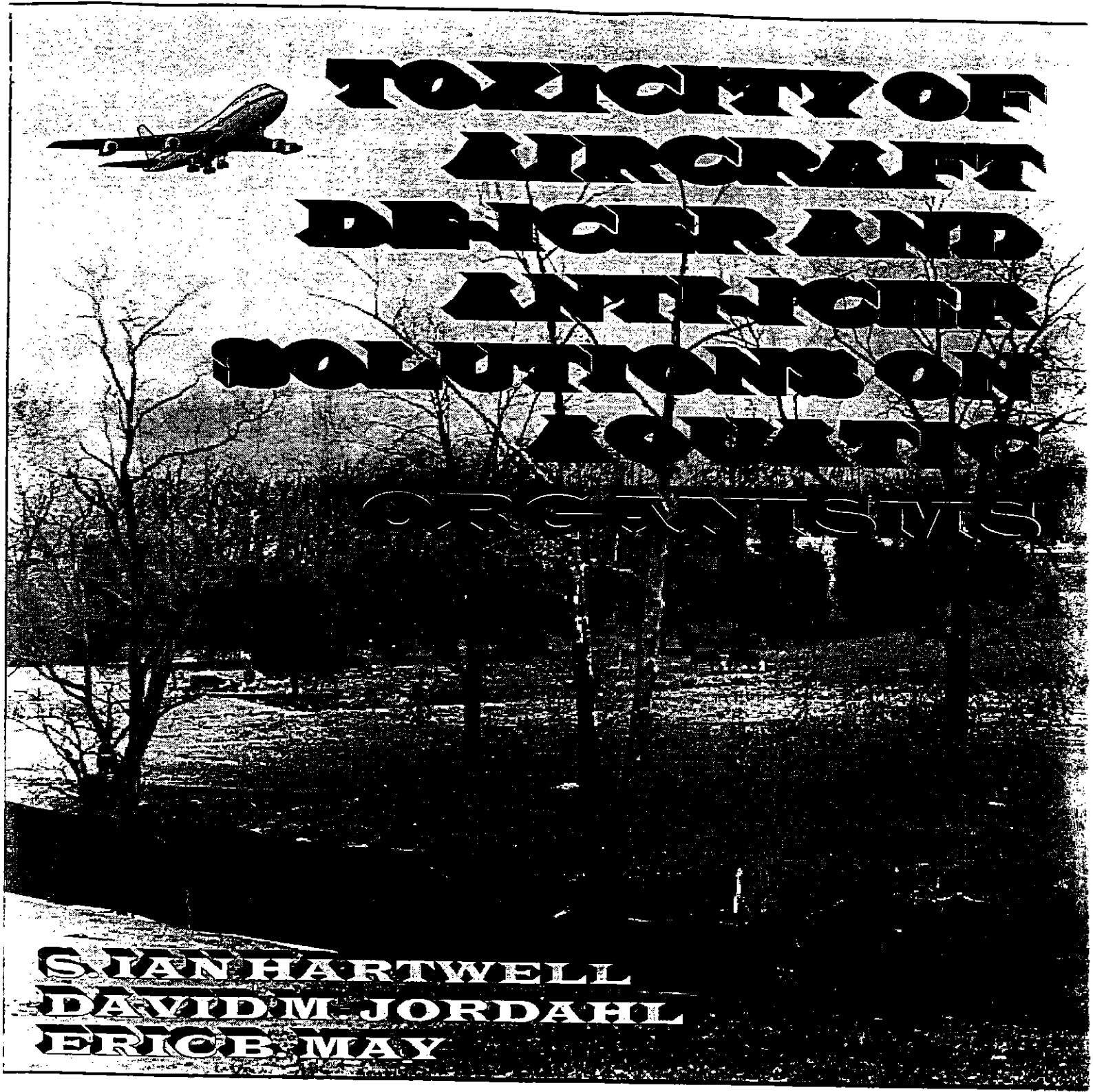

TOXICITY OF AIRCRAFT DEICER AND ANTIICER SOLUTIONS ON AQUATIC ORGANISMS

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Toxicity of Aircraft De-Icer and Anti-Icer Solutions to Aquatic Organisms

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As Secretary of the Maryland Department of Natural Resources, I am convinced that public support of DNR's mission is essential if we are to restore the State's once bountiful natural resources, especially the Chesapeake Bay, to the level which earned the title "America in Miniature". The information in this publication is designed to increase your understanding of our program and of Maryland's natural resources.

Torrey C. Brown, M.D.

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ABSTRACT

Laboratory studies were undertaken to assess the toxicity of industrial mixtures of de-icers and anti-icers used by the aviation industry. Specifically, the two types of anti-freeze solutions were ethylene glycol (de-icer) and propylene glycol (anti-icer), based solutions. Various types of additives and contaminants are also present in these solutions at proportions of ten to twenty percent of the total volume.

Static-renewal toxicity tests were performed at concentrations that bracketed published LC_{50} values for the primary ingredients (9-51 ml glycol/L (9,900-57,000 mg/L)). Water from a stream that receives runoff from a large commercial airport was also tested. These ambient water tests used samples collected during a late winter storm runoff period (March), and a spring baseflow period (April). Fathead minnow (*Pimephales promelas*), *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, and *Photobacterium phosphoreum* (Microtox^R) were used to assess toxicity. Toxic endpoints measured were mortality, reproduction, and bioluminescence. Fathead minnows were further examined for sublethal responses through histological evaluations on freshly killed specimens, exposed at selected concentrations.

The propylene glycol anti-icer solution was found to be more toxic than the ethylene glycol de-icer solution by two orders of magnitude (96 hr LC_{50} range 0.03 - 0.44 ml/L (33-480 mg/L) vs. 3.02 - 13.48 ml/L (3,300-14,800 mg/L), respectively). Both types of solutions exhibited greater toxicity than literature values for the primary ingredients. Toxic effects were observed from the March ambient sample, but not the April sample. Sublethal toxic responses were demonstrated by significant inhibition of reproduction (*C. dubia*) and luminosity (Microtox^R) at all but the lowest concentrations tested. It appears that the additives are the major sources of acute toxicity rather than the glycols.

Histological damage in fathead minnows primarily involved gill, kidney and skin tissue with the most prominent responses seen in fish exposed to the propylene glycol solution. The two solutions caused different histological changes. The ethylene glycol solution elicited respiratory epithelial "disruption" and renal damage, and the propylene glycol solution caused proliferative inflammation and delamination of skin layers.

INTRODUCTION

Ethylene and propylene glycol based defrosting agents are frequently used at airports during winter months to inhibit and prevent ice formation on airplane wings and runways. The glycol-based mixtures contain various types of additives. The percentage composition of glycol in these mixtures is generally between 80 and 90% with the remainder consisting of contaminants, precious metal corrosion inhibitors, rust inhibitors, thickening agents, and surfactants. Contaminants and additives to the ethylene glycol-based de-icers include diethylene glycol, ethylene oxide, dioxane, urea and acetaldehyde. Ethylene oxide is generally two orders of magnitude more toxic than ethylene glycol (Conway et al. 1983). Dioxane and acetaldehyde are suspected carcinogens or teratogens. Ethylene glycol is also a mammalian teratogen. Propylene glycol is not a known carcinogen or teratogen.

There are two distinct kinds of formulations used to remove and/or inhibit ice formation on aircraft. These are referred to as Type 1 and Type 2 in industry terminology. Type 1 formulations are de-icers; they remove ice from the treated surface. Type 2 formulations are anti-icers, which remove ice and stick to the treated surface to inhibit subsequent ice formation while the plane awaits take-off, and during take-off. Both types of formulations can be made with either ethylene or propylene glycol-based solutions. The differences between the two types are in the additives. In addition to rust and precious metal corrosion inhibitors and surfactants, Type 2 anti-icers contain thickening agents (usually polymers) which enable the solution to adhere to the treated surface and therefore inhibit ice formation while the aircraft is stationary. Neutral and anionic polymers are generally not considered to be highly toxic but may cause suffocation or physical damage to gills of aquatic animals at very high concentrations. Cationic polymers, (e.g. polyamines) can be highly toxic to aquatic organisms. The presence of the thickening agents requires a different suite of corrosion inhibitors and surfactants in the solution than those used in Type 1 de-icers. The Type 1 additives interfere with the effectiveness of the thickeners.

