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THE TOXICITY OF NITROGUANIDINE AND PHOTOLYZED NITROGUANIDINE TO FRESHWATER AQUATIC ORGANISMS

WILLIAM H. van der SCHALIE, Ph.D.

U S ARMY MEDICAL BIOENGINEERING RESEARCH & DEVELOPMENT LABORATORY
Fort Detrick
Frederick, Maryland 21701

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The acute toxicity of nitroguanidine (NGu) to ten species of freshwater aquatic organisms was determined. Fish exposed to NGu for 96 hours included fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus), channel catfish (Ictalurus punctatus), and rainbow trout (Salmo gairdneri). Invertebrates, which were tested for 48 hours, included water fleas (Daphnia magna), amphipods (Hyallela azteca and Gammarus minus), midge larvae (Paratanytarsus dissimilis), and aquatic worms (Lumbriculus variegatus). The

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acute toxicity of NGu was very low; less than 50 percent of any of these organisms exposed to NGu were killed at concentrations up to the solubility limit of NGu in water, which ranged from about 1,700 mg/L at 12°C (trout tests) to about 3,000 mg/L at 22°C (most other species). An alga (Selenastrum capricornutum) exposed to NGu at 24°C was only slightly more sensitive; the 120 hr EC50's based on reductions from the controls were 1,937 mg/L for chlorophyll a and 2,146 mg/L for dry weight. The chronic toxicity of NGu was also low. A 42-day rainbow trout early life stage test showed statistically significant differences from the controls only at saturation (1,700 mg/L), with no effects at the other concentrations (857 mg/L and below).

Complete photolyzation of NGu with ultraviolet light greatly increased NGu toxicity. The 96-hr LC50 of NGu for fathead minnows was greater than 2,714 mg/L (the saturation concentration in water), but the LC50 (with 95% confidence limits) for photolyzed NGu (Φ-NGu, expressed as the original NGu concentration) was 34.5 mg/L (22.1 to 61.3). With the alga Selenastrum capricornutum, the 120 hr EC50's (based on dry weight standing crop) were 2,146 mg/L (2,010 to 2,283) for NGu and 32.3 mg/L (26.2 to 38.5) for Φ-NGu. The 48-hr EC50 (based on immobilization) for Daphnia magna and NGu was greater than 2,830 mg/L, but the EC50 for Φ-NGu was 24.6 mg/L (21.1 to 28.7). A second test with daphnids started from the same Φ-NGu stock solution stored for 72 hours at room temperature resulted in an EC50 of 35.0 mg/L (29.6 to 41.5). Thus, the toxicity of Φ-NGu decreased with time but was still much more toxic than NGu. The importance of the increased toxicity of Φ-NGu over NGu will depend on whether the photolysis of NGu can occur at significant rates in sunlight and natural waters and whether such photolysis results in reaction products as toxic as those created under laboratory conditions.

(Phi)

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INTRODUCTION

The US Army Medical Research and Development Command has responsibility for assessing the possible health and environmental hazards associated with munitions-unique pollutants released during the operation of US Army munitions manufacturing facilities. One such potential pollutant is the compound nitroguanidine (NGu), which is being produced at Sunflower Army Ammuntion Plant (SAAP) in DeSoto, Kansas. NGu is used exclusively in M30 triple-base propellant (along with nitrocellulose and nitroglycerin), where it reduces burning temperature and flash but increases thrust and stability.[1] It was originally anticipated that the 45 ton per day manufacturing operation at SAAP would result in no environmental release of NGu. When pilot scale production was recently started, however, some NGu-contaminated wastewater was produced. This wastewater is presently stored in lagoons and holding ponds; release of NGu could result from leakage, overflows, or leaching from the storage facilities.[2] Contaminated water would likely flow into Hansen creek (which originates at SAAP) and then into Kill Creek and the Kansas River.

The potential for release of NGu into streams and rivers means that some information on the toxicity of NGu to aquatic organisms is needed to allow reasonable discharge standards to be established by regulatory authorities. The relatively few data available on NGu suggest a very low degree of toxicity. In a flow-through test with fathead minnows (Pimephales promelas), only 7 of 10 fish were killed at the highest concentration tested (3,650 mg/L), which was near the aqueous solubility of NGu in water. No other toxicant-related deaths or sublethal symptoms of poisoning were observed at lower concentrations. A 42-day test in which mixed algae (and presumably zooplankton) filtered from pond water were exposed to NGu resulted in algal stimulation at 40 mg/L (the lowest concentration tested) and algal inhibition at 130 mg/L and above.[3] Warner et al.[4] found that NGu was nontoxic to fathead minnows (22°C) and Daphnia magna (20°C) in static acute tests at concentrations of 2,000 and 1,175 mg/L, respectively. (These were nominal, not measured, concentrations and were the highest levels tested.)

In addition to the potential problem posed by NGu release, preliminary studies have shown that NGu in water undergoes photolysis in bright sunlight with a half-life of 20 hours.[5] There is, therefore, some concern as to the potential environmental effects of the by-products of NGu photolysis.

The main objective of this study was to determine the toxicity of NGu to freshwater aquatic organisms. Acute toxicity tests were conducted with four species of fish, five species of aquatic invertebrates, and one species of algae. In addition, an early life stage test was conducted with the most sensitive fish species tested, the rainbow trout. Further tests were done to determine whether photolyzed NGu (ϕ-NGu) is more or less toxic than NGu to one species of fish, invertebrate, and algae. The invertebrate ϕ-NGu test was repeated after the ϕ-NGu stock solution had aged for 3 days to see if the toxicity of the photolyzed material was stable with time.

MATERIALS AND METHODS

NGu CHARACTERIZATION AND ANALYTICAL CHEMISTRY

The physical-chemical properties of NGu are given in Table 1. The NGu used in testing was obtained from Aldrich Chemical Company (lot numbers 4709PH and 7621PH). It was purified through recrystallization from boiling water to a purity of 99+ percent, as measured by HPLC. All test solution concentration measurements were done by HPLC; the details of the analytical technique (including precision and accuracy data) are described elsewhere.[6] Average percent recoveries (with standard deviations) for the spike samples which were included with each sample set are as follows: static acute tests - 97 percent (s.d. 5.1 percent, n=10); rainbow trout early life stage test - 100.2 percent (s.d. 2.4 percent, n=13).

