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THE PHARMACOKINETICS OF ETHYLENE GLYCOL IN THE PRESENCE OF STEADY STATE ETHANOL

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THE PHARMACOKINETICS OF ETHYLENE GLYCOL IN THE PRESENCE OF STEADY STATE ETHANOL

by

David Ola Waters

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In the Graduate College

THE UNIVERSITY OF ARIZONA

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This thesis has been approved on the date shown below:

Michael Mayersohn

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Sept. 1, 1982
Date

To my two sons, Eric and Ryan,

with hopes for their future

success and happiness.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Don Perrier, Dr. Mike Mayersohn, and Dr. J. W. Clayton for their continued guidance, patience, and support in completing this project.

Without the love and encouragement of my parents through undergraduate school, completion of this thesis would not have been possible.

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ABSTRACT

The effect of ethanol on the disposition kinetics of ethylene glycol was determined in six greyhound dogs. The dogs were dosed intravenously with ethylene glycol in the absence and presence of steady state ethanol. Blood samples were drawn at appropriate intervals after dosing. These samples were analyzed for ethylene glycol and ethanol using flame ionization detection--gas liquid chromatography procedures adapted for this study. Pre-infusion and terminal serum samples were analyzed for creatinine to determine if the ethylene glycol had adversely affected renal function during the experiment.

The elimination half-life, volume of distribution, and plasma clearance were determined for ethylene glycol from the experimental data. A comparison between the kinetics of ethylene glycol in the absence and in the presence of ethanol indicated that there was no change in the volume of distribution of ethylene glycol, but that ethanol did produce a decrease in the clearance and a corresponding increase in the half-life of ethylene glycol. There was no change in serum creatinine.

CHAPTER 1

REVIEW OF THE LITERATURE

Introduction

Ethylene glycol has been known for many years to be a toxic substance (Pons and Custer 1946). Since it is present in many household products such as antifreeze there are usually several poisonings reported annually (Underwood and Bennett 1973). The most common victims of ethylene glycol poisoning are children between the ages of one and four years and indigent alcoholics who occasionally use ethylene glycol as an alcohol substitute. Ethylene glycol's characteristic sweet taste and ability to produce euphoria may provoke its ingestion. Although the short-term effects may be pleasant, the long-term effects are very serious and are often life threatening (Dreisbach 1977; Peterson et al. 1963).

Intoxication with ethylene glycol produces a well-recognized clinical syndrome which has been widely discussed (Pons and Custer 1946; Berman et al. 1967; Collins et al. 1970; Parry and Wallach 1974). One of the main diagnostic features of ethylene glycol intoxication is a severe metabolic acidosis in which blood pH below 7.0 and bicarbonate concentrations as low as 7 meq/l are frequently observed (Friedman et al. 1962; Moriarty and MacDonald 1974). Serum electrolytes with the exception of bicarbonate have been reported to be within the normal range

during the acute phase of the poisoning, that is, before renal damage becomes apparent (Moriarty and MacDonald 1974). The very low bicarbonate concentrations indicate that approximately 15-20 meg/l of some other anionic species must be present to account for the acidosis.

Metabolism and Pharmacokinetics of Ethylene Glycol

The toxicity of ethylene glycol has been attributed to the compound's metabolites which are produced by the alcohol dehydrogenase enzyme system. The metabolic pathway proposed for ethylene glycol is illustrated in Figure 1.

The acidemia associated with ethylene glycol intoxication has been suggested to be partially due to the acidic metabolites of ethylene glycol, including glycolic, glyoxylic, oxalic, formic, and hippuric acids (Gessner et al. 1961). According to Gessner and Coworkers (1961), the decarboxylation of glyoxylic acid producing formic acid and carbon dioxide is a major pathway of ethylene glycol metabolism. The rapidity with which glycolaldehyde is converted to glycolic acid (Gessner et al. 1961), the rapidity of metabolism of glyoxylic acid, and the relatively slow conversion of glycolic acid to glyoxylic acid (McChesney and Goldberg 1972) suggest that glycolic acid metabolism may be rate limiting in conversion of ethylene glycol to carbon dioxide and oxalate.

It has been suggested that the degree of ethylene glycol toxicity is determined by the extent of its oxidation to oxalate (Milles 1946). However, the fact that ethylene glycol appears to be more toxic

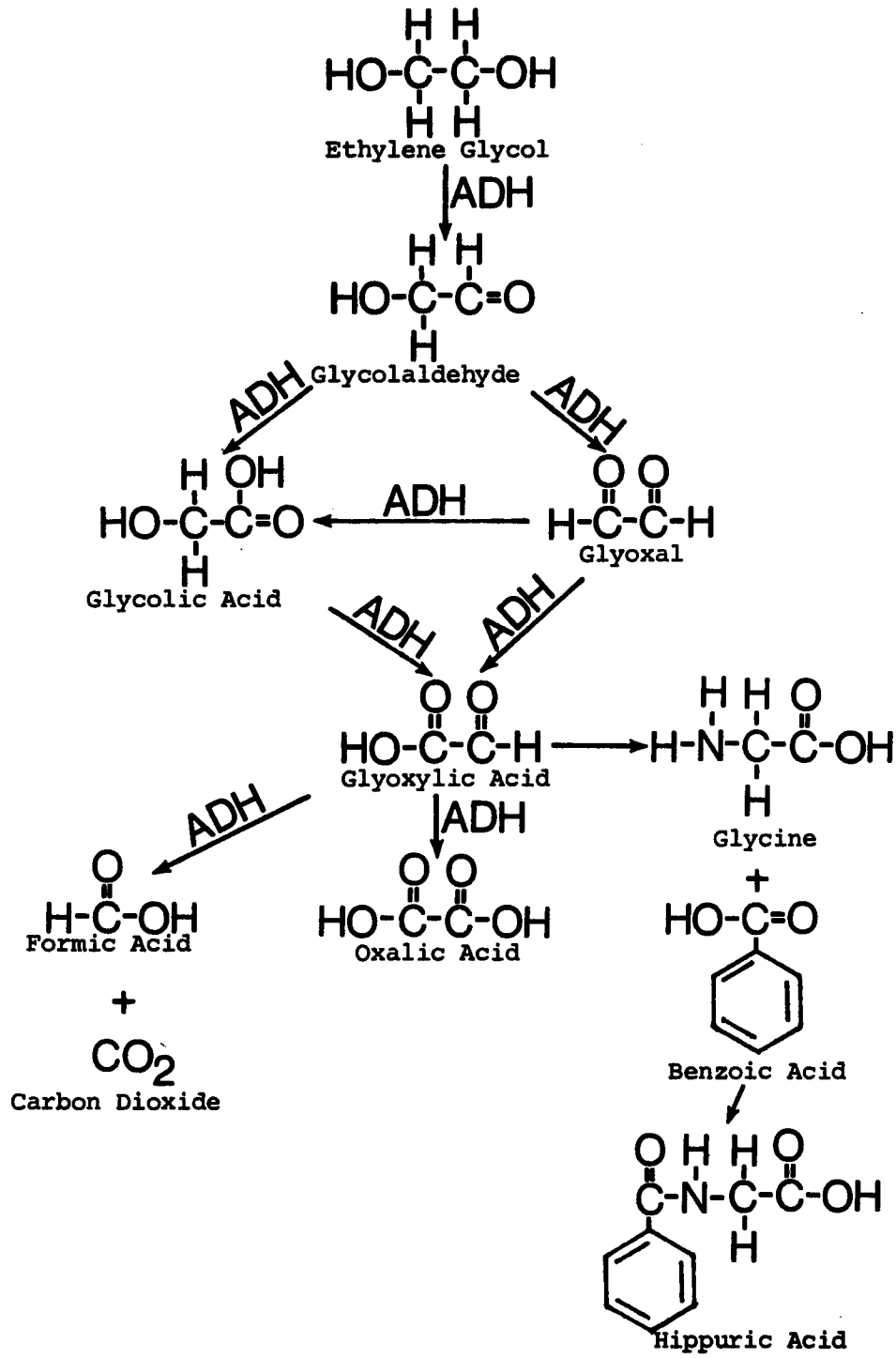

Figure 1. Pathways of ethylene glycol metabolism.

for the monkey, which forms minimal amounts of oxalate from ethylene glycol, than for the rat, which produces large amounts of oxalate (McChesney et al. 1971), does not support this hypothesis. Pons and Custer (1946) observed that if death from acute ethylene glycol poisoning occurred within two days renal damage was minimal, despite the heavy oxalate deposits in the renal tubules. They attributed death to lesions of the central nervous system and observed crystals presumed to be oxalate in the brain and meninges. The deposition of oxalate in the brain in cases of human ethylene glycol poisoning is now well established (Munro and Adams 1967).

The order of increasing toxicity of the main ethylene glycol intermediates in mice, rats, and rabbits is: glycolate < glycoladehyde < glyoxalate (Laborit et al. 1971). Bove (1966) indicates that the toxicity of these oxalate precursors is not due to the mechanical obstruction of the renal tubules by calcium oxalate but is a consequence of their cytotoxicity. All three compounds are potent inhibitors of respiration and glucose metabolism in rat tissues (Lamothe et al. 1971; Rifor et al. 1967). Glycolate increases the NAD:NADH ratio in rat liver (Thuret et al. 1971) but has no toxic action on mitochondrial systems (Bachmann and Goldberg 1971). Glycolaldehyde and glycolate have been reported to have a toxic effect on the central nervous system (Laborit et al 1971). However, since each of these compounds is readily oxidized to oxalate, the possibility exists that their in vivo toxicity is closely related to their conversion to oxalate (Richardson 1973). It should be noted that glycoaldehyde and glyoxylic acid both have been shown to

have very short half-times in vivo (King and Warner 1968), and the numerous pathways available for further metabolism of these substances ensure their oxidation to glycine or oxalate as quickly as glycine or oxalate is formed from glycolate.

Relatively little is known about the kinetics of the ethylene glycol-ethanol interaction. The most comprehensive publication (Peterson et al. 1980) on the subject involved an actual ethylene glycol poisoning case in which a fifty-one-year-old man was treated for ingesting six hundred milliliters of antifreeze (95% ethylene glycol). In this study the elimination half-life ($t_{\frac{1}{2}}$) was three hours when no effective therapy was given. When 20 percent oral ethanol was given the $t_{\frac{1}{2}}$ was prolonged to 17 hours. The calculated volume of distribution for ethylene glycol was V_D = 0.83 l/kg.

McChesney et al. (1970) report a $t_{\frac{1}{2}}$ of 2.7-3.7 hours for ethylene glycol in rhesus monkeys. These workers also offered some information on the percentage of an ethylene glycol dose that is eliminated as parent compound and the percentage eliminated as metabolites. Ethylene glycol was the main excretory product at 17% of the dose, followed by glycolic acid at 11.5% and oxalic acid at 0.3% of the dose. Only 34.1% of the ethylene glycol dose was accounted for in this study on rhesus monkeys.