All corrosion and rust inhibitors are highly reactive substances designed to attach to, and coat metal surfaces to minimize oxidation. This high chemical activity can translate into high biological toxicity. There are many types of chemicals which can be used as rust and corrosion inhibitors.

Surfactants, like corrosion inhibitors, may be made of a wide variety of compounds. They are wetting agents (detergents) which keep the chemicals of the formulations in solution. Surfactants, as a class of compounds, are also very toxic to aquatic organisms. At acutely toxic concentrations, the primary effect would be damage to gill tissue.

Toxicity of ethylene and propylene glycols is well documented for mammals. However, very little information exists regarding their toxicity in the aquatic environment (Shackleford and Keith 1976, Miller 1979) and their effects on aquatic organisms (Johnson and Finley 1980, Mayer and Ellersieck 1986). The few studies that have been performed on aquatic organisms using nearly pure (i.e., reagent grade) ethylene glycol indicate that acute LC_{50} values for aquatic organisms are generally much greater than 10,000 mg/L, which places these compounds in the relatively harmless category according to the classification scheme used by the U.S. Fish and Wildlife Service (FWS 1984). Toxicological data bases from three sources (EPA-Office of Toxic Substances, EPA-Environmental Research Laboratory AQUIRE toxicity data base, and the U.S. Fish and Wildlife Service) indicate that very high concentrations of glycols (i.e., >10,000 mg/L) are required to cause acute toxicological effects. LC_{50} values for ethylene glycol range from 10,000 mg/L (Conway et al. 1983) to 55,000 mg/L (Mayes et al. 1983) for fathead minnow (*Pimephales promelas*), 41,000 to 57,000 mg/L for *Daphnia magna* (Conway et al. 1983; Cowgill et al.

1985) and 10,000 to 29,700 mg/L for *Ceriodaphnia dubia* (Cowgill et al. 1985). Propylene glycol data are more scarce. For relatively pure (i.e., technical grade) solutions, an LC_{50} value of 44,000 mg/L has been reported for rainbow trout (Mayer and Ellersieck 1986). Chronic effects presumably would occur at lower concentrations. However, we have not located relevant data.

One mechanism leading to ethylene glycol toxicity in mammals involves the formation of oxalate crystals and subsequent necrosis of the kidney following ingestion (Jubb and Kennedy 1970). Little published information is available regarding this response in aquatic organisms, although prolonged exposure to sublethal concentrations of ethylene glycol should result in responses similar to those seen in mammals (Jubb and Kennedy 1970). Furthermore, the kidney may be severely damaged and degenerated without any obvious external evidence of toxicity (i.e., mortality or morbidity). We have not located any data that addresses toxicity of propylene glycol from a histological standpoint.

Low molecular weight glycols used in de-icers generally degrade rapidly. Miller (1979) reported that glycol degradation in the natural environment (i.e., outside of sewage treatment plants) could occur rapidly at 25°C and therefore have very low potential to bioaccumulate. Pitter (1976) studied chemical oxygen demand (COD) of various aliphatic organic substances and reported a degradation rate of 42 mg COD/g/hour, which was the most rapidly degraded substance studied other than sugars or alcohols. He cautioned, however, that standard tests such as the one he employed generally provide a more optimum environment for degradation than would normally be found in nature. Watson and Jones (1977) found that low molecular weight glycols such as ethylene and propylene glycol degrade rapidly under optimum conditions (i.e., high temperatures and dissolved oxygen concentrations, ample substrate, and alternative carbon sources) such as would be found at sewage treatment plants. Haines and Alexander (1975) reported that soil bacteria will utilize ethylene glycol as a carbon source during the breakdown of higher molecular weight glycols, however, degradation would occur more rapidly if a variety of other carbon sources were also available.

Both ethylene and propylene glycols are highly soluble in water and therefore have low bioaccumulation potential. Lokke (1984) studied dispersion and degradation of ethylene glycol on various soil types under temperatures that would commonly be encountered under field conditions (6-10°C). Ethylene glycol was found to have a very low potential to adsorb to soil particles and therefore is prone to rapid movement. Degradation occurs rapidly in the presence of adequate levels of dissolved oxygen; however, in hypoxic or anoxic conditions degradation would occur very slowly. Freitag et al. (1985) studied the environmental hazard profiles of various organic substances and also found ethylene glycol to have low bioaccumulation potential, high biodegradation rates, and be rapidly degraded by light (>280 nm). It appears that ethylene glycol will enter streams rapidly under runoff conditions and due to its short residence time in soils would not have time to degrade significantly. Movement of ethylene glycol in groundwater, which generally has low levels of oxygen and absence of light, would appear to be of concern since it would be slowly degraded under such conditions and enter the stream in an undegraded state.

Study Area

Sawmill Creek is a second order Maryland Coastal Plain stream that has been targeted for restoration by a multi-agency (state and federal) task group. The headwaters of this stream are located within the boundaries of Baltimore-Washington International airport (BWI). The airport is located on 1279 hectares (3,158 acres) in Anne Arundel County Maryland, near Baltimore (Figure 1). BWI commonly uses commercially prepared mixtures of de-icers and anti-icers. Aircraft de-icing operations using mobile equipment occur at the gates and on portions of the runway ramps. Estimated annual glycol use at BWI has been 946,250 to 1,059,800 L/yr (250,000 to 280,000

