Pergamon

0045-6535(94)00189-8

Chemosphere, Vol. 29, No. 3, pp. 567-579, 1994
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0045-6535/94 $7.00+0.00

THE ACUTE AND CHRONIC TOXICITY OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) TO THE FATHEAD MINNOW (Pimephales promelas)

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Key Words: RDX, munitions, Pimephales promelas, fathead minnow, acute toxicity, chronic toxicity, ELS, LOEC, NOEC

(Received in Germany 25 March 1994; accepted 20 May 1994)

ABSTRACT

The acute 96-h LC50 for 15- to-17 day old fathead minnow (Pimephales promelas) exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in aqueous solution was 12.7 mg/L at 25°C. A 28-day early life stage (ELS) test with the fathead minnow produced a lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) based on growth (both wet and dry weight) of 2.4 and 1.4 mg/L, respectively. A review of the literature shows that the chronic toxicity of RDX to fathead minnow is similar when evaluated in ELS, partial life cycle, and complete life cycle tests.

INTRODUCTION

RDX is a high explosive used extensively by the military for various applications. The compound may be released to the aquatic environment at munitions plants during manufacturing and blending operations. RDX may also be released to the aquatic environment at load, assembly, and pack plants where the compound is loaded into bombs and projectiles. RDX also has the potential to enter the groundwater from RDX-contaminated sediments (Etnier, 1986).

RDX has been shown to be acutely toxic to fish. The 96-h LC50s, which were based primarily on nominal concentrations of RDX dispersed via dimethyl sulfoxide (DMSO), ranged from 3.6 to >100 mg/L for four species of fish studied under various test conditions (Bentley et al., 1977; Liu et al., 1984). Bentley et al. (1977) conducted chronic toxicity tests with the fathead minnow and channel catfish (Ictalurus punctatus) exposed to RDX dispersed in DMSO. The results of the channel catfish study were not

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conclusive because of an equipment malfunction during the test. The fathead minnow studies suggested that the compound is slightly more toxic under chronic exposure conditions than acute exposures.

The objective of the present study was to verify the acute and chronic toxicity of RDX to freshwater fish using measured concentrations of the material; all RDX was dissolved in aqueous solution rather than dispersed using DMSO. The fathead minnow was selected for study because it is a "standard" test species and toxicity data were available for comparative purposes.

MATERIALS AND METHODS

Test Organism and Rearing Conditions

Fathead minnow (P. promelas) embryos and juveniles were obtained from an inhouse culture maintained at 25 (± 1) °C in non-chlorinated deep well water. The culture procedures were similar to those recommend by Peltier and Weber (1985). The culture was initiated with mature fathead minnows obtained from the U.S. EPA Environmental Monitoring and Support Laboratory - Cincinnati, OH. Briefly, spawning fish were cultured in fiberglass tanks (2.4 x 0.8 x 0.5 m) containing 0.2 m well water held at 25 (± 1) °C. The spawning adults were fed a diet of frozen brine shrimp (Artemia sp.) and TetraMin® staple food (Ramfab Aquarium Products Co., Oak Ridge, TN) twice daily. Excess food was removed daily. Five sets of spawning fathead minnows were maintained in the culture tanks at a ratio of 1 male:3 females. Replacement spawners were rotated at intervals of approximately 3-months. Fathead minnow embryos were collected on spawning substrates (10 cm I.D. x 20 cm long PVC pipe sections cut longitudinally in equal portions). Fry were reared on brine shrimp nauplii (<24 hours old) in 19-L aquaria at 25 (± 1) °C in well water.

No fish embryos were used for testing if they exhibited any signs of disease. No post-hatch fish were used if they exhibited any symptoms of disease within 10 days preceding the start of a test, or if more than 3% died within 48 hours preceding the start of the test. All stages of the fish were reared under a 16-hour light:8-hour dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the tanks).

General Experimental Procedures

Acute and early life stage (ELS) studies were conducted under flow-through test conditions. Test solutions in the flow-through studies were delivered by a solenoid-activated proportional dilutor which was calibrated 24 hours prior to the start of a test and checked and/or recalibrated at a minimum twice daily during the test. The dilutor was constructed of glass; polyethylene fittings and Tygon® tubing were also used. Control and toxicant solutions, which were held at the test temperature, were aerated in their

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All stock concentrations were quantified prior to the start of the test, and each time a new stock solution was prepared during the test. The dilutor was equipped with a counter to monitor the cycling rate as well as to ensure proper function of the dilutor. Saturated solutions of RDX were prepared by physically mixing and aerating the compound in dilution water for several hours at the test temperature before the stocks were used (see below). All stock solutions were kept in the dark to avoid photolysis.