TABLE 1. PHYSICAL-CHEMICAL PROPERTIES OF NGu[a]

Molecular Formula:	$NH_2NH_2C(=N)NO_2$
Molecular Weight:	104.074
Appearance:	Colorless crystals
Melting Point:	Decomposes at 232°C
Density:	1.72 g/cm^3
Vapor Pressure (mm Hg):	Extremely low
Heat of Combustion:	209 kcal/mole
Stability:	Sensitive to ultraviolet light, absorbs at 264 nm Explosive when shocked or exposed to heat or flame
Solubilities:	Water: 4.4 g/L at 25°C 83.5 g/L at 100°C Base (1N KOH): 12 g/L at 25°C Acid (40%H_2SO_4): 80 g/L at 25°C

a. Source: Reference 7.

NGu AND Φ-NGu TEST SOLUTION PREPARATION

For the fish and invertebrate tests, NGu test solutions were prepared by adding appropriate amounts of NGu to aerated well water and allowing the solution to stir 48 hours at room temperature (about 24°C). Saturated solutions were made by adding excess NGu to the well water; approximate saturation concentrations in well water were: 22°C - 3,000 mg/L; 20°C - 2,500 mg/L; 12°C - 1,700 mg/L. Only a few crystals of NGu were present in the

saturated solutions except in the rainbow trout static acute test, where there was a large excess of undissolved crystals. In the algal test with NGu, excess NGu was added to the algal assay medium and stirring was continued for 24 hours. Excess crystals were removed during filter sterilization of the medium prior to test initiation.

Photolyzed NGu was prepared by exposing NGu in an appropriate solution (well water or algal assay medium) to ultraviolet light until the NGu concentration reached non-detectable levels (<0.1 mg/L), as determined by periodic HPLC analysis. The ultraviolet light used was a 450 watt Canrad-Hanovia Model L quartz mercury-vapor immersion lamp, with the spectral energy distribution shown in Table 2. Of the total radiated energy, about 31 watts (7.6 percent) was contributed by wavelengths shorter than those found in natural sunlight (less than 294 nm).

TABLE 2. ENERGY DISTRIBUTION OF THE ULTRAVIOLET LAMP USED IN THE NGu PHOTOLYSIS STUDIES[a]

Portion of Spectrum	Wavelength (nm)	Energy (watts)	Percent of Total Energy
Infrared	1,000-1,400	16.4	9.6
Visible	400-600	75.7	43.1
New Ultraviolet	320-400	28.0	15.9
Middle Ultraviolet	280-320	28.7	16.3
Far Ultraviolet	220-280	27.0	15.4

a. Source: Information sheet distributed by Ace Glass, Inc., Vineland, NJ.

For the tests with fathead minnows, a stock solution of 613 mg/L NGu (measured concentration) in well water took 7 hours to be completely photolyzed. In the initial daphnid static acute test, the NGu stock measured concentration was 198 mg/L and was photolyzed for 5 1/2 hours; this same stock was used after aging 3 days to start the second daphnid static acute test. For the tests with algae, a stock solution of 316 mg/L (measured concentration) was prepared in algal assay medium and photolyzed 5 1/2 hours. The nominal concentrations for the ϕ-NGu tests are given as mg/L NGu and are based on dilutions from the measured (unphotolyzed) stock solution values.

For the fish and daphnid ϕ-NGu tests, controls were prepared by placing groups of organisms into both regular dilution water (well water) and also

into dilution water which had been photolyzed for a period equal to or greater than the NGu solution photolysis period. For the algal test, a preliminary study was conducted to determine if the photolyzed algal assay medium was toxic. The algal medium was photolyzed for 4 1/2 hours and tested at various concentrations after dilution with non-photolyzed algal medium. Procedures followed those described below for algal toxicity testing, except that only one replicate flask was used per treatment, and cell concentrations and mean cell volumes were recorded after allowing a 6-day growth period. The results, shown in Table 3, indicate no apparent effect of photolyzed algal assay medium on growth, as might be expected given the simple inorganic constituents composing the medium.

TABLE 3. GROWTH OF SELENASTRUM CAPRICORNUTUM IN PHOTOLYZED ALGAL ASSAY MEDIUM

Photolyzed Algal Assay Medium (%)	Growth after 6 Days (cells/mL)	Mean Cell Volume (μm^3)
100	3.2×10^6	42
60	3.6×10^6	44
25	3.4×10^6	40
12.5	3.6×10^6	43
6.2	3.4×10^6	44
0	3.3×10^6	41

NGu photolysis increased the acidity of the Φ-NGu stock solutions. While this acidity was part of the toxicity of Φ-NGu, it was felt that pH effects would vary greatly depending on the alkalinity of the water receiving the Φ-NGu and that the pH of NGu wastewater discharge was already controlled by existing discharge regulations. For these reasons, some pH adjustments were made. For the fish and daphnid tests, the stock solution pH was adjusted from an initial pH of 6.4 to a pH of 8.0 - 8.2 through addition of an 0.1 N sodium hydroxide solution. In the algal tests, the pH of the undiluted stock solution was 3.3. Only test solutions having initial pH's below 6.0 were pH-adjusted. In similar algal assay tests, Miller et al.[8] found that the growth of S. capricornutum under control conditions was unaffected by initial pH readings in the range 6.0 to 10.0. The initial pH values for the different treatment levels were as follows: 316 mg/L, pH 3.4; 158 g/L, pH 3.6; 79.0 mg/L, pH 4.3; 39.5 mg/L, pH 6.5; 19.7 mg/L, pH 6.8; 9.87 mg/L, pH 7.4; 0 mg/L, pH 7.4. The pH's were adjusted to near the control level (pH 7.8) at 316, 158, and 79.0 mg/L.

DILUTION WATER QUALITY

The dilution water used in the fish and invertebrate tests was obtained from a 62 meter well. A water softening system was used to reduce the hardness of the water and help prevent calcium carbonate precipitation. Flow reducers were used to deliver a mixture of 60 percent raw well water and 40 percent softened well water through a spray nozzle into a 750 liter reservoir

tank. A second pump then sent the water through a 5-micron cellulose acetate cartridge filter, an ultraviolet sterilizer, and temperature adjustment equipment.

The quality of the well water used in the fish and daphnid acute tests and the rainbow trout early life stage test is shown in Table 4. Test-specific measurements of pH, temperature, and dissolved oxygen concentrations are discussed later in this report. More comprehensive annual analyses of the well water are provided in Table 5. Nearly all the potentially toxic metals and organics were at non-detectable levels. The relatively high levels of sodium are related to the use of the water softener.