Clinical and Pathological Findings in Ethylene Glycol Poisoning

According to Dreisbach (1977) the clinical findings in acute ethylene glycol poisoning are like those seen with alcohol intoxication.

These symptoms soon progress to vomiting, cyanosis, headache, tachypnea, tachycardia, hypotension, pulmonary edema, muscle tenderness, stupor, anuria, prostration, and unconsciousness with convulsions. Hypoglycemia may also occur. Death may occur within the first few hours from respiratory failure or within the first 24 hours from pulmonary edema. Patients who have prolonged coma or convulsions may have irreversible brain damage. Hypocalcemic tetany as a result of calcium precipitation sometimes follows ethylene glycol poisoning.

The pathologic findings in victims of ethylene glycol poisoning are congestion and edema of the brain, focal hemorrhagic necrosis of the renal cortex and hydropic degeneration of the liver and kidneys. Calcium oxalate crystals may be found in the brain, spinal cord, and kidneys.

The spectrum of ethylene glycol poisoning is complex; the extensive damage that ethylene glycol can cause indicates that there is more than one mechanism of toxicity.

Treatment of Ethylene Glycol Poisoning

Currently the treatment of ethylene glycol poisoning involves the following principles.

1. Removal of ethylene glycol by emesis or gastric lavage.
2. Competitive inhibition of the enzyme(s) oxidizing ethylene glycol to its toxic metabolites. This is usually accomplished by administering ethanol.
3. Administration of a suitable base to combat metabolic acidosis. Usually sodium bicarbonate is used for that purpose.

4. Extracorporeal hemodialysis may be used when the clinical symptoms are severe.

The literature evidences several variations on these principles, as might be expected. For instance, success has been reported in using ethanol and citrate (Debray et al. 1968; Joly et al. 1968) in the treatment of ethylene glycol poisoning. Pyrazole and 4-methylpyrazole have also substituted for ethanol as a competitive inhibitor of ethylene glycol metabolism (Mundy, Hall, and Teague 1974) with satisfactory results. However, ethanol has become the most widely used antidote for ethylene glycol poisoning since 1963. There is some controversy concerning the concentration of ethanol which should be maintained during treatment. Freed and coworkers (1981) recommend maintaining a concentration of 50 to 200 mg/dl ethanol, citing the fact that ethanol has a one hundred-fold greater affinity for alcohol dehydrogenase than does ethylene glycol. Peterson (1981), on the other hand, states that there exist multiple forms of alcohol dehydrogenase in the human liver. The saturation of these isoenzymes of alcohol dehydrogenase varies greatly with blood ethanol concentrations. Blood concentrations of less than 100 milligrams per deciliter may provide incomplete enzymatic blockade, increasing the patient's risk of ethylene glycol toxicity (Peterson 1981).

Chemistry and Assay Methods

The physical properties of ethylene glycol are shown in Table 1.

Table 1

Physical Properties of Ethylene Glycol

Molecular Weight	Melting Point	Boiling Point	Density
62.07	-11.5°C	198°C	1.1088 gm/cm^3

Ethylene glycol (1,2 ethanediol) is a heavy, clear, colorless liquid with a sweetish taste. It is very hydrophyllic and has essentially no dissociation in aqueous solutions. The physical and chemical properties of ethylene glycol make it difficult to separate it from an aqueous matrix such as blood plasma. Its high boiling point (low partial vapor pressure) make a headspace technique of separation impractical. The hydrophyllic nature and neutral electrochemical properties of ethylene glycol prevent efficient extraction into immiscible solvents. After considering the chemical and physical properties of ethylene glycol it becomes more evident why a direct injection of diluted plasma on a gas liquid chromatography column designed to accept aqueous samples is the best way to analyze ethylene glycol in biological fluids.

There are three basic procedures in the literature for analyzing ethylene glycol by gas liquid chromatography. One method that we evaluated involves the derivization of ethylene glycol to its dibenzoate ester followed by flame-ionization detection gas liquid chromatography on a 3% OV-17 column (Peterson and Rodgerson 1974). This method is sensitive and specific but has relatively poor precision. Another method involves the use of a 3% Carbowax 20 M on chromosorb 101 column (Kashtock

1979). This column would not resolve ethylene glycol from an endogenous plasma peak. The method of Bost and Sunshine (1979) involves spiking 50 microliters of plasma (sample) with 50 microliters of 2,5-hexanedione as the internal standard and then injecting the diluted plasma onto a 5% Carbowax 20 m on carbopack B column for quantitation. This assay is rapid, specific and has good precision.

Statement of Purpose

Even though ethanol has been used as the antidote for ethylene glycol poisoning for many years, there is still a paucity of information regarding the kinetics of ethylene glycol in the presence of ethanol. Therefore, the primary aim of this study was to provide information, collected under controlled conditions, on the effect of ethanol on ethylene glycol kinetics.

CHAPTER 2

EXPERIMENTAL

Animal Studies

Greyhound dogs were chosen for these studies because of their uniformity in breed in order to achieve a reasonable inter-animal similarity in physiological and anatomical characteristics. In addition these animals are large enough to permit adequate blood sampling over the course of the study.

Each animal served as its own control for each of two experiments which were conducted in a random cross-over design. Each animal received a single intravenous (IV) infusion dose of ethylene glycol alone (control) and the same IV infusion of ethylene glycol during IV ethanol administration (experimental). These experiments were separated by about two weeks' time. The sequence of dosing was randomly determined so that three animals underwent first the control study, followed by the experimental study, and the three other animals underwent the experimental study first, followed by the control study. This design was employed to avoid any effect of the sequence of dosing on the results.

Prior to conducting the studies outlined above it was essential to conduct preliminary experiments in order to answer several questions that were crucial to the ultimate experimental design. The following questions needed to be answered.

1. What dose of ethylene glycol should be used in order to achieve plasma concentrations that are associated with toxicity but which are sublethal in the animal and will not cause irreversible damage?
2. What loading and maintenance doses of ethanol should be used in order to achieve ethanol plasma concentrations desired for the treatment of ethylene glycol toxicity in man and with what frequency should the ethanol maintenance doses be given in order to avoid major fluctuations in those concentrations (i.e., to maintain "therapeutic" ethanol plasma concentrations)?
3. What blood sampling protocol should be used in order to completely characterize ethylene glycol disposition during each experiment and to characterize the ethanol plasma concentration-time profile?

Ethylene glycol disposition was examined in a 25 kg female greyhound dog. A 50 ml aqueous solution containing 56% ethylene glycol was infused via a catheter placed into the right femoral vein at a rate of 1.67 ml/min over 30 minutes. Blood samples were obtained prior to dosing and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, and 24 hours after dosing. These samples were assayed for ethylene glycol.

Ethanol plasma concentration-time profile was examined in a 23.2 kg female greyhound dog. A 50 ml aqueous solution containing 47% ethanol was infused over 12 minutes via a catheter placed into the juglar vein. Blood samples were obtained prior to dosing and at 0.5, 1, 2, 3,

4, 5, 6, 8, 10, 12, 14, 16, 18, and 24 hours after dosing. These samples were assayed for ethanol.

The above data were analyzed in order to develop a precise dosing and sampling protocol. The procedures employed in the study and outlined below are based upon the findings of the preliminary experiments.

Ethylene glycol as a 56% aqueous solution was administered at a dose of 0.7 ml/kg (0.76 g/kg). The solution was manually infused at a rate of 3 to 4 ml/min by a syringe via an indwelling needle placed into the femoral vein. This dose is about equal to one-half of the lethal dose in man (Casserett and Duell 1975).

The solution was administered manually rather than by an infusion pump because it was difficult to restrain the animal for a sufficiently long time to ensure placement of the infusion line. Manual administration permitted use of an indwelling needle and intermittent dosing via a syringe. Exact times of infusion were recorded. Blood samples (3-5 mls) were obtained using 5 ml Vacutainer tubes containing EDTA as an anticoagulant, by multiple venipunctures from the femoral vein of the opposite limb. Blood samples were collected prior to dosing and at the following times after dosing: 5, 15, and 30 min and 1, 2, 4, 6, 8, 10, 12, 18, 24, and 30 hrs. The precise times of collection were recorded.

The experiments requiring ethylene glycol and ethanol coadministration were made more complicated by the fact that sequential doses of ethanol had to be administered in order to maintain plasma concentrations within the desired range. In order to conveniently make the required

number of infusions of ethanol it was necessary to place a catheter in the jugular vein of the dog being used. The hair on the dog's neck was shaved, then the exposed area was scrubbed with an iodine disinfectant solution (Division of Animal Resources disinfectant solution, Arizona Health Sciences Center, Tucson, Arizona). In order to expose the jugular vein the animal was placed on a table in either a standing or a supine position so that the neck could be arched backward. The needle from a 17 ga Bardic inside needle catheter was placed into the jugular vein, and the catheter, inside the needle, was pushed through the needle down into the jugular vein into the thoracic opening of the dog's chest. The needle was withdrawn about one inch and the protective sleeve on the upper portion of the needle was slid down over the end of the needle and taped there with adhesive tape. This procedure prevents the needle from cutting the catheter or sticking the animal if it moves. The steel stylette inside the catheter is removed. A 5 ml syringe is attached to the luer lock at the end of the catheter and 2 to 3 ml of saline are flushed into the catheter. Blood is then pulled back into the syringe to be certain that the catheter is patent. One ml of a heparin solution (Heparin Sodium Injection, 15,000 U.S.P. units/ml) is placed into the catheter to prevent blood from clotting, and a heparin lock is attached to the catheter. The heparin lock is placed so that it protrudes about one-half inch above the neck and the animal's neck is then wrapped with a previously stretched Corban bandage. A hole is cut through the bandage and the heparin lock is placed through that opening. The bandage is snug but not too tight to impede blood circulation and it is checked

periodically to ensure that it is not too tight. A bandage (Ace bandage) is then wrapped around the animal's neck to cover the exposed catheter and heparin lock in order to prevent the animal from removing or accidentally pulling them out of place.

Based upon the results of the preliminary ethanol study in one dog the following dosing procedure was devised to maintain steady state ethanol concentrations between 50 and 200 mg/dl. An initial loading dose of 1.6 g ethanol/kg (2.03 mls/kg) was infused into the jugular over 25-30 min. Maintenance doses of 0.8 g ethanol/kg were given every six hours for the subsequent 36 hours by infusing over 2-5 min into the juglar vein. The ethylene glycol dose of 0.7 ml/kg (0.78 gm/kg) was given at a rate of 3 to 4 mls/min over 10 to 12 min (i.e., 35 mls in 10 to 12 min).

Blood samples (3-5 mls) were obtained from the femoral vein not used for infusing ethylene glycol. These samples were obtained prior to dosing and at the following times after dosing: 5, 15, and 30 minutes and 1, 2, 4, 6, 8, 10, 12, 15, 18, 24, 30, and 42 hours. The specimens were refrigerated pending their analysis by gas liquid chromatography.

