82 mg/kg-day, respectively.

- Incidence×Severity - The effect of EG exposure on the incidence×severity of compound-induced nephropathy was highly significant ($p<0.0001$). The multistage model provided an acceptable fit to the incidence×severity data for compound-induced nephropathy ($p=0.38$). Based upon these data, the BMD05 and BMDL05 for this endpoint were calculated to be 170 and 150 mg/kg-day, respectively.

Visual inspection of the dose-response plots indicate that the multistage model provides a reasonable fit to these data. The fact that BMD values for incidence are lower than those calculated for incidence×severity is not surprising given that the relationship for dose-incidence appears to be relatively steep (rising from 0%-100% across a narrow dose range) compared to the relationship for dose-severity, which increases more gradually with dose.

Task 2: BMD Values for Birefringent Crystals

BMD05 and BMDL05 values were derived from the dose-response data for birefringent crystals assessed in terms of incidence and incidence×severity (Table 2), as summarized below:

- *Incidence* - The effect of EG exposure on the incidence of birefringent crystals was highly significant ($p<0.0001$). The multistage model provided an acceptable fit to the incidence data for birefringent crystals ($p=0.84$). Based upon these data, the BMD05 and BMDL05 for this endpoint were calculated to be 140 and 94 mg/kg-day, respectively.

- *Incidence×Severity* - The effect of EG exposure on the incidence×severity of birefringent crystals was highly significant ($p<0.0001$). The multistage model provided an acceptable fit to the incidence×severity data for birefringent crystals ($p=0.386$). Based upon these data, the BMD05 and BMDL05 for this endpoint were calculated to be 170 and 160 mg/kg-day, respectively.

Visual inspection of the dose-response plots indicate that the multistage model provides a reasonable fit to these data (**Figure 1**). Again, the fact that BMD values for incidence are lower than those calculated for incidence×severity is not surprising given that the relationship for dose-incidence appears to be relatively steep (rising from 0%-100% across a narrow dose range) compared to the relationship for dose-severity, which increases more gradually with dose.

Discussion

The BMD05 value calculated for the incidence of compound-induced nephropathy (120 mg/kg-day) is in general agreement with the values predicted based upon the results of 16-week and 52-week studies in F344 and Wistar rats (99-101 mg/kg-day; The Sapphire Group, 2003). However, the BMDL05 value calculated for the incidence of compound-induced nephropathy (82 mg/kg-day) is considerably higher that the values predicted previously (38 – 45 mg/kg-day) (The Sapphire Group, 2003). This difference reflects the application of the multistage model to data covering a smaller range of doses in the chronic study (50 – 400 mg/kg-day) compared to the broader range of doses (40-1,000 mg/kg-day) tested in the studies used to predict the BMDL05, which resulted in a better fit and tighter confidence limits.

The chronic study of Hard (2005) may be considered an improved basis for human health risk assessment of EG over previously available data sets (Cruzan et al., 2004; DePass et al., 1986) based upon a consideration of: (1) use of the more sensitive test strain (Wistar vs. F344); (2) use of a chronic exposure duration (1 year vs 16 weeks); (3) use of a larger number of animals/group (15 vs. 10 rats); and (4) use of four dose groups over a narrow dose range (50-400 mg/kg-day) to provide a complete characterization of the response range with respect to incidence (0-100%).

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Please feel free to call Chris Kirman (216/514-8430) or me (937/427-4293) with any questions regarding this report.

Sincerely,

Michael L. Gargas

Michael L. Gargas, Ph.D.
Managing Principal

cc: Chris Kirman

Table 1. Incidence of Kidney Effects in Wistar Rats Exposed to EG for 12 Months

	Compound-Induced Nephropathy[1]		Birefringent Crystals[2]	
Dose (mg/kg-day)	Incidence	Incidence × Severity	Incidence	Incidence × Severity
0	0/14	0/70	0/14	0/56
50	0/15	0/75	0/15	0/60
150	0/15	0/75	0/15	0/60
300	12/13	27/65	8/13	16/52
400	10/10	43/50	10/10	34/40

[1] Incidence calculated from the number of animals with a severity grade of 1 or higher from Table 1 of Hard (2005). Incidence×severity scores calculated as the sum of the products of severity score and incidence for each dose group from Table 1 of Hard (2005).

[2] Incidence calculated from the number of animals with a severity grade of 1 or higher from Table 2 of Hard (2005). Incidence×severity scores calculated as the sum of the products of severity score and incidence for each dose group from Table 2 of Hard (2005).

Table 2. Summary of BMD Values Calculated for Kidney Effects in Rats Exposed to EG for 12 Months

Endpoint	Response	Dose-Response[1]	Goodness of Fit[2]	BMD05 (mg/kg-day)	BMDL05 (mg/kg-day)	BMD05: BMDL05
Compound-induced nephropathy	Incidence	<0.0001	0.66	120	82	1.5
	Incidence × Severity	<0.0001	0.38	170	150	1.1
Birefringent crystals	Incidence	<0.0001	0.84	140	94	1.5
	Incidence × Severity	<0.0001	0.39	170	160	1.1

[1] p-value for likelihood ratio test for a dose-response effect using BMDS (version 1.3.2). A value of less than 0.05 indicates a significant, treatment-related response.

[2] p-value for goodness of fit test for multistage model using BMDS (version 1.3.2). A value of greater than 0.05 indicates that the fit of the model is acceptable.

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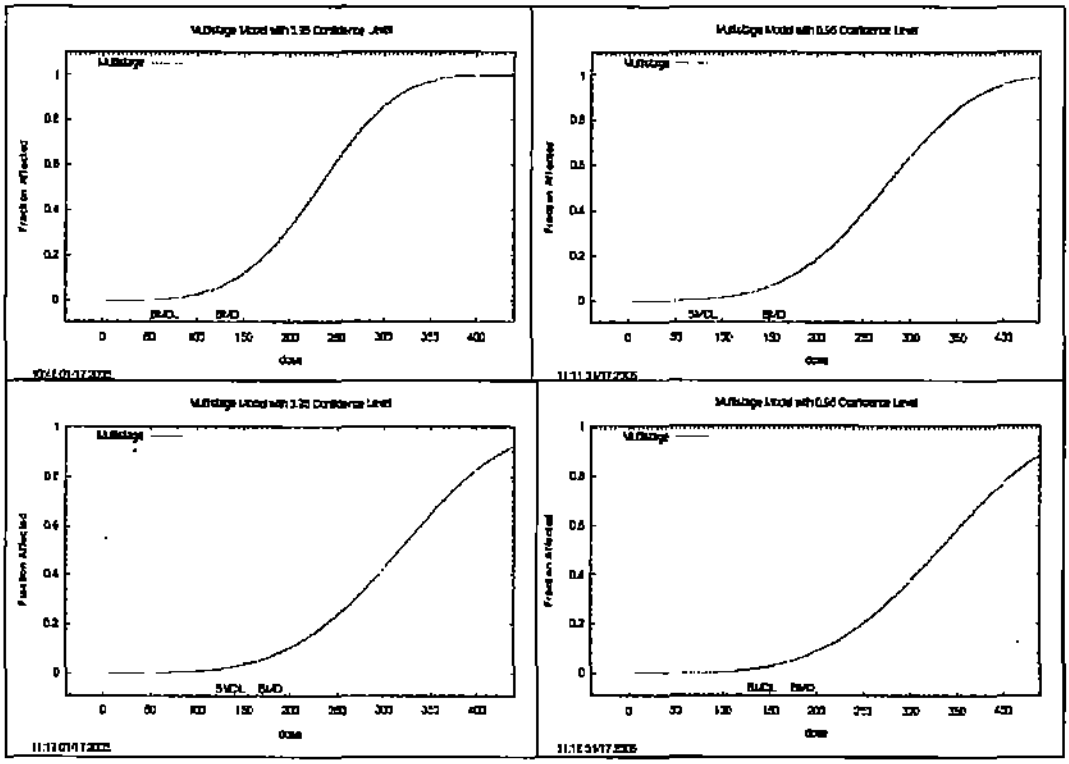
Figure 1. Dose-Response Plots for the Kidney Effects of EG: (A) Incidence of Compound-Induced Nephropathy; (B) Incidence×Severity of Compound-Induced Nephropathy; (C) Incidence of Birefringent Crystals; (D) Incidence×Severity of Birefringent Crystals



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SECTION III

ANNOTATED PAGES FROM THE DRAFT PROFILE DOCUMENT

ANNOTATED PAGES SUBMITTED BY

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How can ethylene glycol affect children?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children are likely to have similar effects as adults	Clinical findings in children who were poisoned by accidentally or intentionally drinking ethylene glycol indicate that it is likely that children would show the same health effects as adults. We do not know whether children differ in their susceptibility to the effects of ethylene glycol.
Birth defects	We do not know whether ethylene glycol causes birth defects in people. Skeletal defects and low birth weights have occurred in newborn animals whose mothers ingested large amounts of ethylene glycol during pregnancy.
Lactation exposure	We do not know whether ethylene glycol can accumulate in breast milk.

How can families reduce the risk of exposure to ethylene glycol?

If your doctor finds that you have been exposed to substantial amounts of ethylene glycol, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Avoid ingestion of antifreeze by careful handling and storage	Antifreeze products should be used with caution and kept out of the reach of children. Open bottles of antifreeze should not be left on or near the ground where children can reach them. Antifreeze should not be stored in anything other than the original container, such as in a cup or soft drink bottle, to avoid someone mistaking it for a beverage. Antifreeze containers should have a child-proof cap, be stored away from food, and be properly marked.
Get medical advice if antifreeze is ingested	Ethylene glycol poisoning can be effectively treated, but early diagnosis is needed to prevent serious injury. Medical attention should be sought as soon as possible in cases of known or suspected antifreeze ingestion.
Limit dermal exposure to products containing ethylene glycol	Minimize skin contact when using antifreeze and other consumer products containing ethylene glycol. Avoid spilling or draining antifreeze on the ground to prevent children from playing in a puddle of ethylene glycol.

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ETHYLENE GLYCOL IN THE UNITED STATES

Ethylene glycol is a colorless, odorless liquid that is ~~miscible~~ mixes completely with water (HSDB 2007). It is released into the environment primarily through industrial emissions and through the use and disposal of ethylene glycol-based automobile antifreeze and airport de-icing formulations (Corsi et al. 2001; EPA 2000; Sills and Blakeslee 1992; Ware 1988). Ethylene glycol that is released into the environment does not persist since it is degraded within days to a few weeks in air, water, and soil (Atkinson 1989; Battersby and Wilson 1989; Conway et al. 1983; Kameya et al. 1995; McGahey and Bouwer 1992; Revitt and Worrall 2003; Schoenberg et al. 2001; Staples et al. 2001). Available monitoring data indicate that ethylene glycol is only found near areas of release. Ethylene glycol vapor concentrations measured in the air at airports during de-icing spray operations ranged from 0.05 to 22 mg/m^3 (Gérin et al. 1997; LA DOTD 1990). Ethylene glycol concentrations as high as 19,000 mg/L have been measured in airport stormwater (Sills and Blakeslee 1992). Background concentrations of ethylene glycol in the environment are not available.

Since ethylene glycol is not expected to be present away from areas where it is released, background exposure of the general population to this substance is not expected to be important. The most common route of exposure to ethylene glycol for the general population is through dermal contact with ethylene glycol-containing automobile antifreeze. However, accidental or intentional ingestion of antifreeze is the most serious route of exposure, resulting in thousands of poisonings reported each year in the United States (Fraser 2002; Leth and Gregersen 2005). Ethylene glycol concentrations in blood, urine, tissue, or breast milk are not available for the general population.

Individuals who live near hazardous waste sites, industrial facilities where ethylene glycol is produced or used, or areas where ethylene glycol-based de-icing formulations are used may be exposed to ethylene glycol through dermal contact with contaminated soil or water, inhalation of ethylene glycol vapor or mist, or ingestion of contaminated groundwater. Occupational exposure through dermal contact and inhalation of ethylene glycol vapor or mist is expected for individuals involved in airport de-icing spray operations. Ethylene glycol has been detected in urine samples collected from airport de-icing workers (Gérin et al. 1997).

Ingestion of ethylene glycol containing antifreeze is a potential route of exposure for children since they are attracted to the bright colors of antifreeze formulations and the sweet taste of ethylene glycol (Leth and Gregersen 2005). Exposure through ingestion is more likely to occur when adults leave opened antifreeze containers within reach or store antifreeze in other types of containers such as beverage bottles. A bittering agent has been added to some ethylene glycol antifreeze formulations in order to deter ingestion; however, caution should still be used since ingestion poisoning has occurred even when a bittering agent was present (Harry et al. 1998; Hogue 2006).

2.2 SUMMARY OF HEALTH EFFECTS

Ethylene glycol is quickly and extensively absorbed through the gastrointestinal tract of many species, but dermal absorption is slow in rodents and is expected to be slow in humans. Limited information is available on absorption of inhaled ethylene glycol, but the existing toxicity studies suggest absorption via the respiratory tract by both humans and rodents. Following absorption, ethylene glycol is distributed in aqueous compartments throughout the body. Ethylene glycol is initially metabolized to glycoaldehyde by alcohol dehydrogenase (with possible contribution from cytochrome P-450 enzymes). Glycolaldehyde is rapidly converted to glycolate and glyoxal by aldehyde oxidase and aldehyde dehydrogenase. Metabolism of glycolate by glycolate oxidase or lactate dehydrogenase results in the formation of glyoxylate, which may be further metabolized to formate, oxalate, glycine, and carbon dioxide. Elimination of ethylene glycol occurs via exhaled carbon dioxide and urinary elimination of both ethylene glycol and glycolic acid. The half-life for elimination in humans has been estimated to be in the range of 2.5–8.4 hours (NTP-CERHR 2004).

The vast majority of information relating to the toxicity of ethylene glycol is from studies of oral exposure. Information on the health effects of oral exposure in humans is largely limited to case reports of acute accidental or intentional ingestion of ethylene glycol. These case reports have identified three stages of acute oral ethylene glycol toxicity in humans. These stages are well documented and occur within 72 hours after ingestion (NTP-CERHR 2004; Robinson and McCoy 1989; Vale 1979). The first stage involves central nervous system depression, metabolic changes (hyperosmolality ~~and acidosis~~), and gastrointestinal upset, and spans the period from 30 minutes to 12 hours. During the second stage (12–24 hours after ingestion), cardiopulmonary symptoms (tachypnea, hyperpnea, tachycardia, cyanosis, pulmonary edema, and/or cardiac failure) ~~due to metabolic acidosis~~ become evident. During stage three, which covers the period 24–72 hours after ethylene glycol ingestion, renal involvement becomes evident. The third stage is characterized by flank pain and oliguria/anuria. Histopathological findings show renal

metabolic acidosis and associated (see Section 3.8.2; pg 91, line 26 f)

tubular necrosis and deposition of calcium oxalate crystals (Vale 1979). Often, the cardiopulmonary effects in the second stage are not evident, so the distinguishing symptoms of ethylene glycol intoxication are central nervous system depression, acidosis, and nephrotoxicity (Jacobsen and McMartin 1986; Karlson-Stiber and Persson 1992). Limited information suggests that a fourth stage involving cranial nerves may occur 6 or more days after exposure (NTP-CERHR 2004). This stage is characterized by neurological symptoms including deafness, facial paralysis, and other sequelae.

Reports of fatalities following ingestion of ethylene glycol indicate that a volume of 150–1,500 mL consumed at one time may ~~be necessary to~~ cause death (Walton 1978). In humans, the lethal dose of ethylene glycol is estimated to be in the range of 1,400–1,600 mg/kg. Based on these estimates, it appears that humans may be more susceptible to the acute lethality of ingested ethylene glycol than other species. In laboratory animals (rats, mice, monkeys), oral doses of ≥4,000 mg/kg were needed to cause death (Clark et al. 1979; Richardson 1973). However, difficulties in quantifying the amounts consumed by persons who have succumbed to the toxic effects lead to uncertainty in the human lethal dose estimates.

A study with human subjects found that inhalation exposure to ethylene glycol vapor at an average concentration of 30 mg/m^3 for 20–22 hours/day for 30 days was well tolerated, with effects that were essentially limited to occasional complaints of mild upper respiratory tract irritation (Wills et al. 1974). There were no indications of renal or other systemic effects as shown by urinalysis, hematology and clinical chemistry evaluations, and neurobehavioral tests throughout the exposure period. Short-term, high-exposure sessions found that respiratory tract irritation became common at approximately 140 mg/m^3, and was tolerated for only 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3, and one or two breaths at 308 mg/m^3. This study was used as the basis for an acute-duration inhalation MRL for ethylene glycol (see Section 2.3).

Animal studies indicate that oral exposure to ethylene glycol can cause effects in a number of different organ systems, although the developing fetus and kidneys are particularly sensitive and well-documented targets of toxicity. Oral effects have also been observed in the central and peripheral nervous systems, heart, liver, hematopoietic system, and immunological and lymphoreticular systems. Available information suggests that the neurological and cardiopulmonary effects stem from metabolic acidosis associated with acute, high-dose exposures. Reported effects on the immunological and lymphoreticular systems are essentially limited to suppressed immune responses in mice given a single near-lethal oral dose (Zabrodskii and Germanchuk 2000; Zabrodskii et al. 2003), and neutrophilia and lymph node hemosiderosis in rats orally exposed for 2 years (DePass et al. 1986a). Effects on hematological

move this portion of these sentences to bottom of 2nd full paragraph on pg 11

parameters have largely been observed at high doses in longer-term studies, and are not consistently reported across studies or across species.

Oral studies in animals have identified the developing fetus as the most sensitive target for acute-duration exposure to ethylene glycol. Gavage exposure of laboratory rodents to ethylene glycol during gestation results in a consistent pattern of developmental effects including reduced fetal body weight and increases in malformations, particularly axial skeletal malformations (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985). Developmental toxicity has also been assessed by the inhalation and dermal routes. Results of the inhalation developmental studies are generally consistent with the oral findings, but are confounded by concurrent oral exposure via ingestion of aerosolized ethylene glycol on the fur of exposed animals (Tyl 1985, 1988a; Tyl et al. 1995a, 1995b). A single study of dermal exposure to ethylene glycol in pregnant mice did not indicate developmental effects (Tyl 1988b; Tyl et al. 1995c).

The kidney is clearly identified as the most sensitive target organ in rats and mice after intermediate-duration oral exposure. Typical renal effects included oxalate crystal deposition and renal tubular dilation, vacuolation, and degeneration. Oxalate, a metabolite of glycolic acid, forms a precipitate in the presence of calcium, and the deposition of these crystals in the renal tubules are hallmarks of ethylene glycol renal toxicity. Additionally, the buildup of glycolic acid in the body can result in metabolic acidosis, leading ultimately to renal failure (LaKind et al. 1999). Males were more sensitive than females, and rats were more sensitive than mice. Chronic oral studies confirm that the kidney is a main target organ in male rats, although liver lesions occurred in female rats (slight fatty metamorphosis) and male mice (hepatocellular hyaline degeneration) at doses lower than those inducing kidney effects (Blood 1965; DePass et al. 1986a; NTP 1993). No hepatic effects were observed in intermediate-duration studies.

There is no indication that ethylene glycol is carcinogenic based on results of a limited renal cancer mortality study in chemical plant workers (Bond et al. 1985) and well-designed chronic oral bioassays in rats (one study) and mice (two studies) (DePass et al. 1986a; NTP 1993).

A more detailed discussion of the developmental and renal effects associated with ethylene glycol exposure follows. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for additional information on these and other health effects.

Note: In the following section, reference should be made to what dose/exposure levels resulted in adverse maternal effects e.g. weight, food consumption

2. RELEVANCE TO PUBLIC HEALTH

Developmental Effects. No studies have addressed the developmental toxicity of ethylene glycol in humans. The developmental toxicity of ethylene glycol in animals has been assessed by inhalation, oral, and dermal exposure in acute-duration studies and by oral exposure in intermediate-duration studies. The acute oral studies indicate that developmental effects (a skeletal variation and total malformations) occur at doses of ≥500 mg/kg/day when administered by gavage during gestation days (Gd) 6–15 to CD-1 mice (Neeper-Bradley et al. 1995; Tyl 1989). Dose-response data for these developmental effects in mice were used to derive an acute-duration oral MRL for ethylene glycol (see Section 2.3). Reduced fetal body weight occurred in mice given gavage doses of ≥750 mg/kg/day (Price et al. 1985). In rats, doses of ≥1,000 mg/kg/day by gavage on Gd 6–15 have resulted in increased incidences of skeletal malformations (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). No teratogenic effects were observed in rabbits exposed to maternally lethal oral doses of 2,000 mg/kg/day during gestation (Tyl et al. 1993). In the only dermal exposure study, no developmental toxicity occurred in pregnant CD-1 mice that were treated with 6-hour daily exposures to ethylene glycol (estimated doses up to 3,549 mg/kg/day) by occluded cutaneous application on Gd 6–15 (Tyl et al. 1993).

Developmental toxicity studies of inhaled ethylene glycol in mice and rats found effects consistent with the oral findings, but all of the studies are confounded by concurrent ingestion of ethylene glycol deposited on the fur. In inhalation studies using whole-body exposure, significant effects on implant viability, weight of live fetuses, and incidence of external, visceral, and skeletal malformations were observed in mice exposed to ≥1,000 mg/m^3 for 6 hours/day on Gd 6–15 (Tyl 1988a; Tyl et al. 1995a). In rats exposed similarly, reduced ossification at some sites in the axial skeleton occurred at ≥1,000 mg/m^3 (Tyl 1985; Tyl et al. 1995a); however, in an Expert Panel Review, the National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR 2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. In a follow-up study aimed at reducing the confounding oral exposure, pregnant CD-1 mice were exposed nose-only to 500–2,500 mg/m^3 aerosolized ethylene glycol (Tyl 1988a; Tyl et al. 1995b). At 2,500 mg/m^3, live fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were also observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at ≥500 mg/m^3. The authors observed that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face (Tyl 1988a; Tyl et al. 1995a). Furthermore, NTP-CERHR (2004) noted that stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol, which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994). Because of the confounding

oral exposure in both the whole-body and nose-only experiments, NTP-CERHR (2004) concluded that the data from these studies were not suitable for evaluation of effect levels from inhalation exposure to ethylene glycol.

Developmental effects of intermediate-duration oral exposure to ethylene glycol include kidney effects in offspring and decreased pup body weights. In mice tested in a continuous breeding assay, pup body weights were reduced in both F_1 and F_2 generations at drinking water doses of ≥897 mg/kg/day (Morrissey et al. 1989; NTP 1986). In a 15-day gestational exposure study, postnatal effects in kidney weights were observed in pups of CD rats exposed to gavage doses of ≥1,250 mg/kg/day *in utero* (NTP 1988). In a three-generation study of rats, no effects on gestation survival or pup body weight through postpartum day (ppd) 21 were observed in F_1 or F_2 pups after parental exposure to dietary doses up to 1,000 mg/kg/day (DePass et al. 1986b).

Recent reviews of mechanistic studies on ethylene glycol developmental toxicity (NTP-CERHR 2004; Slikker et al. 2004) have concluded that glycolic acid, alone or in combination with its downstream metabolites and resultant metabolic acidosis, was likely the proximate toxicant responsible for the developmental effects of ethylene glycol. Using a physiologically based pharmacokinetic (PBPK) model developed for humans, Corley et al. (2005a) estimated that the glycolic acid blood threshold concentration for developmental effects in rodents would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). While the model has been validated against data from acute human oral and inhalation exposures to ethylene glycol (Corley and McMartin 2005; Corley et al. 2005a), it has not been calibrated to the physiological changes associated with pregnancy, which require a different model structure (EPA 2006a); thus, the usefulness of this model in predicting developmental toxicity in humans is limited. Further, NTP-CERHR (2004) noted that additional data were needed to fully delineate the rate of glycolic acid metabolism in humans; such additional data may alter the model predictions of peak glycolic acid concentrations in humans exposed to ethylene glycol.

Renal Effects. The renal toxicity of ethylene glycol in humans is well documented in numerous case reports of accidental or intentional ingestion. Adverse renal effects occur in the third stage of human ethylene glycol poisoning, which occurs 24–72 hours after acute exposure. The hallmark of renal toxicity is the presence of calcium oxalate monohydrate crystals in the renal tubules and urine following ingestion of large amounts of ethylene glycol (Blakeley et al. 1993; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980). Characteristic histopathological changes include renal tubular focal degeneration, atrophy, and interstitial inflammation (Factor and Lava 1987). Renal damage, if untreated, can lead to

renal failure (Chung and Tuso 1989; Gordon and Hunter 1982; Jacobsen et al. 1984; Mallya et al. 1986). With therapy, normal or near-normal renal function can be restored.

Humans who inhaled ethylene glycol showed no indications of impaired renal function. No significant alterations in renal end points were found in volunteers exposed to ethylene glycol aerosol at an average concentration of 30 mg/m^3 for 20–22 hours/day for 30 days (Wills et al. 1974). Evaluations were performed throughout the study and included examination of urine for presence of oxalate crystals and erythrocytes; determinations of urine volume, specific gravity, color, clarity, pH, amino acid nitrogen, and creatinine; and determination of blood urea nitrogen. There also was no indication of renal impairment in aviation workers who were intermittently exposed to ethylene glycol during airplane de-icing operations over a 2-month winter period (Gérin et al. 1997). Ethylene glycol concentrations as high as 22 mg/m^3 for vapor and 190 mg/m^3 for mist were measured, although the vast majority of samples were below the limit of quantification (2.5 mg/m^3 for vapor and 17 mg/m^3 for mist); the frequency and average levels and durations of exposure were not reported. Measurements of urinary albumin, β-*N*-acetyl-glucosaminidase, β-2-microglobulin, and retinol-binding protein were used to assess kidney function.

Renal effects in orally exposed animals are consistent with those observed in humans. In acute-duration studies, effects occurred in the kidneys of rats exposed to 1,250–2,500 mg/kg/day by gavage or 2,615–5,270 mg/kg/day in drinking water for 9–29 days, and rabbits exposed to 2,000 mg/kg/day by gavage for 13 days (Khan et al. 1993; Neeper-Bradley 1990; Neeper-Bradley et al. 1995; NTP 1988; Robinson et al. 1990; Tyl et al. 1993). Evaluation of these animals showed effects that generally included increased kidney weight and renal tubular calcium oxalate deposits, dilation, degeneration, and/or necrosis.

The renal effects of intermediate-duration oral exposure to ethylene glycol are well characterized in a number of studies in rats and mice (Cruzan et al. 2004; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990). These studies indicate that renal toxicity varies with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats. Renal effects in Sprague-Dawley rats that were exposed to ethylene glycol in drinking water for 90 days included renal tubular oxalate crystal deposition, dilation, and degeneration in males at ≥947 mg/kg/day and females at 3,087 mg/kg/day (Robinson et al. 1990). Findings in F344 rats exposed for 13 weeks via diet included renal tubular dilation, necrosis, fibrosis, and oxalate crystal deposition in males at ≥2,500 mg/kg/day, and mild renal lesions (e.g., inflammation and vacuolation) with no crystal deposition in females at 10,000 mg/kg/day (Melnick 1984). Results of 16-week dietary studies showed that male Wistar rats are approximately twice as sensitive as male F344 rats to ethylene glycol

higher than controls) and microscopic examination of kidneys showed no histopathological changes. At 2,500 mg/m^3, live fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at concentrations of ≥500 mg/m^3. The authors observed that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face (Tyl 1988a; Tyl et al. 1995a). Furthermore, stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol (NTP-CERHR 2004; Tyl et al. 1995a), which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994). [address the presence or absence of maternal effects on weight gain and food consumption]

Because of the confounding oral exposures in both the whole-body and nose-only developmental toxicity studies, NTP-CERHR (2004) concluded that the data from these studies were not suitable for evaluation of effect levels from inhalation exposure to ethylene glycol. The available data do, however, provide a conservative estimate of the inhalation no-observed-adverse-effect level (NOAEL), with the caveat that total exposure to ethylene glycol in these studies included intake via ingestion. Collectively, these studies suggest that inhalation exposure to ethylene glycol at a nominal concentration of about 150 mg/m^3 is not associated with developmental toxicity in mice or rats, or renal toxicity in mice (kidney histopathology not assessed in rats). The next highest concentration (500 mg/m^3 in the nose-only study) was associated with developmental effects (increased incidence of skeletal variations), but it is not possible to conclusively relate these effects to inhalation of ethylene glycol.