TEST ORGANISMS

Specific information on the organisms used in testing is given in Table 6. Durotest Optima 50[R]* wide spectrum fluorescent bulbs (color rendering index of 91) were used with all organisms (including fish) during both holding and testing. The diurnal photoperiod was 16 hr light and 8 hr dark. Fish were fed Rangen's trout food pellets (standard US Fish and Wildlife Service formulation) during holding. The loading level in the ϕ-NGu fathead minnow test was high due to the limited amount of ϕ-NGu stock solution available.

Daphnids (Daphnia magna) were raised in an in-house culture unit. Stock daphnids were housed in 2-liter tanks with 10 daphnids per tank. Aerated well water flowed through the tanks at a rate of two tank volumes per day. Temperature was maintained at 20°C (range 19 to 21), and light intensity was 150 to 350 lux. Daphnids were fed twice each day, 7 days per week with vitamin-enriched Ankistrodesmus falcatus, using the methods of Goulden et al.[9] Feeding levels were approximately 2 mg/L (dry weight) in the morning and 4 mg/L in the afternoon. Young were removed from the tanks every Monday, Wednesday, and Friday. Daphnids used for starting a test were not taken from any culture tank having excessive mortality, low reproductive success, or young caught in the surface film.

Eggs of the midge Paratanytarsus dissimilis were obtained from the EPA Environmental Research Laboratory in Duluth, MN. General culturing information may be found in reference 10. Eggs were held in 19 L glass aquaria containing about 3.6 L of well water. The aquaria were fitted with a tight-fitting cover and were gently aerated through a 1 mL disposable glass pipette. Food consisted of the Ankistrodesmus falcatus and a trout chow-yeast suspension which was added daily or as needed to ensure that the midge larvae had not totally cleaned the food from the bottom of the aquarium. Adult midges were transferred to 20 mL scintillation vials containing well water to obtain eggs for starting new cultures. Larvae were raised at temperatures of 21 to 24°C with a light intensity of approximately 150 lux (range 130 to 170). Larvae used in testing were about 12 days old (fourth instar) and were dislodged from their tubes using a stream of water from a pipette.

Aquatic oligochaetes (Lumbriculus variegatus) were obtained from SRI International in Menlo Park, CA. Culture procedures generally followed those

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* Use of Trademarked name does not imply endorsement by the US Army, but is used only to assist in identification of a specific product.

TABLE 4. DILUTION WATER QUALITY

Parameter	NGu Tests			Φ-NGu Tests	
	Fish and Invertebrate Static Acute	Second Midge Static Acute	Rainbow Trout Early Life Stage [Mean (range); No. of Observations]	Fathead Minnow Static Acute	Daphnid Static Acute
pH	8.0	8.0	- (8.0-8.2); n=7	8.2	8.2
Alkalinity (to pH 4.5; mg/L as $CaCO_3$)	207	203	204 (177-229); n=9	207	215
Hardness (mg/L as $CaCO_3$)	161	161	168 (162-178); n=5	156	154
Conductivity (μmhos/cm, 25°C)	650	650	780 (740-825); n=9	706	598
Suspended Solids (mg/L)	<1	<1	<1	<1	<1
Total Organic Carbon (mg/L)	<1	<1	- (<1-1.6); n=6	<1	<1
Unionized Ammonia (μg/L)	<2.2	<2.2	<1.5	<2.2	<2.2

TABLE 5. COMPREHENSIVE DILUTION WATER ANALYSES, 1982 AND 1983

Parameter	Concentration (mg/L) 1982	1983
Ammonia (as N)	0.03	<0.05
Nitrite (as N)	<0.02	—[b]
Nitrate (as N)	0.45	0.16
Chloride	91.5	70.5
Cyanide	—	<0.002
Fluoride	0.33	0.19
Sulfate	46.8	39.3
Sulfide	—	<0.05
Aluminum	<0.002	<0.002
Arsenic	—	<0.002
Barium	0.113	0.089
Boron	—	<0.5
Cadmium	<0.001	<0.0005
Calcium	45.7	47.5
Cobalt	<0.004	<0.002
Copper	<0.003	<0.002
Iron	<0.003	<0.1
Lead	<0.002	<0.002
Magnesium	15.5	14.7
Manganese	—	<0.002
Mercury	<0.0005	<0.0005
Molybdenum	—	<0.003
Nickel	—	<0.002
Phosphorus	—	<0.01
Potassium	1.2	1.25
Selenium	—	<0.002
Silicon	5.4	2.7
Silver	—	<0.0005
Sodium	119.0	93.5
Zinc	<0.02	<0.02

Chlorinated Hydrocarbons	Concentration 1982	1983	Detection Limit (µg/L) 1982	1983
Aldrin	x[a]	x	0.02	0.05
p,p'-DDT	x	x	0.02	0.05
o,p'-DDT	x	x	0.02	0.05
DDD	0.026	x	0.02	0.05
Dieldrin	x	x	0.02	0.05
Endrin	x	x	0.02	0.05
Heptachlor	x	x	0.02	0.05
Heptachlor Epoxide	x	x	0.02	0.05
Lindane	x	x	0.01	0.05
Chlordane	x	x	0.20	0.20
Alpha-BHC	x	x	0.01	0.05
Beta-BHC	x	x	0.02	0.05
Delta-BHC	x	x	0.01	0.05
Toxaphene	x	x	1.0	1.0
Methoxychlor	x	x	0.02	0.05
Polychlorinated Biphenyls	x	x	1.0	1.0
2,4-D	-	x	—	0.05
2,4,5-T	-	x	—	0.05
Silvex	-	x	—	0.05
Diazinon	-	x	—	0.20
Malathion	-	x	—	0.20
Parathion	-	x	—	0.20

a. x = below detection limit; concentrations reported in µg/L.
b. Not measured.