Analytical

The ethylene glycol analysis used for this study was adapted from a publication by Bost and Sunshine (1980). The method involves spiking an aliquot of plasma (50 microliters) with 50 microliters of 2,5-hexanedione as the internal standard (0.1 ml/dl). The mixture is vortexed and then 2.0 microliters of the diluted plasma are injected directly onto a 5% Carbowax 20 m on 80/100 Carbo Pack-B (Supelco Cat. #1-1766) column in a Hewlett-Packard 5700 gas liquid chromatograph

equipped with a flame ionization detector. The instrument parameters were:

Injection Port	250°C
Column Temperature	150°C
Detector Temperature	300°C
Nitrogen Flow	30 ml/min
Hydrogen Flow	30 ml/min
Air Flow	240 ml/min
Chart Speed	1 cm/min
Attenuation	10 x 32

The ethylene glycol and 2,5-hexanedione peaks eluted at 2.3 and 5.2 minutes, respectively. The peaks were manually measured and the peak height ratio obtained using standards was employed to generate a linear regression line which was used to calculate the concentration in the samples.

The ethylene glycol standards were made using J.T. Baker AR Grade ethylene glycol that had been passed through a column filled with dry sodium sulfate. The ethylene glycol stock standard was made by measuring 0.902 ml of water-free theylene glycol into a 100 ml volumetric flask and diluting to the mark with deionized water. The concentration of the stock solution was 10 mg/ml. Dilutions were made from the stock standard to yield concentrations of 250, 150, 100, 50, 25, 10, 5, and 2.5 mg/dl by taking appropriate aliquots of the stock standard and diluting to 10 mls.

Bost and Sunshine (1980) showed that there was essentially no difference between the linearity of curves using standards made up in

water and standards made up in plasma. The slopes for water and plasma matrix curves were 1.189 and 1.187, respectively, and the correlation coefficients for those same curves were 0.997 and 0.998, respectively. We observed that the correlation of peak height ratios of standards made up in water agreed very well with the peak height ratios of standards made up in plasma. Therefore, the standards used for sample analysis were aqueous since aqueous ethylene glycol standards are stable indefinitely while plasma standards are not.

Since it was not possible to immediately analyze the specimens drawn from the experimental animals, it was necessary to evaluate the effect of refrigeration and freezing on the blood samples. In order to do this 35 ml of whole blood (dog) was collected in 5.0 ml lavender top tubes containing EDTA to prevent coagulation. Twenty-five mls of the blood were poured into a 50 ml graduated cylinder equipped with a glass stopper. The blood was spiked with 1.5 mls of 10 mg/ml ethylene glycol standard, then diluted to 30 mls with blood. The graduated cylinder was stoppered and then inverted numerous times to ensure homogeneity. The ethylene glycol concentration of the blood pool was 500 mg/dl.

Three 1.0 ml aliquots were taken from the blood pool and centrifuged, then 50 microliters of the plasma were withdrawn to assay for ethylene glycol. Three more 1.0 ml aliquots were taken from the blood pool and refrigerated at 2-8°C for two weeks, then they were assayed for ethylene glycol.

To evaluate the effect of freezing on the specimens, twelve 1 ml aliquots were pipetted into 12 x 75 mm culture tubes and then frozen.

Three of these frozen samples were analyzed for ethylene glycol every week for four weeks. Hemolysis was noted if it occurred.

The ethanol assay used in this study was a headspace method adapted from a publication by Karnitus and Porter (1972). The method involves placing approximately 0.5 gms of sodium chloride in a 10 ml culture tube and then pipetting 0.5 ml of standard, control, or sample into the tube. To this is added 0.5 ml of acetonitrile, which serves as the internal standard (34.2 mg/dl). The tubes are stoppered and then placed into a heating block at 37°C for 10 minutes. At ten minutes, the tubes are vented by inserting a 20 gauge syringe needle through the rubber stopper and then incubated for an additional 35 minutes.

The instrument used for this analysis was a Hewlett Packard 5700 A gas liquid chromatograph equipped with a flame ionization detector and a 3% Porapak S (100/120 mesh) 6 ft glass column. The instrument conditions for the assay were:

Injection Port Temperature	250°C
Column Temperature	165°C
Detection Temperature	300°C
Nitrogen Carrier Flow Rate	30 ml/min
Air Flow Rate	240 ml/min
H_2 Flow Rate	30 ml/min
Attenuation	10 x 32
Chart Speed	1 cm/min

The syringe used for injecting the headspace of the ethanol specimens was a 1.0 ml tuberculin syringe with a 3 inch luer lock needle attached. The needle was rounded so that it would not cut the GLC septum. A 5000 mg/dl ethanol stock standard was made up by measuring 6.334 ml of absolute ethanol (density = 0.7893 gm/cm^3) into a 100 ml volumetric flask and diluting to the mark with deionized water. Aliquots of 1.0, 2.0, 3.0, and 4.0 ml were taken from the stock ethanol standard and diluted to 100 ml with deionized water, to yield concentrations of 50, 100, 150, and 200 mg/dl, respectively. These standards were used to analyze the experimental specimens for ethanol.

Aliquots of the pre-infusion and terminal blood samples from all the experiments were centrifuged and the plasma frozen, to be analyzed for creatinine after the completion of the animal studies. The frozen creatinine specimens were sent to a commercial laboratory for analysis (Southwest Veterinary Diagnostics, Phoenix, Arizona).

Data Analysis

Ethylene glycol plasma concentrations as a function of time were plotted on semi-logarithmic graph paper. Data in the post-distributive phase were analyzed by linear regression assuming linear decline according to the equation

$$\log C = \log C_o - \frac{Kt}{2.303} \qquad (1)$$

where C is plasma concentration at any time t, C_o is the extrapolated (time zero) concentration, and K is the first-order elimination rate constant. The elimination half-life ($t_{\frac{1}{2}}$) was calculated from

$$t_{\frac{1}{2}} = 0.693/K \tag{2}$$

A model-independent approach was used to determine the other parameters of disposition. The total body or systemic clearance (Cl_s) was determined from

$$Cl_s = \text{dose}/AUC \tag{3}$$

where dose is the intravenous dose administered and AUC is the total area under the plasma concentration versus time curve from time zero to infinity. The area from time zero to the first concentration time point in the post-distribution phase was obtained employing the trapezoidal rule, that is,

$$AUC_{t_1}^{t_2} = (t_2-t_1)(C_2+C_1)/2 \tag{4}$$

where $AUC_{t_1}^{t_2}$ is the area under the curve between two consecutive concentration-time points, $C_2 \cdot t_2$ and $C_1 \cdot t_1$. The area from the first concentration-time point in the post-distribution phase (t) to time ∞ (AUC_t^{∞}) was calculated from the equation,

$$AUC_t^{\infty} = \frac{C_t}{K} \tag{5}$$

This equation is obtained by converting equation (1) into its exponential form and integrating it from time t to ∞.

The concentration used for C_t was the theoretical concentration calculated by the linear regression equation, and not the observed concentration. AUC then equals the sum of the trapezoids plus AUC_t^{∞}, that is,

$$AUC = AUC_0^t + AUC_t^\infty \quad (6)$$

The apparent volume of distribution (V_β) was determined from,

$$V_\beta = Cl_s/K \quad (7)$$

Therefore, the determination of K by linear regression and AUC from equation (6) enables the calculation of $t_{\frac{1}{2}}$, Cl_s, and V_β using equations (2), (3), and (7).

The above pharmacokinetic parameters for ethylene glycol in the presence and absence of steady state ethanol were statistically compared using the paired Students t-test.

CHAPTER 3

RESULTS AND DISCUSSION

Analytical Methodology

Bost and Sunshine (1980) report a coefficient of variation of 6.8% at 11.0 mg/dl (n=3) and a coefficient of variation of 2.1% at 55 mg/dl (n=5) for the ethylene glycol analysis used in this study. We verified this precision by analyzing triplicate samples at 150, 50, and 10 mg/dl; the coefficients of variation were 3.8%, 6.4% and 7.4%, respectively.

The standard curves used in the analysis of the experimental and control specimens are listed in Table 2.

Table 2

Equations of Standard Curves for Ethylene Glycol Obtained by Linear Regression Analysis ($y = m(x) + b$)

Slope (m)	y-Intercept (b)	r^2	n
0.000753	0.122	0.988	7
0.000702	0.033	0.978	7
0.000718	0.089	0.988	7
0.00126	0.091	0.982	7
0.00126	0.039	0.984	7
0.000814	0.038	0.972	7

The points for the standard curves (peak height ratio versus concentration) were fit to a straight line using linear regression analysis. The terms for the equation of a straight line ($y = mx + b$) as used in this study were: y is the peak height ratio (ethylene glycol/internal standard), m is the slope of the ethylene glycol standard curve, x is the ethylene glycol concentration, and b is the y-axis intercept.

The ethylene glycol concentration of specimens taken from the experimental animals was calculated by solving the standard curve, run on the same day, for the concentration term (i.e., $x = [y - b]/m$) and substituting the peak height ratio (y) obtained from the gas liquid chromatographic analysis of the specimen into the latter equation.

The linearity of each ethylene glycol standard curve was good (Table 2). The coefficient of determination (r^2) ranged from 0.972 to 0.988, both of which are an acceptable deviation from perfect linearity ($r^2 = 1.000$). There was some between-day variation among the slopes of the standard curves but not enough to cause concern. Some variation in the slope is normal, because of changes in the column, detector response, and various other factors. The y-axis intercept (b) for each equation in Table 2 was relatively small, which indicates that the ethylene glycol peak was not interfered with by plasma material. Representative chromatograms for the ethylene glycol assay are shown in Figure 2.