The test temperature for both the acute and ELS tests was 25 (±1) °C. Test temperatures were maintained by placing the exposure aquaria in a constant temperature water bath. The temperature in the water bath was monitored and recorded continuously on a strip chart by placing the temperature probe in one of the control chambers. The photoperiod for both tests was 16-hour light:8-hour dark (fluorescent lights; 60-85 foot candles at the surface of the test chambers).

Acute Toxicity Test

The acute toxicity of RDX to the fathead minnow was determined by the procedures given in ASTM Designation E 729-80 (ASTM, 1980). Deviations from the test method are discussed where appropriate. A geometric series of five toxicant concentrations (plus controls), which had a dilution factor of 60%, was used. An attempt was made to include one concentration in the geometric series of five test concentrations that killed 84 to 100% of the test organisms and one concentration that killed 0 to 16% of the test organisms. All treatments including the controls were replicated. A minimum of 10 organisms was run in each replicate. All organism and test chamber assignments were random.

The initial age of the fathead minnows at the start of the 96-hour acute test was 15 to 17 days old. The juveniles were exposed to RDX in 2-L glass aquaria which contained 1.3 L of test solution. All aquaria were supplied with 18.2 volume additions/day. The mean loading at the end of the 4-day exposure period was 0.085 g/L for the control organisms. Feeding was discontinued 24 hours prior to the start of the test.

Dissolved oxygen and pH in the acute test were measured at the beginning of the test and every 24 hours in one replicate of each treatment. Conductivity, alkalinity, and total hardness were measured at the same frequency in one control replicate and one replicate at the highest test concentration. Measurements were made in alternating replicates throughout the test. Routine water quality was determined by procedures given in APHA et al. (1985). Water samples for HPLC analyses were taken at the initiation of the test and every 24 hours in all treatments of one replicate. Samples were taken from alternating replicates.

Early Life Stage Toxicity Test

The early life stage study was conducted by the method given in Draft No. 10 of the ASTM proposed guide for conducting early life-stage toxicity tests with fish (Goodman, 1986). The ELS test was conducted in 10-L glass test chambers containing 6.4 L of test material. The aquaria were supplied with 4.9 volume additions/day. The mean loading at the end of the 28-day post-hatch exposure period was 0.279 g/L for the control organisms. Toxicant concentrations were established in the 10 L test chambers 24 hours prior to the introduction of embryos. During the embryo incubation period, the embryos were held in embryo incubation cups constructed of glass cylinders (50 mm diameter I.D.; 200 mL volume) fitted with Teflon® screens that were attached with silicone sealant. The embryo incubation cups were suspended in the 10-L test chambers on a rocker arm apparatus and reciprocated vertically at 2 rpm to insure good mixing within the embryo cups.

Embryos used in the ELS study were <12 hours old at the start of the exposure. At the start of the test, groups of 5 individual embryos were randomly placed in embryo incubation cups which were held in diluent water maintained at the test temperature. When all the embryo cups were loaded (30 embryos per cup), two embryo incubation cups were randomly placed in each replicate test chamber.

All embryos were observed at least three times per day during the hatching period (≈4 days). Recently hatched larvae were released immediately to the test chambers by lowering the embryo incubation cups below the surface of the test solution allowing the fish to swim free. Embryos with fungus were removed from the test system, counted, and excluded from the calculations of hatching success.

Post-hatch fish were observed daily for developmental abnormalities and mortality. Fathead minnow were fed live brine shrimp (<24 hours old), beginning on day 2 post-hatch, three times daily on weekdays and twice daily on weekends. The amount of ration per feeding was approximately 4% dry food/wet weight fish. Excess food and feces were siphoned from the test aquaria at least daily. At the conclusion of the 28-day post-hatch exposure, total length, blotted wet weight, and dry weight were determined for all fish from each treatment. Dry weight was determine by drying at 60°C for a minimum of 24 hours or longer until a constant weight occurred. Morphometric data were not taken on fish that died while the test was in progress.