TABLE 6. INFORMATION ON ORGANISMS USED IN TESTING

Species	Test	Original Source[a]	Mean Size Used in Testing: Length (mm)	Mean Size Used in Testing: Weight (g)	Age (weeks)	Acclimation (weeks)	Loading in Tests (g fish/L test solution)	Temperature (°C)	Prior Disease Treatments
FISH									
Fathead Minnow (*Pimephales promelas*)	MDu	Kurtz Fish Hatchery, Elverson, PA	21	0.15	9	NA[b]	0.38	22±2	None
	Φ-MDu		28	0.27	15	NA	3.4	22±2	None
Channel Catfish (*Ictalurus punctatus*)	MDu	Kurtz Fish Hatchery, Elverson, PA	46	1.42	14	9	1.01	22±2	Malachite green plus formalin; tetracycline
Bluegill (*Lepomis macrochirus*)	MDu	Kurtz Fish Hatchery	24	0.58	13	9	0.57	22±2	None
Rainbow Trout (*Salmo gairdneri*)	MDu	Fish: Wytheville Federal Fish Hatchery, Wytheville VA	21	0.11	4	3	0.28	12±2	None
	MDu ELS[c]	National Fisheries Center, Leetown, Kearneysville, WV	NA	NA	16 days (eyed eggs)	NA	NA	NA	None
INVERTEBRATES									
Water Flea (*Daphnia magna*)	MDu, Φ-MDu	Carolina Biological Company, Burlington, NC	NA	NA	<24 hours	NA	NA	20±2	NA
Midge Larva (*Paratanytarsus dissimilis*)	MDu	USEPA, Environmental Research Laboratory, Duluth, MN	NA	NA	3rd–4th instar	NA	NA	22±2	NA
Aquatic Worm (*Lumbriculus variegatus*)	MDu	SRI International Menlo Park, CA	22	NA	NA	NA	NA	22±2	NA
Amphipod (*Gammarus minus*)	MDu	Local spring, Frederick, MD	10.7	NA	NA	8	NA	20±2	NA
Amphipod (*Hyalella azteca*)	MDu	Hunt's Pond Norwich, NY	5.00	NA	NA	NA	NA	20±2	NA
ALGAE									
Selenastrum capricornutum	MDu, Φ-MDu	American Type Culture Collection No. 22662	NA	NA	NA	NA	NA	24±1	NA

a. All test organisms except the following were obtained from in-house cultures established from the original source: channel catfish, bluegill, rainbow trout, and the amphipod *C. minus*.
b. NA = Not available or not applicable.
c. ELS = Early life stage test.

described by Bailey and Liu.[11] Oligochaetes were raised in 19 L glass aquaria having approximately a 2-cm bed of fine sand (particle size 1 to 1.5 mm) in which the worms could bury themselves. Well water flowed slowly into the aquarium, and gentle aeration was maintained. Temperature was 21 to 24°C, and light intensity was 225 lux. The worms were provided with the same trout chow-yeast food given the midges, and trout chow pellets were added to the tank once per week to provide additional food for the worms. Worms were removed for testing by dislodging them from the substrate through agitation with water from a large-bore pipette. Worms were then gathered with the pipette and placed in a beaker of well water for transfer to the test chambers.

One amphipod used in testing was Hyalella azteca, which was collected from Hunt's Pond near Norwich, NY. H. azteca was keyed to species using Pennak.[12] The other amphipod tested was Gammarus minus, which was obtained from a spring in the Frederick, MD, area. The species identification was made based on the key by Holsinger.[13,14] Both species were held under similar conditions. The amphipods were raised in aerated 19 L aquaria which had about 4 cm of 2 to 4 mm diameter sand for substrate. Well water flowed into the aquaria at about 10 mL/min, and the temperature was 21 to 24°C. Light intensity was similar to the oligochaetes; about 225 lux. Food was provided ad libitum and was a mixture of trout food, Cerophyll-brand dried grass leaves, vitamins, and dried sugar maple leaves in an agar base, prepared as described by Rach.[15] Both males and females were removed as needed for testing using a wide-bore pipette.

The alga Selenastrum capricornutum was used in testing and was raised and tested in 125 mL DeLong flasks with stainless steel caps containing 60 mL of filter-sterilized EPA algal assay medium prepared as described by Miller et al.[8] The original algal stock was obtained from the American Type Culture Collection (No. 22662), Rockville, MD. Algae were maintained and tested under cool white fluorescent lights (4,300 lux, ±10 percent) at a temperature of 24°C (±1°C) in a Psycrotherm® model 327 incubator-shaker set at a rotational speed of 100 rpm (± 10 percent).

TEST METHODOLOGIES

1. Static Acute Tests. Static acute methods generally followed those recommended by the American Society for Testing and Materials.[16] Fish to be used in testing were acclimated to the well water for the periods indicated in Table 6. Fish were not used in testing if they had any symptoms of disease within 10 days of the start of the test, or if more than 2 percent of the fish died within the 48 hours preceeding the start of the test. Fish were transferred from stock tanks into holding tanks 48 hours prior to the start of a test and were not fed during this time. The fish were then randomly assigned to a test jar in groups of three and four (for channel catfish, with 10 fish per jar) or in groups of five (all other fish, with 15 fish per jar). Test jars were randomly assigned to positions in the water bath.[17]

Test chamber and solution volumes used in testing fish (and invertebrates) are given in Table 7. Temperature was held within one degree (Celsius) of the holding temperature (Table 6) by keeping the test vessels in a water bath. Water bath temperature was monitored with a 7 day temperature recorder, and temperatures were checked daily in one test vessel. Lighting

TABLE 7. TEST CHAMBERS AND TEST SOLUTION VOLUMES

Compound Tested	Species	Test Chamber	Test Solution Volume (L)
NGu	S. gairdneri	19-L Aquarium	6
	I. punctatus	19-L Glass jar	14
	P. promelas	19-L Glass jar	16
	L. macrochirus	19-L Glass jar	10
	All invertebrates	0.25-L Beaker	0.2
Φ-NGu	P. promelas	2.3-L Glass tank	1
	D. magna	0.25-L Beaker	0.2

was of the same quality and photoperiod as was used during holding. The pH and dissolved oxygen concentrations were measured after 0, 48, and 96 hours in one replicate of the control and low, medium, and high toxicant concentrations. Toxicant concentrations were measured at least once during each test in the controls and the low, medium, and high concentrations. During tests with fish, aeration was initiated in all jars if the dissolved oxygen level fell below 40 percent of saturation at the test temperature. Aeration was unnecessary in the invertebrate tests. Other aspects of dilution water quality are summarized in Tables 4 and 5 and were discussed earlier. The total duration of the fish static acute tests was 96 hours.

The testing approach used in the invertebrate static acute tests was, in many ways, identical to that used in the fish static acute tests. Only aspects different from the fish static acute tests are described below. Daphnids to be used in testing were obtained from females isolated from stock cultures less than 24 hours before the start of a test. Algal food was provided to these adults and their neonates up to the time that the young were pooled for testing. Neonates were transferred with an eyedropper having a fire-polished bore at least 2 mm in diameter.

Other invertebrates were obtained from culture tanks as described in the "Test Organisms" section above and pooled into a common well water container for transfer into the test beakers. After pooling, all invertebrates were randomly assigned to positions in the testing water bath.[16] Invertebrates were moved in groups of three, three, and four into 200 mL of test solution in 250 mL borosilicate glass beakers until each beaker contained 10 invertebrates. Three beakers (30 invertebrates total) were used at each treatment level. The duration of the invertebrate tests was 48 hours.