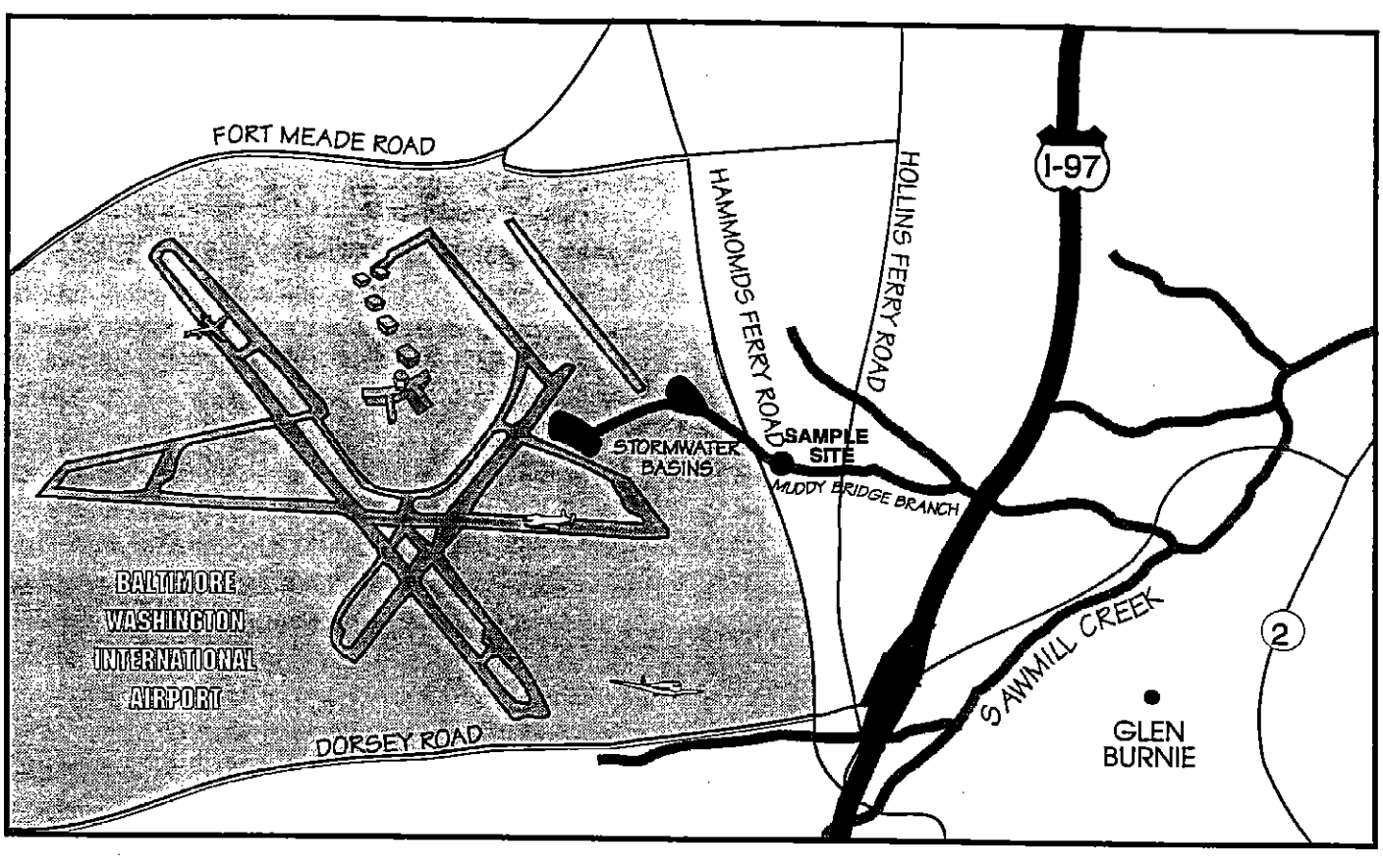

Figure 1. Map of the study area showing airport drainage pattern into Sawmill Creek via Muddy Bridge Branch.

gallons/yr) over the last five years (Greiner Inc. 1991). It is estimated that 80% of the fluids are deposited on the ground due to spray drift, jet blast and wind shear during taxi and take-off. Glycol de-icers have been used in the past on runways and taxiways, but current practices employ salt, sand and urea for ground treatment.

Storm sewer systems drain the gate and ramp areas where aircraft de-icers are used. Some areas also drain to open land where some material is presumably lost to infiltration. The storm drains empty into holding ponds which discharge into Sawmill Creek via Muddy Bridge Branch. The ponds are also fed by perennial creeks within the airport boundaries.

Preliminary Observations

The fish communities of Sawmill Creek are surveyed twice a year by the Maryland Department of Natural Resources (DNR). One of the stream sites appeared to exhibit potential toxic impacts as compared with the other stream sites (Lehman et al. 1992). This site, known as Muddy Bridge Branch, is a tributary of Sawmill Creek and receives runoff directly from the airport ponds. During April 1990 fish specimens of various species were sent to the Fish Health Section of DNR (Oxford, MD) for histopathological examination. In these fish, evidence of kidney damage (i.e., tubular degeneration and necrosis, and the presence of oxalate crystals) was observed. These changes (particularly the presence of oxalate crystals) indicated exposure to sublethal concentrations of ethylene glycol. Furthermore, during a winter runoff event (January 7, 1991) following a snowstorm the previous night, Muddy Bridge Branch appeared unnaturally discolored (pink) and water samples were taken. The concentration of ethylene glycol in the sample was 4800 mg/L, which approaches the lower range of

acutely toxic levels. The following day (January 8) after the runoff had subsided, the concentration of ethylene glycol in the stream had dropped to 300 mg/L.

Concern over the possible toxicological effects from these glycol-based mixtures draining into Sawmill Creek provided the impetus for this study. The objectives were to assess the toxicity of the glycol mixtures to aquatic test organisms using traditional acute and sublethal (chronic) testing methods, compare published toxic concentrations of the primary ingredients to the industrial mixtures, and compare the toxic levels of the mixture to those levels found in Muddy Bridge Branch.

METHODS

Toxicity tests were conducted in the DNR Mobile Bioassay Laboratory stationed at Sandy Point State Park. Two types of test media were used, the glycol mixtures and ambient stream water from Muddy Bridge Branch. The ethylene glycol assay was run during March 1991 and the propylene glycol assay during April 1991. Ambient water grab samples from Muddy Bridge Branch were collected during these months to compare a late winter storm runoff condition (March) with a non-runoff (baseflow) condition (April). The sample location was immediately downstream from the airport boundary. The control water was synthetic freshwater made from deionized water with the appropriate amount of salts added to simulate the basic water chemistry conditions of the water being tested (Table 1), as specified by standard EPA methods (Peltier and Weber 1985, Fisher et al. 1987). Muddy Bridge Branch is classified as having soft to moderately hard water: hardness and alkalinity 40-80 and 30-70 mg/L as $CaCO_3$, respectively; pH range 7.0-7.8; conductivity 200-400 µmhos/cm.

Water collected from Muddy Bridge Branch was transferred to two, 10-L polyethylene carboys, coarse-filtered in the field (to 273 µm), and delivered to the Mobile Bioassay Laboratory immediately (i.e., within one hour) after collection. These ambient samples were then filtered through 73 µm nylon mesh to exclude any potential zooplankton predators or fish parasites. Officials at BWI airport provided the de-icer (ethylene glycol based) and anti-icer (propylene glycol based) solutions, which consisted of a 1:1 proportion of glycol solution and water, the most commonly used formulation. Different proportions of glycol and water may be used depending upon prevailing weather conditions.

The bioassay concentration ranges were selected to bracket the most commonly published LC_{50} values of the glycols (approximately 50,000 mg/L) and the January 7, 1991 ambient ethylene glycol concentration of 4800 mg/L (Table 2). Lower dilutions were added after high mortality occurred in the initial concentrations. Since the solutions tested were not pure compounds, rather a mixture with additives, the concentrations were listed on a nominal volume basis of glycol and cross-referenced with nominal concentrations on a weight basis (Table 2) to compare results with literature values.

Table 1. Means and standard deviations ( ) of physicochemical parameters of test water from Muddy Bridge Branch and synthetic water used in the ambient toxicity tests on fish and zooplankton.

Parameter	March 1991 Ambient	Ethylene Glycol Bioassay	April 1991 Ambient	Propylene Glycol Bioassay	N
			Test Conditions		
Temperature (°C)	21.6 (0.98)	21.6 (0.89)	21.6 (1.27)	21.6 (0.73)	7
Conductivity (µmhos/cm)	210.5 (12.01)	207.2 (40.13)	328.0 (19.31)	265.5 (21.36)	21
Dissolved Oxygen (mg/L)	4.5 (1.06)	5.7 (1.07)	6.4 (0.85)	6.6 (1.61)	21
pH	7.0 (0.32)	7.2 (0.38)	7.4 (0.19)	7.4 (0.10)	7
Alkalinity (mg/L $CaCO_3$)	69.0 (12.97)	90.4 (17.80)	84.2 (3.81)	98.8 (12.06)	7
Ammonia-N (mg/L)	0.003 -	0.145 (0.061)	0.043 (0.018)	0.030 (0.016)	3
			Streamwater Conditions		
[a]Ethylene Glycol (mg/L)	2100	-	[b]<25	-	1
[a]Propylene Glycol (mg/L)	N/A	-	[b]<50	-	1
[a]Discharge (m^3/s)	0.45	-	0.27	-	1

[a]At time of collection (i.e., streamwater grab sample).
[b]Below detectable concentrations.
N/A = Not Analyzed

Juvenile (age ~60d) fathead minnows, three species of zooplankton (*D. magna*, *Daphnia pulex*, and *C.*

Table 2. Dilution series used to assess toxicity of ethylene and propylene glycol solutions to fish and zooplankton. Test solutions were application solutions (1:1 proportion by volume of glycol to water). Nominal volume concentrations are expressed as ml glycol per liter.