As indicated above, the developmental studies (Tyl 1988a; Tyl et al. 1995a, 1995b) collectively suggest that 150 mg/m^3 is a conservative NOAEL for developmental toxicity in rats and kidney toxicity in mice. This concentration is similar to the 140 mg/m^3 lowest-observed-adverse-effect level (LOAEL) for respiratory tract irritation in humans (Wills et al. 1974). The human NOAEL of 23 mg/m^3 is a suitable basis for MRL derivation because it is based on evaluations for renal and other systemic effects as well as local irritation, and is well within the NOAEL range for developmental toxicity in animals.

In the human study, health effects were assessed in 19 male prisoners who voluntarily were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days (Wills et al. 1974). The diameter of the aerosol droplets ranged from 1 to 5 μm. Mean daily and mean weekly concentrations during the first 14 days of the study were 0.8–44.8 and 17–29 mg/m^3, respectively. Mean daily and mean weekly concentrations during the entire 30-day exposure period were 0.8–67 and 17–49 mg/m^3, respectively. The average mean

Calculations of

weekly exposure was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. The average exposure levels did not include brief periods in which the concentration was intentionally raised to higher levels to assess acute responses. A control group consisted of 14 male prisoners; 10 of these men were never exposed to ethylene glycol, whereas the remaining 4 men had been exposed to a mean concentration of 37 mg/m^3 for 20–22 hours/day for 7 days during the week that preceded the start of the study. Subjective responses (symptoms) were monitored throughout the study. During the last 10 days of the study, the concentration of ethylene glycol was occasionally intentionally increased to various high levels (up to 308 mg/m^3) when the volunteers left the exposure chamber during meals; subjective responses to short exposures to the high concentrations were assessed when they reentered the chamber. Complete physical examinations that included slit-lamp, electrocardiographic, and electroencephalographic studies, and a battery of psychological tests designed to reveal effects on simple reaction time, reaction time with discrimination, visual-motor coordination, depth perception, and mental ability (encoding and subtraction accuracy), were conducted on all subjects pre-exposure and after 14 and 30 days of exposure. Blood samples were collected on days 0, 1, 3, 5, 8, 12, 19, 22, 26, and 29 for evaluation of hematology, clinical chemistry (including blood urea nitrogen, serum creatinine, and liver enzymes), and ethylene glycol concentration. Urine was evaluated daily for oxalate crystals, erythrocytes, and ethylene glycol, and twice weekly for volume, specific gravity, color, clarity, pH, amino acid nitrogen, and creatinine. Concentrations of ethylene glycol in the blood and urine were similar in the exposed and control groups. The near-continuous exposure levels (average 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30) were tolerated with effects that were limited to occasional complaints of upper respiratory tract irritation, slight headache, and low backache (incidences and other information not reported). The short-term, high-exposure sessions showed that the irritation became common at approximately 140 mg/m^3, and tolerated for only 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3, and one or two breaths at 308 mg/m^3. Based on these results and those of other trials, the investigators concluded that concentrations of about ≥200 mg/m^3 were intolerable due to strong irritation of the upper respiratory tract that included a burning sensation in the trachea and a burning cough. Because the near-continuous exposures were tolerated with respiratory irritation that was infrequent and not serious, and not accompanied by neurological, hematology, clinical chemistry, or urinalysis findings indicative of renal or other systemic effects, the interim (12–14-day) findings in this study identified a NOAEL of 23 mg/m^3 for acute-duration exposure in humans. The LOAEL in humans was 140 mg/m^3 because brief exposures to this concentration commonly caused respiratory irritation.

The NOAEL of 23 mg/m^3 for respiratory tract irritation and systemic toxicity in humans (Wills et al. 1974) was divided by an uncertainty factor of 10 (for human variability) to derive an MRL of 2 mg/m^3 for

risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

Information regarding health effects of ethylene glycol following inhalation exposure is limited. Health effects in humans were found in only a few studies (Bond et al. 1985; Triosi 1950; Wills et al. 1974). Animal studies were described by Tyl (1985, 1988a).

3.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to ethylene glycol.

Mortality occurred in 1/15 rats, 3/15 guinea pigs, 1/3 rabbits, 0/3 dogs, and 0/3 monkeys that were continuously whole-body exposed to 12 mg/m^3 of ethylene glycol aerosol for 90 days, although none of the affected animals showed "any specific signs of toxicity" (Coon et al. 1970). This concentration is not a reliable LOAEL for mortality because intake of ethylene glycol from ingestion of aerosol deposited on the fur likely significantly contributed to total dose (Tyl et al. 1995a, 1995b). Exposure to 10 or 57 mg/m^3 ethylene glycol aerosol for 8 hours/day, 5 days/week for 6 weeks caused no mortality in rats (15/concentration), guinea pigs (15/concentration), rabbits (3/concentration), dogs (2/concentration), or monkeys (2/concentration) (Coon et al. 1970).

3.2.1.2 Systemic Effects

No studies were located regarding gastrointestinal, musculoskeletal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or respiratory, gastrointestinal, musculoskeletal, dermal, or body weight effects in animals after inhalation exposure to ethylene glycol. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category for ethylene glycol after inhalation exposure are reported in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Tolerable nose and throat irritation were occasional complaints in 19 volunteers (incidence and frequency not reported) who were exposed to ethylene glycol aerosol for 20–22 hours/day for 30 days in a controlled study (Wills et al. 1974). The average mean weekly exposure concentration was 23 mg/m^3 for days 1–14 and 30 mg/m^3 for days 1–30. Sessions in which the concentration was

regimen (Tyl 1985, 1988a; Tyl et al. 1995a). Both the mouse and rat studies were confounded by ingestion of ethylene glycol deposited on the fur of exposed animals and consumed during grooming; the authors estimated that ingestion comprised the majority of exposure. In a companion study, nose-only exposure of CD-1 mice to 500–2,500 mg/m^3 aerosolized ethylene glycol using the same study design resulted in no effects on pre- or postimplantation loss (Tyl 1988a; Tyl et al. 1995a). Although this study was aimed at reducing confounding from concurrent ingestion exposure, the authors noted that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face during nose-only exposure.

As a result of confounding from exposure via ingestion, NTP-CERHR (2004) characterized the developmental toxicity studies as inadequate for the purpose of identifying effect levels for inhalation exposure; thus, there are no reliable NOAEL or LOAEL values.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to ethylene glycol.

Acute-duration developmental toxicity studies of inhaled ethylene glycol in mice and rats are available, but all of the studies are confounded by concurrent ingestion exposure to ethylene glycol deposited on the fur. Groups of 25 pregnant CD-1 mice and CD rats were exposed (whole-body) to target concentrations of 0, 150, 1,000, or 2,500 mg/m^3 aerosolized ethylene glycol (mass median aerodynamic diameter [MMAD] of 2.3 µm) for 6 hours/day on Gd 6–15 (Tyl 1985, 1988a; Tyl et al. 1995a). Fetal evaluations included litter size, fetal weight, and external, visceral, and skeletal malformations. In mice, significant decreases in the number of live fetuses per litter and in the weight of live fetuses, as well as increases in the number of live fetuses per litter and the incidence of external, visceral, and skeletal malformations were observed at target concentrations of ≥1,000 mg/m^3. In rats, reduced ossification at some sites in the axial skeleton was observed with exposure to 1,000 and 2,500 mg/m^3 (Tyl 1985; Tyl et al. 1995a); however, in an Expert Panel Review, NTP-CERHR (2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. This study was confounded by significant ingestion of ethylene glycol deposited on the fur and consumed during grooming; the authors estimated that the ingestion dose comprised the majority of exposure. Address maternal effects.

3.2.2.1 Death

The American Association of Poison Control Centers reported nine fatalities for 1989 and five for 1990 due to ethylene glycol ingestion (Litovitz et al. 1990, 1991). Several other fatal ethylene glycol poisonings have been reported in earlier studies, including seven case reports of deaths resulting from accidental or intentional ingestion of ethylene glycol or antifreeze containing 99% ethylene glycol (Godolphin et al. 1980; Gordon and Hunter 1982; Hewlett et al. 1986; Jacobsen et al. 1984; Siew et al. 1975a; Zeiss et al. 1989). A 22-year-old male who ingested 300 mL of antifreeze (approximately 4,071 mg/kg ethylene glycol) lapsed into a coma 24 hours after hospital admission and died 24 hours later (Siew et al. 1975a). A dose of 7,850 mg/kg can be estimated in the case of a 73-year-old male who consumed 500 mL of 95% ethylene glycol and died of myocardial failure after 68 hours (Gordon and Hunter 1982). In five other fatal cases of accidental or intentional poisoning, the amount of ingested ethylene glycol ranged from 150 to 1,500 mL (2,379–23,786 mg/kg) (Karlson-Stiber and Persson 1992; Walton 1978). Thus, oral dose of ethylene glycol required to cause death in humans is not well defined in the literature. The minimum lethal dose for adults is thought to be 1.4 mL/kg of 95% ethylene glycol, or about 1,330 mg ethylene glycol/kg body weight (Parry and Wallach 1974; Robinson and McCoy 1989; Siew et al. 1975a).

A single dose oral LD_{50} of 4,000 mg/kg was determined in Female F344 rats (Clark et al. 1979). Male Wistar rats administered 12,900 mg/kg ethylene glycol in a single oral dose had 55% mortality within 48 hours (Richardson 1973). Pregnant CD-1 mice given 11,090 mg/kg/day ethylene glycol orally on Gd 7–14 showed 10% mortality (Schuler et al. 1984) and pregnant rabbits exhibited 42% mortality after receiving 2,000 mg/kg/day ethylene glycol orally on Gd 6–19 (Tyl et al. 1993). Cats administered a single 4,440–8,880 mg/kg dose by gavage had 100% mortality within 20–36 hours (Penumarthy and Oehme 1975). A single gavage dose of 4,180–12,540 mg/kg/day caused 17–100% mortality in dogs within 72 hours (Kersting and Nielsen 1965). Dogs administered a single oral dose of 4,880 mg/kg in food had 100% mortality within 6 days (Beckett and Shields 1971).

Intermediate-duration dietary exposure to 1,000 mg/kg/day for 16 weeks caused 20% mortality in male Wistar rats, with no deaths occurring in similarly treated male F344 rats; females were not tested (Cruzan et al. 2004). Male F344/N rats fed 5,000 mg/kg/day ethylene glycol had 40% mortality after 13 weeks, whereas similarly treated females did not die (Melnick 1984). A chronic dietary study of ethylene glycol in Sprague-Dawley found 100% mortality after 12–24 months in males at 750 mg/kg/day and females at

rats

toxicity is the presence of birefringent calcium oxalate monohydrate crystals deposited in renal tubules and their presence in urine after ingestion of relatively high amounts of ethylene glycol (CDC 1987; Blakeley et al. 1993; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980; Heckerling 1987; Parry and Wallach 1974; Rothman et al. 1986; Siew et al. 1975a; Underwood and Bennett 1973). In addition to birefringent oxalate crystals in the tubular lumens, other signs of nephrotoxicity can include focal tubular cell degeneration, atrophy, and tubular interstitial inflammation (Factor and Lava 1987). In a case study of a 38-year-old female who consumed 240 mL of antifreeze (3,454 mg ethylene glycol/kg/day), crystalluria was not present upon hospital admission (about 12 hours after ingestion). Within 5 hours, excretion of calcium oxalate dihydrate crystals was evident, although monohydrate crystals became the primary form in the urine thereafter (2–3 hours) (Jacobsen et al. 1988). In the course of ethylene glycol intoxication, serum creatinine (Factor and Lava 1987; Spillane et al. 1991) and serum blood urea nitrogen (BUN) (Chung and Tuso 1989; Factor and Lava 1987) levels may be increased. If untreated, the degree of renal damage caused by high doses of ethylene glycol progresses and leads to hematuria (CDC 1987; Rothman et al. 1986; Underwood and Bennett 1973), proteinuria (Rothman et al. 1986), decreased renal function, oliguria, anuria (Mallya et al. 1986; Parry and Wallach 1974; Spillane et al. 1991; Woolf et al. 1992; Zeiss et al. 1989), and ultimately renal failure (Chung and Tuso 1989; Gordon and Hunter 1982; Jacobsen et al. 1984; Mallya et al. 1986). These changes in the kidney are linked to acute tubular necrosis (Factor and Lava 1987), but normal or near normal renal function can return with adequate supportive therapy (see Section 3.11, Methods for Reducing Toxic Effects).

In acute-duration studies in rats, kidney effects occurred at doses as low as 1,250 mg/kg/day by gavage and 1,400 mg/kg/day in drinking water. Renal tubular dilation and regeneration were increased in female Sprague-Dawley rats that were exposed to 1,250 or 2,250 mg/kg/day ethylene glycol by gavage on Gd 6–20 and examined on postnatal day (Pnd) 1 (NTP 1988). Increased relative and absolute kidney weights, but no renal histopathology, occurred in female CD rats exposed to 2,500 mg/kg/day by gavage on Gd 6–15 and examined on Gd 21 (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). In a 10-day drinking water systemic toxicity study, the incidence and severity of renal lesions were significantly increased in male Sprague-Dawley rats exposed to 2,615 and 5,270 mg/kg/day, but not at doses ≤1,343 mg/kg/day; lesions included tubular dilation, degeneration, necrosis, and intratubular calcium oxalate crystals (Robinson et al. 1990). Exposure to 1,400 mg/kg/day in the drinking water for 15–29 days caused renal tubular oxalate deposits, but apparently no nephrosis, in male Sprague-Dawley rats (Khan et al. 1993). Mice that were administered doses ≤1,000 mg/kg by gavage for 4 days had no histopathological changes the in kidneys (Hong et al. 1988). Renal toxicity occurred in female New Zealand white rabbits that were exposed to 2,000 mg/kg/day by gavage on Gd 6–19 and examined on Gd 30; lesions that included tubule

Studies in laboratory animals indicate that acute-duration exposure to high doses of ethylene glycol during gestation can affect fetal viability and postimplantation loss. Of 37 pregnancies in CD-1 mice receiving gavage doses of 11,090 mg/kg/day on Gd 7–14, only 15 litters had at least 1 live-born pup, compared with 29/29 control pregnancies (Schuler et al. 1984). In the treated group, there was a significant decrease in the number of live pups per litter and a significant increase in the number of dead pups per litter at birth. Ethylene glycol treatment (up to 2,500 mg/kg/day) of mated female Swiss CD-1 mice during Gd 8–14 did not affect the number of females littering, number of implantation sites, or number of live pups at birth (Harris et al. 1992). The percentage of postimplantation loss per litter was significantly increased in CD rats treated by gavage on Gd 6–15 with 5,000 mg/kg/day and the number of live fetuses per litter was reduced at both 2,500 and 5,000 mg/kg/day (Price et al. 1985). There were no significant effects of treatment on total implantations, preimplantation loss, or litter size when pregnant F344 rats were given ethylene glycol in the diet at target doses of up to 1,000 mg/kg/day on Gd 6–15 (Maronpot et al. 1983). In New Zealand white rabbits given gavage doses of up to 2,000 mg/kg/day ethylene glycol on Gd 6–19, the numbers of pre- or post-implantation losses were not increased in any treatment group, although 42% of the high-dose dams died prior to sacrifice (Tyl et al. 1993).

address whether effects on development occur at doses that are not maternally toxic

The most sensitive indicator of the developmental toxicity of acute oral exposure to ethylene glycol appears to be an increased incidence of malformations, primarily skeletal malformations, in both mice and rats. Available data suggest that malformations appear in mice at lower doses than those that cause malformations in rats. The incidence of skeletal and other malformations was increased at all doses when groups of at least 20 timed-pregnant CD-1 mice were treated by gavage with doses of 0, 750, 1,500, or 3,000 mg/kg/day ethylene glycol on Gd 6–15 (Price et al. 1985). The percentages of malformed fetuses per litter and of litters with one or more malformed fetuses were significantly increased at all doses. The malformations primarily consisted of neural tube, craniofacial, and axial skeletal defects, with skeletal defects comprising the majority. In a later study aimed at identifying a NOAEL for developmental effects in CD-1 mice, an increased incidence of malformations was observed at doses of ≥500 mg/kg/day by gavage on Gd 6–15 (Neeper-Bradley et al. 1995; Tyl 1989). The incidence of total malformations per litter (external, visceral, and skeletal) was significantly increased at both 500 and 1,500 mg/kg/day. There was a significant increase in the incidence of two skeletal malformations (fused ribs or thoracic arches) in the 1,500 mg/kg/day group, and the incidences of 23 skeletal variations were also increased in this group. One of these variations (bilateral extra rib 14) was also significantly increased at 500 mg/kg/day. The incidence of individual external or visceral malformations was not significantly increased in any treatment group relative to the vehicle control; however, exencephaly (a malformation observed by Price

3.2.2.7 Cancer

No studies were located regarding carcinogenicity in humans after oral exposure to ethylene glycol.

Comprehensive histopathological evaluations showed no evidence of carcinogenicity in Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside et al. 1982), B6C3F1 mice exposed to ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside et al. 1982).

3.2.3 Dermal Exposure

Dermal exposure, through activities such as changing antifreeze, is the most likely route of exposure to ethylene glycol, but dermal exposure is not likely to lead to toxic effects.

3.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to ethylene glycol.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, endocrine, or metabolic effects in animals after dermal exposure to ethylene glycol.

The highest NOAEL values for systemic effects in each species and duration category for ethylene glycol after dermal exposure are reported in Table 3-3.

Hepatic Effects. Maternal liver weight was not affected in female CD-1 mice exposed to 3,549 mg/kg/day ethylene glycol for 6 hours/day on Gd 6–15 by occluded dermal application; liver histopathology was not evaluated (Tyl 1988b; Tyl et al. 1995c).

^{14}C-ethylene glycol to both rats and mice, the areas under the ethylene glycol plasma concentration versus time curves were comparable to those observed with equivalent intravenous doses (Frantz et al. 1989, 1991, 1996a).

Results of one study suggest that pregnancy does not alter absorption kinetics in rats dosed once on Gd 10. The time course and peak plasma levels of ethylene glycol did not differ between pregnant and nonpregnant rats given 10 or 2,500 mg/kg by gavage (Pottenger et al. 2001).

3.4.1.3 Dermal Exposure

There are no data quantifying absorption of ethylene glycol after *in vivo* human exposure.

In vivo studies with rats and mice suggest incomplete dermal absorption of ethylene glycol. In rats exposed to occluded dermal doses of 10 or 1,000 mg/kg ^{14}C-ethylene glycol or 1,000 mg/kg of a 50% solution of ^{14}C-ethylene glycol, measurement of radioactivity recovered in body tissues, excreta, and exhaled air suggested apparent absorption of 26–32% of the administered dose (Frantz et al. 1989, 1996b). In the same study, similar treatment of mice with 100 or 1,000 mg/kg ^{14}C-ethylene glycol or 1,000 mg/kg of 50% ^{14}C-ethylene glycol lead to apparent absorption estimates ranging from 60 to 84% (Frantz et al. 1991, 1996b).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Data on the tissue distribution of ethylene glycol in humans exposed via inhalation are not available. Based on plasma concentrations of ethylene glycol in two volunteers who inhaled doses of 0.96 and 1.51 mg/kg, Carstens et al. (2003) estimated the volumes of distribution (Vd) to be 0.78 and 0.91 L/kg.

In rats inhaling ^{14}C-ethylene glycol vapor (32 mg/m^3 for 30 minutes) or aerosol (184 mg/mg^3 for 17 minutes), radioactivity was distributed quickly (Marshall and Cheng 1983). The authors estimated that 60% of ethylene glycol (in either form) was deposited in the respiratory tract, primarily in the nasal cavity, and 75–80% of the initial body burden was distributed throughout the body upon sacrifice immediately after exposure (Marshall and Cheng 1983).

3.4.2.2 Oral Exposure

After oral exposure, ethylene glycol is distributed throughout the body according to total body water. The apparent volume of distribution of ethylene glycol in humans exposed orally has been estimated to be 0.54 - 0.56 L/kg based on clearance data in two patients poisoned with ethylene glycol (Jacobsen et al. 1988). The urine to plasma ethylene glycol concentration ratios in one patient was similar to those of ethanol, indicating distribution with total body water.

In rats, 6–22% of the radioactivity derived from single oral doses of 10 and 1,000 mg/kg of ^{14}C-ethylene glycol were recovered from body tissues and carcass (combined) 96 hours after exposure (Frantz et al. 1989, 1996b, 1996c); mice retained similar percentages (3–11%) in their tissues following single oral doses across the same range (Frantz et al. 1991, 1996b). Among the few tissues examined individually (liver, kidney, brain, fat, and lung), the highest radioactivity was found in the liver of both species (see Table 3-5). In two rhesus monkeys given single oral doses of about 1,100 mg/kg unlabeled ethylene glycol, the parent compound was evenly distributed throughout the tissues 4 hours after exposure; tissue to plasma concentration ratios ranged from 0.85 to 1.91 for the brain, heart, kidney, gastrointestinal tract, liver, lung, muscle, pancreas, and spleen (McChesney et al. 1971).

3.4.2.3 Dermal Exposure

Frantz et al. (1989, 1996b, 1996c) evaluated the distribution of a 10 or 1,000 mg/kg dose of undiluted ^{14}C-ethylene glycol or a 1,000 mg/kg dose of 50% aqueous ^{14}C-ethylene glycol applied dermally to rats under an occlusive bandage. Table 3-6 shows the disposition of radioactivity. The pelt contained the highest radioactivity (5–6% of applied dose) among the tissues examined (liver, kidney, brain, lung, pelt, and remaining carcass) (Frantz et al. 1989, 1996b). Similar experiments in mice at doses of 100 or 1,000 mg/kg undiluted ^{14}C-ethylene glycol or 1,000 mg/kg 50% aqueous solution of ^{14}C-ethylene glycol showed the highest radioactivity in the carcass and pelt combined (~8–15%) (Frantz et al. 1991, 1996b).

3.4.3 Metabolism

The metabolic pathway for ethylene glycol is shown in Figure 3-3. The metabolism of ethylene glycol was reviewed by NTP-CERHR (2004). Ethylene glycol is first converted to glycolaldehyde by nicotinamide adenine dinucleotide (NAD)-dependent alcohol dehydrogenase. Glycolaldehyde has a brief half-life and is rapidly converted to glycolic acid (and to a lesser extent glyoxal) by aldehyde dehydrogenase and aldehyde oxidase. Glycolic acid is oxidized to glyoxylic acid by glycolic acid oxidase

In vitro data provide conflicting comparisons between the rat and human rates of glycolic acid metabolism. Although a comparison of Km values obtained using liver homogenates from female humans and Sprague-Dawley rats (0.19 and 0.79 mM for humans and rats, respectively) (unpublished data of Bartels cited in NTP-CERHR 2004) suggested that humans may metabolize glycolic acid more efficiently than rats, a more recent study suggested the opposite. Booth et al. (2004) reported Km values of 0.43 and 0.28 mM (humans and rats, respectively) from a study using human and rat liver slices; these data suggest less efficient metabolism in humans.

3.4.4 Elimination and Excretion

Little information is available on the elimination of ethylene glycol in humans; most of the elimination data are from humans accidentally poisoned and given therapeutic treatments to reduce the metabolism of ethylene glycol or extract it from the blood. In laboratory animals treated with ^{14}C-ethylene glycol, the primary routes of excretion are exhaled air and urine, regardless of the route of exposure. After oral exposure, saturation of metabolic pathways at higher doses leads to a shift in excretory pattern, with greater urinary excretion (and corresponding decreases in elimination via expired air) at higher doses.

3.4.4.1 Inhalation Exposure

Carstens et al. (2003) evaluated the urinary excretion of ethylene glycol and its two primary metabolites (glycolic and oxalic acids) in two volunteers who inhaled ^{14}C-ethylene glycol at doses estimated by the authors to be 0.96 and 1.51 mg/kg. Urinary excretion of ^{14}C-ethylene glycol up to 30 hours after exposure constituted 6.4–9.3% of the inhaled dose, while ^{13}C-glycolic acid and ^{13}C-oxalic acid together comprised 1–2% of the inhaled dose. However, the dose estimates are highly uncertain, as they were calculated by estimating the loss of ^{14}C-ethylene glycol from an inhalation vessel in which the compound was "warmed". Air concentrations to which the volunteers were exposed were not measured, and the warming temperature was not reported. The authors reported that ^{14}C-ethylene glycol was not detectable in exhaled air, but did not assess expiration of $^{14}CO_2$.

In rats, the major route of elimination for inhaled ethylene glycol is expiration of CO_2. Rats exposed for 30 minutes to ^{14}C-ethylene glycol vapor (32 mg/m^3) or for 17 minutes to ^{14}C-ethylene glycol aerosol (184 mg/m^3) excreted 63% (over 4 days) and 75% (over 6 days), respectively, of the initial body burden as $^{14}CO_2$ (Marshall and Cheng 1971). Urinary excretion constituted 20 and 12% of the initial body

toxicant associated with ethylene glycol exposure, while a downstream metabolite (oxalate) is associated with renal toxicity. There are no data on the tissues most responsible for metabolism of ethylene glycol. Two of the primary enzymes involved in ethylene glycol metabolism (alcohol dehydrogenase and aldehyde dehydrogenase) are also responsible for ethanol metabolism, and ethanol metabolism largely takes place in the liver. Thus, it is likely that the liver is also the primary site of ethylene glycol metabolism; however, other tissues, including the placenta, also produce these enzymes. Pharmacokinetic parameters (e.g., plasma half-life, area under the curve, and peak ethylene glycol concentration) are similar after both oral and intravenous exposure (Frantz et al. 1989, 1991, 1996a, 1996b, 1996c), indicating that a first-pass effect, if any, has a negligible effect on the toxicokinetics.

Excretion. As discussed in more detail in Section 3.4.4, studies in mice and rats of ethylene glycol excretion after oral, dermal, and intravenous exposure indicate that ethylene glycol is principally excreted as expired CO_2 and as both parent compound and glycolic acid in the urine (Frantz et al. 1989, 1991, 1996b, 1996c). At higher doses, oxalate was also excreted at measurable levels. Few data are available with which to evaluate the major excretory pathways for inhaled ethylene glycol.

3.5.2 Mechanisms of Toxicity

The mechanism of action of ethylene glycol can be best explained by describing the main effects that follow its ingestion: increased osmolal gap, metabolic acidosis, and formation of calcium oxalate crystals. The elucidation of ethylene glycol metabolism (Figure 3-3) has helped in the understanding of its mechanism of toxic action.