In all NGu static acute tests, a minimal number of test concentrations were used. Preliminary range-finding tests showed NGu to be virtually nontoxic, so two concentrations (NGu saturation concentration at the test temperature and 50 percent of saturation) were judged to be adequate to demonstrate the lack of toxicity in the definitive tests. Four or five test concentrations were used in each Φ-NGu test so that an LC50 or EC50 could be adequately defined.

2. Fish Early Life Stage (ELS) Test. Since the amount of NGu that would have been required in a flow-through test was prohibitively high and because NGu was very stable in well water, the ELS test with NGu was conducted as a static renewal test rather than the usual flow-through test. Test solutions were prepared fresh weekly. This was frequent enough to prevent test concentrations from dropping more than 5 percent from their initial levels and to maintain good water quality for the trout. Four test concentrations were selected, ranging from 212 mg/L to 1,700 mg/L (the approximate NGu saturation concentration at 12°C). A 50 percent dilution factor was employed.

Sixteen-day-old eyed eggs were obtained from the Leetown National Fisheries Center. They were maintained at 12 (±2)°C during transportation. The eggs were then transferred in groups of five into egg cups suspended in 9.4 L aquaria containing 5 L of test solution, until each egg cup contained 15 eggs. With two replicate aquaria per treatment level, a total of 30 eggs were used per treatment. The egg cups were 11.5 cm lengths of 50 mm ID glass tubing covered at the bottom with 508 micron polyethylene mesh screen. They were kept in motion continuously by a rocker arm apparatus which gave the cups a vertical travel of about 2 cm at a speed of five cycles per minute.

Due to the sensitivity of rainbow trout eggs to light, the eggs were shielded from light until 7 days after hatching was complete. After this time, light intensity ranged from 280 to 390 lux. Temperature was maintained within a degree of 12°C. The pH of fresh test solutions ranged from 7.9-8.3, while the pH of old test solutions (just prior to renewal) ranged from 7.7-8.2. Dissolved oxygen concentrations dropped slowly from the beginning of the test until, on day 24, a 6.6 mg/L minimum was recorded (65 percent of saturation). At this point, aeration was begun in all tanks and was continued through the end of the test. The test lasted 42 days (30 days post-hatch, 18 days after swim-up).

Egg hatching was recorded and dead eggs were removed every day until hatching was complete. The trout were allowed to swim out of the egg cup when they reached the swim-up stage of development. Feeding was then initiated with standard US Fish and Wildlife Service formulation trout food. Fish were fed three time each day. Food formulations were changed as appropriate to match the increasing size of the trout. Excess food and fecal materials were siphoned from the tanks daily. Fish were not fed for the last 48 hours of the test to allow their guts to empty prior to weighing.

3. Algal Test Methods. Algal toxicity test procedures were based on those recommended by Payne and Hall.[18] Test conditions followed those described previously for culturing under "Test Organisms." For both the NGu and the ϕ-NGu algal toxicity tests, two filter-sterilized stock solutions were prepared. One stock was algal assay medium used for dilution of the other stocks, which were either NGu dissolved in algal assay medium or ϕ-NGu which had been photolyzed in algal assay medium. Test solutions were prepared by dispensing appropriate amounts of the stocks into the DeLong flasks under sterile conditions in a Labconco^{R} tissue culture enclosure. An inoculum of S. capricornutum in log-phase growth was added to give an initial cell count of 20,000 cells/mL (± 10%).

Cell counts were determined daily from day 0 through day 5 of the test by aseptically withdrawing 3.5 mL samples from each flask and computing both cell counts and mean cell volumes using a Model ZBI Coulter Counter and a Model MHR Mean Cell Volume Computer. The instrument was calibrated with each use according to procedures described by Miller et al.[8] The conversion factor specified in Miller et al. was used to convert the cell count and cell volume data into dry weight estimates. The daily samples were also used to determine chlorophyll a concentrations relative to the controls by in vivo fluorometric analysis using a Turner Model 112 filter fluorometer equipped for chlorophyll a analysis.[19] To allow accurate comparisons of relative chlorophyll a levels between days during a test, all fluorometer measurements were referenced to a standard curve made from the stable fluorescent material corproporphyrin-1, obtained from Sigma Chemical Company, St. Louis, MO (stock number COP-1-5). Regression analysis was used to convert fluorescence readings to fluorescence expressed as corproporphyrin-1. Fluorometer readings were taken for toxicant solutions without algae to ensure that neither NGu nor Φ-NGu contributed to the observed fluorescence readings.

TEST END POINTS AND DATA ANALYSES

In all tests with NGu, concentration data used in statistical analyses were the mean measured concentrations. In the tests with Φ-NGu, nominal concentrations were used in calculations. These nominal concentrations were based on an original measured concentration of an NGu stock solution which was then completely photolyzed and diluted to the appropriate test concentration. Thus, a 310 mg/L NGu stock solution which was completely photolyzed and diluted 1:10 would be reported as a 31 mg/L Φ-NGu test solution.

A fish was considered dead when ventilatory movements ceased and the fish failed to respond to gentle prodding. Amphipods, midge larvae, and oligochaetes were similarly recorded as dead when they failed to show any movement following gentle agitation. With daphnids, immobilization was used as an end point instead of death, due to the difficulty of determining whether a daphnid was dead or immobilized without microscopic examination. An EC50 based on immobilization was calculated for daphnids rather than an LC50.

LC50's and EC50's (and their 95 percent confidence limits) for mortality were determined using a computer program developed by C. Stephan.[20] The binomial method for estimating LC50's was used when there were less than two concentrations at which mortality was between 0 and 100 percent. Confidence limits generated with this method are actually greater than 95 percent (e.g. 97 or 99 percent); however, they are used here as conservative estimates of the 95 percent limits. Moving average or probit methods were used for determining LC50's when two or more responses between 0 and 100 percent occurred in a test. Probit results were recorded when the goodness of fit probability of the data to the probit model was greater than 0.05. Moving average LC50 estimates were used if the probit goodness of fit was less than 0.05.