[a]Glycol Type Tested	Nominal Volume Concentration (ml glycol/L)	Percent Volume	[b]Nominal Glycol Concentration (mg/L)
P	0.016	0.003	17.2
P	0.031	0.006	34.4
P	0.063	0.013	68.8
P	0.125	0.025	137.5
P,E	0.25	0.05	275.0
P,E	0.5	0.1	550.0
P,E	1.0	0.2	1100.0
P,E	2.0	0.4	2200.0
P,E	4.375	0.875	4812.5
P,E	8.75	1.75	9625.0
P,E	17.5	3.5	19250.0
P,E	35.0	7.0	38500.0
P,E	70.0	14.0	77000.0

[a]P = propylene glycol based anti-icer.
E = ethylene glycol based de-icer.
[b]Density of both glycols ~ 1.1 g/ml.

dubia), and a photoluminescent marine bacterium, *Photobacterium phosphoreum* (Microtox[R]) were the organisms used for toxicity tests. The endpoints for these tests included reduced survival, reproduction, biophotoluminescence and histopathologic effects. Toxicity tests were conducted at a temperature of 21°C (±2°C) for fish and zooplankton, and 15°C for Microtox[R]. Seven day or four day static renewal toxicity tests were performed under the guidelines set forth by EPA (Peltier and Weber 1985, Weber et al. 1989) and the Maryland Department of the Environment (Fisher et al. 1987; Fisher et al. 1988). One exception to the standard protocols was that we used older fathead minnows than recommended since larger fish were needed for histopathologic analysis. The bacterial luminosity tests (Microtox[R]) were run as a dilution series and a non-dilution series assay on selected bioassay glycol solutions, ambient stream water and control water.

For fathead minnows, three replications per dilution level (five fish per replication, due to their size) were tested. The test was run for seven days. The fish were fed brine shrimp nauplii twice a day. Approximately 80% (total volume = 1.5L) of the test media per vessel was renewed daily, shortly after the morning feeding. All surviving fish at the end of the assay were preserved for tissue analysis, as well as moribund fish during the test period. An extra replicate (for certain preselected levels only, Table 3) was run in order to obtain fish for tissue analysis at the beginning (Day 1), middle (Day 4) and end of the test period (Day 7). Fish removed for histological evaluation were preserved whole in Bouin's solution and sent to DNR's Fish Health program at the Cooperative Oxford Laboratory for processing and evaluation of target organs for tissue response (Table 3). In the higher glycol concentrations, unexpectedly high and rapid mortality occurred, which necessitated repeating those levels the following day in order to obtain fish tissue samples prior to death. Time to 100% mortality and median mortality were recorded in hours for those levels in which the fish did not survive beyond the first day.

Table 3. Fathead minnow tissue samples sent to the Department of Natural Resources, Fish Health and Disease Laboratory located at Oxford, Maryland. Samples preserved after less than 24 hours exposure were due to 100% mortality during the first day of testing. Tissues were also preserved following 1, 4 and 7 days exposure to the March and April ambient stream samples.

Nominal Volume Concentration (ml glycol/L)	Days Exposed to Ethylene Glycol De-icer Solution	Days Exposed to PropyleneGlycol Anti-icer Solution
0.0 (control)	**1, 4, 7**	**1, 4, 7**
0.016	-	1, 4, 7
0.031	-	1, 4, 7
0.063	-	1
0.125	-	1
0.25	7	<1
0.50	7	<1
1.0	7	<1
2.0	1, 4, 7	<1
4.375	1, 4, 7	<1
8.75	1, 4, 7	<1
17.5	<1	<1
35.0	<1	<1
70.0	<1	<1

Fish preserved for histological evaluation were processed for paraffin infiltration and embedded, left side down for sectioning. Fish were longitudinally sectioned at 6.0 microns with seven serial sections taken at three levels into the block. Levels were

defined as: 1) at the left eye, 2) immediately internal to the left eye, and 3) at midline. Ribbons were affixed to clean glass slides, and stained using a modified hematoxylin and eosin. Stained sections were sealed using Permount^R and clean cover glass (Luna 1968). Tissues were evaluated using criteria in common use for veterinary pathology (Jones and Hunt 1983) as modified for fish (Ferguson 1989). All major organ systems were scanned in preliminary evaluations to identify target tissues for detailed analysis.

Lesions were tabulated for each fish by organ, organ sublocation, lesion type, lesion distribution within organs, and severity. One or more lesions could apply for each organ. For evaluating the resultant data, target organs were identified for the apparent specificity of glycols toward exposed epithelial tissue (skin and gills) based upon preliminary examination of the slide. In addition, due to the mode of action for ethylene glycol as it is metabolized into oxalate and sequestered into the renal interstitium, the kidney was identified as another target organ. Lesions were classified as mild, moderate or severe. For example, in the gills of a fish showing mild hyperplasia of the respiratory epithelia and severe disruption of gill tissue, the cumulative effect on that individual was recorded as severe, based on disruption.

For both *Daphnid* sp. tests, four replicates per dilution level (ten organisms per replicate) were used. The *Daphnid* tests were run for four days. Organisms were not fed until the second day, when approximately 80% of the test solution was renewed (total volume = 40 ml). The *C. dubia* survival and reproduction test (7 days) required ten replications per level (one organism per vessel). Approximately 80% (total volume = 20 ml) of the test solution was renewed daily. They were fed twice a day, just before (morning) and shortly after solution renewal (afternoon).

The Microtox^R test measures the light output generated by a marine species of photoluminescent bacteria, *P. phosphoreum*. Luminosity was measured at five and fifteen minutes after test initiation. Toxic responses were measured as inhibition (or stimulation) of luminosity over time as compared to a blank sample. Two types of assays were performed, a dilution series and a non-dilution series.

In the dilution series tests, the sample to be tested was diluted along a logarithmicly based concentration gradient to generate an EC_{50}. A dilution series test was performed on control and ambient (March and April stream samples) water, and a stock solution (70 ml glycol/L) of both types of glycol mixtures. Two replications per concentration were performed for each media class (i.e., glycol solutions, ambient and control water).

The non-dilution series assay did not involve a concentration gradient; rather ambient water and selected concentrations of glycol solutions were tested without dilution. Concentrations selected corresponded to those levels used in the fish and zooplankton assays (Table 2). There were four replications per level for the non-dilution series microtox assay.

Probit analysis (SAS 1985) or the Spearman-Karber calculation method (Hamilton et al. 1977) were used (where appropriate) to calculate LC_{50} values for acute effects. A maximum acceptable toxicant concentration (MATC) was calculated to assess chronic, sublethal effects (Horning and Weber 1985) for the *Ceriodaphnia* test. Data were tested for normality with the Shapiro-Wilk's test (SAS 1985). For Microtox^R dilution series assays, the endpoint (decrease in luminosity over time) was compared with rate of light lost in a blank over time, which required a modified probit analysis (Microbics 1988). For non-dilution series tests, comparisons to the control were made with Dunnett's multiple comparison test (Weber et al. 1989). Additionally, comparisons among the treatment groups were made with a Duncan's multiple range test (SAS 1985). Significance was established at $\alpha = 0.05$ for all tests.

RESULTS

Physicochemical parameters of ambient and synthetic test water measured during the bioassays are presented in Table 1. The most apparent differences between the March and April ambient samples were ammonia nitrogen, conductivity and ethylene glycol concentration. The ammonia nitrogen value from the March sample appears to be anomalous. None of the ammonia levels were high enough to bias the tests. Conductivity of the April baseflow sample was nearly twice the value of the March sample. Conversely, ethylene glycol concentrations were below detectable levels during April. The concentration of ethylene glycol (4800 mg/L) associated with an earlier January, 1991 sample (which corresponded with a more intense snowmelt event) was over two times the March value. The pH was slightly lower during March than April, possibly associated with the storm event.

Toxicity Test Results

Table 4 presents calculated LC_{50} and reproductive MATC values for fathead minnows and zooplankton. Propylene glycol (i.e., anti-icer solution) was more toxic than ethylene glycol (de-icer solution) by two orders of magnitude for fish and zooplankton. For both glycol solutions, LC_{50} values for zooplankton decrease by a factor of two to ten times, between the second and fourth day. For fish, this trend occurs with propylene glycol only. The relative degree of sensitivity is time dependent. The LC_{50} values for fish did not decline significantly over time, whereas the values for the daphnids decreased conspicuously over two to four days. *C. dubia* exhibited similar sensitivity to ethylene glycol as the fish after four days exposure, but were more sensitive than the fish after seven days. Concentrations of the ethylene glycol solution associated with reproductive effects are an order of magnitude lower than acute levels. For the propylene glycol solution, short-term chronic and acute levels are similar.