The results of the study on the stability of ethylene glycol blood specimens are summarized in Table 3. This study showed that there is no significant alteration of ethylene glycol concentration due to

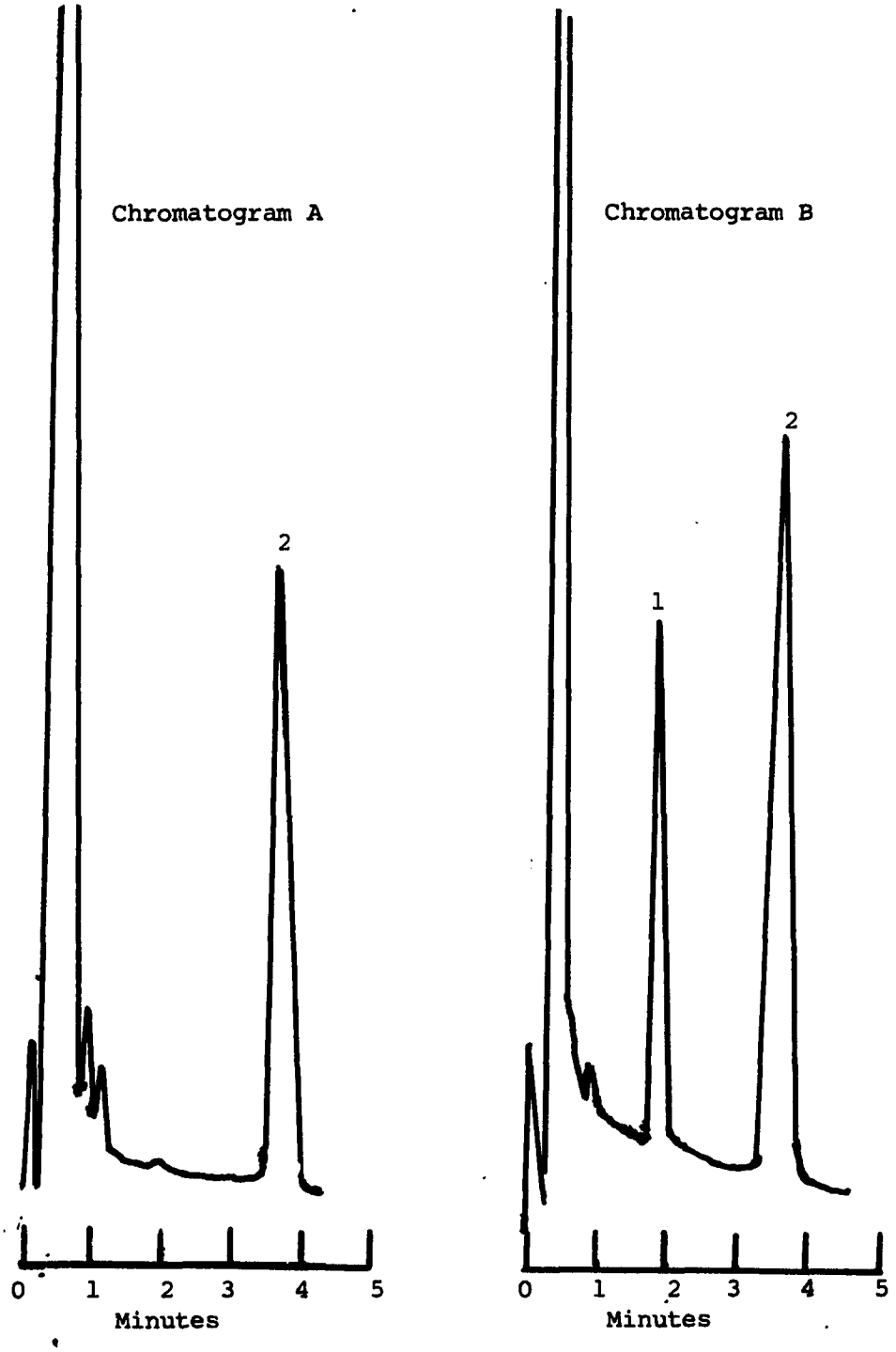

Figure 2. Ethylene glycol chromatograms.

The peak numbered 1 is an ethylene glycol peak and the peaks numbered 2 are 2,5-hexanedione internal standard peaks. Chromatogram A is a pre-infusion plasma sample taken from a dog. Chromatogram B is a 3.0 hour post-infusion plasma sample from a dog (ethylene glycol concentration 79.5 mg/dL).

Table 3

Evaluation of Ethylene Glycol Stability During Refrigeration and Freezing
(Ethylene Glycol Plasma Concentrations in mg/dl)

	No Refrigeration (Assayed Immediately)	2 Weeks Refrigeration at 2-8°C	Frozen 1 Week at -10°C	Frozen 2 Weeks at -10°C	Frozen 3 Weeks at -10°C	Frozen 4 Weeks at -10°C
	47.7	48.3	50.4	49.6	54.0	50.7
	53.9	55.4	50.1	50.8	50.3	54.2
	53.2	47.8	46.0[a]	46.6	53.8	51.7
Mean	51.6	50.5	48.8	49.0	52.7	52.2
Coefficient of Variation	6.6	8.4	5.0	4.4	3.9	3.5

[a]Specimen hemolyzed.

refrigeration of a specimen at 2-8°C for two weeks or freezing an ethylene glycol blood specimen for up to four weeks. The overall coefficient of variation for all 18 specimens was 5.6% and the mean for all 18 specimens was 50.8 mg/dl. The blood pool used for the ethylene glycol stability study was spiked with ethylene glycol stock standard to yield a concentration of 50.0 mg/dl, as mentioned previously.

The precision and accuracy of the headspace ethanol analysis is well documented. This method is the method of choice for medico-legal blood alcohol analyses for many states including Arizona (Karnitus and Porter 1972). We verified the method's reproducibility by analyzing triplicate injections of each standard concentration used in the assay; the results are shown in Table 4. The accuracy of the assay was verified by analyzing a 150 mg/dl College of American Pathologists certified ethanol standard in triplicate. The mean and the coefficient of variation were 149.3 mg/dl and 1.7%. A representative chromatogram is presented in Figure 3.

The standard curves used in the ethanol analysis of the experimental specimens are listed in Table 5. The points for the standard curves (peak height ratio versus concentration) were fit to a straight line using linear regression analysis. The equation for a straight line ($y = mx + b$), obtained by linear regression analysis of the ethanol standard data, was used to calculate the ethanol concentration of blood specimens taken from the experimental animals. This was accomplished by solving the above equation for the concentration term (i.e.,

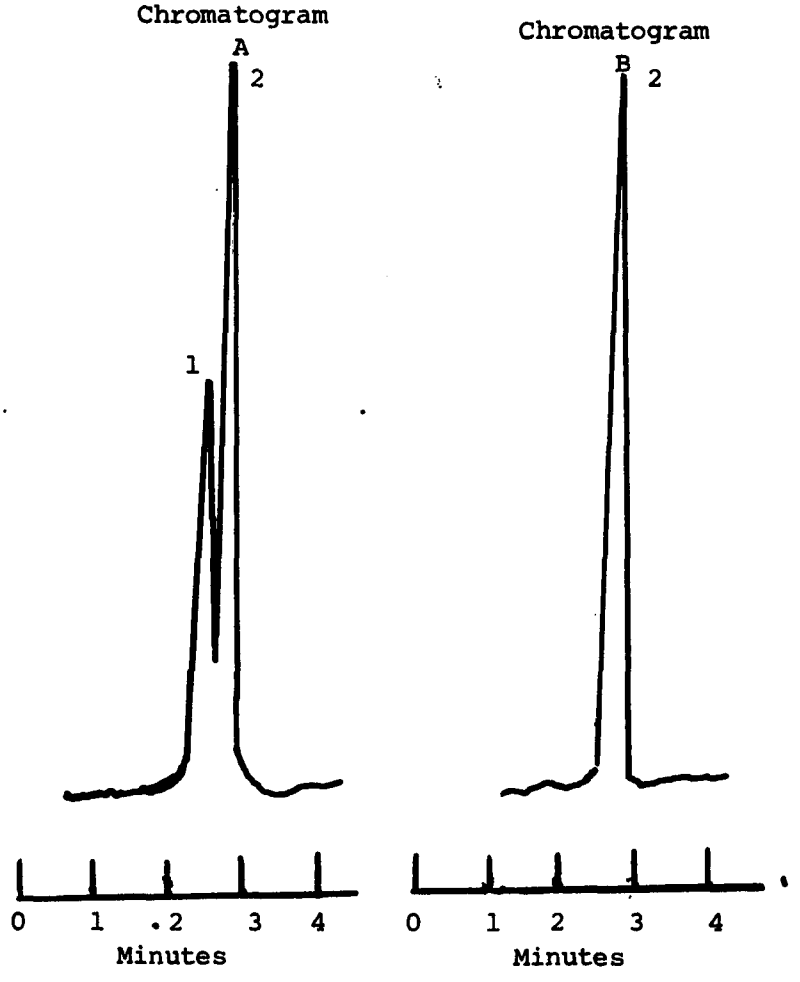

Figure 3. Ethanol chromatograms.

The peak labeled 1 is an ethanol peak, the peaks labeled 2 are the acetonitrile internal standard peaks. Chromatogram A is a blood sample taken from a dog 3 hours after infusing ethanol. The ethanol concentration is 102 mg/dL for this sample. Chromatogram B is a pre-infusion blood sample showing no peak for ethanol.

x = [y - b]/m) and substituting the peak height ratio (y) of the ethanol sample into the latter equation.

Table 4

Reproducibility of the Ethanol Assay

	50 mg/dl	100 mg/dl	150 mg/dl	200 mg/dl
Coefficient of Variation	6.4% (n=3)	2.4% (n=3)	4.4% (n=3)	3.2% (n=3)

Table 5

Equations of Standard Curves for Ethanol Analysis Obtained by Linear Regression Analysis (y = mx + b)

Slope (m)	y-Intercept (b)	r^2
0.00494(x)	-0.0765	0.98
0.00515(x)	-0.198	0.98
0.00551(x)	-0.1075	0.99

The linearity of the standard curves for the ethanol analysis was good in all cases. The coefficients of determination were 0.98, 0.98, and 0.99, all of which are reasonably close to perfect linearity (r^2 = 1.000). The between-day variability of the slopes (m) of the ethanol standard curves was acceptable, and the y-axis intercepts were not too large to cause any concern. Therefore, the standard curves for the ethanol assay were considered to be acceptable.

The overall evaluation of the ethylene glycol and the ethanol assays proved them both to be well suited for the analysis of specimens

in this study. Both of these assays gave us the specificity, precision, and accuracy we required.

Animal Studies

The dogs used in the study all tolerated the intravenous doses of ethanol and ethylene glycol during the course of the experiment very well. Dog #3, however, had to be sacrificed approximately three weeks after the last infusion due to a sloughing of the skin and vasculature in the vicinity of the jugular vein. Evidently, some of the 47% ethanol solution used for the ethanol infusion accidentally infiltrated into the tissue of the neck and this later caused the sloughing. A necropsy was ordered. The gross pathology of the internal organs showed nothing remarkable, and the histopathology of the liver and kidneys showed a mild cholangihepatitis, and a mild chronic infectious nephritis. These findings were not attributed to ethylene glycol toxicity.

The response of the dogs to ethylene glycol in the absence of ethanol, in this study, was consistent with our expectations for a dose of 0.7 mls/kg ethylene glycol. This dose represents half the lethal dose of ethylene glycol for man (1.4 mls/kg) and 1/9 the lethal dose for the dog (6.3 mls/kg) (Casserrett and Doull 1975). There were no observed adverse effects in the animals other than an instance of hematouria noticed at the 8 hr post-infusion sampling period (dog #4), and the symptoms resembling alcoholic intoxication that are associated with ethylene glycol intoxication.

