The Fate of Ethylene Glycol in the Environment

(Final Report)

by
A. A. Abdelghani
A. C. Anderson
G. A. Khoury
S. N. Chang
Tulane University

TECHNICAL REPORT STANDARD PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
FHWA/LA-90/228		

4. Title and Subtitle	5. Report Date
FATE OF ETHYLENE GLYCOL IN THE ENVIRONMENT	JANUARY 1990
	6. Performing Organization Code
7. Author(s)	8. Performing Organization Report No.
A.A. ABDELGHANI, A.C. ANDERSON G.A. KHOURY AND S.N. CHANG	N/A
9. Performing Organization Name and Address	10. Work Unit No.
TULANE UNIVERSITY SCHOOL OF PUBLIC HEALTH & TROPICAL MEDICINE 1430 TULANE AVENUE NEW ORLEANS, LA 70112	11. Contract or Grant No. NO. 87 - 1M(B)
	13. Type of Report and Period Covered 1987 - 1989
12. Sponsoring Agency Name and Address	
LOUISIANA DEPARTMENT OF TRANSPORTATION AND DEVELOPMENT	14. Sponsoring Agency Code

15. Supplementary Notes

THE STUDY WAS CONDUCTED IN COOPERATION WITH THE UNITED STATES DEPARTMENT OF TRANSPORTATION FEDERAL HIGHWAY ADMINISTRATION.

16. Abstract

The Louisiana Department of Transportation and Development uses ethylene glycol (EG) as a deicing agent on bridges. This study was undertaken to assess the impact of ethylene glycol on workers and the environment after spraying. The objectives of the project were:

1. To determine the level of exposure of workers spraying EG on bridges;
2. To monitor the level of EG in the atmosphere above sprayed bridges;
3. To determine the aqueous concentrations of EG due to runoff of the chemical from sprayed bridges to the aquatic environment;
4. To determine the effect of EG in the aquatic environment including sorption capacity to soil, acute toxicity to bluegill sunfish, crawfish, and microorganisms, bioaccumulation in crawfish, and biodegradation by soil microorganisms.

Some conclusions include:

1. Air samples collected above sprayed bridges contained far less EG than the American Conference of Governmental Industrial Hygienists (ACGIH) recommended values;
2. EG concentrations in sediment and water collected from areas under sprayed bridges were below detection limits. EG did not adsorb to soils in laboratory sorption studies;
3. Common soil microorganisms readily degraded EG;
4. Acute toxicity values for crawfish, bluegill sunfish and soil microrganisms were far above the expected environmental concentration resulting from normal applications;
5. In a bioaccumulation study, crawfish did not concentrate EG to levels above the water concentration. The amount of EG taken up in crawfish edible tissues does not pose acute health effects to humans. One would have to consume 63,900 contaminated crawfish or 384 kg of crawfish edible tissues at one time to be affected by EG toxicity;
6. In a depuration study, crawfish were able to completely eliminate the accumulated EG within 5 to 6 days.

Recommendations:

1) While concentrations and toxicity of EG were low, care should still be taken in handling the compound. For example: a) all applicators should stay inside the cab of the spray rig and windows should be kept closed; b) Care should be taken to protect the hands wearing gloves during handling of concentrated EG; c) It is advisable to stand upwind of the prevailing wind direction when mixing EG to avoid aerosol inhalation; d) Spills and direct application of EG to water should be avoided.
2) Acute studies on juvenile crawfish and other aquatic species could be done to determine potential acute effects on more sensitive stages of the organisms.

17. Key Words		18. Distribution Statement
ETHYLENE GLYCOL ACCUMULATION ACUTE TOXICITY BIODEGREDATION	DEPURATION ABSORPTION BREATHING ZONE AEROSOL VAPOR	Unrestricted. This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
UNCLASSIFIED	UNCLASSIFIED	108	

Form DOT F 1700.7 (8-69)

THE FATE OF ETHYLENE GLYCOL IN THE ENVIRONMENT

FINAL REPORT

by

A. A. ABDELGHANI, A. C. ANDERSON, G. A. KHOURY
AND S. N. CHANG
DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES
SCHOOL OF PUBLIC HEALTH AND TROPICAL MEDICINE
TULANE UNIVERSITY
1430 TULANE AVE.
NEW ORLEANS, LA 70112

CONDUCTED FOR

LOUISIANA DEPARTMENT OF TRANSPORTATION AND DEVELOPMENT
STATE PROJECT #736-12-67
NUMBER 87-1M(B)

DECEMBER, 1989

ACKNOWLEDGEMENTS

We sincerely appreciate the contribution of the following persons for their constructive role in this research project:

A. Arman, S. Cumbaa, N. Kensella, P. Landry, B. Naghavi, J. Ray, J. Rapol, M. Rasoulian, S. Shah, and W. Temple.

Special thanks to K. Kepper and his crew for assisting in the field portion of the project.

ABSTRACT

The Louisiana Department of Transportation and Development uses ethylene glycol (EG) as a deicing agent on bridges. This study was undertaken to assess the impact of ethylene glycol on workers and the environment after spraying.

Air samples collected above sprayed bridges showed that time-weighted average EG values ranged from <0.05 to 0.33 mg/m^3 for aerosols and <0.05 to 10.4 mg/m^3 for vapor. Air samples collected from the breathing zone of workers applying the ethylene glycol indicated ranges between <0.05 to 2.33 mg/m^3, and < 0.05 to 3.37 mg/m^3 for aerosol and vapor respectively. All air samples contained far less than the American Conference of Governmental Industrial Hygienists' (ACGIH) recommended values of 10 mg/m^3 EG aerosol and 125 mg/m^3 EG vapor.

Ethylene glycol concentrations in samples of soils, sediment and water collected from areas under sprayed bridges were below detection limits. In addition, ethylene glycol did not adsorb to soils collected from these sites in laboratory sorption studies.

Common soil microorganisms (Serratia, Citrobacter and Pseudomonas) degraded ethylene glycol within 3 days with a rate of biodegradation of 0.5 µg/l/hr for 1% and 3% ethylene glycol concentrations. Concentrations of ethylene glycol higher than 5% exerted toxic effects on the microbial population.

Acute toxicity studies on crawfish and bluegill sunfish showed a 96-hour LC_{50} of 91,430 mg/l for crawfish and 27,540 mg/l for bluegills. The toxicity to a mixed population of soil microorganisms was also determined. The average toxic end point (LC_{50}) for microorganisms was 114,300 mg/l. The acute toxic values of EG found in these studies were far higher than the expected environmental concentration resulting from normal Department of Transportation and Development applications.

In a bioaccumulation study, crawfish were exposed to EG at three concentrations (50 µg/ml, 200 µg/ml and 1000 µg/ml) for 61 days and were subsequently transferred to clean water for a 67-day decontamination phase. During the uptake and loss phases, samples were analyzed for EG content in gills,

muscle, gastrointestinal tract and hepatopancreas. An open, one-compartment mathematical model was developed to describe the uptake and loss phases data.

Bioaccumulation was dependent upon the concentration of EG to which crawfish were exposed. The tissues did not concentrate EG to levels above the water concentration. The order of bioaccumulation among tissues was: gastrointestinal tract > abdominal muscle ≈ hepatopancreas > gills. The accumulation study showed that the amounts of EG taken up in edible crawfish tissues (abdominal muscles and hepatopancreas) do not pose acute health effects to humans. One would have to consume 63,900 contaminated crawfish or 384 kg of edible crawfish tissues at one time to be affected by EG toxicity.

The depuration study showed that crawfish were able to completely eliminate the accumulated ethylene glycol within 5 days for animals exposed to 50 μg/ml EG and 6 days for those exposed to 200 μg/ml EG and 1000 μg/ml EG.

IMPLEMENTATION STATEMENT

While ethylene glycol showed low toxicity and low environmental concentrations, care should be taken in its application.

1) In this study ethylene glycol was detected in the air inside the spraying truck at low levels. Although the concentration was much below the ACGIH recommended level, precautions should be taken. All applicators should stay inside the cab and windows should be kept closed.

2) Although there are few reports of adverse effects from direct contact with the skin, care should be taken to protect the hands by wearing gloves during handling of concentrated ethylene glycol.

3) It is advisable to stand upwind of the prevailing wind direction when mixing ethylene glycol to avoid aerosol inhalation. Spraying rigs could be modified (if possible) so the nozzles are at the back of the truck.

4) Results of ethylene glycol testing on crawfish and bluegills showed low acute toxicity; however, this does not preclude toxicity to other aquatic species. Therefore, spills and direct application of ethylene glycol to water should be avoided.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Ethylene glycol (EG) is a colorless, odorless, viscous, water-soluble liquid with a bitter sweet taste (Merck Index, 1983). In 1983, 4.5 billion pounds were produced in the United States (USITC Publication #1183). Ethylene glycol is used for several purposes. It is used as an antifreeze, a deicing agent on bridges and airport runways, and as a solvent in the plastic industry in manufacturing fibers (Merck Index, 1983).

Human exposures to ethylene glycol by ingestion (Goodman et al., 1980; Terlinsky, 1980; Grant, 1974), inhalation (Trofsi, 1950; Dubeikovska et al., 1973) and by dermal exposure (Dawson, 1976) are reported in the literature. Ethylene glycol enters the environment through effluents coming from manufacturing industries, spills and through its use as a deicing agent on bridges, airplanes and airport runways.

This study was undertaken to assess the fate of ethylene glycol in the environment including: 1) potential exposure of workers applying EG, 2) potential contamination levels in water, soil and sediment under sprayed bridges, 3) its toxicity to aquatic organisms and soil microorganisms, and 4) bioaccumulation by aquatic organisms.

OBJECTIVES

The objectives of the project were:

1. To determine the level of exposure of workers spraying ethylene glycol on bridges as a deicing agent.
2. To monitor the level of ethylene glycol in the atmosphere above sprayed bridges.
3. To determine the aqueous concentrations of ethylene glycol due to runoff of the chemical from sprayed bridges to the aquatic environment.
4. To determine the effect of ethylene glycol in the aquatic environment including:
    a. sorption capacity to soil
    b. acute toxicity to bluegill sunfish, crawfish, and microorganisms
    c. bioaccumulation in crawfish
    d. biodegradation by soil microorganisms

LITERATURE REVIEW

Ethylene glycol is used as an antifreeze in cooling and heating systems, in hydraulic brake fluids and as a solvent in the paint and plastics industries. It is used in the formulation of printers' inks, stamp pad inks, and inks for ball-point pens. It serves as a softening agent for cellophane and as a stabilizer for soybean foam used to extinguish oil and gasoline fires. It is used in the synthesis of safety explosives, glyoxal, plasticizers, elastomers, synthetic fiber, and synthetic waxes (Merck Index, 1983).

The Louisiana Department of Transportation and Development uses ethylene glycol as a deicing agent on highway bridges (Kepper, 1989). Environmental contamination may result when bridges are sprayed with ethylene glycol and runoff reaches surface water and soil. Industrial waste, spills, spent antifreeze, and application of EG to airport runways and aircraft could also contribute to environmental contamination.

The toxicity of ethylene glycol depends upon the susceptibility of the species; for example, EG is five times more toxic to humans than to poultry. (Beasley, 1980). Ingestion of ethylene glycol by humans can result in toxicity resembling alcoholic intoxication with ataxia, drowsiness, and slurred speech, and possibly coma, convulsions, and death (Parry, M. F., 1974; Berman, L. B., 1957). Drinking antifreeze fluid causes transient stimulation of the central nervous system followed by depression,

vomiting, drowsiness, coma, respiratory failure, convulsions, and renal damage, which may proceed to anuria, uremia, and death (Merck, 1983). A fatal case was reported in which a 1/4 to 1/2 pint of antifreeze solution was ingested; acute meningoencephalitis occurred followed by anuria. Death from renal failure resulted after 12 days (Clay, 1982).

Human plasma clearance half-lives of ethylene glycol following oral administration range from 2 to 6 hours (Reif, 1950; Winek, 1975; Peterson et al., 1981). Some work has been done on the acute toxicity of EG in other species. Toxicity of ethylene glycol to soil microorganisms (Pseudomonas) was studied by Bringmann and Khun (1980), who reported a toxicity threshold of > 10,000 mg/l. The 24-hour media tolerance limit (TLM) to brine shrimp and crawfish was found to be > 20,000 and 169,000 mg/l respectively (Price et al., 1974). The LC_{50} for common shrimp and rainbow trout were reported to be > 100,000 mg/l (Bachmann, 1974) and > 18,500 (Jank et. al., 1974) respectively.

METHODOLOGY

FIELD SAMPLING OF AIR, WATER AND SEDIMENT

A truck equipped with a spraying rig was used to apply a 50% ethylene glycol concentration on three designated bridges. The spray rig, mounted under the front bumper of the truck, consisted of a ten-foot bar fitted with spray nozzles which directed an overlapping-fan pattern to the street. These bridges were selected because they are sprayed with 50% ethylene glycol on a routine basis during freeze conditions and because of their close proximity to each other. A fourth bridge in the same vicinity that had no history of spraying was used as a control. Selected bridges were above running streams so that water and sediment samples could be collected.

Air samples were collected from the atmosphere above the sprayed bridges and from the breathing zone of the workers mixing and spraying the chemical. Water, sediment and soil samples were also collected from under the sprayed bridges.

AIR SAMPLING

Air samples above the bridge were collected using an air sampling train as follows: a glass tube, 8 cm by 6 mm ID, contained two sections of 20/40 mesh silica gel (front=520 mg, back=260 mg) separated by a 3 mm urethane foam plug. A 13 mm glass fiber filter, free of binders in a millipore filter holder, precedes the front section. A sampling SKC pump was connected to this tube and accurately calibrated at a flow rate of 0.2 liters per minute (see Figure 1). The glass fiber filter takes up ethylene glycol aerosol and the silica gel accumulates the ethylene glycol vapor. The concentration of ethylene glycol determined on both the filter and silica gel is the total EG in the air sample (NIOSH, 1977).

Sampling units were placed at three locations on the curb of each bridge at a height of 8 inches (ends and middle of each bridge). Wind velocity was 6 miles per hour and the temperature was 42° F. Air samples were taken at about 2-hour intervals for approximately 8 hours following spraying. Air from the breathing zone of the spray-rig driver and a passenger were drawn through the same type of air sampling train (Figure 1) for 15 minutes for ceiling concentrations. Eight persons were monitored on two separate spraying occasions to give a total of 16 worker exposure samples. The driver's window was closed while the passenger window was open during the spraying and sampling. Normally both windows are closed due to the cold weather when spraying takes place.

Filters and silica gel tubes were replaced every 2 hours on the sampling train. As soon as the air samples were collected, the glass filters were preserved in a vial containing 1 ml of 2% propanol. The open ends of the silica gel tubes were capped, each section (front and back) was collected in a vial and 1 ml of 2% propanol was added to preserve the samples for analysis (NIOSH, 1977).

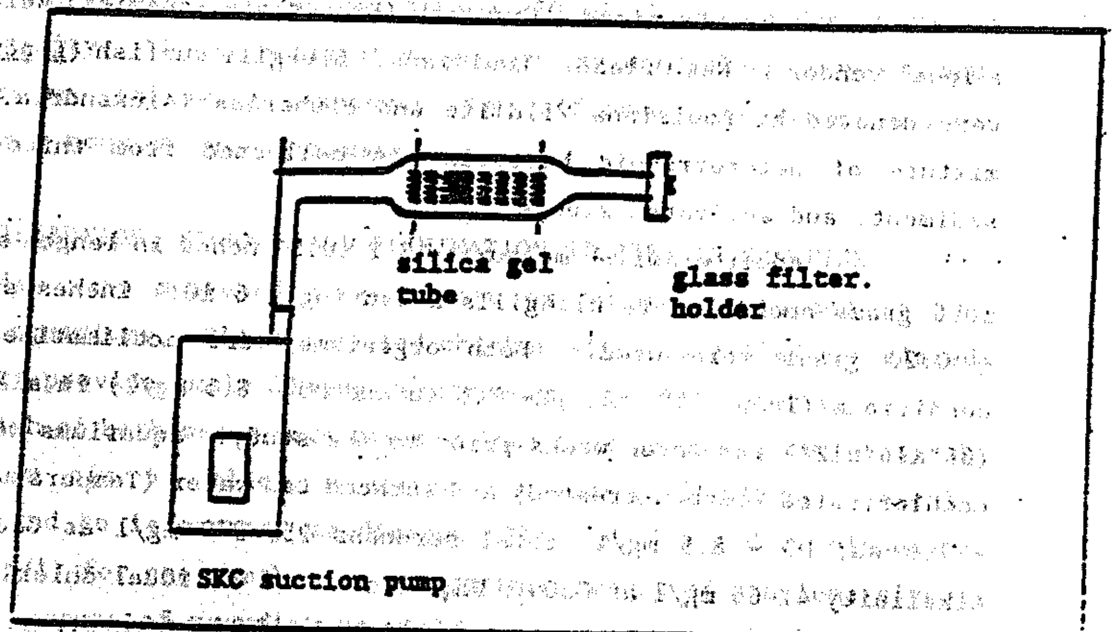


Figure 1. Apparatus of the air sampling train

WATER, SEDIMENT AND SOIL

Water, sediment and soil samples were collected from under the sprayed bridges at three locations: upstream, underbridge and downstream. Water samples were collected from a depth of 6" to 12" and placed in clean glass containers (acid washed and rinsed with distilled water). Sediment samples were collected from the same areas, placed in clean glass containers and properly capped.

ACUTE TOXICITY TO CRAWFISH, BLUEGILLS AND SOIL MICROORGANISMS

Ethylene Glycol was purchased from Shell Oil Company (Geismar, Louisiana). The material, 99.9% pure, with a specific gravity of 1.115, was used for all test concentrations. Crawfish (Procambarus species) were purchased from a local vendor in New Orleans, Louisiana. Bluegill sunfish (Lepomis macrochirus) were donated by Louisiana Wildlife and Fisheries (Alexandria, Louisiana). A mixture of heterotrophic bacteria was collected from three sources: soil, sediment, and activated sludge.

Adult crawfish measuring 3.2 ±0.5 inches in length and weighing 13.6 ±0.6 grams and juvenile bluegills measuring 1.6 ±0.4 inches and weighing 0.85 ± 0.20 grams were used. Both organisms were acclimatized to laboratory conditions (Temp. 21°C ±1, pH- 7.5 su and DO - 8.5 mg/l) in all glass aquariums (36"x16"x12") for three weeks prior to the study. Aquariums were supplied with dechlorinated (carbon-treated) and aerated tap water (Temperature, 21°C ±1.0; pH - 7.5 su; DO - 8.5 mg/l; total hardness 250-270 mg/l as $CaCO_3$; pH-7.5 ±0.2; Alkalinity 47-65 mg/l as $CaCO_3$; NH_3-N and total residual chlorine were below the detection limits). A 14-hour dark and 10-hour light photoperiod was simulated.

Toxicity tests were conducted according to Standard Methods (1985) and the EPA method for static-tank acute toxicity tests (EPA, 1985). Five test concentrations and a control were used. A duplicate at each concentration was used to evaluate variability.

Dissolved oxygen, pH and temperature were recorded daily. Dead animals were counted and removed daily. The ethylene glycol concentration was measured (Appendix A, A-1 through A-10). Tests for both crawfish and bluegills were run for 96 hours.

The LC_{50} was estimated using the EPA probit analysis computer program version 1.4 for calculating effective concentrations (EC).

Bacterial toxicity assessment was done following the methods described by Alsop et al., (1980), and Bauer et al., (1981). The Alsop Assay measures turbidity as an indication of bacterial growth. Several concentrations of ethylene glycol and a control were used. The degree of growth inhibition was determined by measuring the turbidity (optical density at 530 nm) of the test medium at various concentrations after 16 hours of exposure. The measured optical density value was calculated as a percentage of the control system. The percent of control values were then plotted against the log of test sample concentration. The test concentration corresponding to a 50% reduction in optical density, termed "50% inhibition concentration" (IC_{50}), was taken as the end point of toxicity.

The Bauer Assay utilizes short-term (20 min.) oxygen depletion as the measure of toxicity. The kinetics of dissolved oxygen depletion by a mixed microbial population following exposure to different ethylene glycol concentrations and a control were evaluated.

ACCUMULATION, DISTRIBUTION, STORAGE AND ELIMINATION STUDIES IN CRAWFISH

Crawfish were declawed to eliminate predation and placed in 50-gallon, all-glass aquariums. Plastic-coated chicken wire was coiled and placed in each aquarium to provide ample living requirements for the crawfish.

Crawfish of both sexes were divided into four groups of 500 crawfish each and placed in six 50-gallon aquariums (measuring 12"x18"x48" each). Crawfish were adapted to laboratory conditions (21°C, pH 7.4 ± 0.2, dissolved oxygen 7.3 ± 0.2 mg/l) for one month. An appropriate amount of ethylene glycol was added to three groups of crawfish to yield concentrations of 50 µg/l, 200 µg/l and 1,000 µ/l EG. The fourth group was used as a control and no ethylene glycol was added. A flow through system was used. The total volume of water in aquariums was replaced once every two days. Ethylene glycol was added by using a peristaltic pump adjusted to deliver the proper concentration of ethylene glycol when mixed with incoming fresh tap water. Total volume of water in the aquariums was filtered once every six hours and was aerated continuously. Crawfish were fed Quaker Oats daily. Excess food and crawfish excreta were siphoned out daily using a plastic hose.

Following 61 days of continuous exposure of crawfish for the uptake study, aquariums were emptied, rinsed well and refilled with dechlorinated tap water. The remaining crawfish were placed in the clear aquarium to start the depuration phase. The same filtration rate and flow-through system of water were used as in the contamination phase. This phase was carried on for 67 days. Three crawfish were randomly sampled daily from each system and rinsed with tap water, followed by deionized distilled water. Crawfish were dissected into gills, hepatopancreas, G.I. tract and abdominal muscle. Tissues were extracted and analyzed for ethylene glycol content using gas chromatography as indicated in the section "Sample Preparation and Analysis."

BIODEGRADATION

Biodegradation of ethylene glycol was followed by a mineral salt medium (Table 1) containing ethylene glycol as the sole carbon source for bacteria. The system was spiked with radiolabelled [1,2-^{14}C] ethylene glycol (10 mCi $mmol^{-1}$), which was obtained from ICN Radiochemicals (Irvine, California).