Table 4. Calculated LC_{50} and MATC endpoints from toxicity tests of commercial aircraft de-icers (ethylene glycol based) and anti-icers (propylene glycol based) to fish and zooplankton. LC_{50} with confidence intervals ( ) and reproductive MATC values are expressed as ml glycol/L.

	Ethylene Glycol (de-icer)			Propylene Glycol (anti-icer)		
Species	48h	96h	168h	48h	96h	168h
Fathead Minnow	9.82 (8.30 11.63)	9.82 (8.30 11.63)	9.82 (8.30 11.63)	0.07 (0.04 0.09)	0.03 (0.02 0.04)	0.03 0.02 0.03)
Daphnia magna	13.48 (9.68 18.76)	3.83 (2.32 6.35)	-.-	0.24 (0.19 0.30)	0.05 (0.04 0.06)	-.-
Daphnia pulex	8.44 (6.39 11.15)	4.25 (3.15 5.74)	-.-	0.27 (0.23 0.33)	0.06 (0.04 0.08)	-.-
Ceriodaphnia dubia	12.85 (9.66 15.85)	8.95 (6.28 13.02)	3.02 (1.72 5.22)	0.44 (0.19 0.81)	0.12 (0.07 0.20)	0.07 (0.05 0.11)
C. dubia Reproduction MATC	-.-	-.-	0.38	-.-	-.-	0.05

All fish died within a few hours in the highest concentrations of ethylene glycol and most concentrations of propylene glycol tested (Table 5). Fish exposed to these solutions exhibited immediate signs of stress and tended to stay near the surface, gulping air. Loss of equilibrium occurred shortly before death. For ethylene glycol, 100% mortality occurred in concentrations as low as 17.5 ml glycol/L. One third of the fish died by the second day (with no

Table 5. Time (hours) to median and total mortality for fathead minnows exposed to various concentrations of ethylene and propylene glycol solutions.

Nominal Volume Concentration (ml glycol/L)	Ethylene Glycol (de-icer)		Propylene Glycol (anti-icer)	
	Median	Total	Median	Total
0.016	-.-[a]	-.-[a]	N/O[b]	N/O[c]
0.031	-.-[a]	-.-[a]	84	N/O[c]
0.063	-.-[a]	-.-[a]	48	96
0.125	-.-[a]	-.-[a]	36	72
0.25	N/O[b]	N/O[c]	12	48
0.5	N/O[b]	N/O[c]	3.25	6.0
1.0	N/O[b]	N/O[c]	2.0	3.25
2.0	N/O[b]	N/O[c]	1.0	3.0
4.375	N/O[b]	N/O[c]	0.5	2.5
8.75	N/O[b]	N/O[c]	N/A[d]	2.0
17.5	2.5	4.5	N/A[d]	1.0
35.0	1.5	2.5	N/A[d]	0.5
70.0	N/A[d]	0.75	N/A[d]	0.25

[a]Concentrations of ethylene glycol solution that were not tested (i.e., below test levels).
[b]N/O = not observed, less than 50% mortality at test termination.
[c]N/O = not observed, less than 100% mortality at test termination.
[d]N/A = not applicable, all fish died at the same time.

further mortality after this day) in the 8.75 ml glycol/L concentration, and all fish survived in the 4.375 ml glycol/L and lower concentrations (Appendix Table 1). A similar trend occurred with propylene glycol, however, total mortality occurred at lower concentrations. All fish died after four days in propylene glycol concentrations as low as 0.063 ml glycol/L. After seven days, 60% of the fish were dead in the 0.031 ml glycol/L concentration. One fish died in the lowest concentration tested, 0.016 ml glycol/L.

With zooplankton, more partial mortalities (less than 100% mortality per replicate) occurred across the concentration gradient as compared to fish (Appendix Tables 2 and 3). Mortality occurred in lower concentrations of propylene glycol than ethylene glycol. Significant inhibition of *C. dubia* neonate production occurred at ethylene glycol levels as low as 0.5 ml glycol/L, whereas significant inhibition of reproduction occurred at 0.063 ml glycol/L for propylene glycol (Appendix Table 4).

There were no fish mortalities in either of the two ambient stream samples collected from Muddy Bridge Branch (Table 6). There was significant mortality for *D. magna* and *D. pulex* after two and four days for the March ambient sample (Table 6). For *C. dubia*, three adults died after seven days in the March ambient group, but no deaths occurred in the April group (Table 6). A significant decrease in neonate production also occurred with the March ambient test, but a significant increase in reproduction occurred for the April ambient test (Appendix Table 4).

Table 6. Cummulative percent survival of fathead minnows *Daphnia magna*, *Daphnia pulex* and *Ceriodaphnia dubia* from toxicity tests of ambient water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow conditions (April), respectively.

Species	March (winter storm)			April (spring baseflow)		
	48h	96h	168h	48h	96h	168h
Fathead Minnow	100	100	100	100	100	100
Daphnia magna	*65	*55	-.-	100	92	-.-
Daphnia pulex	*78	*70	-.-	95	92	-.-
Ceriodaphnia dubia	100	100	70	100	100	100

*Significant reduction in survival or reproduction as compared to control (Dunnett's test, $\alpha = 0.05$).

Histological Evaluation

Dilution Series - Acute Results

At the 70 ml/L ethylene glycol concentration, the main response was a combination of respiratory cell necrosis and disruption of the respiratory epithelium. This was consistent with observed behavioral responses of fish during the experiments (i.e., staying near the surface and gulping air). These gill changes were consistent through dilutions of 2.0 ml/L ethylene glycol de-icer (Table 7). Renal (kidney) change was seen as interstitial congestion at high concentrations. One fish exhibited moderate tubal necrosis

Table 7. Histological responses of fathead minnows to varying concentrations of ethylene glycol de-icer and propylene glycol anti-icer after 1, 4 and 7 days exposure. Five fish per concentration were examined.

Nominal Volume Concentration (ml glycol/L)	Lesion Distribution By Day								
	Ethylene Glycol (de-icer)								
	1			4			7		
	Skin	Gill	Kidney	Skin	Gill	Kidney	Skin	Gill	Kidney
0.25	0	0	0	0	0	0	0	**	0
0.5	0	0	0	0	0	0	0	**	*
1.0	0	0	0	0	0	0	0	***	***[b]
2.0	0	****	**	0	***	***[b]	0	***	***[b]
4.375	0	****	***[b]	0	***	***[b]	0	***	***[b]
8.75	0	****	*	0	****	*[b]	0	***	***[b]
[a]17.5	0	****	0	-	-	-	-	-	-
[a]35.0	0	****	*	-	-	-	-	-	-
[a]70.0	0	****	****	-	-	-	-	-	-
	Propylene Glycol (anti-icer)								
0.016	0	**	0	0	*	0	0	***	0
0.031	0	*	0	***	****	0	*	****	0
[a]0.063	0	****	0	-	-	-	-	-	-
[a]0.125	****	****	0	-	-	-	-	-	-
[a,c]0.25	****	****	0	-	-	-	-	-	-

[a]No survivors past day 1 exposure (<24 hrs).
[b]Oxalate crystals observed.
[c]No survival at higher concentrations, lesions identical to those observed at 0.25 ml/L.

KEY

0 = no lesions.
* = lesions mild in <3 fish
** = lesions mild in 3 or more fish
*** = lesions moderate in 3 or more fish
**** = lesions severe in 3 or more fish

at 35 ml/L ethylene glycol. Concentrations of 8.75, 4.375, 2.0, 1.0, 0.5 and 0.25 ml/L glycol are considered under short-term chronic results as fish survived to study termination after seven days exposure (see below).

Exposure to concentrations of 70.0 through 0.5 ml/L of the propylene glycol solution resulted in 100% mortality in less than 24 hours. The primary lesions observed following exposure to 0.25 ml/L propylene glycol were on the gills and skin (Table 7). Gill lesions manifested as a severe proliferative inflammation which obliterated interlamellar spaces and may have effectively stopped respiration. Skin lesions manifested as delamination of the epidermis from the dermis. At a test concentration of 0.063 ml/L only the gill lesions were seen. Concentrations of 0.031 and 0.016 ml/L are considered under short-term chronic results as fish survived to study termination after seven days exposure (below).

