0514

Environmental Toxicology and Chemistry, Vol. 10, pp. 1073–1081, 1991
Printed in the USA. Pergamon Press plc

0730-7268/91 $3.00 + .00
Copyright © 1991 SETAC

Ref

THE ACUTE AND CHRONIC TOXICITY OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) TO THREE FRESHWATER INVERTEBRATES

GREGORY T. PETERS,* DENNIS T. BURTON, ROBERT L. PAULSON and STEVEN D. TURLEY
Johns Hopkins University, Applied Physics Laboratory, Environmental Sciences Group, Shady Side, Maryland 20764

(*Received* 2 *February* 1990; *Accepted* 20 *December* 1990)

Abstract—The acute and chronic toxicities of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to a daphnid (*Ceriodaphnia dubia*), a hydra (*Hydra littoralis*), and a midge (*Paratanytarsus parthenogeneticus*) were examined. RDX concentrations at the solubility limit of the compound under specific test conditions (17.0 mg RDX/L, *Ceriodaphnia dubia* at 25°C; 32.3 mg RDX/L, *Hydra littoralis* at 22°C; and 29.2 mg RDX/L, *Paratanytarsus parthenogeneticus* at 22°C) were not acutely toxic to any of the three tested invertebrates. In a 7-d survival and reproduction test with *Ceriodaphnia dubia*, RDX caused no significant effect on survival, but reduced reproductive success. The no-observed-effect concentration, lowest-observed-effect concentration, and chronic value for *Ceriodaphnia dubia* were 3.64, 6.01, and 4.68 mg/L, respectively. In an egg-to-egg life cycle test with *Paratanytarsus parthenogeneticus*, survival, growth, egg production, and hatching success were unaffected by RDX. Although not statistically significant, reductions in emergence success were observed at concentrations as low as 6.78 mg/L.

Keywords—RDX Aquatic toxicity *Ceriodaphnia dubia* *Hydra littoralis* *Paratanytarsus parthenogeneticus*

INTRODUCTION

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is an explosive used by the military in a variety of munitions applications. RDX may be released into the aquatic environment at munitions production plants during manufacturing and blending operations and at load, assembly, and pack (LAP) plants where the compound is loaded into bombs and projectiles.

One study has been conducted on the toxicity of RDX to freshwater invertebrates. In 48-h tests with a cladoceran (*Daphnia magna*), an isopod (*Asellus militaris*), an amphipod (*Gammarus fasciatus*), and a midge (*Chironomus tentans*), Bentley et al. [1] observed no toxicant-related effects. The EC50s (based on immobilization) were reported as being higher than the highest tested concentrations (15 mg/L in flow-through tests and 100 mg/L in static tests; nominal concentrations). Chronic studies with *D. magna* and *C. tentans* [1] showed that RDX exerts few, if any, toxic effects on invertebrate growth, development, and reproduction.

In contrast, RDX is acutely toxic to freshwater fish. The 96-h LC50s (based on nominal concentrations) for bluegill (*Lepomis macrochirus*), rainbow trout (*Salmo gairdneri*), channel catfish (*Ictalurus punctatus*), and fathead minnows (*Pimephales promelas*) range from 4.1 to 6.0 mg/L in 96-h static tests and from 6.6 to 13 mg/L in 96-h flow-through tests [1]. Liu et al. [2] obtained a 96-h LC50 for fathead minnows of 4.5 mg RDX/L under static test conditions. Based on these initial tests with six out of eight families of aquatic organisms required to establish U.S. Environmental Protection Agency (EPA) water quality criteria [3], an interim final acute value of 5.18 mg RDX/L was established [4].

The objective of the present study was to verify the acute and chronic toxicity of RDX to freshwater invertebrates. The midge *Paratanytarsus parthenogeneticus*, the daphnid *Ceriodaphnia dubia*, and the hydra *Hydra littoralis* were selected for study to (a) confirm the toxicity of RDX to selected invertebrates and (b) provide data to complete the invertebrate data base for establishing definitive water quality criteria.

*To whom correspondence may be addressed.
The current address of G.T. Peters is Wildlife International Ltd., 305 Commerce Drive, Easton, Maryland 21601.