In the initial stages after ingestion, ~~of~~ the ethylene glycol, ~~its~~ concentration in extracellular fluids increases, leading to increased osmolality. This increased osmolality (hyperosmolarity) ~~further leads to~~ causes an increased osmolal gap, one of the hallmarks of ethylene glycol intoxication. Osmolal gap is defined as a difference between the measured and calculated osmolality. Osmolality (calculated) can be estimated from the formula that takes into account normal serum concentrations of sodium, glucose, and BUN. This calculated osmolality is then compared to the serum osmolality measured following ethylene glycol ingestion; a difference >10 indicates an increased osmolal gap (Fligner et al. 1985). The increased osmolal gap is not ~~solely characteristic of~~ specific to ethylene glycol intoxication and can occur when any osmotically active, non-measured solute (e.g., mannitol) is present in the serum. In dogs given oral doses of 10,743 mg/kg ethylene glycol, serum osmolality peaked (460 milliosmoles/kg) at 3–6 hours, and the osmolal gap peaked (134 milliosmoles/kg) at 3 hours, coinciding with peak serum ethylene glycol levels

Clinical signs of neurotoxicity similar to those in humans summarized above occurred in rats, dogs, and cats following administration of large oral bolus doses of ethylene glycol (Beckett and Shields 1971; Clark et al. 1979; Dial et al. 1994; Grauer et al. 1987; Penumarthy and Oehme 1975). No clinical signs of neurotoxicity or histopathological changes in brain, spinal cord, or peripheral nerve tissue were observed in rats or mice exposed to ethylene glycol in the diet or drinking water in acute-, intermediate-, or chronic-duration studies (Blood 1965; DePass et al. 1986a; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998). Tests of neurobehavioral function have not been conducted in orally-exposed animals. Although there were no effects on neurobehavioral function in humans exposed by inhalation (Wills et al. 1974), neurobehavioral testing in orally-exposed animals is needed to adequately assess the neurotoxic potential of lower doses of ethylene glycol.

Epidemiological and Human Dosimetry Studies. A limited amount of epidemiological data on ethylene glycol is available from two studies of workers mainly exposed by inhalation with possible secondary exposure by the dermal route. One of these occupational studies evaluated kidney function in a small number of aviation workers who were intermittently exposed to ethylene glycol during airplane de-icing operations over a 2-month winter period (Gérin et al. 1997). Personal exposures to ethylene glycol vapor and aerosol were measured, but most samples were below the detection limit and average levels were not reported. This study found no indication of renal impairment based on a limited number of urinary end points (albumin, β-*N*-acetyl-glucosaminidase, β-2-microglobulin, and retinol-binding protein). The other study assessed renal cancer mortality in 1;666 chemical plant employees and found no increase in a small number of workers exposed to unmeasured levels of ethylene glycol (Bond et al. 1985). Epidemiological studies of orally-exposed humans are not available, although numerous clinical case reports of intentional or accidental ingestion have documented neurological, renal, and other effects of high acute doses of ethylene glycol. The available information suggests that ethylene glycol is likely to cause effects in humans similar to those found in animals. Additional epidemiological studies investigating dose-response relationships between ethylene glycol exposure and likely target organ toxicity would be useful. Background exposure of the general population is not expected to be important because ethylene glycol is rapidly degraded in air, water, and soil, and available monitoring data indicate that it is only found near areas of release (Atkinson 1989; Battersby and Wilson 1989; Conway et al. 1983; Kameya et al. 1995; McGahey and Bouwer 1992; Revitt and Worrall 2003; Schoenberg et al. 2001; Staples et al. 2001). ~~Populations likely to show effects of ethylene glycol~~ Potential study populations include individuals exposed through dermal contact with ethylene glycol-containing automobile antifreeze and individuals who live near hazardous waste sites, industrial facilities where ethylene glycol is produced or used, or areas where ethylene glycol-based de-icing formulations are used and may be exposed through dermal contact with

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contaminated soil or water, inhalation of ethylene glycol vapor or mist, or ingestion of contaminated groundwater. Additionally, occupational exposure through inhalation of ethylene glycol vapor or mist and dermal contact is expected for individuals involved in airport de-icing spray operations.

Biomarkers of Exposure and Effect.

Exposure. The only biomarker of exposure that is specific to ethylene glycol is parent compound in the blood and urine. Based on the relatively short half-life of ethylene glycol in the blood and urine (Eder et al. 1998; Jacobsen et al. 1988; Peterson et al. 1981), parent compound would likely be detectable only within a few hours to 1 day following acute ingestion. Rapid methods for determining ethylene glycol in serum and urine are available for use in the clinical setting (Aarstad et al. 1993; Blandford and Desjardins 1994), but may not be readily available in emergency situations.

Other identified biomarkers of exposure are not specific to ethylene glycol. They include ethylene glycol metabolites such as glycolic, lactic, and oxalic acids in blood and/or urine; and calcium oxalate monohydrate crystals in renal tubules and/or urine.

Based on available information regarding the toxicokinetics of ethylene glycol and its metabolites, and available methods for identifying parent compound and metabolites in body fluids, it appears that ethylene glycol poisoning can be adequately diagnosed in most cases. Additional studies to assess additional potential biomarkers of exposure for ethylene glycol do not appear necessary at this time.

Effect. Biomarkers of effects exist for ethylene glycol poisoning, but none are specific to ethylene glycol. These include clinical manifestations of central nervous system, cardiopulmonary, and renal toxicity, and laboratory findings of metabolic acidosis and calcium oxalate crystalluria. Clinical manifestations progress in three main stages. Signs of central nervous system toxicity appear within 0.5–12 hours following acute ingestion, although manifestations suggestive of cranial nerve damage may appear as late as 1–2 weeks after exposure (CDC 1987; Cheng et al. 1987; Chung and Tuso 1989; Factor and Lava 1987; Hess et al. 2004; Leth and Gregersen 2005; Lewis et al. 1997; Mallya et al. 1986; Parry and Wallach 1974; Rothman et al. 1986; Spillane et al. 1991; Underwood and Bennett 1973; Zeiss et al. 1989). Cardiopulmonary manifestations generally develop after 12–24 hours and renal failure occurs after 24–72 hours (Godolphin et al. 1980; Hess et al. 2004; Leth and Gregersen 2005; Parry and Wallach 1974; Siew et al. 1975a; Vale 1979; Zeiss et al. 1989). Ethylene glycol-induced metabolic acidosis occurs approximately 12–24 hours following ingestion and is characterized by pronounced serum osmolal

responsible for metabolizing ethylene glycol and glycolic acid, inter-individual variability in metabolic parameters (e.g., polymorphisms in genes encoding these isozymes), and developmental ontogeny of these isozymes are needed to better characterize species differences and identify sensitive subpopulations. In addition, further information is needed on species differences in metabolic rates and saturation points, as available data provide conflicting information on the relative sensitivity of humans and laboratory rodents.

Because most human exposure has been associated with acute accidental or intentional poisoning incidents, there are few data on the elimination kinetics of ethylene glycol after oral exposure in humans. Most of the available estimates of plasma elimination half-lives have been confounded by concurrent therapeutic treatments such as ethanol administration or hemodialysis that modify elimination kinetics. Elimination of orally-administered ethylene glycol across a broad dose range has been thoroughly studied in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c), and to a more limited extent in monkeys (McChesney et al. 1971).

No data describing the kinetics of *in vivo* human dermal exposure were found in the literature. The *in vitro* permeability of human skin to ethylene glycol has been studied, with widely varying results. Using full-thickness cadaver skin, Loden (1986) estimated a percutanous absorption rate of 118 $\mu g/cm^2$/hour with a steady-state concentration of 0.97 mg/cm^2, while Driver et al. (1993) estimated absorption rates of 0.09–0.25 $\mu g/cm^2$/hour for three different skin samples. The absorption, distribution, metabolism, and elimination of ethylene glycol administered dermally has been thoroughly studied in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c).

All of the toxicokinetic data in humans and animals were collected after acute exposures to ethylene glycol; there are no data on toxicokinetics after intermediate- or chronic-duration exposures. Intermediate- and chronic-duration data are needed in order to adequately assess absorption, metabolism, and elimination with prolonged exposure. Acute duration studies following dermal exposure in humans are needed to characterize kinetics following this route of exposure.

Comparative Toxicokinetics. Species differences in *in vivo* toxicokinetics are not well characterized. While there are high quality toxicokinetic data comparing absorption, distribution, metabolism, and excretion in mice and rats (Frantz et al. 1989, 1991, 1996a, 1996b, 1996c), available data in other species (Hewlett et al. 1989; McChesney et al. 1971) are more limited; in many cases, only single dose levels were used, the numbers of animals per dose were small, and mass balance information was

Ethylene glycol has been identified in soil and sediment samples collected at 2 and 1 of the 37 NPL hazardous waste sites, respectively, where it was detected in some environmental media (HazDat 2007).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Ethylene glycol has a low vapor pressure (0.089 mm Hg at 25 °C) and is miscible with water (see Table 4-2). If released to the atmosphere (e.g., as vapors generated at elevated temperatures), ethylene glycol should exist almost entirely in the vapor phase (Eisenreich et al. 1981). The high solubility of ethylene glycol in water ensures that at least partial removal of the compound will occur by wet deposition. The low Henry's law constant value for this compound (6.00×10^{-8} atm-m^3/mole, see Table 4-2) suggests that ethylene glycol released to surface water will not partition to the atmosphere via volatilization (Simmons et al. 1976; Thomas 1990). Ethylene glycol is not expected to adsorb to sediment or soil particulates based on an estimated K_{oc} value of 1 (see Table 4-2). Based on the low K_{oc} value (see Table 4-2), ethylene glycol is expected to have a very high mobility in soil and could leach into groundwater (Swann et al. 1983).

The low octanol/water partition coefficient (K_{ow}) value of -1.36 (see Table 4-2) ~~suggest~~ suggests that bioconcentration and biomagnification of ethylene glycol are not likely to occur. Laboratory testing with this compound confirms insignificant bioconcentration in fish (Freitag et al. 1985). The bioconcentration factor (BCF) for ethylene glycol in fish (Golden ide) was 10 after 3 days of exposure.

Ethylene glycol is expected to be highly mobile, particularly in moist soils, and it may leach into groundwater upon release to surface soils. In laboratory studies, ethylene glycol was found to percolate rapidly through soil columns with little or no adsorption (LA DOTD 1990; Lokke 1984); however, rapid biodegradation is expected to limit the extent of leaching through soil (see Section 6.3.2.3). The compound may also volatilize from dry surface soils (EPA 1979, 1987a; Hine and Mookerjee 1975). In dry soils, ethylene glycol liquid can enter the soil system and travel through the porous media before contacting free water. Amoozegar et al. (1986) reported that in dry soils (<1% water) the rate of ethylene glycol movement was the slowest of 6 organic liquids tested (toluene, xylene, kerosene, acetone, and isopropyl alcohol).

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What happens to ethylene glycol when it enters the environment?

Released into air, water, and soil	The primary source of ethylene glycol in the environment is from run-off at airports where is used in de-icing agents for runways and airplanes. Ethylene glycol can also enter the environment through the disposal of products that contain it.
Quickly broken down	*Air:* Ethylene glycol in air will break down in about 10 days *Water and soil:* Ethylene glycol in water and in soil will breakdown within several days to a few weeks.

See Chapters 5 and 6 for more information on ethylene glycol in the environment.

How might I be exposed to ethylene glycol?

Antifreeze	The general public can be exposed to ethylene glycol through skin contact when using automobile antifreeze. Accidental or intentional ingestion can occur because antifreeze is a sweet tasting, brightly colored liquid.
Air, water, soil	Background concentrations of ethylene glycol in air, surface water, groundwater, drinking water, soil, and sediment have not been reported. Exposure to ethylene glycol in air, drinking water, or soil is not expected.
Workplace air	People who work in industries that use ethylene glycol may be exposed by touching products containing this substance Workers can also be exposed to low levels from ethylene glycol-containing products that have been sprayed into the air.

See Chapter 6 for more information on exposure to ethylene glycol.

How can ethylene glycol enter and leave my body?

Enters your body after ingestion, inhalation, or dermal contact	Ingested ethylene glycol is quickly and extensively absorbed. There is some information suggesting that inhaled ethylene glycol is also absorbed. It can also slowly enter your bloodstream through your skin if you come in direct contact with it and do not wash it off. More Toxic 1
Typically leaves your body within 1–2 days	Once in your body, most of the ethylene glycol is broken down into other chemicals and excreted in the urine. Some ethylene glycol is also excreted unchanged in the urine.

Is there a medical test to determine whether I have been exposed to ethylene glycol?

Analysis of blood and urine Ethylene glycol and its ~~metabolites~~ effects can be measured in blood and urine. The metabolites cause characteristic chemical changes in the blood and urine that help to diagnose ethylene glycol poisoning.

You should have these tests done within a few hours after exposure occurs because ethylene glycol leaves the body very quickly and early diagnosis is necessary for effective treatment.

The presence of crystals in the urine may indicate kidney damage.

Refer to Chapters 3 and 7 for more information on these tests.

What recommendations has the federal government made to protect human health?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it.

3.2.2.1 Death

OLD DATA - See comment sheet

~~The American Association of Poison Control Centers reported nine fatalities for 1989 and five for 1990 due to ethylene glycol ingestion (Litovitz et al. 1990, 1991~~). Several other fatal ethylene glycol poisonings have been reported in earlier studies, including seven case reports of deaths resulting from accidental or intentional ingestion of ethylene glycol or antifreeze containing 99% ethylene glycol (Godolphin et al. 1980; Gordon and Hunter 1982; Hewlett et al. 1986; Jacobsen et al. 1984; Siew et al. 1975a; Zeiss et al. 1989). A 22-year-old male who ingested 300 mL of antifreeze (approximately 4,071 mg/kg ethylene glycol) lapsed into a coma 24 hours after hospital admission and died 24 hours later (Siew et al. 1975a). A dose of 7,850 mg/kg can be estimated in the case of a 73-year-old male who consumed 500 mL of 95% ethylene glycol and died of myocardial failure after 68 hours (Gordon and Hunter 1982). In five other fatal cases of accidental or intentional poisoning, the amount of ingested ethylene glycol ranged from 150 to 1,500 mL (2,379–23,786 mg/kg) (Karlson-Stiber and Persson 1992; Walton 1978). Thus, oral dose of ethylene glycol required to cause death in humans is not well defined in the literature. The minimum lethal dose for adults is thought to be 1.4 mL/kg of 95% ethylene glycol, or about 1,330 mg ethylene glycol/kg body weight (Parry and Wallach 1974; Robinson and McCoy 1989; Siew et al. 1975a).

A single dose oral LD_{50} of 4,000 mg/kg was determined in Female F344 rats (Clark et al. 1979). Male Wistar rats administered 12,900 mg/kg ethylene glycol in a single oral dose had 55% mortality within 48 hours (Richardson 1973). Pregnant CD-1 mice given 11,090 mg/kg/day ethylene glycol orally on Gd 7–14 showed 10% mortality (Schuler et al. 1984) and pregnant rabbits exhibited 42% mortality after receiving 2,000 mg/kg/day ethylene glycol orally on Gd 6–19 (Tyl et al. 1993). Cats administered a single 4,440–8,880 mg/kg dose by gavage had 100% mortality within 20–36 hours (Penumarthy and Oehme 1975). A single gavage dose of 4,180–12,540 mg/kg/day caused 17–100% mortality in dogs within 72 hours (Kersting and Nielsen 1965). Dogs administered a single oral dose of 4,880 mg/kg in food had 100% mortality within 6 days (Beckett and Shields 1971).

Intermediate-duration dietary exposure to 1,000 mg/kg/day for 16 weeks caused 20% mortality in male Wistar rats, with no deaths occurring in similarly treated male F344 rats; females were not tested (Cruzan et al. 2004). Male F344/N rats fed 5,000 mg/kg/day ethylene glycol had 40% mortality after 13 weeks, whereas similarly treated females did not die (Melnick 1984). A chronic dietary study of ethylene glycol in Sprague-Dawley found 100% mortality after 12–24 months in males at 750 mg/kg/day and females at

Goldfrank LR, Flomenbaum NE, Lewin NA, et al. 2002. Goldfrank's toxicologic emergencies. 7th ed. New York, NY: McGraw-Hill Companies, Inc., 980-1003.

Mégarbane B, Borron SW, Baud FJ. 2005. Current recommendations for treatment of severe toxic alcohol poisonings. Intensive Care Med 31(2):189-195.

Scalley RD, Ferguson DR, Piccaro JC, et al. 2002. Treatment of ethylene glycol poisoning. Am Fam Physician 66(5):807-812.

White ML, Liebelt EL. 2006. Update on antidotes for pediatric poisoning. Pediatr Emerg Care 22(11):740-749.

3.11.1 Reducing Peak Absorption Following Exposure

or activated charcoal (oral dose one gram/kg)

No studies were found describing methods to reduce peak absorption of ethylene glycol after inhalation exposure. After oral exposure, gastric lavage may be of benefit in reducing absorption, but only if performed within 1–2 hours following ingestion (Barceloux et al. 1999; Egbert and Abraham 1999; Leth and Gregersen 2005). Administration of syrup of ipecac is contraindicated due to central nervous system depression (Barceloux et al. 1999; Leth and Gregersen 2005). ~~Activated charcoal is not effective at reducing the absorption of ingested ethylene glycol because it does not bind clinically significant amounts of ethylene glycol, which is well absorbed within 30 minutes of ingestion when taken in large quantities.~~ Dermal absorption can be minimized through washing the skin with soap to remove any existing ethylene glycol.

3.11.2 Reducing Body Burden

Clinical procedures for treating ethylene glycol poisoning focus on reduction of the body burden of ethylene glycol and its toxic metabolites, interference with toxic metabolite formation (which results in increased urinary excretion of parent compound), increased elimination of toxic metabolites produced, reduction of metabolic acidosis, and prevention of kidney failure. Procedures include administration of antidotes (ethanol or fomepizole), intravenous bicarbonate and hydration for profound acidemia, and hemodialysis for refractory acidosis (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005; Scalley et al. 2002).

Antidotes for ethylene glycol include the alcohol dehydrogenase inhibitors, ethanol and fomepizole, which act to decrease the alcohol dehydrogenase-catalyzed metabolism of ethylene glycol, thus effectively increasing the urinary excretion of ethylene glycol. Ethanol competes with ethylene glycol for alcohol dehydrogenase receptor sites and fomepizole acts as a potent inhibitor of alcohol dehydrogenase

(Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005). Antidotal therapy is indicated if ethylene glycol blood levels exceed 200 mg/L (Gardner et al. 2004). Fomepizole treatment has repeatedly been demonstrated to be an effective therapy for ethylene glycol poisoning without obviating hemodialysis (Boyer et al. 2001; Caravati et al. 2004; Harry et al. 1998; Pizon and Brooks 2006; White and Liebelt 2006).

Intravenous fluid administration may be initiated early to increase urine output, which effectively increases the excretion of ethylene glycol and toxic metabolites such as glycolic and oxalic acids (Egbert and Abraham 1999). Sodium bicarbonate infusion is used to correct metabolic acidosis, increase elimination of renal glycolic acid, and inhibit the precipitation of calcium oxalate crystals, although the latter benefit has not been demonstrated in clinical trials (Egbert and Abraham 1999; Leth and Gregersen 2005; Scalley et al. 2002).

Hemodialysis is indicated when serum ethylene glycol levels exceed ~~20~~ 50 mg/dL or when ingestion of ethylene glycol results in refractory acidosis, deteriorating clinical status, or renal compromise (Egbert and Abraham 1999). Hemodialysis can effectively remove ethylene glycol and the acid metabolites, glycolic and oxalic acids, because they have low molecular weights and do not exhibit protein binding (Egbert and Abraham 1999). Hemodialysis is also effective in treating metabolic acidosis (Leth and Gregersen 2005; Scalley et al. 2002).

Thiamine (vitamin B_1), and pyroxidine (vitamin B_6), ~~and magnesium~~ are co-factors for the metabolism of ethylene glycol. Thiamine is believed to reduce the formation of toxic oxalic acid by shifting glyoxylic acid metabolism to the less toxic α-hydroxy-β-ketoadipic acid (Egbert and Abraham 1999; Goldfrank 2002). Pyroxidine, in the presence of magnesium, may promote the conversion of glyoxylic acid to glycine and benzoic acid, which also results in reduced toxic oxalic acid formation (Egbert and Abraham 1999; Gardner et al. 2004; Goldfrank 2002; Leth and Gregersen 2005; Scalley et al. 2002). However, the efficacy of treatment with thiamine, pyroxidine, ~~and magnesium~~ and has not been demonstrated in human cases of ethylene glycol poisoning.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

There are no documented methods for interfering with mechanisms of action for toxic effects of ethylene glycol and its potent metabolites. As described in Section 3.11.2, clinical procedures for treating ethylene glycol poisoning consist of measures focused on reduction of the body burden of parent compound and its

incomplete. Available data in humans are limited to acute, high-dose exposures, with toxicokinetic data often confounded by the effects of therapeutic interventions.

Using a PBPK model for humans, Corley et al. (2005a) estimated that the threshold glycolic acid concentration for developmental effects in rodents (considered by the authors to be a peak of 2 mM) would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). However, the human model has not been calibrated to the physiological changes associated with pregnancy.

Slikker et al. (2004) reported that there are species-specific differences in the transfer of glycolic acid, the primary metabolite and putative developmental toxicant associated with ethylene glycol exposure, from maternal blood to conceptus. NTP-CERHR (2004) noted that the inverted yolk sac placenta that develops in both mice and rats tends to concentrate weak acids including glycolic acid; neither humans nor rabbits develop a yolk sac placenta, and a preliminary study by Carney and coworkers showed that glycolic acid does not concentrate in rabbit embryonic fluids. In addition, fetal and/or placental differences in expression of enzymes metabolizing ethylene glycol and glycolic acid over the course of gestation will affect local concentrations of glycolic acid to which the developing conceptus is exposed, yet little is known about species differences in the ontogeny of these enzymes (NTP-CERHR 2004).

Additional data are needed to reduce uncertainty in the saturation concentration of glycolic acid in humans. Although a comparison of Km values obtained using liver homogenates from female humans and Sprague-Dawley rats suggested that humans may metabolize glycolic acid more efficiently than rats (0.19 and 0.79 mM for humans and rats, respectively; Bartels 2001), a more recent study suggested the opposite. Booth et al. (2004) reported Km values of 0.43 and 0.28 mM (humans and rats, respectively) from a study using human and rat liver slices; these data suggest less efficient metabolism in humans.

Methods for Reducing Toxic Effects. No studies were found describing methods to reduce peak absorption of ethylene glycol after inhalation exposure. After oral exposure, gastric lavage may be of benefit in reducing absorption, but only if performed within 1–2 hours following ingestion (Barceloux et al. 1999; Egbert and Abraham 1999; Leth and Gregersen 2005). Dermal absorption can be minimized through washing the skin with soap to remove any existing ethylene glycol.

Clinical procedures for treating ethylene glycol poisoning focus on reducing the body burden of ethylene glycol and its toxic metabolites, interference with toxic metabolite formation (which results in increased

urinary excretion of parent compound), increased elimination of toxic metabolites produced, reduction of metabolic acidosis, and prevention of kidney failure. Procedures include administration of antidotes (ethanol or fomepizole), intravenous bicarbonate and hydration for profound acidemia, and hemodialysis for refractory acidosis (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005; Scalley et al. 2002).

Antidotes for ethylene glycol include the alcohol dehydrogenase inhibitors, ethanol and fomepizole, which act to decrease the alcohol dehydrogenase-catalyzed metabolism of ethylene glycol, thus effectively increasing the urinary excretion of ethylene glycol (Barceloux et al. 1999; Egbert and Abraham 1999; Gardner et al. 2004; Leth and Gregersen 2005).

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Hemodialysis can effectively remove ethylene glycol and the acid metabolites, glycolic and oxalic acids, because they have low molecular weights and do not exhibit protein binding (Egbert and Abraham 1999).

Thiamine (vitamin B1), pyroxidine (vitamin B6), ~~and magnesium~~ are co-factors for the metabolism of ethylene glycol and may reduce toxicity by assisting in the formation of relatively nontoxic metabolites (Egbert and Abraham 1999; Gardner et al. 2004; Goldfrank 2002; Leth and Gregersen 2005; Scalley et al. 2002). However the efficacy of treatment with thiamine, pyroxidine, ~~and magnesium~~ has not been demonstrated in human cases of ethylene glycol poisoning.

There are no documented methods for interfering with mechanisms of action for toxic effects of ethylene glycol and its potent metabolites.

Additional information that might be useful in treating ethylene glycol poisoning include studies designed to identify additional methods to reduce the body burden of ethylene glycol and its toxic metabolites and studies designed to elucidate methods for interfering with mechanisms of action.

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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ETHYLENE GLYCOL IN THE UNITED STATES

Ethylene glycol is a colorless, odorless liquid that is miscible with water (HSDB 2007). It is released into the environment primarily through industrial emissions and through the use and disposal of ethylene glycol-based automobile antifreeze and airport de-icing formulations (Corsi et al. 2001; EPA 2000; Sills and Blakeslee 1992; Ware 1988). Ethylene glycol that is released into the environment does not persist since it is degraded within days to a few weeks in air, water, and soil (Atkinson 1989; Battersby and Wilson 1989; Conway et al. 1983; Kameya et al. 1995; McGahey and Bouwer 1992; Revitt and Worrall 2003; Schoenberg et al. 2001; Staples et al. 2001). Available monitoring data indicate that ethylene glycol is only found near areas of release. Ethylene glycol vapor concentrations measured in the air at airports during de-icing spray operations ranged from 0.05 to 22 mg/m^3 (Gérin et al. 1997; LA DOTD 1990). Ethylene glycol concentrations as high as 19,000 mg/L have been measured in airport stormwater (Sills and Blakeslee 1992). Background concentrations of ethylene glycol in the environment are not available.

Since ethylene glycol is not expected to be present away from areas where it is released, background exposure of the general population to this substance is not expected to be important. The most common route of exposure to ethylene glycol for the general population is through dermal contact with ethylene glycol-containing automobile antifreeze. However, accidental or intentional ingestion of antifreeze is the most serious route of exposure, resulting in thousands of poisonings reported each year in the United States (~~Fraser 2002; Leth and Gregersen 2005~~). Ethylene glycol concentrations in blood, urine, tissue, or breast milk are not available for the general population. Litovitz et al 2002

Individuals who live near hazardous waste sites, industrial facilities where ethylene glycol is produced or used, or areas where ethylene glycol-based de-icing formulations are used may be exposed to ethylene glycol through dermal contact with contaminated soil or water, inhalation of ethylene glycol vapor or mist, or ingestion of contaminated groundwater. Occupational exposure through dermal contact and inhalation of ethylene glycol vapor or mist is expected for individuals involved in airport de-icing spray operations. Ethylene glycol has been detected in urine samples collected from airport de-icing workers (Gérin et al. 1997).

Ingestion of ethylene glycol containing antifreeze is a potential route of exposure for children since they are attracted to the bright colors of antifreeze formulations and the sweet taste of ethylene glycol (Leth and Gregersen 2005). Exposure through ingestion is more likely to occur when adults leave opened antifreeze containers within reach or store antifreeze in other types of containers such as beverage bottles. A bittering agent has been added to some ethylene glycol antifreeze formulations in order to deter ingestion; however, caution should still be used since ingestion poisoning has occurred even when a bittering agent was present (Harry et al. 1998; Hogue 2006).

2.2 SUMMARY OF HEALTH EFFECTS

Ethylene glycol is quickly and extensively absorbed through the gastrointestinal tract of many species, but dermal absorption is slow in rodents and is expected to be slow in humans. Limited information is available on absorption of inhaled ethylene glycol, but the existing toxicity studies suggest absorption via the respiratory tract by both humans and rodents. Following absorption, ethylene glycol is distributed in aqueous compartments throughout the body. Ethylene glycol is initially metabolized to glycoaldehyde by alcohol dehydrogenase (with possible contribution from cytochrome P-450 enzymes). Glycolaldehyde is rapidly converted to glycolate and glyoxal by aldehyde oxidase and aldehyde dehydrogenase. Metabolism of glycolate by glycolate oxidase or lactate dehydrogenase results in the formation of glyoxylate, which may be further metabolized to formate, oxalate, glycine, and carbon dioxide. Elimination of ethylene glycol occurs via exhaled carbon dioxide and urinary elimination of both ethylene glycol and glycolic acid. The half-life for elimination in humans has been estimated to be in the range of 2.5–8.4 hours (NTP-CERHR 2004).