TABLE 1

COMPOSITION AND CONCENTRATION OF GROWTH MEDIA

Constituent	Concentration (mgl^{-1})
K_2HPO_4	1300
KH_2PO_4	820
$(NH_4)_2SO_4$	1000
$CoCl_2.6H_2O$	1
NaCL	50
$MgSO_4.7H_2O$	50
$FeSO_4.7H_2O$	10
$CuSO_4.5H_2O$	10
$MnCl_2.4H_2O$	1

Microorganisms for the biodegradation study were collected from three sources: soil, pond water, and the influent of a municipal waste water treatment plant. The microorganism mixture was acclimated to 3% ethylene glycol. The culture was then used in 1%, 3%, and 5% ethylene glycol concentrations. Each concentration was prepared in triplicate and placed in sterile gas washing bottles.

Biodegradation was followed by measuring the isolation of $^{14}CO_2$ produced. The evolved $^{14}CO_2$ was trapped in a mixture of monoethanolamine and 2-methoxy ethanol (10:7 v/v) (9,10). The growth media was slowly aerated with filtered compressed air which continuously flushed $^{14}CO_2$ into the trapping solution. At selected time intervals, 1 ml sample of the CO_2 trapping solution was taken for analyses.

Radioactivity was measured by liquid scintillation using a 3 channel Beckman LS-150 liquid scintillation instrument. Samples were placed in 20-ml polypropylene scintillation vials from Beckman (Houston, Texas) containing 14 ml of CytoScint, a biodegradable-nontoxic liquid scintillation cocktail (ICN Biomedical, Inc. Irvine, California).

The trapping solution was changed at each sampling time interval. The controls had the same growth media with the labelled ethylene glycol and 3% unlabelled ethylene glycol, but no bacteria were added. Sterile conditions were maintained. Two controls were used: one in the light and the other covered with aluminum foil to simulate degradation in the dark. This was done to check for possible photodegradation. The experiment was followed for 15 days at room temperature.

SOIL ADSORPTION-DESORPTION

Five soils were used, four were taken from fields near bridges that are frequently sprayed with ethylene glycol during winter. The fifth soil was montmorillonite, a laboratory clay (Dressier, Inc., Houston, Texas). One hundred grams of each soil was heat-dried at 103°C. The dried soil was then powder-ground in a blender. The grounded soil was passed through a 50 mesh screen. Ten-gram samples of the soil were placed in 250 ml Erlenmeyer flasks, which were acid-washed and rinsed. The flasks were then covered with cotton and aluminum foil and sterilized in an autoclave. One hundred ml of selected concentrations of labelled ethylene glycol were then added to each flask containing 10 grams of sterile soils. A duplicate flask for each sample/concentration was used to establish the time when samples reach equilibrium or steady state. Contents of flasks were mixed using a mechanical wrist shaker. Every hour the "equilibrium" flask was removed, contents centrifuged and 1 ml of the supernatant counted by liquid scintillation (as described below) until two consecutive samples gave the same reading. At that time the test was terminated and all samples were centrifuged and counted for ^{14}C. The supernatant of each soil was then decanted into a container for proper disposal.

For desorption, 100 ml of clean autoclaved distilled water was added to the precipitated soil and shaken for the same time required for samples to reach equilibrium during the adsorption study. The samples were then centrifuged and 1 ml of the supernatant was counted by a liquid scintillator.

Radioactivity was counted by liquid scintillation using a 3 channel Beckman LS-150 instrument. Analysis was performed on $^{14}CO_2$. Samples were placed in 20-ml polypropylene scintillation vials from Beckman (Houston, Texas) containing 14 ml of CytoScint, a biodegradable-nontoxic liquid scintillation cocktail (ICN Biomedical, Inc., Irvine, California).

SAMPLE PREPARATION AND ANALYSIS

Soil, Sediment and Water:

One hundred grams of soil and sediment samples were extracted with an equal volume of 2% propanol. The extracted samples were centrifuged at a speed of 2000 rpm. The supernatant was then analyzed by chromatography. The water samples were centrifuged at 13,000 rpm and the supernatant was analyzed.

Air:

Glass filters, the front and the back of the silica gel tubes, were extracted with 1 ml portions of 2:98 2-propanol-water (v/v). The extract was combined and analyzed.

Crawfish Tissue:

Each sampled crawfish was rinsed with tap water, then with distilled water, and dissected into gills, hepatopancreas, gastrointestinal tract and muscle tissue. Each tissue was then individually weighed and homogenized in a microblender with 5 ml of 2% propanol and centrifuged. The supernatant was then analyzed.

Soil, sediment, water, air and crawfish extracted samples were analyzed using a gas chromatography method recommended by NIOSH (1977) with slight modification. The Carbowax packed glass column was replaced with a DB-wax megabore with an ID of 0.53 μ and 15 meters long. The detection limit was enhanced three times with a detection limit of .1 mg/l.

The operating conditions for gas chromatography were as follows:

Carrier Gas:	Helium
Temperature of column:	165°C
Temperature of injection port:	250°C
Temperature of detector:	300°C
Flow rate of helium:	13 ml/min
Flow rate of hydrogen:	44 ml/min
Flow rate of air:	304 ml/min
Retention time:	Approx. 3.6 min

QUALITY CONTROLS AND ASSURANCE

See Appendix B.

DISCUSSION OF RESULTS

FIELD STUDIES

Air, Water and Sediment:

Tables 2 and 3 represent the data from air samples collected over bridges sprayed with a 50% ethylene glycol concentration on February 15, 1989, and March 10, 1988. The time- weighted average (TWA) values for aerosols or particulates ranged from less than 0.05 to 0.33 mg/m^3 and from less than 0.05 to 10.34 mg/m^3 for vapor.

TABLE 2

TIME WEIGHTED AVERAGE (TWA) FOR AIR SAMPLES COLLECTED ON FEBRUARY 15, 1989 FROM BRIDGES SPRAYED WITH ETHYLENE GLYCOL (EG)

Bridge	Bridge Location	Sampling Time* (min.)	Conc. of EG Aerosol (mg/m^3)	Conc. of EG Vapor (mg/m^3)	Total Conc. of EG in Air Sample (mg/m^3)
Hwy. 61	Front	385	0.05	0.37	0.427
	Middle	385	0.08	1.52	1.604
	End	385	0.10	1.50	1.617
Joor Rd. Bridge 1	Front	350	0.31	2.35	2.636
	Middle	350	0.14	2.35	2.495
	End	350	<0.05	2.93	2.930
Greenwell Springs Rd.	Front	335	0.03	1.49	1.527
	Middle	335	0.32	2.85	3.183
	End	335	0.13	4.18	4.319
Joor Rd. Bridge 2 Control	Front	406	<0.05	0.05	0.058
	Middle	406	<0.05	0.05	0.051
	End	406	<0.05	0.07	0.074

Air temperature during spraying was 84°F

*Sampling pump was calibrated at a flow rate of 0.2 l/min.

TABLE 3

TIME WEIGHTED AVERAGE (TWA) FOR AIR SAMPLES COLLECTED ON MARCH 10, 1988 FROM BRIDGES SPRAYED WITH ETHYLENE GLYCOL (EG)

Bridge	Bridge Location	Sampling Time* (min.)	Conc. of EG Aerosol (mg/m^3)	Conc. of EG Vapor (mg/m^3)	Total Conc. of EG in Air Sample (mg/m^3)
Hwy. 61	Front	545	0.12	2.61	2.73
	Middle	545	0.20	3.92	4.12
	End	545	0.23	0.57	0.81
Joor Rd. Bridge 1	Front	420	0.03	3.30	3.33
	Middle	420	0.13	5.68	5.81
	End	420	0.18	10.38	10.57
Greenwell Springs Rd.	Front	445	0.10	2.62	2.72
	Middle	445	0.29	1.80	2.10
	End	445	0.07	4.23	4.31
Joor Rd. Bridge 2 Control	Front	445	<0.05	<0.05	<0.05
	Middle	445	<0.05	<0.05	<0.05
	End	445	<0.05	<0.05	<0.05

Air temperature during spraying was 44°F

*Sampling pump was calibrated at a flow rate of 0.2/1 min.

Tables 4 and 5 represent ceiling values for air samples collected from persons spraying bridges with 50% EG on February 15, 1989, and March 10, 1988. The ceiling values ranged from less than 0.05 to 2.33 mg/m^3 aerosol and from less than 0.05 to 3.37 mg/m^3 vapor. All values in Tables 2-5 are much less than the recommended ACGIH ceiling level of 10 mg/m^3 for aerosol and 125 mg/m^3 for vapor.

TABLE 4

CEILING VALUES FOR AIR SAMPLES COLLECTED ON FEBRUARY 15,1989 FROM PERSONS SPRAYING ETHYLENE GLYCOL (EG) ON BRIDGES

Bridge	Bridge Location	Sampling Time* (min.)	Conc. of EG Aerosol (mg/m^3)	Conc. of EG Vapor (mg/m^3)	Total Conc. Of EG In Air Sample (mg/m^3)
Hwy. 61	Passenger	15	<0.05	2.36	2.36
	Driver	15	2.33	2.20	4.53
Joor Rd. Bridge 1	Passenger	15	<0.05	<0.05	<0.05
	Driver	15	1.27	<0.05	1.27
Greenwell Springs Rd.	Passenger	15	1.83	3.36	5.20
	Driver	15	0.96	1.73	2.70
Joor Rd. Bridge 2 Control	Passenger	15	<0.05	<0.05	<0.05
	Driver	15	<0.05	<0.05	<0.05

Air temperature during spraying was 84°F

*Sampling pump was calibrated at a flow rate of 0.2 l/min.

TABLE 5

CEILING VALUES FOR AIR SAMPLES COLLECTED ON MARCH 10, 1989
FROM PERSONS SPRAYING ETHYLENE GLYCOL (EG) ON BRIDGES

Bridge	Bridge Location	Sampling Time* (min.)	Conc. Of EG Aerosol (mg/m^3)	Conc. of EG Vapor (mg/m^3)	Total Conc. Of EG In Air Sample (mg/m^3)
Hwy. 61	Passenger	15	<0.05	<0.05	<0.05
	Driver	15	1.20	<0.05	1.20
Joor Rd. Bridge 1	Passenger	15	1.50	1.00	2.50
	Driver	15	<0.05	0.90	0.90
Greenwell Springs Rd.	Passenger	15	<0.05	0.73	0.73
	Driver	15	0.50	<0.05	0.50
Joor Rd. Bridge 2 Control	Passenger	15	<0.05	<0.05	<0.05
	Driver	15	<0.05	<0.05	<0.05

Air temperature during spraying was 84°F

*Sampling pump was calibrated at a flow rate of 0.2 l/min.

Potential toxic concentrations from inhalation are unlikely at room temperature or colder temperatures due to ethylene glycol's vapor pressure. Vapor poisoning usually occurs only if the liquid is heated or aerosolized (Marshall, 1988). A group of volunteers exposed to 30 mg/m^3 EG for 20 hours per day over two weeks complained of throat irritation, mild headache and low backache. These complaints became more marked when concentrations of EG were increased to above 140 mg/m^3 for part of one day (ACGIH, 1980). The lowest published lethal dose (LDLo) for human toxicity by inhalation is reported at 10,000 mg/m^3 with the toxic effect on the eye and the pulmonary system (NIOSH, 1986).

From these results, spraying crews do not appear to be in danger from ethylene glycol vapor or aerosol during spraying. However, the potential for exposure to higher levels still exists, and spraying crews should take precautionary measures when handling, mixing and spraying.

Tables 6 and 7 show the data on water, sediment and soil samples collected under the sprayed bridges. These tables show that ethylene glycol was not detected in any of the samples collected. This could be due to the small volume of compound applied to bridges combined with the high dilution of water in receiving streams or runoff water from rain or melting ice. Table 8 shows the areas of sprayed bridges and rate of application.

TABLE 6

WATER, SEDIMENT AND SOIL SAMPLES COLLECTED UNDER BRIDGES SPRAYED WITH ETHYLENE GLYCOL ON FEBRUARY 15, 1989

		Concentration of Ethylene Glycol (mg/l)		
Bridge Name	Location	Water	Sediment	Soil
Hwy. 61	Upstream	< 1.0	< 1.0	< 1.0
	Under Bridge	< 1.0	< 1.0	< 1.0
	Downstream	< 1.0	< 1.0	< 1.0
Greenwell Springs Rd.	Upstream	< 1.0	< 1.0	< 1.0
	Under Bridge	< 1.0	< 1.0	< 1.0
	Downstream	< 1.0	< 1.0	< 1.0

TABLE 7

WATER, SEDIMENT AND SOIL SAMPLES COLLECTED UNDER BRIDGES
SPRAYED WITH ETHYLENE GLYCOL ON FEBRUARY 15, 1989

		Concentration of Ethylene Glycol (mg/1)		
Bridge Name	Location	Water	Sediment	Soil
Hwy. 61	Upstream	< 1.0	< 1.0	< 1.0
	Under Bridge	—	—< 1.0	
	Down Stream	< 1.0	< 1.0	< 1.0
Joor Rd. Bridge	Upstream	< 1.0	< 1.0	< 1.0
	Under Bridge	—	—< 1.0	
	Downstream	< 1.0	< 1.0	< 1.0
Greenwell Springs Rd.	Upstream	< 1.0	< 1.0	< 1.0
	Under Bridge	—	—	< 1.0
	Downstream	< 1.0	< 1.0	< 1.0
Joor Rd. Bridge	Under Bridge	< 1.0	< 1.0	< 1.0
Control				

TABLE 8

BRIDGE DIMENSION AND SPRAYING TIME AND RATE

Bridge Name	Bridge Surface (feet)	Application Rate (gal/min)	Total Spraying Time (sec)
Hwy. 61	165 X 24	20	34
Joor Rd.	360 X 26	20	52
Greenwell Springs Rd.	300 X 28	20	52

ACUTE TOXICITY OF ETHYLENE GLYCOL TO CRAWFISH, BLUEGILL SUNFISH AND SOIL MICROORGANISMS

Results of the acute toxicity of ethylene glycol to crawfish are found in Table 9 and Figure 2. The 96-hr. LC_{50} was 91,430 mg/l. The literature cites a 48-hr. and 96-hr. LC_{50} for EG to common shrimp of 100,000 mg/l and 50,000 mg/l, respectively (Blackman, 1974). The 48-hr. LC_{50} for Daphnia magna is 41,000 mg/l (Gersich, 1986). The crawfish seem to be more resistant to ethylene glycol than species cited in the literature. This high resistance could be related to the difference in the age and species of the tested organisms.

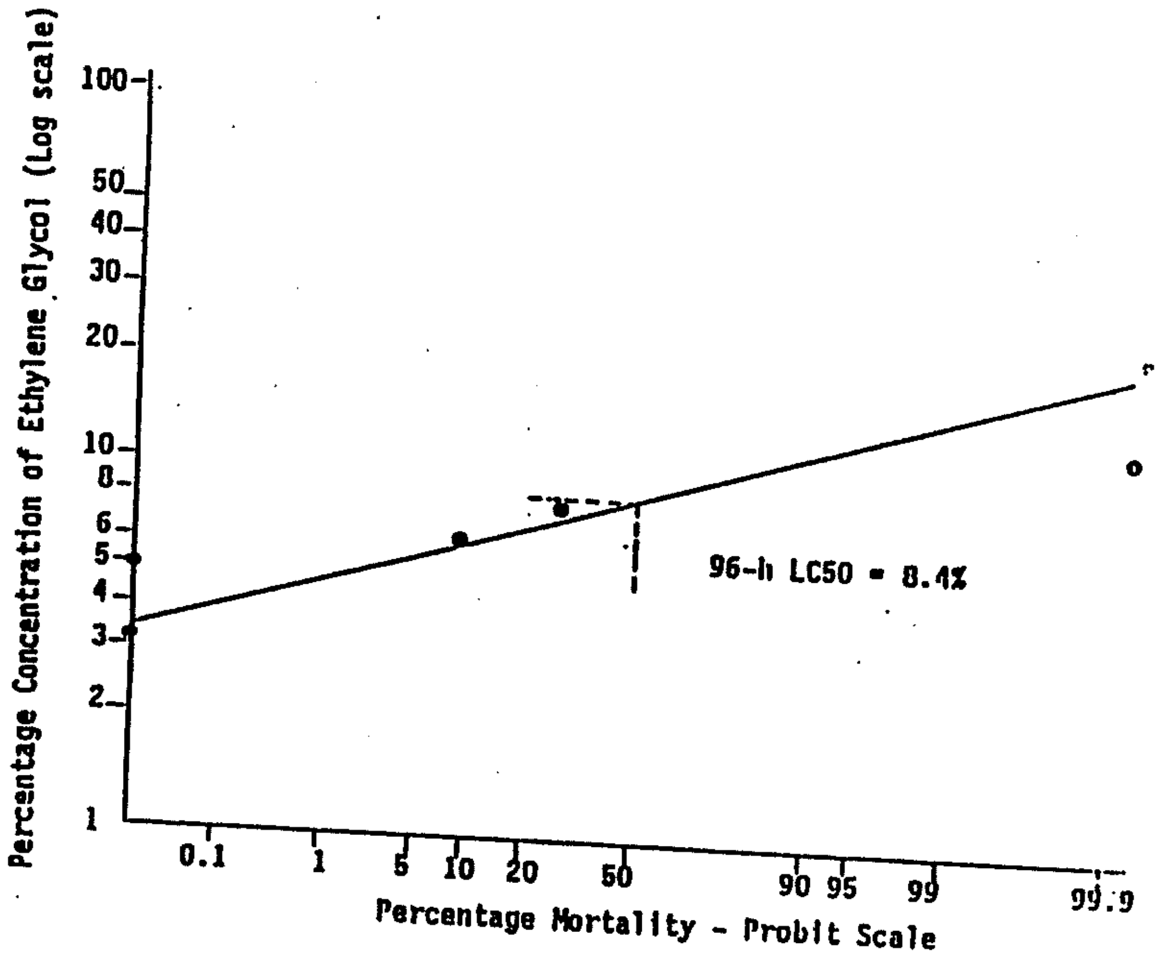


Figure 2. The medial lethal concentration of ethylene glycol to crawfish by probit analysis and line of best fit (EPA probit analysis program Version 1.3)

TABLE 9

EXPERIMENTAL DATA FROM ACUTE TOXICITY TEST OF ETHYLENE GLYCOL (EG) ON CRAWFISH (PROCAMCARUS SP.)

Concentration of EG % by Volume	Number of Test Crawfish	Number of Test Crawfish Death of EG	
		48 hr.	96 hr.
12.6	20	6	20
7.9	20	2	7
6.3	20	1	2
5.0	20	0	1
3.2	20	0	0
0.0	20	0	0
LC_{50}, %, estimated by probit analysis		16.3	8.2
95% confidence limits		—	7.5
		—	9.2
Slope of probit line		—	10.1

The acute toxicity of ethylene glycol to bluegill sunfish is shown in Table 10 and Figure 3. The 96-hr. LC_{50} for bluegills was 27,540 mg/l. The literature cites a 96-hr. LC_{50} of 28,000 mg/l for guppies (Koneman, 1981). The 24-hr., 48-hr., and 96-hr. LC_{50} for fathead minnows were all greater than 10,000 mg/l (Conway et al., 1983). These values compare well with this study.

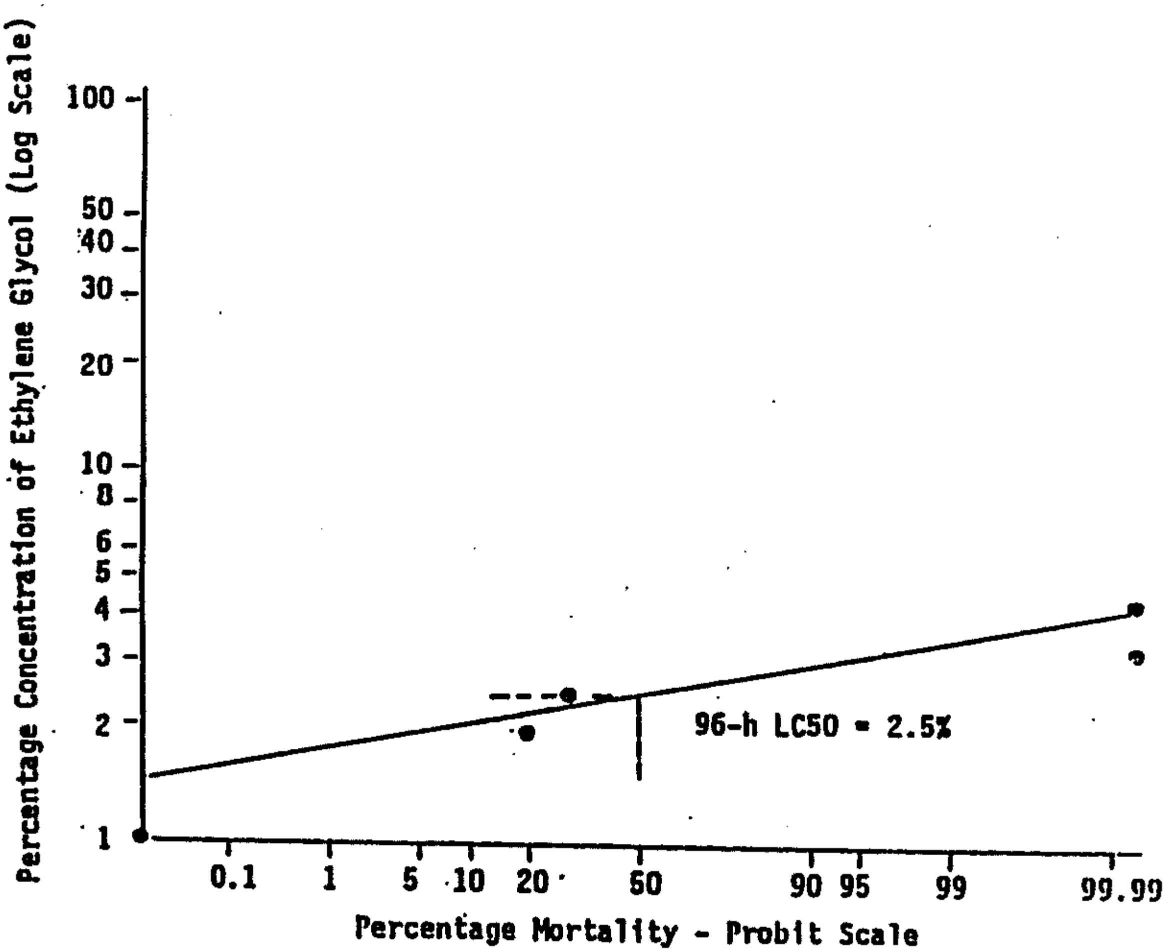


Figure 3. The median lethal concentration of ethylene glycol to blue gills (*Lepomis macrochirus*) by probit analysis and line of best fit

TABLE 10

EXPERIMENTAL DATA FROM ACUTE TOXICITY TEST OF ETHYLENE GLYCOL (EG) TO BLUEGILL SUNFISH (<u>LEPOMIS MACROCHIRUS</u>)

Concentration of EG % by Volume	No. of Test Bluegills	Number of Test Bluegills Dead at	
		48 hr.	96 hr.
4.5	20	10	20
3.5	20	6	17
2.5	20	3	9
2.0	20	2	6
1.0	20	0	0
0.0	20	0	0
LC_{50}, %, estimated by probit analysis		4.57	2.47
95% confidence limits		3.7	2.2
		7.6	2.7
Slope of probit line		3.9 7.6	

The Bauer Assay was used for measuring the toxicity of ethylene glycol to a mixed population of heterotrophic bacteria derived from soil, sediment and activated sludge. Table 11 and Figure 4 indicate that 10%-20% is moderately toxic to the culture based on an activity quotient (AQ) of 0.50 - 0.70. At 30% and above, the toxicity of ethylene glycol is extreme (AQ = < 0.5).