9537

MATERIALS AND METHODS

Toxicant

RDX (CAS Registry No. 121-82-4) was obtained from the U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Maryland. The chemical purity of the material used in the study was >99%. In static renewal tests, concentrated toxicant solutions were prepared by mixing RDX with diluent water for 24 h with gentle heating. Toxicant solutions were cooled to the test temperature before use in the tests. In flow-through tests, toxicant solutions were prepared by mixing RDX for 24 h with dilution water held in a proportional diluter headbox at test temperature. The maximum concentrations of RDX tested in the flow-through experiments were lower than the maximum concentrations used in the static renewal tests at the same experimental temperature because the stock solutions were not heated during preparation.

Test organisms and culture procedures

The chironomid *P. parthenogeneticus* is a widely distributed aquatic insect, commonly found in lotic and lentic waters throughout North America. This easily cultured organism has a relatively short life cycle and is an excellent test organism for use in both acute and chronic toxicity tests. *Paratanytarsus parthenogeneticus* was obtained for culture from the Center for Lake Superior Environmental Studies, University of Wisconsin–Superior.

Paratanytarsus parthenogeneticus was cultured at 22°C in 38-L glass aquaria filled with Johns Hopkins University Applied Physics Laboratory (JHU/APL) nonchlorinated well water (alkalinity = 156 mg/L as $CaCO_3$; hardness = 180 mg/L as $CaCO_3$; conductivity = 290 μmhos; pH = 7.6) to depths of 5 to 10 cm. The midge was reared on a diet of dried grass and trout chow (0.5 g Cerophyl® + 10 g trout chow/300 ml well water; Cerophyl Laboratories, Inc.). The mixture was blended at high speed for 5 min, allowed to stand at 4°C for 24 h, filtered to remove large particulates, and stored at 4°C prior to use. Feeding rates were adjusted according to consumption in order to maximize growth and discourage excessive fungal growth in the cultures. When extensive areas near larval tubes were cleared of food, 5 to 10 ml of the food mixture were added to the aquaria. When the immediate areas surrounding larval tubes appeared to be ungrazed, the feeding rate was reduced to 1 to 5 ml per aquarium per day, or feeding was postponed until grazing was evident. Feeding rates were reduced during periods of pupation and emergence.

The cladoceran *C. dubia* has been used extensively in acute and short-term chronic toxicity tests. *Ceriodaphnia dubia* was cultured in well water amended with selenium (2 μg Se/L). The diet consisted of a mixture of Cerophyl (prepared as above) and the green alga *Selenastrum capricornutum* added to the daphnid cultures to achieve final concentrations of 120 μg Cerophyl/ml and 6.7×10^5 *S. capricornutum* cells/ml. Starter cultures of *C. dubia* were obtained from the Center for Lake Superior Environmental Studies, University of Wisconsin–Superior.

Hydra littoralis is a coelenterate that inhabits cool, clean fresh water in pools and streams. *Hydra littoralis* was obtained from Carolina Biological Supply, Inc. (Burlington, NC) and cultured in a 50:50 mixture of JHU/APL well water and deionized water on a diet of freshly hatched brine shrimp. Excess food and debris were removed from the culture on a daily basis, and 50% of the medium was renewed.

Experimental procedures

Forty-eight-hour acute tests. The 48-h static renewal toxicity tests were conducted with techniques outlined in ASTM's Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians [5]. Acute tests with *C. dubia* and *H. littoralis* were conducted at 25 (±1)°C and 22 (±1)°C, respectively, using 10 to 12 organisms per replicate. Tests with *C. dubia* were conducted in 400-ml glass beakers (test solution volume = 100 ml), whereas tests with *H. littoralis* were conducted in 600-ml glass beakers (test solution volume = 200 ml). A photoperiod of 16:8 h light:dark was provided in all tests, using cool-white, fluorescent lighting (60–85 ft-c).

The *P. parthenogeneticus* acute test was conducted at 22 (±1)°C under flow-through conditions in glass crystallizing dishes (75 mm high; 150 mm diameter; 1,325-ml volume) fitted with drainage tubes (5-mm-diameter glass tubes mounted in neoprene stoppers). Five toxicant concentrations and a control were delivered to replicate experimental chambers from a proportional diluter that was calibrated at the beginning and end of the test. Control and toxicant solutions were aerated in their respective headboxes with air supplied by an oil-free pump. Before the start of the test, 10 larvae (age 6 d) were placed in each of the test chambers, which contained 600 ml clean well water and 1.2 ml midge food. After midges were allowed to

construct larval tubes for 24 h, toxicant and diluent (control) solutions were introduced to the test chamber by the proportional diluter. The 48-h test was begun after one 90% replacement with test solution had occurred. A minimum of five volume additions per day were delivered to each test chamber during the test. By allowing midges to construct tubes before the delivery of test solutions, the probability of midges escaping via the overflow was reduced.

