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Toxicity of Formulated Glycol Deicers and Ethylene and Propylene Glycol to *Lactuca sativa, Lolium perenne, Selenastrum capricornutum,* and *Lemna minor*

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Abstract. Laboratory studies were conducted to determine the toxicity of ethylene glycol (EG) and propylene glycol (PG) as well as two formulated glycol aircraft deicing/anti-icing fluids (ADAFs) to lettuce (*Lactuca sativa*), perennial ryegrass (*Lolium perenne*), a green alga (*Selenastrum capricornutum*), and duckweed (*Lemna minor*). Seedling emergence, root length, and shoot length were measured in lettuce and ryegrass; cell growth of the alga and frond growth, chlorophyll *a,* and pheophytin *a* of the duckweed were measured. While both the ADAFs and pure glycols were toxic to the test species, there were substantial differences in how the organisms responded to the test materials. ADAFs affected emergence in ryegrass more than in lettuce. However, when considering the sublethal endpoints of root and shoot length, the ADAFs were significantly more toxic to lettuce. The root length 120-h IC_{25}s for lettuce were 2,710 and 21,270 mg EG/L for the ADAF and pure EG compound, respectively; the root length 120-h IC_{25}s for ryegrass were 4,150 and 3,620 mg EG/L for the ADAF and pure EG compound, respectively. EG and PG ADAFs were more toxic than pure EG or PG to *L. minor.* To *S. capricornutum,* EG ADAF toxicity was similar to EG toxicity, however, PG ADAF was substantially more toxic to the alga than pure PG. The greater toxicity of ADAFs is reflective of other studies using animals and suggests that although glycols no doubt contribute to toxicity in deicer formulations, other compounds in the mixtures also contribute to the toxicity of the deicers. However, differences in responses between the four plant species suggest differences in modes of action and/or how the plants metabolize the compounds.

Type I and Type II ethylene glycol (EG)– and propylene glycol (PG)–based aircraft deicing/anti-icing fluids (ADAFs) are commonly used and are generally very effective in snow and ice removal. Type I ADAFs are unthickened fluids used to remove ice and snow as well as to provide some protection against refreezing (Sills and Blakeslee 1992). Type II ADAFs are thickened fluids that, by adhering to airplane surfaces, provide a greater (relative to Type I) protection against refreezing. Type I ADAFs generally contain a minimum of 80% glycols; Type II ADAFs contain a minimum of 50% glycols, with the balance of the constituents including water as well as additives such as corrosion inhibitors, wetting agents, surfactants, and thickening agents that enhance ADAF effectiveness.

Many of the studies on glycols have dealt with mammalian toxicology (Kesten *et al.* 1939; Thomas *et al.* 1949; Weil *et al.* 1971; Gaunt *et al.* 1972; Holman *et al.* 1979; US EPA 1987). Some studies have concluded that propylene glycol–based coolants, antifreezes, and deicers are environmentally safer because they are less toxic than ethylene glycol–based compounds to terrestrial mammals (Wray 1995). As a result, some PG antifreezes have been marketed as an environmentally friendly alternative to EG antifreezes. However, Pillard (1995) showed that pure propylene glycol was more toxic than ethylene glycol to some aquatic animals. In addition, toxicity of the glycol ADAFs to aquatic animals is exacerbated by the presence of chemicals that are added to improve end-product performance. Recent studies by Fisher *et al.* (1995), Hartwell *et al.* (1995), and Pillard (1995) reported that glycol-based deicing compounds exhibit substantially greater toxicity to aquatic animals than could be explained by the toxicity of glycols alone. The increased toxicity is probably due to additives contained in deicing solutions. One additive that is common in many ADAFs is 4(5)-methylbenzotriazole (MeBT), which is a flame retardant/corrosion inhibitor. MeBT (also referred to as tolyltriazole) was identified as a significant toxicant to Microtox® bacteria by Cancilla *et al.* (1997) and Cornell *et al.* (1998). PG plus MeBT was also found to be more toxic than PG alone to invertebrates and fish, although it had less of an effect than the additive package found in PG ADAF (Cornell *et al.* 1998). The additive package, or AdPack, contains the remainder of agents used to enhance ADAF effectiveness and meet performance criteria. The formulation of the AdPack is propietary, and therefore it is difficult to associate environmental effects with specific chemicals.