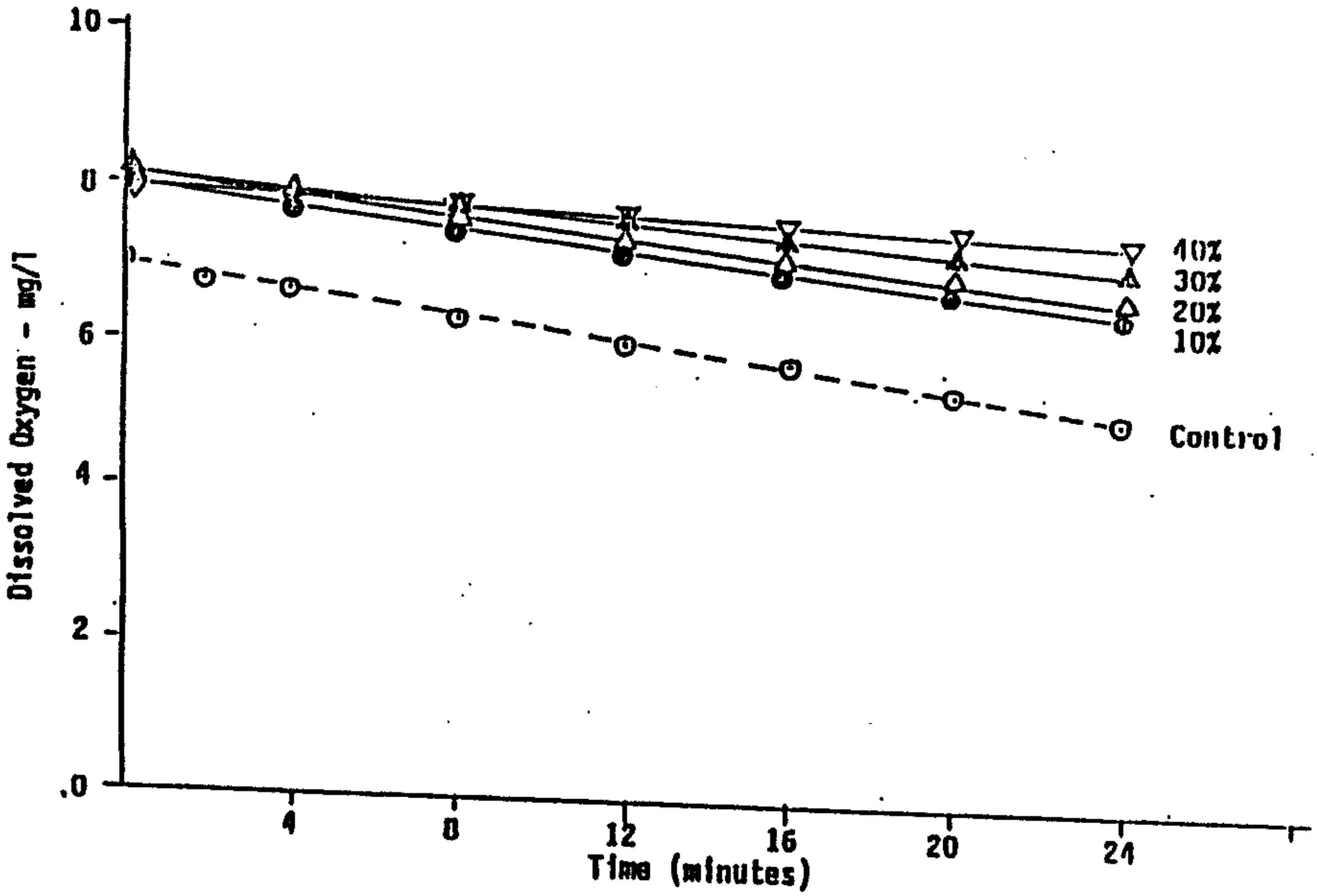


Figure 4. Toxicity of ethylene glycol to microorganisms based on the Bauer Assay

TABLE 11

BAUER ASSAY: T_{50} AND ACTIVITY QUOTIENTS FOR MICROBES EXPOSED TO ETHYLENE GLYCOL

	% Ethylene Glycol (vol./vol.)				
	0	10	20	30	40
T_{50}*	44.50	64.50	68.90	96.50	--
AQ**	1.00	0.69	0.65	0.46	≈0.0

*	T_{50}	-	Time (min.) required for 50% depletion in dissolved oxygen.
**	AQ	-	T-50 control/T-50 test
	AQ 1.00	-	no toxicity
	AQ 0.8-0.94	-	slightly toxic
	AQ 0.5-0.79	-	moderately toxic
	AQ < 0.50	-	extremely toxic

The toxicity test done on microorganisms by the Alsop Assay measured the inhibition concentration (IC_{50}) that caused a 50% reduction in optical density (Figure 5). Table 12 shows the turbidity (optical density) data as compared to the control for different concentrations of ethylene glycol. The IC_{50} was found at 10.25% or 114,300 mg/l. This toxicity level to the microorganisms agrees with that found in the literature. The IC_{50} for activated sludge microorganisms was >10,000 mg/l (Conway et al., 1983). The toxicity threshold for bacteria (Pseudomonas putida) was greater than 100,000 mg/l (Verschueren, 1983).

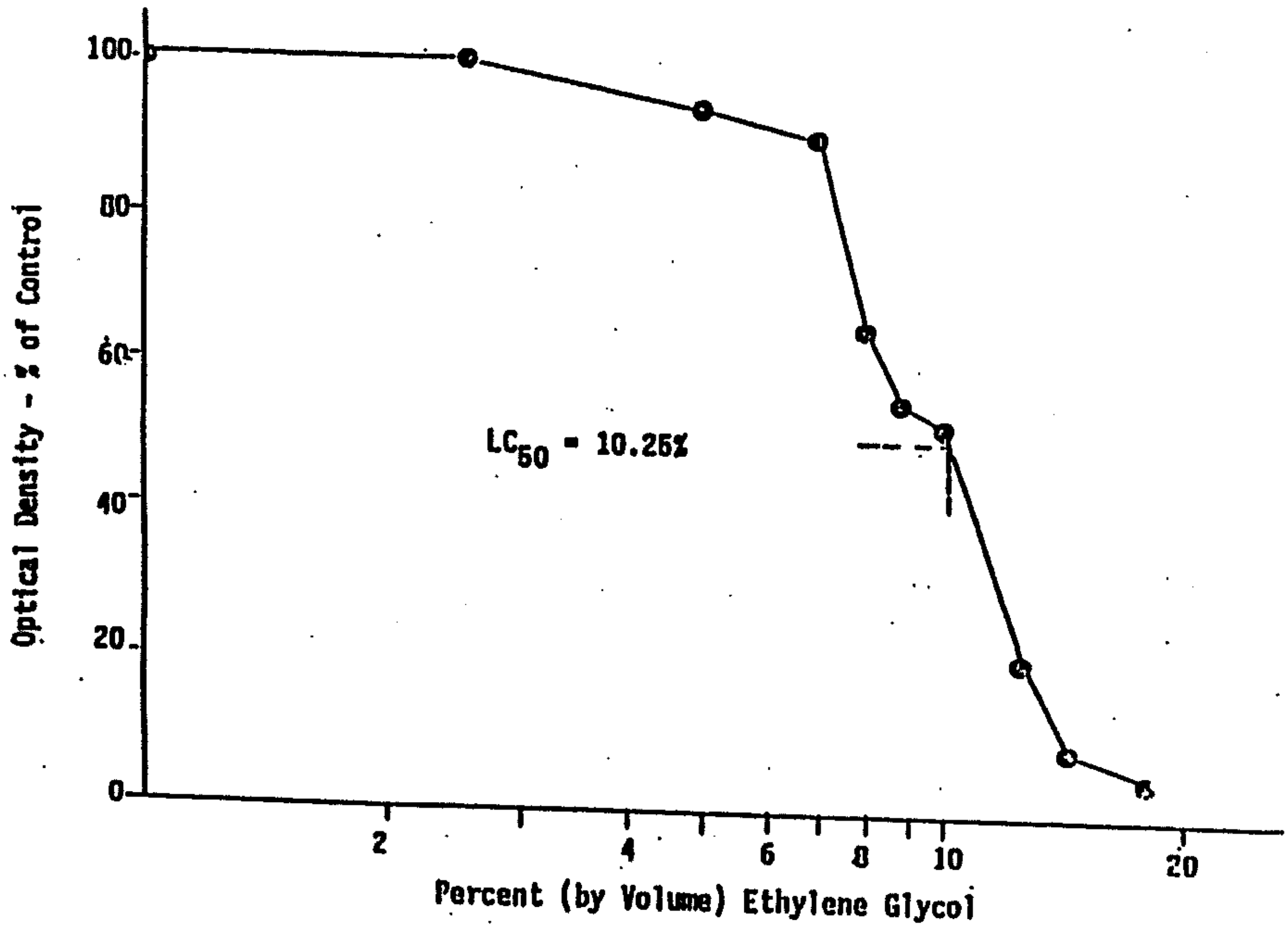


Figure 5. Toxicity of ethylene glycol to microorganisms based on the Alsop Assay

TABLE 12

TOXICITY OF ETHYLENE GLYCOL (EG) TO SOIL MICROORGANISMS AS MEASURED BY TURBIDITY (OPTICAL DENSITY)

% EG (V/V)	0.0	2.5	5.0	7.0	8.0	9.0	10.0	12.0	14.0	16.0	18.0
OD	0.8	0.8	0.75	0.73	0.52	0.44	0.42	0.16	0.08	0.06	0.04
* % of Control	100.0	100.0	93.8	90.6	65.0	54.4	51.9	20.0	9.4	7.5	5.3

* % of Control = $\frac{\text{Optical density (OD) of test material}}{\text{OD of control}} \times 100$

It can be concluded that the concentration of ethylene glycol used as a deicing agent on bridges, combined with the high volume of water in receiving streams, will dilute ethylene glycol sufficiently to pose no danger to crawfish or to bluegill sunfish. Ethylene glycol at normal application rates (50 µl of 50% ethylene glycol per square inch) will not exert a toxic effect on bacterial flora. Bacteria in water will degrade the chemical almost completely within 3-4 days (Evans et al., 1974).

BIOACCUMULATION OF ETHYLENE GLYCOL (EG) BY CRAWFISH ORGANS

Figures 6 through 9 for System I (50 µg/ml EG), System II (200 µg/ml EG) and System III (100 µg/ml EG) are the uptake data for ethylene glycol (µg EG/g tissues) in crawfish gills, muscles, gastrointestinal tract (G.I.) and hepatopancreas (Appendix A, A-11 through A-13). Each data point represents the mean of three measurements taken from three crawfish exposed to the same ethylene glycol concentration during the uptake phase for Systems I, II and III

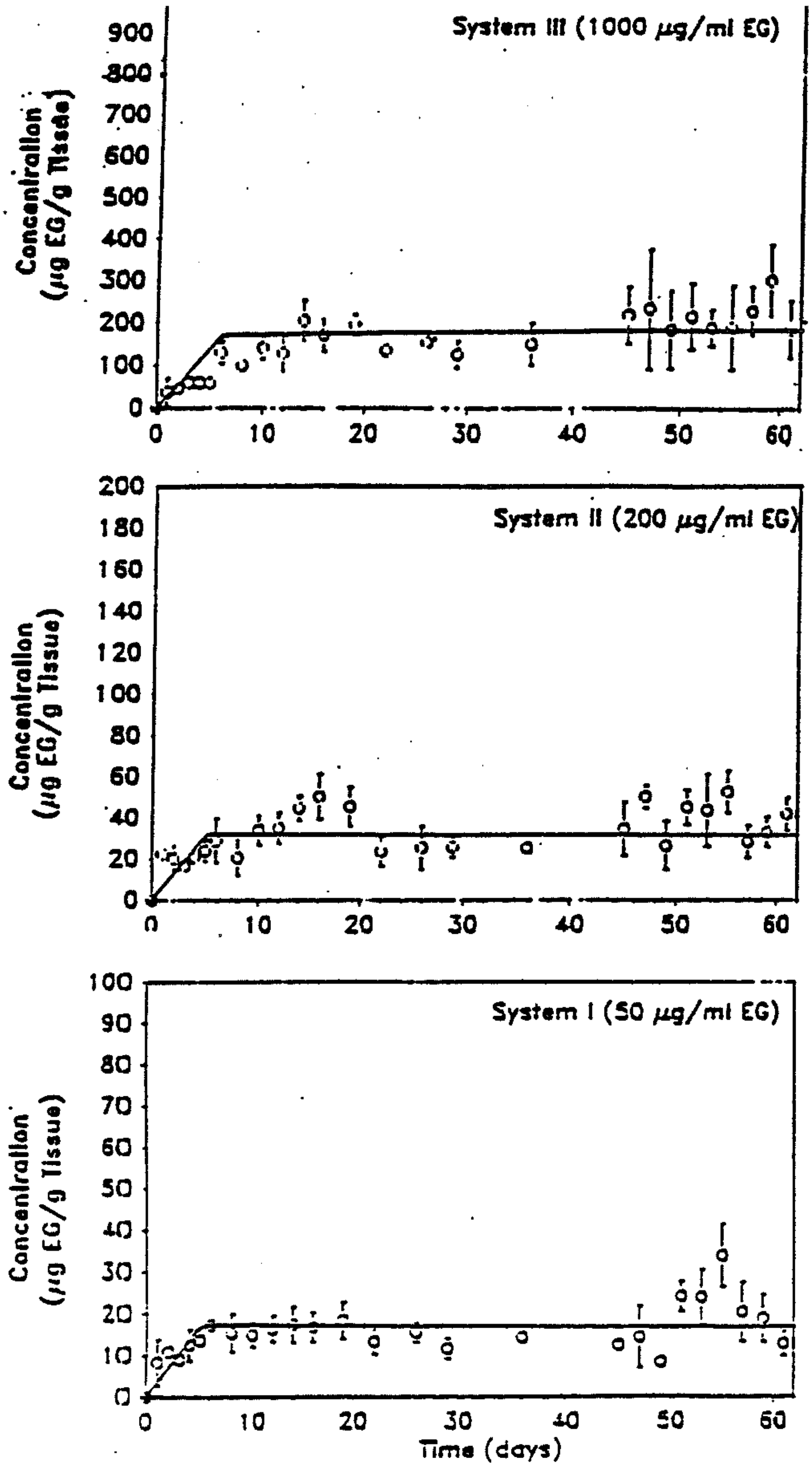


Figure 6. Average bioconcentration of EG by gills during continuous exposure for 61 days to 50, 200 & 1000 µg/ml EG

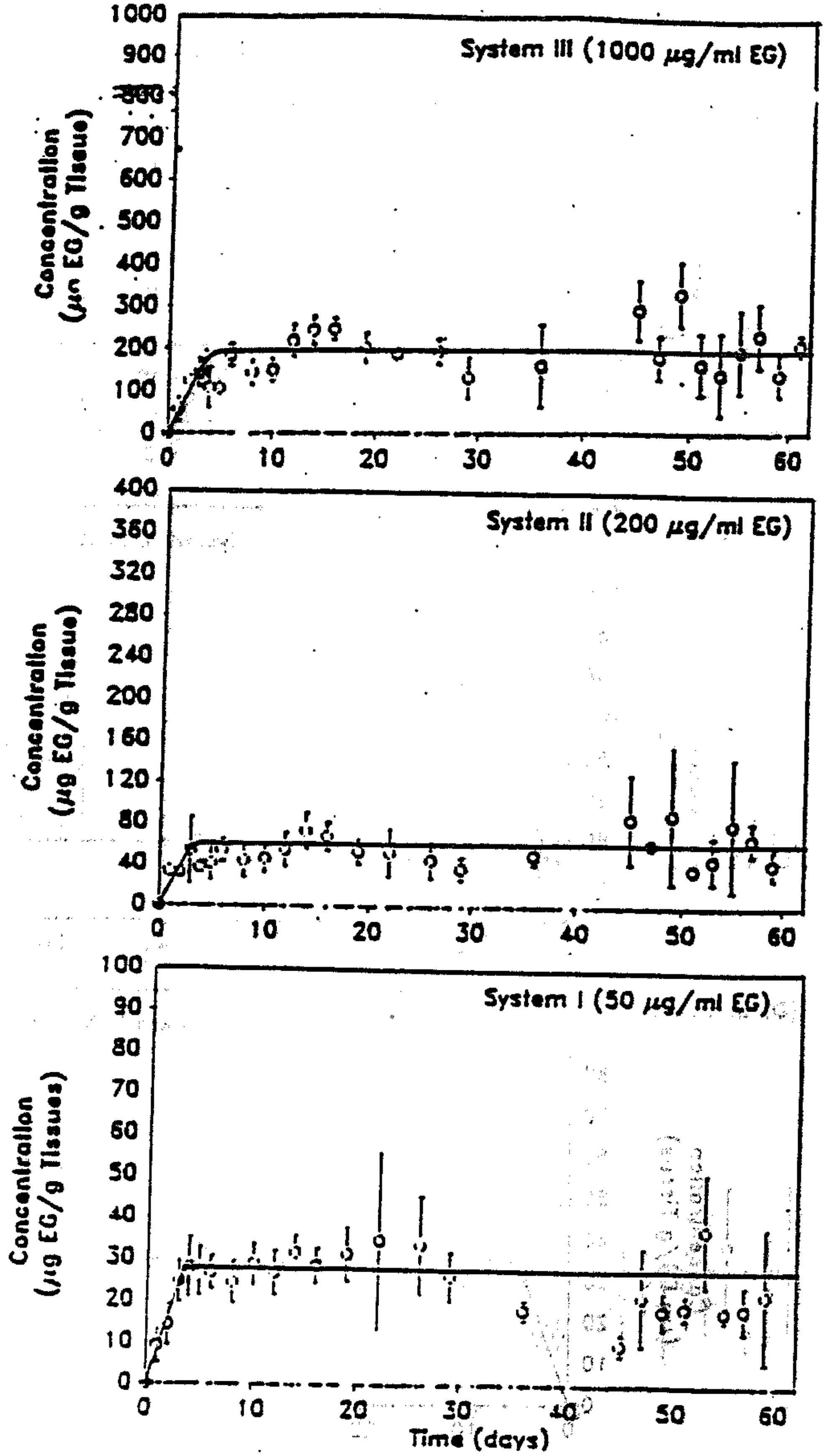


Figure 7. Average bioconcentration of EG by muscles during continuous exposure for 61 days to 50, 200 & 1000 μ g/ml EG