There were no observed differences between the responses of the dogs dosed with ethylene glycol only and the responses of those infused

with both ethylene glycol and ethanol, except for the more pronounced CNS depressant effects in the latter group.

Plots of ethylene glycol plasma concentration-time data are presented in Figures 4 to 9. The linear regression analysis of the terminal ethylene glycol concentration vs. time data in the absence and presence of ethanol for each dog is shown in Table 6.

Table 6

Summary of the Linear-Regression Analysis of Ethylene Glycol Disposition in the Absence and Presence of Ethanol

Equations of Log Linear-Regression Curves in the Absence of Ethanol			
Animal	Slope	Intercept	r^2
1	-0.0442	1.78766	0.97
2	-0.0937	2.13730	0.97
3	-0.0730	2.14000	0.98
4	-0.0677	2.04418	0.98
5	-0.1226	2.02750	0.95
6	-0.0700	2.001192	0.95
Equations of Log Linear-Regression Curves in the Presence of Ethanol			
Animal	Slope	Intercept	r^2
1	-0.0328	1.91631	0.97
2	-0.0385	1.9969	0.96
3	-0.0341	2.0689	0.96
4	-0.0355	2.0757	0.98
5	-0.0394	2.0639	0.93
6	-0.0274	1.96137	0.97

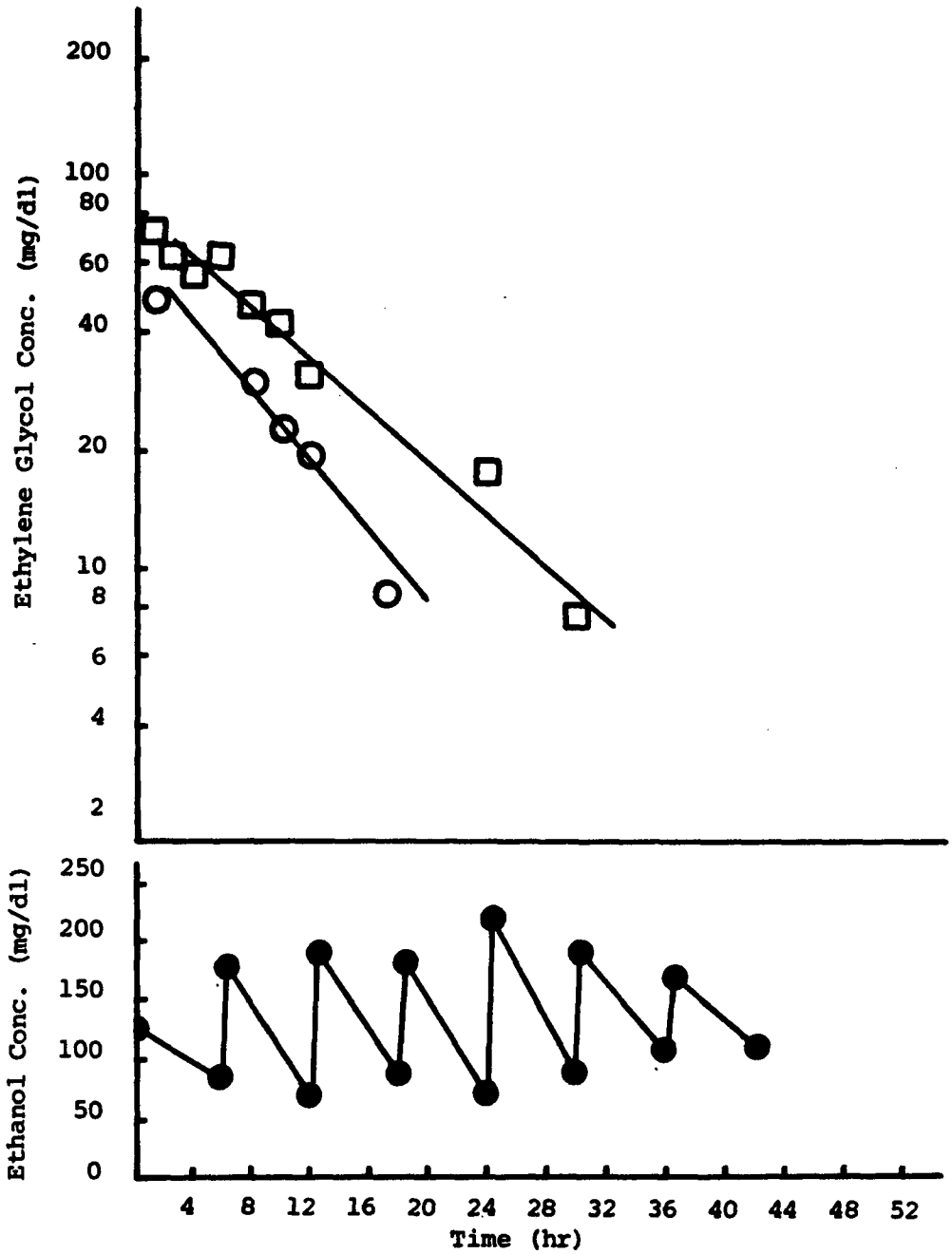

Figure 4. Ethylene glycol and ethanol concentrations as a function of time in dog #1.

Symbols: ethylene glycol in the presence of ethanol (□); ethylene glycol in the absence of ethanol (○); ethanol concentrations produced after multiple dosing (●).

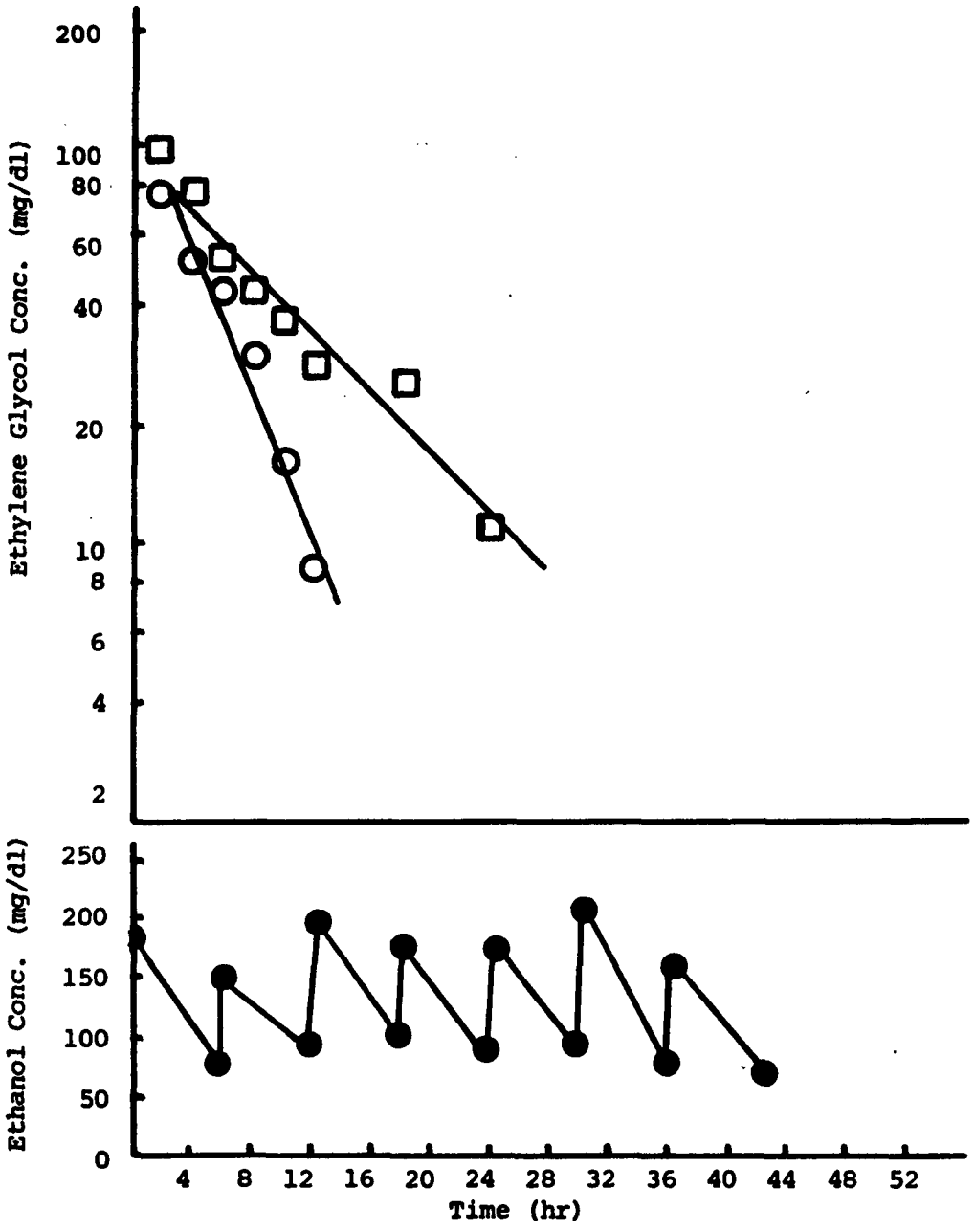

Figure 5. Ethylene glycol and ethanol concentrations as a function of time in dog #2.

Symbols: ethylene glycol in the presence of ethanol (□); ethylene glycol in the absence of ethanol (○); ethanol concentrations produced after multiple dosing (●).