Dilution Series - Short-Term Chronic Results

Fish survived seven days exposure to the ethylene glycol de-icer solution at concentrations of 8.75 through 0.25 ml/L. At 8.75 and 4.375 ml/L, gill lesions included edema, lamellar disruption, and respiratory cell hypertrophy and necrosis. Lesions following 24 hours were severe but appeared to diminish to moderate after four and seven days exposure (Table 7). Seven days exposure to test concentrations of 0.25 through 2.0 ml/L caused respiratory epithelial necrosis of the gills. Additional edema and

hypertrophy resulted at 0.5 through 2.0 ml/L exposure. These latter concentrations did not cause disruption of the respiratory epithelia as seen with 4.375 and 8.75 ml/L exposure concentrations.

Renal lesions appeared in fish exposed to ethylene glycol de-icer after one day at 8.75, 4.375 and 2.0 ml/L concentrations. The lesions manifested as acute tubular necrosis and the appearance of oxalate crystals in two individuals examined. Lesions at 0.5 through 2.0 ml/L exposure consistently manifested as acute tubular necrosis, however oxalate crystal formation did not occur below 1.0 ml/L. Crystal appearance was not consistently observed in all fish specimens.

Severe and moderate proliferative inflammation in the gills resulted following seven days of exposure to 0.031 and 0.016 ml/L propylene glycol, respectively (Table 7). In addition, at a test concentrations of 0.016 ml/L mild to moderate edema and respiratory cell hypertrophy were also seen.

Following four and seven days of exposure to a concentration of 0.031 ml/L the skin was seen to exhibit focal detachment of the epidermis similar, but not identical, to that seen in the skin of fish exposed to higher concentrations of propylene glycol.

Controls and Ambient Stream Exposure

Mild lamellar congestion and edema and mild gill epithelial hypertrophy were present in fish exposed to March control water after 24 hours, returning to normal after four days, with no responses seen at four and seven days exposure (Table 8). Lesions were not observed in the kidneys or skin. No responses were seen in skin and kidney at any time from exposure to control water during the April tests.

The gills of fish exposed to the April ambient stream sample consistently exhibited moderate lamellar fusion, proliferative inflammation, and lamellar edema after 24 hours of exposure (Table 8). These effects remained moderate throughout seven days exposure with the exception of edema which receded by the seventh day. The March ambient test resulted in severe necrosis and congestion of the respiratory epithelium of all fish exposed for 24 hours, and mild edema in gills of two of five fish after four and seven days. After four and seven days exposure, the necrosis and congestion receded to moderate but was present in all five fish, and the response also included edema, epithelial hypertrophy and lamellar fusion. These changes were qualitatively more severe

Table 8. Histological responses of fathead minnows to control and ambient stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow conditions (April), respectively. Five fish per treatment were examined.

	Lesion Distribution By Day								
	1			4			7		
Treatment	Skin	Gill	Kidney	Skin	Gill	Kidney	Skin	Gill	Kidney
March Control	0	*	0	0	0	0	0	0	0
March Ambient	0	****	0	0	***	*	0	***	***a
April Control	0	0	0	0	*	0	0	*	0
April Ambient	0	***	0	0	***	0	0	***	0

[a]Oxalate crystals observed.

KEY
0 = no lesions
* = lesions mild in < 3 fish
** = lesions mild in 3 or more fish
*** = lesions moderate in 3 or more fish
**** = lesions severe in 3 or more fish

in comparison to fish exposed to control and the April ambient water.

The kidneys responded after only four days of exposure to March ambient water, exhibiting mild tubular necrosis in two of five fish, and increasing in severity to moderate tubular necrosis in all five fish exposed for seven days, with crystals refractive to polarized light (indication of presence of oxalate) seen in interstitial renal tissue and subtubular zones in four of five fish. No effects of skin tissue were seen in any of the ambient stream samples.

Microtox Response

Dilution Series

A dose-response trend began to appear after fifteen minutes incubation with the ethylene glycol solution, while a trend was readily apparent with the propylene glycol solution after only five minutes. The EC_{50} for ethylene glycol de-icer was greater than the EC_{50} for propylene glycol anti-icer in these assays (Table 9).

Table 9. EC_{50} values from dilution series Microtox^R assays for ethylene and propylene glycol solutions and stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow conditions (April), respectively. EC_{50} values are expressed as ml glycol/L.

Test	5 minutes	15 minutes
Control	NR	NR
March ambient	NR	NR
April ambient	NR	NR
Ethylene glycol (de-icer)	NR	32.2
Propylene glycol (anti-icer)	53.2	21.7

NR = no response

An EC_{50} was not reached for either the March or April ambient water samples. However, a dose-response trend appeared after fifteen minutes which was one of stimulation rather than inhibition. There was no trend across the concentration gradient for the control water.

Non-dilution Series

Statistically significant decreases in luminosity were observed in the ethylene glycol de-icer assays at all test concentrations in both five and fifteen minute incubations (Table 10). The propylene glycol anti-icer dilution series included lower test concentrations than the ethylene glycol de-icer series. Significant decreases in luminosity were observed at concentrations above 2.0 and 0.125 ml glycol/L at five and fifteen minute incubations respectively. After five minute incubations, propylene glycol anti-icer stimulated the bacteria at 0.016, 0.031, and 0.25 ml glycol/L. No stimulation was observed in the fifteen minute incubation series. At the intermediate concentration of 0.25 ml/L, stimulation after five minutes was followed by significant inhibition after fifteen minutes exposure.

Table 10. Results of Duncan multiple comparison tests for non-dilution series Microtox^R assays for selected concentrations of ethylene and propylene glycol solutions, and stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow conditions (April), respectively. Concentrations with the same letter are not significantly different at $\alpha = 0.05$.

Treatment	5 minute	15 minute
Control	A	A
March Ambient	*AA	B
April Ambient	*AA	*AA
Nominal Volume Concentration (ml glycol/L)		
	Ethylene Glycol (de-icer)	
0.25	B	B
2.0	C	C
8.75	D	D
17.5	D	D
70.0	E	E
	Propylene Glycol (anti-icer)	
0.016	*AA	A
0.031	*AA	A
0.063	*AA	A
0.125	A	B
0.25	*AA	C
2.0	B	D
8.75	C	D
17.5	C	D
70.0	D	D

*AA = Stimulation, significant enhancement of luminosity as compared to controls.

DISCUSSION

Both ethylene glycol de-icer and propylene glycol anti-icer solutions were more toxic than literature values for the pure glycol compounds. The higher toxicity was presumably due to the additives present in these solutions. The propylene glycol based anti-icer is more toxic than the ethylene glycol based de-icer by one to two orders of magnitude, depending on test species. This is supported by histological observations where acute lesions of a lethal nature were seen at all but the lowest concentrations of the propylene glycol solution. It is likely that the higher toxicity of the propylene glycol solution is due to the presence of thickening agents and/or the particular suite of additives in this type of formulation.

Some examples of the various types of additives and contaminants contained in the de-icer and anti-icer solutions include diethylene glycol, ethylene oxide, acetaldehyde, dioxane, high molecular weight polymers, polyamines, triazoles and urea, all of which may be more toxic than either ethylene or propylene glycols. For example, Conway et al. (1983) compared the toxicity of ethylene glycol and ethylene oxide to fathead minnows and *D. magna*. He reported LC_{50} values greater than 10,000 mg/L of ethylene glycol for both organisms, however the LC_{50} values for ethylene oxide were 90 mg/L for fathead minnow and 200-300 mg/L for *D. magna*. In addition to the additives previously mentioned, other types of corrosion and rust inhibitors are routinely added to industrial glycol solutions. Lokke (1984) reported that sodium nitrite, sodium benzoate, borax, and benzotriazole are commonly used as rust and corrosion inhibitors. These compounds may also be more toxic than pure glycol solutions. It appears that the additives are either highly toxic and/or synergistic with the glycols.