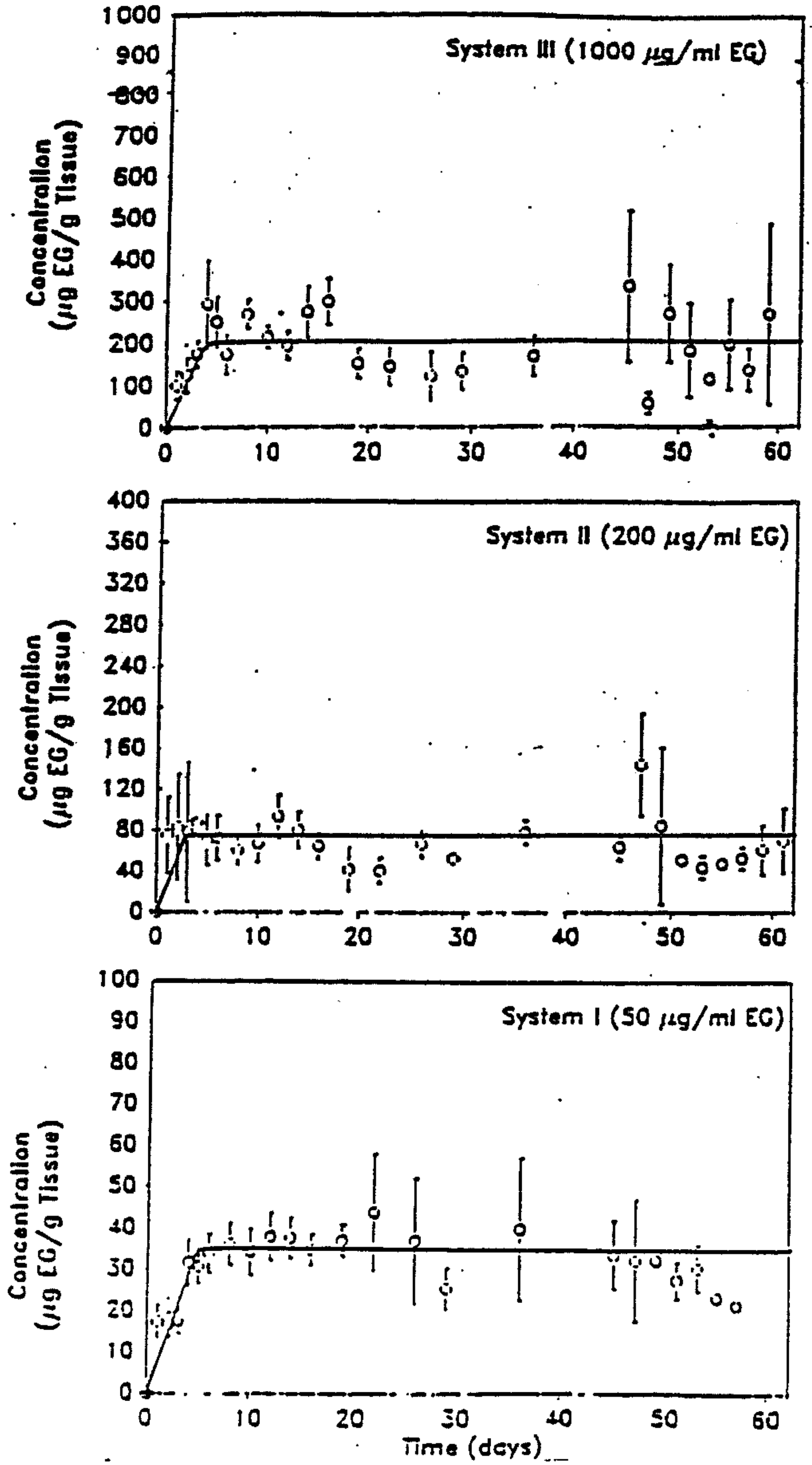


Figure 8. Average bioconcentration of EG by G.I. during continuous exposure for 61 days to 50, 200 & 1000 µ g/ml EG

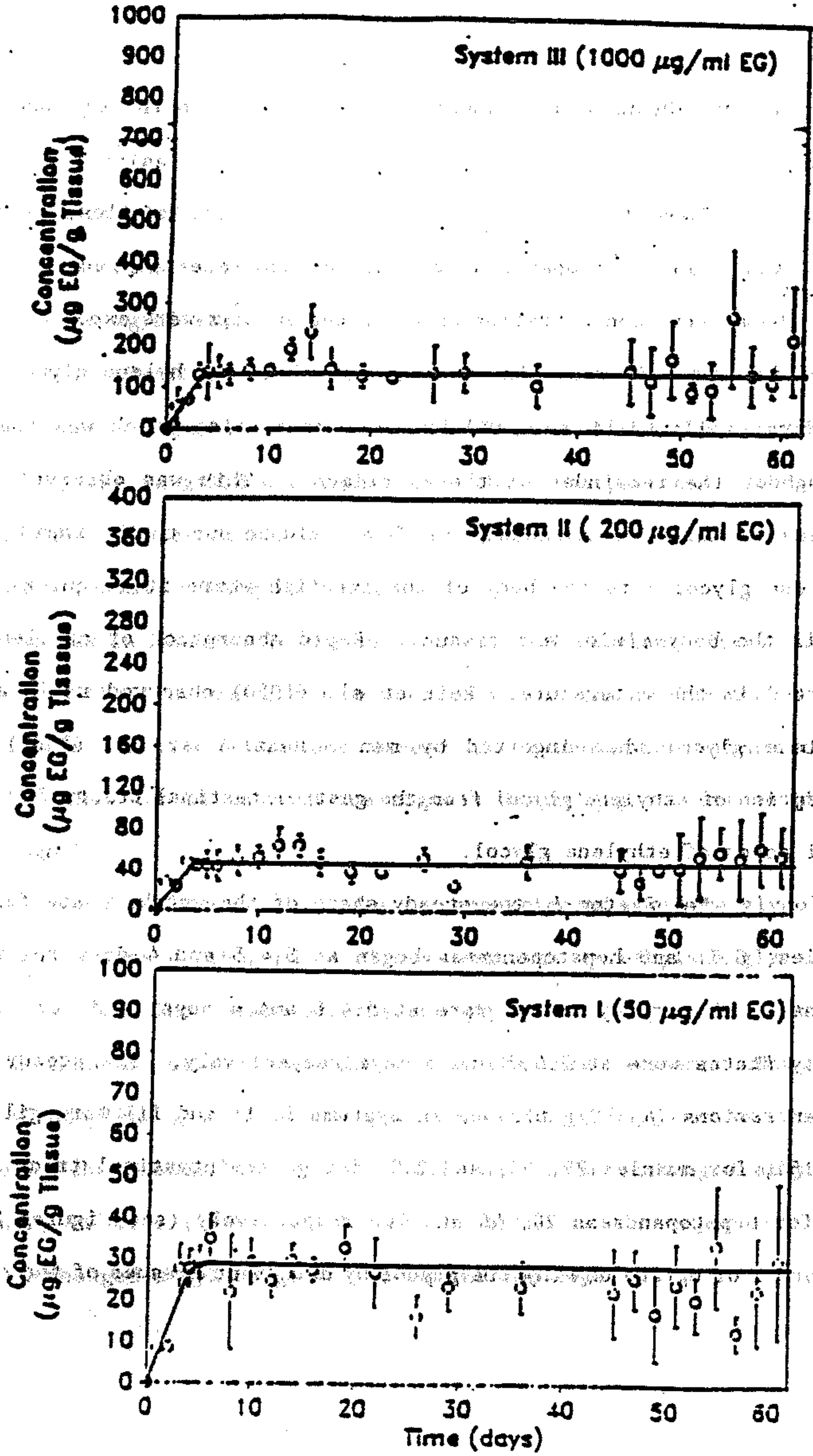


Figure 9. Average bioconcentration of EG by hepta. during continuous exposure for 61 days to 50, 200 & 100 μ g/ml EG

respectively. Means plus standard deviation error bars were used as plotting points.

These figures show that crawfish gills, muscles, gastrointestinal tract (G.I.) and hepatopancreas did not concentrate ethylene glycol to levels above the aqueous concentration to which the animals were exposed. The data show an initial rapid increase in the concentration of ethylene glycol in the first few days, followed by an equilibrium concentration which was then maintained throughout the remainder of the experiment. This was observed in all three systems and in all tissues tested. This could be due to the rapid absorption of ethylene glycol into the body of the crawfish where it is quickly distributed in all the body fluids and tissues. Rapid absorption of ethylene glycol was reported in the literature. Reif et al. (1950) observed rapid absorption of ethylene glycol when ingested by man. Hanzlik et al. (1939) found rapid absorption of ethylene glycol from the gastrointestinal tract of dogs when given small doses of ethylene glycol.

For System I, the steady state of the uptake phase for the gills, muscles, G.I. and hepatopancreas began at 5,4,5 and 4 days respectively; for System II the steady states were at 5,4,6 and 4 days; and for System III the steady states were at 7,5,7 and 5 days respectively. The steady state uptake concentrations (μg EG/g tissue) in Systems I, II and III for gills were 19,36 and 154, for muscles 29, 53, and 210, for gastrointestinal tract 37,70 and 215 and for hepatopancreas 28, 56 and 140 respectively (see Figures 2 through 5). The order of uptake of ethylene glycol by different tissues of the crawfish were:

System I (50 μg/ml EG):

G.I. > Muscles ≈ Hepatopancreas > Gills

System II (200 μg/ml EG):

G.I. > Muscles ≈ Hepatopancreas > Gills

System III (1000 μg/ml EG):

G.I. > Muscles > Gills > Hepatopancreas

The order of uptake was the same for Systems I & II but in System III the order of uptake in gills was higher than the hepatopancreas. This may be due to the higher concentration of ethylene glycol in the medium and its relatively greater contact with gill tissue.

There was in increase in the concentration of ethylene glycol in the selected tissues as the ethylene glycol concentration in the medium increased; however, the levels in tissue did not exceed the aqueous concentration of ethylene glycol. This can be seen in the figures describing the uptake phase (Figures 6 through 9).

Table 13 shows the final uptake concentration of ethylene glycol in selected crawfish tissues at the end of 61 days exposure to ethylene glycol in Systems I, II, and III. The uptake of ethylene glycol by the gastrointestinal tract was higher than the other organs in each of the three systems. The high concentration of ethylene glycol in the gastrointestinal tract may be related to the G.I. being the predominant route of elimination of unchanged ethylene glycol. Gessner, et al., (1961) found that the urine is the major route of elimination of unchanged ethylene glycol in humans and dogs.

TABLE 13

CONCENTRATION OF ETHYLENE GLYCOL (μg/g) IN SELECTED CRAWFISH TISSUES AT THE END OF A 61 DAY EXPOSURE FOR THE SYSTEMS

Tissue	System I μg/g	System II μg/g	System III μg/g
Gills	19.0	36.0	194.0
Abdominal muscles	22.0	42.0	210.0
Gastrointestinal tract	30.5	70.0	268.0
Hepatopancreas	29.0	55.0	223.0

Uptake by the gills was lowest among the selected tissues in systems I & II and next to lowest among selected tissues system III. The gills have a large surface area and an efficient blood supply. Almost 90% of the water entering a crawfish does so through the gills surfaces (Holdich, 1988). This quick diffusion of ethylene glycol through the gills could explain the low uptake of ethylene glycol by the gills.

LOSS PHASE

Figures 10 through 13 represent the loss or depuration phase in System I, II and III. Each data point (Appendix A, A-14 through A-16) was drawn by plotting the average of three measurements taken from three crawfish during the loss phase for Systems I, II and III respectively. Means plus standard deviation error bars were used as plotting points. These figures represent the depuration of ethylene glycol from crawfish tissues after crawfish were transferred to clean water following ethylene glycol exposure for 61 days.

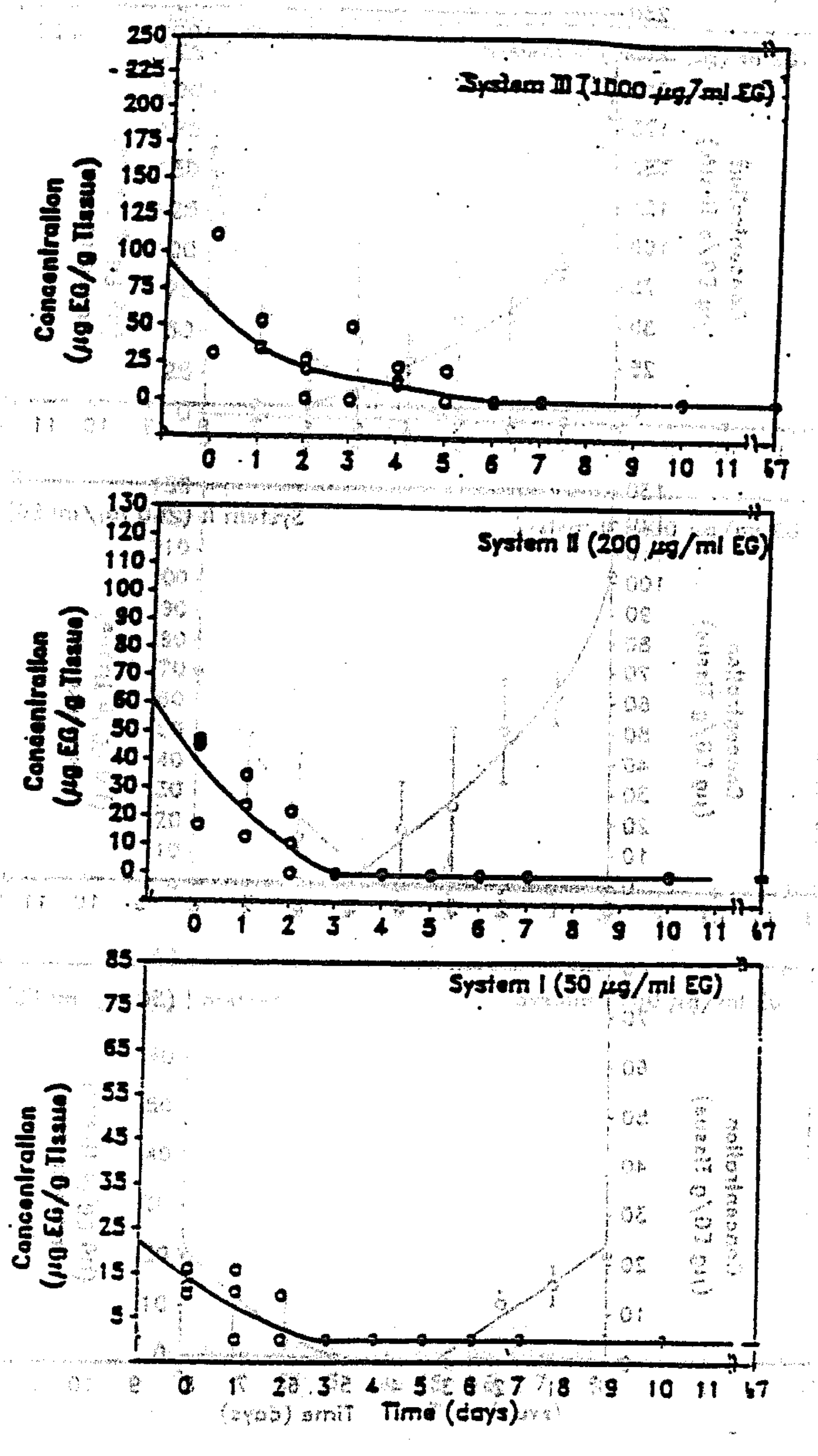


Figure 10. Depuration of EG by gills during loss phase for 67 days in Systems I, II & III

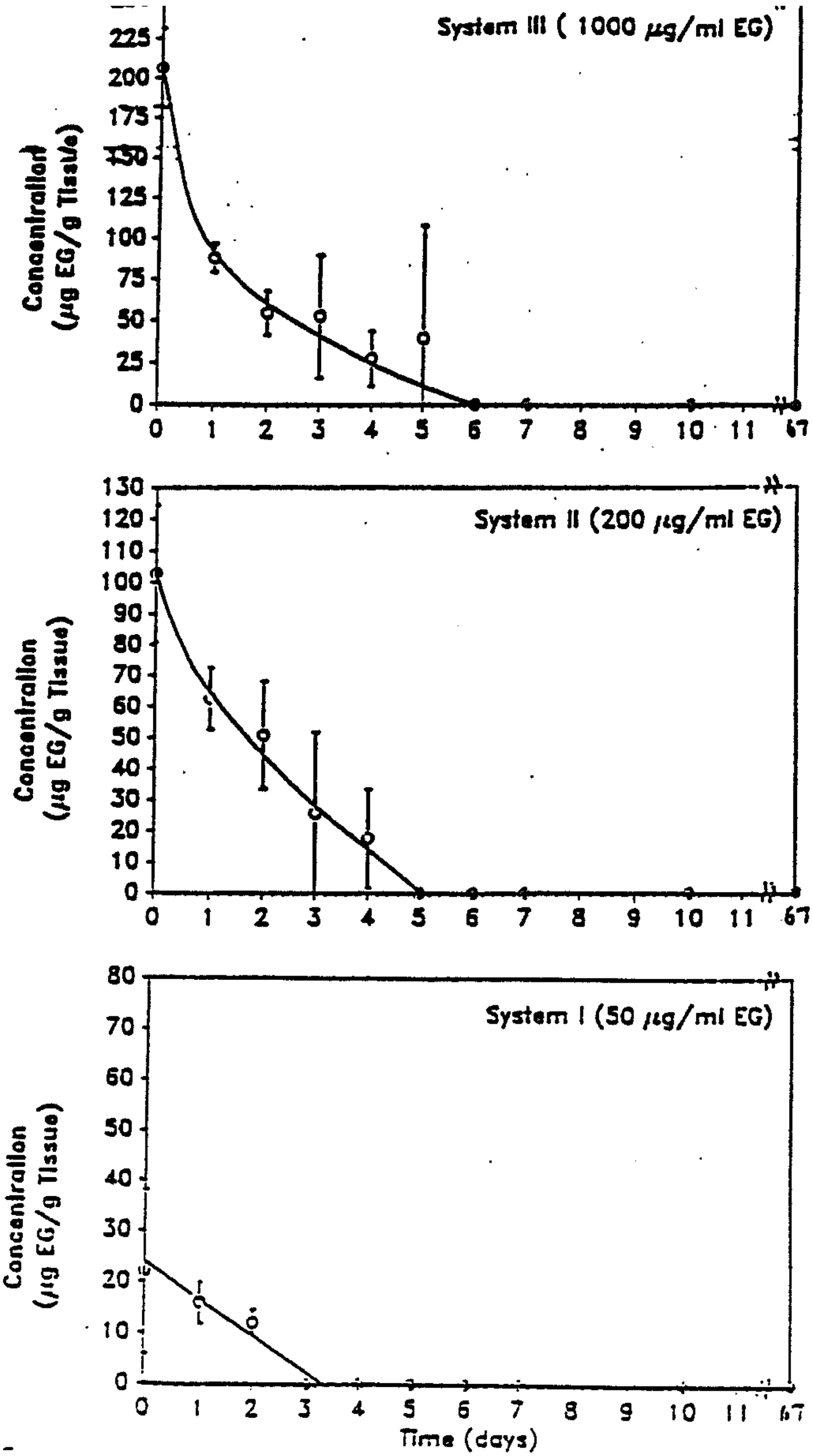


Figure 11. Average depuration of EG by muscles during loss phases for 67 days in Systems I, II & III

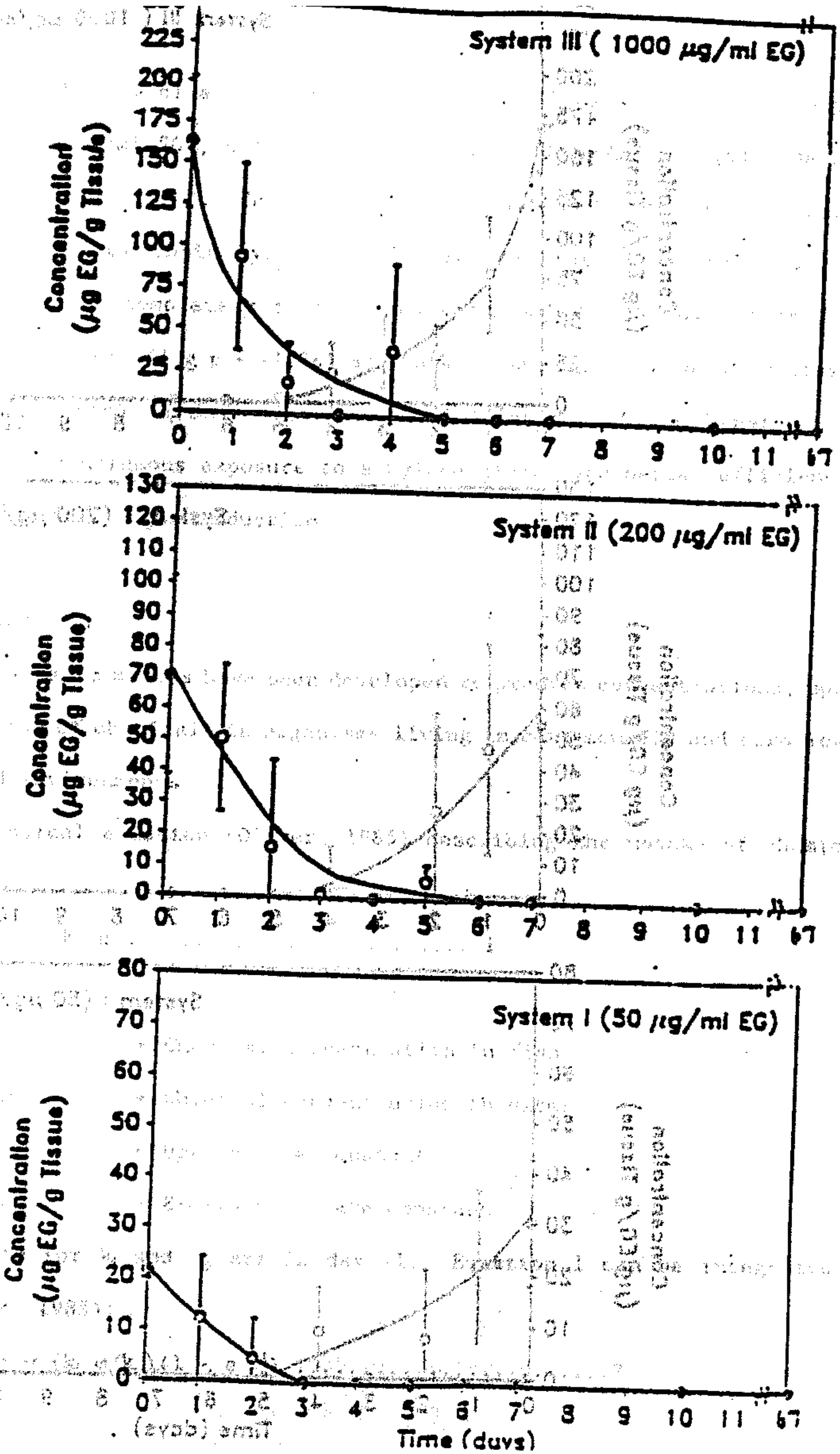


Figure 12. Average depuration of EG by G.I. during loss phase for 67 days in System i, II & III.