The vast majority of information relating to the toxicity of ethylene glycol is from studies of oral exposure. Information on the health effects of oral exposure in humans is largely limited to case reports of acute accidental or intentional ingestion of ethylene glycol. These case reports have identified three stages of acute oral ethylene glycol toxicity in humans. These stages are well documented and occur within 72 hours after ingestion (NTP-CERHR 2004; Robinson and McCoy 1989; Vale 1979). The first stage involves central nervous system depression, metabolic changes (hyperosmolality and acidosis), and gastrointestinal upset, and spans the period from 30 minutes to 12 hours. During the second stage (12–24 hours after ingestion), cardiopulmonary symptoms (tachypnea, hyperpnea, tachycardia, cyanosis, pulmonary edema, and/or cardiac failure) due to metabolic acidosis become evident. During stage three, which covers the period 24–72 hours after ethylene glycol ingestion, renal involvement becomes evident. The third stage is characterized by flank pain and oliguria/anuria. Histopathological findings show renal

parameters have largely been observed at high doses in longer-term studies, and are not consistently reported across studies or across species.

Oral studies in animals have identified the developing fetus as the most sensitive target for acute-duration exposure to ethylene glycol. Gavage exposure of laboratory rodents to ethylene glycol during gestation results in a consistent pattern of developmental effects including reduced fetal body weight and increases in malformations, particularly axial skeletal malformations (Neeper-Bradley 1990; Neeper-Bradley et al. 1995; Price et al. 1985). Developmental toxicity has also been assessed by the inhalation and dermal routes. Results of the inhalation developmental studies are generally consistent with the oral findings, but are confounded by concurrent oral exposure via ingestion of aerosolized ethylene glycol on the fur of exposed animals (Tyl 1985, 1988a; Tyl et al. 1995a, 1995b). A single study of dermal exposure to ethylene glycol in pregnant mice did not indicate developmental effects (Tyl 1988b; Tyl et al. 1995c).

The kidney is clearly identified as the most sensitive target organ in rats and mice after intermediate-duration oral exposure. Typical renal effects included oxalate crystal deposition and renal tubular dilation, vacuolation, and degeneration. Oxalate, a metabolite of glycolic acid, forms a precipitate in the presence of calcium, and the deposition of these crystals in the renal tubules are hallmarks of ethylene glycol renal toxicity. ~~Additionally, the buildup of glycolic acid in the body can result in metabolic acidosis, leading ultimately to renal failure (LaKind et al. 1999)~~. Males were more sensitive than females, and rats were more sensitive than mice. Chronic oral studies confirm that the kidney is a main target organ in male rats, although liver ~~lesions~~ occurred in female rats (slight fatty metamorphosis) ~~and male mice (hepatocellular hyaline degeneration)~~ at doses lower than those inducing kidney effects (~~Blood 1965~~; DePass et al. 1986a; ~~NTP 1993~~). No hepatic effects were observed in intermediate-duration studies.

There is no indication that ethylene glycol is carcinogenic based on results of a limited renal cancer mortality study in chemical plant workers (Bond et al. 1985) and well-designed chronic oral bioassays in rats (one study) and mice (two studies) (DePass et al. 1986a; NTP 1993).

A more detailed discussion of the developmental and renal effects associated with ethylene glycol exposure follows. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for additional information on these and other health effects.

Developmental Effects. No studies have addressed the developmental toxicity of ethylene glycol in humans. The developmental toxicity of ethylene glycol in animals has been assessed by inhalation, oral, and dermal exposure in acute-duration studies and by oral exposure in intermediate-duration studies. The acute oral studies indicate that developmental effects (a skeletal variation and total malformations) occur at doses of ≥500 mg/kg/day when administered by gavage during gestation days (Gd) 6–15 to CD-1 mice (Neeper-Bradley et al. 1995; Tyl 1989). Dose-response data for these developmental effects in mice were used to derive an acute-duration oral MRL for ethylene glycol (see Section 2.3). Reduced fetal body weight occurred in mice given gavage doses of ≥750 mg/kg/day (Price et al. 1985). In rats, doses of ≥1,000 mg/kg/day by gavage on Gd 6–15 have resulted in increased incidences of skeletal malformations (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). No teratogenic effects were observed in rabbits exposed to maternally lethal oral doses of 2,000 mg/kg/day during gestation (Tyl et al. 1993). In the only dermal exposure study, no developmental toxicity occurred in pregnant CD-1 mice that were treated with 6-hour daily exposures to ethylene glycol (estimated doses up to 3,549 mg/kg/day) by occluded cutaneous application on Gd 6–15 (Tyl et al. 1993).

Developmental toxicity studies of inhaled ethylene glycol in mice and rats found effects consistent with the oral findings, but all of the studies are confounded by concurrent ingestion of ethylene glycol deposited on the fur. In inhalation studies using whole-body exposure, significant effects on implant viability, weight of live fetuses, and incidence of external, visceral, and skeletal malformations were observed in mice exposed to ≥1,000 mg/m^3 for 6 hours/day on Gd 6–15 (Tyl 1988a; Tyl et al. 1995a). In rats exposed similarly, reduced ossification at some sites in the axial skeleton occurred at ≥1,000 mg/m^3 (Tyl 1985; Tyl et al. 1995a); however, in an Expert Panel Review, the National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR 2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. In a follow-up study aimed at reducing the confounding oral exposure, pregnant CD-1 mice were exposed nose-only to 500–2,500 mg/m^3 aerosolized ethylene glycol (Tyl 1988a; Tyl et al. 1995b). At 2,500 mg/m^3, live fetal body weight was significantly reduced, and there was a significant increase in the one type of skeletal malformation (fused ribs). Increases in some skeletal variations were also observed at 2,500 mg/m^3, and one type (extra ossification sites in the sagittal suture) was significantly increased at ≥500 mg/m^3. The authors observed that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face (Tyl 1988a; Tyl et al. 1995a). Furthermore, NTP-CERHR (2004) noted that stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol, which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994). Because of the confounding

oral exposure in both the whole-body and nose-only experiments, NTP-CERHR (2004) concluded that the data from these studies were not suitable for evaluation of effect levels from inhalation exposure to ethylene glycol.

Developmental effects of intermediate-duration oral exposure to ethylene glycol include kidney effects in offspring and decreased pup body weights. In mice tested in a continuous breeding assay, pup body weights were reduced in both F_1 and F_2 generations at drinking water doses of ≥897 mg/kg/day (Morrissey et al. 1989; NTP 1986). In a 15-day gestational exposure study, postnatal effects in kidney weights were observed in pups of CD rats exposed to gavage doses of ≥1,250 mg/kg/day *in utero* (NTP 1988). In a three-generation study of rats, no effects on gestation survival or pup body weight through postpartum day (ppd) 21 were observed in F_1 or F_2 pups after parental exposure to dietary doses up to 1,000 mg/kg/day (DePass et al. 1986b).

Recent reviews of mechanistic studies on ethylene glycol developmental toxicity (NTP-CERHR 2004; Slikker et al. 2004) have concluded that glycolic acid, alone or in combination with its downstream metabolites and resultant metabolic acidosis, was likely the proximate toxicant responsible for the developmental effects of ethylene glycol. Using a physiologically based pharmacokinetic (PBPK) model developed for humans, Corley et al. (2005a) estimated that the glycolic acid blood threshold concentration for developmental effects in rodents would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). While the model has been validated against data from acute human oral and inhalation exposures to ethylene glycol (Corley and McMartin 2005; Corley et al. 2005a), it has not been calibrated to the physiological changes associated with pregnancy, which require a different model structure (EPA 2006a); ~~thus~~, ~~the usefulness of~~ this model in predicting developmental toxicity in humans ~~is limited~~. ~~Further~~, NTP-CERHR (2004) noted that additional data were needed to fully delineate the rate of glycolic acid metabolism in humans; such additional data may alter the model predictions of peak glycolic acid concentrations in humans exposed to ethylene glycol.

because these changes are not likely to alter EG kinetics (Pottenger et al., 2001)

However

may be useful

Renal Effects. The renal toxicity of ethylene glycol in humans is well documented in numerous case reports of accidental or intentional ingestion. Adverse renal effects occur in the third stage of human ethylene glycol poisoning, which occurs 24–72 hours after acute exposure. The hallmark of renal toxicity is the presence of calcium oxalate monohydrate crystals in the renal tubules and urine following ingestion of large amounts of ethylene glycol (Blakeley et al. 1993; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980). Characteristic histopathological changes include renal tubular focal degeneration, atrophy, and interstitial inflammation (Factor and Lava 1987). Renal damage, if untreated, can lead to

nephrotoxicity (Cruzan et al. 2004), and that kidney lesions in male Wistar rats occurred at doses ~~as low~~ ≥ 180 mg/kg/day (Gaunt et al. 1974). [Dose-response data for kidney lesions in male Wistar rats (~~Gaunt et al. 1974~~ Cruzan et al. 2004) were used to derive an intermediate-duration oral MRL for ethylene glycol (see Section 2.3).] Disagree as discussed In a 13-week dietary study in B6C3F1 mice, effects were observed in the kidneys (minimal to mild tubule dilation, cytoplasmic vacuolation, and regenerative hyperplasia, without tubular oxalate crystal deposition) of males at ≥6,450 mg/kg/day, with no renal effects in females at doses ≤16,000 mg/kg/day (Melnick 1984; NTP 1993).

Chronic toxicity studies provide information on renal effects in rats and mice exposed to ethylene glycol in the diet for up to 2 years. Males were more sensitive than females and rats were more sensitive than mice, ~~although none of the studies tested Wistar rats, the strain shown to be particularly sensitive to ethylene glycol nephrotoxicity following intermediate duration exposure~~. Renal effects ~~in rats~~ also included oxalate crystal deposition and apparent tubular degenerative changes in Sprague-Dawley male rats at ≥375 mg/kg/day and females at ≥750 mg/kg/day, (Blood 1965), and oxalate nephrosis (and consequent mortality) in F344 males at 1,000 mg/kg/day, with changes in F344 females at this dose limited to increased kidney weight and crystalluria without histopathology (DePass et al. 1986a). No kidney histopathology occurred in male or female CD-1 mice exposed to 1,000 mg/kg/day (DePass et al. 1986a) or female B6C3F1 mice exposed to ≤12,000 mg/kg/day (NTP 1993), and effects in male B6C3F1 mice were limited to small numbers of oxalate-like crystals and/or calculi in the renal tubules, urethrae, and urinary bladder in a few animals at 6,000 mg/kg/day (NTP 1993).

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for ethylene glycol. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

(BMR) lower than 10%; thus, an extra risk incidence of 10% above controls was selected as the BMR. The multistage and quantal linear models converged on the same model providing the best fit to the data on total malformations; these models both predicted a BMD_{10} of 113.84 mg/kg/day and a $BMDL_{10}$ of 75.59 mg/kg/day. For the data on bilateral extra rib 14, the probit model provided the best fit, and predicted a BMD_{10} of 99.35 mg/kg/day and a $BMDL_{10}$ of 75.56 mg/kg/day. Modeling of both the malformation and skeletal variation end points resulted in the same $BMDL_{10}$, indicating that an acute oral MRL based on this point of departure should provide protection against both effects. The $BMDL_{10}$ of 76 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive an MRL of 0.8 mg/kg/day for acute-duration oral exposure to ethylene glycol.

Although some mechanistic information suggests that humans may be less sensitive than rodents to the developmental effects of ethylene glycol, the available data are not adequate to support a lower interspecies uncertainty factor; thus, a full 10-fold uncertainty factor was used for interspecies extrapolation. ~~While one~~ Although studies suggested that humans metabolize glycolic acid (the proximate developmental toxicant) more efficiently than rats (~~Bartels 2001~~ Corley et al. 2005a; ~~other data conflict with this finding~~ Booth et al. 2004), NTP-CERHR (2004) observed that the data supporting the glycolic acid metabolic rate in humans are limited. In addition, NTP-CERHR (2004) reviewed preliminary data indicating that the inverted yolk sac placenta, a stage in placental development that does not exist in humans, tends to concentrate weak acids such as glycolic acid in the embryonic fluids. These data suggest enhanced sensitivity to ethylene glycol developmental effects in rodents compared with humans; however, NTP-CERHR (2004) characterized the available data as inconclusive. A 10-fold uncertainty factor for human variability was also used. Ethylene glycol metabolism is known to involve alcohol dehydrogenase and aldehyde dehydrogenase, and may also involve cytochrome p450 isozymes (NTP-CERHR 2004). Polymorphisms in the genes encoding these enzymes may lead to wide variability in the production and elimination of glycolic acid and other metabolites in humans exposed to ethylene glycol, but data quantifying the range of variability are not currently available (NTP-CERHR 2004). In addition, fetal and/or placental differences in expression of these enzymes over the course of gestation will affect local concentrations of glycolic acid and other metabolites to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004).

A human PBPK model for ethylene glycol has been developed (Corley et al. 2005a), but the model has not been calibrated to the physiological changes of pregnancy ~~and thus is not suitable for use in deriving an acute oral MRL based on developmental toxicity~~. In addition, Corley et al. (2005a) published a PBPK

model for rats, but no model has yet been developed for mice, the species used in the study selected for MRL derivation. Finally, NTP-CERHR (2004) noted ~~a critical~~ some uncertainty in the database on ethylene glycol toxicokinetics: there are limited data to delineate the glycolic acid metabolic rate in humans. As a result, available data do not support the use of PBPK modeling to derive an acute oral MRL for ethylene glycol based on developmental toxicity in mice.

A key uncertainty in the acute-duration oral MRL stems from the use of gavage administration in the critical study. Bolus doses from gavage administration ~~can~~ lead to higher peak concentrations of glycolic acid in the blood than ~~would~~ occur with slower dose-rates associated with equal doses at environmentally-relevant exposures (Carney et al. 2001; NTP-CERHR 2004). Because the key study used gavage administration, the dose at which effects were observed ~~may have been~~ is lower than would be observed with non-bolus dosing (Maronpot et al, 1983; Neeper-Bradley et al, 1985)

- An MRL of 0.7 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to ethylene glycol.

Information on the toxicity of intermediate-duration oral exposure to ethylene glycol essentially consists of several well-designed studies in rats (Cruzan et al. 2004; Gaunt et al. 1974; Melnick 1984; Robinson et al. 1990) and mice (Melnick 1984; NTP 1993). Based on generally comprehensive evaluations that included body and organ weights, food and water consumption, hematology, blood chemistry, urinalysis, and histopathology in adequate numbers of animals, these studies consistently showed that the kidney is the predominant and most sensitive target of ethylene glycol toxicity. As summarized below, renal toxicity varied with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats.

Renal effects in Sprague-Dawley rats that were exposed to ethylene glycol in drinking water for 90 days included renal tubular oxalate crystal deposition, dilation, and degeneration in males at ≥947 mg/kg/day and females at 3,087 mg/kg/day (Robinson et al. 1990). Key findings in F344 rats exposed for 13 weeks via diet consisted of renal tubular dilation, necrosis, fibrosis, and oxalate crystal deposition in males at ≥2,500 mg/kg/day, mortality in males at 5,000 mg/kg/day, and mild renal lesions (e.g., inflammation and vacuolation) with no crystal deposition or mortality in females at 10,000 mg/kg/day (Melnick 1984). Results of 16-week dietary studies showed that male Wistar rats are approximately twice as sensitive as male F344 rats to ethylene glycol nephrotoxicity (Cruzan et al. 2004), and that kidney lesions in male Wistar rats occurred at doses as low as ~~1,000~~ 500 mg/kg/day (~~Gaunt et al. 1974~~). In a 13-week dietary study in B6C3F1 mice, effects were observed in the kidneys (minimal to mild tubule dilation, cytoplasmic

1,000 mg/kg/day. Due to the higher incidence and greater severity of the crystal nephropathy, as well as the accompanying impairment of kidney function (i.e., compromised kidney water regulation as indicated by increased urine volume and decreased urine specific gravity leading to increased water consumption), 500 mg/kg/day is a serious LOAEL in the Wistar rats. The only effect observed at doses lower than 500 mg/kg/day was calcium oxalate crystals in the urine of both strains at 150 mg/kg; this is a NOAEL because excretion of crystals in the urine reflects a detoxification process and is not considered adverse in the absence of crystal deposition in the renal tubule epithelium and associated histopathology.

The 16-week study by Gaunt et al. (1974) exposed male and female weanling Wistar rats to diets containing 0, 0.05, 0.1, 0.25, or 1.0% ethylene glycol for 2 weeks (5/sex/dose), 6 weeks (5/sex/dose), or 16 weeks (15/sex/dose). Reported calculated average daily chemical intakes were 35, 71, 180, and 715 mg/kg/day in males, and 0, 38, 85, 185, and 1,128 mg/kg/day in females. Survival, clinical signs, food and water intake, and body weight were evaluated throughout the exposure period. Hematology (hemoglobin, hematocrit, packed cell volume, total erythrocytes, reticulocytes, total and differential leukocytes), serum chemistry (urea, glucose, protein, albumin, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and lactic dehydrogenase), organ weights (including kidneys, liver, spleen, brain, heart, stomach, small intestines, caecum, adrenals, pituitary, thyroid, and gonads), and histology (organs that were weighed and 19 additional tissues) were evaluated at the 2-, 6-, and 16-week sacrifices. Urinalysis (glucose, ketones, bile salts, blood, protein, and presence of oxalic acid crystals, cells and other microscopic constituents) and renal function (urine concentration and dilution tests measuring volume and specific gravity, and cell excretion) were evaluated at weeks 2 and 16. Urine was additionally analyzed for oxalic acid at weeks 2, 6, 12, 14, and 16. There were no clear exposure-related effects on survival, clinical signs, body weight, hematology, or serum chemistry. Urinary excretion of oxalic acid was significantly increased in males at 715 mg/kg/day at weeks 2–16 and in females at 1,128 mg/kg/day at weeks 6–16, with the magnitude of the effect markedly greater in males (100–500% of control levels) than females (40–100% of control values). Increased absolute kidney weight, oxalic acid crystals in urine, and excretion of a larger volume of urine with a lower specific gravity after a prolonged period (16 hours) without water were observed in the 715 mg/kg/day males at week 16. Exposure-related histopathologic changes occurred only in the kidneys. Incidences of kidney lesions were statistically significantly increased in males at ≥180 mg/kg/day. Specific renal histopathologic findings in the males at 16 weeks included individual nephrons with degenerative changes (incidences of 0/15, 1/15, 1/15, 2/15, and 5/15 [$p<0.05$] in the control to high-dose groups), individual nephrons with degenerative changes and occasional oxalate crystals (0/15, 0/15, 0/15, 1/15, and 4/15 [$p<0.05$]), and generalized tubular damage and heavy oxalate crystals (0/15, 0/15, 0/15, 0/15, and 4/15 [$p<0.05$]). At 0,

35, 71, 180, and 715 mg/kg/day, the total incidence of male rats with oxalate crystals was 0/15, 0/15, 0/15, 1/15, and 10/15 ($p<0.001$), and the total incidence of male rats with renal tubular damage was 0/15, 1/15, 1/15, 4/15 ($p<0.05$), and 15/15 ($p<0.001$). Females had an increased incidence of renal tubular damage at 1,128 mg/kg/day, but the increase was not statistically significant. The histological evaluations of the kidneys in the five rats/sex/dose exposed for 2 or 6 weeks showed no statistically significant increases in incidences of specific changes, although the total incidence of animals with tubular damage was significantly increased in the 715 mg/kg/day males at 6 weeks. Based on the 16-week kidney histopathology data in male Wistar rats, this study identified a NOAEL of 71 mg/kg/day and LOAEL of 180 mg/kg/day for intermediate-duration exposure.

The 16-week studies of Cruzan et al. (2004) ~~and Gaunt et al. (1974) are~~ is appropriate for MRL consideration because ~~they~~ it provide dose-response data for the critical effect in the most sensitive species, strain and sex (i.e., kidney lesions in male Wistar rats). The NOAEL and LOAEL were 150 and 500 mg/kg/day in the Cruzan et al. (2004) study and 71 and 180 mg/kg/day in the Gaunt et al. (1974) study. The ~~Gaunt et al. (1974)~~ Cruzan et al (2004) study is selected as the basis for MRL derivation because it identified the ~~lowest LOAEL and is better suited for BMD analysis due to a larger number of dose levels in the lower dose range (below the 150–180 mg/kg/day threshold region)~~ highest NOAEL and was a study with fewer confounding factors.

insert sentences

this will have to be changed for the Cruzan study

To derive a point of departure for MRL derivation, BMD dose modeling was conducted using incidences of kidney lesions in male Wistar rats from the Gaunt et al. (1974) study. All available dichotomous models in the EPA Benchmark Dose Software (version 1.4.1) were fit to the data and predicted doses associated with a 10% extra risk were calculated. All models adequately fit the incidence data, although the best fit was provided by the probit model, which predicted a BMD_{10} of 107.75 mg/kg/day and a $BMDL_{10}$ of 74.51 mg/kg/day. The $BMDL_{10}$ was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to derive an intermediate-duration oral MRL of 0.7 mg/kg/day for ethylene glycol.

An MRL has ~~not~~ been derived for chronic-duration oral exposure (365 days or more) to ethylene glycol. The chronic oral toxicity of ethylene glycol was evaluated in ~~two~~ three studies in rats (Blood 1965; DePass et al. 1986a; Wilson et al., 2005) and two studies in mice (DePass et al. 1986a; NTP 1993). As summarized below, the kidney and liver were the main target organs in both species, and rats were more sensitive than mice. ~~Chronic testing has not been conducted in Wistar rats, a strain shown to be particularly sensitive to ethylene glycol nephrotoxicity following intermediate-duration exposure.~~

In the female F344 rats, effects occurred in kidneys and lymph nodes at 1,000 mg/kg/day and liver at ≥200 mg/kg/day (DePass et al. 1986a). Renal effects in females were limited to increased kidney weight and calcium oxalate crystals and uric acid crystals in the urine at 1,000 mg/kg/day; no kidney histopathology or mortality occurred as in males (DePass et al. 1986a). Hemosiderosis in the mesenteric lymph nodes was increased at 1,000 mg/kg/day. Hepatic effects included increases in mononuclear cell infiltrates at 1,000 mg/kg/day and fatty metamorphosis (slight) at ≥200 mg/kg/day. Total incidences of liver fatty metamorphosis in the 0, 40, 200, and 1,000 mg/kg/day females were 34/256, 16/129, 27/125, and 35/128, respectively; the increases at 200 and 1,000 mg/kg/day were statistically significant. [A NOAEL of 40 mg/kg/day and LOAEL of 200 mg/kg/day were identified in female F344 rats based on liver histopathology.] see comments about possible changes.

CD-1 mice (80/sex/dose) were fed ethylene glycol in approximate dietary doses of 0, 40, 200, or 1,000 mg/kg/day for up to 2 years (DePass et al. 1986a). Evaluations were limited to clinical signs, body weight, food consumption, and comprehensive histopathology. No clear treatment-related effects were observed in either sex, indicating that this study identified a NOAEL of 1,000 mg/kg/day and no LOAEL in CD-1 mice. In the other mouse study, B6C3F1 mice (60/sex/dose) were exposed to ethylene glycol in the diet for up to 2 years (NTP 1993). Estimated average doses were 0, 1,500, 3,000, and 6,000 mg/kg/day in males and 0, 3,000, 6,000, and 12,000 mg/kg/day in females. Evaluations included hematology, clinical chemistry, organ weights (limited), and comprehensive histopathology. Effects were essentially limited to increased incidences of hepatocellular hyaline degeneration in males at ≥3,000 mg/kg/day and females at 12,000 mg/kg/day, and medial hyperplasia of the pulmonary arterioles in females at ≥3,000 mg/kg/day; the biological significance of the pulmonary lesion was unclear (NTP 1993). Small numbers of oxalate-like crystals and/or calculi were noted in the renal tubules, urethrae, and urinary bladder in a few males at 6,000 mg/kg/day. A NOAEL of 1500 mg/kg/day and LOAEL of 3,000 mg/kg/day for liver histopathology were identified in male B6C3F1 mice.

Insert paragraph re. Wilson et al. (2005) here.

Key findings in the chronic toxicity studies were kidney lesions (oxalate crystal deposition and implied degenerative changes) at ≥375 mg/kg/day and mortality at 750 mg/kg/day in male Sprague-Dawley rats (Blood 1965), kidney lesions (oxalate nephrosis) and mortality at 1,000 mg/kg/day in male and female F344 rats (DePass et al. 1986a), ~~liver lesions (fatty metamorphosis) in female F344 rats at ≥200 mg/kg/day (DePass et al. 1986a)~~ Insert here, no kidney or liver histopathology in male or female CD-1 mice at 1,000 mg/kg/day (DePass et al. 1986a), and liver lesions (hepatocellular hyaline degeneration) in male B6C3F1 mice at ≥3,000 mg/kg/day (NTP 1993). The mortality and kidney and liver lesions in rats occurred at doses that

were NOAELs in mice, showing that rats were more sensitive than mice and the most appropriate species for MRL consideration.

insert

Effect levels in male rats are based on kidney lesions and mortality; these included a NOAEL of 200 mg/kg/day and serious LOAEL of 1,000 mg/kg/day in F344 males (DePass et al. 1986a), and a NOAEL of 150 mg/kg/day and serious LOAEL of 750 mg/kg/day in Sprague-Dawley males (Blood 1965). An apparent increase in kidney lesions without mortality occurred in Sprague-Dawley males at 375 mg/kg/day (Blood 1965), suggesting that this dose was a less serious LOAEL for renal effects. The 150 mg/kg/day NOAEL for renal effects in Sprague-Dawley males (Blood 1965) and Wistar males (Wilson et al, 2005) is consistent with the 200 mg/kg/day NOAEL for renal effects in F344 males (DePass et al. 1986a), ~~but 200 mg/kg/day is also a LOAEL for liver effects in female F344 rats (DePass et al. 1986a)~~. as noted above

Although chronic NOAELs of 150–200 mg/kg/day were identified for kidney toxicity in Sprague-Dawley and F344 male rats, no information is available on effects of chronic exposure in Wistar rats, a strain shown to be approximately twice as sensitive as F344 rats to kidney toxicity in a 16-week study (Cruzan et al. 2004), and the strain used to derive the intermediate-duration oral MRL. The intermediate-duration LOAEL for kidney toxicity in Wistar males is 180 mg/kg/day (Gaunt et al. 1974), which is in the range of the chronic NOAELs for kidney toxicity in F344 and Sprague-Dawley males. Additionally, the 180 mg/kg/day intermediate-duration LOAEL for kidney toxicity in Wistar males is lower than the 200 mg/kg/day chronic LOAEL for liver toxicity (fatty metamorphosis) in female F344 rats (DePass et al. 1986a). The chronic NOAEL for liver toxicity in F344 females is 40 mg/kg/day. Although 40 mg/kg/day is also a chronic NOAEL for kidney effects in F344 males, it is not known if it is a chronic NOAEL for kidney effects in Wistar males. The lack of chronic data in Wistar rats precludes derivation of a chronic MRL because it is not known whether an MRL based on the liver toxicity data in F344 rats would be protective of kidney toxicity.

Delete and use data from Wilson study for MRL.

