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ACUTE TOXICITY OF MONOETHANOLAMINE, DIETHANOLAMINE, DIETHYLENE GLYCOL AND TRIETHYLENE GLYCOL TO RAINBOW TROUT, *DAPHNIA MAGNA*, AND *HYALELLA AZTECA*

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EXECUTIVE SUMMARY

The acute toxicity of monoethanolamine (MEA), diethanolamine (DEA), diethylene glycol (DEG), and triethylene glycol (TEG) were selectively tested using the following test methods and species:

	Amines		**Glycols**	
	MEA	**DEA**	**DEG**	**TEG**
Acute 96-hr Rainbow Trout Survival Test (EPS 1/RM/9)	✓	✓	✓	
Acute 48-hr *Daphnia magna* Survival Test (EPS 1/RM/11)	✓		✓	
Acute 96-hr *Hyalella azteca* Survival Test (EPS 1/RM/33)	✓	✓	✓	✓

The LC50s and 95% confidence limits, based on nominal concentrations, were as follows:

	Amines (mg/L)		**Glycols (g/L)**	
	MEA	**DEA**	**DEG**	**TEG**
96-hr Rainbow Trout	105 (85, 145)	460 (250, 625)	66 (50, 100)	N/A
48-hr *Daphnia magna*	66.7 (60.1-74.1)	N/A	62.63 (57.63-68.07)	N/A
96-hr *Hyalella azteca*	170 (163-177)	344 (312-377)	65.98 (61.69-70.56)	43.5 (39.4, 48.1)

The measured concentrations at test initiation and completion were generally within 20% of nominal concentrations, indicating that the test substance concentrations were satisfactorily maintained during the exposure periods. The ranges of values (%measured/nominal) are presented below:

	MEA	**DEA**	**DEG**	**TEG**
96-hr Rainbow Trout	93-101%	96-110%	83-106%	N/A
48-hr *Daphnia magna*	85-118%	N/A	92-105%	N/A
96-hr *Hyalella azteca*	79-104%	93-100%	84-91%	83-90%

ACUTE RAINBOW TROUT TESTS (96-HR, STATIC)

Acute lethality tests were conducted with rainbow trout (*Oncorhynchus mykiss*) according to the Environment Canada method, Biological Test Method: Acute Lethality Test Using Rainbow Trout, EPS 1/RM/9 (EC 1990/1996). The tests were conducted with monoethanolamine (MEA), diethanolamine (DEA), and diethylene glycol (DEG).

Five nominal test concentrations and a control were tested with each chemical. The stock and test solutions were prepared in dechlorinated and hardened Vancouver city tap water. Water hardness was 14 mg/L as $CaCO_3$ (measured by EDTA titration). Test solutions were aerated for 30 minutes prior to the addition of the fish. Test solutions were not renewed during the tests.

There was one replicate per treatment, which consisted of 10 fish in a total volume of 12 L or 15 L control/dilution water in a plastic-lined glass aquarium. The mean fish length was 3.9 cm or 3.6 cm, depending on the test, with a mean weight of 0.49 g or 0.38 g, respectively. Loading densities were 0.33 g/L or 0.32 g/L. The fish were not fed during the tests, and were starved for at least 12 h prior to test initiation. The test chambers were covered with a plexiglass sheet and aeration was provided during the test. The tests were conducted at a daily mean water temperature of 15 ± 1°C, with a photoperiod of 16L:8D.

The test chambers were monitored daily for number of dead fry. Measurements of dissolved oxygen concentrations, temperature, and pH, were taken at the start and end of the test. Conductivity was measured at test initiation. Samples from the control, low, medium and high treatments were collected at the start and end of each test, and analysed by GC_FID (glycols) or HPLC (amines).

The 96-hr LC50 with 95% confidence limits were calculated for each chemical using the binomial option in “Bioassay Program”, a Lotus Notes-based application which implements a BASIC computer program obtained from Environment Canada that is based on calculations in Stephan, 1977, Methods for Calculating an LC50, in: *Aquatic Toxicology and Hazard Evaluation (ASTM STP 634)*, Mayer and Hamelink (eds.), ASTM, Philadelphia, PA, pp. 65-84.

The tests were considered valid as none of control fish died or displayed loss of equilibrium or atypical swimming behaviour. Reference toxicant (positive control) tests were conducted with phenol with each batch of fish used, and the resulting LC50s were within two standard deviations of previous tests.

The following raw data is presented after this summary: toxicity test reports, benchsheets, measured concentrations and analytical reports, and reference toxicant control charts.

ACUTE *DAPHNIA MAGNA* TESTS (48-HR, STATIC)

Acute lethality tests were conducted with the freshwater cladoceran, *Daphnia magna*, according to the Environment Canada method, Biological Test Method: Acute Lethality Test Using *Daphnia* spp, EPS 1/RM/11 (EC 1990/1996). The tests were conducted with monoethanolamine (MEA) and diethylene glycol (DEG).