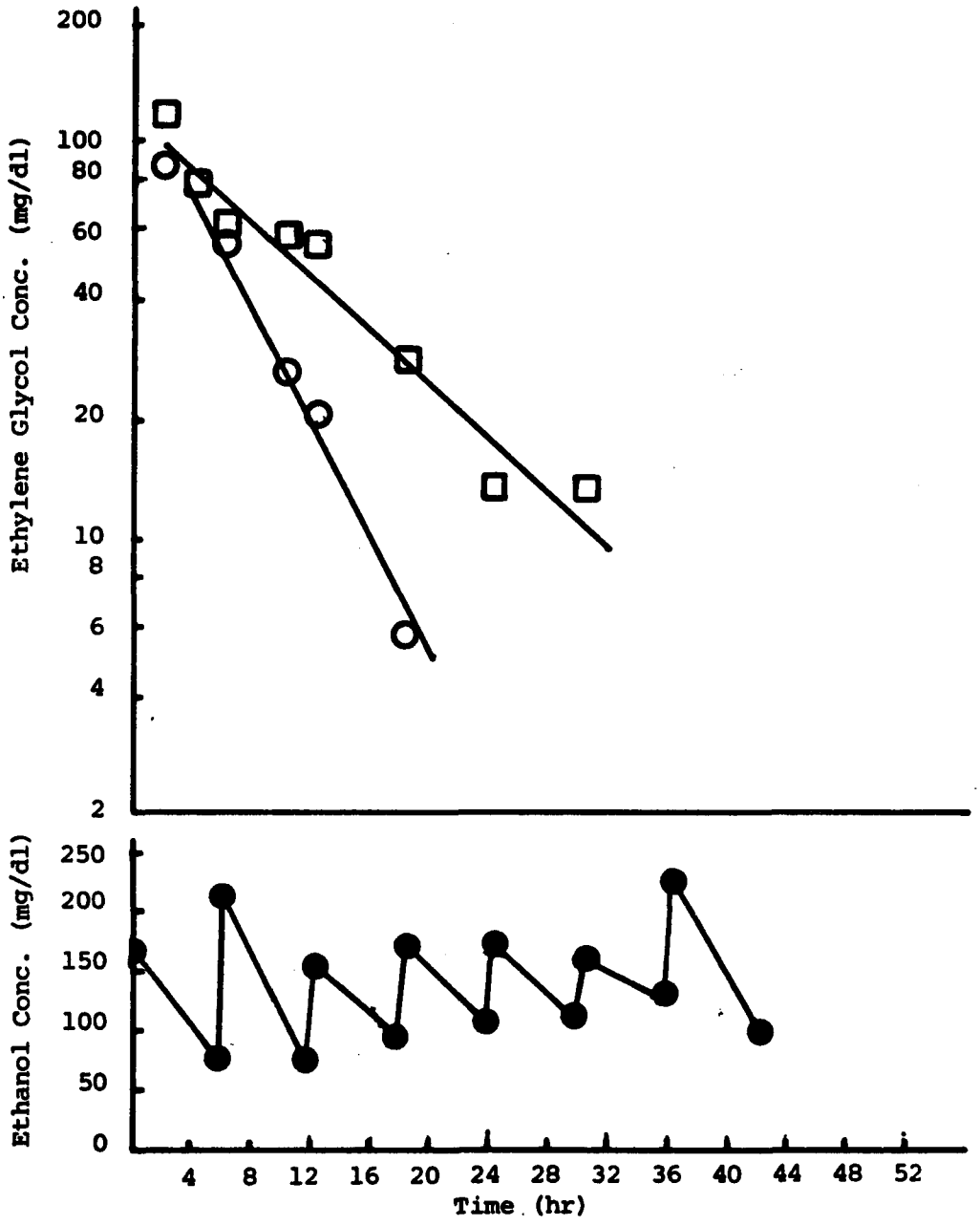

Figure 6. Ethylene glycol and ethanol concentrations as a function of time in dog #3.

Symbols: ethylene glycol in the presence of ethanol (□); ethylene glycol in the absence of ethanol (○); ethanol concentrations produced after multiple dosing (●).

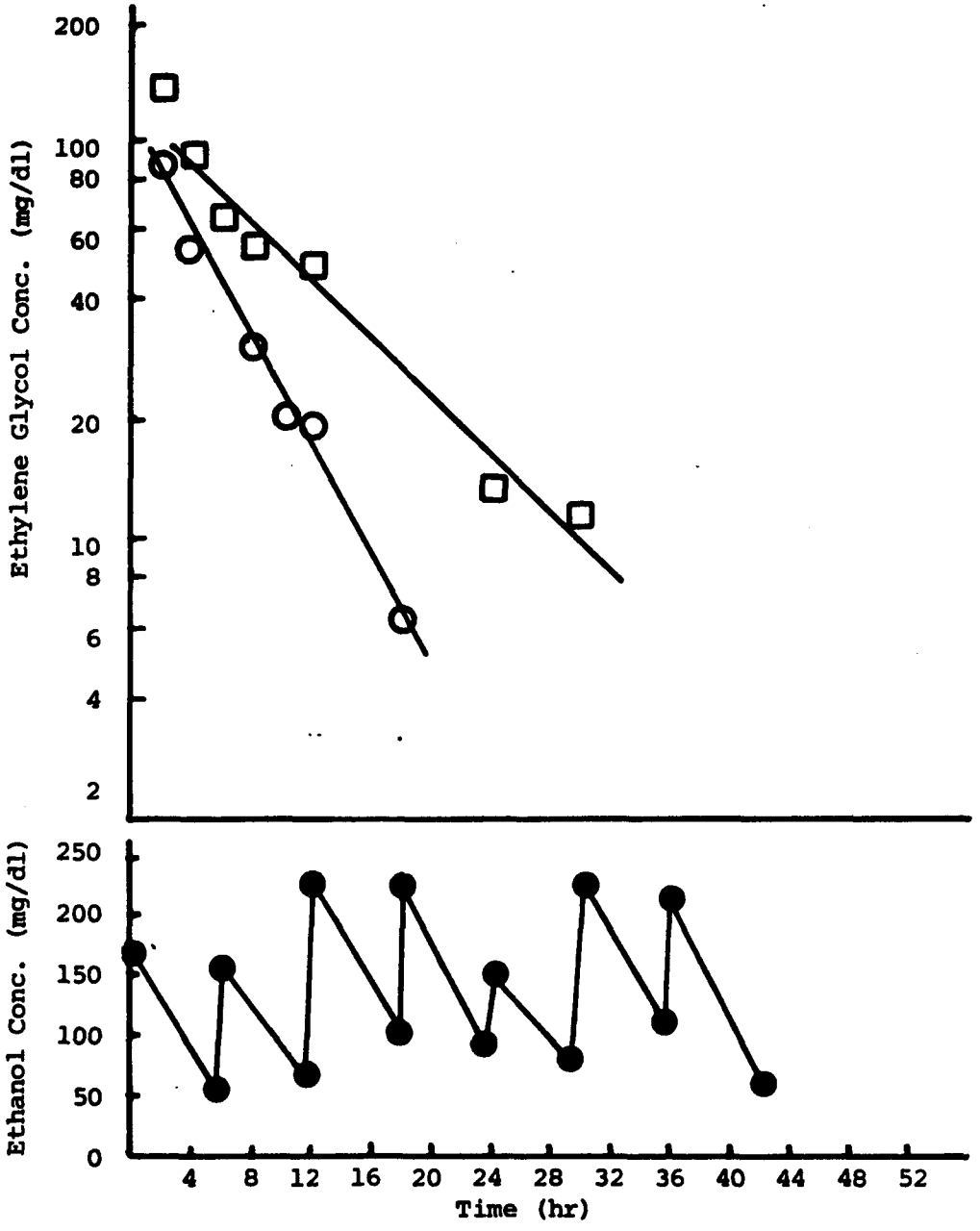

Figure 7. Ethylene glycol and ethanol concentrations as a function of time in dog #4.

Symbols: ethylene glycol in the presence of ethanol (□); ethylene glycol in the absence of ethanol (○); ethanol concentrations produced after multiple dosing (●).

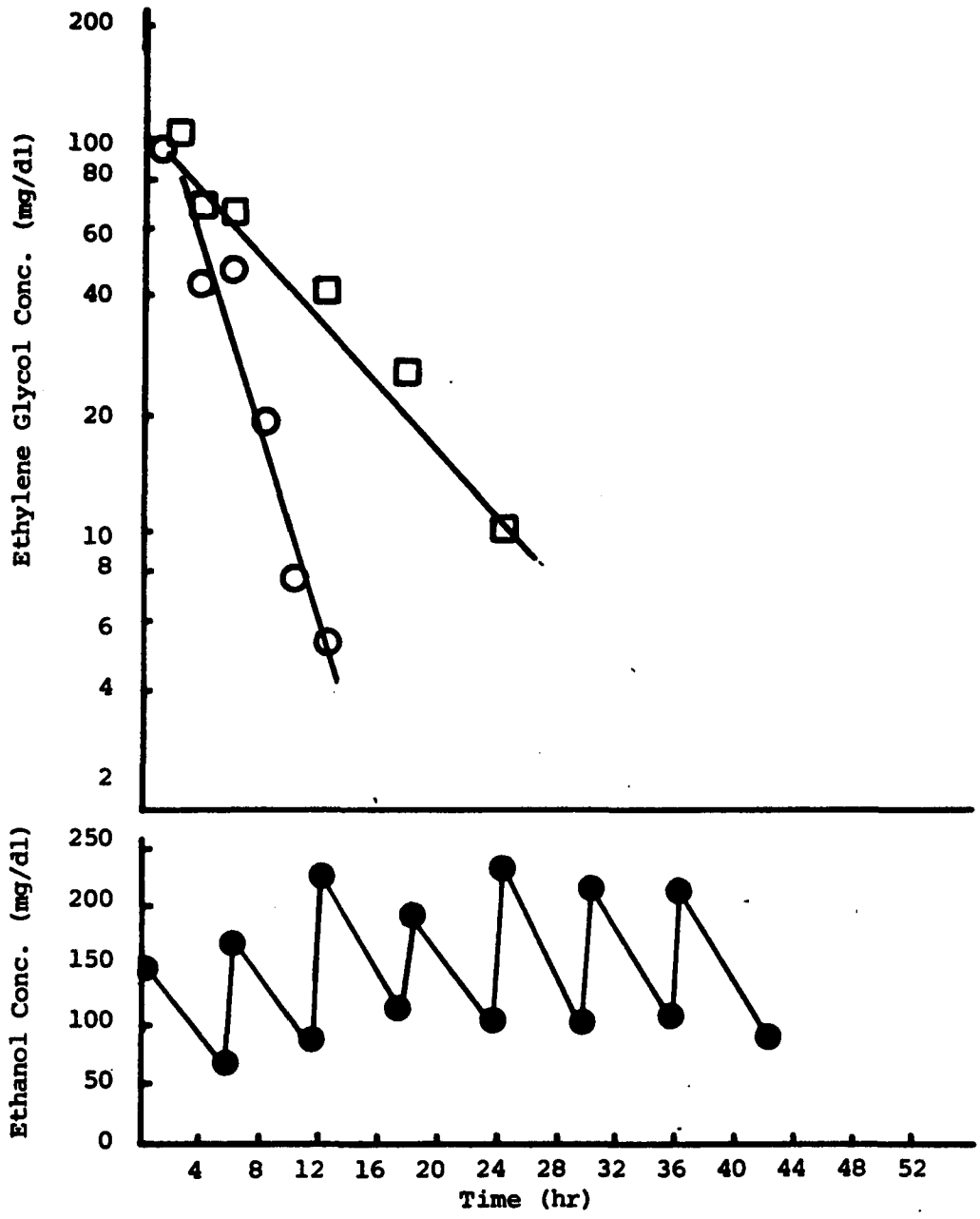

Figure 8. Ethylene glycol and ethanol concentrations as a function of time in dog #5.

Symbols: ethylene glycol in the presence of ethanol (□); ethylene glycol in the absence of ethanol (○); ethanol concentrations produced after multiple dosing (●).