~~[redacted]~~

$$\text{BMDL}_{05} = \frac{150 \text{ mg/kg/day}}{100 \text{ UF}} = 1.5 \text{ mg/kg/day}$$

4 10 x 10

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of ethylene glycol. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

The general population may be exposed to ethylene glycol. Ethylene glycol is widely sold in grocery stores and in automobile supply, discount, drug, and other stores throughout the United States for general use as an antifreeze/coolant in automobile radiators. Additionally, it is used in the manufacturing or blending of polyester products; aircraft and runway de-icing fluids; heat transfer fluids used in heating, ventilation, and air conditioning systems; polyester resins; humectants; alkyd-type resins; plasticizers; electrolytic capacitors; low freeze dynamite; and brake and shock solutions (Wiener and Richardson 1988). Ethylene glycol is also used in the production of artificial mists or fogs (NIOSH 1994).

Dermal exposure, through activities such as changing antifreeze, is the most likely route of exposure to ethylene glycol, but dermal exposure is not likely to lead to toxic effects. Only oral exposure, through accidental or intentional ingestion, is likely to lead to such effects, and then only if a sufficient amount is swallowed at one time. A review of the literature for ethylene glycol indicated that the stages of oral ethylene glycol poisoning in humans are well understood and documented. There is adequate knowledge of ethylene glycol metabolism to permit successful treatment of ethylene glycol intoxication, and substantial information concerning pathology and pathophysiology of the organ systems involved is available. Although the majority of the studies in humans represent descriptions of case studies of accidental or intentional poisoning, or exposure in industrial settings, they have been collected for a period of >60 years. Animal studies corroborate human findings and were used to provide quantitative data to support observations made in humans.

regimen (Tyl 1985, 1988a; Tyl et al. 1995a). Both the mouse and rat studies were confounded by ingestion of ethylene glycol deposited on the fur of exposed animals and consumed during grooming; the authors estimated that ingestion comprised the majority of exposure. In a companion study, nose-only exposure of CD-1 mice to 500–2,500 mg/m^3 aerosolized ethylene glycol using the same study design resulted in no effects on pre- or postimplantation loss (Tyl 1988a; Tyl et al. 1995a). Although this study was aimed at reducing confounding from concurrent ingestion exposure, the authors noted that the animals in the nose-only experiment were also exposed by ingestion of ethylene glycol deposited on the face during nose-only exposure.

As a result of confounding from exposure via ingestion, NTP-CERHR (2004) characterized the developmental toxicity studies as inadequate for the purpose of identifying effect levels for inhalation exposure; thus, there are no reliable NOAEL or LOAEL values.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to ethylene glycol.

Acute-duration developmental toxicity studies of inhaled ethylene glycol in mice and rats are available, but all of the studies are confounded by concurrent ingestion exposure to ethylene glycol deposited on the fur. Groups of 25 pregnant CD-1 mice and CD rats were exposed (whole-body) to target concentrations of 0, 150, 1,000, or 2,500 mg/m^3 aerosolized ethylene glycol (mass median aerodynamic diameter [MMAD] of 2.3 µm) for 6 hours/day on Gd 6–15 (Tyl 1985, 1988a; Tyl et al. 1995a). Fetal evaluations included litter size, fetal weight, and external, visceral, and skeletal malformations. In mice, significant decreases in the number of live fetuses per litter and in the weight of live fetuses, as well as increases in the number of live fetuses per litter and the incidence of external, visceral, and skeletal malformations were observed at target concentrations of ≥1,000 mg/m^3. In rats, reduced ossification at some sites in the axial skeleton was observed with exposure to 1,000 and 2,500 mg/m^3 (Tyl 1985; Tyl et al. 1995a); however, in an Expert Panel Review, NTP-CERHR (2004) concluded that the relationship of this effect to treatment was uncertain due to the lack of a dose-response relationship. This study was confounded by significant ingestion of ethylene glycol deposited on the fur and consumed during grooming; the authors estimated that the ingestion dose comprised the majority of exposure.

3.2.2.1 Death

The American Association of Poison Control Centers reported nine fatalities for 1989 and five for 1990 due to ethylene glycol ingestion (Litovitz et al. 1990, 1991). Several other fatal ethylene glycol poisonings have been reported in earlier studies, including seven case reports of deaths resulting from accidental or intentional ingestion of ethylene glycol or antifreeze containing 99% ethylene glycol (Godolphin et al. 1980; Gordon and Hunter 1982; Hewlett et al. 1986; Jacobsen et al. 1984; Siew et al. 1975a; Zeiss et al. 1989). A 22-year-old male who ingested 300 mL of antifreeze (approximately 4,071 mg/kg ethylene glycol) lapsed into a coma 24 hours after hospital admission and died 24 hours later (Siew et al. 1975a). A dose of 7,850 mg/kg can be estimated in the case of a 73-year-old male who consumed 500 mL of 95% ethylene glycol and died of myocardial failure after 68 hours (Gordon and Hunter 1982). In five other fatal cases of accidental or intentional poisoning, the amount of ingested ethylene glycol ranged from 150 to 1,500 mL (2,379–23,786 mg/kg) (Karlson-Stiber and Persson 1992; Walton 1978). Thus, oral dose of ethylene glycol required to cause death in humans is not well defined in the literature. The minimum lethal dose for adults is thought to be 1.4 mL/kg of 95% ethylene glycol, or about 1,330 mg ethylene glycol/kg body weight (Parry and Wallach 1974; Robinson and McCoy 1989; Siew et al. 1975a).

A single dose oral LD_{50} of 4,000 mg/kg was determined in female F344 rats (Clark et al. 1979). Male Wistar rats administered 12,900 mg/kg ethylene glycol in a single oral dose had 55% mortality within 48 hours (Richardson 1973). Pregnant CD-1 mice given 11,090 mg/kg/day ethylene glycol orally on Gd 7–14 showed 10% mortality (Schuler et al. 1984) and pregnant rabbits exhibited 42% mortality after receiving 2,000 mg/kg/day ethylene glycol orally on Gd 6–19 (Tyl et al. 1993). Cats administered a single 4,440–8,880 mg/kg dose by gavage had 100% mortality within 20–36 hours (Penumarthy and Oehme 1975). A single gavage dose of 4,180–12,540 mg/kg/day caused 17–100% mortality in dogs within 72 hours (Kersting and Nielsen 1965). Dogs administered a single oral dose of 4,880 mg/kg in food had 100% mortality within 6 days (Beckett and Shields 1971).

Intermediate-duration dietary exposure to 1,000 mg/kg/day for 16 weeks caused 20% mortality in male Wistar rats, with no deaths occurring in similarly treated male F344 rats; females were not tested (Cruzan et al. 2004). Male F344/N rats fed 5,000 mg/kg/day ethylene glycol had 40% mortality after 13 weeks, whereas similarly treated females did not die (Melnick 1984). A chronic dietary study of ethylene glycol in Sprague-Dawley found 100% mortality after 12–24 months in males at 750 mg/kg/day and females at

3,000 mg/kg/day (Blood 1965). Male F344 rats given 1,000 mg/kg/day ethylene glycol in the feed all died within 16 months (DePass et al. 1986a; Woodside 1982). Add Wilson et al (2005) mortality data here and to Table 2-1 & Fig 3-2.

All reliable LOAEL and LD_{50} values for death in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2, and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding hematological, musculoskeletal, endocrine, hepatic, dermal, ocular, or body weight effects in humans after oral exposure to ethylene glycol.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2 and Figure 3-2.

Respiratory Effects. Respiratory system involvement occurs 12–24 hours after ingestion of sufficient amounts of ethylene glycol and is considered to be a second stage in ethylene glycol poisoning (Vale 1979). The symptoms include hyperventilation (Godolphin et al. 1980; Gordon and Hunter 1982), shallow rapid breathing (Woolf et al. 1992; Zeiss et al. 1989), and generalized pulmonary edema with calcium oxalate crystals occasionally present in the lung parenchyma (Vale 1979). Respiratory failure was observed in a woman who had consumed 9,771 mg/kg ethylene glycol (as antifreeze) (Blakeley et al. 1993). It appears that respiratory system involvement is dose-dependent and occurs concomitantly with cardiovascular changes. Symptoms related to acidosis such as hyperpnea and tachypnea are frequently observed; however, major respiratory morbidities such as pulmonary edema rarely occur, having been reported in only 5 of 36 severely poisoned cases (Karlson-Stiber and Persson 1992).

Pulmonary hyperemia and edema were frequent findings in dogs that ingested unknown lethal amounts of ethylene glycol in cases of antifreeze poisoning (Kersting and Nielsen 1965). A generalized soft tissue mineralization that included the lungs (interstitial) occurred in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside et al. 1982). Histological examinations of the lungs showed no effects in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), Sprague-Dawley rats exposed to

A centrilobular degenerative change occurred in the liver of male B6C3F1 mice exposed to ethylene glycol in estimated dietary doses of 6,450 or 12,900 mg/kg/day for 13 weeks (Melnick et al. 1984; NTP 1993). This effect was characterized by the accumulation of a non-birefringent eosinophilic hyaline material in the cytoplasm of hepatocytes adjacent to or close to the central veins, and was not observed in females similarly exposed to ≤16,000 mg/kg/day (Melnick et al. 1984; NTP 1993). No liver lesions or changes in liver weight were observed in CD-1 mice exposed to ≤2,500 mg/kg/day by gavage for 17 days (Harris et al. 1992) or ≤2,826 mg/kg/day in the diet for one or two generations (Bolon et al. 1997; Morrissey et al. 1989; NTP 1986), Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 33 days (Schladt et al. 1998) or ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to ≤10,000 mg/kg/day in the diet for 13 weeks (Melnick 1984), or Sprague-Dawley rats exposed to ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990). There were no effects on serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AP), lactate dehydrogenase (LDH), cholesterol, and/or bilirubin in the 33- and 90-day studies in rats (Robinson et al. 1990; Schladt et al. 1998); clinical chemistry was not evaluated in the other intermediate-duration studies.

A 2-year study of ethylene glycol in B6C3F1 mice found significantly increased incidences of centrilobular hepatocyte hyaline degeneration in males at estimated dietary doses of 3,000 and 6,000 mg/kg/day (45 and 67% compared to 0% in controls) and females at 12,000 mg/kg/day (52% compared to 0% in controls) (NTP 1993). The lesions appeared similar to the hyaline degeneration in the 13-week study by the same investigators (Melnick et al. 1984; NTP 1993) and consisted of cytoplasmic accumulations of non-birefringent, eosinophilic, granular to globular material resembling erythrocytes in size, shape, and tinctorial properties. Severity did not increase with dose. In another chronic study, CD-1 mice and F344 rats of both sexes were exposed to doses as high as 1,000 mg/kg/day in the diet for up to 2 years (DePass et al. 1986a; Woodside et al. 1982). There were no effects on liver weight or histopathology in mice of either sex or male rats. The female F344 rats had significantly increased incidences of slight liver fatty metamorphosis at ≥200 mg/kg/day and liver mononuclear cell infiltrates at 1,000 mg/kg/day; the incidences of fatty metamorphosis were 13% (34/256), 12% (16/129), 22% (27/125), and 27% (35/128) at 0, 40, 200, and 1,000 mg/kg/day, respectively. A 2-year dietary study in Sprague-Dawley rats found no effects on liver weight or histopathology in males at ≤375 mg/kg/day (higher doses caused early mortality) or females at ≤3,000 mg/kg/day.

Renal Effects. Adverse renal effects after ethylene glycol ingestion in humans can be observed during the third stage of ethylene glycol toxicity 24–72 hours after acute exposure. The hallmark of renal

toxicity is the presence of birefringent calcium oxalate monohydrate crystals deposited in renal tubules and their presence in urine after ingestion of relatively high amounts of ethylene glycol (CDC 1987; Blakeley et al. 1993; Chung and Tuso 1989; Factor and Lava 1987; Godolphin et al. 1980; Heckerling 1987; Parry and Wallach 1974; Rothman et al. 1986; Siew et al. 1975a; Underwood and Bennett 1973). In addition to birefringent oxalate crystals in the tubular lumens, other signs of nephrotoxicity can include focal tubular cell degeneration, atrophy, and tubular interstitial inflammation (Factor and Lava 1987). In a case study of a 38-year-old female who consumed 240 mL of antifreeze (3,454 mg ethylene glycol/kg/day), crystalluria was not present upon hospital admission (about 12 hours after ingestion). Within 5 hours, excretion of calcium oxalate dihydrate crystals was evident, although monohydrate crystals became the primary form in the urine thereafter (2–3 hours) (Jacobsen et al. 1988). In the course of ethylene glycol intoxication, serum creatinine (Factor and Lava 1987; Spillane et al. 1991) and serum blood urea nitrogen (BUN) (Chung and Tuso 1989; Factor and Lava 1987) levels may be increased. If untreated, the degree of renal damage caused by high doses of ethylene glycol progresses and leads to hematuria (CDC 1987; Rothman et al. 1986; Underwood and Bennett 1973), proteinuria (Rothman et al. 1986), decreased renal function, oliguria, anuria (Mallya et al. 1986; Parry and Wallach 1974; Spillane et al. 1991; Woolf et al. 1992; Zeiss et al. 1989), and ultimately renal failure (Chung and Tuso 1989; Gordon and Hunter 1982; Jacobsen et al. 1984; Mallya et al. 1986). These changes in the kidney are linked to acute tubular necrosis (Factor and Lava 1987), but normal or near normal renal function can return with adequate supportive therapy (see Section 3.11, Methods for Reducing Toxic Effects).

In acute-duration studies in rats, kidney effects occurred at doses as low as 1,250 mg/kg/day by gavage and 1,400 mg/kg/day in drinking water. Renal tubular dilation and regeneration were increased in female Sprague-Dawley rats that were exposed to 1,250 or 2,250 mg/kg/day ethylene glycol by gavage on Gd 6–20 and examined on postnatal day (Pnd) 1 (NTP 1988). Increased relative and absolute kidney weights, but no renal histopathology, occurred in female CD rats exposed to 2,500 mg/kg/day by gavage on Gd 6–15 and examined on Gd 21 (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). In a 10-day drinking water systemic toxicity study, the incidence and severity of renal lesions were significantly increased in male Sprague-Dawley rats exposed to 2,615 and 5,270 mg/kg/day, but not at doses ≤1,343 mg/kg/day; lesions included tubular dilation, degeneration, necrosis, and intratubular calcium oxalate crystals (Robinson et al. 1990). Exposure to 1,400 mg/kg/day in the drinking water for 15–29 days caused renal tubular oxalate deposits, but apparently no nephrosis, in male Sprague-Dawley rats (Khan et al. 1993). Mice that were administered doses ≤1,000 mg/kg by gavage for 4 days had no histopathological changes in the kidneys (Hong et al. 1988). Renal toxicity occurred in female New Zealand white rabbits that were exposed to 2,000 mg/kg/day by gavage on Gd 6–19 and examined on Gd 30; lesions that included tubule

dilatation and regeneration, epithelial necrosis, and intraluminal oxalate crystal deposition were increased at this dose level, but not at doses ≤1,000 mg/kg/day (Tyl et al. 1993).

Limited data are available on acute renal effects in other species. A single oral dose of 4,440 mg/kg in cats (Penumarthy and Oehme 1975) or 4,880 or 10,743 mg/kg in dogs (Beckett and Shields 1971; Grauer et al. 1987) caused kidney damage leading to oliguria and renal failure. Dogs administered a single dose of 10,600 mg/kg ethylene glycol as antifreeze or as reagent-grade ethylene glycol in feed exhibited polyuria, azotemia, and renal failure (Dial et al. 1994). Serum BUN and creatinine were not increased in two dogs given a single gavage dose of approximately 1,000 mg/kg/day, suggesting that renal function was not altered (Hewlett et al. 1989). Insert here In male macaque monkeys exposed to ethylene glycol in drinking water, five of seven animals receiving doses ranging from 1,665 to 146,520 mg/kg/day for 6–13 days had calcium oxalate crystals and evidence of necrosis in the kidneys (Roberts and Seibold 1969).

The renal effects of intermediate-duration oral exposure to ethylene glycol are well characterized in a number of studies in rats and mice (Cruzan et al. 2004; Gaunt et al. 1974; Melnick 1984; NTP 1993; Robinson et al. 1990). As summarized below, the results of these studies indicate that renal toxicity varies with sex, species, and strain, with males more sensitive than females, rats more sensitive than mice, and Wistar rats more sensitive than other strains of rats.

In a 90-day drinking water study with Sprague-Dawley rats (Robinson et al. 1990), incidences of renal lesions were significantly increased in males at ≥947 mg/kg/day and females at ≥3,087 mg/kg/day. Males showed a greater number and severity of lesions than females; lesions included tubular dilation and degeneration, acute and subacute inflammation, calcium oxalate crystals in tubules and pelvis epithelium, dilation of urinary pelvis, and hyperplasia and degeneration of pelvis epithelium. The male rats also had increases in relative kidney weight and serum creatinine at ≥947 mg/kg/day and BUN at 3,134 mg/kg/day. A 13-week dietary study in F344 rats (Melnick 1984) found renal effects that included increased relative kidney weight at ≥2,500 mg/kg/day in males and ≥5,000 mg/kg/day in females, increased BUN and serum creatinine in males at ≥2,500 mg/kg/day, and histopathology in males ≥2,500 mg/kg/day and females at 10,000 mg/kg/day. The lesions were more severe in the males (e.g., dilation, necrosis, fibrosis, and crystal deposition in renal tubules) than in the females (e.g., inflammation and vacuolation without crystal deposition). The NOAELs for renal toxicity in this study were 1,250 mg/kg/day in males and 2,500 mg/kg/day in females.

In a 16-week dietary study in Wistar rats (Gaunt et al. 1974), renal findings in males included no effects at 71 mg/kg/day, increased incidences of kidney lesions at ≥180 mg/kg/day, and oxalic acid crystals in urine, increased absolute kidney weight, increased urine volume, and decreased urine specific gravity at 715 mg/kg/day. The lesions ranged from degenerative changes in individual nephrons with occasional oxalate crystals to generalized tubular damage with heavy crystal deposition. At the 0, 35, 71, 180, and 715 mg/kg/day dose levels for the male rats in this study, the overall incidence of renal tubular damage was 0/15, 1/15, 1/15, 4/15, and 15/15, respectively. The only effect observed in females was a non-statistically significant increase in kidney lesions at 1,128 mg/kg/day, the highest tested dose. Based on the renal tubular lesions in male Wistar rats, an intermediate-duration oral MRL of 0.5 mg/kg/day was derived as indicated in the footnote to Table 3-2 and discussed in Chapter 2 and Appendix A.

Need to change Table 3-2 accordingly

In another 16-week dietary study (Cruzan et al. 2004), male Wistar and male F344 rats were exposed to dose levels of 0, 50, 150, 500, or 1,000 mg/kg/day. Effects included calcium oxalate crystals in the urine of both strains of rats at ≥150 mg/kg/day and increased absolute and relative kidney weights, increased water intake, increased urine volume, and decreased urine specific gravity at ≥500 mg/kg/day in Wistar rats and 1,000 mg/kg/day in F344 rats. No treatment-related increases in alpha 2-μ-globulin were observed in the kidneys of either strain of rats. No histological effects occurred in the kidneys of either strain of rats at 50 or 150 mg/kg/day. At higher doses, histopathological findings included calcium oxalate crystal deposition in the renal tubules with associated nephropathy in all Wistar rats (10/dose) at ≥500 mg/kg/day. Histological findings in the F344 rats included crystals in the tubules without nephropathy in 6/10 animals at 500 mg/kg/day, and crystal nephropathy in 1/10 animals at 500 mg/kg/day and 10/10 animals at 1,000 mg/kg/day. The severity of the crystal nephropathy in the Wistar rats at 500 mg/kg/day was approximately equivalent to that in the F344 rats at 1,000 mg/kg/day. Although the male Wistar rats were more sensitive than the male F344 rats, the LOAEL for kidney toxicity was 500 mg/kg/day in both strains. The NOAEL in both strains of rats is 150 mg/kg/day because the only effect at this dose, crystalluria, reflects a detoxification process and is not adverse in the absence of crystal deposition in the renal tubule epithelium and associated histopathology.

Information on the intermediate-duration renal toxicity of ethylene glycol is also available in mice. In a 13-week dietary study in B6C3F1 mice, effects were observed in the kidneys of males at ≥6,450 mg/kg/day (minimal to mild tubule dilation, cytoplasmic vacuolation, and regenerative hyperplasia, without tubular oxalate crystal deposition), with no effects on kidney histology or urinalysis in females at doses ≤16,000 mg/kg/day (Melnick 1984; NTP 1993). No histopathological changes were observed in the kidneys of male CD-1 mice that were administered doses as high as 2,500 mg/kg/day by

gavage for 17 days (Harris et al. 1992). Kidney weight and histology were evaluated in F_0 and F_1 parental male and female CD-1 mice that were exposed to 2,826 mg/kg/day in the drinking water in a two-generation reproduction study (Bolon et al. 1997; Morrissey et al. 1989; NTP 1986). The exposure period of both generations included 14 weeks of cohabitation through gestation and lactation. Kidney lesions occurred in 60% of the F_0 male mice; the lesions included tubular degeneration, dilation, and regeneration, as well as a low incidence of oxalate crystal deposition (3/20 treated vs. 0/21 controls). There was no effect on kidney weight in the F_0 males or on kidney weight or histology in the F_0 females or F_1 males or females.

Two-year studies in rats (Blood 1965; DePass et al. 1986a) and mice (DePass et al. 1986a; NTP 1993) provide information on chronic renal toxicity of ethylene glycol. Males were more sensitive than females and rats were more sensitive than mice, ~~although chronic testing has not been performed in~~ and Wistar rats, ~~a~~ appear to the most ~~strain shown to~~ be ~~particularly~~ strain sensitive to ethylene glycol nephrotoxicity ~~following intermediate duration exposure (Cruzan et al. 2004)~~. A chronic toxicity study in Wistar rats (Wilson et al, 2005) has ~~been~~ shown renal toxicity. [Describe results in further detail here].
In Sprague-Dawley rats that were fed ethylene glycol for 2 years, effects included increased water consumption, proteinuria, and mortality in males at ≥750 mg/kg/day and females at 3,000 mg/kg/day. Incidences of calcification (oxalate crystal deposition) in the kidneys were increased in both sexes at ≥750 mg/kg/day, and oxalate-containing calculi were increased in males at ≥750 mg/kg/day and females at 3,000 mg/kg/day. The incidences of oxalate crystal deposition in the males were 0/7, 0/12, 0/10, 4/10, 7/7, and 15/15 at 0, 75, 150, 375, 750 and 3,000 mg/kg/day; the increase at 375 mg/kg/day was not statistically significant. The report implied, but did not adequately document, that many of the animals with crystal deposition in the renal tubules also had degenerative changes (mainly cytoplasmic vacuolation) in the tubular epithelium. Due to the insufficiently reported histopathology findings and lack of a clear (statistically significant) increase in oxalate crystal deposition at 375 mg/kg/day due to small numbers of animals, this study provides limited evidence that 375 mg/kg/day was a chronic LOAEL for kidney toxicity in male Sprague-Dawley rats.

F344 rats (130/sex/dose) were fed ethylene glycol in the dietary concentrations that yielded reported approximate doses of 0, 40, 200, or 1,000 mg/kg/day for up to 2 years (DePass et al. 1986a). No treatment-related or statistically significant changes occurred in the male rats at 40 or 200 mg/kg/day. A number of renal effects were observed in the 1,000 mg/kg/day males after 12 months (subsequent sacrifices at this dose level were precluded by early mortality), including increased water consumption and urine volume, decreased urine specific gravity and pH, increased urinary calcium oxalate crystals,

NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside et al. 1982). None of these studies included assessments of endocrine function.

Dermal Effects. Histological examinations of the skin showed no effects in Sprague-Dawley rats exposed to ≤7,327 mg/kg/day in drinking water for 10 days or ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside et al. 1982), B6C3F1 mice exposed to ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside et al. 1982).

Ocular Effects. Histological examinations of the eyes showed no effects in Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), or in F344 rats or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 1–2 years (DePass et al. 1986a; Woodside et al. 1982).

Body Weight Effects. In an acute-duration study, male Sprague-Dawley rats exposed to 5,279 mg/kg/day ethylene glycol in the diet for 10 days experienced 13% body weight loss; no effect occurred in females at doses as high as 7,327 mg/kg/day (Robinson et al. 1990). Administration of ethylene glycol by gavage during gestation (Gd 6–15 or 6–20) caused 17–31% decreases in maternal body weight gain in CD and Sprague-Dawley rats exposed to 1,250–2,500 mg/kg/day and B6C3F1 mice exposed to 1,500 mg/kg/day (Marr et al. 1992; Neeper-Bradley 1990, Neeper-Bradley et al. 1995; NTP 1988; Price et al. 1985). Body weight gain corrected for gravid uterine weight was generally similar to controls, indicating that intrauterine loss was a significant contributor to the reduced maternal weight gain during pregnancy. New Zealand white rabbits showed no changes in maternal body weight after gavage exposure to 2,000 mg/kg/day ethylene glycol on Gd 6–19 (Tyl et al. 1993).

In intermediate-duration studies, body weight gain was 13–30% lower than controls in F344, Wistar, and Sprague-Dawley rats exposed to ~~750~~ 500–3,134 mg/kg/day in the diet or drinking water for 13–16 weeks (Blood 1965; Melnick et al. 1984; Robinson et al. 1990). No adverse effects on body weight occurred in Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to 1,000 mg/kg/day in a three-generation reproduction study (DePass et al. 1986b), CD-1 mice exposed to 2,500 mg/kg/day by gavage for 17 days (Harris et al. 1992) or B6C3F1 mice exposed 16,000 mg/kg/day in the diet for 13 weeks (Melnick 1984; NTP 1993).

and 89% less than controls in male Wistar rats at 300 mg/kg/day (Wilson et al 2005)

Chronic (2-year) dietary studies of ethylene glycol found decreased body weight gain (15% less than controls) in male F344 rats at 1,000 mg/kg/day, but not in male F344 or Sprague-Dawley rats at 200–375 mg/kg/day (Blood 1965; DePass et al. 1986b); decreased body weight gain in female Sprague-Dawley rats at 3,000 mg/kg/day, but not in female Sprague-Dawley or F344 rats at 750–1,000 mg/kg/day (Blood 1965; DePass et al. 1986b); and no effects on body weight in CD-1 or B6C3F1 mice at 1,000–12,000 mg/kg/day (DePass et al. 1986a; Melnick 1984; NTP 1993; Woodside et al. 1982).

Metabolic Effects. One of the major adverse effects following acute oral exposure of humans to ethylene glycol involves metabolic changes. These changes occur as early as 12 hours after ethylene glycol exposure. Ethylene glycol intoxication at doses of 1,628 mg/kg/day is accompanied by metabolic acidosis which is manifested by decreased pH and bicarbonate content of serum and other bodily fluids caused by accumulation of excess glycolic acid (CDC 1987; Berger and Ayyar 1981; Blakeley et al. 1993; Cheng et al. 1987; Chung and Tuso 1989; Gordon and Hunter 1982; Heckerling 1987; Jacobsen et al. 1988; Parry and Wallach 1974; Siew et al. 1975a; Spillane et al. 1991; Woolf et al. 1992; Zeiss et al. 1989). There is an inverse relationship between the decreasing plasma pH and increasing plasma glycolic acid concentrations (Clay and Murphy 1977). The normal level of bicarbonate of 24 mmol/L can be depleted in cases of severe ethylene glycol intoxication to reach concentrations as low as 2 mmol/L (Jacobsen et al. 1984). This decrease in base concentration indicates that a similar quantity of acid has to be present to achieve such a depletion. Glycolic acid is the only acidic metabolite present in such quantities. Humans highly intoxicated with ethylene glycol had glycolate concentrations of 17–29 and <1 mmol of glyoxalate and oxalate, respectively (Jacobsen et al. 1984). Similar observations were made in animals. Metabolic acidosis due to glycolate accumulation was observed after acute oral exposure of dogs to 1,000–1,360 mg/kg of ethylene glycol (Hewlett et al. 1989) and of rats to 1,000 mg/kg (Marshall 1982). These results indicate that glycolic acid is the major toxic metabolite causing metabolic acidosis, and that its high serum levels are likely responsible for systemic toxicity observed after ethylene glycol exposure.