Dissolved oxygen and pH measurements were taken in one replicate of each treatment at the beginning and end of the ELS test and at least weekly during the test. Conductivity, alkalinity, and total hardness were measured at the same frequency in one control replicate and one replicate at the highest test concentration. Water samples for HPLC analyses were taken in one replicate of each treatment at the beginning and end of the test and at least weekly during the test. Water chemistry measurements and

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Toxicant

RDX (CAS No. 121-82-4) was obtained from the Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory, Ft. Detrick, Frederick, MD. The compound was recrystallized to a purity of >99%, as measured by HPLC, and stored in amber glass bottles in the dark at room temperature. Saturated stock solutions were prepared by dissolving appropriate amounts of the compound in aerated diluent water; no solvents were used to disperse the compound. The stock solutions were stirred in the dark for periods up to 24 hours at room temperature. All stocks were prepared in amber glass containers.

Analytical Chemistry

Aqueous concentrations of RDX were determined by the isocratic HPLC method of Brueggeman (1983) with minor exceptions in the operating conditions as described below. A Waters HPLC system (Waters Associates, Milford, MA) was used which consisted of the following components: dual M45 pumps with Model 680 gradient controller, Model 780 data module (integrator), U6K injector, Model 481 variable wavelength UV detector, Z-Module radial compression column system, and Model 712 Waters intelligent sample processor (WISP). A Waters μBondapak C_{18} reverse phase column was used. The mobile phase, delivered at a rate of 1.0 mL/minute, was 55% methanol:45% water. The effluent was monitored at 240 nm, 0.1 absorbance units full scale (a.u.f.s.). The injection volume was 2-200 μL depending on the concentration of material in solution.

HPLC grade methanol was used; the water was deionized glass distilled. Standard solutions of RDX were prepared by dilution of a stock solution freshly prepared each day of analysis. Precision and accuracy analyses of the HPLC method were conducted prior to the start of the bioassays. The analyses were performed with RDX dissolved in well water (diluent water) which was used for the bioassays. Precision was evaluated by injecting a sample three times on three separate days. Mean relative standard deviations for the low and high concentrations of RDX routinely used to generate standard curves for evaluating test concentrations were 2.01 and 0.43%, respectively. The accuracy of the method was evaluated by quantifying the concentration of RDX in an unknown sample prepared by an independent chemist. Accuracy, expressed as percent error, was 0.93%.

Aqueous samples from all bioassays were injected directly into the HPLC after filtration to remove particles >0.45 μm. In the cases where samples could not be analyzed immediately following filtration, the filtered samples were stored at 4 °C in amber glass vials fitted with Teflon-lined caps and analyzed

within 24 hours from the time the samples were originally taken from the test aquaria. Periodic HPLC analyses showed that little, if any, decomposition of the samples occurred during storage.

Test Endpoints and Statistical Analyses

All concentration data used in the statistical analyses were mean measured concentrations. The test endpoint for the acute toxicity of RDX to the fathead minnow was mortality. A probit analysis was initially used to determine the 96-h LC50 and it 95% fiducial limits. However, the data set could not be analyzed by the probit method because the goodness of fit probability was <0.05. Thus, the moving average angle method was used to determine the fathead minnow 96-h LC50 (Stephen, 1978).

The test endpoints for the ELS test were hatching success of the embryos after ≈4 days of exposure; survival of the fish after 28 days of exposure; and total length, wet weight, and dry weight after 28 days of exposure. The percent hatching success and percent survival data were arc-sine square root transformed before further data analyses were performed. The arc-sine square root transformed hatching success and survival data as well as the total length, wet weight, and dry weight data were then analyzed by hypothesis testing.

The no-observed-effect concentrations (NOEC) and lowest-observed-effect concentrations (LOEC) were determined by Dunnett's test. Dunnett's test consists of an analysis of variance (ANOVA) to determine the error term, which was then used in a multiple comparison to compare each of the treatment means with the control mean. The assumptions upon which the use of Dunnett's test is contingent are that the observations within treatments are independent and normally distributed, with homogeneity of variance. The chi-square test for normality and Bartlett's test for homogeneity of variances were performed before the Dunnett's test was used. The above statistical tests were perform using Toxstat (Gulley et al., 1989). A minimum probability level of 0.05 was used for all tests.

