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Reduction of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) Toxicity to the Cladoceran *Ceriodaphnia dubia* Following Photolysis in Sunlight

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Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a high explosive used extensively by the military in a number of applications. The compound may enter the aquatic environment via wastewater during manufacturing activities and blending operations at load, assembly, and pack (LAP) plants. RDX has been shown to be toxic to a number of aquatic organisms including algae, some invertebrates, and fish at concentrations well below its solubility limit in water (Etnier 1986; Burton et al. 1993).

Several studies have shown that RDX is decomposed via photolysis in aqueous solutions at UV wavelengths shorter than ≈290 nm and at longer wavelengths above ≈290 nm which can occur from irradiance in natural sunlight (Kubose and Hoffsommer 1977; Glover and Hoffsommer 1979; Spanggord et al. 1980). Liu et al. (1984) found that exposure of composition B type LAP waste (1.6:1 mixture of 2,4,6-trinitrotoluene and RDX) to simulated sunlight (filtered UV light) reduced toxicity to several aquatic organisms. Photolyzed 2,4,6-trinitrotoluene (no RDX present) was also less toxic. The photolysis of RDX alone was not studied by Liu et al. (1984). The current study was initiated to verify whether or not photolyzed RDX may be less toxic than the parent compound. A 7-d chronic test with the cladoceran, *Ceriodaphnia dubia*, was conducted in order to compare the data to those of Peters et al. (1991) who exposed the organism to the parent compound under the same test conditions.

MATERIALS AND METHODS

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, Maryland. The compound was recrystallized to a purity of >99 percent as measured by HPLC and stored in amber glass bottles in the dark at room temperature. Photolyzed RDX was prepared by exposing the compound in non-chlorinated well water (water quality given below) to sunlight (38° 51′ N latitude; 76° 31′ W longitude) until the parent compound reached non-detectable levels as determined by periodic HPLC analysis.

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RDX concentrations were analyzed by the HPLC method of Brueggeman (1983) with minor exceptions in the operating conditions as described in Peters et al. (1991).

A 5-L stock solution of 10.0 mg/L RDX (measured concentration) was photolyzed for a total of 28 hr in sunlight in early October until RDX could not be measured from background. The stock solution of RDX, which was photolyzed in a rectangular glass container (40 x 58 x 82 cm) with no top, was covered each evening with black plastic so that the actual number of hours of exposure to sunlight (i.e., 28 hr) could be calculated. Light intensity was not estimated by the use of a chemical actinometer since measurement of the reaction quantum yield was not necessary for the study.

The RDX photolyzed stock was used for the entire 7-d cladoceran test. Nominal concentrations of the photolyzed RDX were used in the study. The nominal concentrations were based on measured concentrations of the stock solution which was held in sunlight until the parent compound reached non-detectable levels. The dilutions used in the toxicity tests were made from the completely photolyzed stock solution and are labeled as photolyzed (Φ) concentrations. The 10.0 mg/L stock solution of RDX completely photolyzed is labeled 10.0 mg/L Φ-RDX.

Ceriodaphnia dubia was cultured at 25 (± 0.5) °C in 600 mL glass beakers filled with 400 mL non-chlorinated well water amended with selenium (2 μg Se/L as Na_2SeO_3) as recommended by Winner (1989). The diet consisted of a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, Missouri) and the green alga, *Selenastrum capricornutum*, added to the culture to achieve final concentrations of 120 μg Cerophyl®/mL and 6.7 x 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.

The chronic toxicity of photolyzed RDX to *Ceriodaphnia* was determined by the method given in Draft No. 3 of the ASTM proposed guide for conducting three brood, renewal toxicity tests (Waller and Lazorchak 1986). All neonates used in the 7-d survival and reproduction test were produced by cladocerans in culture that had released at least three broods. The initial age of the neonates at the start of the test was 4 to 6 hr old. The test chambers were 50-mL glass beakers; the test volume was 30 mL. The exposures were conducted in an environmental chamber at 25 (±0.5)°C under a 16-hr light:8-hr dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the test vessels). All test organisms were fed daily as described above after each 24-hr renewal.