The following end points were monitored during the rainbow trout early life stage test: egg hatching success, days to 50 percent hatch, days to 50 percent swim-up (measured from the 50 percent hatch day), fry survival, overall survival, standard length and weight (measured at the end of the test), fry deformities (percent), and behavioral effects. Times to hatch and

swim-up and behavioral effects were not analyzed statistically. When statistical tests were utilized, an initial chi square test for heterogeneity was applied to the data from replicate exposure tanks to determine if the replicates could be pooled. For the growth parameters, sample size was adjusted downward when heterogeneity was detected according to the methods of Feder.[21]

For the count variables including egg hatchability, fry survival, overall survival, and fry deformities, a multiple comparison statistical approach was used to determine which treatment groups were significantly different from the controls. A succession of 2 x 2 contingency table tests of homogeneity between each treatment group and the controls were done based on a one-tailed Fisher's exact test. Bonferroni's method was used to adjust for simultaneity. Quadratic regression analysis was performed for length and weight measurements using the General Linear Models program of the Statistical Analysis System.[22] This program provided pairwise comparisons between the treatment and control groups at a 0.05 significance level. Bonferonni's method was again used to correct for simultaneity.

In the algal toxicity test, the end point monitored was growth, measured both as dry weight (cells/mL x cell volume x conversion factor) and as chlorophyll a concentration (relative to controls, measured in vivo using a fluorometer). A series of end points was evaluated as described below. The end points are given in order from most to least toxic. A log(10) transformation was used for all growth measurements and, when a single point estimate of a treatment effect was required, the geometric mean of the three replicate flasks was used. A 0.05 significance level was used, and significance levels were adjusted for simultaneity using Bonferonni's correction when appropriate.

1. Algicidal concentration. This is the lowest concentration tested which causes an apparent algistatic effect after 5 days and which prevents cells transferred to clean media from resuming logarithmic growth.

2. Algistatic concentration. The minimum algistatic concentration can be determined by an inverse regression technique as described by Payne and Hall.[17] In this study, an algistatic effect was said to have occurred if, after the 5-day growth period, cell counts did not increase significantly from the initial inoculum level.

3. EC50 based on growth rate. Growth rate trends were analyzed using a mulitvariate analysis of variance approach. Changes in growth were recorded as the multiplicative change in log(10) growth from day to day during test days 1 to 5. Effects of treatment level, flask within treatment level, day of exposure, and day/treatment interactions were tested using the SAS MANOVA procedure.[22] Khatri's techniques[23] were then used to fit the concentration-response data to a polynomial model. Growth rate EC50's could not be determined if the model was not linear. If the model was linear, the growth rates at each treatment level (the slope of each line) were expressed as percentages of the control growth rate, and the log(10) of these values were then regressed against the log(10) of concentration. Feder's inverse regression methods[24] were to be used to determine the EC50 for growth rate and the associated 95 percent confidence limits.

4. EC50 based on 5-day standing crop. The 5-day dry weight and relative chlorophyll a data were modeled using the SAS General Linear Models

program.[22] EC50's and 95 percent confidence limits were determined from these results using the inverse regression techniques of Feder.[24]

5. No observed effect concentration (NOEC). The NOEC is the highest concentration tested which causes no statistically significant differences from control growth. For overall growth rate trends, Khatri's techniques[23] were used in conjunction with a multivariate analysis of variance to provide pairwise tests between treatments and the controls. A classical analysis of variance approach was used to compute 5-day standing crop NOEC's. In this case, the SAS ANOVA procedure was utilized.[22] This procedure also provided pairwise comparisons between the controls and each treatment level.

RESULTS AND DISCUSSION

Results from the static acute toxicity tests with NGu and fish and invertebrates are given in Table 8. During these tests, pH levels varied less than 0.4 units from initial levels, which ranged from 8.0 to 8.4. No LC50's or EC50's could be determined, since in no case were more than 50 percent of the organisms affected at the top (saturation) concentration of NGu. Except in the bluegill static acute test, control mortality never exceeded 3 percent. Bluegill controls had 13.3 percent mortality, but the test was not repeated due to the lack of any mortality at higher concentrations. The rainbow trout was the most sensitive fish species to NGu in these tests, while the amphipod Hyalella azteca was the most sensitive of the invertebrates tested.

The response of S. capricornutum to NGu after 5 days of exposure is shown in Tables 9 through 11. At the top concentration, which was the approximate saturation concentration of NGu in algal assay medium at 24°C, no algicidal or algistatic effects were observed. A 120 hour EC50 based on growth rates could not be calculated since, as shown in Table 9, neither dry weight nor relative chlorophyll a growth rates were reduced below 50 percent of the control levels. A 120 hour EC50 based on standing crop dry weight and relative chlorophyll a levels could be calculated (see data in Tables 10 and 11) and was found to be approximately 2,000 mg/L for both dry weight and relative chlorophyll a measurements. Both growth end points were significantly different from the controls at all the NGu concentrations tested, so an NOEC could not be determined. These results are summarized in Table 12.

TABLE 8. ACUTE TOXICITY OF NGu TO FISH AND INVERTEBRATES

Species	Length of Exposure (hr)	Mean Measured Concentration[a] (mg/L)	Percent Mortality or Immobilization
Rainbow Trout (Salmo gairdneri)	96	1638	20
		901	0
		<1.45	0
Fathead Minnow (Pimephales promelas)	96	2714	0
		1690	0
		<1.45	0
Bluegill (Lepomis macrochirus)	96	2634	0
		1671	0
		<1.45	13.3
Channel Catfish (Ictalurus punctatus)	96	2636	0
		1644	0
		<1.45	0
Water Flea (Daphnia magna)	48	2838	16.7
		1485	0
		<1.49	0
Amphipod (Gammarus minus)	48	2720	0
		1412	0
		<1.49	0
Amphipod (Hyallela azteca)	48	2730	46.7
		1424	0
		<1.49	0
Midge Larva (Paratanytarsus dissimilis)	48	3395	10[b]
		1839	0
		<1.56	3
Oligochaete (Lumbriculus variegatus)	48	2868	0
		1785	0
		<1.49	0

a. The high, low, and control NGu concentrations are listed for each test. The top concentration tested was approximately at saturation with well water at the test temperature.
b. One larva emerged and died.

TABLE 9. GROWTH RATES OF SELENASTRUM CAPRICORNUTUM EXPOSED TO NGu

Mean Measured Concentration (mg/L)	Growth Rate[a]	
	Dry Weight	Relative Chlorophyll a
3,753	1.50 (40.9)[b]	1.45 (-43.7)
2,002	2.26 (11.0)	2.19 (-14.9)
990	2.47 (2.8)	2.40 (-6.8)
508	2.46 (3.1)	2.44 (-5.1)
<1.45 (control)	2.54	2.58

a. Multiplicative change per day. Geometric means of three replicate flasks are reported.
b. Percent difference from control levels.