Phytotoxicity studies on glycol are limited in number. EC_{50}s of 19,000 and 7,900 ppm for PG and EG, respectively, were calculated using the freshwater green alga *Selenastrum capricornutum* (Ward *et al.* 1992). The same study reported 96-h EC_{50}s of 19,100 and 44,200 mg/L for PG and EG, respectively, for the marine alga *Skeletonema costatum. Scenedesmus quadricauda* (a freshwater green alga) was found to have a toxicity threshold of greater than 10,000 mg/L EG (Bringmann and Kühn 1980).

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Other researchers studying the effects of EG on *S. capricornutum* reported an IC_{25} and IC_{50} of 7,925 and 11,730 µl/L, respectively (G. Craig, personal communication). Reynolds (1977), using *Lactuca sativa*, reported germination EC_{50}s of 54,560 mg/L and 50,540 mg/L for EG and PG, respectively.

Very large volumes of ADAFs are used annually at airports worldwide, and most of these airports have no system for capturing and reclaiming the material. Even at airports that do have sophisticated collection systems (*e.g.,* Denver International Airport), deicing fluid can run off aircraft as they proceed down the runway and be lost through leaks and inefficient systems. Because current assessments concerning the potential environmental impacts of ADAFs on plants are based primarily on toxicity data generated with only the major active ingredients (glycol) and not with the end-use products, this study was initiated to contribute to the available phytotoxicity data related to formulated ethylene and propylene glycol ADAFs, which constitute a major source of glycols in ecosystems near airports.

Materials and Methods

Test Material and Test Organisms

Formulated ethylene glycol and propylene glycol Type I ADAFs were obtained from Stapleton International Airport (SIA), Denver, CO. The propylene glycol ADAF was Octagon ADF Plus® (ARCO Chemical Company). The ethylene glycol ADAF was an EG mixture from more than one manufacturer. Although specific manufacturers could not be identified, it was felt that this EG mixture was representative of EG ADAFs typically used at SIA. The ADAF stock mixtures obtained from SIA were analytically determined using US EPA Method 8015, gas chromatography for nonhalogenated organic compounds (US EPA 1986), to contain 500,000 mg/L EG and 980,000 mg/L PG, respectively. Pure product EG and PG were purchased from Sigma Chemical Co. (St Louis, MO).

Lactuca sativa (lettuce var. buttercrunch) seeds were obtained from the Rocky Mountain Seed Company in Denver, CO (lot #7578). Prior to testing, seeds were placed in a seed blower at the National Seed Storage Laboratory (NSSL) at Colorado State University in Fort Collins, CO, to remove empty hulls, damaged seeds, and other debris. *Lolium perenne* (perennial ryegrass var. New Zealand) seeds were obtained from the NSSL in Fort Collins. As the seeds were supplied by the NSSL and appeared very clean, they were not blown prior to use. Seeds were stored in darkness at 4°C. *S. capricornutum* was obtained from in-house cultures. The alga was grown in artesian water purchased from a local supermarket, to which Alga-Gro® (Carolina Biological Supply, Burlington, NC) was added. Five to seven days prior to test initiation, cells were isolated in US EPA medium (US EPA 1989). On the day of test initiation, cells were diluted using US EPA growth medium to a density of 6.7×10^5 cells/ml. Duckweed was obtained from Florida Bioassay Supply, Inc. (Gainesville, FL).

L. sativa *and* L. perenne *Studies*

Studies using *L. sativa* and *L. perenne* were conducted in general accordance with US EPA protocols (Greene *et al.* 1989). Tests were conducted in 100 × 15 mm plastic petri dishes. Fifty grams of medium-grain (16–40 mesh) washed silica sand were placed into each dish. The sand was evenly dispersed in the dish and 10 ml (75% of the water holding capacity) of the appropriate test concentration were added to each dish; four replicates were tested per concentration. Test concentrations were prepared by diluting the stock concentration with deionized (Milli-Q) water. After the test solution had been added, 10 seeds were placed in a circular pattern in the dish at least 1 cm from the edge of the dish. Forty grams of a larger-grain sand (10–20 mesh) were then spread evenly over the medium grain sand, covering all seeds. The covers of the petri dishes were then put in place.

