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Acute Toxicity of Hydrazine Hydrate to the Fathead Minnow (*Pimephales promelas*) and Daphnid (*Daphnia pulex*)

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Utilities that depend on steam generation for the production of electricity have benefitted greatly from the use of hydrazine (H_2NNH_2). The chemical is a strong antioxidant that reacts with oxygen in water to form water and nitrogen gas. Deoxygenated boiler water helps reduce oxidation inside boilers and steam systems, thereby increasing their efficiency and longevity. Hydrazine compounds are additionally beneficial because they do not increase the total dissolved solids in treated boiler water as do other oxygen scavenging chemicals (Becker and Thatcher 1973).

Excess hydrazine breaks down under extreme temperature and pressure conditions to form ammonia which is corrosive to copper and copper alloys used in some heated water systems. The most effective means of treating active boiler water with hydrazine therefore is to consistently maintain a low hydrazine residual. Idle boilers, however, are protected with layup solutions of hydrazine in concentrations as high as 200 mg/L (American Society for Testing and Materials 1980). Hydrazine may enter the aquatic environment as controlled industrial discharge or as a result of an accidental spill.

Pure hydrazine is a fuming, oily liquid that has explosive properties and is used commercially as a rocket fuel. The aquatic toxicity of pure hydrazine and its methylated derivatives has been studied extensively (Slonim 1977; Fisher et al. 1980a, b; Hunt et al. 1981; Henderson et al. 1981, 1983). Hydrazine hydrate ($H_2NNH_2 \cdot H_2O$) is a fuming refractive liquid that is miscible with water. It is far less hazardous to store and handle than pure hydrazine; therefore, it is the chemical form that is most commonly used to deoxygenate boiler systems. Data on the aquatic toxicity of hydrazine hydrate, however, are lacking.

Hydrazine has been reported to be only moderately toxic to aquatic organisms (Becker and Thatcher 1973), but Slonim (1977) suggested that hydrazine is highly toxic to fish. This paper addresses the acute toxicity of hydrazine hydrate to a freshwater fish (fathead minnow, Pimephales promelas) and invertebrate (daphnid, Daphnia pulex).

MATERIALS AND METHODS

A commercial preparation of hydrazine hydrate (54.4% H_2NNH_2)(Olin Chemicals, Lake Charles, Louisiana) was used as the test toxicant. All exposure, lethal, and effective concentrations are reported as hydrazine (the active component of hydrazine hydrate).

A spectrophotometric procedure was used to determine the hydrazine exposure concentrations in the tests (American Society for Testing and Materials 1964). This colorimetric method for the determination of hydrazine in water gave a detectable range between 0.005 and 0.15 mg hydrazine/L, and mean precision of 0.0017 mg hydrazine/L. Samples with hydrazine concentrations greater than the detectable range were diluted with deionized water before analysis.

Two 48-h static renewal tests were used to expose D. pulex to hydrazine hydrate. Most of the procedures used were from the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). The test vessels were 250-mL glass beakers, and eight hydrazine concentrations and a control were tested in duplicate. The D. pulex were obtained from a laboratory culture that is maintained in reconstituted water. The daphnids were fed daily a blended diet of yeast, trout chow, and dehydrated plant material. First-instar daphnids less than 24 h old were used as the test organisms. Nominal hydrazine concentrations were prepared in soft reconstituted water (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). The reconstituted water used for the tests was prepared by adding the following quantities of salts to 1 L of deionized water: 48 mg $NaHCO_3$, 30 mg $MgSO_4$, 30 mg $CaSO_4$, and 2 mg KCl. Ten daphnids were placed in each beaker, giving a total of 20 daphnids per treatment. They were not fed for 24 h before, or during, the exposure to hydrazine. The exposure volume was 150 mL and the exposure beakers were kept in a 20 $\pm$ 0.5 °C water bath. The test temperature and dissolved oxygen (DO) were measured daily; pH and alkalinity were measured initially and at the end of the 48-h exposure. The photoperiod was maintained at 16 h light and 8 h dark by the method of Drummond and Dawson (1970).

The highest nominal hydrazine exposure concentrations were 5.00 and 4.00 mg/L in the first and second daphnid tests, respectively. A dilution factor of 0.5 was used to prepare the remaining concentrations in both tests. The renewal was accomplished at 24 h by preparing a second set of exposure beakers with the same initial hydrazine concentrations and transferring the surviving daphnids to them using a disposable Pasteur pipet. Initial hydrazine exposure concentrations were measured at 0 and 24 h, and the renewal concentrations were measured at 24 and 48 h. The actual exposure concentrations are reported as the average of those measurements.

The daphnids were observed at 3, 6, 12, 24, 36, and 48 h for immobilization. A small probe was used to stimulate any daphnids

that appeared motionless. Immobile organisms were immediately siphoned from the exposure beakers with a glass pipet and recorded. The immobility data were used to determine the median effective concentration (EC50) for different exposure durations.