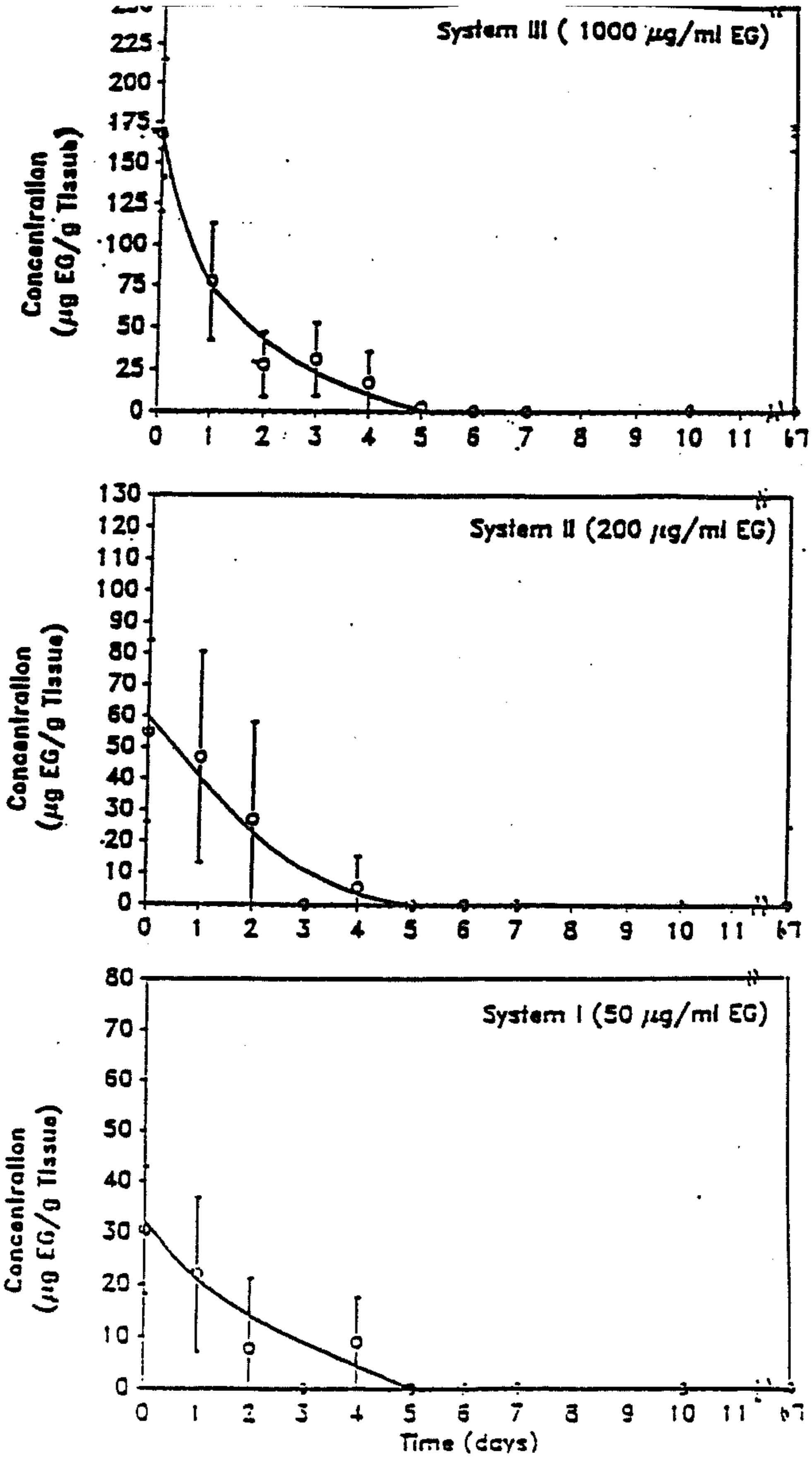


Figure 13. Average depuration of EG by hepta. during loss phase for 67 days in Systems I, II & III

Crawfish were able to clear ethylene glycol within 5 days for animals exposed in System I (50 μg/ml EG), 6 days for System II (200 μg/ml EG) and 7 days for System III (1000 μg/ml EG). The capability of crawfish to completely eliminate ethylene glycol from their system may be due to ethylene glycol's physical properties. It is completely miscible in water and has a low octanol/water partition coefficient (log p = -1.36) and thus is not strongly bound to tissues. Clearance could also be due to an increased tolerance of the hepatopancreas because of the continuous exposure to ethylene glycol and better efficiency in detoxification via enzyme induction.

MATHEMATICAL MODEL

Kinetic models have been developed to predict concentrations, uptake and elimination of chemicals in organisms living in both acutely and chronically contaminated environments.

The classical equation (Oliver, 1985) describing the uptake of chemicals by fish is:

$$dm/dt = k_i C - k_o m \qquad \text{......................... 1}$$

Where:

c	= Chemical concentration in fish
m	= Chemical concentration in water
k_i	= Uptake rate constant
k_o	= Elimination rate constant

The units for k_i and k_o are in day -1. Equation 1 can be integrated to yield (Oliver, 1985):

$$c = (k_i m/k_o)(1 - e^{-k_o t}) \qquad \text{.........................2}$$

or

$$c/m = k_i/k_o (1 - e^{-k_o t}) \qquad \text{......................3}$$

Equation 2 represents an open, one-compartment model and can be used in the interpretation of chemical uptake by aquatic organisms. Knowing k_o, Equation 3 can be linearized by plotting c/m versus $(1 - e^{-k_o t})$ to yield a straight line with a slope equal to ki/ko.

Data developed in this study were found to fit the equation for the one-compartment model. This was determined by an analysis of variance (ANOVA). The results of this test are shown in Appendix C, C-1 through C-21. Figure 14 is a plot of the one- compartment model (on arithmetic paper) for accumulation of xenobiotic in the medium (Ruzik, 1972).

Figures 10 through 13 show loss of ethylene glycol by crawfish gills, muscles, gastrointestinal tract and hepatopancreas. The loss phase followed a single compartment exponential decay model.

The equation governing the loss is:

$Ct = C_i * e^{-k_o t}$ 4

Where:

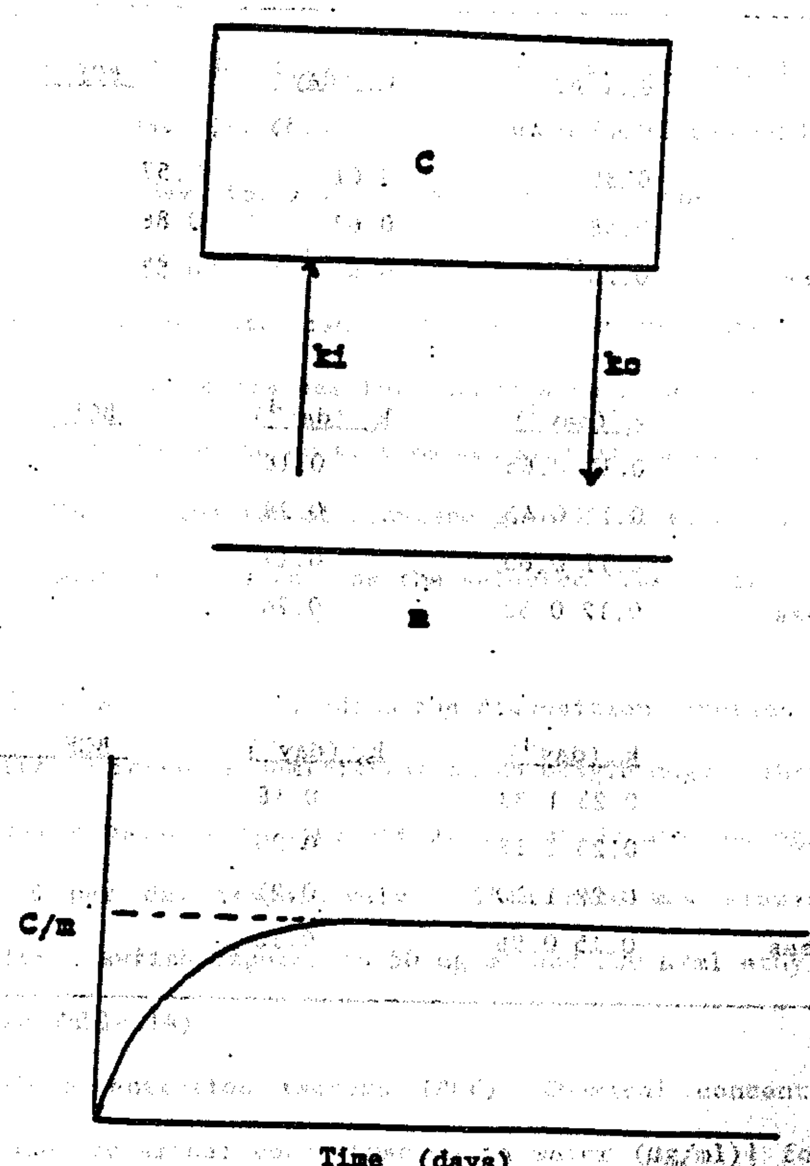
C_i = initial value.

k_o = elimination rate constant.

C_t = concentration of EG at time t.

t = time in days.

The elimination rate constants, k_o, were determined for each of the selected tissues and are represent in Table 14. Kinetic parameters (k_i, k_o) and bioconcentration factor (BCF) are presented in Table 14. The bioconcentration factor for the three systems did not exceed 1.



$$C/m = \frac{\text{Concentration of EG in tissue}}{\text{Concentration of EG in water}}$$

Figure 14. Schematic representation of the mathematical model used for data analysis (Ruzic, 1972); (Anderson, 1988).

TABLE 14

KINETIC PARAMETERS (K_i,K_o) AND
BCF OBTAINED USING MATHEMATICAL MODEL

System I			
	k_i (day^{-1})	k_o (day^{-1})	BCF
Gills	0.18 0.46	0.33	
Muscles	0.35	0.61	0.57
G.I. tract	0.58	0.67	0.86
Hepatopancreas	0.24	0.45	0.53
System II			
	k_i (day^{-1})	k_o (day^{-1})	BCF
Gills	0.12 0.65	0.18	
Muscles	0.13 0.45	0.28	
G.I.	0.11 0.65	0.17	
Hepatopancreas	0.12 0.52	0.24	
System III			
	k_i (day^{-1})	k_o (day^{-1})	BCF
Gills	0.23 1.33	0.18	
Muscles	0.23 1.18	0.20	
G.I.	0.22 1.10	0.21	
Hepatopancreas	0.15 0.96	0.16	

The uptake rate constant (k_i) for System I ranged between 0.18 day^{-1} for gills and 0.58 day^{-1} for gastrointestinal tract. The uptake rate constant for System II ranged between 0.11 day^{-1} and for System III the k_i ranged between 0.16 day^{-1} for hepatopancreas and 0.24 day^{-1} for muscles.

The uptake rate constants (k_i) for the selected tissues in Systems I, II and III were tested statistically using one-way Analysis of Variance. The variation in the uptake with a p value = 0.30. Also the variation in the uptake rate constants within each selected tissue for Systems I, II and III was not significant (p value = 0.27).

The elimination rate constants (k_0) for System I ranged between 0.46 day $^{-1}$ for gills and 0.67 day^{-1} for G.I. The k_0 for System II ranged between 0.45 day^{-1} for muscles and 0.65 day^{-1} for gills. For System III, the k_0 ranged between 0.97 day^{-1} for hepatopancreas and 1.33 day^{-1} for gills.

The elimination rate constants (k_0) did not show significant differences among the selected tissues for each system p value = 0.67, but the variation within each tissue for three systems was statistically significant with a p value = 0.000. The elimination of ethylene glycol for each selected tissue in System III was almost twice as fast as the selected tissues in Systems I and II (see Table 14).

Martis, et al., (1982) studied the disposition kinetics of ethylene glycol following its intravenous administration to beagle dogs. They estimated the elimination rate constants of ethylene glycol at 35 and 106 mg/kg dosages were 5.76 and 4.18 per day respectively. This rate was slower than the elimination rate for crawfish exposed to 50 µg/ml and 200 µ/ml ethylene glycol concentrations (see Table 14).

The bioconcentration factors (BCF) [Chemical concentra-tion in crawfish (µg/g tissue)/chemical concentration in water (µg/ml)] for System I ranged between 0.33 for gills and 0.86 for G.I. In System II, the k_0 ranged between 0.17 for G.I. and 0.28 for muscles. For System III, the k_0 ranged between 1.16 for hepatopancreas and 0.21 for G.I.

The variation in the bioconcentration factors (BCF) among tissues for each system was a statistically insignificant p-value = 0.31 (Table 14). But the variation within each tissue in the three systems was a significant p value = 0.07 (Table 14). The bioconcentration factor System I was almost twice the bioconcentration factor for Systems II and III.

The biological half-life of ethylene glycol in the selected crawfish tissues was determined by:

$$T_{1/2} = 0.693/k_0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 5$$

where k_0 is the elimination rate constant.

Table 15 represents the half-life values as determined by Equation 5. The half-life value for Systems I and II were almost the same. They ranged between 1 and 1.5 days. The half-life for System III did not exceed one day for all selected tissues. This is due to the higher rate of elimination of ethylene glycol by the tissues for System III.

TABLE 15

BIOLOGICAL HALF-LIFE (DAYS) OF ETHYLENE GLYCOL IN SELECTED TISSUES OF CRAWFISH FOLLOWING EXPOSURE FOR 61 DAYS TO 50 μg/ml, 200 μg/ml AND 1000 μg/ml EG

Tissue	Concentration of exposure μg/ml EG		
	50	200	1000
Gills	1.27	1.060.52	
Muscle	1.13	1.530.58	
Gastrointestinal tract	1.03	1.060.63	
Hepatopancreas	1.53	1.320.72	

The variation in half-lives among the selected tissues for Systems I, II and III were statistically analyzed using one-way Analysis of Variance. The variation was not statistically significant (p-value = 0.28), but the variation within each tissue for the three systems was statistically significant at a p value = 0.07.

McChesney et al., (1971) found that the plasma half-life of ethylene glycol in a study done on monkeys was 2.7 to 3.7 hours. Human plasma clearance half-lives of ethylene glycol following oral intoxication range from 2 to 6 hours (Reif, 1950; Winek, 1975; Peterson, et al., 1981). These half-lives are shorter than the half-lives of ethylene glycol in the crawfish tissues.

POTENTIAL HEALTH EFFECTS ON MAN

The ultimate importance of bioaccumulation of ethylene glycol in crawfish is the possible health effects on man following consumption of contaminated crawfish. The lethal dose of ethylene glycol for an average individual weighing 70 kg is 100 ml of ethylene glycol (Gessner et al., 1961). This study found that the maximum concentrations of ethylene glycol in edible crawfish tissues were 331 µg/g for the abdominal muscle and 278 µg/g for hepatopancreas. Because the average crawfish contains approximately 2 g of hepatopancreas and 4 g of abdominal muscle, the combined amount of ethylene glycol present in edible tissue is approximately 1.8 mg EG/crawfish. To reach the acute lethal dose of 115 g of ethylene glycol one would have to consume 63,900 contaminated crawfish (or 384 kg of crawfish edible tissues) at one time. This scenario was developed considering the worst possible conditions; that is, that the crawfish are continuously in direct contact with a high concentration of ethylene glycol (1000 mg/l).

BIODEGRADATION

Table 16 shows the cumulative average $^{14}CO_2$ level generated from three concentrations of ethylene glycol and $^{14}CO_2$ values in the two controls. There was no apparent difference in biodegradation pattern at the three concentrations. However, a toxic effect could be observed with increasing concentration. Rapid degradation was observed for the first 72 hours. Table 17 shows the slope values for both the growth phase and stationary phase at all three concentrations. The slope, or the rate of degradation, in the growth phase decreases with an increase of concentration, indicating inhibitory or toxic effects of ethylene glycol on the microorganisms as depicted in Figure 15. There was no difference between the controls (dark and light), indicating no photodegradation. The figure also shows the sharp increase in CO_2 production for the first 72 hours, indicating a high rate of biodegradation of the ethylene glycol.

TABLE 16
CUMULATIVE $^{14}CO_2$ LEVELS GENERATED FROM THE BIODEGRADATION OF ETHYLENE GLYCOL TEST RUN (EG) (CUMULATIVE AVERAGE IN $\mu g/l$)

Time (hr)	Control light($\mu g/l$)	Control dark ($\mu g/l$)	1% EG ($\mu g/l$)	3% EG ($\mu g/l$)	5% EG ($\mu g/l$)
2	0.03	0.05	2.80	2.32	1.99
4	0.09	0.09	3.99	3.19	2.75
6	0.09	0.09	5.19	4.07	3.32
12	--	--	9.96	7.59	5.46
24	0.23	0.21	20.10	16.13	9.25
48	0.35	0.36	31.10	29.85	22.98
72	0.43	0.46	34.83	33.89	29.31
96	0.43	0.55	6.60	36.09	31.98
120	0.46	0.64	37.51	37.08	33.42
144	0.59	0.77	38.31	37.26	34.42
168	0.71	0.88	38.91	37.85	34.73
240	0.99	1.14	41.58	39.02	35.54
360	1.31	1.59	44.03	41.41	36.71

TABLE 17

RATE DATA FOR MICROBIAL DEGRADATION OF ETHYLENE GLYCOL (EG)

	Control Light	Control Dark	1% EG	3% EG	5% EG
Growth Phase					
$k, \mu g l^{-1} h^{-1}$	0.005	0.0059	0.500.497	0.414	
half-life, h	120	117	1.4	1.4	1.7
r^*	0.978	0.982	0.971	0.982	0.994
Stationary Phase					
$k, \mu g l^{-1} h^{-1}$	0.0033	0.0039	0.031	0.020	0.021
half-life, h	210	178	22.434.7	33.0	
r^*	0.989	0.999	0.984	0.954	0.853

* r = correlation coefficient

The microorganisms responsible for this degradation were isolated and identified as Gram negative, rod- shaped bacteria, belonging to the Pseudomonas, Serratia and Klebsiella species.

These soil and water microorganisms, commonly found in the environment, will biodegrade ethylene glycol to a significant level over 3 days. The rate of biodegradation for the first 3 days is 0.5 µg/l/h for 1% and 3% ethylene glycol. Concentrations higher than 5% tend to exert inhibitory or toxic effects.

In the results reported here (see Table 4) first-order rate constants (k) were determined by using least-squares methods to obtain the regression and correlation coefficients. The half-lives are determined from $T_{1/2} = 0.693/k$.

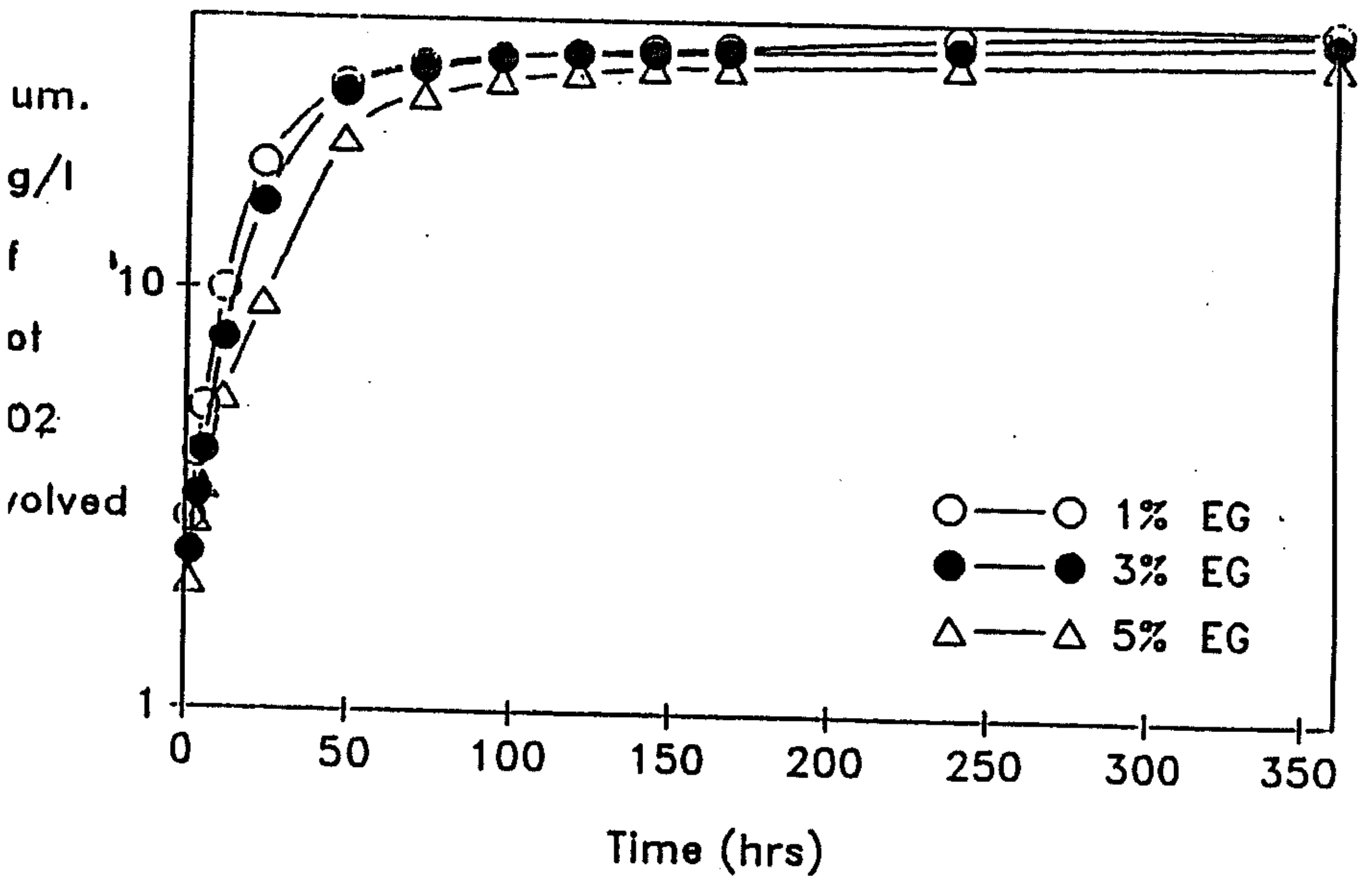


Figure 15. Cumulative average of $^{14}CO_2$ of three triplicate concentrations of ethylene glycol

SOIL SORPTION STUDIES

Table 18 shows the adsorption/desorption capacities of ethylene glycol to montmorillonite (a three dimensional clay) and to soils collected from four different locations in Louisiana. Soils were characterized by a local engineering laboratory. Two were clay, (Joor Road and Joor Road Control) while the other two (Highway 61 and Greenwell Springs Road) were sandy clay. Total organic carbon was also measured for all collected soils and is given in the table.