Other characteristic metabolic effects of ethylene glycol poisoning are increased serum anion gap, increased osmolal gap, and hypocalcemia. Serum anion gap is calculated from concentrations of sodium, chloride, and bicarbonate and is elevated after ethylene glycol ingestion (Chung and Tuso 1989; Factor and Lava 1987; Heckerling 1987; Spillane et al. 1991; Zeiss et al. 1989). The increase in the anion gap correlates with the elevation in plasma glycolate levels (Jacobsen et al. 1984). Osmolal gap represents the difference between the measured and calculated osmolalities and is also elevated during ethylene glycol intoxication. The amount of ethylene glycol causing these effects ranged from 1,628 to 12,840 mg/kg/day

13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside et al. 1982).

Leukocyte counts were generally unaffected in the acute-, intermediate- and chronic-duration studies of ethylene glycol cited above. Exceptions included statistically significant decreased total leukocyte counts in female Sprague-Dawley rats exposed to 7,327 mg/kg/day for 10 days (34.8% less than controls) or 597–5,744 mg/kg/day for 90 days (30–50% less than controls) (Robinson et al. 1990), and significantly increased neutrophil count (38% higher than controls) in male F344 rats exposed to 1,000 mg/kg/day for 1 year (DePass et al. 1986a).

The highest NOAEL values and all reliable LOAEL values for immunological and lymphoreticular effects in rats after intermediate-duration oral exposure to ethylene glycol are reported in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Adverse neurological reactions are among the first symptoms to appear in humans after ethylene glycol ingestion. These early neurotoxic effects are also the only symptoms attributed directly to ethylene glycol. Together with metabolic changes, they occur during the period of 30 minutes to 12 hours after exposure and are considered to be part of the first stage in ethylene glycol intoxication (Robinson and McCoy 1989; Vale 1979). In cases of acute intoxication, in which a large amount of ethylene glycol is ingested over a very short time period, there is a progression of neurological manifestations which, if not treated, may lead to convulsions and coma (Zeiss et al. 1989). Ataxia, slurred speech, and somnolence are common during the initial phase of ethylene glycol intoxication (CDC 1987; Parry and Wallach 1974; Zeiss et al. 1989), as are irritation, restlessness, and disorientation (Cheng et al. 1987; Factor and Lava 1987; Gordon and Hunter 1982; Rothman et al. 1986; Woolf et al. 1992). In a fatal case of ethylene glycol poisoning, a 22-year-old man was admitted to the hospital in a state of stupor 6 hours after ingesting 4,071 mg/kg of ethylene glycol. He vomited several times prior to admission, lost consciousness, and became comatose (Siew et al. 1975a).

Crystalline deposits of calcium oxalate in the walls of small blood vessels in the brain were found at autopsy in a man who died after acute ethylene glycol poisoning (Zeiss et al. 1989). Other neurological symptoms commonly encountered in cases of acute oral human exposure to ethylene glycol are semiconsciousness (Underwood and Bennett 1973) and unresponsiveness (Blakeley et al. 1993; Chung

above, a similar effect occurred in ~~a man~~ two humans who died from acute ethylene glycol poisoning (Zeiss et al. 1989). ; Froberg et al., 2006)

There were no clinical signs of neurotoxicity or histopathological changes in nervous system tissue in other intermediate- or chronic-duration studies of ethylene glycol in rats or mice. As indicated in Table 3-2, the histopathological evaluations included brain, spinal cord, and/or sciatic nerve in Wistar rats exposed to ≤2,000 mg/kg/day by gavage for 4 weeks (Schladt et al. 1998), Sprague-Dawley rats exposed ≤5,744 mg/kg/day in drinking water for 90 days (Robinson et al. 1990), Wistar rats exposed to ≤1,128 mg/kg/day in the diet for 16 weeks (Gaunt et al. 1974), F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside et al. 1982), Sprague-Dawley rats exposed to ≤3,000 mg/kg/day in the diet for 2 years (Blood 1965), B6C3F1 mice exposed to ≤16,000 mg/kg/day in the diet for 13 weeks or ≤12,000 mg/kg/day in the diet for 2 years (Melnick 1984; NTP 1993), or CD-1 mice exposed to ≤1,000 mg/kg/day in the diet for 2 years (DePass et al. 1986a; Woodside et al. 1982).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2, and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to ethylene glycol.

Ethylene glycol treatment did not affect gestational length in CD rats given 2,500 mg/kg/day ethylene glycol by gavage administration on Gd 6–15 (Marr et al. 1992). Testis and uterine weights and histopathology were not affected in B6C3F1 mice treated with ethylene glycol for 4 consecutive days at doses up to 250 mg/kg/day and evaluated 1 day later (Hong et al. 1988).

Reproductive function after intermediate-duration oral exposure to ethylene glycol has been tested in three multi-generation studies (one in rats and two in mice) and several shorter studies (15–20 days in rats and mice). In these studies, effects on fertility, fetal viability, and male reproductive organs were observed in mice, while the only effect in rats was an increase in gestational duration.

In a continuous breeding study in which CD-1 mice were exposed to ethylene glycol in drinking water, there were slight, but statistically significant, reductions in the number of litters per fertile pair and in the mean number of live pups per litter at 1,640 mg/kg/day of the F_0 generation (Lamb et al. 1985; Morrisey et al. 1989). In mated F_1 offspring, there were no differences between high-dose and control groups in fertility or live litter size. In a follow-up to this study using the same overall protocol, the number of live female pups and the number of live pups per litter were significantly reduced at 2,826 mg/kg/day in the F_0 generation of mice, but there were no effects on reproductive parameters in the F_1 generation (Morrissey et al. 1989; NTP 1986). Ethylene glycol treatment did not affect mating or fertility rate in either generation, or in F_0 parents used in a crossover mating trial (20/sex high dose mice mated to 20/sex controls) (Morrissey et al. 1989; NTP 1986). Female Swiss CD-1 mice given ethylene glycol at 2,500 mg/kg/day by gavage for 20 days including a 5-day mating period (days 8–12) with concurrently treated males had significantly fewer live and significantly more dead implants as well as complete resorption of two of six litters (Harris et al. 1992). Total number of implantation sites was not affected.

In a three-generation reproductive toxicity and dominant lethality study in F344 rats exposed via the diet, no treatment-related effects on fertility index, gestation index, gestation survival index, or days from first mating to litter were observed in any generation at doses up to 1,000 mg/kg/day (DePass et al. 1986b). Number of implantation sites was not affected at doses up to 2,250 mg/kg/day in timed pregnant CD rats given gavage doses of ethylene glycol on Gd 6–20 (NTP 1988).

Effects on the male reproductive system, manifested mainly as changes in sperm parameters and testicular lesions, occurred in CD-1 mice exposed to ethylene glycol in drinking water in a continuous breeding study (Morrissey et al. 1989; NTP 1986). Sperm number was decreased in F_1 males at doses as low as 897 mg/kg/day, but the effect did not exhibit a dose-response relationship. Sperm motility, absolute seminal vesicle weight, relative epididymis weight, and absolute and relative testis weights were significantly reduced in F_1 males at ≥1,798 mg/kg/day. Effects at 2,826 mg/kg/day included increased incidence of abnormal sperm and decreased sperm motility in F_0 males, and increased incidence and severity of testicular and epididymal lesions in F_0 males (seminiferous tubule degeneration, loss of spermatozoa, spermatic, spermatogonia and spermocytes, vacuolization of epithelial cells, and interstitial cell hyperplasia) and F_1 males (seminiferous tubule degeneration and intersititial cell hyperplasia). An Expert Panel review of this study (NTP-CERHR 2004) concluded that, while this study provided some evidence for testicular changes and effects on sperm parameters, the high incidence of testicular effects in the control animals limited the ability to draw conclusions about the relationship of this effect to treatment. Ethylene glycol treatment did not affect testis weight, epididymis weight, sperm count, sperm

spelling?

Studies in laboratory animals indicate that acute-duration exposure to high doses of ethylene glycol during gestation can affect fetal viability and postimplantation loss. Of 37 pregnancies in CD-1 mice receiving gavage doses of 11,090 mg/kg/day on Gd 7–14, only 15 litters had at least 1 live-born pup, compared with 29/29 control pregnancies (Schuler et al. 1984). In the treated group, there was a significant decrease in the number of live pups per litter and a significant increase in the number of dead pups per litter at birth. Ethylene glycol treatment (up to 2,500 mg/kg/day) of mated female Swiss CD-1 mice during Gd 8–14 did not affect the number of females littering, number of implantation sites, or number of live pups at birth (Harris et al. 1992). The percentage of postimplantation loss per litter was significantly increased in CD rats treated by gavage on Gd 6–15 with 5,000 mg/kg/day and the number of live fetuses per litter was reduced at both 2,500 and 5,000 mg/kg/day (Price et al. 1985). There were no significant effects of treatment on total implantations, preimplantation loss, or litter size when pregnant F344 rats were given ethylene glycol in the diet at target doses of up to 1,000 mg/kg/day on Gd 6–15 (Maronpot et al. 1983). In New Zealand white rabbits given gavage doses of up to 2,000 mg/kg/day ethylene glycol on Gd 6–19, the numbers of pre- or post-implantation losses were not increased in any treatment group, although 42% of the high-dose dams died prior to sacrifice (Tyl et al. 1993).

The most sensitive indicator of the developmental toxicity of acute oral exposure to ethylene glycol appears to be an increased incidence of malformations, primarily skeletal malformations, in both mice and rats. Available data suggest that malformations appear in mice at lower gavage doses than those that cause malformations in rats. The incidence of skeletal and other malformations was increased at all doses when groups of at least 20 timed-pregnant CD-1 mice were treated by gavage with doses of 0, 750, 1,500, or 3,000 mg/kg/day ethylene glycol on Gd 6–15 (Price et al. 1985). The percentages of malformed fetuses per litter and of litters with one or more malformed fetuses were significantly increased at all doses. The malformations primarily consisted of neural tube, craniofacial, and axial skeletal defects, with skeletal defects comprising the majority. In a later study aimed at identifying a NOAEL for developmental effects in CD-1 mice, an increased incidence of malformations was observed at doses of ≥500 mg/kg/day by gavage on Gd 6–15 (Neeper-Bradley et al. 1995; Tyl 1989). The incidence of total malformations per litter (external, visceral, and skeletal) was significantly increased at both 500 and 1,500 mg/kg/day. There was a significant increase in the incidence of two skeletal malformations (fused ribs or thoracic arches) in the 1,500 mg/kg/day group, and the incidences of 23 skeletal variations were also increased in this group. One of these variations (bilateral extra rib 14) was also significantly increased at 500 mg/kg/day. The incidence of individual external or visceral malformations was not significantly increased in any treatment group relative to the vehicle control; however, exencephaly (a malformation observed by Price

et al. [1985] at higher doses) was observed in two fetuses in the 500 mg/kg/day group and in three fetuses of the 1,500 mg/kg/day dose group (Neeper-Bradley et al. 1995; Tyl 1989).

In rats, gestational doses of at least 1,000 mg/kg/day were required to induce malformations. The number of litters with malformations, number of malformed fetuses per litter, and number of litters with skeletal malformations were increased at doses of ≥2,500 mg/kg/day in CD rats treated by gavage on Gd 6–15 (Price et al. 1985). At 5,000 mg/kg/day, the number of litters with fetuses having external and visceral malformations (primarily neural tube and craniofacial defects) was also increased. The authors reported a significant increase in visceral malformations at 1,250 mg/kg/day, but NTP-CERHR (2004) classified the observed effects (hydroureter, hydronephrosis, and great artery anomalies) as variations rather than malformations, and characterized the 1,250 mg/kg/day dose as a developmental NOAEL. In later studies using lower doses, the incidence of litters with fetuses having two skeletal malformations (missing thoracic arch and missing ribs) was increased in CD rats exposed by gavage to ≥1,000 mg/kg/day on Gd 6–15 (Neeper-Bradley 1990; Neeper-Bradley et al. 1995). The incidences of total skeletal malformations and skeletal variations (delayed ossification) were also significantly increased at ≥1,000 mg/kg/day. The highest dose (2,500 mg/kg/day) was associated with increased frequencies of visceral and external malformations, including gastroschisis, hydrocephaly, lateral ventricle dilation, umbilical hernia, and atelectasis (Neeper-Bradley 1990; Neeper-Bradley et al. 1995).

Reduced ossification of the vertebral centra was observed in the 1,000 mg/kg/day dose group when F344 rats were given ethylene glycol in the diet on Gd 6–15 (Maronpot et al. 1983). However, an Expert Panel Review of this study (NTP-CERHR 2004) identified the high dose (1,000 mg/kg/day) as a developmental NOAEL, noting the lack of other findings (change in body weights or consistent alterations in skeletal integrity) to support the authors' suggestion that reduced ossification was indicative of minimal embryotoxicity.

When developmental effects were assessed over the course of postnatal development, there were significant reductions in percentages of total ossification, sternebral ossification, and vertebral centra ossification on Gd 20 and at all postnatal evaluations up to ppd 63 in CD rats given 2,500 mg/kg/day ethylene glycol by gavage administration on Gd 6–15 (Marr et al. 1992). The percent of malformed fetuses per litter was also significantly increased at all scheduled sacrifice times other than ppd 63. The percent of litters with skeletal malformations (primarily skeletal axial defects) was 100% in the treated litters at all time points other than ppd 63 (Marr et al. 1992).

and vertebral defects) at 2,250 mg/kg/day ethylene glycol when CD rats were given gavage doses on Gd 6–20; the authors noted that 9/443 pups in this group also had hydrocephaly (NTP 1988).

Average pup weight was reduced in the F_0 generation at 1,640 mg/kg/day in a continuous breeding study in CD-1 mice (Lamb et al. 1985; Morrisey et al. 1989), but female pup body weights and pup weight adjusted for litter size were significantly reduced at doses as low as 897 mg/kg/day in both F_0 and F_1 generations in a follow-up study (Morrissey et al. 1989; NTP 1986). In a crossover mating trial using the F_0 parents, pup body weight were reduced when 2,826 mg/kg/day females were mated to control males (Morrissey et al. 1989; NTP 1986). In studies on the postnatal effects of intrauterine exposure, average pup body weights were not affected on ppd 4, 14, or 21 in F344 rats exposed via the diet to doses up to 1,000 mg/kg/day in a three-generation reproductive toxicity study (DePass et al. 1986b); however, pup body weights were lower than controls at various times between ppd 1 and 22 when CD rats were given gavage doses of 2,250 mg/kg/day ethylene glycol on Gd 6–20 (NTP 1988). Postnatal decreases in kidney weight (1,250 and 2,250 mg/kg/day groups) and brain weight (2,250 mg/kg/day group), without corresponding histopathology changes, have also been observed in the offspring of rats exposed in utero (Gd 6–20) to ethylene glycol (NTP 1988).

Dams exposed to 2,500 mg/kg/day ethylene glycol had significantly fewer live implants and significantly more dead implants as well as complete resorption of two of six litters in a study exposing female Swiss CD-1 mice by gavage at doses up to 2,500 mg/kg/day for 20 days including a period of mating to concurrently treated males (Harris et al. 1992). In a study of postnatal effects of intrauterine exposure, cumulative pup mortality was significantly higher on ppd 1 and 4 in CD rats exposed to gavage doses of 2,250 mg/kg/day ethylene glycol on Gd 6–20 (NTP 1988).

In summary, there is a substantial database demonstrating developmental toxicity at ethylene glycol doses that are not maternally toxic. Mice appear to be more vulnerable to the developmental effects of ethylene glycol, responding at lower doses than rats. Skeletal and other malformations appear to be the most sensitive indicators of toxicity, with effects observed at doses of ≥500 mg/kg/day in mice and ≥1,000 mg/kg/day in rats. Effects on fetal body weight and fetal viability occur at higher doses. The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category for ethylene glycol after oral exposure are reported in Table 3-2 and plotted in Figure 3-2.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

^{13}C = stable isotope
• not radiolabel

Limited information suggests that ethylene glycol is absorbed across the human respiratory tract. When two male volunteers inhaled ^{13}C-labeled ethylene glycol vapor (estimated to result in inhaled doses of 0.96 and 1.51 mg/kg body weight), ~~radio~~labeled ethylene glycol and glycolic acid were detected in the plasma and urine, providing evidence of absorption (Carstens et al. 2003). No increase, as compared to controls, in serum or urinary levels of ethylene glycol was recorded in men exposed to 17–49 mg/m^3 ethylene glycol aerosol for 30 days (Wills et al. 1974). However, in a review of this study, NTP-CERHR (2004) noted that the analytical techniques used for serum and urine analysis of ethylene glycol may not have been adequately sensitive to detect a difference.

In rats exposed nose-only for 30 minutes to ^{14}C-labeled ethylene glycol vapor (32 mg/mg^3) or for 17 minutes to ^{14}C-ethylene glycol aerosol (184 mg/m^3) on gallium oxide particles, between 75 and 85% of the deposited radiolabel was found to be distributed throughout the body regardless of the form of the compound (Marshall and Cheng 1983). In its review, NTP-CERHR (2004) estimated that 60–90% of the inhaled dose was absorbed in this study.

3.4.1.2 Oral Exposure

Indirect evidence of the oral absorption of ethylene glycol by humans is available from case reports of clinical symptoms in persons accidentally or intentionally ingesting ethylene glycol (Hewlett et al. 1986; Jacobsen et al. 1988; Robinson and McCoy 1989; Walton 1978). Measurements of the plasma concentration of ethylene glycol after acute poisoning (studies report levels ranging from 1 to 40 mmol/L; Hewlett et al. 1986; Jacobsen et al. 1988) provide additional evidence; however, because the amounts ingested in these events were generally unknown, and blood analyses were performed at varying times after exposure, the data are not useful for quantifying the rate or extent of oral absorption in humans.

In rats, ingested ethylene glycol is rapidly absorbed, usually reaching peak blood levels within 1 hour after single gavage doses of 150–20,000 mg/kg (Frantz et al. 1989, 1996a, 1996c; Pottenger et al. 2001; Winek et al. 1978). Absorption is equally rapid in other species, with peak blood levels reached within 1–3 hours after gavage exposure in mice, monkeys, and dogs (Frantz et al. 1991, 1996a, 1996b; Grauer et al. 1987; Hewlett et al. 1989; McChesney et al. 1971). In addition, available data suggest near complete absorption of ingested ethylene glycol in both rats and mice. After gavage doses of 10 and 1,000 mg/kg

or lactic dehydrogenase. Glyoxylic acid can be metabolized to formate, glycine, or malate, all of which may be further broken down to generate respiratory CO_2, or to oxalic acid, which is excreted in the urine. In excess, oxalic acid can form calcium oxalate crystals. Rate-limiting steps in the metabolism of ethylene glycol include the initial formation of glycolaldehyde and the conversion of glycolic acid to glyoxylic acid, both of which are saturable processes.

Both glycolic and oxalic acids are found in the blood and urine of unexposed individuals as a result of normal metabolism of proteins and carbohydrates (NTP-CERHR 2004). The ranges of background levels of glycolic acid are 0.0044–0.0329 mM (plasma) and 0.075–0.790 mM (urine) (NTP-CERHR 2004). For oxalic acid, the background ranges are 0.002–0.0233 mM (plasma) and 0.086–0.444 mM (urine) (NTP-CERHR 2004).

In two volunteers who inhaled ^{14}C-ethylene glycol for 4 hours, glycolic acid concentrations in the plasma peaked at about 4–5 hours after the commencement of exposure (Carstens et al. 2003). About 1% of the estimated dose of 0.96–1.51 mg/kg was excreted in the urine as glycolic acid, and 0.08–0.28% was excreted as oxalic acid over 30 hours. Expired CO_2 was not measured in this study.

Plasma glycolate levels of 12.2 and 15.4 mmol/L were reported upon hospital admission of an infant female and an adult male, respectively, with ethylene glycol intoxication after oral exposure (Hewlett et al. 1986). The infant survived, while the adult male died, probably due to delayed treatment. In a case report of six adult male patients with ethylene glycol intoxication, one of whom died, plasma glycolate levels on admission ranged from 17.0 to 29.3 mmol/L (Jacobsen et al. 1984).

Glycolic acid was the major metabolite in the plasma of male rats exposed orally to single gavage doses of 10, 100, or 1,000 mg/kg ^{14}C-ethylene glycol (Frantz et al. 1989, 1996c). During the first 12 hours after dosing, no oxalate was detected in the plasma at any dose, but glyoxylate and glyoxal, as well as trace amounts of glycoaldehyde, were detected in plasma samples from the lower dose groups (100 and 1,000 mg/kg). In the 10 mg/kg group, gyloyxylate levels exceeded glycolate levels throughout the 12 hours postdosing. glyoxylate

In rats given 2,000 mg/kg ethylene glycol by gavage, peak plasma levels of ethylene glycol occurred 2 hours after administration, while plasma glycolate levels peaked 6 hours after dosing (Hewlett et al. 1989). Dogs receiving 1,000 or 1,360 mg/kg ethylene glycol by gavage exhibited peak plasma ethylene

In vitro data provide conflicting comparisons between the rat and human rates of glycolic acid metabolism. Although a comparison of Km values obtained using liver homogenates from female humans and Sprague-Dawley rats (0.19 and 0.79 mM for humans and rats, respectively) (unpublished data of Bartels cited in NTP-CERHR 2004) suggested that humans may metabolize glycolic acid more efficiently than rats, a more recent study suggested the opposite. Booth et al. (2004) reported Km values of 0.43 and 0.28 mM (humans and rats, respectively) from a study using human and rat liver slices; these data suggest less efficient metabolism in humans.

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3.4.4 Elimination and Excretion

Little information is available on the elimination of ethylene glycol in humans; most of the elimination data are from humans accidentally poisoned and given therapeutic treatments to reduce the metabolism of ethylene glycol or extract it from the blood. In laboratory animals treated with ^{14}C-ethylene glycol, the primary routes of excretion are exhaled air and urine, regardless of the route of exposure. After oral exposure, saturation of metabolic pathways at higher doses leads to a shift in excretory pattern, with greater urinary excretion (and corresponding decreases in elimination via expired air) at higher doses.

3.4.4.1 Inhalation Exposure

Carstens et al. (2003) evaluated the urinary excretion of ethylene glycol and its two primary metabolites (glycolic and oxalic acids) in two volunteers who inhaled ^{14}C-ethylene glycol at doses estimated by the authors to be 0.96 and 1.51 mg/kg. Urinary excretion of ^{14}C-ethylene glycol up to 30 hours after exposure constituted 6.4–9.3% of the inhaled dose, while ^{13}C-glycolic acid and ^{13}C-oxalic acid together comprised 1–2% of the inhaled dose. However, the dose estimates are highly uncertain, as they were calculated by estimating the loss of ^{14}C-ethylene glycol from an inhalation vessel in which the compound was "warmed". Air concentrations to which the volunteers were exposed were not measured, and the warming temperature was not reported. The authors reported that ^{14}C-ethylene glycol was not detectable in exhaled air, but did not assess expiration of $^{14}CO_2$.

In rats, the major route of elimination for inhaled ethylene glycol is expiration of CO_2. Rats exposed for 30 minutes to ^{14}C-ethylene glycol vapor (32 mg/m^3) or for 17 minutes to ^{14}C-ethylene glycol aerosol (184 mg/m^3) excreted 63% (over 4 days) and 75% (over 6 days), respectively, of the initial body burden as $^{14}CO_2$ (Marshall and Cheng 1971). Urinary excretion constituted 20 and 12% of the initial body

burden after vapor and aerosol exposures, respectively, while fecal excretion was 3% and 1% (Marshall and Cheng 1971).

3.4.4.2 Oral Exposure

These data are not representative since they reflect elimination kinetics during dialysis, which is > than normal elimination

~~The approximate serum half-life of ethylene glycol was 1.5–3.0 hours in a child treated with hemodialysis and mannitol therapy (Rothman et al. 1986), and 2.7 hours in an adult male during hemodialysis and intravenous ethanol treatment (Cheng et al. 1987).~~ In untreated adults, the serum half-life has been estimated to be between 3.0 and 8.4 hours (Jacobsen et al. 1988; Peterson et al. 1981). Insert here.

In laboratory animals, the elimination half-lives for ethylene glycol in the plasma have been estimated at 1.4 - 2.5 hours in rats given between 10 and 2,000 mg/kg; 0.3–1.1 hours in mice given doses between 10 and 1,000 mg/kg; 3.5 hours in dogs given 1,000–1,360 mg/kg; and 2.7–3.7 hours in monkeys given 1,110 mg/kg (Frantz et al. 1989, 1991, 1996a, 1996c; Hewlett et al. 1989; McChesney et al. 1971). The plasma elimination half-life for ethylene glycol was similar (1.4–1.7 hours) in pregnant rats treated with single oral doses of 10 or 2,500 mg/kg on Gd 10 (Pottenger et al. 2001). Data from intravenous administration of ethylene glycol show similar elimination half-lives (Frantz et al. 1989, 1991, 1996a, 1996c; Martis et al. 1982).

Frantz et al. (1989, 1991, 1996b, 1996c) treated rats and mice with single oral doses of ^{14}C-ethylene glycol between 10 and 1,000 mg/kg and measured radioactivity in exhaled air, excreta, tissues, and carcass up to 96 hours after exposure. Table 3-7 shows the disposition of radioactivity. In male and female rats, the major excretory routes were via CO_2 exhalation (27–48% of the administered radioactivity) and urinary elimination (21–43%); 2–4% was excreted via the feces (Frantz et al. 1989, 1996b, 1996c). Female mice showed a similar profile when exposed over the same dose range, exhaling 22–55% of the dose as CO_2 and 3–11% as exhaled volatile organic compounds (VOCs), while excreting 24–56% in the urine and 5–16% in the feces (Frantz et al. 1991, 1996b). In mice, the majority of the exhaled radioactivity was eliminated during the first 12 hours after dosing (Frantz et al. 1991, 1996b). Both mice and rats exhibited a dose-dependent shift in excretory patterns, as shown in the data in Table 3-7. An increase in urinary excretion of radioactivity was evident between 10 and 100 mg/kg in female mice, between 10 and 400 mg/kg in female rats, and between 800 and 1,000 mg/kg in male rats. In its review of these data, NTP-CERHR (2004) noted that the increased urinary excretion of radioactivity probably resulted from saturation of the enzymes that metabolize glycolic acid, leading to increased excretion of this metabolite in the urine. Pottenger et al. (2001) provided data on urinary levels of

If PBPK models for ethylene glycol exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

PBPK models are available for ethylene glycol and its intermediate metabolite, glycolic acid, in rats and humans (Corley et al. 2005a). The models include the inhalation, oral, dermal, intravenous, and subcutaneous routes of exposure. Models for ethylene glycol consist of eight compartments connected by blood flow (lungs, richly perfused tissues, poorly perfused tissues, fat, skin, gastrointestinal tract, liver, and kidney); models for glycolic acid have a similar structure except that the lung is included in the richly perfused tissue group. Gastrointestinal tract, lung, and skin were included separately in order to permit simulation of different exposure routes. Models for both compounds assume instantaneous dispersion of the compound through each compartment based on blood perfusion rates and partition coefficients. The models for ethylene glycol and glycolic acid are connected via a saturable metabolic route in the liver, and renal elimination of both compounds was modeled.