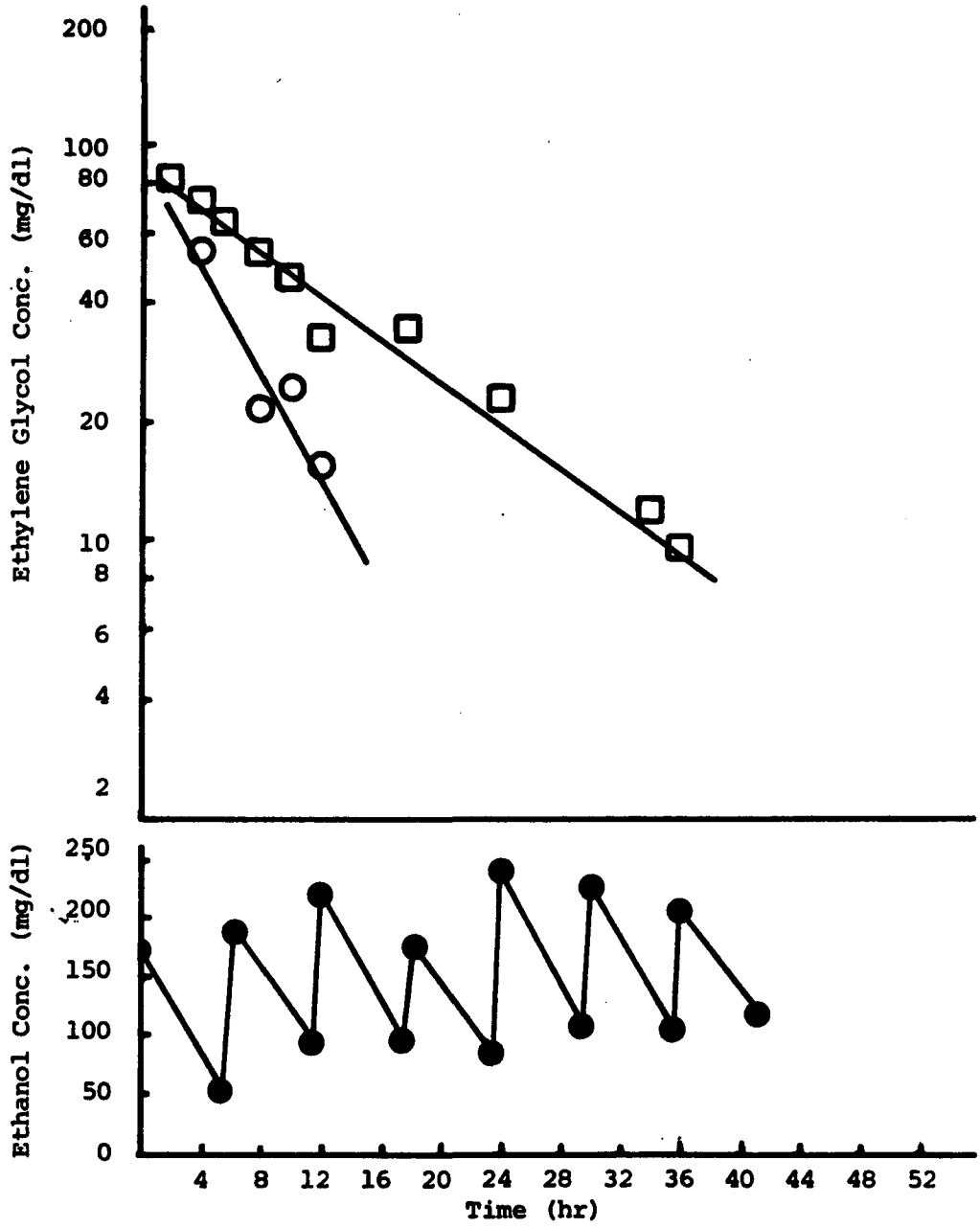

Figure 9. Ethylene glycol and ethanol concentrations as a function of time in dog #6.

Symbols: ethylene glycol in the presence of ethanol (□); ethylene glycol in the absence of ethanol (○); ethanol concentrations produced after multiple dosing (●).

The pharmacokinetic parameters obtained from the analysis of the ethylene glycol concentration-time data are shown in Tables 7 and 8. The data from which the pharmacokinetic values were obtained, the AUC_0^∞ and the dose used in each infusion are summarized in Appendix A. Ethanol concentration-time data are summarized in Appendix A. As can be seen by reference to Figures 4 to 9, these concentrations were within the 50 to 200 mg/dl range in all cases.

Table 7

Summary of the Pharmacokinetic Parameters of Ethylene Glycol in the Absence of Ethanol

Animal	$t_{\frac{1}{2}}$ (hr)	Cl_s (mls/min/kg)	V_β (l/kg)	K (hr^{-1})
(1)[a]	6.86	2.13	1.26	0.101
(2)	3.21	2.21	0.62	0.216
(3)	4.12	1.67	0.60	0.168
(4)[b]	4.42	1.90	0.73	0.157
(5)	2.46	2.54	0.54	0.282
(6)	4.30	2.11	0.79	0.161
Mean:	3.83[c]	2.09	0.76	0.181
± SD:		0.29	0.26	0.062
% CV:		14.0	35.0	34.0

[a]Animals 1-3 received infusions of ethanol and ethylene glycol followed by an infusion of ethylene glycol only, two weeks later.

[b]Animals 4-6 first received infusions of ethylene glycol only, followed by an infusion of ethanol and ethylene glycol two weeks later.

[c]Harmonic mean.

Table 8

Summary of the Pharmacokinetic Parameters of Ethylene Glycol in the Presence of Ethanol

Animal	$t_{\frac{1}{2}}$ (hr)	Cl_s (mls/min/kg)	V_β (l/kg)	K(hr^{-1})
(1)[a]	9.17	1.16	0.92	0.076
(2)	7.82	1.19	0.81	0.089
(3)	8.83	0.87	0.67	0.079
(4)[b]	8.46	0.91	0.67	0.082
(5)	7.24	1.15	0.72	0.096
(6)	11.00	0.90	0.85	0.060
Mean:	8.60[c]	1.03	0.77	0.081
± SD:		0.15	0.10	0.011
% CV:		14.6	13.5	14.0

[a]Animals 1-3 received an infusion of ethanol and ethylene glycol followed by an infusion of ethylene glycol only two weeks later.

[b]Animals 4-6 first received an infusion of ethylene glycol only, followed by an infusion of ethanol and ethylene glycol two weeks later.

[c]Harmonic mean.

In the presence of ethanol the mean volume of distribution (V_β) for ethylene glycol was 0.77 l/kg. In the absence of ethanol this value was 0.76 l/kg. There was no statistically significant difference in volumes of distribution of ethylene glycol in the absence and presence of ethanol (paired t-test, df = 5, p = N.S.)(Table 10). The values for V_β obtained in this study agree well with a volume of distribution of

0.83 l/kg determined by Peterson (1980) in a human subject. The volume of distribution of ethylene glycol is not affected by the presence of ethanol, probably because neither of these substances is bound to plasma proteins and they are distributed evenly through total body water.

The mean plasma clearance of ethylene glycol in the absence of ethanol was 2.09 mls/min/kg. This decreased to 1.03 mls/min/kg in the presence of ethanol. There was a significant difference in the plasma clearance of ethylene glycol in the absence and presence of steady state ethanol (paired t-test, $df = 5$, $p < 0.001$) (Table 10). The decrease in plasma clearance of ethylene glycol in the presence of ethanol is a consequence of the competitive inhibition of ethylene glycol metabolism exerted by ethanol. When a dose of ethylene glycol is administered to an animal in the presence of ethanol, the major portion of the ethylene glycol dose must be excreted as the parent compound. On the other hand, when a dose of ethylene glycol is administered to an animal without ethanol coadministration the ethylene glycol is excreted as the major metabolites, such as glycolic and oxalic acids, as well as the parent compound. This process is much faster, therefore the plasma clearance of ethylene glycol in the absence of ethanol is greater than the plasma clearance of ethylene glycol in the presence of ethanol.

In order to be sure that renal function was not adversely affected during the experiments, pre-infusion and terminal plasma samples taken from dogs infused with ethylene glycol were analyzed for creatinine. The creatinine results are shown in Table 9.

Table 9

Plasma Creatinine Concentrations (mg/dl)

Animal	Infusion with Ethanol and Ethylene Glycol		Infusion with Ethylene Glycol Only	
	Pre-Infusion Creatinine	Terminal Creatinine	Pre-Infusion Creatinine	Terminal Creatinine
1[a]	1.2	0.8	1.3	0.9
2	1.0	0.8	0.9	0.9
3	1.2	0.9	1.0	1.1
4[b]	1.1	0.9	1.0	1.0
5	1.3	1.1	1.4	1.1
6	1.3	0.7	1.2	1.1

[a]Animals 1-3 were infused with ethanol and ethylene glycol first, followed by an infusion of ethylene glycol two weeks later.

[b]Animals 4-6 were infused with ethylene glycol first, followed by an infusion of ethanol and ethylene glycol two weeks later.

There was no increase in the terminal creatinine plasma concentrations compared to the pre-infusion values when dogs were infused with ethylene glycol only or when ethylene glycol and ethanol were coadministered. Therefore, the renal function of the dogs was evidently unimpaired even though exposed to the toxic metabolites of ethylene glycol when no ethanol was present.

The harmonic mean of the disposition half-life ($t_{\frac{1}{2}}$) of ethylene glycol was 3.83 hrs in dogs in the absence of ethanol. This value agrees well with the $t_{\frac{1}{2}}$ of 3.0 hrs reported by Peterson et al. (1980) in a

human subject, and the $t_{\frac{1}{2}}$ range of 2.7-3.7 hrs reported by McChesney et al. (1971) in rhesus monkeys. It should be noted that McChesney et al. (1971) suggested measurable differences in the $t_{\frac{1}{2}}$ in younger monkeys and the $t_{\frac{1}{2}}$ in older monkeys. The $t_{\frac{1}{2}}$ of younger monkeys tended to be shorter and approached a $t_{\frac{1}{2}}$ of 2.7 hrs, while the older monkeys had a longer $t_{\frac{1}{2}}$ which approached a value of 3.7 hrs.

The harmonic mean of the elimination $t_{\frac{1}{2}}$ of ethylene glycol in the presence of steady state ethanol was 8.60 hrs. The disposition $t_{\frac{1}{2}}$ of ethylene glycol in the absence and presence of ethanol was statistically tested and there was a significant difference (paired t-test, df = 5, $p < 0.01$) (Table 10). This significant increase in $t_{\frac{1}{2}}$ is a consequence of an increase in ethylene glycol clearance since the volume of distribution did not change.

Peterson et al. (1980) report a $t_{\frac{1}{2}}$ of 17 hrs for ethylene glycol in a human subject after treatment with 20% oral ethanol. The disparity between these results and the present findings may be due to interspecies differences in metabolism and/or the fact that the human subject in Peterson's study ingested 600 mls of antifreeze (95% ethylene glycol) and was not treated for 3 to 3.5 hrs post-ingestion. This amount of ethylene glycol could have caused renal function impairment which would decrease the overall renal clearance, thereby prolonging the elimination $t_{\frac{1}{2}}$ (Tozer 1981).