The data derived from the Microtox^R assay also confirms that the propylene glycol anti-icer solution was more toxic than the ethylene glycol de-icer solution. Toxicity of the propylene glycol solution occurred almost immediately whereas some time was required for the ethylene glycol solution to induce toxicity. Intermediate concentrations and high levels of both glycol solutions showed significant inhibition of bacterial luminosity. Stimulatory responses appeared at very low levels of propylene glycol initially, followed by inhibition with the passage of time. The intermediate concentration (0.25 ml glycol/L) initially exhibited stimulation followed by significant inhibition. This effect appears consistent with metabolic processes that are generally stimulated by low levels of toxicants or short exposure times, followed by subsequent inhibition at higher levels or longer exposure times (Pritchard and Bourquin 1985, Nyholm et al. 1992, Microbics 1991). This type of stimulatory/inhibition effect did not occur with the ethylene glycol solution.

There were no acute toxicological effects to fathead minnows exposed to ambient (Muddy Bridge Branch) stream water. Ambient water collected in March did cause acute toxic effects in *D. magna* and *D. pulex*. There was also a significant reduction in the number of neonates produced by *C. dubia* exposed to the March ambient sample. The ambient levels were as toxic to zooplankton as similar concentrations of ethylene glycol de-icer solution tested in the laboratory. The March ambient stream sample also showed toxic effects in the Microtox^R tests. While the effect did not reach an EC_{50} level of effect, the decrease in luminosity (relative to controls) was statistically significant after fifteen minutes incubation. Histological responses in the gills and kidneys of fish were similar to those observed in exposure tests using the ethylene glycol solution mixed with control water in the laboratory bioassays. This suggests that glycol and additive levels during winter storm runoff periods are sufficient to cause acute and chronic toxicity. The April ambient sample did not induce toxicity in any test organism, which corresponded with undetectable levels of either ethylene or propylene glycol. There was significant enhancement of

reproduction by *C. dubia* in the April sample. Enhancement in reproduction may be due to increased availability of micronutrients and possibly a greater variety of food (even after 73 µm filtration) in stream water as compared to synthetic control water (Tester and Turner 1990, Stottrup and Jensen 1990). Cowgill et al. (1985) reported enhanced reproductive performance and increased resistance to toxicants in zooplankton (*D. magna*) with an increase in the variety of natural foods. This is consistent with stimulation of luminosity in the Microtox^{R} tests using ambient water samples. Significant stimulation in the Microtox^{R} tests did occur but since this was not followed by inhibition it does not appear to be a toxic effect. Rather the ambient water probably provided more micronutrients and alternative carbon sources for the microbes as compared with the control water (synthetic freshwater). Histological lesions in fish tested with April ambient water were minimal, typical of nutrient enriched systems (Ferguson 1989, May et al. 1985). The lesions involved fusion and edema of the secondary lamellae, as opposed to those seen from the March ambient tests which included tissue necrosis and hyperplastic changes (cell proliferation), which are more severe degrees of tissue damage.

It appears that since toxicity was not detected during non-runoff periods, elevated levels of ethylene and propylene glycol solutions and their additives were at least partly responsible for the toxicity observed during runoff periods. This does not preclude the possibility of other contaminants from airport operations (e.g., fuel spills) from exerting a toxic impact on receiving streams.

Chronicity is a measure of the relative difference between acute and chronic toxicity levels. The acute to chronic ratio (chronicity) of the ethylene glycol deicer solution is larger than the ratio for the propylene glycol anti-icer solution. While the toxic concentration is of greater concern for the propylene glycol solution, larger levels of chronicity of the ethylene glycol solution indicate that sublethal impacts of ethylene glycol and or the additives on resident biota are also of concern in Muddy Bridge Branch.

Since the mode of sublethal toxic action for ethylene glycol in renal tissue is via metabolism into oxalates which then become sequestered into tissue surrounding the kidney tubules, chronicity becomes of great concern. During April 1990, fish from Sawmill Creek were sampled and the kidneys of these fish examined for signs of damage. In tessellated darters (*Etheostoma olmstedi*), oxalate crystals appeared in the interstitial tissue of the kidneys and basal layers of the tubules, with apparent loss of kidney tubules (Evans 1990). American eels (*Anguilla rostrata*) exhibited kidney lesions consistent with oxalate damage, but crystals were not present (Evans 1990). This was over a month since the last de-icing operation of the 1990 winter season. Thus, the chronic effects may be long lasting and could result in permanent debilitation.

Since the present study was conducted for only seven days, it is not possible to verify these findings. However, from Table 7 it can be seen that exposure to ethylene glycol for seven days results in oxalate crystal formation at all but the two lowest concentrations. Crystals formed after four days exposure to glycol concentrations in the 2.0 - 8.75 ml/L range. The observed lesions were consistent with those seen in darters from the earlier (April 1990) field samples. Thus, it appears that chronic responses to ethylene glycol do occur in the kidneys of fish but it is unknown if the lesions disappear and recovery results, or if these lesions are permanent.

The renal lesions seen in fish exposed to ethylene glycol are consistent with the formation of oxalate which is toxic to tubular epithelia of the proximal segments of the kidney in mammals. The location of the crystals is not entirely consistent with crystals reported in mammals exposed to ethylene glycol (Jones and Hunt 1983). The mechanism of renal response to ethylene glycol toxicity in fish needs further study.

Two different modes of action of the glycols and/or the additives appear to be operating with respect to the gills. The ethylene glycol solution appears to directly affect the respiratory epithelia causing cell

necrosis and breakdown of cell to cell connection (disruption). The propylene glycol solution stimulates a severe proliferative response of the sub-basal tissue below the respiratory epithelium obliterating the inter-lamellar space. While the end result is loss of respiratory function in both cases, this comes about by two different mechanisms. Whether this is a function of ethylene vs. propylene glycol difference, or a function of the additives in anti-icing vs. de-icing mixtures cannot be determined by this study. Skin lesions resulting from exposure to propylene glycol solutions are unique and need further study.

CONCLUSIONS

Ethylene and propylene glycol based anti-freeze solutions are more toxic than pure ethylene and propylene glycol, respectively. Additives and contaminants are implicated as the cause of increased toxicity relative to the glycols themselves. The low percentage composition of the additives and contaminants (10% to 20%) and the higher toxicity we found as compared to published data for pure solutions of both ethylene and propylene glycol supports this conclusion. Some synergism between the additives and/or the glycols may also be involved. The comparatively higher toxicity of the propylene glycol solution may be due to the specialized type of additives (i.e., thickening and adhering agents) and the corresponding rust and corrosion inhibitors used with them. Although ethylene and propylene glycols are degraded in the environment under suitable conditions, the degradation of the additives is unknown since their composition is proprietary. Furthermore, during winter runoff periods the glycols may not have sufficient time to degrade and toxic effects from the glycols themselves as well as the additives may occur. At high glycol levels it appears that damage to fish kidneys would occur and, depending upon the magnitude, intensity, and frequency of glycol runoff pulses into the stream, the kidneys of resident fish could become irreversibly damaged. However, since the short-term chronic assays terminated at seven days, long term responses are not known. Acute (*Daphnia* sp.) and chronic (*C. dubia* and fathead minnow) toxic effects were observed during a seven day exposure at ambient levels (2,100 mg/L, March) which are well below levels reported in Muddy Bridge Branch two months earlier (4,800 mg/L, January 1991) before this study commenced.

To better assess the environmental risk of the de-icer and anti-icer solutions, a future investigation will need to run parallel tests with these solutions and pure solutions of ethylene and propylene glycol to address the question of toxicity of additives. Daily monitoring of glycol levels in both types of dilution series (i.e., pure solutions versus the de-icer, anti-icer solutions) and ambient samples would allow us to determine whether or not the glycols are being degraded rapidly enough to be of concern. Moreover, it may allow us to infer the degradation rate of the additives. Monitoring glycols in the stream during runoff events would provide better information on the concentrations and length of exposures of resident biota. Also, no data currently exists on sediment toxicity, which could be an important sink for the additives.

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Table A-1. Results of acute toxicity tests exposing juvenile fathead minnows (approximately 60 days old) to ethylene glycol de-icer and propylene glycol anti-icer solutions, and stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow (April) conditions, respectively. Results are expressed as cumulative mean percent survival, N=3 per treatment level or concentration.