In the acute tests with *H. littoralis* and *C. dubia*, dissolved oxygen and pH were measured in one replicate of each treatment at the beginning and end of each 24-h renewal period. In the flow-through test with *P. parthenogeneticus*, dissolved oxygen and pH were measured in one replicate per treatment on a daily basis. Conductivity, hardness, and alkalinity were measured in one replicate per treatment at the beginning and end of all tests.

Seven-day survival and reproduction test with C. dubia. The procedure employed for this test generally followed the specifications outlined in "Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms" [6]. The primary end points of the test were survival of first-generation (F_0) daphnids and total number of live young produced by each F_0 daphnid.

The test was conducted in an environmental chamber maintained at 25 (±1)°C. Griffin beakers (50-ml) were used as test chambers. Test solutions (30-ml) were made with selenium-amended JHU/APL well water (2 μg Se/L) and renewed daily. Tests were started with 4- to 6-h-old neonates produced by daphnids in culture that had released at least three broods. *Ceriodaphnia dubia* was fed daily as described in the section Test Organisms and Culture Procedures, and water chemistry parameters were evaluated at the beginning and end of at least two renewal periods.

Life cycle test with P. parthenogeneticus. The flow-through, life cycle toxicity test with *P. parthenogeneticus* was conducted by using the methods outlined in Anderson [7]. The test system was identical to that described for the acute test, with the following exceptions: The diluter was calibrated before the test and at weekly intervals during the test. In addition to aerating the toxicant solution and diluent water in their respective headboxes, air lines fitted with glass Pasteur pipettes were used to aerate test solutions in the test chambers when delivery of test solution was suspended.

Approximately 5 h before the test, delivery of test solutions to the chambers was initiated, and the diluter was allowed to cycle 10 times. The flow of test solution was then suspended, and eggs <12 h old were counted and randomly placed in two replicate test chambers per treatment. On day 2 of the study, food was added to the chambers so that newly hatched larvae could begin feeding and constructing larval cases (tubes).

Because newly hatched larvae are planktonic, delivery of the test solution was resumed only after larval cases were observed in all test chambers to reduce the loss of larvae from test chambers via the overflow tubes. Food was administered at a rate governed by larval consumption during the previous 24-h period (as evidenced by larval grazing). Typically, 1.2 ml of the Cerophyl/trout chow solution was added per chamber per day (final concentration = 2 ml Cerophyl/trout chow solution per L).

Paratanytarsus parthenogeneticus larvae were measured on days 6, 8, and 10 by placing the test chambers under a dissecting microscope equipped with a calibrated ocular micrometer. Whenever possible, each larva in a chamber was measured. At the onset of pupation, delivery of test solution to the chambers was again suspended so that the surface of the water would not be disturbed during emergence. This procedure also eliminated the possible loss of emerging adults via the overflow tubes.

Fiberglass screen covers (1-mm^2 mesh) were placed on the chambers to capture midges that emerged successfully. To evaluate egg production, adult midges were aspirated into screw-cap vials containing 2.5 ml of test solution. The vials were placed in an environmental chamber maintained at similar temperature and photoperiod and inspected daily for egg production. When adult midges oviposited, egg strands were transferred to petri plates, counted, and held until eclosion or death (generally 2–3 d). Test chambers were also examined on a daily basis for strands of eggs that were deposited by adults before being aspirated.

Dissolved oxygen was measured in one replicate of each treatment at the beginning and end of the test and at least weekly during the test. Conductivity, hardness, alkalinity, and pH were measured at similar intervals in one control replicate and one replicate of the highest toxicant concentration.