Fathead minnows were tested using a flow-through acute test procedure. A proportional diluter (Lemke et al. 1978) was used to expose the juvenile fathead minnows to hydrazine hydrate. The proportional diluter used well water to provide five different hydrazine concentrations and a control to duplicate exposure tanks.

The well water was sterilized with ultraviolet light (35000 μWsec/cm^2), and was brought to equilibrium with atmospheric gases by passing it through a degassing column. The exposure tanks were constructed of glass, 60 cm X 30 cm X 30 cm (length X width X depth), and were fitted with standpipes that maintained a constant volume in each tank of approximately 49 L. The diluter cycled every 4 min and delivered 1 L of exposure water to each tank per cycle. That flow rate resulted in approximately 7.3 exposure tank volume replacements per 24 h. The exposure tanks were kept within a flow-through water bath that was maintained at the same temperature as the influent exposure water, 20 $\pm$ 0.5 °C.

Bioassay-grade fathead minnows (i.e., a defined genetic strain of individuals which are free of parasites and diseases) were obtained from Sea Plantations, Inc. (Salem, Massachusetts). The fathead minnows were held in a 200-L circular fiberglass tank for a two-week acclimation period. A dry, finely ground commercial fish food was given ad libitum twice per day during the acclimation period. The fathead minnows were not fed for 48 h before, or during, the test. The eight-week old test fish had a mean weight of 0.14 g (standard deviation = 0.07 g) and a mean length of 26 mm (standard deviation = 3.2 mm). Ten fish were stocked in each exposure tank; therefore, 20 fish were exposed to each concentration and the control.

A stock solution of hydrazine hydrate was prepared for delivery to the diluter system. A Micromedic® Automatic Pipette (Micromedic Systems, Inc., Horsham, Pennsylvania) was used to automatically deliver 2 mL of hydrazine hydrate stock solution per cycle to the diluter. The stock solution was held in an amber 4-L glass bottle and was maintained under an atmosphere of nitrogen gas to prevent the hydrazine from reacting with atmospheric oxygen. The diluter used the stock hydrazine hydrate solution to produce the following nominal exposure concentrations: 1.12, 2.25, 4.50, 9.00, and 18.0 mg hydrazine/L. The actual exposure concentrations were measured at 0, 48, and 96 h during the test.

The photoperiod was maintained at 16 h light and 8 h dark. Temperature and dissolved oxygen were measured daily, and pH and alkalinity were determined at the beginning and end of the 96-h exposure. The test fish were observed at 3, 6, 18, 24, 48, 72, and 96 h, and dead

fathead minnows were removed and recorded. The mortality data were used to determine the median lethal concentrations (LC50) for different durations of exposure.

The moving average angle method (Harris 1959; Finney 1971) was used to calculate the EC50 and LC50 values for the daphnid and fathead minnow tests, respectively. Average measured hydrazine exposure concentrations from each test were used in the respective calculations. The procedure provided a median lethal value (median effective value for D. pulex) and the associated 95% confidence limits. The two 48-h EC50 values for daphnids were compared for significant difference using a standard error formula (American Public Health Association et al. 1980).

RESULTS AND DISCUSSION

The 48-h EC50 values for first instar D. pulex exposed to hydrazine hydrate were 0.19 and 0.16 mg/L. The EC50 values for 24, 36, and 48 h are given for both tests (Table 1). Test conditions for the first and second daphnid tests were: temperature, 20 $\pm$ 0.3 and 20 $\pm$ 0.4 °C; pH, 7.2 and 7.1; alkalinity, 36.3 and 34.8 mg/L as $CaCO_3$; DO, 8.2 $\pm$ 0.2 and 8.2 $\pm$ 0.3 mg/L. Averaged measured hydrazine exposure concentrations are given in Table 2. There was no significant difference ($P \leq 0.05$) between the 48-h EC50 values determined for the two daphnid tests.

The 96-hr LC50 value was 5.98 mg hydrazine/L for juvenile fathead minnows. Median lethal concentrations for several exposure durations are given in Table 1. The test temperature was 20 $\pm$ 0.4 °C, the well water that served as dilution water had pH of 6.95, and alkalinity was 31.2 mg/L as $CaCO_3$. Dissolved oxygen ranged from 7.9 mg/L in the highest hydrazine concentration to 8.3 mg/L in the control. The average measured exposure concentrations of hydrazine during the fathead minnow test are presented in Table 2. At all observation periods during the test, the range of hydrazine concentrations that fell between 'no effect' and 'total effect' was narrow (i.e., 'total effect' was only 4 to 8 times the 'no effect' concentration). This trend was also observed during the daphnid exposure.