	[a]March			[b]April		
Treatment	48h	96h	168h	48h	96h	168h
Control	100	100	100	100	100	100
Ambient	100	100	100	100	100	100
Nominal Volume Concentration (ml glycol/L)	Ethylene Glycol (de-icer)			Propylene Glycol (anti-icer)		
0.016	-[c]	-[c]	-[c]	93	93	93
0.031	-[c]	-[c]	-[c]	80	*53	*40
0.063	-[c]	-[c]	-[c]	*40	0	0
0.125	-[c]	-[c]	-[c]	*47	0	0
0.25	100	100	100	0	0	0
0.5	100	100	100	0	0	0
1.0	100	100	100	0	0	0
2.0	100	100	100	0	0	0
4.375	100	100	100	0	0	0
8.75	67	67	67	0	0	0
17.5	0	0	0	0	0	0
35.0	0	0	0	0	0	0
70.0	0	0	0	0	0	0
LC_{50} (ml/L)	9.82	9.82	9.82	0.067	0.030	0.027

*Significant reduction in survival as compared to control (Dunnett's test, α=0.05).

[a]March ambient sample tested in conjunction with ethylene glycol de-icer assay (March 1991).
[b]April ambient sample tested in conjunction with propylene glycol anti-icer assay (April 1991).
[c]Concentrations of ethylene glycol de-icer not tested (below test levels).

Table A-2. Results of acute toxicity tests exposing *Daphnia magna* and *Daphnia pulex* to ethylene glycol de-icer and propylene glycol anti-icer solutions, and stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow (April) conditions, respectively. Results are expressed as cumulative mean percent survival, N=4 per treatment level or concentration.

	[a]March				[b]April			
	D. magna		D. pulex		D. magna		D. pulex	
Treatment	48h	96h	48h	96h	48h	96h	48h	96h
Control	95	85	100	100	100	95	100	90
Ambient	*65	*55	*78	*70	100	92	95	92
Nominal Volume Concentration (ml glycol/L)	Ethylene Glycol (de-icer)				Propylene Glycol (anti-icer)			
0.016	-[c]	-[c]	-[c]	-[c]	80	80	92	70
0.031	-[c]	-[c]	-[c]	-[c]	85	*60	90	*68
0.063	-[c]	-[c]	-[c]	-[c]	82	*55	92	*55
0.125	-[c]	-[c]	-[c]	-[c]	80	*5	88	*0
0.25	85	75	95	90	*52	*0	*68	*0
0.5	88	70	90	80	*10	*0	*8	*0
1.0	82	*62	88	*72	-[d]	-[d]	-[d]	-[d]
2.0	82	*60	*78	*68	-[d]	-[d]	-[d]	-[d]
4.375	78	*60	*75	*68	-[d]	-[d]	-[d]	-[d]
8.75	*60	*40	*45	*42	-[d]	-[d]	-[d]	-[d]
17.5	*55	*25	*35	*22	-[d]	-[d]	-[d]	-[d]
35.0	*28	*5	*30	*12	-[d]	-[d]	-[d]	-[d]
70.0	*5	*0	*0	*0	-[d]	-[d]	-[d]	-[d]
LC_{50} (ml/L)	13.48	3.83	8.44	4.25	0.24	0.05	0.27	0.06

*Significant reduction in survival as compared to control (Dunnett's test, α=0.05).

[a]March ambient sample tested in conjunction with ethylene glycol de-icer assay (March 1991).
[b]April ambient sample tested in conjunction with propylene glycol anti-icer assay (April 1991).
[c]Concentrations of ethylene glycol de-icer not tested (below test levels).
[d]Concentrations of propylene glycol anti-icer not tested (above test levels).

Table A-3. Results of acute toxicity tests exposing *Ceriodaphnia dubia* to ethylene glycol de-icer and propylene glycol anti-icer solutions, and stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow (April) conditions, respectively. Results are expressed as cumulative mean percent survival, N=10 per treatment level or concentration.

Treatment	[a]March 48h	96h	168h	[b]April 48h	96h	168h
Control	100	100	100	100	100	100
Ambient	100	100	70	100	100	100
Nominal Volume Concentration (ml glycol/L)	Ethylene Glycol (de-icer)			Propylene Glycol (anti-icer)		
0.016	-[c]	-[c]	-[c]	90	90	90
0.031	-[c]	-[c]	-[c]	80	80	80
0.063	-[c]	-[c]	-[c]	100	80	80
0.125	-[c]	-[c]	-[c]	80	*40	*30
0.25	100	100	90	70	*30	*0
0.50	100	100	90	*30	*0	*0
1.0	100	100	90	-[d]	-[d]	-[d]
2.0	100	100	*60	-[d]	-[d]	-[d]
4.375	100	100	*60	-[d]	-[d]	-[d]
8.75	90	100	*60	-[d]	-[d]	-[d]
17.5	*10	*10	*10	-[d]	-[d]	-[d]
35.0	*0	*0	*0	-[d]	-[d]	-[d]
70.0	*0	*0	*0	-[d]	-[d]	-[d]
LC_{50} (ml/L)	12.85	8.95	3.02	0.44	0.12	0.07

*Significant reduction in survival as compared to control (Fisher's exact test, α=0.05).

[a]March ambient sample tested in conjunction with ethylene glycol de-icer assay (March 1991).
[b]April ambient sample tested in conjunction with propylene glycol anti-icer assay (April 1991).
[c]Concentrations of ethylene glycol de-icer not tested (below test levels).
[d]Concentrations of propylene glycol anti-icer not tested (above test levels).

Table A-4. Results of short-term chronic toxicity tests exposing *Ceriodaphnia dubia* to ethylene glycol de-icer and propylene glycol anti-icer solutions, and stream water from Muddy Bridge Branch, representative of winter storm runoff (March) and spring baseflow (April) conditions, respectively. Results are expressed as six reproductive parameters (key). Chronic test endpoints expressed as ml glycol/L.

	[a]March						[b]April					
Treatment	A	B	C	D	E	F	A	B	C	D	E	F
Control	118	11.8	10	0	0	19	130	13.0	10	0	0	20
Ambient	*65	9.3	7	1	1	14	**170	17.0	10	0	0	34
Nominal Volume Concentration (ml glycol/L)	Ethylene Glycol						Propylene Glycol					
	A	B	C	D	E	F	A	B	C	D	E	F
0.016	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	122	13.6	9	0	0	25
0.031	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	107	13.4	8	0	0	25
0.063	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	*54	9.0	6	2	0	12
0.125	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]	*50	10.0	5	1	3	14
0.250	87	10.9	8	2	1	14	*21	10.5	2	0	2	11
0.5	*62	10.3	6	4	1	14	*0	0	0	0	0	0
1.0	*63	9.0	7	1	2	16	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
2.0	*56	8.0	7	0	1	13	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
4.375	*36	9.0	4	2	0	14	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
8.75	*17	5.7	3	1	1	7	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
17.5	*7	7.0	1	0	0	7	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
35.0	*0	0	0	0	0	0	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
70.0	*0	0	0	0	0	0	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]

Chronic Toxicity Test Endpoint

	Ethylene Glycol De-Icer	Propylene Glycol Anti-Icer
NOEC	0.25	0.031
LOEC	0.5	0.063
MATC	0.375	0.047

Key
A = Total number of neonates produced over the seven day test period.
B = Mean number of neonates produced per adult.
C = Number of reproducing individuals.
D = Number of non-reproducing survivors.
E = Number of adults that did not survive after reproducing.
F = Maximum number of neonates produced by one individual.

*Significant change in parameter, as compared to control. (Dunnett's test, α=0.05).
Significant **enhancement in neonates produced as compared to control (Dunnett's test, α=0.05).

[a]March ambient sample tested in conjunction with ethylene glycol assay (March 1991).
[b]April ambient sample tested in conjunction with propylene glycol assay (April 1991).
[c]Concentrations of ethylene glycol de-icer not tested (below test levels).
[d]Concentrations of propylene glycol anti-icer not tested (above test levels).