The petri dishes were placed in a plastic tray lined with wet paper towels (to maintain humidity). The tray covers were taped into place and the trays placed into black garbage bags, which were also taped shut. The covered trays were placed in an environmental chamber set at 25 ± 1°C. After 48 h the trays were removed from the black plastic bags, the petri dish covers were taken off, and the uncovered dishes were placed back into the trays. The trays (without covers) were then put into clear plastic bags for another 72 h. Lighting in the environmental chamber was 50 to 100 foot-candles with a 16 h:8 h light:dark cycle.

At the end of the test all chambers were examined for seedlings that emerged through the cover soil. Although this is generally referred to as a germination study, seeds can actually be germinated but not visible through the cover soil. Therefore, this endpoint is referred to as ''emergence'' in this study. Emerged plants were carefully removed from the sand with forceps and rinsed in deionized water. The root length (length of the radicle from the node to the radicle tip) and shoot length (length of the hyptocotyl from the node to the end of the stem, excluding the leaves) were measured to the nearest mm.

S. capricornutum *Studies*

S. capricornutum test procedures were in accordance with US EPA methods (US EPA 1989). Aliquots of actively growing cultures of *S. capricornutum* were centrifuged. The resulting pellet was then resuspended in US EPA culture medium (without EDTA). One hundred milliliters of each test concentration were added to sterilized 250-ml Erlenmeyer flasks. Test concentrations were prepared by diluting the stock concentration with US EPA culture medium (without EDTA). Four replicates were tested per treatment, and five concentrations plus a control were tested for each material. To each flask, 1.5 ml of stock algal solution were added to create an initial density of 1.0×10^4 cells per ml (verified by counting under a compound microscope). Flasks were capped with foam plugs and placed on a shaker table set at approximately 100 cycles per minute. The flasks were kept in constant motion for 4 days in an environmental chamber set at 25 ± 1°C under continuous lighting (approximately 400 ft-c). After 4 days the density of cells in each flask was either determined immediately or cells were preserved with Lugol's solution for later counting (APHA/AWWA/WPCF 1989). Cell density was determined by counting under a compound microscope using a hemacytometer.

Lemna minor *Studies*

L. minor test methods followed those described by Taraldsen and Norberg-King (1990). To initiate the duckweed tests, healthy fronds (*i.e.,* no frond damage or discoloration) were removed from the culture aquarium and roots were cut to approximately 2 to 3 mm on each frond. Ten duckweed plants, having two fronds each, were placed in the test chambers (30-ml plastic cups) containing approximately 15 ml of water. Four replicates were tested per treatment, and five or six concentrations plus a control were tested for each material. Test concentrations were prepared by diluting the stock concentration with duckweed growth medium, which was US EPA algal culture medium (without EDTA) plus 10 g/L of sterile soil (Taraldsen and Norberg-King 1990). Test chambers were placed in an environmental chamber set at 25 ± 1°C under continuous lighting (400 ft-c).

After 4 days the fronds in each chamber were counted. All fronds were counted, regardless of size, unless they were visually moribund, as evidenced by a tan or brown color. Surviving fronds were placed in a

borosilicate vial with 5 ml of 100% acetone. Vials were capped, covered with aluminum foil, and placed in a dark freezer at −6°C. After 72 h the fronds were removed from the freezer and macerated by hand with mortar and pestle. The macerated material was placed back in the vials with a total of 10 ml of 100% acetone, covered with aluminum foil, and placed back into the freezer.

After an additional 48 h to allow for extraction of pigments, the clear extract (with no visible particles) was used to determine the concentration of chlorophyll *a* and pheophytin *a*. A Perkin-Elmer UV/VIS model Lambda 3 spectrophotometer was used to measure light transmission according to Standard Methods Method 10200 H (APHA/AWWA/WPCF 1989). Three milliliters of clear extract were transferred to a clean, 1-cm cuvette, and the optical density of each solution was first measured at 750 and 664 nm. The liquid in each cuvette was then acidified with 0.1 ml of 0.1 N HCl, and optical density was remeasured at 750 and 665 nm. Chlorophyll and pheophytin concentrations (µg per replicate) were calculated according to the equations described in Standard Methods (APHA/AWWA/WPCF 1989).