RESULTS AND DISCUSSION

The routine water quality data for the acute and ELS tests are summarized in Table 1. The mean and range of dissolved oxygen, pH, and temperature for both tests fell within the limits specified in the study guidelines. All test concentrations of RDX were below the solubility limit of the compound in water. The solubility of RDX in water at 25 °C has been reported by Banerjee et al. (1980) and Glennon et al. (1977), to be 60 and 76 mg/L, respectively.

Acute Toxicity

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TABLE 1. MEAN TEMPERATURES AND WATER QUALITY (RANGE IN PARENTHESES) OF THE RDX TOXICITY TESTS

Parameter	96-h Test	ELS Test
Temperature (°C)	25.3 (24.6-25.9)	25.1 (24.7-25.3)
pH (Std. Units)	7.9 (7.4-8.1)	7.6 (7.1-8.1)
Dissolved Oxygen (mg/L)	7.6 (7.0-8.1)	7.6 (7.0-8.1)
Conductivity (μmhos/cm)	290 (270-310)	324 (290-370)
Alkalinity (mg/L as $CaCO_3$)	121 (115-128)	100 (65-125)
Hardness (mg/L as $CaCO_3$)	165 (156-176)	221 (192-240)

Acute Toxicity

A 96-h LC50 of 12.7 (95% confidence limits = 11.14 to 14.97) mg/L was obtained for 15- to 17-day old fathead minnow in the current study using measured concentrations of RDX. Bentley et al. (1977) obtained 96-h LC50s of 3.8 and 16 mg/L (nominal concentrations using DMSO) for 7- and 30-day old fathead minnow, respectively. Liu et al. (1984) reported a 96-h LC50 of 4.5 mg/L for 2- to 7-day old fathead minnow. Acute 96-h LC50s of 5.8 and 6.6 mg/L (nominal) have been reported for adult fathead minnow (Bentley et at., 1977). Similar acute toxicity has been reported by Bentley et al. (1977) for other freshwater fish. The 96-h LC50 for fingerling rainbow trout (Oncorhynchus mykiss) is 6.4 mg/L (nominal); 4.1 and 13 mg/L (nominal) for juvenile channel catfish (I. punctatus); and 6.0 and 6.4 mg/L (nominal) for juvenile bluegill (Lepomis macrochirus).

The acute toxicity of RDX (nominal concentrations using DMSO) to various life stages of the fathead minnow has been addressed by Bentley et al. (1977). The 96-h LC50s varied as follows: >100 mg/L for embryos; 43 mg/L for 1-hour post-hatch larvae; 3.8 mg/L for 7-day old larvae; 16 mg/L for 30-day old juveniles; and 11 mg/L for 60-day old fish. The embryo was the most tolerant life stage. This has been observed for a number of species exposed to several classes of toxicants (for ex., see Woltering, 1984). With the exception of the 1-hour old post-hatch LC50 which is high relative to the other post-hatch stages, the LC50s for the 7-, 30-, and 60-day old life stages were similar to those obtained in other studies.

The effect of water quality on the acute toxicity of RDX to fish was determined by Bentley et al.

(1977) using juvenile bluegill at 20 °C. Water hardness did not affect toxicity. The 96-h LC50s for RDX in water hardnesses of 35, 100, and 250 mg/L as $CaCO_3$ were 3.8, 5.3, and 3.0 mg/L (nominal), respectively. Based on these data, the hardness of the water used in the present study (≈165 mg/L as $CaCO_3$) would be expected to have little or no effect on the observed toxicity of RDX.

Likewise, pH had no affect on toxicity (Bentley et al., 1977). The 96-h LC50s for RDX ranged from 3.6 to 3.9 mg/L (nominal) for pH which ranged from 6.0 to 8.0. Temperature appeared to play a role in toxicity; but, its effect was difficult to ascertain because the data were not analyzed statistically. As would be expected for most toxicants, toxicity appeared to be slightly reduced at the lowest temperature tested. At a constant pH of 7.0 and a hardness of 35 mg/L as $CaCo_3$, the 96-h LC50s were 4.1, 5.1, and 8.4 mg/L (nominal) at 25, 20, and 15 °C, respectively (Bentley et al., 1977).