Physiological parameters used in the model are shown in Table 3-8. Tissue volumes were scaled to body weight; alveolar ventilation and cardiac output were scaled as (body weight)$^{0.75}$; blood flows were scaled to cardiac output; and kidney parameters (glomerular filtration, tubule urine volume, and urine production) were scaled as a fraction of kidney weights. Partition coefficients used in the model are given in Table 3-9. Blood:air partition coefficients were measured *in vitro* using human and female Sprague-Dawley rat blood; tissue:blood coefficients were measured in rats, and human partition coefficients were assumed to equal those of rats.

A simplified metabolic pathway simulating metabolism of ethylene glycol to glycolic acid and from glycolic acid to glyoxylic acid (the rate-limiting steps) with saturable Michaelis-Menten kinetics was used in the model. Metabolic rate constants were estimated from *in vitro* data. Elimination via the kidneys was initially simulated as a first-order equation, but was modified to allow for reabsorption of glycolic acid in the renal tubules by a saturable Michaelis-Menten-like process in order to better predict elimination of this metabolite at low doses (<200 mg/kg). Table 3-10 shows the metabolic and renal elimination parameters used in the study.

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The model was validated against several pharmacokinetic studies in rats and humans (Corley and McMartin 2005; Corley et al. 2005a). In the examples reported by Corley et al. (2005a), the model

predictions provided reasonably good fit to measured plasma concentrations of ethylene glycol and glycolic acid after oral exposure to ethylene glycol in female Sprague-Dawley rats and intraperitoneal exposure to male Wistar rats, although predictions of glycolic acid concentrations after low-dose (10 mg/kg) oral exposure were not as reliable. The authors suggested that differences in analytical methods used to measure glycolic acid in the dataset used to determine model parameters and the validation dataset may have contributed to the less reliable prediction after low-dose exposure. Validation against human data was complicated by the need to incorporate effects of therapeutic interventions on blood levels of ethylene glycol and glycolic acid in humans acutely poisoned with ethylene glycol. With modifications to simulate these effects, the model provided reasonably good predictions of blood levels reported in several clinical case reports over a broad range of oral doses (Corley and McMartin 2005).

Using the model for humans, Corley et al. (2005a) estimated that the threshold glycolic acid concentration for developmental effects in rodents (considered by the authors to be a peak of 2 mM) would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). However, it is important to note that the human model has not been calibrated to the physiological changes associated with pregnancy, which require a different model structure (EPA 2006a). Furthermore, uncertainty in the glycolic acid saturation concentration in humans limits the usefulness of this model for predicting developmental toxicity in human embryos.

Data from a single study (Pottenger et al. 2001) suggested that pregnancy status did not affect the time course of ethylene glycol, glycolic acid, or oxalic acid pharmacokinetics in maternal blood and urine (including peak concentration, time of peak concentration, area under the concentration vs. time curve, or elimination half-time) when groups of pregnant and nonpregnant rats were treated by gavage with doses of 10 or 2,500 mg/kg ethylene glycol (pregnant rats treated on Gd 10). However, these data are not adequate to suggest that a pharmacokinetic model based on nonpregnant humans can be used to predict exposure to a developing human fetus. NTP-CERHR (2004) observed that pregnancy-related changes in metabolism would not be captured in this study due to the narrow exposure window (Gd 10). In addition, the study measured maternal, not fetal, levels of ethylene glycol and its metabolites. Slikker et al. (2004) reported that there are species-specific differences in the transfer of glycolic acid from maternal blood to conceptus. Likewise, fetal and/or placental differences in expression of enzymes metabolizing ethylene glycol and glycolic acid over the course of gestation will affect local concentrations of glycolic acid to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004). ~~Differences between rats and humans in the ontogeny of these enzymes and in the contribution of~~

maternal levels of glycolic acid to embryonic exposure limit the usefulness of this model for predicting developmental toxicity in humans. Model is useful - see comments.

Additional data are needed to reduce uncertainty in the saturation concentration of glycolic acid in humans, and such data may alter the model predictions of peak glycolic acid concentrations in humans exposed to ethylene glycol. Although NTP-CERHR (2004) suggested that humans may metabolize glycolic acid more efficiently than rats, based on a comparison of unpublished Km values obtained by Bartels using liver homogenates from female humans and Sprague-Dawley rats (0.19 and 0.79 mM for humans and rats, respectively), the authors noted the limited data supporting this finding. A more recent study published after the NTP review (Booth et al. 2004) reported Km values of 0.43 and 0.28 mM (humans and rats, respectively) from a study using human and rat liver slices; these data suggest less efficient metabolism in humans. As a result of the uncertainty in this critical parameter, predictions of peak human glycolic acid concentrations made using the existing PBPK model are likewise rendered uncertain. this has to be revised - see comments

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies investigating the mechanism by which ethylene glycol is absorbed from the lung, gastrointestinal tract, or skin were located.

Distribution. As discussed in more detail in Section 3.4.2, there are limited data on the distribution of ethylene glycol after inhalation exposure. Studies in rats, mice, and monkeys, as well as limited data in humans, suggest that ethylene glycol is distributed according to total body water (Frantz et al. 1989, 1991, 1996b, 1996c; Jacobsen et al. 1988). There are no data on the sites of ethylene glycol metabolism or on the distribution of its primary metabolite (glycolic acid) in the body. The inverted yolk sac placenta, which develops in both mice and rats, tends to concentrate weak acids such as glycolic acid; neither humans nor rabbits develop a yolk sac placenta, and a preliminary study showed that glycolic acid does not concentrate in rabbit embryonic fluids (NTP-CERHR 2004). insert No additional data are available to characterize the mechanisms by which ethylene glycol is transported to the kidneys or developing fetus, the primary sites of toxic action.

Metabolism. As discussed in more detail in Section 3.4.3, ethylene glycol metabolism has been well characterized. Glycolic acid has been identified as the primary metabolite and putative developmental

at 3 hours (Grauer et al. 1984). In these animals, the anion gap was also significantly increased at 3 hours (19 Meq/L).

The second characteristic of ethylene glycol intoxication is metabolic acidosis. Ethylene glycol itself has low toxicity (Godolphin et al. 1980; Jacobsen and McMartin 1986), but it is metabolized to a variety of toxic metabolites such as glycolaldehyde, glycolic acid (glycolate), glyoxylic acid (glyoxylate), and oxalic acid (oxalate) (Jacobsen et al. 1988; Parry and Wallach 1974; Vale 1979; Wiener and Richardson 1988). In general, the accumulation of acids leads to acidosis, a state that is characterized by actual or relative decrease of alkali in body fluids in relation to the acid content. In the case of ethylene glycol, metabolic processes that follow ethylene glycol ingestion lead to the accumulation of glycolic and lactic acids resulting in metabolic acidosis. The assumption that ethylene glycol toxicity is due to its metabolic products is made because there is a latent period before the symptoms of acidosis appear, because there is no correlation between observed toxicity and ethylene glycol blood concentration, and because inhibition of ethylene glycol oxidation prevents toxicity (Jacobsen and McMartin 1986). Furthermore, glycolic acid is the most abundant of all ethylene glycol metabolites (Jacobsen et al. 1984). Following ingestion of high doses of ethylene glycol, glycolic acid tends to accumulate because it is a substrate for lactic dehydrogenase and/or glycolic acid oxidase.

The accumulation of metabolites such as glycolic acid, oxalate, and lactic acid leads to an increased anion gap and metabolic acidosis, which are responsible for toxicity observed after ethylene glycol ingestion. While lactate levels increase in some human cases up to 5–7 mmol (Jacobsen et al. 1984, 1988; Parry and Wallach 1974), glycolate levels range up to 20–25 mmol, thus accounting for a greater portion of the anion gap. The serum anion gap is calculated by subtracting the sum of the serum chloride and bicarbonate ions from serum sodium ions. In dogs given oral doses of 10,743 mg/kg ethylene glycol, the anion gap was significantly increased at 3 hours (19 Meq/L) coinciding with peak serum ethylene glycol levels (Grauer et al. 1984). The maximum production of metabolites occurs 6–12 hours after ethylene glycol ingestion and coincides with development of respiratory & cardiovascular symptoms ~~neurotoxicity~~. Ethylene glycol metabolites inhibit oxidative phosphorylation, respiration, glucose metabolism, protein synthesis, deoxyribonucleic acid (DNA) replication, ribosomal ribonucleic acid (RNA) synthesis, central nervous system respiration, and serotonin metabolism (Vale 1979). Glycolic acid and lactic acid are the major and minor contributors, respectively, to the production of metabolic acidosis, one of the hallmarks of acute ethylene glycol intoxication.

Nephrotoxicity and neurotoxicity can follow because oxalate can produce renal and brain damage as it chelates with calcium ions forming insoluble calcium oxalate monohydrate crystals, another characteristic

of ethylene glycol poisoning (Jacobsen et al. 1988). This may lead to hypocalcemia and imbalance of serum divalent ion concentrations (Zeiss et al. 1989). Although the mechanism of ethylene glycol neurotoxicity is not completely understood, the available information on humans suggests that it occurs in two stages, an early one (30 minutes to 12 hours after exposure) and a late one (several days after exposure). The early-stage symptoms are due to the direct toxicity of ethylene glycol, while the late-stage neurotoxicity is due to metabolic acidosis caused by the accumulation of ethylene glycol metabolites, primarily glycolic acid, which leads to metabolic acidosis. Additional evidence for this late neurotoxicity is crystalline deposits of calcium oxalate in the walls of small blood vessels found in ~~the brain of a man~~ the brain of humans who died of acute ethylene glycol poisoning (Zeiss et al. 1989; Froberg et al, 2006). Similar effects were observed in rats fed 2,500 mg/kg/day ethylene glycol for 13 weeks (Melnick 1984). The role of calcium in ethylene-glycol-induced neurotoxicity is not known but the formation of calcium oxalate crystals may cause perturbation of intracellular calcium homeostasis causing membrane abnormalities generally associated with cell injury and cell death. A generalized soft tissue mineralization that included the heart (vessels and muscle), lungs (interstitial), stomach, and vascular system occurred in male F344 rats exposed to 1,000 mg/kg/day in the diet for 1 year (DePass et al. 1986a; Woodside et al. 1982). These histopathological changes may be the result of altered calcium metabolism (Rajagopal et al. 1977).

The presented data indicate that glycolic acid is the major toxic metabolite contributing to metabolic acidosis, which is a primary cause of systemic toxicity following exposure to ethylene glycol. Glycolic acid has also been identified as the proximate cause of developmental effects observed with ethylene glycol exposure (NTP-CERHR 2004; Slikker et al. 2004). A number of mechanistic studies have ruled out both ethylene glycol itself and other metabolites as the primary developmental toxicants, while metabolic acidosis was shown to interact with glycolic acid at high doses to enhance developmental effects. The available data suggest that peak concentrations in the range of 2–3 mM glycolic acid are necessary for developmental toxicity to occur in rodents (Carney et al. 2001; NTP-CERHR 2004; Slikker et al. 2004; Corley et al, 2002).

Klug et al. (2001) compared the effects of several ethylene glycol metabolites on rat whole embryos (Gd 9.5) in culture, observing that only glycolic acid affected embryonic development at metabolite concentrations observed in *in vivo* studies of ethylene glycol. Ethylene glycol and other metabolites did not affect development except at much higher concentrations than have been seen *in vivo*.

Using rat whole embryos (Gd 10) exposed to either ethylene glycol or glycolic acid for 46 hours *in vitro*, Carney et al. (1996) showed that concentrations up to 50 mM ethylene glycol did not cause

morphological changes, while glycolic acid caused changes in the skeletal and craniofacial regions at concentrations of ≥12 mM. These changes are consistent with the dysmorphogenesis observed in rats after *in vivo* exposure to ethylene glycol. In the same study, the role of medium acidification in the observed effects was investigated by comparing the effects of 12.5 mM glycolic acid (pH 6.7), 12.5 mM sodium glycolate (pH 7.4), and control medium (pH 7.4 or 6.7) on rat whole embryos in culture. The incidence of affected embryos was 67% in the glycolic acid group, 58% in the sodium glycolate group, 8% in the pH 6.7 controls, and 0% in the pH 7.4 controls. The authors concluded that glycolic acid was the primary developmental toxicant, and that medium acidification was a minor contributor to the observed effects.

In vivo studies have shown similar results. When glycolic acid was administered to CD rats via gavage on Gd 6–15, the observed effects on offspring were similar to those observed after ethylene glycol exposure (Munley et al. 1999). In an effort to determine the extent to which metabolic acidosis contributed to the developmental effects induced by glycolic acid, Carney et al. (1999) treated time-mated Sprague-Dawley rats with ethylene glycol (2,500 mg/kg) or glycolic acid (650 mg/kg) via gavage or sodium glycolate via subcutaneous injection on Gd 6–15. Metabolic acidosis was induced in both the ethylene glycol and glycolic acid groups, but not in the sodium glycolate treatment group. Upon sacrifice on Gd 21, fetal body weights were decreased and malformations were increased in all three groups, indicating that glycolate was capable of inducing effects in the absence of metabolic acidosis. The authors reported that developmental toxicity was enhanced by an interaction between metabolic acidosis and glycolate at high doses (Carney et al. 1999).

3.5.3 Animal-to-Human Extrapolations

Toxicokinetic and mechanistic data suggest that humans may be less sensitive than rodents to systemic and developmental effects of ingested ethylene glycol. ~~An~~ *In vitro* studies by ~~Bartels cited by NTP-CERHR (2004)~~ Booth et al (2004) and Corley et al (2005a) found that human liver tissue was more effective than liver tissue from rats and rabbits in metabolizing glycolic acid to glyoxylic acid, suggesting that humans are less likely to accumulate glycolic acid (the proximate developmental toxicant), ~~while other data conflict with this finding (Booth et al. 2004)~~. In addition, NTP-CERHR (2004) reviewed preliminary data by Carney and coworkers indicating that the inverted yolk sac placenta, a stage in placental development that does not exist in humans or rabbits, tends to concentrate weak acids such as glycolic acid in the embryonic fluids. Insert here These data suggest enhanced sensitivity to ethylene glycol developmental effects in rodents compared with humans; ~~however, NTP-CERHR (2004) characterized the available data as inconclusive.~~

Insufficient information is available to adequately assess the endocrine disruptor potential of ethylene glycol. No studies were located regarding endocrine disruption in humans after exposure to ethylene glycol.

No histopathological changes occurred in endocrine organs of rats or mice in acute-, intermediate- and chronic-duration oral studies of ethylene glycol. As discussed in the Endocrine Effects subsection of Section 3.2.2.2, histological examinations in these studies included the adrenals, pancreas, pituitary, thyroid, and/or parathyroids (Blood 1965; DePass et al. 1986a; Hong et al. 1988; Melnick 1984; NTP 1993; Robinson et al. 1990; Schladt et al. 1998; Woodside et al. 1982). Assessments of endocrine function (e.g., hormone levels) were not conducted in these or other studies of ethylene glycol.

Reproductive toxicity studies showed that oral exposure to high doses of ethylene glycol affected fertility and fetal viability in mice and rats (Harris et al. 1992; Lamb et al. 1985; Morrisey et al. 1989; NTP 1986; Price et al. 1985; Schuler et al. 1984), and possibly male reproductive function in mice (Morrissey et al. 1989; NTP 1986) and gestational duration in rats (NTP 1988).

Ethylene glycol had no estrogenic or antiestrogenic activity in an *in vitro* MVLN cell-based transactivation assay (Freyberger and Schmuck 2005). MVLN cells constitutively express the estrogen receptor (ER) and are stably trasfected with the luciferase reporter gene and the corresponding hormone responsive element derived from the *Xenopus* Vitellogenin A2 gene. Evaluations included cytotoxicity and luciferase gene expression in the absence and presence of estradiol stimulation, as well as ER-α binding affinity.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Young children are susceptible to ethylene glycol poisoning through the accidental ingestion of antifreeze because it is a brightly colored, sweet tasting liquid that can be mistaken for a beverage (Leth and Gregersen 2005). Many ethylene glycol poisonings occur when an antifreeze bottle is in use or when antifreeze is not kept in its original container (e.g., if it is poured into a cup or soft drink bottle), because children can ingest ethylene glycol from an accessible open container (EPA 2004b; Leth and Gregersen 2005). Children also may play in a puddle of antifreeze that has been spilled or drained onto the ground. Children and adolescents comprise a significant percentage of ethylene glycol acute intoxications from accidental or intentional ingestion. For example, in a total of 4,938 exposures voluntarily reported to U.S. poison control centers in 2001, 1,404 (28%) were younger than 19 years old and 713 (14%) were younger than 6 years old (Litovitz et al. 2002). Similarly, of 735 total exposures reported in 2003, 150 (20%) were younger than 19 years old and 84 (11%) were younger than 6 years old (Watson et al. 2004). It has been reported that ingestion of as little as 10–15 mL ethylene glycol can be fatal in small children (White and Liebelt 2006).

See comment

A limited amount of information on health effects of ethylene glycol in children is available from several case reports of patients admitted to hospitals for treatment of acute oral poisoning. A 4-year-old girl (14 kg) who accidentally ingested an unknown amount of antifreeze containing 41% ethylene glycol vomited and was admitted to a hospital 4 hours later, where drowsiness, hypotonia, and metabolic acidosis subsequently developed (Harry et al. 1998). A 13-year-old girl (80 kg) who intentionally ingested approximately 4 fluid ounces of antifreeze (ethylene glycol concentration not reported) was brought to a hospital approximately 30 minutes after ingestion with no evidence of intoxication, but subsequently developed ataxia, dysarthria, metabolic acidosis, and oxalate crystals in the urine (Boyer et al. 2001). An 8-month-old boy (7.7 kg) who drank up to 120 mL ethylene glycol (95%) was taken to a hospital where he appeared lethargic; metabolic acidosis, increased osmolal gap, and oxalate crystals in the urine were detected 3–4 hours post-ingestion (Baum et al. 1999). Six children ranging in age from 22 months to 14 years were admitted to a hospital for treatment of ethylene glycol poisoning over a 4-year period (Caravati et al. 2004). Four of the children (7–13 years old, 22–50 kg) ingested between 30 and 120 mL (alleged doses) of antifreeze (ethylene glycol concentration not reported); the amounts ingested by the other two children were unknown. Presenting symptoms included dizziness, slurred speech, nausea, ataxia, and lethargy. Varying degrees of metabolic acidosis were also observed, but renal function was normal.

mutations in orally-exposed rats (DePass et al. 1986b) and was consistently negative in *in vitro* genotoxicity assays in a variety of test systems, indicating that it is unlikely to affect DNA in parental germ cells.

No studies are available that describe potential differences in the toxicokinetics or the mechanism of action of ethylene glycol in children. As discussed in Section 3.5.2, Mechanisms of Toxicity, glycolic acid is the major toxic metabolite contributing to metabolic acidosis, which is a primary cause of systemic toxicity in children as well as adults following exposure to ethylene glycol. Glycolic acid has also been identified as the proximate cause of the developmental effects in animals observed with ethylene glycol exposure (NTP-CERHR 2004; Slikker et al. 2004).

Ethylene glycol metabolism is known to involve alcohol dehydrogenase and aldehyde dehydrogenase, and may also involve cytochrome P450 isozymes (NTP-CERHR 2004). Polymorphisms in the genes encoding these enzymes may lead to wide variability in the production and elimination of glycolic acid and other metabolites in humans exposed to ethylene glycol, but data quantifying the range of variability are not currently available (NTP-CERHR 2004). In addition, fetal and/or placental differences in expression of these enzymes over the course of gestation will affect local concentrations of glycolic acid and other metabolites to which the developing conceptus is exposed, yet little is known about these differences (NTP-CERHR 2004).

A PBPK model for ethylene glycol in adult humans has been developed and has been used to estimate that the threshold glycolic acid concentration for developmental effects in rodents would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female) (Corley et al. 2005a). However, ~~the human model has not been calibrated to the physiological changes associated with pregnancy, and~~ no models are available for children or lactating women. A PBPK model has also been developed for rats (Corley et al. 2005a), but there is no model for mice, which are more sensitive than rats to ethylene glycol developmental toxicity. Biomonitoring data for children, including levels of ethylene glycol in placental tissue, cord blood, neonatal blood, meconium fluid, or breast milk, have not been located.

→ delete for reasons noted several times above

absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Ethylene Glycol

The presence of parent compound in the blood and urine serves as the only biomarker of exposure that is specific to ethylene glycol. The half-life of ethylene glycol in plasma is estimated to be 3–7 hours in laboratory animals (Marshall 1982; Winek et al. 1978). Available human data indicate a similar half-life for ethylene glycol in human plasma (Eder et al. 1998). The elimination half-life of ethylene glycol ~~in the urine of~~ acutely intoxicated humans ranges from 3.0 to 8.4 hours (Jacobsen et al. 1988; Peterson et al. 1981). Based on the relatively short half-life in the blood and urine, the presence of parent compound would serve as a reliable biomarker of exposure only within the first day following exposure. Rapid methods for determining ethylene glycol in serum and urine are available for use in the clinical setting (Aarstad et al. 1993; Blandford and Desjardins 1994), but may not always be readily available in emergency situations.

Other biomarkers of exposure are typically used in conjunction with serum and urinary ethylene glycol levels to assist in confirmation and quantitation of ethylene glycol intoxication. However these other biomarkers are not specific to ethylene glycol. For example, levels of glycolic, lactic, and oxalic acid metabolites of ethylene glycol may be useful indicators of ethylene glycol-induced toxicity, but they are not specific to ethylene glycol. As discussed in detail in Section 3.4, ethylene glycol is rapidly metabolized to glycolic acid, which accumulates in the blood and causes metabolic acidosis (Gabow et al. 1986; Jacobsen et al. 1984). Glycolic acid blood levels have been more closely correlated to clinical symptoms than ethylene glycol blood levels (Hewlett et al. 1986). Due to the rapid formation of glycolic acid in the body and its correlation to clinical symptoms of ethylene glycol poisoning, measurements of both parent compound and glycolic acid levels are important in diagnosis and treatment (Hess et al. 2004). Lactic acid may contribute to metabolic acidosis, whereas oxalic acid forms calcium oxalate crystals that are considered to be the cause of ethylene glycol-induced nephrotoxicity (Jacobsen and McMartin 1986; Wiley 1999).

The presence of calcium oxalate monohydrate crystals is an indicator of possible ethylene glycol intoxication, although not specific to ethylene glycol. The crystals can be found in renal tubules and/or urine after exposure to relatively large amounts of ethylene glycol (CDC 1987; Chung and Tuso 1989;

Andrus 1962). Vitamin B6 deficiency can cause inhibition of ethylene glycol's oxidation to carbon dioxide and thus cause an increase in ethylene glycol toxicity. Magnesium may prevent renal deposition of calcium oxalate by altering solvent characteristics of oxalate in urine (Browning 1965; Gershoff and Andrus 1962; Khan et al. 1993).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to ethylene glycol than will most persons exposed to the same level of ethylene glycol in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of ethylene glycol, or compromised function of organs affected by ethylene glycol. Populations who are at greater risk due to their unusually high exposure to ethylene glycol are discussed in Section 6.7, Populations with Potentially High Exposures.

Individuals deficient in vitamin B6 could be more sensitive to toxic effects of ethylene glycol because vitamin B6 may increase (increase) the accumulation of toxic metabolites (Browning 1965; Gershoff and Andrus 1962). Similarly, magnesium deficiency appears to encourage calcium oxalate deposition in the renal tubules, especially in the presence of high calcium levels (Ebisuno et al. 1987). Thus, individuals who are deficient in magnesium and/or ingest high levels of calcium may be more sensitive to the toxic effects of ethylene glycol.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to ethylene glycol. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to ethylene glycol. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to ethylene glycol:

Egbert PA, Abraham K. 1999. Ethylene glycol intoxication: Pathophysiology, diagnosis, and emergency management. ANNA J 26(3):295-300.

Ellenhorn MJ, Schonwald S, Ordog G, et al, eds. 1997. Ellenhorn's medical toxicology. Diagnosis and treatment of human poisoning. Baltimore, MD: Williams & Wilkins, 1152-1156.

See comments

et al. (2004) study and 71 and 180 mg/kg/day in the Gaunt et al. (1974) study. The Gaunt et al. (1974) study was used for MRL derivation because it identified the lowest LOAEL and is better suited for BMD analysis due to a larger number of dose levels in the lower dose range (below the 150–180 mg/kg/day threshold region). Additional testing could increase confidence in the MRL by addressing limitations in the critical study (e.g., relatively small group sizes and incomplete compliance with current test guidelines due to age of study) and providing additional dose-response data in the lower dose range.

No information is available on the intermediate-duration dermal toxicity of ethylene glycol. Studies using the dermal route would be useful because absorption and systemic distribution of ethylene glycol has been shown in dermal toxicokinetic studies in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c).

Chronic-Duration Exposure and Cancer. Information on the health effects of chronic inhalation exposure to ethylene glycol is essentially limited to the negative results of an epidemiologic study on renal cancer mortality in humans (Bond et al. 1985). This study is not suitable for assessing chronic inhalation toxicity because it lacks noncancer end points, measured exposure concentrations, and other relevant information. Chronic testing in animals is needed to provide a basis for chronic inhalation MRL derivation and to adequately assess the potential for inhalation carcinogenicity.

The chronic oral toxicity and carcinogenicity of ethylene glycol was evaluated in three studies in rats (Blood 1965; DePass et al. 1986a; Wilson et al, 2005) and two studies in mice (DePass et al. 1986a; NTP 1993). None of the studies provided evidence of carcinogenicity. The kidney and liver were the main targets of toxicity in both species, and rats were more sensitiye than mice. Effects in the rats included kidney lesions at ≥300 mg/kg/day in male Wistar rats, kidney lesions at ≥375 mg/kg/day and mortality at 750 mg/kg/day in male Sprague-Dawley rats (Blood 1965), kidney lesions and mortality at 1,000 mg/kg/day in male F344 rats (DePass et al. 1986a), and liver lesions in female F344 rats at ≥200 mg/kg/day (DePass et al. 1986a). Chronic NOAELs of 150–200 mg/kg/day were identified for kidney lesions in the Sprague-Dawley and F344 male rats, but no information is available on effects of chronic exposure in Wistar rats, a strain shown to be approximately twice as sensitive as F344 rats to kidney toxicity in a 16-week study (Cruzan et al. 2004), and the strain used to derive the intermediate-duration oral MRL. The intermediate-duration NOAEL for kidney toxicity in Wistar males is 150 mg/kg/day (Gaunt et al. 1974), which is in the range of the 150–200 mg/kg/day chronic NOAELs for kidney toxicity in F344 and Sprague-Dawley males. Additionally, the 180 mg/kg/day intermediate-duration LOAEL for kidney toxicity in Wistar males is lower than the 200 mg/kg/day chronic LOAEL for liver lesions in female F344 rats (DePass et al. 1986a). The chronic NOAEL for liver toxicity in F344 females is 40 mg/kg/day. Although 40 mg/kg/day is also a chronic-

Change this

~~NOAEL for kidney effects in F344 males, it is not known if it is a chronic NOAEL for kidney effects in Wistar males. The lack of chronic data in Wistar rats precludes derivation of a chronic MRL because it is not known whether an MRL based on the liver toxicity data in F344 rats would be protective of kidney toxicity. A chronic oral toxicity study in Wistar rats therefore is needed to provide a suitable basis for a chronic oral MRL. This study could also be used to confirm the lack of carcinogenicity in the available studies.~~

Genotoxicity. Human genotoxicity data were not located for ethylene glycol. A single *in vivo* study was located in which ethylene glycol did not produce dominant lethality in orally-exposed rats (DePass et al. 1986b). Available *in vitro* assays in a variety of test systems consistently provide negative results for genotoxicity (Abbondandolo et al. 1980; Clark et al. 1979; Griffiths 1979, 1981; Hastwell et al. 2006; Kubo et al. 2002; McCann et al. 1975; McCarroll et al. 1981; McGregor et al. 1991; Miller et al. 2005; Pfeiffer and Dunkelberg 1980; Storer et al. 1996; Zeiger et al. 1987). Additional *in vivo* animal studies could be conducted to more completely assess the genotoxicity of ethylene glycol, although available data do not indicate that the compound is of genotoxicity concern.