TABLE 10. EFFECT OF NGu ON THE GROWTH OF SELENASTRUM CAPRICORNUTUM: DRY WEIGHT MEASUREMENTS

Mean Measured Concentration (mg/L)	Test Day[a]			
	0	1	2	5
3,753	0.315[b]	0.597	0.853	2.92[c] (-92.9)[d]
2,002	0.347	0.811	2.36	22.8[c] (-44.4)
990	0.361	0.875	2.80	35.4[c] (-13.7)
508	0.323	0.939	2.82	35.9[c] (-12.4)
<1.45 (controls)	0.365	0.917	2.90	41.0

a. No data available for test days 3 and 4.
b. Geometric mean of three replicate flasks; mg/L.
c. Significantly different from the controls ($p < 0.05$). Only day 5 data was tested.
d. Percent difference from control levels.

TABLE 11. EFFECT OF NGu ON THE GROWTH OF SELENASTRUM CAPRICORNUTUM: RELATIVE CHLOROPHYLL a MEASUREMENTS

Mean Measured Concentration (mg/L)	Test Day[a] 0	1	2	5
3,753	21.8[b]	40.7	86.2	211[c] (-93.6)[d]
2,002	22.9	53.0	175	1,600[c] (-51.8)
990	22.3	57.7	210	2,500[c] (-24.7)
508	22.7	61.0	210	2,670[c] (-19.6)
<1.45 (controls)	22.3	54.5	212	3,320

a. No data available for test days 3 and 4.
b. Geometric mean of three replicate flasks; mg/L as corproporhyrin.
c. Significantly different from the controls ($p < 0.05$). Only day 5 data was tested.
d. Percent difference from control level.

TABLE 12. SUMMARY OF NGu TOXICITY TO SELENASTRUM CAPRICORNUTUM

End Point	Growth Measurement: Dry Weight[a]	Relative Chlorophyll a[b]
Minimum algicidal concentration	>3,753[c]	>3,753
Minimum algistatic concentration	>3,753	>3,753
120 Hr EC50 (growth rate)	>3,753	>3,753
120 Hr EC50 (standing crop)	2,146 (2,010-2,282)[d]	1,937 (1,734-2,140)
120 Hr no observed effect concentration	<508[e]	<508

a. Milligrams per liter.
b. Milligrams per liter as corproporhyrin.
c. Highest concentration tested (approximate NGu saturation concentration at 24°C).
d. Ninety-five percent confidence limits.
e. Lowest concentration tested.

The rainbow trout early life stage test exposure concentrations are given in Table 13. The mean measured concentrations were very close to nominal levels, and the variation in concentration levels was relatively small. As stated previously, the one week interval between test solution changes did not seem to affect the NGu concentrations, as the concentrations of NGu in the old solutions were never more than 5 percent lower than the concentrations of the fresh solutions.

TABLE 13. EXPOSURE CONCENTRATIONS DURING THE RAINBOW TROUT EARLY LIFE STAGE TEST

Nominal Concentration (mg/L)	Mean Measured Concentration (mg/L)	Standard Deviation	Range	Number of Observations
1,700	1,703	46.0	1,642 - 1,787	13
850	857	24.7	812 - 888	13
425	418	13.1	398 - 435	13
212	197	8.4	177 - 209	13
0	BDL[a]	—	—	13

a. Below detection limit (1.5 mg/L).

Biological data from the trout test are reported in Table 14. The only statistically significant differences between the controls and the treated fish occurred at the top concentration (1,700 mg/L) for weight (16.3 percent below the controls), length (3.7 percent below the controls), and deformities (spinal curvature and the presence of a raised area on the dorsal surface of the head). The length effect at 418 mg/L is most likely an artifact, since there is no corresponding significant effect on weight at this concentration, and there is no apparent effect on weight or length at the next higher concentration (857 mg/L). The biological significance of the raised dorsal area on the head is unclear; this effect did not seem to be related to effects on either survival or growth.

TABLE 14. RESULTS OF THE EARLY LIFE STAGE TEST WITH RAINBOW TROUT AND NGu

Mean Measured Concentration (mg/L)	Hatching Success (%)	Time to Hatch (days)[a]	Time to Swim-Up (days)[a]	Fry Survival (%)	Overall Survival (%)	Fry Deformities[b] (%)	Fry Length (mm)	Fry Weight (g)
1,703	100	28	12	90.0	90.0	90.0[c] (90.0)	23.7[c]	0.149[c]
8[illegible]7	96.7	28	12	82.8	80.0	27.6 (17.2)	24.5	0.170
418	96.7	28	12	89.7	86.7	27.6 (20.7)	23.6[c]	0.164
197	100	28	12	76.7	76.7	20.0 (10.0)	24.3	0.167
<1.5	93.3	28	12	92.9	86.7	10.7 (0)	24.6	0.178

a. These end points were not analyzed statistically.
b. Deformities included spinal curvature and a raised area on the dorsal surface of the head. The figures in parentheses include the latter deformity only.
c. Statistically different from the controls ($p<0.05$).

In summary, the acute toxicity of NGu to the 10 species of aquatic organisms tested was uniformly low. LC50's (or EC50's) for all the fish and invertebrate species were greater than the saturation concentration of NGu at the test temperatures used. The 120-hr standing crop EC50 for S. capricornutum was only slightly lower at 2,000 mg/L.

Photolysis of NGu greatly increased its toxicity. This can be seen in the results of algal toxicity test with Φ-NGu (Tables 15 through 17). One inconsistency is the high degree of growth reduction at 39.5 mg/L, which does not follow the overall concentration-response trend. This treatment is the highest Φ-NGu concentration tested without initial pH adjustment, although, as stated previously, the initial pH of 6.5 was well above levels known to inhibit growth. It is possible that some components of the Φ-NGU solution were made more toxic at acidic pH.

Although it is clear that the 120-hour standard crop EC50 lies between 19.7 and 39.5 mg/L, the inconsistent growth response at 39.5 mg/L affected the statistical analysis of the data in such a way that a meaningful EC50 could not be calculated. The 39.5 mg/L treatment was therefore excluded from the data analysis so that an EC50 estimate could be made. The resulting EC50 represents a conservative estimate of toxicity, i.e. the "actual" EC50 could be somewhat lower but would not be greater than this.