Analyses of RDX concentrations

RDX concentrations were analyzed in one replicate per treatment at the beginning and end of each renewal period during the acute tests with *C. dubia* and *H. littoralis* and during the 7-d survival

and reproduction test with *C. dubia*. In the 48-h flow-through test with *P. parthenogeneticus*, RDX samples were taken at 0, 24, and 48 h. In the life cycle test with the midge, samples were taken at the start of the test, at least weekly during the test, and at the end of the test. Samples for toxicant analyses were filtered to remove particulates >0.45 μm and stored until analysis at 4°C in amber-glass vials fitted with Teflon®-lined caps.

RDX concentrations were determined by using a Waters Associates (Milford, MA) high-pressure liquid chromatograph equipped with a C_{18} reversed-phase column and a UV detector (wavelength = 240 nm; 0.1 absorbance units, full scale). The mobile phase, delivered at a rate of 1.0 ml/min, was 55% methanol : 45% glass-distilled, deionized water. Precision was evaluated by injecting RDX standards in triplicate on three dates. 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%.

Statistical analyses

The results of the acute test were analyzed by (a) probit analysis whenever there were two or more treatments with between 0 and 100% mortality; (b) the moving average-angle method whenever there was only one treatment with between 0 and 100% mortality; and (c) the binomial method whenever there were no treatments between 0 and 100% mortality.

Data concerning survival and young production of first-generation daphnids that were generated in the 7-d survival and reproduction test with *C. dubia*, and survival, hatching success, length, and egg production of midges in the life cycle test with *P. parthenogeneticus* were analyzed statistically as follows: Raw data were transformed by using arc-sine (daphnid and midge survival data and midge hatching success data) or square-root (daphnid young production data, midge length data, and midge egg production data) transformations whenever they enhanced the normal distribution or improved the homogeneity of variances. The data were then subjected to chi-square tests of normality and Bartlett's tests for homogeneity of variances.

When survival and hatching success data sets failed to meet the assumptions of normality or homogeneity of variances, the nonparametric Kruskal-Wallis procedure was used to compare differences between sample means. Whenever the data met the assumptions of normality and homogeneity of variances, a parametric procedure was used. Dunnett's test was used when the number of replicates was constant among treatments, and a *t*- test with Bonferroni adjustment of error rate was performed when the number of replicates was not constant among treatments.

Whenever daphnid young production data, midge length data, and midge egg production data failed the assumptions of normality and homogeneity of variances, the nonparametric Steel's Many-One Rank test was used to compare differences between sample means for an equal number of replicates per treatment. A Wilcoxon Rank Sum test with Bonferroni adjustment of error rate was used when the number of replicates was unequal. Whenever the data met the assumptions of normality and homogeneity of variances, a parametric procedure was used. Dunnett's test was used when the number of replicates was constant among treatments, and a *t* test with Bonferroni adjustment of error rate was performed when the number of replicates per treatment was unequal.

RESULTS AND DISCUSSION

Mean temperature and water quality parameters for each toxicity test are given in Table 1. In all tests, the highest tested toxicant concentration was the solubility limit of RDX in JHU/APL well water at the test temperature (Table 2). As shown in Table 2, the solubility of RDX decreases as temperature increases. Environmental fate studies by Spanggord et al. [8], Sikka et al. [9], and Patterson et al. [10] confirm this observation.

Acute toxicity tests

RDX was not acutely toxic to any of the three invertebrates tested at the solubility limit of RDX in water. In addition, sublethal effects such as immobilization were not evident in short-term exposures. Bentley et al. [1] observed no toxicant-related effects in 48-h tests with four species of invertebrates: the cladoceran *D. magna*, the isopod *A. militaris*, the amphipod *G. fasciatus*, and the midge *C. tentans*. Bentley et al. [1] reported their EC50s (based on immobilization) as being higher than the highest tested concentrations (15 mg/L in flow-through tests and 100 mg/L in static tests; nominal concentrations).