Routine water quality measurements were taken daily over the 7-d exposure period. Temperature was monitored and recorded continuously in one of the

control replicates via a strip chart. Dissolved oxygen and pH were measured in one replicate of each treatment at the beginning of each 24-hr renewal and at the end of the 24-hr period before the next daily renewal. Conductivity, alkalinity, and total hardness were measured at the same frequency in one control replicate and one replicate at the highest test concentration. The mean (range) water quality during culturing and exposure to photolyzed RDX was pH = 7.8 (7.5-8.1) std. units; conductivity = 376 (320-420) μmhos/cm; alkalinity = 68 (35-110) mg $CaCo_3$/L; hardness = 196 (152-230) mg $CaCO_3$/L: dissolved oxygen = 6.1 (5.0-7.9) mg/L; and temperature = 25.1 (24.9-25.3) °C.

The test end points for the bioassay with *Ceriodaphnia* were survival and young production. The statistical analyses of the photolyzed RDX data were conducted as follows. The raw cladoceran survival data were not analyzed because only one death occurred in the study (Table 1). A *t*-test with Bonferroni adjustment of error rate was used to detect differences in the treatment data sets relative to the control data set. The assumptions upon which the use of the Bonferroni *t*-test are 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 Bonferroni *t*-test was used. The above statistical tests were performed using Toxstat (Gulley et al. 1989). A minimum probability level of 0.05 was used.

RESULTS AND DISCUSSION

The photolyzed RDX chronic toxicity data for *C. dubia* neonate production are summarized in Table 1. Photolyzed RDX did not affect survival of the cladocerans up to a nominal concentration of 10 mg/L Φ-RDX; one organism died at 10 mg/L Φ-RDX. Likewise, photolyzed RDX up to a nominal concentration of 10 mg/L Φ-RDX did not affect neonate production (ANOVA calculated test statistic = 1.86; critical value = 2.45; α = 0.05). In contrast, Peters et al. (1991) found that the parent compound was toxic to *C. dubia* at concentrations below 10 mg/L when the cladoceran was exposed to RDX in the same laboratory under similar experimental conditions. The lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) for the cladoceran exposed to RDX were 6.0 and 3.6 mg/L, respectively (Peters et al. 1991). The data obtained in this study in conjunction with those of Peters et al. (1991) show that photolysis eliminates the toxicity of RDX to *C. dubia*. This study confirms the indirect evidence by Liu et al. (1984) which suggested that photolysis may reduce the toxicity of RDX to several other aquatic organisms.

Several photoproducts have been reported for photolyzed RDX. Kubose and Hoffsomer (1977) found the following products for RDX photolyzed in tap water at wavelengths between 280 and 1367 nm: nitrate, nitrite, ammonia, formaldehyde, and 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane (the mono-

Table 1. Mean (± S.D.) number of cladocerans produced after 7 days of exposure to photolyzed RDX (n = 10 for all treatments except the concentrations of 1.3 Φ-RDX and 10.0 Φ-RDX in which 1 organism was lost and 1 organism died, respectively).

Concentration (mg/L)	Brood No. 1	Brood No. 2	Brood No. 3	Total Young
Control	4.0 (±0.67)	11.8 (±1.32)	14.3 (±1.34)	30.2 (±1.23)
1.3 Φ-RDX	3.4 (±1.35)	10.9 (±1.45)	14.7 (±1.12)	29.7 (±1.58)
2.2 Φ-RDX	3.5 (±0.71)	10.2 (±1.40)	14.9 (±1.60)	28.6 (±2.27)
3.6 Φ-RDX	3.5 (±0.85)	9.9 (±1.20)	16.3 (±1.25)	29.4 (±2.46)
6.0 Φ-RDX	3.5 (±0.53)	9.9 (±1.60)	15.3 (±1.89)	28.7 (±2.41)
10.0 Φ-RDX	3.3 (±0.50)	8.4 (±3.32)	15.2 (±1.56)	27.7 (±1.87)

nitroso analog of RDX). Nitrate, nitrite, and formaldehyde were also reported by Spanggord et al. (1980) as photoproducts when RDX was photolyzed in sunlight or at 313 nm in distilled and filter-sterilized natural water.

Spanggord et al. (1980) could not identify the N-nitroso analog of RDX reported by Kubose and Hoffsomer (1977) in acidic aqueous solution. Burrows et al. (1989), who reviewed the literature on RDX photoproducts, showed that a number of additional photoproducts can be formed when wavelengths below those normally present in the solar spectrum are used. Depending on the reaction system employed, at least two reaction pathways may occur at the shorter UV wavelengths.