In comparing the kinetics of ethylene glycol in the absence and in the presence of steady state ethanol it was found that there was no change in the volume of distribution but that ethanol did produce a

substantial decrease in the plasma clearance and a corresponding increase in the elimination $t_{\frac{1}{2}}$ of ethylene glycol. This was expected due to ethanol's competitive inhibition of alcohol dehydrogenase which is responsible for metabolism of ethylene glycol. Therefore, the change in ethylene glycol $t_{\frac{1}{2}}$ is primarily a reflection of altered metabolic clearance.

Table 10

Statistical Analysis of Ethylene Glycol Pharmacokinetic Parameters

Parameters	V_{β}	Cl_s	$t_{\frac{1}{2}}$	K
t	-0.2156	12.6037	4.569	4.569
df	5.00	5.00	5.00	5.00
p	N.S.[a]	<0.001	<0.01	<0.01

[a]N.S. = not significant.

CHAPTER 4

SUMMARY AND RECOMMENDATIONS

This investigation has described the pharmacokinetic characteristics of ethylene glycol in dogs, in the presence and absence of steady state ethanol. The data, collected under controlled conditions, support most of the available information regarding the elimination half-life and volume of distribution reported in previous evaluations of ethylene glycol kinetics. Some disparity in the elimination half-life ($t_{\frac{1}{2}}$) in the presence of ethanol, reported in a previous study in man, and the mean elimination half-life, in the presence of ethanol, found in this study was observed. The variation was attributed to interspecies differences and possible renal function impairment of the single human subject involved in the previous study (Peterson et al. 1980). In addition, we report the total body clearance (Cl_s) of ethylene glycol in the absence and presence of ethanol, in dogs.

In order to collect the data mentioned above, it was necessary to adapt analytical methods for the analysis of ethylene glycol in blood plasma and the analysis of ethanol in blood. The method for the analysis of ethanol in blood used in this study is relatively straightforward and presented no problem in adapting it for our purposes.

The ethylene glycol procedure used in this study was not decided on until other methods for the analysis of ethylene glycol were tried and

found to be lacking in precision or specificity. The assay used in this study involved injection of plasma containing ethylene glycol and 2,5-hexanedione as the internal standard onto a 5% Carbowax 20 m on 60/80 Carbopack B Column. This assay proved to be a superior assay for the analysis of ethylene glycol in plasma. The precision, accuracy, specificity and sensitivity of the ethylene glycol assay used in this study were very good.

In order to develop a suitable protocol for dosing the dogs with ethylene glycol and ethanol two preliminary experiments were done. One experiment was designed to find out what dose of ethylene glycol should be given the dog to achieve plasma ethylene glycol concentrations associated with toxicity yet be sublethal and which would not impair renal function. In this study, 0.7 mls/kg of ethylene glycol was the dose chosen. A preliminary experiment was also done to determine what loading dose and maintenance doses of ethanol would be required to achieve plasma ethanol concentrations that would effectively prevent ethylene glycol metabolism.

In this study, an ethanol concentration of 50 to 200 mg/dl was found to be effective in significantly reducing the clearance of ethylene glycol. Associated with the ethanol infusion was the venous catheterization of the dogs to facilitate the infusion of comparatively large volumes of solutions.

The experiments involving the infusion of ethylene glycol in the absence and presence of steady state ethanol employed a random cross-over design so that each dog served as its own control. This was done

in order to minimize the effects the order of dosing might have on the results.

Blood specimens were collected at appropriate intervals throughout the experiments and refrigerated or frozen pending analysis by gas liquid chromatography. The plasma and blood samples obtained were analyzed for ethylene glycol, and ethanol (when ethanol was present) by gas liquid chromatography. The analysis of the data obtained from the assays of the specimens taken from the dogs yielded the desired pharmacokinetic parameters of disposition.

In the presence of ethanol the mean volume of distribution was 0.77 l/kg. In the absence of ethanol this value was 0.76 l/kg. This was consistent with our expectations for the volume of distribution of ethylene glycol with and without ethanol coadministration, and agreed well with the volume of distribution (0.83 l/kg) reported in a previous study (Peterson et al. 1981).

The mean plasma clearance of ethylene glycol in the absence of ethanol was 2.09 mls/min/kg. This decreased to 1.03 mls/min/kg in the presence of ethanol. The decrease in the clearance rate in the presence of ethanol is a consequence of the competitive inhibition of ethylene glycol metabolism exerted by ethanol. In the presence of ethanol, there is essentially no metabolism of ethylene glycol, and it must be excreted intact. This is a slower process than the excretion of ethylene glycol plus its metabolites when no ethanol is present. There was no significant difference in serum creatinine concentrations of the pre-infusion and terminal samples taken from dogs infused with ethylene glycol with

and without ethanol coadministration. This indicates that renal function was not adversely affected by the toxic metabolites of ethylene glycol.

The harmonic mean of the disposition half-life ($t_{\frac{1}{2}}$) of ethylene glycol was 3.83 hrs in dogs in the absence of ethanol. The harmonic mean of the elimination $t_{\frac{1}{2}}$ in the presence of steady state ethanol was 8.60 hrs. These values for $t_{\frac{1}{2}}$ are inversely related to the plasma clearance of ethylene glycol in the absence and presence of ethanol, discussed previously. That is, as the $t_{\frac{1}{2}}$ of ethylene glycol is increased by the presence of ethanol, the plasma clearance of ethylene glycol must decrease proportionally, and vice versa in the absence of ethanol. This is due to the competitive inhibition of ethylene glycol metabolism exerted by ethanol.

Considerations for further research on the kinetics of ethylene glycol with and without ethanol coadministration should include the collection of data on the kinetics of the major toxic metabolites of ethylene glycol, particularly glycolic acid. Several workers have attributed the most damaging effects of ethylene glycol intoxication, such as metabolic acidosis, renal tubule degeneration, and direct cytotoxicity, to glycolic acid (Bove 1966; Clay and Murphy 1974; McChesney et al. 1971; Pons and Custer 1946).

However, glycolic acid determinations in biological fluids have not been attempted extensively because of the general unsuitability of most of the assays in biological systems. There is presently an assay with the specificity, sensitivity, precision, and accuracy required for

a study on the kinetics of glycolic acid, but it requires the use of a GC/MS system (Clay and Murphy 1974).

The use of 4-methylpyrazole as a competitive inhibitor of ethylene glycol metabolism has been reported to be as effective as ethanol in treating ethylene glycol intoxication (Mundy, Hall and Teague 1974).

It may therefore be useful to study the kinetics of ethylene glycol in the absence and presence of 4-methylpyrazole and compare those results with the results found in this study. The obvious advantages to using 4-methylpyrazole in place of ethanol are that 4-methylpyrazole will not have an additional CNS depressant effect with ethylene glycol as does ethanol and the infusion volumes required would not be as large as the infusion volumes required for ethanol.

APPENDIX A

Table A.1

Plasma Ethylene Glycol Concentrations (mg/dl) in the Presence of Ethanol

Sampling Time (hr)	Dog #1	Dog #2	Dog #3	Dog #4	Dog #5	Dog #6
Pre-infusion	0.0	0.0	0.0	0.0	0.0	0.0
0.083	74.2	69.5	81.4	70.2	99.6	93.0
0.250	92.7	75.4	101.0	[a]	99.7	103.7
0.500	101.0	82.7	90.8	[a]	93.5	94.9
1.000	93.7	85.0	104.5	100.4	104.2	82.9
2.000	73.9	90.9	117.4	107.2	101.8	81.3
3.000	62.0	79.5	92.7	100.1	75.0	83.2
4.000	56.7	76.1	70.8	90.9	67.3	72.7
6.000	53.2	53.3	68.1	65.9	66.0	64.1
8.000	46.0	44.0	61.0	52.6	[a]	54.3
10.000	41.7	38.7	57.1	[a]	66.3	46.1
12.000	30.3	28.9	55.1	49.0	40.2	32.3
18.000	[a]	25.7	28.0	[a]	26.8	35.4
24.000	18.0	11.3	13.2	14.5	10.4	23.6
30.000	6.9	[a]	13.4	11.5	[a]	12.0
36.000						4.7
AUC_0^∞ (mgxmin/ml)	669.05	649.51	892.30	851.17	673.34	871.21
Dose (gm)	22.120	19.714	17.541	20.024	18.473	22.974

[a] Sample missed.

Table A.2

Plasma Ethylene Glycol Concentrations
in the Absence of Ethanol (mg/dl)

Sampling Time (hr)	Dog #1	Dog #2	Dog #3	Dog #4	Dog #5	Dog #6
Pre-infusion	0.00	0.0	0.0	0.0	0.0	0.0
0.083	65.1	79.9	67.0	95.5	69.1	89.2
0.250	67.1	91.0	104.7	100.1	81.6	84.9
0.500	63.3	93.2	115.4	92.1	95.2	87.3
1.000	57.6	91.7	92.6	63.7	103.0	64.9
2.000	49.8	78.8	89.3	86.4	90.6	81.8
3.000	39.9	74.6	74.9	60.9	61.1	66.1
4.000	38.4	52.7	66.5	57.3	43.0	50.4
6.000	36.5	43.4	55.1	50.7	46.7	[a]
8.000	30.0	29.4	44.6	30.7	19.4	21.4
10.000	22.7	16.2	26.4	20.7	7.7	24.2
12.000	19.6	8.7	20.2	19.8	5.3	15.5
18.000	8.8	[a]	5.7	6.4	[a]	[a]
AUC_0^∞ (mgxmin/ml)	365.06	351.23	391.46	409.10	305.34	367.73
Dose (gm)	28.888	18.628	17.541	21.733	17.386	19.249

Table A.3

Ethanol Concentration Versus Time Data

Sampling Time (hr)	Dog #1	Dog #2	Dog #3	Dog #4	Dog #5	Dog #6
Pre-infusion	0	0	0	0	0	0
0.083	97	136	143	173	151	178
0.250	122	180	166	126	149	86
3.000	104	108	123	102	86	67
6.000[a]	88	74	79	57	60	47
6.250[b]	183	148	219	163	174	199
12.000[a]	65	91	68	63	74	94
12.250[b]	194	201	156	232	231	227
18.000[a]	85	96	96	107	102	92
18.250[b]	187	174	175	229	188	180
24.000[a]	69	80	107	82	97	82
24.250[b]	216	180	179	150	242	243
30.000[a]	87	87	111	71	84	103
30.250[b]	195	212	164	237	219	229
36.000[a]	100	77	121	106	101	94
36.250[b]	172	166	231	244	215	216
42 hr.	111	82	104	69	93	127

[a]Blood sample taken immediately prior to infusion of ethanol maintenance dose.

[b]Blood sample taken 15 min (±5 min) after start of infusion of ethanol maintenance dose.

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