The percent adsorption ranged from 0-0.5% for all tested soils. Desorption was almost complete at the end of the 4-hour experiment for the soils and the montmorillonite. Due to the high water solubility and the low vapor pressure of ethylene glycol, it rapidly partitions to water in the environment.

Ethylene glycol showed insignificant adsorption to tested soils. This agrees with Verschueren (1977), who showed that only 14 µg/g of ethylene glycol adsorbed to carbon and 93% was desorbed. It can be concluded that ethylene glycol does not adsorb on tested soils. Table 18 also shows that the ethylene glycol concentration, the soil/clay content, and the total organic carbon have no effect on increasing or decreasing the adsorption/desorption capacity of ethylene glycol on tested soils.

TABLE 18

ETHYLENE GLYCOL SORPTION STUDY DATA

Soil sample location	Soil type	Toc mg/kg	Conc mg/l	Adsorption µg/g	Adsorption y.	Desorption µg/g	Desorption y.
Hwy. 61	sand/clay	1310	1	0.05	0.50	0.05	100
			5	0.06	0.12	0.05	100
			10	0.03	0.03	0.03	100
			50	0.03	0.01	0.03	100
			100	0.03	0.01	0.03	100
Greenwell Springs Rd.	sand/clay	1440	1	0.02	0.20	0.02	100
			5	0.02	0.04	0.02	100
			10	0.02	0.02	0.02	100
			50	0.03	0.01	0.02	67
			100	0.03	0.00	0.00	--
Joor Road	clay	1600	1	0.01	0.10	0.01	100
			5	0.01	0.20	0.01	100
			10	0.03	0.03	0.01	33
			50	0.03	0.01	0.02	67
			100	0.03	0.003	0.013	43
Monmorillinite	clay		1	0.05	0.5	0.05	100
			5	0.06	0.12	0.05	83
			10	0.03	0.03	0.05	100
			50	0.03	0.01	0.03	100
			100	0.03	0.01	0.03	100
Joor Road Control	clay	3240	1	0.01	0.1	0.01	100
			5	0.01	0.02	0.01	100
			10	0.03	0.03	0.01	33
			50	0.03	0.006	0.02	67
			100	0.03	0.003	0.02	67

CONCLUSIONS

1) The highest concentrations of ethylene glycol aerosol (2.33 mg/m^3) and vapor (3.37 mg/m^3) found in the breathing zone of workers in this study were below the ACGIH recommended levels of 10 mg/m^3 and 125 mg/m^3, respectively. However, care should be taken while spraying due to aerosolization of ethylene glycol caused by the spraying rig.

2) Ethylene glycol was not detected in water, soil and sediment samples taken from areas under treated bridges.

3) The 96-hour LC_{50} of ethylene glycol for crawfish and bluegill sunfish were 91,430 mg/l and 27,540 mg/l, respectively. The average toxic end point (IC_{50}) for a mixed population of soil microorganisms was 114,300 mg/l. The concentration of ethylene glycol used as a deicing agent on bridges combined with rain, melting ice and water in the receiving stream will dilute ethylene glycol sufficiently to pose no danger to crawfish or to bluegills. Ethylene glycol at normal DOTD application rates will not exert a toxic effect on bacterial flora.

4) Bioaccumulation by crawfish tissue was found to be dependent upon the concentration of ethylene glycol in the water. Ethylene glycol levels in crawfish tissues in all cases did not exceed the EG water concentration to which crawfish were exposed.

The crawfish were capable of completely depurating ethylene glycol from their system within a maximum period of 6 days at the highest concentration (0.1%) used in this study. The half-lives of ethylene glycol in the selected tissues ranged between 0.52-1.53 a day.

Crawfish bioconcentrated minimal amounts of ethylene glycol following exposure to 50 μg/ml, 200 μg/ml and 1000 μg/ml ethylene glycol for 61 days. The maximum levels of EG found in crawfish edible tissue (abdominal muscle < 210 μ/g, hepatopancreas < 140 μ/g) do not pose an acute health effect to humans. One would have to consume 384 kg of ethylene glycol contaminated edible crawfish tissues at one time to result in acute toxicity.

5) Common soil or water microorganisms found in the environment will biodegrade ethylene glycol significantly in the first 3 days of exposure. The rate of biodegradation for the first 3 days is 0.5 μg/l/h for 1% and 3% ethylene glycol concentrations. However, concentrations of ethylene glycol higher than 5% will begin to exert inhibitory or toxic effects. The microorganisms responsible for this degradation were isolated and identified as Gram negative, rod-shaped bacteria, belonging to the Pseudomonas, Serratia, and Citrobacter species.

6) Due to the high solubility of ethylene glycol in water, adsorption to tested soils (Louisiana clay, clay/sand and laboratory clay/soil) was negligible.

RECOMMENDATIONS

1) In this study, ethylene glycol was detected in the air inside the spraying truck at low levels. Although the concentration was much below the ACGIH recommended level, precautions should be taken. All applicators should stay inside the cab and windows should be kept closed.

2) Although there are few reports of adverse effects from direct contact with the skin, care should be taken to protect the hands by wearing gloves during handling of concentrated ethylene glycol.

3) It is advisable to stand upwind of the prevailing wind direction when mixing ethylene glycol to avoid aerosol inhalation. Spraying rigs could be modified (if possible) so the nozzles are at the back of the truck.

4) Results of ethylene glycol testing on crawfish and bluegills showed low acute toxicity; however, this does not preclude toxicity to other aquatic species. Therefore, spills and direct application of ethylene glycol to water should be avoided.

5) Acute studies on juvenile crawfish and other aquatic species could be done to determine potential acute effects to more sensitive stages of the organisms.

REFERENCES

Alsop, G.M., Waggy, G.T. and & Conway, R.A. 1980. Bacterial Growth Inhibition Test. Journal of Water Pollution Control Federation 52 (10), 2452-2456.

Bauer, N.J., Seidler, R.F. and Knittel, M.D. 1981. "A Simple Rapid Bioassay for Detecting Effects of Pollutants on Bacteria." Bulletin of Environmental Contamination and Toxicology 27, 577-582.

Bachmann, E. and Goldberg, L. 1971. "Reappraisal of the Toxicology of Ethylene Glycol. III Mitochondrial Effects." Food and Cosmetics Toxicology, 9:39-55.

Beasley, V.R. and Buck, W.B. 1980. "Acute Ethylene Glycol Toxicosis: A Review." Veterinary and Human Toxicology, 22(4):255-263.

Bedard, R.G. 1976. "Biodegradability of Organic Components." NTIS PB- 264-707, pp 84.

Berman, L.B., Schreiner, G.E. and Feys, J. 1957. "The Nephrotic Toxic Lesion of Ethylene Glycol." Ann Int Med 46:611-619.

Blackman, R.A. 1974. "Toxicity of Oil Sinking Agents." Mar. Poll. Bull, 5:116-118.

Bringmann, G. and Kuhn, R. 1980. "Comparison of the Toxicity Thresholds of Water Pollutants to Bacteria, Algae, and Protozoa in the Cell Multiplication Inhibition Test." Water Research, 14:231-241.

Clay, K.L. and Murphy, R.C. 1977. "On the Metabolic Acidosis of Ethylene Glycol Intoxication." Toxicol. Appl. Pharmacol, 39:39-49.

Conway, R.A., Waggy, G. T., Spiegel, M. H. and Berglund, R.L. 1983. "Environmental Fate and Effects of Ethylene Oxide." Environmental Sciences and Technology 17(2), 107-112.

Dawson, T.A. 1976. "Ethylene Glycol Sensitivity." Contact Dermatitis, 2(4):233.

Dubeikovska, L.S., Asanove, T.P., Rozina, G.Y., Budanova, L.F., Zenkevich, E.S., Revnova, N.V. and Gorn, L.E. 1973. "Hygienic Significance of Ethylene Glycol in the Manufacture of Some Radio Components." Gig. Truda i. Prof. Zabol., 10:1-4.

Evans, W.H. and David, E.J. 1974. "Biodegradation of Mono-, Di-, and Triethylene Glycols in River Waters Under Controlled Laboratory Conditions." Water Res., 8(2):97-100.

Gersich, F.M. 1986. "The Precision of Daphnid (Daphnid magna straus-, 1820) Static Acute Toxicity Tests." Arch. Environ. Contam. Toxicol. 15, 741.

Gessner, P.K., Parke, D.V. and Williams, R.T. 1961. "Studies in Detoxication 86 the Metabolism of ^{14}C Labelled Ethylene Glycol." Biochem. J., 79:482-489.

Goodman, L. and Gilman, A. 1980. The Pharmacological Basis of Therapeutics. Macmillan Publishing Co. N. Y., 6th ed., p. 1646.

Grant, W. 1974. Toxicology of the Eye: Drugs, Chemicals, Plants, Venoms. Springfield IL, Thomas Co., 2nd ed., p. 476.

Hanzlik, P.J., Seidenfeld, M.A. and Johnson, C.C. 1931. "General Properties, Irritant and Toxic Actions of Ethylene Glycol." J. Pharmacol. Exptl. Therap., 41:387-406.

Holdich, D.M. 1988. Freshwater Crayfish: Biology, Management and Exploitation. Portland OR.

Jank, B.E., Guo, H.M. and Cairns, V.W. 1974. "Activated Sludge Treatment of Airport Wastewater Containing De-icing Fluids." Water Res., 8:875-880.

Kepper, K. 1989. Louisiana Department of Transportation and Development. Personal communication.

King, J.S. Jr. and Wainer, A. "Glyoxylate Metabolism in Normal and Stone Forming Humans and the Effect of Allopurinol Therapy." (3321-9).

Koneman, H. 1981. "Quantitative Structure Activity Relationships in Fish Toxicity Studies, Part 1;, Relationship for 50 Industrial Pollutants." Toxicology, 19(3). 209-221.

Marshall, T.C. and Cheng, Y.S. 1983. "Deposition and Fate of Inhaled Ethylene Glycol Vapor and Condensation Aerosol in the Rat. Fund." Appl. Toxicol., 3:175-181.

Martis, L., Kroes, R., Darby, T.D. and Woods, E.F. 1982. "Disposition Kinetics of Ethylene Oxide, Ethylene Glycol and 2-Chlorethanol in the Dog." J. Toxicol. Environ. Hlth., 10:847-856.

McChesney, E.W., Goldberg, L., Parekh, C.K., Russel, J.C. and Min, B.H. 1971. "Reappraisal of Toxicology of Ethylene Glycol. II. Metabolism Studies in Laboratory Animals." Food Cosmet. Toxicol., 9:21-38.

McChesney, E.W., Goldberg, L. and Harris, E.S. 1972. "Reappraisal of the Toxicology of Ethylene Glycol. IV. The Metabolism of Labelled Glycolic and Glyoxylic Acids in the Rhesus Monkey." Food Cosmet. Toxicol., 10:655-670.

Merck Index. 1983. Rahway, N. J. , U. S. A. Merk and Co. Inc., 10th ed., pp. 550.

Miller, L.M. 1979. Investigation of Selected Potential Environmental Contaminants: Ethylene Glycol, Propylene Glycols and Butylene Glycols. Prepared for EPA NTIS PB 80 109119.

NIOSH National Institute for Occupational Safety and Health. Registry of Toxic Effects of Chemical Substances (RETCS) on line file, current file 86/8512.

NIOSH. 1977. NIOSH Method No P&CAM 338. Ethylene Glycol. In NIOSH Manual of Analytical Methods, 2nd ed., Vol. 1, Cincinnati, Ohio. DHEW (NIOSH) publ. No. 77-157-4.

Parry, M.F. and Wallach, R. 1974. "Ethylene Glycol Poisoning." Amer. J. Med., 57:143-150.

Peterson, C.C., Collins, A.J., Hines, J.M., Bullock, M.L. and Keane, W.F. 1981. "Ethylene Glycol Poisoning. Pharmacokinetics During Therapy with Ethanol and Hemodialysis." N. Engl. J. Med., 304:21-23.

Reif, G. 1950. "Selbstveruche mit Athylen Glycol." Pharmazie., 5:2-76-278.

Ruzik, I. 1972. "Two Compartment Model of Radionuclide Accumulation into Marine Organisms." Mar-Biol., 15:105-112.

Standard Methods for the Examination of Water and Wastewater. 1985. American Public Health Association, American Water Works Association, Water Pollution Control Federation, 16th ed.

Terlinsky, A.S. 1980. "Monohydrate Calcium Oxalate Crystalluria in Ethylene Glycol Poisoning." New England J. Med., 302:922.

Troisi, F.M. 1950. "Chronic Intoxication by Ethylene Glycol Vapor." Brit. J. Industry. Med., 7:65-69.

United States International Trade Commission. 1984. Synthetic Organic Chemicals: United States Production and Sales. United States Government Printing Office, Washington, D.C. USITC Publication No. 1183.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals. Van Nostrand Reinhold Company, NY, 646-647.

Winek, C.L. 1975. "Ethylene Glycol Poisoning. Letter to the Editor." New England J. Med., 292:928-929.

APPENDICES

APPENDIX A

Water parameters data: ethylene glycol concentration in water, pH, dissolved oxygen alkalinity and temperature during uptake and loss phases of the study.

Uptake and loss data for individual crawfish tissue.

TABLE A-1
DISSOLVED OXYGEN DATA FOR THE ACUTE TOXICITY
OF ETHYLENE GLYCOL (EG) ON CRAWFISH
(*Procambarus sp.*) GROUP 1 & 2

Concentration of EG % by Volume	D.O. (mg/l) at				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
12.6	8.6	5.3	5.5	5.0	5.0
7.9	8.5	4.5	3.5	3.5	3.0
6.3	8.5	5.0	4.5	3.5	3.0
5.0	8.4	4.2	4.0	3.2	3.2
3.2	8.4	5.5	4.2	3.0	3.0
0.0	8.3	5.0	4.4	4.0	3.0
GROUP 2:					
12.6	8.9	5.4	5.0	4.3	3.9
7.9	8.7	5.0	4.2	3.5	3.2
6.3	8.7	4.6	4.0	3.3	3.1
5.0	8.6	5.5	4.6	4.0	3.5
3.2	8.6	5.4	4.8	4.2	3.6
0.0	8.6	5.2	4.3	3.0	3.0

TABLE A-2
pH DATA FOR THE ACUTE TOXICITY TEST OF ETHYLENE GLYCOL (EG) ON CRAWFISH (*Procambarus sp.*) GROUP 1 & 2

Concentration of EG % by Volume	pH (s.u.) at				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
12.6	7.9	6.9	6.9	7.0	7.1
7.9	7.8	6.9	6.9	6.8	6.8
6.3	7.8	7.0	6.9	6.8	6.8
5.0	7.8	7.0	6.9	6.8	6.7
3.2	7.7	6.9	6.7	6.7	6.7
0.0	7.7	6.8	6.7	6.7	6.7
GROUP 2:					
12.6	7.8	6.9	6.9	7.0	7.0
7.9	7.8	7.0	7.0	6.9	6.9
6.3	7.8	7.1	7.1	6.9	7.0
5.0	7.8	6.9	6.9	6.9	7.0
3.2	7.7	7.1	7.0	6.9	7.0
0.0	7.7	7.3	7.1	7.0	7.0

TABLE A-3
WATER TEMPERATURE DATA FOR THE ACUTE TOXICITY TEST OF ETHYLENE GLYCOL (EG) ON CRAWFISH IN GROUP 1 & 2

Concentration of EG % by Volume	Temperature in Degrees Centigrade at				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
12.6	23.0	20.5	21.5	21.0	22.0
7.9	23.0	20.5	21.5	21.0	22.0
6.3	23.0	21.0	21.5	21.0	22.0
5.0	22.0	21.0	21.5	21.0	22.0
3.2	22.0	21.0	21.5	21.0	21.5
0.0	22.0	20.5	21.5	21.0	22.0
GROUP 2:					
12.6	23.5	20.0	20.5	22.0	22.0
7.9	23.0	20.5	20.5	20.5	21.5
6.3	23.0	20.0	20.5	20.5	21.5
5.0	22.0	20.5	20.5	20.5	20.5
3.2	22.0	20.0	20.5	21.0	21.5
0.0	22.0	20.0	20.5	21.0	21.5

TABLE A-4
WATER QUALITY FOR THE DILUTION WATER USED IN THE TOXICITY TEST OF ETHYLENE GLYCOL ON CRAWFISH (*Procambarus sp.*)

Parameter Tested	Value
pH	7.9 s.u.
Alkalinity	47 mg/l as $CaCO_3$
Total Residual Chlorine	0.0 mg/l
Total Hardness	270 mg/l as $CaCO_3$
Dissolved Oxygen	8.5 mg/l
Ammonia Nitrogen	0.0 mg/l
Chemical Oxygen Demand (COD)	8.0 mg/l

TABLE A-5
CONCENTRATION OF ETHYLENE GLYCOL (EG) IN THE TEST CHAMBERS
OF THE ACUTE TOXICITY TEST RUN ON CRAWFISH GROUP 1 & 2[a]

Concentration of EG % by Volume	Concentration of EG in mg/l (dilution factor 100 X)				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
12.6	1366.97	1182.30	--	--	1170.00
7.9	848.99	749.20	--	--	747.00
6.3	570.37	561.25	--	--	559.00
5.0	470.09	445.84	--	--	434.00
3.2	301.28	297.63	--	--	295.00
0.0	0.0	--	--	--	0.0
GROUP 2:					
12.6	1264.22	--	--	--	1168.00
7.9	771.56	--	--	--	781.84
6.3	612.84	--	--	--	615.93
5.0	493.03	--	--	--	492.21
3.2	310.92	--	--	--	289.43
0.0	0.0	--	--	--	0.0
STANDARD	1090.00	1064.00	--	--	1097.00

[a] Ethylene glycol was measured by Gas Chromatography

TABLE A-6
WATER QUALITY FOR THE DILUTION WATER USED IN THE TOXICITY TEST OF ETHYLENE GLYCOL (EG) ON BLUE GILLS (*Lepomis macrochirus*) GROUP 1 & 2

Parameter Tested	Value
pH	7.5 s.u.
Alkalinity	65 mg/l as $CaCO_3$
Total Residual Chlorine	0.0 mg/l
Total Hardness	255 mg/l as $CaCO_3$
Dissolved Oxygen	8.6 mg/l
Ammonia Nitrogen	0.0 mg/l
Chemical Oxygen Demand (COD)	6.5 mg/l

TABLE A-7
DISSOLVED OXYGEN DATA FOR THE ACUTE TOXICITY OF
ETHYLENE GLYCOL (EG) ON BLUE GILLS
(*Lepomis macrochirus*) GROUP 1 & 2

Concentration of EG % by Volume	D.O. (mg/l) at				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
4.5	8.5	6.8	5.0	5.0	4.8
3.5	8.4	7.8	6.4	6.0	5.6
2.5	8.4	7.5	5.5	5.0	5.0
2.0	8.4	6.8	6.0	5.5	5.2
1.0	8.4	7.4	6.4	5.2	5.0
0.0	8.4	7.7	7.6	7.6	7.6
GROUP 2:					
4.5	8.3	7.0	6.2	5.8	5.0
3.5	8.4	6.6	6.0	5.4	5.0
2.5	8.3	6.6	5.8	5.0	4.8
2.0	8.3	7.0	6.0	5.5	5.3
1.0	8.3	6.9	6.5	5.7	5.2
0.0	8.4	7.8	7.4	7.3	7.6

TABLE A-8
pH DATA FOR THE ACUTE TOXICITY TEST OF
ETHYLENE GYLCOL (EG) ON BLUE GILLS
(*Lepomis macrochirus*) GROUP 1 & 2

Concentration of EG % by Volume	pH (s.u.) at				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
4.5	7.6	6.5	6.5	6.6	6.7
3.5	7.5	6.5	6.5	6.3	6.4
2.5	7.5	6.6	6.5	6.4	6.4
2.0	7.4	6.6	6.5	6.4	6.4
1.0	7.4	6.5	6.3	6.3	6.3
0.0	7.4	6.4	6.4	6.4	6.5
GROUP 2:					
4.5	7.8	6.5	6.4	6.6	6.7
3.5	7.6	6.5	6.5	6.4	6.4
2.5	7.6	6.6	6.5	6.5	6.4
2.0	7.5	6.5	6.4	6.5	6.5
1.0	7.4	6.6	6.4	6.3	6.3
0.0	7.4	6.4	6.5	6.5	6.5

TABLE A-9
WATER TEMPERATURE DATA FOR THE ACUTE TOXICITY TEST OF
ETHYLENE GYLCOL (EG) ON BLUE GILLS
(*Lepomis macrochirus*) GROUP 1 & 2

Concentration of EG % by Volume	Temperature in Degrees Centigrade at				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
4.5	21.0	20.0	20.5	20.0	21.0
3.5	21.0	20.0	20.5	20.0	21.0
2.5	21.0	20.5	20.5	20.0	21.0
2.0	20.5	20.0	20.5	20.5	20.5
1.0	21.0	21.0	20.5	20.5	21.0
0.0	21.0	20.0	20.5	20.0	21.0
GROUP 2:					
4.5	21.0	20.5	20.5	20.5	21.0
3.5	21.0	20.5	20.5	21.0	21.0
2.5	21.0	21.0	21.0	20.5	20.5
2.0	20.5	20.5	20.5	21.0	21.0
1.0	21.0	20.5	20.5	21.0	21.0
0.0	21.0	20.5	20.5	20.5	21.0

TABLE A-10
CONCENTRATION OF ETHYLENE GLYCOL (EG) IN THE TEST CHAMBERS
OF THE ACUTE TOXICITY TEST RUN ON BLUE GILLS
(*Lepomis macrochirus*) GROUP 1 & 2

Concentration of EG % by Volume	Concentration of EG in mg/l (dilution factor 100 X)				
	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.
GROUP 1:					
4.5	470.30	--	--	--	441.80
3.5	340.46	--	--	--	330.00
2.5	279.53	--	--	--	270.65
2.0	193.68	--	--	--	190.95
1.0	110.00	--	--	--	101.54
0.0	0.00	--	--	--	0.00
GROUP 2:					
4.5	452.40	--	--	--	--
3.5	339.62	--	--	--	340.90
2.5	246.78	--	--	--	248.40
2.0	196.00	--	--	--	186.27
1.0	93.29	--	--	--	95.98
0.0	0.0	--	--	--	0.00