The acute toxicity of hydrazine hydrate varied greatly between the test species. Daphnids were far more sensitive to hydrazine than were fathead minnows during equivalent lengths of exposure. After 48 h the LC50 value for fathead minnows (5.98 mg/L) was approximately 33 times greater than the mean 48-h EC50 value for daphnids (0.18 mg/L).

There was no significant difference ($P \leq 0.05$) between the 48-hr EC50 values determined from the two daphnid tests. Daphnid Test 2 was conducted to confirm the results of Test 1 because daphnids were clearly the more sensitive of the two species tested.

Table 1. Test type, median lethal (effective) concentrations for various exposure durations, and the associated 95% confidence limits for the daphnid and fathead minnow exposures to hydrazine hydrate.

Species	Test	Time (hr)	LC50 (EC50) (mg/L)	Confidence Limits (95%)
Daphnia	Static-renewal	48	0.19	0.16- 0.23
	(test 1)	36	0.28	0.22- 0.36
		24	1.01	0.85- 1.21
	(test 2)	48	0.16	0.13- 0.19
		36	0.18	0.15- 0.22
		24	0.51	0.39- 0.71
Fathead Minnow	Flow-through	96	5.98	4.83- 7.40
		72	5.98	4.83- 7.40
		48	6.19	5.01- 7.69
		24	7.63	6.20- 9.66
		18	8.98	7.24-11.6

Table 2. Average measured hydrazine exposure concentrations ± standard deviations, and the nominal high exposure concentration for each test.

Exposure Tank	Hydrazine Concentration (mg/L)[a] ± Standard Deviation		
	Daphnid Test 1	Daphnid Test 2	Fathead Minnow Test
0	<0.002 ± 0.000	<0.002 ± 0.000	0
1	0.04 ± 0.006	0.03[b] ± 0.007	0.92 ± 0.121
2	0.08 ± 0.008	0.06[b] ± 0.002	2.18 ± 0.146
3	0.16 ± 0.006	0.13 ± 0.006	3.99 ± 0.234
4	0.31 ± 0.024	0.26 ± 0.010	8.95 ± 0.216
5	0.66 ± 0.030	0.53[c]	18.6 ± 0.500
6	1.26 ± 0.060	1.07[c]	
7	2.75 ± 0.144	2.17[b] ± 0.119	
8	5.36 ± 0.245	4.35[c]	
Nominal High Concentration	5.00	4.00	18.0

[a] Concentrations are means of six measurements (except as noted).
[b] Concentration is mean of four measurements.
[c] Concentration derived from one measurement.

The measured hydrazine replicates and the percentage deviations of the measured concentrations from the nominal concentrations were all within the limits of deviation specified by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Hydrazine loss was minor during the daphnid static exposures. The mean hydrazine loss was 2.7% for the 24-h periods preceding and following renewal.

Dissolved oxygen concentrations in the exposure chambers were affected only slightly by the hydrazine concentrations tested. During the fathead minnow flow-through test, hydrazine may have caused a gradual reduction of DO in the well water (8.3 to 7.9 mg/L) from the low to high concentrations. The DO variability in the reconstituted water daphnid static test beakers was random (8.2 $\pm$ 0.3 mg/L). In both tests the DO concentration remained above 90% saturation in all chambers; therefore, reduced DO was not considered a factor contributing to the mortalities that were observed.

Hydrazine decays readily in the environment due to its reactivity; however, the rate of degradation is closely related to the temperature, receiving water chemistry, and the amount of suspended particulate material present (Slonim and Gisclard 1976). Hydrazine toxicity is also related to temperature and water chemistry. Hunt et al. (1981) determined 96-h LC50 values for juvenile bluegills (Lepomis macrochirus) to be 1.6, 1.0, and 1.2 mg/L at 10, 15.5, and 21 ^{o}C, respectively. Slonim (1977) reported that guppies (Lebistes reticulatus) exposed under static conditions to hydrazine gave 96-h LC50 values of 0.61 and 3.85 mg/L in 'soft' and 'hard' water, respectively. It is expected that hydrazine illicited responses near maximal sensitivity from the species tested in this study. Soft waters and relatively warm temperatures (20 $\pm$ 0.4 ^{o}C) would act to increase the toxicity of hydrazine to daphnids and fathead minnows.

The mode of direct toxic action of hydrazine to aquatic organisms has not been determined. Given the reactivity and simplicity of molecular hydrazine, and of the degradation products of hydrazine (H_2O, N_2, NH_3), it is improbable that absorbed molecular hydrazine could survive metabolic chemical processes and accumulate in living tissues. It is apparent that hydrazine hydrate is acutely toxic to daphnids, and less so to fathead minnows. Also, the range between 'no effect' and 'total effect' is narrow. When hydrazine concentrations reach levels capable of harming aquatic life, an increase of only 4 to 8 times could be sufficient to cause 100% mortality.

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