Chronic toxicity tests

Seven-day survival and reproduction test with C. dubia. RDX had no significant effect on the

Table 1. Mean temperatures and water quality parameters (ranges in parentheses) for toxicity tests involving *Ceriodaphnia dubia* (48-h acute test and 7-d, three-brood survival and reproduction test), *Paratanytarsus parthenogeneticus* (48-h, flow-through acute test and life cycle test), and *Hyra littoralis* (48-h acute test)

	C. dubia		*P. parthenogeneticus*		*H. littoralis*
	48-h	7-d	48-h	Life cycle	48-h
Temperature (°C)	25.0 (24.5–25.5)	25.0 (24.4–25.4)	22.3 (21.4–22.9)	22.0 (20.9–24.0)	22.0 (21.5–22.5)
Dissolved oxygen (mg/L)	8.3 (8.1–8.8)	7.3 (6.6–7.9)	7.6 (7.1–8.2)	7.7 (6.9–8.4)	8.1 (7.9–8.4)
pH	8.3 (8.0–8.4)	8.1 (7.6–8.4)	7.9 (7.4–8.1)	7.8 (7.4–8.2)	7.5 (7.2–7.9)
Conductivity (μm,hos)	307 (260–340)	322 (280–390)	241 (210–275)	281 (244–317)	294 (285–310)
Alkalinity (mg/L)	114 (110–120)	92 (70–130)	106 (105–110)	134 (110–145)	150 (136–162)
Hardness (mg/L)	168 (156–176)	154 (144–200)	165 (156–170)	186 (164–216)	161 (148–170)

survival of neonates of *C. dubia* exposed for 7 d ($\alpha = 0.05$) (Table 3). However, the number of neonates produced per surviving F_0 daphnid was significantly ($\alpha = 0.05$) reduced at concentrations ≥6.01 mg/L (Tables 3 and 4). Whereas control *C. dubia* produced an average of 36.8 neonates during the 7-d test, daphnids exposed to 6.01 mg RDX/L produced 19.0% fewer neonates (mean 29.8), and daphnids exposed to 16.4 mg RDX/L produced 48.9% fewer young (mean = 18.8). The no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), and chronic values calculated for *C. dubia* based on impairment of reproduction were 3.64, 6.01, and 4.68 mg/L, respectively.

Bentley et al. [1] conducted a chronic study of the effect of RDX on two generations of the daphnid *D. magna* and found that nominal RDX concentrations ranging from 0.9 to 15 mg/L had no effect on survival. However, the number of neonates produced by first-generation adults was significantly reduced at nominal RDX levels of 3.7, 7.5, and 15 mg/L between days 7 and 14 of the first 21-d exposure. Reproduction in the second generation was unaffected by RDX. Although reproduction was significantly affected in the first generation at RDX concentrations as low as 3.7 mg/L, Bentley et al. [1] suggested that, for an undisclosed reason, this may have been due to a higher concentration of RDX in solution between days 7 and 14. These data are difficult to interpret, because the reduction in reproductive success occurred only in the first-generation exposure for a limited time and because the RDX concentrations were nominal rather than measured.

Life cycle test with P. parthenogeneticus. In the life cycle test, midges were tested at concentrations ranging from 2.4 to 20.8 mg/L. Hatching success in the F_0 generation was not calculated because unhatched eggs quickly decomposed and their remains were easily lost in the food and detritus that accumulated on the bottoms of test chambers. Be-

Table 2. Solubility concentrations of RDX in JHU/APL well water for each toxicity test

Test species/type of test	Temperature (°C)	Solubility concn (mg/L)
Hydra littoralis		
48-h acute test	22	32.3
Ceriodaphnia dubia		
48-h acute test	25	17.0
7-d survival and reproduction test	25	16.4
Paratanytarsus parthenogeneticus		
48-h acute test	22	29.2
life cycle test	22	20.8

Table 3. Number of young produced per day, total number of young, number of broods, and mean number of young per brood in the 7-d survival and reproduction test with *Ceriodaphnia dubia*