TABLE A-11
UPTAKE DATA FOR INDIVIDUAL CRAWFISH ORGANS FOR
SYSTEM I (50 μg/ml EG)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
0	1	M	0.0	0.0	0.0	0.0
0	2	M	0.0	0.0	0.0	0.0
0	3	F	0.0	0.0	0.0	0.0
1	1	M	6.4	13.8	13.4	8.8
1	2	F	4.0	7.4	21.5	9.6
1	3	F	14.5	6.3	17.3	7.0
2	1	F	12.0	20.0	20.0	9.5
2	2	F	9.2	12.1	16.0	9.2
2	3	F	11.4	10.9	14.3	7.3
3	1	M	10.1	24.0	20.0	27.8
3	2	M	9.0	30.0	19.0	33.5
3	3	F	8.0	20.0	14.0	21.6
4	1	F	8.7	20.0	28.0	24.0
4	2	F	12.5	30.0	38.0	26.5
4	3	F	16.4	34.0	29.0	32.8
5	1	M	13.0	21.6	26.9	28.2
5	2	M	11.8	33.2	35.1	33.7
5	3	F	15.9	26.5	30.1	25.9
6	1	F	15.6	23.4	29.6	36.7
6	2	F	18.9	25.7	32.8	28.6
6	3	M	17.0	31.2	38.9	40.0
8	1	F	14.6	28.9	31.0	37.2
8	2	M	20.2	25.2	41.3	19.2
8	3	M	11.1	19.1	35.8	9.9
10	1	F	11.5	34.2	29.2	35.1
10	2	M	17.3	28.0	32.9	29.2
10	3	F	16.0	24.3	40.3	24.3
12	1	F	19.2	27.1	43.2	25.3
12	2	F	17.0	21.3	31.7	20.7
12	3	M	12.9	32.0	38.6	28.9
14	1	F	18.2	35.7	38.2	25.6
14	2	M	12.8	27.1	42.1	30.7
14	3	F	21.4	31.9	32.3	33.5
16	1	M	13.1	32.4	35.3	28.3
16	2	F	17.1	29.2	38.1	30.2
16	3	M	20.2	24.0	30.6	24.4
19	1	M	23.1	30.6	41.2	39.1
19	2	M	17.8	38.1	33.8	31.5
19	3	F	14.3	25.1	35.7	28.5

TABLE A-11 (continued)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
22	1	M	10.0	74.8	48.0	34.7
22	2	F	16.0	84.5	85.3	18.1
22	3	M	14.0	32.1	28.0	28.4
26	1	M	18.2	25.4	26.3	10.6
26	2	M	14.9	19.7	30.0	20.2
26	3	M	13.6	58.9	54.4	18.9
29	1	F	14.0	22.7	20.0	19.0
29	2	F	9.0	32.3	26.0	22.0
29	3	M	12.0	46.2	30.0	30.0
36	1	M	14.0	32.0	23.6	26.0
36	2	F	16.0	26.0	78.0	16.5
36	3	M	13.0	20.5	38.0	28.5
45	1	M	11.9	20.4	43.2	22.9
45	2	F	14.3	15.7	30.3	32.9
45	3	M	11.7	18.6	27.6	11.4
47	1	M	23.1	12.9	130.0	21.7
47	2	F	11.4	7.7	45.7	21.4
47	3	M	9.0	9.4	71.1	34.0
49	1	F	9.6	34.3	31.2	9.1
49	2	M	7.2	11.4	18.2	30.6
49	3	F	8.4	18.9	67.7	12.7
51	1	F	23.5	22.4	32.4	28.0
51	2	F	28.2	13.6	30.7	13.3
51	3	M	21.0	18.6	34.0	32.0
53	1	F	20.8	22.0	26.0	12.2
53	2	M	31.5	18.6	24.0	26.7
53	3	F	19.7	16.3	32.5	23.5
55	1	F	26.9	53.0	125.5	25.4
55	2	F	41.9	27.1	50.8	25.4
55	3	F	32.9	32.7	56.6	50.8
57	1	M	16.1	15.7	19.8	8.8
57	2	M	12.1	20.1	21.4	17.4
57	3	M	10.3	18.2	23.2	13.0
59	1	F	14.5	13.5	23.3	8.7
59	2	M	17.0	17.8	24.9	25.7
59	3	F	25.3	24.6	21.7	34.2
61	1	F	25.7	24.0	36.2	20.1
61	2	F	23.3	17.5	30.3	30.9
61	3	M	12.3	30.6	24.9	38.5

TABLE A-12
UPTAKE DATA FOR INDIVIDUAL CRAWFISH ORGANS FOR
SYSTEM II (200 μg/ml EG)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
0	1	M	0.0	0.0	0.0	0.0
0	2	M	0.0	0.0	0.0	0.0
0	3	M	0.0	0.0	0.0	0.0
1	1	F	21.4	42.5	48.0	20.6
1	2	M	26.3	29.3	60.0	34.6
1	3	M	19.1	28.1	117.6	27.0
2	1	F	21.1	31.6	30.0	20.0
2	2	F	26.0	34.0	134.0	31.8
2	3	F	14.0	32.7	85.0	19.6
3	1	M	20.0	65.0	43.0	41.0
3	2	M	15.0	78.0	35.0	51.0
3	3	M	14.9	17.0	157.0	51.0
4	1	M	19.0	46.0	94.0	53.0
4	2	F	18.0	31.0	82.0	41.0
4	3	F	25.0	33.0	85.0	37.8
5	1	F	22.3	40.8	88.3	59.3
5	2	M	29.9	24.6	78.7	40.6
5	3	M	19.6	56.5	42.2	34.5
6	1	F	40.6	65.8	96.7	59.3
6	2	M	19.3	44.1	65.3	39.7
6	3	F	26.2	51.3	54.9	30.5
8	1	F	29.6	59.8	76.7	53.1
8	2	F	19.8	28.9	53.3	32.7
8	3	M	12.3	41.2	50.9	60.3
10	1	M	26.9	56.3	83.8	63.3
10	2	M	41.6	49.3	47.5	41.8
10	3	M	33.3	30.7	68.9	50.3
12	1	M	43.5	73.4	73.8	83.1
12	2	F	29.3	43.0	89.4	58.2
12	3	F	32.2	47.2	116.0	49.0
14	1	F	51.0	92.3	98.0	75.2
14	2	M	43.5	65.9	62.4	61.3
14	3	F	39.2	59.5	79.3	54.4
16	1	M	39.2	82.1	78.6	58.3
16	2	M	61.3	55.7	53.7	33.9
16	3	F	50.2	64.2	61.9	48.2
19	1	M	55.7	62.3	65.3	49.2
19	2	M	43.2	57.6	23.2	33.1
19	3	M	37.1	38.8	36.4	30.8

TABLE A-12 (continued)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
22	1	F	19.0	48.2	26.0	40.9
22	2	M	32.0	31.2	50.0	37.1
22	3	F	20.0	77.0	46.0	35.0
26	1	M	26.9	29.4	52.5	50.3
26	2	M	14.0	42.0	68.2	40.7
26	3	F	35.1	63.8	79.6	62.6
29	1	F	29.0	25.9	44.0	30.3
29	2	M	20.0	48.0	60.0	20.0
29	3	F	28.0	36.8	54.0	23.7
36	1	F	25.6	42.7	73.0	36.7
36	2	F	22.5	61.0	69.8	42.7
36	3	M	27.2	47.3	92.0	67.3
45	1	F	45.1	64.3	60.2	62.3
45	2	M	38.6	135.7	53.0	40.0
45	3	F	20.0	57.4	77.3	21.4
47	1	M	56.4	69.0	185.0	21.2
47	2	M	48.0	57.8	160.0	47.5
47	3	F	46.0	58.0	87.8	19.0
49	1	M	39.8	166.6	172.9	32.6
49	2	F	22.0	50.6	42.1	48.0
49	3	M	17.6	52.5	38.8	45.0
51	1	F	47.2	40.1	57.9	16.8
51	2	M	51.9	41.6	48.2	31.5
51	3	F	35.7	28.6	47.9	82.6
53	1	M	53.6	47.6	55.8	26.9
53	2	M	23.1	67.7	43.2	35.8
53	3	F	53.6	23.4	33.6	100.0
55	1	M	42.3	24.5	39.8	67.3
55	2	F	63.1	151.5	54.8	31.0
55	3	M	52.3	66.2	47.8	79.5
57	1	M	20.5	181.5	62.3	94.3
57	2	M	28.2	42.1	55.5	47.7
57	3	F	36.3	14.3	41.0	16.5
59	1	M	41.0	53.0	37.8	101.2
59	2	M	26.5	84.3	86.1	55.4
59	3	M	31.3	62.8	59.7	31.3
61	1	M	32.5	25.9	104.3	85.6
61	2	M	47.1	51.6	81.4	58.1
61	3	F	45.3	49.5	64.6	51.3

TABLE A-13
UPTAKE DATA FOR INDIVIDUAL CRAWFISH ORGANS FOR
SYSTEM III (1000 μg/ml EG)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
0	1	M	0.0	0.0	0.0	0.0
0	2	F	0.0	0.0	0.0	0.0
0	3	F	0.0	0.0	0.0	0.0
1	1	M	75.5	89.2	48.0	20.6
1	2	M	26.4	35.5	60.0	34.6
1	3	M	12.6	45.5	117.6	27.0
2	1	F	66.5	122.0	30.0	20.0
2	2	F	43.9	118.9	134.0	31.8
2	3	F	29.9	133.4	85.0	19.6
3	1	M	50.9	144.0	43.0	41.0
3	2	M	50.9	109.0	35.0	51.0
3	3	M	76.4	174.0	157.0	51.0
4	1	M	54.0	112.0	94.0	53.0
4	2	F	60.2	58.2	82.0	41.0
4	3	F	66.5	153.8	85.0	37.8
5	1	F	69.2	130.1	88.3	59.3
5	2	M	45.7	89.8	78.7	40.6
5	3	F	59.1	100.3	42.2	34.5
6	1	F	159.7	214.3	96.7	59.3
6	2	F	119.2	166.9	65.3	39.7
6	3	M	110.3	173.3	54.9	30.5
8	1	M	123.1	168.1	76.7	53.1
8	2	F	82.4	109.0	53.3	32.7
8	3	F	95.4	150.0	50.9	60.3
10	1	M	167.2	178.9	83.8	63.3
10	2	M	142.7	127.6	47.5	41.8
10	3	M	113.9	136.3	68.9	50.3
12	1	M	177.4	259.3	73.8	83.1
12	2	M	110.1	188.1	89.4	58.2
12	3	F	98.2	201.1	116.0	49.0
14	1	F	201.1	278.3	98.0	75.2
14	2	M	160.7	226.9	62.4	61.3
14	3	M	253.9	216.7	79.3	54.4
16	1	M	214.0	266.3	78.6	58.3
16	2	F	141.1	215.7	53.7	33.9
16	3	F	156.1	250.1	61.9	48.2
19	1	F	170.7	237.6	65.3	49.2
19	2	F	195.5	169.8	23.2	33.1
19	3	M	218.5	190.3	36.4	30.8

TABLE A-13 (continued)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
22	1	F	138.0	207.0	96.0	105.4
22	2	F	113.0	181.0	186.0	138.0
22	3	F	155.0	170.0	145.5	132.2
26	1	M	148.0	160.0	156.0	211.0
26	2	F	147.2	194.0	150.8	111.0
26	3	M	165.6	220.0	54.0	84.2
29	1	M	126.0	105.1	101.2	126.5
29	2	F	156.6	185.5	181.6	186.0
29	3	F	93.2	102.1	110.7	98.6
36	1	M	207.0	269.0	209.0	166.0
36	2	M	126.0	110.2	115.0	85.2
36	3	F	117.0	96.4	175.0	71.7
45	1	F	288.0	251.0	396.0	106.0
45	2	M	154.0	835.7	130.0	235.0
45	3	M	216.0	57.4	477.0	96.0
47	1	F	120.0	191.0	63.0	77.0
47	2	M	392.2	222.0	29.0	216.0
47	3	F	189.7	122.3	77.0	72.1
49	1	M	291.0	409.0	395.0	281.0
49	2	M	124.0	254.0	243.0	125.0
49	3	F	140.0	330.0	167.2	120.0
51	1	M	239.9	124.8	266.6	78.2
51	2	F	129.0	118.8	220.9	94.7
51	3	F	280.7	248.0	55.3	120.6
53	1	F	153.2	115.5	124.8	170.7
53	2	M	236.4	58.1	92.6	39.4
53	3	F	181.2	248.0	126.0	101.3
55	1	M	307.3	308.5	317.6	228.5
55	2	M	132.7	147.0	146.3	463.5
55	3	M	135.0	128.0	122.1	144.0
57	1	F	173.2	282.9	63.0	73.0
57	2	M	231.3	144.9	586.1	212.9
57	3	M	288.8	270.7	148.0	129.7
59	1	M	286.5	172.6	193.3	125.7
59	2	F	228.2	82.9	106.4	150.0
59	3	F	396.5	160.8	106.2	82.6
61	1	F	161.0	185.0	268.0	213.0
61	2	F	213.0	212.0	317.0	268.0
61	3	F	240.0	234.0	220.0	189.0

TABLE A-14
LOSS DATA FOR INDIVIDUAL CRAWFISH ORGANS FOR
SYSTEM I (50 μg/ml EG)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
0	1	F	16.1	14.0	19.8	17.9
	2	F	12.1	11.5	21.4	30.9
	3	M	10.3	40.6	23.2	42.6
1	1	M	15.7	19.0	24.2	8.0
	2	F	0.0	17.5	0.0	20.3
	3	F	10.8	11.3	12.5	87.2
2	1	M	10.0	9.0	13.6	23.3
	2	M	0.0	13.0	0.0	0.0
	3	M	0.0	14.0	0.0	0.0
3	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
4	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
5	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
6	1	F	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
7	1	F	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
10	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
12	1	F	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
18	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
25	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
32	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0

TABLE A-14 (continued)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
39	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
46	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
67	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0

TABLE A-15
LOSS DATA FOR INDIVIDUAL CRAWFISH ORGANS FOR
SYSTEM II (200 μg/ml EG)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
0	1	F	16.9	125.9	104.3	85.6
	2	F	47.1	82.6	41.4	28.1
	3	M	45.3	99.5	64.6	51.3
1	1	F	23.7	73.0	74.0	18.3
	2	M	34.6	53.3	50.9	84.0
	3	M	12.6	61.3	26.7	38.4
2	1	M	21.8	52.7	0.0	20.2
	2	M	0.0	32.6	48.0	61.0
	3	M	10.4	67.1	0.0	0.0
3	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	24.8	0.0	0.0
	3	F	0.0	52.3	4.1	0.0
4	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	22.3	0.0	0.0
	3	F	0.0	30.6	0.0	17.0
5	1	M	0.0	0.0	7.4	0.0
	2	M	0.0	0.0	10.2	0.0
	3	M	0.0	0.0	0.0	0.0
6	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
7	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
10	1	F	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
12	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
18	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
25	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
32	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0

TABLE A-15 (continued)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
39	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
46	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
67	1	F	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0

TABLE A-16
LOSS DATA FOR INDIVIDUAL CRAWFISH ORGANS FOR
SYSTEM III (1000 μg/ml EG)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
0	1	F	111.0	185.0	168.0	113.0
	2	F	441.1	712.0	517.0	368.0
	3	F	31.0	234.0	120.0	189.0
1	1	F	52.2	81.8	158.4	46.0
	2	M	53.4	98.0	60.7	70.3
	3	F	34.3	84.4	61.8	116.6
2	1	F	0.54	42.8	2.1	36.6
	2	M	20.5	52.5	8.5	5.8
	3	F	26.8	68.8	45.8	40.4
3	1	M	0.0	47.1	0.0	0.0
	2	F	49.2	19.2	0.0	6.7
	3	F	0.0	92.2	0.0	37.4
4	1	F	12.9	25.6	0.0	5.5
	2	F	10.0	12.0	18.4	37.8
	3	F	21.8	44.6	98.0	7.3
5	1	F	20.0	118.5	0.0	0.0
	2	M	0.0	0.0	10.2	0.0
	3	M	0.0	0.0	0.0	0.0
6	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
7	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
10	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
12	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
18	1	F	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
25	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0
32	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0

TABLE A-16 (continued)

Time Day	Animal No.	Sex	Gills	Muscles	G.I.	Hepta.
39	1	F	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
46	1	M	0.0	0.0	0.0	0.0
	2	M	0.0	0.0	0.0	0.0
	3	M	0.0	0.0	0.0	0.0
67	1	M	0.0	0.0	0.0	0.0
	2	F	0.0	0.0	0.0	0.0
	3	F	0.0	0.0	0.0	0.0

APPENDIX B

Monitoring Water Parameters:

Dissolved oxygen and pH meters were calibrated before each use. Alkalinity and total hardness were determined according to procedures in Standard Methods for Testing Water and Waste Water. All glassware were acid washed and rinsed with deionized, distilled water.

Ethylene Glycol Standards:

Preparation and analysis

A stock solution of 1000 mg/l ethylene glycol concentration was prepared by adding 0.9 ml of pure ethylene glycol in a liter of distilled water. The stock solution of ethylene glycol was used to prepare standards of different ethylene glycol concentrations. Ethylene glycol standards were prepared daily and analyzed before sample analysis (see Table B-1).

TABLE B-1
STANDARDS FOR ETHYLENE GLYCOL CONCENTRATION
ANALYZED BY GAS CHROMATOGRAPH

Expected Value mg/l	Observed Value mg/l
40	40.0
30	30.5
30	30.8
25	25.4
40	42.0
30	30.5
40	29.8
30	22.5
40	28.2
30	24.7
30	30.5
30	30.0

Field and Laboratory Samples:

Field samples including water, sediment, soil and air filters were collected in acid washed glass containers, preserved with 2% propanol and refrigerated until analysis.

Crawfish tissues in the laboratory were immediately extracted following dissection. They were centrifuged and the supernatant was then analyzed.

Recovery of Ethylene Glycol and Crawfish Tissues:

Known concentrations of ethylene glycol were added to ethylene glycol free crawfish tissues and were treated exactly the same as sample and analyzed. Percent recovery was determined (Table B-2).

TABLE B-2
PERCENT RECOVERY OF ETHYLENE GLYCOL FROM CRAWFISH TISSUES

Tissues	% Recovery					
	1	2	3	4	5	Average
Gills	60	62	75	83	73	71
Muscles	80	66	83	84	79	78
G.I.	69	72	77	65	66	70
Hepatopancreas	69	67	85	89	84	79

Recovery of Ethylene Glycol from Feed (Quaker Oats):

Ten grams of commercial Quaker Oats, which was used as crawfish feed, were placed in known concentration of ethylene glycol, treated exactly the same as samples, and analyzed. Percent recovery was determined (Table B-3).

TABLE B-3
PERCENT RECOVERY OF ETHYLENE GLYCOL FROM QUAKER OATS

Expected EG Concentration (mg/l)	Observed EG Concentration (mg/l)	% Recovery
0.0	0.0	
10.0	10.0	100
50.0	49.0	98
100.0	101.0	100

Spike samples:

Samples were spiked with known ethylene glycol concentrations, treated exactly the same as samples, and analyzed (Table B-4).

TABLE B-4
PERCENT RECOVERY OF ETHYLENE GLYCOL FROM SPIKED CRAWFISH TISSUES

Tissue	Expected Value mg/l	Observed Value mg/l	% Recovery
Gill	40.0	36.4	91
Muscle	45.6	41.5	91
G.I.	30.0	23.5	78
Hepatopancreas	46.8	37.8	81

Figure B-1 represents the standard curve of ethylene glycol concentrations when analyzed by gas chromatograph. The least-square best fit line was drawn. This curve was used to determine the concentrations of ethylene glycol in the analyzed samples.

Recovery of Ethylene Glycol from Soil, Water and Sediment:

Known concentrations of ethylene glycol were added to ethylene glycol free water, sediment and soil samples, treated exactly the same as samples, and analyzed. Percent recovery was determined (Table B-5).