Reproductive Toxicity. Studies have not addressed the reproductive toxicity of ethylene glycol in humans. Reproductive testing in animals includes three multigeneration studies (one in rats and two in mice) and several shorter studies (15–20 days in rats and mice) by the oral route (DePass et al. 1986b; Harris et al. 1992; Lamb et al. 1985; Morrisey et al. 1989; NTP 1986. 1988). The only effect in rats was an increase in gestational duration, whereas fertility and fetal viability were affected in mice. Mice also showed some changes in sperm parameters, as well as testicular and epididymal lesions (Morrissey et al. 1989; NTP 1986); however, the incidence of testicular effects was high in the control group, so the relationship to ethylene glycol exposure is uncertain. Additional reproductive testing may not be needed because several multigeneration studies have been conducted, and most studies suggest that reproductive effects occur at higher doses than developmental effects.

Developmental Toxicity. Studies have not addressed the developmental toxicity of ethylene glycol in humans. The developmental toxicity of oral exposure to ethylene glycol has been studied in rats, mice and rabbits over a wide range of doses (DePass et al. 1986b; Harris et al. 1992; Lamb et al. 1985; Maronpot et al. 1983; Marr et al. 1992; Morrissey et al. 1989; Neeper-Bradley 1990; Neeper-Bradley et al. 1995; NTP 1986; Price et al. 1985; Schuler et al. 1984; Tyl 1989; Tyl et al. 1993), indicating that further evaluation of the developmental toxicity of orally-administered ethylene glycol toxicity may not be warranted. The most sensitive indicator of developmental toxicity appears to be an increased

incidence of malformations, primarily skeletal malformations, in both mice and rats. Available data suggest that malformations appear in mice at lower doses than those which cause malformations in rats. As indicated in the discussion of data needs for Acute-Duration Exposure, the acute oral MRL for ethylene glycol is based on developmental effects in mice exposed to ethylene glycol daily by gavage on Gd 6–15 (Neeper-Bradley et al. 1995; Tyl 1989). An uncertainty in the acute-duration oral MRL that may need to be addressed stems from the use of gavage administration in the MRL study. Bolus doses from gavage administration ~~can~~ lead to higher peak blood concentrations of glycolic acid (the proximate developmental toxicant) than ~~would~~ occur with slower dose-rates associated with environmentally-relevant exposures (~~Carney et al. 2001~~ Corley et al. 2002; NTP-CERHR 2004). Because the MRL study used gavage administration, the dose at which effects were observed ~~may have been~~ is lower than would be observed with non-bolus dosing (Carney et al., 2001)

Developmental toxicity has also been assessed in rats and mice by the inhalation route. Results of the inhalation developmental studies are generally consistent with the oral findings, but are confounded by concurrent oral exposure via ingestion of aerosolized ethylene glycol on the fur of exposed animals (Tyl 1985, 1988a; Tyl et al. 1995a, 1995b). The studies included a nose-only inhalation study in mice aimed at reducing the confounding oral exposure, but these animals had exposure by ingestion of ethylene glycol deposited on the face (Tyl 1988a; Tyl et al. 1995a). Additionally, stress from restraint in the nose-only exposure study may have contributed to the developmental effects observed with ethylene glycol (NTP-CERHR 2004), which were similar in nature to effects observed in a study of restrained nose-only exposure to water vapor (Tyl et al. 1994). Because of the confounding oral exposure in both the whole-body and nose-only studies, as well as the confounding effect of stress due to restraint in the nose-only study, additional testing is needed to adequately evaluate developmental effect levels from inhalation exposure to ethylene glycol. Given the problems of oral exposure from deposition of ethylene glycol on the fur, the feasibility of conducting an adequate inhalation study is unclear.

A single well-designed study of dermal gestational exposure to ethylene glycol found no developmental toxicity in mice (Tyl 1988b; Tyl et al. 1995c). Additional dermal testing could confirm the apparent low potential for developmental toxicity by this route of exposure.

Immunological and Lymphoreticular Effects. A limited amount of information on immunological and lymphoreticular effects of ethylene glycol is available from oral studies in animals. There were no histopathological alterations in the spleen, lymph nodes, or thymus, or consistent changes in leukocyte counts in rats or mice in acute-, intermediate-, and chronic-duration oral studies of ethylene

contaminated soil or water, inhalation of ethylene glycol vapor or mist, or ingestion of contaminated groundwater. Additionally, occupational exposure through inhalation of ethylene glycol vapor or mist and dermal contact is expected for individuals involved in airport de-icing spray operations.

Biomarkers of Exposure and Effect.

Exposure. The only biomarker of exposure that is specific to ethylene glycol is parent compound in the blood and urine. Based on the relatively short half-life of ethylene glycol in the blood and urine (Eder et al. 1998; Jacobsen et al. 1988; Peterson et al. 1981), parent compound would likely be detectable only within a few hours to 1 day following acute ingestion. Rapid methods for determining ethylene glycol in serum and urine are available for use in the clinical setting (Aarstad et al. 1993; Blandford and Desjardins 1994), but ~~may~~ are often not be readily available in emergency situations.

However, increased blood glycolate above normal human background levels is strongly indicative of exposure

Other identified biomarkers of exposure are not specific to ethylene glycol, They include ethylene glycol metabolites such as glycolic, lactic, and oxalic acids in blood and/or urine; and calcium oxalate monohydrate crystals in renal tubules and/or urine. Insert here

Based on available information regarding the toxicokinetics of ethylene glycol and its metabolites, and available methods for identifying parent compound and metabolites in body fluids, it appears that ethylene glycol poisoning can be adequately diagnosed in most cases. Additional studies to assess additional potential biomarkers of exposure for ethylene glycol do not appear necessary at this time.

Effect. Biomarkers of effects exist for ethylene glycol poisoning, but none are specific to ethylene glycol. These include clinical manifestations of central nervous system, cardiopulmonary, and renal toxicity, and laboratory findings of metabolic acidosis and calcium oxalate crystalluria. Clinical manifestations progress in three main stages. Signs of central nervous system toxicity appear within 0.5–12 hours following acute ingestion, although manifestations suggestive of cranial nerve damage may appear as late as 1–2 weeks after exposure (CDC 1987; Cheng et al. 1987; Chung and Tuso 1989; Factor and Lava 1987; Hess et al. 2004; Leth and Gregersen 2005; Lewis et al. 1997; Mallya et al. 1986; Parry and Wallach 1974; Rothman et al. 1986; Spillane et al. 1991; Underwood and Bennett 1973; Zeiss et al. 1989). Cardiopulmonary manifestations generally develop after 12–24 hours and renal failure occurs after 24–72 hours (Godolphin et al. 1980; Hess et al. 2004; Leth and Gregersen 2005; Parry and Wallach 1974; Siew et al. 1975a; Vale 1979; Zeiss et al. 1989). Ethylene glycol-induced metabolic acidosis occurs approximately 12–24 hours following ingestion and is characterized by pronounced serum osmolal

responsible for metabolizing ethylene glycol and glycolic acid, inter-individual variability in metabolic parameters (e.g., polymorphisms in genes encoding these isozymes), and developmental ontogeny of these isozymes are needed to better characterize species differences and identify sensitive subpopulations. In addition, further information is needed on species differences in metabolic rates and saturation points, as available data provide ~~conflicting~~ information on the relative sensitivity of humans and laboratory rodents. inadequate

Because most human exposure has been associated with acute accidental or intentional poisoning incidents, there are few data on the elimination kinetics of ethylene glycol after oral exposure in humans. Most of the available estimates of plasma elimination half-lives have been confounded by concurrent therapeutic treatments such as ethanol administration or hemodialysis that modify elimination kinetics. Elimination of orally-administered ethylene glycol across a broad dose range has been thoroughly studied in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c), and to a more limited extent in monkeys (McChesney et al. 1971).

No data describing the kinetics of *in vivo* human dermal exposure were found in the literature. The *in vitro* permeability of human skin to ethylene glycol has been studied, with widely varying results. Using full-thickness cadaver skin, Loden (1986) estimated a percutanous absorption rate of 118 $\mu g/cm^2$/hour with a steady-state concentration of 0.97 mg/cm^2, while Driver et al. (1993) estimated absorption rates of 0.09–0.25 $\mu g/cm^2$/hour for three different skin samples. The absorption, distribution, metabolism, and elimination of ethylene glycol administered dermally has been thoroughly studied in rats and mice (Frantz et al. 1989, 1991, 1996b, 1996c).

All of the toxicokinetic data in humans and animals were collected after acute exposures to ethylene glycol; there are no data on toxicokinetics after intermediate- or chronic-duration exposures. Intermediate- and chronic-duration data are needed in order to adequately assess absorption, metabolism, and elimination with prolonged exposure.

Comparative Toxicokinetics. Species differences in *in vivo* toxicokinetics are not well characterized. While there are high quality toxicokinetic data comparing absorption, distribution, metabolism, and excretion in mice and rats (Frantz et al. 1989, 1991, 1996a, 1996b, 1996c), available data in other species (Hewlett et al. 1989; McChesney et al. 1971) are more limited; in many cases, only single dose levels were used, the numbers of animals per dose were small, and mass balance information was

incomplete. Available data in humans are limited to acute, high-dose exposures, with toxicokinetic data often confounded by the effects of therapeutic interventions.

Using a PBPK model for humans, Corley et al. (2005a) estimated that the threshold glycolic acid concentration for developmental effects in rodents (considered by the authors to be a peak of 2 mM) would only be reached in human females ingesting doses of 350 mg/kg (assuming a 58-kg female). ~~However, the human model has not been calibrated to the physiological changes associated with pregnancy~~.

Slikker et al. (2004) reported that there are species-specific differences in the transfer of glycolic acid, the primary metabolite and putative developmental toxicant associated with ethylene glycol exposure, from maternal blood to conceptus. NTP-CERHR (2004) noted that the inverted yolk sac placenta that develops in both mice and rats tends to concentrate weak acids including glycolic acid; neither humans nor rabbits develop a yolk sac placenta, ~~and~~ A preliminary study by Carney and coworkers showed that glycolic acid does not concentrate in rabbit embryonic fluids. In addition, fetal and/or placental differences in expression of enzymes metabolizing ethylene glycol and glycolic acid over the course of gestation will affect local concentrations of glycolic acid to which the developing conceptus is exposed, yet little is known about species differences in the ontogeny of these enzymes (NTP-CERHR 2004).

Additional data are needed to reduce uncertainty in the saturation concentration of glycolic acid in humans. ~~Although a comparison of Km values obtained using liver homogenates from female humans and Sprague-Dawley rats suggested that humans may metabolize glycolic acid more efficiently than rats (0.19 and 0.79 mM for humans and rats, respectively; Bartels 2001), a more recent study suggested the opposite. Booth et al. (2004) reported Km values of 0.43 and 0.28 mM (humans and rats, respectively) from a study using human and rat liver slices; these data suggest less efficient metabolism in humans,~~

Methods for Reducing Toxic Effects. No studies were found describing methods to reduce peak absorption of ethylene glycol after inhalation exposure. After oral exposure, gastric lavage may be of benefit in reducing absorption, but only if performed within 1–2 hours following ingestion (Barceloux et al. 1999; Egbert and Abraham 1999; Leth and Gregersen 2005). Dermal absorption can be minimized through washing the skin with soap to remove any existing ethylene glycol.

Clinical procedures for treating ethylene glycol poisoning focus on reducing the body burden of ethylene glycol and its toxic metabolites, interference with toxic metabolite formation (which results in increased

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

A limited amount of information on health effects of ethylene glycol in children is available from several case reports of patients admitted to hospitals for treatment of acute oral poisoning (Baum et al. 1999; Boyer et al. 2001; Caravati et al. 2004; Harry et al. 1998). The effects in these pediatric patients were largely consistent with the first stage of ethylene glycol poisoning in adults (e.g., central nervous system depression, metabolic changes, gastrointestinal upset). Treatment with fomepizole (4-methylpyrazole), alone or in combination with other methods, generally mitigated the progression of the clinical course to the second and third stages of ethylene glycol poisoning (pronounced metabolic acidosis, cardiopulmonary compromise, and renal insufficiency) and led to full recovery. The case reports are consistent with an expectation that health effects in children and adults are similar. Although there are no known differences in the toxicity of ethylene glycol between adults and children, there is no evidence to substantiate the presumption. There is no evidence to indicate that children are likely to be exposed to higher or lower amounts of ethylene glycol from everyday living, suggesting that children are perhaps equally at risk for non-accidental/non-intentional acute oral exposure and potential toxic side effects. Information is lacking on the toxicity of longer duration exposures in children, as well as on developmental effects in children.

No studies are available that describe potential differences in the toxicokinetics or the mechanism of action of ethylene glycol in children. Glycolic acid is the major toxic metabolite contributing to metabolic acidosis, which is a primary cause of systemic toxicity in children as well as adults following exposure to ethylene glycol. Glycolic acid has also been identified as the proximate cause of the developmental effects in animals observed with ethylene glycol exposure (NTP-CERHR 2004; Slikker et al. 2004).

Limited mechanistic information suggests that humans may be less sensitive than rodents to the developmental effects of ethylene glycol. Two in vitro studies (Barth et al, 2004; Corley et al 2005a) ~~An unpublished study by Bartels cited by NTP-CERHR (2004)~~ suggested that humans metabolize glycolic acid more efficiently than rats, ~~while other data conflict with this finding (Booth et al. 2004),~~ although the data supporting the glycolic acid metabolic rate in humans are limited (NTP-CERHR 2004). Additionally, NTP-CERHR (2004) reviewed preliminary data indicating that the inverted yolk sac placenta, a stage in placental development that occurs in rats and mice but does not exist in humans or rabbits, tends to concentrate weak acids such as glycolic acid in the

2004; Rebsdat and Mayer 2005). A second method was introduced in 1937, the direct oxidation of ethylene to ethylene oxide followed by hydrolysis to ethylene glycol (Brown et al. 1980; Forkner et al. 2004). This soon became the primary method for the production of ethylene glycol and is currently the only method used in the United States (Brown et al. 1980; Forkner et al. 2004; Rebsdat and Mayer 2005).

Other methods that have been used to manufacture ethylene glycol include the direct oxidation of ethylene and synthesis from carbon monoxide, methanol, hydrogen, and formaldehyde (Forkner et al. 2004; Rebsdat and Mayer 2005). The methanol and formaldehyde used in the latter method is obtained from syngas, which is originally obtained from coal.

Ethylene oxide is converted to ethylene glycol through uncatalyzed neutral hydrolysis (pH 6–10) in the presence of a large excess of water at high temperatures and pressures (Forkner et al. 2004; Rebsdat and Mayer 2005). Selectivity of ethylene glycol is 89–91% in this process. The primary byproduct is diethylene glycol with higher glycols such as triethylene and tetraethylene glycols formed in smaller amounts. The product mixture is fed through a series of evaporators to remove the water and then through vacuum distillation for separation and refinement of the individual glycols.

5.2 IMPORT/EXPORT

Both U.S. imports and exports of ethylene glycol have increased since the 1970s. Annual ethylene glycol imports rose from 29,300 metric tons in 1977 to 289,000 metric tons in 2006, while annual exports rose from 56,800 metric tons in 1978 to 573,000 metric tons in 2006 (HSDB 2007; U.S. Department of Commerce 2007). From 2000 to 2006, the average annual U.S. import and export quantities were 317,000 and 556,000 metric tons, respectively (U.S. Department of Commerce 2007). Annual U.S. ethylene glycol import and export quantities reported for different years are listed in Table 5-3. Over 70% of the ethylene glycol imported into the United States during 2006 was imported from Saudi Arabia (114,846 metric tons) and Canada (93,669 metric tons) (U.S. Department of Commerce 2007).

5.3 USE

Ethylene glycol has been used in a wide variety of industrial applications because of its unique chemical and physical properties. Ethylene glycol dissolves in water and is miscible in alcohol and acetone, has the capacity to hold large amounts of heat before boiling, and lowers the freezing point of water (Lewis 2001; O'Neil et al. 2001; Rebsdat and Mayer 2005). In addition, ethylene glycol is hygroscopic (has the ability to absorb twice its weight in water), is suitable for use as an industrial humectant (drying agent), and

1992). At an initial substrate concentration of 111 mg/L (ppm), naturally occurring microorganisms in groundwater biodegraded ethylene glycol with a calculated half-life of <1 day following a lag phase of <3 days.

Ethylene glycol is not expected to undergo significant abiotic transformation in surface waters via hydrolysis or oxidation (EPA 1979; Harris 1990). Glycols are resistant to hydrolysis (Harris 1990). Ethylene glycol is not expected to undergo direct photolysis in sunlit waters since alcohols do not absorb UV light at environmental wavelengths (above 295 nm) (Boethling and Mackay 2000). However, indirect photolysis of ethylene glycol sorbed to goethite (a common natural constituent of surface water sediments) by near ultraviolet radiation (300–400 nm) has been demonstrated in the laboratory. Formaldehyde and glycolaldehyde were detected as degradation products (Cunningham et al. 1985).

6.3.2.3 Sediment and Soil

Biodegradation under both aerobic and anaerobic conditions is also the most important transformation process for ethylene glycol in soils, with a half-life similar to or less than that in surface waters (EPA 1987a).

The rate of biodegradation of ethylene glycol in simulated subsurface soils is dependent on substrate concentrations, soil types, and ambient soil temperatures (McGahey and Bouwer 1992). Greater than 95% removal was consistently accomplished in <5 days and 7 days at ethylene glycol concentrations of 100 and 1,000 ppm, respectively; however, substrate concentrations of 10,000 ppm showed negligible loss of ethylene glycol. The rate of degradation was higher in soils with high organic matter. A doubling in the degradation rate was also observed with a 10 °C increase in soil temperature. McGahey and Bouwer (1992) concluded that microorganisms naturally occurring in soils and groundwater are effective in biodegrading ethylene glycol with the half-life ranging from 0.2 to 0.9 days. Approximately 23–26% of ethylene glycol at 2.25 ppm was biodegraded in anaerobic sandy till soil grab sample tests run for 86 and 140 days (Lokke 1984).

Klecka et al. (1993) studied the biodegradation of aircraft de-icing fluids in soils adjacent to airport runways at various ethylene glycol concentrations and at various temperatures ranging from -2 to 25 °C. Generally, the rate of biodegradation of ethylene glycol was faster in soils with low glycol concentrations, high organic carbon content, and higher ambient soil temperatures. Ethylene glycol present in soils at concentrations <6,000 mg/kg (ppm) biodegraded at an average rate of 3.0 mg/kg (ppm) soil/day at -2 °C,

6.4.4 Other Environmental Media

Ethylene glycol has been found to migrate into a number of foods from regenerated cellulose films containing triethylene glycol and polyethylene glycol as softening agents. Ethylene glycol was detected in fruit cakes at 27–34 mg/kg (ppm) after 84–336 days of storage, in meat pies at <10 mg/kg (ppm) after 3–7 days of storage, in toffee at <10–22 mg/kg (ppm) after 168–450 days of storage, in madeira cake at <10–22 mg/kg (ppm) after 21–28 days storage, and in boiled sweets at 14–34 mg/kg (ppm) after 168–450 days storage (Castle et al. 1988a). According to Kashtock and Breder (1980), ethylene glycol can migrate into food simulants from polyethylene terephthalate (PET) bottles used in the packaging of carbonated beverages. The compound was detected at a concentration of about 100 ppb (0.1 ppm) in a 3% acetic acid solution used as a food simulant after 6 months of storage at 32 °C (Kashtock and Breder 1980). These authors stated that the source of ethylene glycol in this food simulant is the small amount of unreacted ethylene glycol in the polyethylene terephthalate polymer. More recent information regarding levels of ethylene glycol in food is not available.

Ethylene glycol has been identified in negligible amounts in the water-soluble component of cigarette smoke (Schumacher et al. 1977).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Ethylene glycol concentrations in blood, urine, tissue, or breast milk are not available for the general population in the United States. The most common route of human exposure to ethylene glycol for members of the general population is dermal contact with ethylene glycol-based automobile antifreeze. However, intentional or accidental ingestion of antifreeze is the most serious type of exposure, resulting in thousands of ethylene glycol poisonings including several deaths reported each year in the United States (~~Fraser 2002; Leth and Gregersen 2005~~). Litovitz 2002; Watson 2004

Exposure to ethylene glycol through consumption of foods or drinks stored in plastics made from this chemical may be possible if the plastic contains unreacted ethylene glycol that can migrate into the food (Kashtock and Breder 1980). However, current levels of ethylene glycol in food have not been located; therefore, evidence is not available to indicate that this as an important route of human exposure to ethylene glycol.

Background concentrations of ethylene glycol in air, surface water, groundwater, drinking water, soil, and sediment are not available. Ethylene glycol is not expected to be found in the environment away from

positive results for ethylene glycol be confirmed using another method (Ochs et al. 1988; Ryder et al. 1986). The enzymatic method has been modified to eliminate some of the interference problems present in the earlier methods (Blandford and Desjardins 1994).

Thin-layer chromatography (TLC) with a chloroform solvent has been used to detect ethylene glycol and its metabolites in urine or renal tissue (Riley et al. 1982). Metabolites of ethylene glycol in the blood may be detected by analytical isotachophoresis using a system equipped with both a conductivity detector and an ultraviolet detector. Blood and serum samples should not have been previously treated with oxalate, citrate, or ethylene diamine tetracetic acid. This technique may be of value when ethylene glycol poisoning is suspected but sufficient time has elapsed for metabolism of the compound to have occurred (Ovrebo et al. 1987). A simple and rapid colorimetric method that uses chromatropic acid has been proposed for the quantitation of glycolic acid, the major toxic metabolite of ethylene glycol (Fraser and MacNeil 1993).

No information was located on detecting ethylene glycol in feces, adipose tissue, or human milk.

7.2 ENVIRONMENTAL SAMPLES

As with biological samples, GC is the major technique used to determine ethylene glycol concentrations in environmental samples whether in air, water, food, drugs, or other substances. Capillary gas chromatography with FID or ECD, possibly followed by MS, generally gives good quantitative results down to the ppm range with recovery usually >80%. The determination of ethylene glycol in air requires adsorption onto a surface and subsequent extraction. Water samples may be analyzed without preparation (EPA 1995a, 1995b). Detection of ethylene glycol in foods and drugs may be accomplished by chromatography of the sample; for substances with a high fat content, extraction with hexane may be used to remove the fat. Table 7-2 is a summary of some of the most commonly used methods reported in the literature for detecting ethylene glycol in environmental samples. The specific techniques used for each analytical method are listed in the table if that information was provided by the author(s).

Air sampling for ethylene glycol is performed by adsorption onto a resin column such as Amberlite XAD-2. Although activated charcoal filters have some utility, recovery is greater with the Amberlite, and it is the preferred adsorption medium. Ethylene glycol is then solvent-extracted with recovery of 98%. If activated charcoal is used for adsorption, 5% methanol in dichloromethane is the best solvent with maximum recovery of 84% (Andersson et al. 1982, 1984). An alternative method for sampling ethylene

MINIMAL RISK LEVEL WORKSHEET

Need to change as per comments

Chemical Name:	Ethylene glycol
CAS Number:	107-21-1
Date:	May 2007
Profile Status:	First Draft Pre Public Comment
Route:	[ ] Inhalation [X] Oral
Duration:	[ ] Acute [X] Intermediate [ ] Chronic
Graph Key:	59
Species:	Rat

MRL: 0.7 [X] mg/kg/day [ ] ppm

References: Gaunt IF, Hardy J, Gangolli SD, et al. 1974. Short-term toxicity of monoethylene glycol in the rat. BIBRA International: Carshalton, Surrey, UK, 1-31. (Research Report 4/1974).

Experimental design: Male and female weanling Wistar rats were fed diets containing 0, 0.05, 0.1, 0.25, or 1.0% ethylene glycol for 2 weeks (5/sex/dose), 6 weeks (5/sex/dose), or 16 weeks (15/sex/dose). Reported calculated average daily chemical intakes were 35, 71, 180, and 715 mg/kg/day in males, and 0, 38, 85, 185, and 1,128 mg/kg/day in females. Survival, clinical signs, food and water intake, and body weight were evaluated throughout the exposure period. Hematology (hemoglobin, hematocrit, packed cell volume, total erythrocytes, reticulocytes, total and differential leukocytes), serum chemistry (urea, glucose, protein, albumin, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and lactic dehydrogenase), organ weights (including kidneys, liver, spleen, brain, heart, stomach, small intestines, caecum, adrenals, pituitary, thyroid, and gonads), and histology (organs that were weighed and 19 additional tissues) were evaluated at the 2-, 6-, and 16-week sacrifices. Urinalysis (glucose, ketones, bile salts, blood, protein, and presence of oxalic acid crystals, cells and other microscopic constituents) and renal function (urine concentration and dilution tests measuring volume and specific gravity, and cell excretion) were evaluated at weeks 2 and 16. Urine was additionally analyzed for oxalic acid at weeks 2, 6, 12, 14, and 16.

Effects noted in study and corresponding doses: There were no clear exposure-related effects on survival, clinical signs, body weight, hematology, or serum chemistry. Urinary excretion of oxalic acid was significantly increased in males at 715 mg/kg/day at weeks 2–16 and in females at 1,128 mg/kg/day at weeks 6–16, with the magnitude of the effect markedly greater in males (100–500% of control levels) than females (40–100% of control values). Increased absolute kidney weight, oxalic acid crystals in urine, and excretion of a larger volume of urine with a lower specific gravity after a prolonged period (16 hours) without water were observed in the 715 mg/kg/day males at week 16. Exposure-related histopathologic changes occurred only in the kidneys. Incidences of kidney lesions were statistically significantly increased in males at ≥180 mg/kg/day. Specific renal histopathologic findings in the males at 16 weeks included individual nephrons with degenerative changes (incidences of 0/15, 1/15, 1/15, 2/15, and 5/15 [$p<0.05$] in the control to high-dose groups), individual nephrons with degenerative changes and occasional oxalate crystals (0/15, 0/15, 0/15, 1/15, and 4/15 [$p<0.05$]), and generalized tubular damage and heavy oxalate crystals (0/15, 0/15, 0/15, 0/15, and 4/15 [$p<0.05$]). At 0, 35, 71, 180, and 715 mg/kg/day, the total incidence of male rats with oxalate crystals was 0/15, 0/15, 0/15, 1/15, and 10/15 ($p<0.001$), and the total incidence of male rats with renal tubular damage was 0/15, 1/15, 1/15, 4/15 ($p<0.05$), and 15/15 ($p<0.001$). Females had an increased incidence of renal tubular damage at 1,128 mg/kg/day, but the increase was not statistically significant. The histological evaluations of the kidneys in the five rats/sex/dose exposed for 2 or 6 weeks showed no statistically significant increases in incidences of specific changes, although the total incidence of animals with tubular damage was significantly increased in the 715 mg/kg/day males at 6 weeks. Based on the 16-week kidney