Statistical Analysis

All data were tested for normality and homogeneity of variance ($\alpha = 0.01$) before statistical analysis. Data sets meeting the assumptions of normality and homogeneity of variance were analyzed using parametric ANOVA and Dunnett's test ($\alpha = 0.05$). Data sets not meeting the parametric assumptions were analyzed using Steel's many-one rank test ($\alpha = 0.05$). All hypothesis testing was performed using Toxstat® Version 3.4 (West and Gulley 1994). No observed effect concentrations (NOECs), lowest observed effect concentrations (LOECs), and chronic values (ChVs, the geometric mean of the NOEC and LOEC) were determined. In addition to hypothesis testing, the 25% and 50% inhibition concentrations (IC_{25} and IC_{50}) were also calculated (Norberg-King 1993). For tests with *L. sativa* and *L. perenne*, ICps were calculated using the mean shoot or root length per the number of seeds germinated at the end of the test. Cell growth was examined in the *S. capricornutum* tests. *L. minor* frond growth and chlorophyll *a* and pheophytin *a* concentrations were evaluated as well.

Results

L. sativa

Control emergence in all treatments was >80%. In the highest EG and EG ADAF treatments none of the seeds emerged while in the PG treatments there was always partial emergence (Table 1). Root length in the control plants was generally greater than 30 mm in length, although the control plants in the PG ADAF study had a mean length of 24 mm. Shoots were generally slightly shorter than roots, with control shoots ranging from 27 mm to 33 mm.

Statistical endpoints for the lettuce tests are presented in Table 2. Emergence was reduced more by EG than EG ADAF. However, lettuce root and shoot length were more sensitive to EG ADAF. For example, the 120-h emergence IC_{25}s were 31,650 and 21,750 mg/L for EG ADAF and EG, respectively; the 120-h root length IC_{25}s were 2,710 and 21,270 mg/L for EG ADAF and EG, respectively. Emergence and root length were consistently more sensitive to PG ADAF than PG. Shoot length, however, appeared slightly more sensitive to PG than PG ADAF, based on 120-h IC_{25}s.

L. perenne

Control emergence in all treatments was >80%. No seeds emerged in the highest EG or EG ADAF treatments; there was some emergence in all PG and PG ADAF treatments (Table 1). Control root and shoot length were greater than 35 mm in all tests. Although emergence in most of the treatments, including the controls, was fairly constant, except for the highest concentrations, root and shoot length tended to decrease consistently with increasing glycol concentration.

Emergence in ryegrass was only slightly more sensitive to the ADAF treatments than either EG or PG and the toxicity of all of the glycol treatments to ryegrass was generally similar when examining root and shoot length (Table 2). There were few major differences in the statistical endpoints for root and shoot length, although PG and PG ADAF tended to be slightly more toxic than EG or EG ADAF. The root length IC_{25}s for EG ADAF, EG, PG ADAF, and PG were 4,150, 3,620, 2,720, and 2,850 mg glycol/L, respectively.

S. capricornutum

Cell growth in the controls in each treatment ranged between approximately 800,000 to over 1,200,000 cells/ml. In studies with the pure EG, there was a consistent decrease in cell density as glycol concentration increased (Table 3). In the EG ADAF and both PG studies, cell growth in at least the lowest glycol treatment was greater than in the control, a fairly common response (hormesis) in algae growth tests. In the PG ADAF study, cell growth in all but the highest concentration of deicer was higher than in the control. In all tests, however, cell growth was significantly ($\alpha = 0.05$) reduced in at least the highest treatment.

The toxicity of EG ADAF was similar to the toxicity of pure EG (Table 4). For example, the EG ADAF IC_{25} was 7,610 mg/L, while the pure EG IC_{25} was 5,340 mg/L. *S. capricornutum* was more sensitive to the PG ADAF than to the EG ADAF. The lowest calculated IC_{25} was 1,750 mg PG/L.

L. minor

The mean number of fronds in the controls of all treatments ranged from 32.3 to 39.3, chlorophyll *a* concentrations ranged from 20.2 to 27.2 µg/treatment, and pheophytin *a* concentrations ranged from 8.5 to 14.4 µg/treatment (Table 3). These values are very similar to the control results reported by Taraldsen and Norberg-King (1990), who used similar extraction and analytical methods. In both pure EG and PG treatments, all of the *L. minor* fronds were dead in the highest concentration. In the highest concentrations of the ADAFs, some fronds were still considered alive, though not necessarily in good health. Although fronds in the controls were generally large, with a bright green color, fronds in the glycol treatments that were considered alive were often smaller and pale in color. Frequently, parts of the frond were completely absent of green color, with a yellowish, translucent appearance. In all tests, chlorophyll *a* concentrations decreased as glycol exposure increased. Pheophytin *a* concentrations increased, relative to the control, in some of the lower glycol treatments, although