TABLE 15. EFFECT OF Φ-NGu ON THE GROWTH OF SELENASTRUM CAPRICORNUTUM: DRY WEIGHT MEASUREMENTS

Nominal[a] Concentration	Test Day					
	0	1	2	3	4	5
316	0.319[b]	0.365	0.469	0.464	0.575	0.465 (-98.7)[c]
158	0.294	0.428	0.611	0.762	0.849	0.762 (-97.9)
79.0	0.277	0.558	1.050	1.620	2.641	3.398 (-90.8)
39.5[d]	0.286	0.307	0.335	0.346	0.384	0.438 (-98.9)
19.7	0.286	0.740	2.793	8.776	24.148	36.601 (-0.6)
9.87	0.312	0.704	2.839	8.661	23.631	38.494 (+4.5)
0 (control)	0.302	0.752	2.463	7.213	23.554	36.829

a. Concentrations are reported as NGu (mg/L) prior to photolyzation.
b. Geometric mean of three replicate flasks; mg/L.
c. Percent difference from control levels (day 5).
d. This treatment was excluded from the data analysis. (See text for explanation.)

TABLE 16. EFFECT OF Φ-NGu ON THE GROWTH OF SELENASTRUM CAPRICORNUTUM: RELATIVE CHLOROPHYLL a MEASUREMENTS

Nominal[a] Concentration	Test Day 0	1	2	3	4	5
316	18.42[b]	26.98	40.72	47.60	54.31	54.30 (-98.2)[c]
158	16.32	24.19	41.45	56.86	73.06	85.18 (-97.1)
79.0	17.94	33.63	49.35	153.04	274.12	388.58 (-86.9)
39.5[d]	17.00	18.49	19.84	22.22	30.52	40.45 (-98.6)
19.7	17.49	46.72	212.34	785.70	1,721.54	2,972.56 (+0.6)
9.87	16.66[e]	43.81[e]	212.85	784.07[e]	1,760.63	2,828.97 (-4.3)
0 (control)	17.26	46.65	176.66	621.17	1,601.98	2,956.18

a. Concentrations are reported as NGu (mg/L) prior to photolyzation.
b. Geometric mean of three replicate flasks; mg/L as corproporphyrin.
c. Percent difference from control levels (day 5).
d. This treatment was excluded from the data analysis. (See text for explanation.)
e. Geometric mean of two observations only.

TABLE 17. SUMMARY OF Φ-NGu TOXICITY TO SELENASTRUM CAPRICORNUTUM

End Point	Growth Measurement	
	Dry Weight[a]	Relative Chlorophyll a[b]
Minimum algicidal concentration	>316[c]	>316
Minimum algistatic concentration	>316	>316
120 hr EC50[d] (standing crop)	32.3 (26.2 - 38.5)[e]	34.3 (26.3 - 42.3)
No observed effect concentration		
growth rate	19.7	19.7
120 hr standing crop	19.7	19.7

a. Milligrams per liter.
b. Milligrams per liter as corproporphyrin.
c. Highest concentration tested (mg/L); all concentrations reported as NGu (mg/L) prior to photolyzation.
d. Growth rate EC50 was not available to non-linear growth rates at some treatment levels.
e. Ninety-five percent confidence limits.

Analysis of the dry weight and chlorophyll a data (Tables 15 and 16) showed that neither algicidal nor algistatic effects were obtained. Growth rate EC50's could not be computed due to non-linear growth rates at high treatment levels. There are large and statistically significant reductions in both growth parameters at all concentrations above 19.7 mg/L, based on pairwise comparisons between the toxicant treatments and the controls using both the multivariate growth trend and the day 5 standing crop analysis of variance techniques. This level of toxicity (see summary, Table 17) is much higher than that observed for S. capricornutum and NGu (Table 12).

The increase in toxicity caused by the photolyzation of NGu is evident in acute tests with other aquatic organisms besides algae (Table 18). The Φ-NGu LC/EC50 levels were quite similar for all three species tested and represent increases of from 66 to over 115 times the LC/EC50's of the parent NGu material. The test with aged (3 day old) Φ-NGu was conducted to evaluate the toxicological stability of the Φ-NGu solution. Although the EC50 of the aged material appears to be significantly higher than the EC50 of fresh Φ-NGu, the magnitude of the difference is small enough that it could be due to variability in the sensitivity of the batches of daphnids used in the two tests rather than to a decrease in toxicity of the aged Φ-NGu solution.

TABLE 18. THE EFFECT OF PHOTOLYZATION ON THE ACUTE TOXICITY OF NGu

Species	End Point	Toxicant Concentrations (mg/L) NGu	Toxicant Concentrations (mg/L) Φ-NGu[a]	Relative Toxicity Ratio[b], Φ-NGu:NG
Fathead Minnow (Pimephales promelas)	96 hr LC50	>2,714[c]	34.5 (22.1 - 61.3)[d]	>78
Water Flea (Daphnia magna)	48 hr EC50	>2,838[e]	24.6 (21.1 - 28.7)	>115
		Aged Φ-NGu[f]:	35.0 (29.6 - 41.5)	> 81
Algae (Selenastrum capricornutum)	120 hr EC50[g]	2,146 (2,010 - 2,283)	32.3 (26.2 - 38.5)	66.4

a. Concentrations reported as NGu (mg/L) prior to photolyzation. No mortality was observed in the fish and daphnid photolyzed well water control tanks.
b. NGu EC50 divided by the corresponding Φ-NGu EC50.
c. No mortality at 2,714 mg/L.
d. Confidence limits from binomial LC50 estimation are greater than 95%; other confidence limits reported are 95% limits.
e. Immobilization was 16.7% at this concentration.
f. Test used the same Φ-NGu solution as the first D. magna test after it was stored for 3 days at room temperature.
g. Based on dry weight standing crop.

CONCLUSIONS

The toxicity of NGu to the freshwater fish, invertebrates, and algae tested was uniformly low. In addition, there was little difference between the acute and chronic toxicity of NGu in the tests conducted with rainbow trout. Based on these results, NGu appears to present little hazard to aquatic organisms except at concentrations approaching its solubility limit in water.

Photolysis increased the acute toxicity of NGu to the three species tested by factors ranging from 66 to over 115, and aging the Φ-NGu for 72 hours had little effect on its toxicity. This indicates the possibility that the toxicity of NGu-containing wastewaters could increase dramatically if left in holding ponds exposed to sunlight. On the other hand, the present studies were done with NGu photolyzed in a short period of time using a high intensity ultraviolet light which had some wavelengths not found in natural sunlight. In addition, there is no definitve information available on the rate of photolysis of NGu in natural waters. Thus, the importance of the increased toxicity of Φ-NGu will depend on the extent to which NGu photolysis is a significant environmental fate process in "real world" situations.

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