Concn (mg/L)	Replicate	Number of young 96 h	120 h	144 h	168 h	192 h	Total young	Broods	Survival at end of test
Control	1	4		10	17		31	3	Yes
	2	3		12		21	36	3	Yes
	3	5		11	19		35	3	Yes
	4	5		11		18	34	3	Yes
	5	5		10	18		33	3	Yes
	6	4		12		21	37	3	Yes
	7	6		14		24	44	3	Yes
	8	7		10		24	41	3	Yes
	9	4		11		25	40	3	Yes
	10	6		10		21	37	3	Yes
2.15	1	4		11	20		35	3	Yes
	2	6		11	18	1	35	3	Yes
	3	4		12	18	17	35	4	Yes
	4	2		12	17	22	48	4	Yes
	5	5		12	15		54	4	Yes
	6	4		14	16	1	34	3	Yes
	7	3		10	19	5	33	4	Yes
	8	4		11	18		38	4	Yes
	9	5		10	18		33	3	Yes
	10	4		10	18		32	3	Yes
3.64	1	4		10		21	35	3	Yes
	2	3		11		18	32	3	Yes
	3	3		11		21	35	3	Yes
	4	3		9	20		32	3	Yes
	5	3		9		25	37	3	Yes
	6	4		11		17	32	3	Yes
	7	4		11	21		36	3	Yes
	8	6		12		20	38	3	Yes
	9	5		12	23		40	3	Yes
	10	4		12	19		35	3	Yes
6.01	1	4		11		15	30	3	Yes
	2	4		14		16	34	3	Yes
	3	3		11		11	25	3	Yes
	4	4		12		15	31	3	Yes
	5	33		12		10	25	3	Yes
	6	4			3		7	2	No
	7	6		11	19		36	3	Yes
	8	4		13		15	32	3	Yes
	9	4		9		16	29	3	Yes
	10	3		10		13	26	3	Yes
9.74	1	4		11		19	34	3	Yes
	2	5	1	11		14	31	4	Yes
	3	3		12		17	32	3	Yes
	4	4		11		10	25	3	Yes
	5	3	1	14		12	30	4	Yes
	6	3		11		17	31	3	Yes
	7	4		11		16	31	3	Yes
	8	3		11		14	28	3	Yes
	9	6		10		17	33	3	Yes
	10	3	2	11		15	31	4	Yes
16.4	1	1	1	6		7	15	4	Yes
	2	4		9		10	23	3	Yes
	3	3		6		9	18	3	Yes
	4	3		10		6	19	3	Yes
	5	3		6		11	20	3	Yes
	6	4		6		9	19	3	Yes
	7		4	8		10	22	3	Yes
	8		3	9		8	20	3	Yes
	9	4		4		9	17	3	Yes
	10		4	6		5	15	3	Yes

Table 4. Statistical analysis of total neonate production in the 7-d survival and reproduction test with *Ceriodaphnia dubia*

Concentration (mg/L)	Mean no. of neonates[a]	SD	Range
Control	36.8	3.94	31–44
2.15	37.7	7.33	32–54
3.64	35.2	2.70	32–40
6.01	29.8[b]	3.93	25–36
9.74	30.6[b]	2.55	25–34
16.4	18.8[b]	2.66	15–23

[a]Mean number of neonates produced per surviving adult.
[b]Significant difference from control neonate production; Dunnett's test ($\alpha = 0.05$; minimum detectable difference = 4.78, or 13.8% of the control).

cause it is possible that larvae escaped during the experiment via the drain tubes, the number of larvae observed during the experiment could not be used to estimate the total number of eggs that hatched.

Midge lengths, expressed as mean midge length for each treatment group, ranged from 1.46 to 1.72 mm on day 6, from 2.22 to 3.06 mm on day 8, and from 3.35 to 5.27 mm on day 10 (Table 5). Length estimates for each of the treatment groups were expressed as a percentage of mean midge length in the controls to better illustrate the effects of RDX on growth. Mean midge length in each of the treatment groups expressed as a percentage of mean control midge length (relative midge length) ranged from 74 to 117%. On days 6, 8, and 10 there were no significant differences in midge length between the control group and any treatment group ($\alpha = 0.05$). Thus, RDX concentrations as high as 20.82 mg/L had no significant effect on midge growth, relative to controls, at any time during the test.

Adult emergence success, expressed for each replicate as percent emergence, ranged from 16.7% in one replicate of the highest RDX concentration tested to 100% in the lowest two treatment levels and the controls (Table 6). Percent emergence was calculated as

$$\frac{\text{number of larvae that emerged successfully}}{\text{maximum number of larvae observed}} \times 100\%.$$

Despite an apparent concentration–response relationship where emergence was reduced at RDX levels as low as 6.78 mg/L, there was no significant difference in emergence success between the controls and any tested RDX concentration ($\alpha = 0.05$).