Table 1. Seedling emergence, root length, and shoot length in lettuce and ryegrass exposed to ethylene glycol (EG) and propylene glycol (PG), and EG and PG aircraft deicing/anti-icing fluids (ADAFs)

Test Material	Glycol Concentration (mg/L)	Lettuce Mean per Test Treatment (SE)			Ryegrass Mean per Test Treatment (SE)		
		Percent Emergence	Root Length (mm)	Shoot Length (mm)	Percent Emergence	Root Length (mm)	Shoot Length (mm)
EG	0	90 (7.1)	38.9 (1.5)	27.6 (2.4)	95 (2.9)	42.4 (1.6)	40.6 (3.1)
	1,200	90 (4.1)	36.8 (1.7)	26.2 (0.8)	95 (2.9)	36.7 (2.9)	38.4 (3.6)
	4,000	88 (6.3)	35.5 (2.2)	22.8 (0.8)	98 (2.5)	31.1 (1.0)[a]	32.2 (1.1)
	14,000	73 (2.5)	34.7 (2.1)	18.7 (0.8)[a]	88 (4.8)	28.1 (1.2)[a]	16.8 (2.7)[a]
	45,000	53 (4.8)[a]	11.1 (1.9)[a]	6.7 (0.5)[a]	3 (2.5)[a]	11.0 (2.8)[a]	7.0 (1.8)[a]
	150,000	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
EG ADAF	0	85 (6.5)	39.0 (1.5)	30.1 (1.2)	93 (2.5)	42.4 (0.6)	41.9 (1.8)
	600	90 (4.1)	36.4 (2.3)	25.6 (1.2)	93 (2.5)	36.0 (3.1)	38.9 (3.5)
	2,000	98 (2.5)	32.5 (3.1)[a]	23.3 (1.8)[a]	88 (2.5)	34.2 (3.8)	37.4 (4.9)
	7,000	90 (4.1)	9.4 (0.7)[a]	18.0 (0.9)[a]	90 (4.1)	28.7 (2.3)[a]	27.9 (2.0)[a]
	22,500	83 (4.8)	4.0 (0.4)[a]	10.1 (0.4)[a]	53 (4.8)[a]	7.4 (1.2)[a]	7.3 (0.5)[a]
	75,000	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
PG	0	90 (0)	34.5 (1.3)	33.4 (1.1)	83 (10.3)	37.8 (1.2)	39.5 (1.3)
	405	93 (4.8)	26.8 (0.2)[a]	26.4 (1.3)[a]	83 (2.5)	34.9 (1.6)	39.1 (0.9)
	1,350	90 (4.1)	32.7 (1.9)	24.8 (1.2)[a]	85 (5.0)	31.2 (1.2)[a]	35.4 (1.8)
	4,500	80 (4.1)	28.0 (0.8)[a]	24.5 (0.9)[a]	73 (8.5)	25.3 (1.1)[a]	25.2 (1.2)[a]
	15,000	78 (6.3)[a]	23.9 (2.5)[a]	20.5 (1.8)[a]	78 (4.8)	21.2 (1.6)[a]	14.6 (1.4)[a]
	50,000	45 (9.6)[a]	3.3 (0.4)[a]	2.9 (0.2)[a]	28 (6.3)[a]	2.2 (0.3)[a]	0.9 (0.2)[a]
PG ADF	0	88 (4.8)	23.5 (3.8)	27.3 (3.3)	95 (2.9)	35.6 (2.3)	36.8 (2.4)
	397	88 (4.8)	32.4 (1.5)	29.3 (1.9)	95 (2.9)	35.5 (1.8)	35.2 (2.3)
	1,323	90 (4.1)	26.0 (1.1)	21.9 (1.1)	88 (2.5)	33.9 (2.2)	36.4 (2.5)
	4,410	80 (10.8)	15.6 (1.9)[a]	15.4 (1.0)[a]	100 (2.5)	18.2 (1.9)[a]	29.3 (1.4)[a]
	14,700	60 (8.2)[a]	4.1 (0.2)[a]	11.1 (1.3)[a]	88 (2.5)[a]	5.0 (0.7)[a]	10.7 (1.2)[a]
	49,000	35 (9.6)[a]	1.5 (0.2)[a]	3.1 (0.4)[a]	10 (0)[a]	1.0 (0.4)[a]	2.5 (0.3)[a]

[a] Significantly ($\alpha = 0.05$) different from the control

Table 2. Results of lettuce and ryegrass toxicity tests using ethylene glycol (EG) and propylene glycol (EG) and EG and PG aircraft deicing/anti-icing fluids (ADAFs)

Endpoint (mg/L)	Lettuce				Ryegrass			
	EG ADAF	EG	PG ADAF	PG	EG ADAF	EG	PG ADAF	PG
NOEC								
Emergence	22,500	14,000	4,410	4,500	7,000	14,000	4,410	15,000
Root length	600	14,000	1,323	1,350	2,000	1,200	1,323	405
Shoot length	600	4,000	1,323	<405	2,000	4,000	1,323	1,350
LOEC								
Emergence	75,000	45,000	14,700	15,000	22,500	45,000	14,700	50,000
Root length	2,000	45,000	4,410	4,500	7,000	4,000	4,410	1,350
Shoot length	2,000	14,000	4,410	405	7,000	14,000	4,410	4,500
ChV								
Emergence	41,079	25,100	8,052	8,216	12,550	25,100	8,052	27,386
Root length	1,095	25,100	2,415	2,465	3,742	2,191	2,415	739
Shoot length	1,095	7,483	2,415	NA[a]	3,742	7,483	2,415	2,465
120-h IC25								
Emergence	31,650	21,750	11,480	24,760	15,280	19,700	21,890	24,210
Root length	2,710	21,270	2,820	9,880	4,150	3,620	2,720	2,850
Shoot length	2,690	8,960	1,660	1,190	5,120	5,100	5,250	3,120
120-h IC50								
Emergence	46,100	60,000	36,420	49,330	28,750	28,440	32,400	39,560
Root length	4,810	34,030	5,850	26,240	12,410	22,450	4,680	19,110
Shoot length	12,760	26,530	7,370	22,570	12,210	11,730	10,360	9,890

[a] Cannot be calculated

pheophytin concentrations in the highest glycol treatments were always significantly ($\alpha = 0.05$) lower than in the controls.

ADAFs were more toxic than the pure materials to *L. minor* (Table 4). For example, the frond growth NOECs for pure EG and PG were 5,000 and 2,500 mg/L, respectively, while the frond growth NOECs for EG and PG ADAFs were 2,500 and 1,470 mg/L, respectively. ADAF IC_{25}s and IC_{50}s were also lower than for the pure glycols. In tests using the ADAFs,

Table 3. Cell growth of *Selenastrum capricornutum* and frond growth, chlorophyll *a*, and pheophytin *a* concentrations in *Lemna minor* exposed to ethylene glycol (EG) and propylene glycol (PG), and EG and PG aircraft deicing/anti-icing fluids (ADAFs)