TABLE B-5
PERCENT RECOVERY OF ETHYLENE GLYCOL FROM WATER, SOIL AND SEDIMENT SAMPLES

Sample	Expected EG Concentration (mg/l)	Observed EG Concentration (mg/l)	% Recovered
Water	0.0	0.0	
	10.0	10.0	100
	30.0	30.6	100
	60.0	60.0	100
	100.0	100.0	100
Soil	0.0	0.0	
	10.0	11.0	100
	30.0	30.0	100
	60.0	63.0	100
	100.0	98.0	98
Sediment	0.0	0.0	
	10.0	10.0	100
	50.0	50.5	100

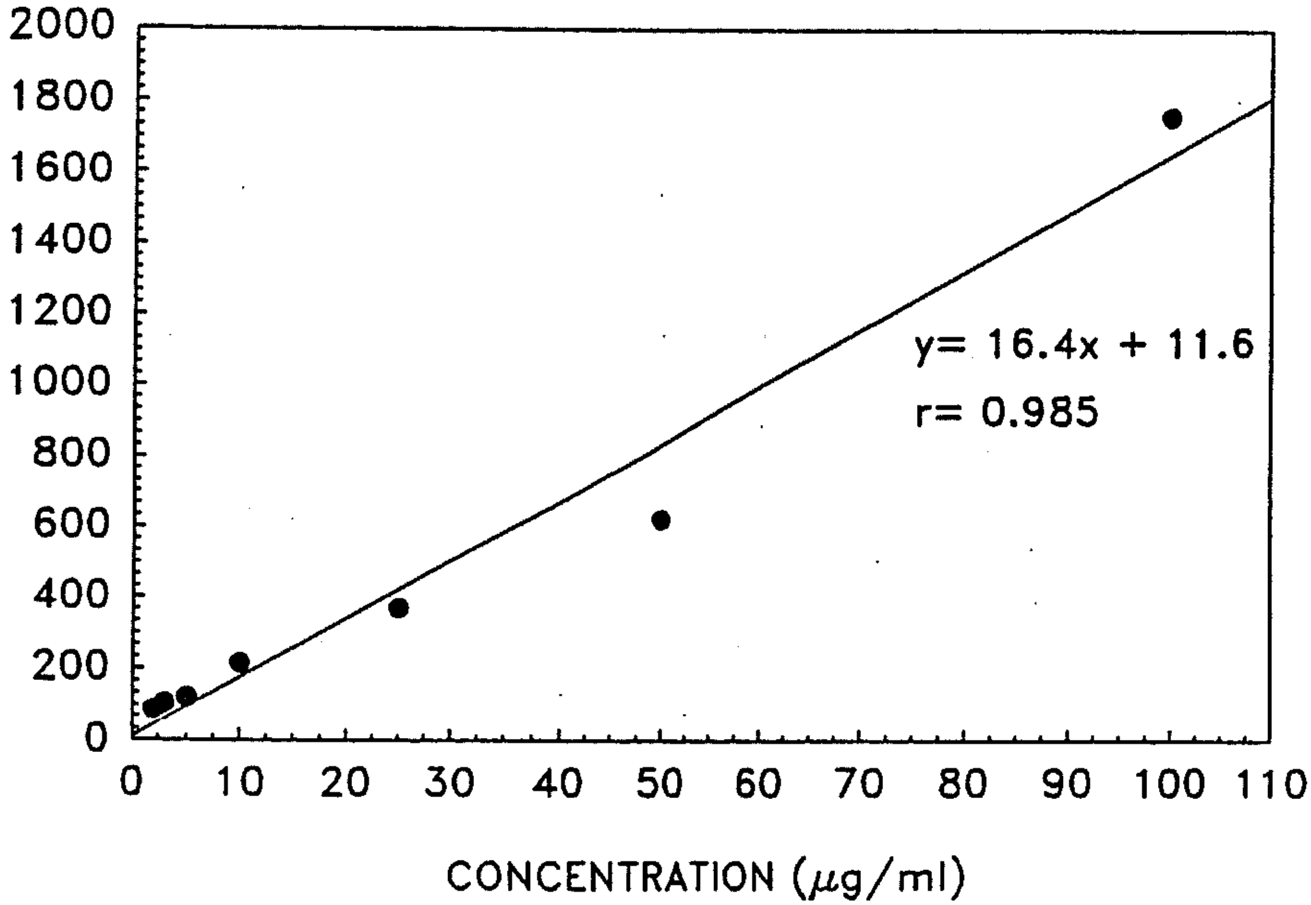


Figure B-1. Standard curve of ethylene glycol using a gas chromatograph

TABLE B-6
RESULTS OF STANDARD SAMPLES ANALYZED EVERY TEN SAMPLES
TO CHECK FOR THE ACCURACY OF THE ANALYSIS DURING THE UPTAKE PHASE

Time	Expected (mg/l)	Observed (mg/l)
Day 1	5.0	5.4
	5.0	4.9
	5.0	6.1
	5.0	5.2
Day 2	5.0	5.0
	5.0	5.5
	5.0	5.8
	5.0	5.2
Day 3	5.0	4.8
	10.0	9.2
	5.0	5.6
	10.0	10.4
Day 4	5.0	4.2
	5.0	4.9
	5.0	5.2
	5.0	5.2
Day 5	5.0	4.8
	10.0	12.4
	5.0	4.6
	10.0	10.0
Day 6	5.0	4.9
	5.0	5.1
	10.0	12.0
	10.0	10.4
Day 8	5.0	4.8
	10.0	11.4
	5.0	4.9
	10.0	10.6
Day 10	5.0	5.3
	10.0	10.6
	5.0	4.9
	10.0	10.2
Day 12	5.0	4.8
	10.0	10.4
	5.0	5.4
	10.0	10.4

TABLE B-6 (continued)

Time	Expected (mg/l)	Observed (mg/l)
Day 14	5.0	4.9
	10.0	9.8
	5.0	4.9
	10.0	10.6
Day 16	5.0	5.2
	10.0	10.6
	5.0	5.8
	10.0	10.4
Day 19	5.0	5.2
	5.0	4.9
	5.0	5.5
	5.0	5.0
Day 22	5.0	5.4
	10.0	4.9
	5.0	5.4
	10.0	5.8
Day 26	5.0	4.1
	10.0	8.9
	5.0	4.9
	10.0	5.2
Day 29	5.0	4.8
	10.0	9.8
	5.0	5.4
	10.0	9.9
Day 36	5.0	4.6
	5.0	4.4
	10.0	10.8
	10.0	12.2
Day 45	5.0	5.5
	10.0	11.2
	5.0	5.8
	5.0	5.2
Day 47	5.0	4.9
	5.0	5.4
	5.0	5.2
	5.0	5.6
Day 49	5.0	4.8
	5.0	5.2
	5.0	5.5
	5.0	5.6

TABLE B-6 (continued)

Time	Expected (mg/l)	Observed (mg/l)
Day 51	10.0	10.4
	5.0	5.4
	10.0	11.2
	5.0	4.9
Day 53	5.0	4.8
	5.0	5.6
	5.0	5.8
	5.0	5.0
Day 55	5.0	4.9
	5.0	5.8
	10.0	11.0
	10.0	10.4
Day 57	5.0	5.5
	5.0	5.8
	5.0	4.8
	5.0	4.1
Day 59	5.0	5.4
	5.0	5.8
	5.0	4.9
	5.0	4.6
Day 61	5.0	4.8
	10.0	9.0
	10.0	11.0
	5.0	4.8

TABLE B-7
RESULTS OF STANDARD SAMPLES ANALYZED EVERY TEN SAMPLES
TO CHECK FOR THE ACCURACY OF THE ANALYSIS DURING THE LOSS PHASE

Time	Expected (mg/l)	Observed (mg/l)
Day 1	5.0	4.6
	5.0	4.4
	10.0	10.8
	10.0	12.2
Day 2	5.0	5.5
	10.0	11.2
	5.0	5.8
	5.0	5.2
Day 3	5.0	4.9
	5.0	5.4
	5.0	5.2
	5.0	5.6
Day 4	5.0	4.8
	5.0	5.2
	5.0	5.5
	5.0	5.6
Day 5	10.0	10.4
	5.0	5.4
	10.0	11.2
	5.0	4.9
Day 6	5.0	4.8
	5.0	5.6
	5.0	5.8
	5.0	5.0
Day 7	5.0	4.9
	5.0	5.8
	10.0	11.0
	10.0	10.4
Day 10	5.0	5.5
	5.0	5.8
	5.0	4.8
	5.0	4.1
Day 12	5.0	5.4
	5.0	5.8
	5.0	4.9
	5.0	4.6

TABLE B-7 (continued)

Time	Expected (mg/l)	Observed (mg/l)
Day 18	5.0	4.8
	10.0	9.0
	10.0	11.0
	5.0	4.8
Day 25	5.0	5.2
	10.0	10.6
	5.0	5.8
	10.0	10.4
Day 32	5.0	5.2
	5.0	4.9
	5.0	5.5
	5.0	5.0
Day 39	5.0	5.4
	10.0	4.9
	5.0	5.4
	10.0	5.8
Day 46	5.0	4.1
	10.0	8.9
	5.0	4.9
	10.0	5.2
Day 67	5.0	4.8
	10.0	9.8
	5.0	5.4
	10.0	9.9

APPENDIX C

Data fitting of the results in a one compartment model for the selected tissues.

Model fitting showing effect of weight and sex of crawfish on the concentration of ethylene glycol in selected tissues.

One-way ANOVA for uptake and loss kinetics, bioconcentration factor and half-lives.

One Compartment Model Equation:

C/m = ki/ko (1-exp(-ko*time))

Where:

C = concentration of the chemical in animal tissue

m = concentration of the chemical in the medium

ki = rate of the chemical uptake

ko = rate of the chemical elimination

A plot of C/m vs. 1-exp(-ko*time) is a straight line with slope equal to ki/ko.

TABLE C-1
DATA ANALYSIS OF GILLS SAMPLES FOR ANIMALS EXPOSED TO
SYSTEM I (50 μg/ml EG BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	74	1.006		
Regression	1	0.426	0.426	53.6
Residual	73	0.580	0.008	
Lack of Fit	23	0.214	0.0093	1.27 (not significant at a = 0.05)
Pure Error	50	0.366	0.0073	

Slope = ki/ko = 0.33
ko = 0.547
ki = 0.18

TABLE C-2
DATA ANALYSIS OF MUSCLE SAMPLES FOR ANIMALS EXPOSED TO SYSTEM I (50 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	74	4.670		
Regression	1	1.202	1.202	25.3
Residual	73	3.468	0.0475	
Lack of Fit	23	0.979	0.0426	0.86 (not significant at a = 0.05)
Pure Error	50	2.489	0.0498	

Slope = ki/ko = 0.572
ko = 0.616
ki = 0.352

TABLE C-3
DATA ANALYSIS OF G.I. SAMPLES FOR ANIMALS EXPOSED TO SYSTEM I (50 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	74	13.557		
Regression	1	2.626	2.626	17.5
Residual	73	10.930	0.1497	
Lack of Fit	23	4.775	0.207	1.68 (not significant at a = 0.05)
Pure Error	50	6.156	0.123	

Slope = ki/ko = 0.859
ko = 0.672
ki = 0.577

TABLE C-4
DATA ANALYSIS OF HEPTA. SAMPLES FOR ANIMALS EXPOSED TO SYSTEM I (50 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	77	4.566		
Regression	1	1.189	1.189	26.8
Residual	76	3.376	0.0444	
Lack of Fit	24	1.496	0.0623	1.72 (not significant at a = 0.05)
Pure Error	52	1.88	0.0362	

Slope = ki/ko = 0.532
ko = 0.453
ki = 0.241

TABLE C-5
DATA ANALYSIS OF GILLS SAMPLES FOR ANIMALS EXPOSED TO SYSTEM II (200 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	77	0.373		
Regression	1	0.113	0.113	33
Residual	76	0.259	0.003	
Lack of Fit	24	0.115	0.0047	1.72 (not significant at a = 0.05)

TABLE C-6
DATA ANALYSIS OF MUSCLE SAMPLES FOR ANIMALS EXPOSED TO SYSTEM II (200 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	77	1.756		
Regression	1	0.333	0.333	17.8
Residual	76	1.423	0.018	
Lack of Fit	24	0.41	0.0171	0.88 (not significant at a = 0.05)
Pure Error	52	1.013	0.0195	

Slope = ki/ko = 0.282
ko = 0.454
ki = 0.130

TABLE C-7
DATA ANALYSIS OF HEPTA. SAMPLES FOR ANIMALS EXPOSED TO SYSTEM II (200 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	73	1.330		
Regression	1	0.1336	0.1336	8.03
Residual	72	1.197	0.017	
Lack of Fit	21	0.4388	0.0209	1.39 (not significant at a = 0.05)
Pure Error	51	0.7582	0.0150	

Slope = ki/ko = 0.182
ko = 0.490
ki = 0.09

TABLE C-8
DATA ANALYSIS OF G.I. SAMPLES FOR ANIMALS EXPOSED TO SYSTEM II (200 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	77	0.849		
Regression	1	0.219	0.219	26.4
Residual	76	0.630	0.0083	
Lack of Fit	24	0.218	0.0091	1.15 (not significant at a = 0.05)
Pure Error	52	0.412	0.0079	

Slope = ki/ko = 0.236
ko = 0.524
ki = 0.124

TABLE C-9
DATA ANALYSIS OF GILLS SAMPLES FOR ANIMALS EXPOSED TO SYSTEM III (1000 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	77	0.534		
Regression	1	0.092	0.092	15.8
Residual	76	0.442	0.0058	
Lack of Fit	24	0.2063	0.0085	1.8 (not significant at a = 0.05)
Pure Error	52	0.2357	0.00453	

Slope = ki/ko = 0.175
ko = 1.327
ki = 0.232

TABLE C-10
DATA ANALYSIS OF MUSCLES SAMPLES FOR ANIMALS EXPOSED TO SYSTEM III (1000 μg/ml EG) BY ANOVA
(SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	75	0.472		
Regression	1	0.121	0.121	25.5
Residual	74	0.351	0.0047	
Lack of Fit	24	0.142	0.0059	1.4 (not significant at a = 0.05)
Pure Error	50	0.209	0.0042	

Slope = ki/ko = 0.199
ko = 1.185
ki = 0.236

TABLE C-11
DATA ANALYSIS OF G.I. SAMPLES FOR ANIMALS EXPOSED TO SYSTEM III (1000 μg/ml EG) BY ANOVA (SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	72	0.7125		
Regression	1	0.1304	0.1304	15.9
Residual	71	0.5822	0.0082	
Lack of Fit	21	0.127	0.0060	0.66 (not significant at a = 0.05)
Pure Error	50	0.455	0.0091	

Slope = ki/ko = 0.205
ko = 1.108
ki = 0.227

TABLE C-12
DATA ANALYSIS OF HEPTA. SAMPLES FOR ANIMALS EXPOSED TO SYSTEM III (1000 μg/ml EG) BY ANOVA (SHOWING LACK-OF-FIT)

Source	df	SS	MS	F ratio
Total	77	0.398		
Regression	1	0.083	0.083	20
Residual	71	0.3149	0.0041	
Lack of Fit	24	0.123	0.0051	1.38 (not significant at a = 0.05)
Pure Error	52	0.192	0.0037	

Slope = ki/ko = 0.161
ko = 0.969
ki = 0.156

TABLE C-13
MODEL FITTING RESULTS SHOWING EFFECT OF WEIGHT
ANS SEX OF CRAWFISH ON ETHYLENE GLYCOL CONCENTRATION
IN GILLS SYSTEM I

Independent Variable	Coefficient	Std. Error	T-value	Sig. Level
Constant	0.21	0.08	2.63	0.01
Time	0.002	0.0008	2.4	0.018
Sex	0.0048	0.034	0.14	0.89
Weight	0.0046	0.052	0.87	0.38

R-SQRD (ADJ.) = 0.0615

ANOVA for variables in the order fitted

Source	SS	df	MS	F-Ratio	P-value
Time	0.144	1	0.144	6.99	0.01
Sex	0.0018	1	0.0018	0.09	0.769
Weight	0.016	1	0.016	0.77	0.393

Pearson correlation matrix for the coefficient variables.

	Constant	Time	Sex	Weight
Constant	1.000	-0.142	-0.504	-0.69
Time	-0.142	1.000	0.043	-0.19
Sex	-0.504	0.043	1.000	-0.172
Weight	-0.694	-0.190	-0.172	1.000

TABLE C-14
MODEL FITTING RESULTS SHOWING EFFECT OF WEIGHT ANS SEX OF CRAWFISH ON ETHYLENE GLYCOL CONCENTRATION IN GILLS SYSTEM II

Independent Variable	Coefficient	Std. Error	T-value	Sig. Level
Constant	0.093	0.044	2.09	
Time	0.00091	0.0004	2.16	
Sex	0.0023	0.017	0.136	
Weight	0.024	0.0029	0.825	

R-SQRD (ADJ.) = 0.0615

ANOVA for variables in the order fitted

Source	SS	df	MS	F-Ratio	P-value
Time	0.032	1	0.032	5.93	0.017
Sex	0.00068	1	0.00068	0.01	0.912
Weight	0.00369	1	0.0369	0.68	0.42

Pearson correlation matrix for the coefficient variables.

	Constant	Time	Sex	Weight
Constant	1.000	-0.119	-0.61	0.75
Time	-0.119	1.000	0.093	-0.245
Sex	-0.607	0.093	1.000	0.0285
Weight	-0.746	-0.243	0.285	1.000

TABLE C-15
MODEL FITTING RESULTS SHOWING EFFECT OF WEIGHT
ANS SEX OF CRAWFISH ON ETHYLENE GLYCOL CONCENTRATION
IN GILLS SYSTEM III

Independent Variable	Coefficient	Std. Error	T-value	Sig. Level
Constant	0.09	0.04	2.24	0.028
Time	0.002	0.0004	5.6	0.000
Sex	-0.012	0.017	-0.688	0.494
Weight	-0.0009	0.026	0.036	0.971

R-SQRD (ADJ.) = 0.288

ANOVA for variables in the order fitted

Source	SS	df	MS	F-Ratio	P-value
Time	0.18	1	0.18	32.5	0.000
Sex	0.0027	1	0.0027	0.49	0.495
Weight	0.00007	1	0.000007	0.00	0.972

Pearson correlation matrix for the coefficient variables.

	Constant	Time	Sex	Weight
Constant	1.000	-0.096	-0.544	-0.693
Time	-0.096	1.000	0.022	-0.213
Sex	-0.539	0.022	1.000	0.123
Weight	-0.693	-0.213	-0.123	1.000

TABLE C-16
MODEL FITTING RESULTS SHOWING EFFECT OF WEIGHT
ANS SEX OF CRAWFISH ON ETHYLENE GLYCOL CONCENTRATION
IN MUSCLES SYSTEM II

Independent Variable	Coefficient	Std. Error	T-value	Sig. Level
Constant	0.199	0.093	2.133	0.036
Time	0.0012	0.0008	1.489	0.141
Sex	-0.03	0.03	-0.88	0.38
Weight	0.027	0.0337	0.808	0.422

R-SQRD (ADJ.) = 0.0178

ANOVA for variables in the order fitted

Source	SS	df	MS	F-Ratio	P-value
Time	0.064	1	0.064	30.8	0.084
Sex	0.0127	1	0.0127	0.61	0.44
Weight	0.0135	1	0.0135	0.65	0.43

Pearson correlation matrix for the coefficient variables.

	Constant	Time	Sex	Weight
Constant	1.000	-0.143	-0.435	-0.785
Time	-0.142	1.000	0.125	-0.182
Sex	-0.435	0.0125	1.000	-0.134
Weight	-0.785	-0.0182	-0.134	1.000

TABLE C-17
MODEL FITTING RESULTS SHOWING EFFECT OF WEIGHT
ANS SEX OF CRAWFISH ON ETHYLENE GLYCOL CONCENTRATION
IN G.I. SYSTEM I

Independent Variable	Coefficient	Std. Error	T-value	Sig. Level
Constant	0.426	0.216	1.97	0.052
Time	0.0038	0.0025	1.51	0.135
Sex	-0.0246	0.11	-0.223	0.824
Weight	2.45	1.71	1.43	0.157

R-SQRD (ADJ.) = 0.0221

ANOVA for variables in the order fitted

Source	SS	df	MS	F-Ratio	P-value
Time	0.525	1	0.525	2.57	0.114
Sex	0.011	1	0.011	0.05	0.818
Weight	0.419	1	0.419	2.05	0.157

Pearson correlation matrix for the coefficient variables.

	Constant	Time	Sex	Weight
Constant	1.000	-0.293	-0.534	-0.541
Time	-0.293	1.000	0.029	-0.061
Sex	-0.534	0.029	1.000	-0.311
Weight	-0.541	-0.061	-0.311	1.000

TABLE G-18

ONE-WAY ANALYSIS OF VARIANCE (ANOVA) FOR THE UPTAKE RATE CONSTANTS FOR SYSTEMS I (ki1), II (ki2) & III (ki3)

Tissues	ki1	ki2	ki3	Total
Gills	0.18	0.12	0.232	0.532 = P1
Muscles	0.353	0.130	0.236	0.718 = P2
G.I.	0.577	0.114	0.227	0.918 = P3
Hepta.	0.241	0.124	0.156	0.521 = P4
	1.35 T1	0.488 T2	0.851 T3	2.869 = G

Source of Variation	SS	df	MS	F Ratio
Between Tissues	0.034547	3	0.011516	1.118
Within Tissues	0.155019	8	0.01938	1.88
ki	0.0932	2	0.0466	4.52
Residual	0.061819	6	0.01030	

TABLE C-19
ONE-WAY ANALYSIS OF VARIANCE (ANOVA) FOR THE
ELIMINATION RATE CONSTANTS FOR SYSTEMS I (ko1),
II (ko2) & III (ko3)

Tissues	ko1	ko2	ko3	Total
Gills	0.457	0.654	1.327	2.438 - P1
Muscles	0.616	0.454	1.185	2.255 - P2
G.I.	0.672	0.654	1.108	2.434 - P3
Hepta.	0.453	0.524	0.969	1.946 - P4
	2.198 T1	2.286 T2	4.589 T3	9.073 - G

Source of Variation	SS	df	MS	F Ratio
Between Tissues	0.0532	3	0.01773	1.318
Within Tissues	0.9997	8	0.12496	9.29
ko	0.919	2	0.4595	34.2
Residual	0.0807	6	0.01345	

TABLE C-20
ONE-WAY ANALYSIS OF VARIANCE (ANOVA) FOR THE BIOCONCENTRATION FACTOR (BCF) FOR SYSTEMS I (BCF1), II (BCF2) & III (BCF3)

Tissues	BCF1	BCF2	BCF3	Total
Gills	0.33	0.177	0.175	0.682 - P1
Muscles	0.572	0.282	0.199	1.236 - P2
G.I.	0.859	0.172	0.205	1.236 - P3
Hepta.	0.532	0.236	0.161	0.929 - P4
	2.293 T1	0.867 T2	0.74 T3	3.9 - G

Source of Variation	SS	df	MS	F Ratio
Between Tissues	0.054	3	0.018	1.10
Within Tissues	0.4697	8	0.0587	3.60
BCF	0.3718	2	0.1859	11.40
Residual	0.0979	6	0.0163	

TABLE C-21
ONE-WAY ANALYSIS OF VARIANCE (ANOVA) FOR THE BIOLOGICAL HALF-LIVES OF CRAWFISH TISSUE IN SYSTEMS I (D1), II (D2) & III (D3)

Tissues	D1	D2	D3	Total
Gills	1.27	1.06	0.52	2.85 = P1
Muscles	1.13	1.53	0.58	3.24 = P2
G.I.	1.03	1.06	0.63	2.72 = P3
Hepta.	1.53	1.32	0.72	3.57 = P4
	4.96 T1	4.97 T2	2.45 T3	12.38 = G

Source of Variation	SS	df	MS	F Ratio
Between Tissues	0.1511	3	0.0504	1.86
Within Tissues	1.2229	8	0.15286	3.63
BCF	1.06	2	0.53	19.52
Residual	0.1629	6	0.02715	