In contrast to fish, Peters et al. (1991) showed that RDX was not acutely toxic to the following invertebrates at concentrations at or near the solubility limits of the compound in water: hydra (Hydra littoralis), cladoceran (Ceriodaphnia dubia), and chironomid (Paratanytarsus parthenogeneticus). Bentley et al. (1977) also found that RDX was not acutely toxic to the water flea Daphnia magna, scud Asellus militaris, and the midge Chironomous tentans at concentrations >100 mg/L (nominal concentrations using DMSO). Similar to invertebrates, RDX is not acutely toxic to several species of algae at concentrations near the solubility limit of RDX in water (Bentley et al., 1977).

Chronic Toxicity

The toxicity data for fathead minnow exposed to RDX during the ELS study are summarized in Tables 2 and 3. RDX did not affect the hatching success of embryos after 4 days of exposure to concentrations as high as 9.8 mg/L (Table 2), the highest concentration tested. A significant ($\alpha = 0.05$) reduction in survival relative to the controls occurred at 9.8 mg/L after 28 days of exposure (Table 2). With regard to the morphometric data (Table 3), a significant ($\alpha = 0.05$) reduction in both wet and dry weight was observed at all concentrations ≥2.4 mg/L. No reduction in wet or dry weight, relative to the controls, was observed at 1.4 mg/L. The LOEC and NOEC for fathead minnow based on both wet and dry weights are 2.4 and 1.4 mg/L, respectively. A total of 3 fish with spinal curvature occurred in RDX concentrations of up to 9.8 mg/L. The incidence of spinal curvature did not appear to be related to exposure to RDX.

Bentley et al. (1977) conducted a 30-day ELS test with channel catfish and fathead minnow exposed to RDX dispersed via DMSO at 22 °C. Hatching success of the channel catfish embryos was not affected at measured concentrations ≤2.3 mg/L, the highest concentration tested; however, fry survival was reduced at 1.2 mg/L. Because a dilutor malfunction occurred on day 10 in the channel catfish study, the authors

TABLE 2. HATCHING SUCCESS OF FATHEAD MINNOW EMBRYOS AFTER 4 DAYS OF EXPOSURE AND SURVIVAL OF JUVENILES AFTER 28 DAYS OF POST-HATCH EXPOSURE TO RDX IN THE ELS TEST

Concentration (mg/L)	Rep	Hatching Success (%)[1]	Juvenile Survival (%)
Control	1	100.0	91.7
	2	66.7	100.0
1.35	1	95.2	90.0
	2	86.4	94.7
2.36	1	88.9	95.8
	2	87.5	95.2
3.42	1	88.4	87.0
	2	80.0	80.0
6.42	1	79.5	100.0
	2	82.9	86.2
9.83	1	92.3	45.8[2]
	2	67.9	36.8[2]

[1] RDX did not affect hatching success at any treatment.
[2] Mean percent survival of the treatment is significantly different ($\alpha = 0.05$) from the mean percent survival of the control.

speculated that the concentrations in the treatments may have increased significantly. Thus, the fry results appear to be suspect during the 30-day post-hatch exposure. In the Bentley et al. (1977) fathead minnow study, hatching success of the embryos and survival of the fish after 30 days of exposure were not affected at concentrations ≤5.8 mg/L, the highest concentration tested. A significant ($\alpha = 0.05$) reduction in total length, however, occurred at 5.8 mg/L after 30 days of exposure. Thus, the LOEC and NOEC for fathead minnow based on total length were 5.8 and 3.0 mg/L, respectively.

A partial life cycle and a complete life cycle test were also conducted with the fathead minnow at 25 °C by Bentley et al. (1977). The first test was designed to be a complete life cycle test; however, the test was terminated at 140 days when all fish were accidentally killed when being treated for external parasites. In the first test, no significant ($\alpha = 0.05$) effect was observed for percent hatch, juvenile survival, and total length after 30 days at the maximum measured test concentration of 4.9 mg/L. At 60 days, survival of the fish exposed to 4.9 mg/L was significantly ($\alpha = 0.05$) lower than that of the controls. Total length of the fathead minnow exposed to concentrations ≤4.9 mg/L was not affected at 60 days. According to the authors, all fish survived and appeared to develop normally between days 63 and 140. The LOEC and NOEC based on survival were 4.9 mg/L and 2.7 mg/L, respectively.