	S. capricornutum Mean per Test Treatment (SE)		*L. minor* Mean per Test Treatment (SE)			
Test Material	Glycol Concentration (mg/L)	Number of Cells/ml	Glycol Concentration (mg/L)	Fronds per Replicate	Chl *a* (µg) per Replicate	Pheo *a* (µg) per Replicate
EG	0	1,215,000 (190,897)	0	32.2 (0.8)	21.2 (0.4)	8.5 (1.0)
	10,000	645,750 (78,415)	2,500	31.8 (1.4)	20.6 (1.6)	10.4 (0.7)
	20,000	240,625 (72,423)[a]	5,000	31.0 (0.4)	19.2 (1.5)	10.5 (0.2)
	35,000	89,350 (3,561)[a]	10,000	26.5 (1.0)[a]	18.1 (0.9)	10.4 (0.7)
	50,000	23,650 (2,231)[a]	20,000	23.2 (0.5)[a]	15.9 (1.4)[a]	6.1 (1.4)
	100,000	0[a]	40,000	20.0 (0)[a]	9.4 (0.8)[a]	4.3 (1.0)[a]
			80,000	0[a]	0[a]	0[a]
EG ADAF	0	974,250 (91,836)	0	39.2 (1.8)	26.6 (1.4)	14.4 (1.4)
	5,000	1,422,500 (88,164)	1,250	41.2 (1.2)	24.7 (1.6)	14.6 (1.4)
	10,000	625,000 (37,009)[a]	2,500	39.2 (0.8)	21.3 (1.7)*	11.2 (0.9)
	17,500	63,100 (8,734)[a]	5,000	30.8 (1.7)*	15.8 (0.8)*	6.1 (0.2)*
	25,000	8,750 (2,976)[a]	10,000	23.2 (1.1)*	14.8 (0.7)*	6.0 (0.5)*
	50,000	3,125 (1,573)[a]	20,000	21.0 (0.4)*	10.9 (0.5)*	4.0 (0.6)*
			40,000	19.5 (0.9)*	9.1 (1.1)*	2.1 (0.8)*
PG	0	811,250 (141,729)	0	35.0 (0.7)	20.2 (0.6)	13.9 (1.0)
	10,000	1,498,000 (378,793)	2,500	32.8 (0.8)	19.2 (1.4)	13.4 (1.9)
	20,000	595,000 (162,547)	5,000	29.5 (1.0)[a]	19.4 (2.2)	11.7 (2.0)
	35,000	174,750 (11,729)[a]	10,000	26.8 (1.1)[a]	18.4 (1.0)	10.7 (1.0)
	50,000	1,875 (1,197)[a]	20,000	24.2 (1.0)[a]	15.8 (0.8)[a]	9.1 (0.9)[a]
	100,000	0[a]	40,000	19.5 (0.9)[a]	9.5 (0.8)[a]	3.7 (0.1)[a]
			80,000	0[a]	0[a]	0[a]
PG ADAF	0	838,875 (92,875)	0	35.8 (1.3)	27.2 (1.2)	11.9 (1.4)
	294	3,275,000 (547,258)	735	35.0 (1.1)	27.1 (3.5)	15.8 (2.9)
	882	3,642,500 (361,026)	1,470	34.2 (1.2)	26.6 (1.6)	11.8 (1.4)
	1,470	2,755,000 (142,449)	2,940	30.0 (0.8)[a]	19.7 (0.8)[a]	8.1 (0.6)
	2,058	1,235,500 (365,199)	5,880	26.2 (1.2)[a]	16.8 (1.8)[a]	6.9 (1.1)
	2,646	156,500 (28,209)[a]	11,760	24.5 (0.9)[a]	12.2 (0.8)[a]	6.5 (1.5)

[a] Significantly ($\alpha = 0.05$) different from the control

Table 4. Results of *S. capricornutum* and *L. minor* toxicity tests using ethylene glycol (EG) and propylene glycol (EG) and EG and PG aircraft deicing/anti-icing fluids (ADAFs). Concentrations are mg/L of glycol

	S. capricornutum					*L. minor*				
	Endpoint	EG ADAF	EG	PG ADAF	PG	Endpoint	EG ADAF	EG	PG ADAF	PG
NOEC	Cell growth	5,000	10,000	2,058	20,000	Frond growth	2,500	5,000	1,470	2,500
						Chl *a*	1,250	10,000	1,470	10,000
						Pheo *a*	2,500	20,000	11,760	10,000
LOEC	Cell growth	10,000	20,000	2,646	35,000	Frond growth	5,000	10,000	2,940	5,000
						Chl *a*	2,500	20,000	2,940	20,000
						Pheo *a*	5,000	40,000	>11,760	20,000
ChV	Cell growth	7,071	14,142	2,334	26,458	Frond growth	3,536	7,071	2,079	3,536
						Chl *a*	1,768	14,142	2,079	14,142
						Pheo *a*	3,536	28,284	NA[a]	14,142
96-h IC_{25}	Cell growth	7,610	5,340	1,750	15,160	Frond growth	5,380	17,120	5,440	12,000
						Chl *a*	3,100	19,850	2,780	21,880
						Pheo *a*	2,670	16,470	2,040	12,000
96-h IC_{50}	Cell growth	10,340	10,940	2,020	20,630	Frond growth	31,670	47,750	NA[a]	44,100
						Chl *a*	13,770	36,290	9,940	38,240
						Pheo *a*	4,460	33,130	6,300	28,000

[a] Cannot be calculated

chlorophyll *a* and pheophytin *a* were generally the more sensitive test endpoints. In tests with pure material, however, frond growth was nearly as sensitive an endpoint as chlorophyll or pheophytin. EG and PG appeared equally toxic to duckweed when tested as either the parent compound or as an ADAF.

Discussion

Unlike studies with aquatic animals, where EG and PG glycol ADAFs appear to be consistently more toxic than chemically pure EG and PG, plant response is not so clearly defined. In

these studies there generally was not a substantial difference between the response of ryegrass to pure compound or ADAFs. For lettuce, on the other hand, glycol ADAFs were generally more toxic than pure glycol, as indicated by the IC_{25}s and IC_{50}s. For both species, root and shoot lengths were more sensitive than germination.

Lettuce test results compare favorably to the results from the limited number of other studies. Reynolds (1977), for example, reported germination EC_{50}s for lettuce of 54,560 and 50,540 mg/L for EG and PG, respectively, which are very similar to the emergence IC_{50}s of 60,000 and 49,330 mg/L for EG and PG determined in this study. HydroQual (1995) tested lettuce and calculated a mean (of three tests) root elongation EC_{25} and EC_{50} of 32,000 and 42,000 mg EG/L, respectively. The root elongation IC_{25} and IC_{50} for lettuce in the current study were 21,270 and 34,030 mg EG/L, respectively.

The results of the current *S. capricornutum* studies with pure glycols are similar to those reported by other investigators. For example, an ethylene glycol NOEC, LOEC, IC_{25}, and IC_{50} of 6,930, 13,860, 8,787, and 13,006 mg/L, respectively, were reported by Beak Consultants (G. Craig, personal communication). Considering differences in test concentrations, as well as expected variations between studies, these values compare favorably with those reported in this study: 10,000, 20,000, 5,340, and 10,940 mg/L, respectively. Ward *et al.* (1992) determined that EG was more toxic than PG to *S. capricornutum.* They reported 96-h EC_{50}s of 19,000 and 7,900 mg/L for PG and EG, respectively; 96-h IC_{50}s of 20,630 and 10,940 mg/L were calculated in the current study for the same materials.

Unlike *Selenastrum*, *L. minor* consistently showed greater sensitivity to the ADAFs than to the pure glycols, regardless of the test, or statistical, endpoint examined. Frond growth was generally the least sensitive endpoint; neither chlorophyll *a* nor pheophytin *a* was clearly more sensitive than the other. In an aquatic ecosystem, reduced pigment concentrations could result in diminished population health of aquatic plants, an effect that may not be immediately evident through more gross endpoints. There was little difference in toxicity to *L. minor* between EG and PG, or between EG and PG ADAFs. The differences in responses between the alga and duckweed could be related to significant physiological differences between single-celled organisms and multicellular (albeit very small) angiosperms. Thomas *et al.* (1996) reported that low concentrations of EG may actually stimulate growth of a related duckweed, *L. gibba.* In their study, EG appeared to enhance the uptake of substances, such as nutrients, from the growth medium. However, scanning electron microscopic examination of *L. gibba* fronds exposed to EG also showed that the cuticle of the upper surface was disrupted, which may adversely impact the hydrophobicity of the plant. In addition, intercellular holes were created between the individual cells that surround the intercellular spaces. When *L. gibba* was simultaneously exposed to EG and other toxicants, the toxicity of these other substances increased (as evidenced by a lower effective concentration). The disruption of the cuticle and intercellular spaces by EG and the subsequent increase in permeability may partially explain the greater toxicity of glycol ADAFs. Although ADAF additives, such as surfactants and corrosion inhibitors, may be toxic by themselves, there may be interactive effects when they are combined with glycols.

While there is a general consensus that chemicals in ADAFs other than the active deicing compounds (glycols) are probably responsible for much of the higher toxicity to some species observed in ADAFs, what exactly those other chemicals are has yet to be completely resolved. ADAF formulations are proprietary, thus complicating efforts to link toxicity to specific chemical agents. The work of Cancilla *et al.* (1997) and Cornell *et al.* (1998) confirm that MeBT is a significant toxicant, particularly to bacteria. However, because of the substantial physiological and biochemical differences between prokaryotes and eukaryotes, toxicity to bacteria may not necessarily be indicative of toxicity to multicellular plants or animals. Cornell *et al.* (1998) indicated that AdPack chemicals may be more important than MeBT in causing adverse effects to freshwater invertebrates and fish. The actual cause of toxicity in any specific ADAF may vary depending on the formulation. Not only will toxicity change as new formulations are developed but, as evidenced by the responses of ryegrass in this study, not all organisms are demonstrably more sensitive to the formulated ADAFs.

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