Five nominal test concentrations and a control were tested with each chemical. The stock and test solutions were prepared in moderately hard reconstituted water. This water was prepared by adding 1.144 g $MgSO_4$, 1.67 g $CaSO_4 \cdot 2H_2O$, 2.112 g $NaHCO_3$, 0.088 g KCl, 10 mL of a 4 mg/L Vitamin B12 (as cyancobalamin) solution, and 40 mL of a 1 mg/L selenium solution to 19-20 L of deionised water. The water was aerated at test temperature at least overnight prior to use in the test. Water hardness was 104 mg/L as $CaCO_3$ (measured by EDTA titration). Test solutions were not renewed during the tests.

There were four replicates per treatment; each replicate consisted of 10 neonates in a total volume of 200 mL control/dilution water in a 250 mL glass beaker. The neonates were <24 h old at test initiation, and were collected from a brood that had 8.5% parental mortality in the 7 days preceding test initiation. The neonates were not fed during the tests, but were fed a mixture of *Selenastrum* and *Chlorella* prior to use in the tests. The test chambers were covered with a plexiglass sheet and no aeration was provided during the test. The tests were conducted at a daily mean water temperature of 20 ± 2°C, with a photoperiod of 16L:8D.

The test chambers were monitored daily for number of dead neonates. Measurements of dissolved oxygen concentrations, temperature, and pH, were taken at the start and end of the test. Conductivity was measured at test initiation. Samples from the control, low, medium and high treatments were collected at the start and end of each test, and analysed by GC_FID (glycols) or HPLC (amines).

The 48-hr LC50 with 95% confidence limits were calculated for each chemical using the trimmed Spearman-Karber (0% trim) in the statistical program, ToxCalc™ (Version 5.0.23j), a Microsoft Excel-based software application (Tidepool Scientific Software 1994-2006).

The tests were considered valid as none of control neonates died or displayed atypical or stressed behaviour. Reference toxicant (positive control) tests were conducted with zinc sulphate. The resulting LC50 (1.06 mg Zn/L with 95% confidence limits of 0.63 and 1.85 mg/L) were within two standard deviations of previous tests (0.44 and 1.07 mg Zn/L).

The following raw data is presented after this summary: statistical analysis, benchsheets, mean measured concentrations and analytical reports, and reference toxicant control charts.

ACUTE *HYALELLA AZTECA* TESTS (96-HR, STATIC)

Acute lethality tests were conducted with the freshwater amphipod, *Hyalella azteca*, according to the Environment Canada method, Biological Test Method: Test for Survival and Growth in Sediment Using the Freshwater Amphipod *Hyalella azteca*, EPS 1/RM/33 (EC 1997). Specifically, the guidance for conducting 96-hr water-only reference toxicant tests was used. The tests were conducted with monoethanolamine (MEA), diethanolamine (DEA), diethylene glycol (DEG), and triethylene glycol (TEG).

Five nominal test concentrations and a control were tested with each chemical. The stock and test solutions were prepared in SAM-5S reconstituted water as per Borgmann, 1996, Systematic analysis of aqueous ion requirements of *Hyalella azteca*: A standard artificial medium including the essential bromide ion. *Archives of Environmental Contamination and Toxicology*, 30: 356-363. This water was prepared by adding 8.82 g $CaCl_2$•$2H_2O$, 1.81 g $MgSO_4$, 0.06 g NaBr, 5.04 g $NaHCO_3$, 0.22 g KCl to 60 L of deionised water. The water was aerated at test temperature at least overnight prior to use in the test. Water hardness was 124 mg/L as $CaCO_3$ (measured by EDTA titration) and alkalinity was 53 mg/L as $CaCO_3$. Test solutions were not renewed during the tests.

There were four replicates per treatment; each replicate consisted of 10 amphipods in a total volume of 200 mL control/dilution water in a 250 mL glass beaker. The amphipods were purchased from an outside supplier, and were held at test conditions for 5 days prior to use in the tests. During this acclimation/holding period, 20-50% of the culture water was renewed and the amphipods were fed 10 mL each of YCT and Tetrafin slurry twice daily. Total mortality during this period was 1.25%.

The amphipods were fed 1.3 mL YCT on Day 0 and 0.5 mL on Day 2 of the test. The test chambers were covered with a plexiglass sheet and aeration was not provided during the test. The tests were conducted at a daily mean water temperature of 23 ± 2°C, with a photoperiod of 16L:8D.

The test chambers were monitored on Days 2 and 4 for number of dead neonates. Measurements of dissolved oxygen concentrations, temperature, and pH, were taken at the start and end of the test. Conductivity was measured at test initiation. Samples from the control, low, medium and high treatments were collected at the start and end of each test, and analysed by GC_FID (glycols) or HPLC (amines).

The 96-hr LC50 with 95% confidence limits were calculated for each chemical using the trimmed Spearman-Karber (0-10% trim) method or maximum likelihood probit method in the statistical program, ToxCalc™ (Version 5.0.23j), a Microsoft Excel-based software application (Tidepool Scientific Software 1994-2006).

The tests were considered valid as none of control amphipods died or displayed atypical or stressed behaviour during the test. A reference toxicant (positive control) test was conducted with copper sulphate. The resulting LC50 (203 µg Cu/L with 95% confidence limits of 177 and 233 µg/L) were within two standard deviations of previous tests (35 and 389 µg/L).

The following raw data is presented after this summary: statistical analysis, benchsheets, mean measured concentrations and analytical reports, and reference toxicant warning chart.