Egg production, expressed as the mean number of eggs produced per adult for each replicate, ranged from 58 to 226 (Table 7). Because oviposition occurred before the removal of adults from the screened enclosures, several egg strands produced by an unknown number of adults were routinely found in the test chambers after the collection of emergent adults. Consequently, the exact number of eggs produced by a given adult could not be determined with certainty. The mean number of eggs produced per adult was calculated for each replicate as the total number of eggs produced, including eggs removed from the bottom of the test chamber, divided by the total number of adults that emerged successfully. Thus, for each treatment, there were two observations of egg production. No significant differences in egg production were observed (minimum significant difference in egg production = −44.5%; $\alpha = 0.05$) between the controls and treatment groups.

Because egg strands could not be associated with individual adults, hatching success was calculated for each replicate as the total number of eggs that hatched successfully divided by the total number of eggs produced. Mean hatching success for each treatment group ranged from 90.5% in the 12.67-mg/L exposure to 97.8% in the controls

Table 5. Midge length, expressed as mean length (mm ± 1 SD) and as relative length (percent of control midge length) for measurements taken on days 6, 8, and 10 of the life cycle test with *Paratanytarsus parthenogeneticus*

	Day 6		Day 8		Day 10	
Concn (mg/L)	Mean length (mm)	Relative length (%)	Mean length (mm)	Relative length (%)	Mean length (mm)	Relative length (%)
Control	1.55 (0.21)		2.62 (0.47)		4.50 (0.48)	
2.42	1.51 (0.41)	97	2.54 (1.02)	97	3.79 (1.60)	84
3.91	1.72 (0.21)	111	3.06 (0.36)	117	5.27 (0.63)	117
6.78	1.55 (0.22)	100	2.98 (0.66)	114	4.59 (0.76)	102
12.67	1.71 (0.63)	110	3.00 (0.80)	115	4.48 (0.73)	100
20.82	1.46 (0.41)	94	2.22 (0.34)	85	3.35 (0.64)	74

Table 6. Emergence success in the life cycle toxicity test with *Paratanytarsus parthenogeneticus*

Concn (mg/L)	Replicate	No. of adults that emerged (test day) 13	14	15	16	17	Total emergence	Maximum no. of larvae observed	Percent emergence[a]
Control	1		4	1			5	5	100.00
	2		1		4	1	6	6	100.00
2.42	1	6	1		2	2	11	10	100.00
	2				1	4	5	4	100.00
3.91	1	1	5		1		7	7	100.00
	2	6	1				7	10	70.00
6.78	1	3	4	1			8	10	80.00
	2			2		1	3	6	50.00
12.67	1	1	2	1			4	8	50.00
	2					1	1	8	12.50
20.82	1			1	2	1	4	8	50.00
	2		1				1	6	16.67

[a]In replicates 2.42-1 and 2.42-2, the number of larvae that emerged successfully was greater than the maximum number of larvae observed. One larva in each chamber constructed its tube in a location that was hidden from view (e.g., below the overflow tube). Emergence success was therefore corrected to 100% for 2.42-1 and 2.42-2.

(Table 8). Statistical analysis indicated that there was no significant deleterious effect on hatching success in the F_1 generation (Dunnett's test; minimum significant difference in hatching success = −9.9%; $\alpha = 0.05$).

By comparison, nominal RDX concentrations ranging from 0.9 to 15 mg/L had no significant effect on survival or adult emergence of *C. tentans* in a test conducted by Bentley et al. [1]. However, egg production was nonexistent at 7.5 mg/L, and no fertile eggs were produced at concentrations as low as 0.9 mg/L. Eggs produced by the controls in the Bentley et al. [1] experiment were exposed to the same RDX concentrations for a second test. In this exposure, adult emergence was reduced at 1.8 mg/L. However, the authors did not consider this phenomenon to be biologically significant because emergence success at higher RDX concentrations was comparable to that of controls. Larval survival was reduced at nominal RDX concentrations of 0.9 and 3.7 mg/L, and pupal and adult survival were reduced at 0.9 mg RDX/L. However, the authors questioned these results, stating that if those concentrations were indeed toxic, the response should have been evident in the first test.