TABLE 3. TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF JUVENILE FATHEAD MINNOW AFTER 28 DAYS OF POST-HATCH EXPOSURE TO RDX IN THE ELS TEST

Concentration (mg/L)	Rep	Mean Length (mm)[1]	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	24 (22-26)	145.1 (96.0-186.6)	30.2 (18.3-41.8)
	2	24 (17-27)	163.2 (62.0-224.9)	34.1 (10.5-46.5)
1.35	1	23 (18-27)	146.1 (92.5-210.0)	30.2 (18.7-42.5)
	2	22 (18-27)	138.7 (92.7-208.3)	28.9 (18.0-43.3)
2.36	1	22 (20-25)	104.9 (59.4-174.2)[2]	18.8 (9.5-32.5)[2]
	2	23 (18-26)	121.3 (67.9-195.8)[2]	22.7 (12.2-37.2)[2]
3.42	1	22 (19-25)	107.8 (65.6-161.1)[2]	20.2 (10.5-32.0)[2]
	2	22 (17-25)	100.6 (47.3-148.6)[2]	19.9 (9.1-34.6)[2]
6.42	1	21 (16-25)	83.5 (27.5-165.4)[2]	15.3 (7.3-33.7)[2]
	2	21 (14-25)	84.3 (25.7-140.2)[2]	15.1 (5.3-28.3)[2]
9.83[3]				

[1] RDX did not affect total length at the highest concentration evaluated.
[2] Mean weight of the treatment is significantly different ($\alpha = 0.05$) from the mean weight of the control.
[3] Lost replicate while conducting morphometric analyses; therefore, no statistical analyses were performed on this data set.

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During the complete life cycle test, Bentley et al. (1977) found that hatching success was not affected at concentrations ≤6.3 mg/L. Survival was significantly ($\alpha = 0.05$) affected at 6.3 mg/L on day 30 and day 60; survival was not affected at concentrations below 3.0 mg/L. Total lengths of the fish were not affected at 30 or 60 days at concentrations ≤6.3 mg/L. Survival, wet weight, and total length of both males and females were not affected from days 60 to 240 at concentrations ≤6.3 mg/L. Egg production by first generation fish and hatching success were not affected at concentrations ≤6.3 mg/L. The survival and growth of the second generation fish after 30 days of exposure were not affected by exposure to RDX concentrations ≤6.3 mg/L. The LOEC and NOEC based on survival were 6.3 and 3.0 mg/L (measured), respectively, during the complete life cycle test.

The chronic toxicity of RDX to fathead minnows is similar to chronic RDX toxicity in invertebrates. Peters et al. (1991) found that the LOEC and NOEC for the cladoceran C. dubia were 6.0 and 3.6 mg/L (based on reduced neonate production), respectively. Peters et al. (1991) also found that while survival, growth, emergence success, egg production, and hatching success of the midge P. parthenogeneticus were not significantly affected by RDX, a trend toward reduced emergence success occurred in the midge at concentrations as low as 6.8 mg/L. Chronic effects of RDX on algae also occur at concentrations that are toxic to fish and invertebrates (Sullivan et al., 1979 and Burton et al., in press). For example, Burton et al. (in press) showed that the LOEC and NOEC for the green alga Selenastrum capricornutum were 4.8 and 0.5 mg/L (reduction in cell density), respectively.

SUMMARY

The data in this study support earlier findings that RDX is toxic to the fathead minnow (slightly to moderately toxic as defined by Zucker, 1985). A 96-h LC50 of 12.7 mg/L (measured concentration) was obtained for 15- to 17-day old fathead minnow. This LC50 is similar to other 96-h LC50s for the fathead minnow which range from 3.6 to 16.0 mg/L (nominal concentration) for fish 7 to 30 days old. Other 96-h LC50s for juvenile channel catfish, rainbow trout, and bluegill range from 4.1 to 13 mg/L (nominal).

Although the LOEC and NOEC endpoints differ between studies, ELS, partial life cycle, and complete life cycle evaluations of the chronic toxicity of RDX to the fathead minnow yield similar results. In the current ELS study, the LOEC and NOEC based on growth (both wet and dry weight) were 2.4 and 1.4 mg/L, respectively. The LOECs obtained by Bentley et al. (1977) in several chronic tests with the fathead minnow ranged from 4.9 to 6.3 mg/L based on survival. The NOECs for survival obtained in the same tests ranged from 2.7 to 3.0 mg/L. Chronic toxicity data are not available for other species of fish. With the exception of the fathead minnow embryos (96-h LC50 >100 mg/L) exposed to RDX dispersed in DMSO in the Bentley et al. (1977) study, the acute and chronic toxicity concentrations of RDX all fall well below the solubility limits of the compound in water.