The photolysis experiments with RDX conducted in this study and those of Spanggord et al. (1980) were followed by HPLC analysis of the photolyzed solutions by direct aqueous injection. No photoproducts were observed in the HPLC chromatograms using UV detection at 254 nm in the current study or by Spanggord et al. (1980) who also used HPLC with UV detection at 254. Similarly, Harvey et al. (1991) did not find any photoproducts in a 7-d bioaccumulation study of a plant exposed to RDX in a hydroponic system under constant growth-chamber lights using HPLC with UV detection at 243 nm. The photoproducts identified by Kubose and Hoffsomer (1977) and

Spanggord et al. (1980) were identified by the use of various analytical techniques other than HPLC with UV detectors.

The concentrations of nitrate, nitrite, formaldehyde, and ammonia that may have been produced in the current study can be estimated from the yield data in Kubose and Hoffsomer (1977). Assuming that no changes occurred in the concentrations of the products (nitrite and formaldehyde concentrations would be lower because of the length of the study), the concentrations of nitrate, nitrite, formaldehyde, and ammonia would be approximately 2.0, 4.1, 0.8, and 0.5 mg/L, respectively. With the possible exception of nitrite which would not normally occur in concentrations as high as 4 mg/L in surface waters, the concentrations of the four photoproducts are not particularly toxic to aquatic organisms (McKee and Wolf 1971). Thus, it is not surprising that the photoproducts of RDX were not toxic to *C. dubia* in this study.

The photolysis of RDX in distilled water and in natural water samples follows first order kinetics when RDX is photolyzed in sunlight or at 313 nm (Spanggord et al. 1980). In a comparison of photolysis in distilled water and natural waters (filter-sterilized pond and river water), Spanggord et al. (1980) found that only slight light-screening occurred in natural water. Thus, they concluded that indirect photolysis of RDX due to natural substances does not appear to be an important mechanism in aquatic environments. Several studies have shown that indirect photolysis of xenobiotics can occur in natural aquatic systems (Zepp 1982; Weiner and Goldberg 1985).

The measured half-life of RDX (half-life based on 24-hr days; calculated by first order regression) in the current study in well water exposed to natural sunlight (38° 51′ N latitude; 76° 31′ W longitude) in early October was ≈12 hr (Table 2). Spanggord et al. (1980) calculated the half-lives (half-lives calculated as 24-hr days) for RDX in sunlight at 40° N latitude in summer, fall, winter, and spring in distilled water to be 1.2, 2.6, 5.0, and 1.5 d, respectively. The half-life measured in this study is similar to that calculated by Spanggord et al. (1980) from quantum yield and absorption data. The difference between the measured half-life of our study and the calculated half-life by Spanggord et al. (1980) may be due to differences in solar irradiance which can be variable, depending on factors such as time, location, and cloud cover.

The primary physical mechanism that degrades RDX in aqueous solution is photolysis (Burrows et al. 1989); however, the role of photolysis in the environmental fate of RDX in aquatic systems is not clear. Spanggord et al. (1980) state that RDX may be a persistent chemical in the aquatic environment for the following reasons: 1) they estimated half-lives up to 13 d could occur in some natural waters during the winter; 2) the compound is not rapidly biotransformed by microorganisms in natural waters under aerobic conditions; and 3) sediment sorption does not lead to significant RDX loss in the aquatic environment. The sediment sorption partition coefficient, K_p, for

RDX varies from 0.8 for a sandy loam to 5.5 for a highly organic sediment (Spanggord et al. 1980). The K_ps indicate that RDX is only weakly adsorbed to sediments. Adsorption does not appear to increase with extended contact times (Spanggord et al. 1980). Spanggord et al. (1980) concluded that the major environmental fate of RDX in natural waters would be dilution. Etnier (1986) has made the point that hydrolysis and volatilization should not significantly influence the environmental fate of RDX since these processes proceed very slowly relative to photolysis.

Table 2. HPLC measurements of RDX as a function of time in sunlight.

Hours Exposed to Sunlight	RDX (mg/L)
0	10.00
2	9.06
4	8.32
6	7.57
8	6.41
8	6.38
10	5.42
13	4.21
16	3.26
18	2.43
18	2.07
20	1.16
22	0.42
24	0.11
26	0.06
28	0.03

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