SUMMARY

The data in this study support previous findings that RDX is not acutely toxic to a number of freshwater invertebrates when tested at the solubility limits of the compound in water. However, the reproductive success of *C. dubia* was affected at con-

Table 7. Egg production in the life cycle toxicity test with *Paratanytarsus parthenogeneticus*

Concn (mg/L)	Replicate	No. of adults captured for eggs	No. of eggs produced	No. of eggs per adult	Mean no. of eggs per adult
Control	1	5	941	188	197
	2	4	823	206	
2.42	1	11	2,484	226	225
	2	5	1,120	224	
3.91	1	7	981	140	167
	2	7	1,357	194	
6.78	1	8	1.077	135	97
	2	3	173	58	
12.67	1	4	677	169	187
	2	1	205	205	
20.82	1	4	461	115	132
	2	1	149	149	

Table 8. Hatching success in the F_1 generation of the life cycle toxicity test with *Paratanytarsus parthenogeneticus*

Concn (mg/L)	Replicate	Total no. of eggs produced	Total no. of eggs hatched	Hatching success (%)	Mean hatching success (%)
Control	1	941	909	96.6	97.8
	2	823	814	98.9	
2.42	1	2,484	2,378	95.7	97.6
	2	1,120	1,113	99.4	
3.91	1	981	905	92.3	94.0
	2	1,357	1,297	95.6	
6.78	1	1,077	1,029	95.5	97.2
	2	173	171	98.8	
12.67	1	677	580	85.7	90.2
	2	205	194	94.6	
20.82	1	461	442	95.8	95.9
	2	149	143	95.9	

centrations as low as 6.01 mg/L, and at 16.4 mg/L the production of young was reduced by 48.9%. The NOEC, LOEC, and chronic value for *C. dubia*, based on impairment of reproduction, were 3.64, 6.01, and 4.68 mg/L, respectively. RDX had no adverse effect on survival of *C. dubia* during the chronic study. Although the survival, growth, emergence success, egg production, and hatching success of *P. parthenogeneticus* were not significantly affected by RDX during the full life cycle exposure, a trend toward reduced emergence success occurred at concentrations as low as 6.78 mg/L.

Acknowledgement—We thank S.H. Poirer, University of Wisconsin—Superior, for providing *P. paratanytarsus* and *C. dubia*, and Drs. M.A. Major and W.H. van der Schalie of the U.S. Army Biomedical Research and Development Laboratory, Ft. Detrick, Frederick, Maryland, for reviewing the manuscript. This work was funded by the U.S. Army Biomedical Research and Development Laboratory, Ft. Detrick, Frederick, Maryland.

REFERENCES

1. **Bentley, R.E., J.W. Dean, S.J. Ells, T.A. Hollister, G.A. Leblanc, S. Sauter** and **B.H. Sleight III.** 1977. Laboratory evaluation of the toxicity of RDX to aquatic organisms. Technical Report. Contract No. DAMD-17-74-C-4101. U.S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD.
2. **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. Technical Report. Contract No. DAMD17-75-C-5056. U.S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD.
3. **Stephen, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman** and **W.A. Burgs.** 1985. Guidelines for deriving national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. National Technical Information Service, Springfield, VA.
4. **Etnier, E.L.** 1986. Water Quality Criteria for Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). ORNL-6178. Oak Ridge National Laboratory, Oak Ridge, TN.
5. **American Society for Testing and Materials.** 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-80. In *Annual Book of ASTM Standards*, Vol. 11.04. Philadelphia, PA, pp. 276–296.
6. **Horning, W.V. II** and **C.I. Weber.** 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA 600/4-85-014. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
7. **Anderson, R.L.** 1980. Chironomidae toxicity tests—Biological background and procedures. In *Aquatic Invertebrate Bioassays*. STP 715. American Society for Testing and Materials, Philadelphia, PA, pp. 70–80.
8. **Spanggord, R.J., T. Mill, T.W. Chou, W.R. Mabey** and **J.H. Smith.** 1980. Environmental fate studies on certain munition wastewater constituents: Phase II, laboratory studies. AD-A099 256. Defense Technical Information Center, Alexandria, VA.
9. **Sikka, H.C., S. Banerjee, E.J. Pack** and **H.T. Appleton.** 1980. Environmental fate of RDX and TNT. Technical Report. Contract No. DAMD17-77-C-7026. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.
10. **Patterson, J., I.N. Shapira, J. Brown, W. Duckert** and **J. Polson.** 1976. State-of-the-art: Military explosives and propellants production industry. Vols. 1-3. EPA 600/2-76-213a–c. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.