ACKNOWLEDGEMENTS

The study was supported by the U.S. Army Medical Research and Development Command. The opinions, interpretations, conclusions, and recommendations of the study are those of the authors and are not necessarily endorsed by the U.S. Army. We acknowledge the Research Methods Branch/Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory for supporting the study through U.S. Army Contract No. MIPR 88MM8501 via U.S. Navy Contract No. N00039-89-C-0001 to The Johns Hopkins University Applied Physics Laboratory (JHU/APL). We acknowledge JHU/APL for providing a subcontract to the University of Maryland System, Agricultural Experiment Station, Wye Research and Education Center to complete the study. This paper is Scientific Article No. A6566, Contribution No. 8778 from the Maryland Agricultural Experiment Station.

REFERENCES

APHA et al. (American Public Heath Association, American Water Works Association, and Water Pollution Control Federation). 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, DC.

ASTM (American Society for Testing and Materials). 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Annual Book of ASTM Standards, Part 46, Designation: E 729-80, American Society for Testing and Materials, Philadelphia, PA.

Banerjee, S., S.H. Yalkowsky, and S.C. Valvani. 1980. Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility-partition coefficient correlation. Environ. Sci. Technol. 14:1227-1229.

Bentley, R.E., J.W. Dean, S.J. Ells, T.A. Hollister, G.A. LeBlanc, S. Sauter, and B.H. Sleight. 1977. Laboratory evaluation of the toxicity of cyclotrimethylene trinitramine (RDX) to aquatic organisms. Final Report, AD A061730. EG&G Bionomics, Wareham, MA.

Brueggemann, E.E. 1983. HPLC analysis of SEX, HMX, TAX, RDX, and TNT in wastewater. Technical Report 8206, AD A127348. U.S. Army Medical Bioengineering Research and Development Laboratory, Ft. Detrick, Frederick, MD.

Burton, D.T., S.D Turley, and G.T. Peters. In press. The toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the freshwater green alga Selenastrum capricornutum. Water Air Soil Pollut.

Etnier, E.L. 1986. Water quality criteria for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Final Report ORNL-6178, AD A169506. Oak Ridge National Laboratory, Oak Ridge, TN.

Glennon, J.P., J.G. Pearson, J.J. Barkley, J.C. Dacre, M.C. Warner, and D.H. Rosenblatt. 1977. Munitions environmental quality standards research status report. Technical Report 7709. U.S. Army Medical Bioengineering Research and Development Laboratory, Ft. Detrick, Frederick, MD.

Goodman, L.R. 1986. Proposed new standard guide for conducting early life-stage toxicity tests with

fishes. Draft No. 10. American Society for Testing and Materials, Philadelphia, PA.

Gulley, D.H., A.M. Boelter, and H.L. Bergman. 1989. Toxstat release 3.0. University of Wyoming, Laramie, WY.

Liu, D.H.W., R.J. Spanggord, H.C. Bailey, H.S. Javitz, and D.C.L. Jones. 1984. Toxicity of TNT wastewaters to aquatic organisms. Vol. I. Acute toxicity of LAP wastewater and 2,4,6-trinitrotoluene. Final Report, AD A142144. SRI International, Menlo Park, CA.

Peltier, W.H. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed. EPA/600/4-85/013. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, OH.

Peters, G.T., D.T. Burton, R.L. Paulson, and S.D. Turley. 1991. The acute and chronic toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to three freshwater invertebrates. Environ. Toxicol. Chem. 10:1073-1081.

Stephen, C.E. 1978. EPA statistics program, version 1.4. U.S. Environmental Protection Agency, Environmental Research Laboratory - Duluth, Duluth, MN.

Sullivan, J.H., Jr., H.D. Putnam, M.A. Keirn, B.C. Pruitt, J.C. Nichols, and J.T. McCleave. 1979. A summary and evaluation of aquatic environmental data in relation to establishing water quality criteria for munitions-unique compounds. Final Report, AD A087683. Water and Air Research, Inc., Gainesville, FL.

Woltering, D.M. 1984. The growth response in fish chronic and early life stage toxicity tests: A critical review. Aquatic Toxicol. 5:1-21.

Zucker, E. 1985. Hazard evaluation division standard evaluation procedure acute toxicity test for freshwater fish. EPA-540/9-85-006. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC.