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TOXICITY OF NITROGUANIDINE, NITROGLYCERIN, HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX), AND 2,4,6-TRINITROTOLUENE (TNT) TO SELECTED FRESHWATER AQUATIC ORGANISMS

FINAL REPORT

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The primary objective of the study was to conduct the necessary toxicity tests to complete the existing data base for deriving U.S. EPA numerical water quality criteria for freshwater organisms exposed to nitroguanidine (NQ), nitroglycerin (NG), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4,6-trinitrotoluene (TNT). A secondary objective of the study was to determine what effect photolysis may have on the toxicity of NQ and RDX. The acute toxicity of NQ was established for the hydra (Hydra littoralis), cladoceran (Ceriodaphnia dubia), rainbow trout (Oncorhynchus mykiss), and fathead minnow (Pimephales promelas). The following NQ chronic tests were performed: 7-d cladoceran, 28-d ELS rainbow trout, and 28-d ELS fathead minnow. Photolyzed NQ was 100-fold more toxic to the cladoceran than the parent compound. NG acute toxicity was determined for the green alga (Selenastrum capriocornutum), hydra, cladoceran, midge (Paratanytarsus parthenogeneticus), and fathead minnow. The following chronic tests were conducted with NG: 7-d cladoceran, 60-d ELS rainbow trout, and 28-d ELS fathead minnow. RDX acute toxicity was established for green alga, hydra, midge, cladoceran, and fathead minnow. The following chronic tests were conducted with RDX: 7-d cladoceran,

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midge life cycle, and 28-d ELS fathead minnow. Photolyzed RDX was less toxic than the parent compound to the cladoceran. The acute toxicity of TNT was determined for duckweed, cladoceran, midge, and fathead minnow. A complete life cycle test was run with the fathead minnow.

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EXECUTIVE SUMMARY

This study was conducted at the request of the U.S. Army Biomedical Research and Development Laboratory to complete the existing data base for deriving U.S. Environmental Protection Agency (EPA) numerical national water quality criteria (Stephen et al., 1985) for freshwater organisms exposed to nitroguanidine (NQ), nitroglycerin (NG), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4,6-trinitrotoluene (TNT).

Various toxicity data, which were acceptable for use in deriving EPA's numerical water quality criteria for freshwater organisms, were available for the above compounds. The primary objective of the study was to conduct the necessary toxicity tests to complete the existing data base for deriving numerical water quality criteria for freshwater organisms exposed to NQ, NG, RDX, and TNT. A secondary objective of the study was to determine what effect photolysis may have on the toxicity of NQ and RDX.

Toxicity of NQ

The acute toxicity of NQ to the organisms needed to complete the EPA numerical water quality criteria data base is as follows. The 48-h LC50s for the hydra (Hydra littoralis) and cladoceran (Ceriodaphnia dubia) were 2,061 and 2,698 mg/L, respectively. No mortality occurred to the rainbow trout (Oncorhynchus mykiss) or fathead minnow (Pimephales promelas) in 96 h at the aqueous solubility limits of the compound which were 1,550 mg/L for rainbow trout at 12° and 3,320 mg/L at 25°C for the fathead minnow.

The chronic toxicity of NQ to the cladoceran was determined using a 7-d survival and reproduction test. The lowest-observed-effect-concentration (LOEC) and no-observed-effect-concentration (NOEC) for the cladoceran, based on reduction in neonate production, were 440 and 260 mg/L, respectively. NQ was not toxic to rainbow trout during an early life stage (ELS) test at the solubility limit of the compound in water. A 7-d exposure to NQ concentrations up to 1,520 mg/L did not affect hatching success of the rainbow trout embryos. A 28-d post-hatch exposure to concentrations up to 1,520 mg/L did not affect fry survival, total length, wet weight, or dry weight. The LOEC and NOEC for the fathead minnow during a 28-d ELS, based on a reduction in total length were 2,030 and 1,050 mg/L, respectively.

The toxicity of photolyzed NQ was evaluated in a cladoceran 7-d survival and reproduction test. The LOEC and NOEC for the cladoceran, based on survival of the adults, were 3.6 mg/L Φ-NQ and 2.2 mg/L Φ-NQ (nominal concentrations), respectively. Photolyzed NQ was approximately two orders of magnitude more

toxic to the cladoceran than the parent compound under the same test conditions. The environmental fate of NQ in surface waters has been shown to be dominated by photolysis; thus, photolysis should be considered in an hazard evaluation of NQ discharged to the aquatic environment.

Toxicity of NG

The acute 96-h EC50 (cell density) for the green alga Selenastrum capricornutum exposed to NG was 1.15 mg/L. The 48-h LC50 for the hydra, cladoceran, and midge (Paratanytarsus parthenogenticus) were 17.43, 17.83, and 34.93 mg/L, respectively. The 96-h LC50 for rainbow trout was 1.90 mg/L. The 96-h LC50 for the fathead minnow was 3.58 mg/L

The chronic toxicity of NG was determined for the green alga by analyzing the 96-h growth data as chronic data. When the data were treated as chronic data rather than acute data, the LOEC and NOEC for S. capricornutum growth were 0.59 and 0.37 mg/L, respectively. The LOEC and NOEC for the cladoceran during a 7-d survival and reproduction test, were 5.48 and 3.23 mg/L, respectively, based on a reduction in neonate production. The LOEC and NOEC for rainbow trout during a 60-d ELS test were 0.06 and 0.03 mg/L (based on a reduction in dry weight). A 28-d ELS with fathead minnow produced a LOEC and NOEC of 0.20 and 0.12 mg/L (based on a reduction in hatching success).

Toxicity of RDX

RDX was not acutely toxic to the green alga S. capricornutum when tested at the solubility limit of the compound in algal assay media. A maximum reduction of 38% in cell density occurred after a 96-h exposure to 36.69 mg/L; thus, an EC50 could not be determined. RDX was not acutely toxic to the hydra and midge when tested at the solubility limits of the compound in water which were 32.67 and 29.22 mg/L, respectively. RDX was not acutely toxic to the cladoceran at a concentration of 17.04 mg/L which was the highest concentration tested. RDX may prove to be acutely toxic if tested at concentrations approaching the solubility of the compound in water.

The chronic LOEC and NOEC for the alga S. capricornutum were 4.81 and 0.47 mg/L (reduction in cell density), respectively, when the data were treated as chronic data rather than acute data. The LOEC and NOEC for the cladoceran exposed to RDX during a 7-d survival and reproduction test were 6.01 and 3.64 mg/L (based on neonate production), respectively. RDX was not toxic to the midge during a complete life cycle test conducted at concentrations up to 20.82 mg/L. An apparent concentration-response relationship occurred in total emergence success at concentrations as low as 6.78 mg/L. However, a statistically significant difference (α=0.05) was not found in total emergence

success between the controls and the RDX treatment groups when the non-parametric Kruskal-Wallis statistical test was used. A 28-d ELS test with the fathead minnow produced a NOEC and LOEC based on both wet and dry weight of 2.36 and 1.35 mg/L.

The toxicity of photolyzed RDX was evaluated in a cladoceran 7-d chronic survival and reproduction test. Photolyzed RDX did not affect survival or neonate production of the cladoceran up to a nominal concentration of 10 mg/l ϕ-RDX. In contrast, cladocerans exposed to the parent compound were affected at concentrations below 10 mg/L. The LOEC and NOEC for the cladoceran exposed to the parent compound were 6.01 and 3.64 mg/L. Thus, photolysis reduced the toxicity of RDX. Indirect evidence in a study by Liu et al. (1984) also suggests that photolysis reduces the toxicity of RDX to aquatic organisms.

Toxicity of TNT

The acute 96-h EC50 (reduction in frond production) of TNT to the duckweed (Lemna minor) was 1.59 mg/L. The 48-h LC50s for the cladoceran and midge exposed to TNT were 4.03 and 42.90 mg/L, respectively. The 96-h LC50 for the fathead minnow was 2.66 mg/L.

The chronic LOEC and NOEC for the duckweed were 1.21 and 0.59 mg/L (reduction in frond production), respectively, when the TNT test results were treated as chronic data rather than acute data. The LOEC and NOEC for the cladoceran during a 7-d survival and reproduction test, were 2.71 and 1.64 mg/L, respectively, based on a reduction in neonate production. The LOEC and NOEC obtained during a 9-month TNT life cycle test with the fathead minnow were 0.014 and 0.005 mg/L based on a reduction in total length of the parental females after spawning was completed.

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SECTION 1

INTRODUCTION

The U.S. Army Biomedical Research and Development Laboratory (USABRDL) has the responsibility for assessing the possible environmental hazards associated with munitions-unique pollutants released during manufacturing activities and deployment in the field. The Health Effects Research Division of USABRDL expressed interest in completing the existing data base for deriving U.S. Environmental Protection Agency (EPA) numerical national water quality criteria (Stephen et al., 1985) for freshwater organisms exposed to nitroguanidine (NQ), nitroglycerin (NG), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4,6-trinitrotoluene (TNT).

Nitroguanidine is used primarily in M30 triple-base propellant along with nitrocellulose and nitroglycerin (Small and Rosenblatt, 1974). Nitroglycerin is used as an explosive and, more frequently, with other materials (e.g., nitroglycol) to make explosives such as dynamite, smokeless gunpowders, and blasting gels (Smith, 1986). It is also used in rocket propellants and the treatment of various medical disorders. RDX and TNT are explosives used extensively by the military for various applications (U.S. Army, 1984).

Various toxicity data (see Section 2), which were acceptable for use in deriving EPA's numerical water quality criteria for freshwater organisms, were available for the above compounds. The primary objective of the study was to conduct the necessary toxicity tests to complete the existing data base for deriving numerical water quality criteria for freshwater organisms exposed to NQ, NG, RDX, and TNT. A secondary objective of the study was to determine what effect photolysis may have on the toxicity of NQ and RDX.

SECTION 2

NUMERICAL WATER QUALITY CRITERIA REQUIREMENTS

The minimum data needed for EPA's numerical aquatic water quality criteria are briefly reviewed in this section as a means to facilitate the review of literature discussion for each compound. The toxicity data for NQ, NG, RDX, and TNT, which were acceptable for use in deriving EPA's water quality criteria at the initiation of the present study, are summarized below for each compound. Finally, the minimum studies needed to complete the criterion data base for each material are discussed below and summarized in Tables 1-4. It should be noted that this study was conducted to provide the minimum data needed to complete the numerical water quality criteria for each compound. The final criterion as defined by EPA (Stephen et al., 1985) for each compound was not the objective of this study and consequently was not determined for each compound in this study.

2.1 EPA Water Quality Criterion Requirements

The EPA numerical aquatic water quality criterion for a material consists of two concentrations which are the Criterion Maximum Concentration and the Criterion Continuous Concentration (Stephen et al., 1985). The following minimum data are required to derive the Criterion Maximum Concentration and Criterion Continuous Concentration.

2.1.1 Criterion Maximum Concentration

The Criterion Maximum Concentration is equal to one-half of the Final Acute Value. The Final Acute Value is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values (EC50s, where they exist) for at least one species in eight different families such that all of the following are included: 1) family Salmonidae in the class Osteichthyes, 2) second family in the class Osteichthyes, preferably a commercially or recreationally important warm water species, 3) third family in the phylum Chordata which may be a fish or other aquatic Chordata, 4) planktonic crustacean, 5) benthic crustacean, 6) insect, 7) family in a phylum other than Arthropoda or Chordata, and 8) family in any order of insect or any phylum not already represented.

2.1.2 Criterion Continuous Concentration

The Criterion Continuous Concentration is equal to the lowest of the 1) Final Chronic Value, 2) Final Plant Value, and 3) Final Residue Value unless 4) other data show that a lower value should be used. The Final Chronic Value may be calculated

either in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-Chronic Ratio for animals in at least three different families provided that one is a fish; one is an invertebrate; and one is an acutely sensitive fish or invertebrate.

The Final Plant Value is the lowest result obtained in a 96-h EC50 test conducted with an alga or a chronic test conducted with an aquatic vascular plant. The Final Residue Value is the lowest of the residue values that are obtained by dividing the maximum permissible tissue concentration as defined in the EPA guidelines by appropriate bioconcentration factors. Other data (category 4 above) may be pertinent information (e.g., flavor impairment, behavioral changes, etc.) that could not be used above concerning adverse effects on aquatic organisms. If the data show that a lower value occurs than those described in 1, 2, and 3 above, then the lower value should be used.

The EPA guidelines state that the Criterion Continuous Concentration is equal to the lowest value in the above four categories. However, in many cases data are not available for one or more of the categories. There is no requirement in the guidelines that acceptable toxicity data must be available for categories 1, 2, and 3. According to Stephan (1987), if no acceptable toxicity data exist for a material, priority should be given to establishing the Final Chronic Value. Stephan (1987) considers the Final Plant Value to be less important than the Final Chronic Value because no commercially or recreationally important freshwater algae or vascular plants exits.

With regard to the Final Residue Value, the EPA guidelines state that data concerning bioaccumulation by aquatic organisms are only required if relevant data are available concerning the significance of residues in aquatic organisms. If maximum permissible tissue concentration data are not available and evidence exists (e.g., low octanol-water partition coefficient) which shows that a material will not bioconcentrate, then the Final Residue Value does not have to be established.

2.2 NQ Water Quality Criterion Data Base

The acute toxicity of NQ to freshwater aquatic organisms was studied by van der Schalie (1985). Based on a review of the literature and the study by van der Schalie, data were available for seven of the eight families required for the Final Acute Value. Acute data were needed for a family in any order of insect or any phylum not represented in the existing data base to complete the Criterion Maximum Concentration. Hydra (Hydra littoralis) was used to complete the acute data base (Table 1).

An early life stage (ELS) test with rainbow trout (Oncorhynchus mykiss) was conducted by van der Schalie (1985);

however, the data were not acceptable for use in determining the Criterion Continuous Concentration because the test was conducted under static renewal conditions rather than flow-through test conditions (Stephan et al., 1985). With the exception of the rainbow trout study, which was not acceptable under the EPA guidelines, no other data existed for a Final Chronic Value. Thus, the rainbow trout ELS test had to be re-run as defined in the EPA guidelines along with two other species. The two additional tests were a 7-d chronic cladoceran (Ceriodaphnia dubia) test and a fathead minnow (Pimephales promelas) ELS test (Table 2). Acute tests were also run with each species to provide acceptable data for acute-chronic ratios (Table 3).

A Final Plant Value could be determined from a 120-h EC50 determined by van der Schalie (1985) for growth and standing crop of the green alga Selenastrum capricornutum. Data did not exist to determine a Final Residue Value. Since the log P (0.15) of NQ is very low (Spanggord et al., 1985), there was no reason to believe the material would bioconcentrate in biological systems. Thus, no further work in this area was warranted.

The photolysis of NQ in surface water exposed to August and March sunlight (≈ 40°N latitude) was reported by Spanggord et al. (1985) and Dennis (1982), respectively, to have half-lives of approximately 20 and 50 hours, respectively. The calculated half-lives of NQ in surface water at 40°N latitude were 0.8, 0.6, 1.3, and 2.3 days, respectively, for the spring, summer, fall, and winter seasons (Spanggord et al., 1987). The complete photolyzation of NQ with ultraviolet light in the laboratory was shown by van der Schalie (1985) to increase the toxicity of NQ to fathead minnow, water flea (Daphnia magna), and S. capricornutum. Of the total radiated energy used in the laboratory study by van der Schalie, about 17.6% was contributed by wavelengths shorter than those found in natural sunlight (<294 nm). Because of the possibility of increased toxicity of photolyzed NQ (ϕ-NG) to aquatic organisms, the question of whether or not toxic photolytic product(s) were created at wavelengths found in natural sunlight or at wavelengths below natural sunlight used in van der Schalie's study was addressed in a chronic cladoceran study (Table 4).

2.3 NG Water Quality Criterion Data Base

Smith (1986) completed a literature review of the environmental aspects of NG in an attempt to determine a water quality criterion for the compound. Sufficient data were not available to calculate the Criterion Maximum Concentration or the Criterion Continuous Concentration for a final aquatic criterion.

The acute toxicity of NG was determined by Bentley et al. (1978) for six of the eight families required by the EPA guidelines. Nominal concentrations, which are acceptable for

most test materials if measured concentrations are not available for acute tests (Stephan et al., 1985), were used for the study. Bentley et al. (1978) examined the stability of NG over time (0-96 h) under a variety of water quality conditions and concluded that mean measured concentrations were essentially identical to nominal concentrations. Thus, acute data were needed for a species from a phylum other than Arthropoda and Chordata and for a species in any order of insect or phylum not already represented. The hydra and midge (Paratanytarsus parthenogenticus) were used to complete the data base (Table 1).

Several life cycle tests were conducted by Bentley et al. (1978); however, none were acceptable under the EPA guidelines because all concentrations of NG used in all tests were nominal concentrations. Thus, three chronic studies had to be performed to meet the minimum EPA requirements for a Final Chronic Value. The three chronic tests were a rainbow trout ELS test, fathead minnow ELS test, and a 7-d cladoceran test (Table 2). Acute tests were also conducted on the three species in order to provide data for acute-chronic ratios (Table 3).

Bentley et al. (1978) determined a 96-h EC50 (reduction in cell numbers) for S. capricornutum; however, the value was based on nominal concentrations of NG. The EPA guidelines state that the concentrations of test material used in toxicity tests for the Final Plant Value must be measured. Therefore, a 96-h EC50 for growth with the same green alga using measured concentrations of NG was determined (Table 4).

The bioconcentration of NG (eight days) in several species was studied by Bentley et al. (1978). The BCFs ranged from 8-15; however, it was not clear whether or not the tissue concentrations attained a steady state. Thus, the data were not acceptable under EPA guidelines. However, since the log P of NG is relatively low (2.35; Leo et al., 1971), the data by Bentley et al. (1978) indicated that NG was not bioconcentrated to any degree, and no maximum permissible tissue concentration data exist, further work in this area was not required.

2.4 RDX Water Quality Criterion Data Base

The environmental effects of RDX to aquatic organism were reviewed by Etnier (1986) in an attempt to calculate the Criterion Maximum Concentration and Criterion Continuous Concentration for a final aquatic criterion.

The acute toxicity of RDX dispersed in dimethyl sulfoxide (DMSO) was studied by Bentley et al. (1977). Aquatic organisms from six of the eight families required by EPA were studied. As with Bentley et al.'s (1978) NG studies, nominal concentrations of RDX were used. The authors stated that RDX was stable in water and that nominal concentrations of RDX very closely

approximated the measured concentrations over the course of some long-term flow-through studies. Liu et al. (1983) also determined an acute LC50 value for one of the same species studied by Bentley et al. (1977). To complete the acute data base, tests were conducted with hydra and a midge (Table 1).

Bentley et al. (1977) conducted chronic studies on four species; however, none of the studies satisfied EPA's guideline requirements. As discussed in Section 2.1.2, chronic tests with a fish, invertebrate, and an acutely sensitive fish or invertebrate are required to formulate a criterion. The acute data by Bentley et al. (1977) showed that fish were more sensitive than invertebrates. However, the chronic studies by Bentley et al. indicated that invertebrates (D. magna and Chironomus tentans) may be as sensitive or more sensitive than fish (P. promelas and Ictalurus punctatus). Thus, a second invertebrate rather than a fish was studied. Ceriodaphnia, Paratanytarsus, and the fathead minnow were used to complete the chronic data base (Tables 2 and 3).

The toxicity of RDX to four algal species was studied by Bentley et al. (1977) using nominal concentrations which do not meet guideline requirements. A review of the literature showed that no chronic data were available for a vascular plant. To establish a Final Plant Value, a 96-h growth study was conducted with the green alga, S. capricornutum (Table 4).

No maximum permissible tissue concentration data exist for RDX (Etnier, 1986). Bentley et al. (1977) conducted 28-d BCF studies with three species of fish and found that the 28-d BCFs for edible tissue ranged from 2.9 in channel catfish (I. punctatus) to only 5.9 in fathead minnow. Liu et al. (1983) also found very low 4-d BCFs which ranged from 1.6 in water fleas to 3.0 in the aquatic oligochaete (Lumbricus variegatus). The above data coupled with the fact that the log P is approximately 0.87 (Banerjee et al., 1980), showed that RDX is not bioconcentrated to any degree. Thus, further work to establish a Final Residue Value was not necessary.

Several studies showed that the primary mechanism that degrades RDX in aqueous solution is photolysis. The range of UV wavelengths that causes photolytic reactions with RDX is generally between 240 and 350 (Etnier, 1986). Smetana and Bulusu (1977) studied the degradation of aqueous solutions of RDX by UV photolysis at 254, 300, and 350 nm. They achieved very rapid rates of RDX disappearance at 254 nm (<1 h); the higher wavelengths were much less effective. Spanggord et al. (1980) found that photolysis of RDX in distilled water and in natural water samples followed first order kinetics when photolyzed in sunlight or at 313 nm. The calculated half-lives for RDX at 40° N latitude in summer, fall, winter, and spring in distilled water were 1.2, 2.6, 5.0, and 1.5 d, respectively.

The toxicity of RDX to aquatic organisms appears to decrease after photolysis. Liu et al. (1984) found that exposure of composition B type LAP wastewater (1.6:1 mixture of TNT and RDX) and a laboratory-prepared TNT-RDX mixture (1.6:1) to simulated sunlight (filtered UV light) reduced their toxicity to several aquatic organisms. Photolyzed TNT (no RDX present) was also less toxic. The photolysis of RDX alone was not studied by Liu et. (1984). Because the toxicity of photolyzed RDX alone was not studied, we conducted one photolysis study using natural sunlight to verify whether or not photolyzed RDX may be less toxic than the parent compound. A chronic Ceriodaphnia test was conducted in order to compare the results to the chronic Ceriodaphnia test conducted as part of the Final Chronic Value (Table 4).

2.5 TNT Water Quality Criterion Data Base

Ryon (1987) reviewed the literature on the effects of TNT to aquatic organisms in order to determine a water quality criterion. Sufficient data were not available to calculate the Criterion Maximum Concentration or Criterion Continuous Concentration for a final aquatic criterion.

A number of acute studies were conducted with various aquatic organisms. Data were available for seven of the eight families required by the EPA guidelines. Paratanytarsus was tested to complete the data base for the Final Acute Value (Table 1).

Data existed for rainbow trout and daphnids (Bailey et al., 1985) to calculate two of the three acute to chronic ratios required by the guidelines. The Bailey et al. (1985) daphnid data had some inconsistencies within and between two series of test; therefore, a chronic Ceriodaphnia test was run to provide an additional chronic test (Tables 2 and 3). Bailey et al. also conducted an excellent full life-cycle study with the fathead minnow. However, the study did not determine the concentration at which no observable effect occurred which, unfortunately, did not meet the EPA guideline requirements. Thus, to complete the required data base, a full life-cycle study with the fathead minnow was run to determine the no-observed effect level for TNT (Tables 2 and 3).

Ryon (1987) concluded that sufficient algal data were available to determine a Final Plant Value for TNT. While we do not disagree with his conclusion, we observed that in several studies cited by Ryon, TNT concentrations decreased to some extent over the course of the experiments. The decreases were most likely the result of photolysis (for ex, see Bailey, 1982; Liu et al., 1984; Burrows et al., 1989). Thus, we concluded that the plant values may not reflect the true toxicity of TNT to plants. Therefore, an acute test with duckweed (Lemna minor) was performed to provide further phytotoxicity data using a flowering

plant rather than algae. Duckweed was selected because the test solutions could be renewed during the test and reduce the potential for photolysis; test solutions are not normally renewed during standard algal tests. In addition, Schott and Worthley (1974) conducted a study with the duckweed, L. perpusilla, which provided a basis for comparison with L. minor.

A Final Residue Value could not be calculated because data on maximum permissible tissue concentrations were not available. Limited data by Liu et al. (1984) showed that 4-d BCFs (non-steady state) for three animal species ranged from 9.5 in bluegill (Lepomis macrochirus) muscle to 209 in whole body daphnids. The log P for TNT is 2.03 (Liu et al., 1984) which also indicates that TNT should not be bioconcentrated to any degree in aquatic organisms. Thus, further work in this area was not necessary.

The primary physical mechanism that degrades TNT munitions in dilute aqueous solution is photolysis (Burrows et al., 1989). Spanggord et al. (1980) reported the half-lives of TNT exposed to sunlight in natural water ranged from 3 to 22 h. Liu et al. (1984) showed that the photolysis of TNT reduced the toxicity of TNT to fathead minnows and daphnids. Thus, photolysis did not appear to be an important issue with TNT toxicity. Further toxicity research in this area did not appear to be necessary.

SECTION 3

MATERIALS AND METHODS

3.1 Analytical Chemistry

3.1.1 Chemicals

NQ (CAS No. 556-88-7) was obtained from the U.S. Naval Ordnance Station (NOS), Indian Head, MD. The high density NQ (Lot No. 985-1) had no solvents or stabilizers present. The chemical purity of the high density NQ was 99.998%. NQ was stored in the original plastic-lined shipping containers at room temperature.

Two shipments of NG (CAS No. 55-63-0) were obtained from NOS. The first shipment was 10% NG in absolute ethanol (no lot number). A second shipment of NG dissolved in diluent water rather than ethanol was obtained in order to conduct bioassays without the presence of a solvent. The second shipment of NG (no lot number) was a 1 g/L solution of NG dissolved in JHU/APL well water (see Section 3.3) used for the bioassays with no other solvents or stabilizers present. The purity of both NG shipments met NOS's nitrogen nitration specification number N248B; NOS does not determine percent purity for NG. No reverse phase high performance liquid chromatography (HPLC)-detectable impurities were observed during the study. NG was stored at 4 °C in glass bottles placed in metal flasks.

RDX (CAS No. 121-82-4) and TNT (CAS No. 118-96-7) were obtained from the Health Effects Research Division of USABRDL (no lot numbers). Both compounds were recrystallized to a purity of 99+ percent as measured by HPLC. Both compounds were stored in amber glass bottles in the dark at room temperature.

Known standards of NQ, RDX, and TNT were supplied by the Health Effects Research Division of USABRDL. Two NG standards were supplied by NOS (see below). HPLC grade methanol was used for HPLC analyses. Water used for the HPLC was deionized glass distilled.

3.1.2 HPLC Analyses

Analyses of aqueous NQ concentrations were performed by HPLC using the operating conditions described by Burrows et al. (1984a,b). Aqueous concentrations of RDX and TNT were determined by the HPLC method of Brueggeman (1983) with minor exceptions in the operating conditions as shown below. NG concentrations were also determined by the method of Bruggemann (1983) with the exception that the UV detector was operated at 215 nm. A mobile phase of 80% methanol:20% water was originally used for NG but

was switched to 55% methanol:45% water because we achieved better separation of NG from trace background organics in the bioassay test media.

A Waters HPLC system (Waters Associates, Milford, MA) was used for all compounds 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). The following HPLC operating conditions were used:

NQ

Column:	DuPont Zorbax C_8
Mobile Phase:	100% water
Method:	Isocratic
Flow Rate:	1.0 mL/min
Detector:	UV 235 nm, 0.05 absorbance units full scale (AUFS)
Injection Volume:	2-50 µL depending on the concentration of material in solution

NG

Column:	Waters µBondapak C_{18}
Mobile Phase:	55% methanol:45% water
Method:	Isocratic
Flow Rate:	1.0 mL/min
Detector:	UV 215 nm, 0.02 AUFS
Injection Volume:	25-200 µL depending on the concentration of material in solution

RDX

Column:	Waters µBondapak C_{18}
Mobile Phase:	55% methanol:45% water
Method:	Isocratic
Flow Rate:	1.0 mL/min
Detector:	UV 240 nm, 0.1 AUFS
Injection Volume:	2-200 µL depending on the concentration of material in solution

TNT

Column:	Waters µBondapak C_{18}
Mobile Phase:	55% methanol:45% water
Method:	Isocratic
Flow Rate:	1.0 mL/min
Detector:	UV 240 nm, 0.1 AUFS
Injection Volume:	10-200 µL depending on the concentration of material in solution

Standard solutions of NQ, RDX, and TNT were prepared by dilution of a stock solution freshly prepared each day of analysis. The standard solutions with concentrations of 10.0, 5.0, 1.0, and 0.5 µg/L were used for NQ; 1,098, 549, 275, 110, and 11 ng/L for RDX; and 4,667, 2,334, 1,167,and 47 ng/L for high concentrations of TNT; and 40.0, 10.0, 5.0, and 1.0 ng/L for low concentrations of TNT.

Two stock solutions of NG were used during the study. The first stock solution of NG was 1 g NG dissolved in 1 L of ethanol (with no stabilizer present) adjusted to pH 7.0. This stock solution was used for the acute bioassays with hydra (H. littoralis), midge (P. parthenogenticus), and rainbow trout (O. mykiss). The second stock solution was 1 g NG dissolved in JHU/APL well water (see Section 3.3) which was used for all other bioassays. Both NG stock solutions were keep in the dark at 4 °C in glass bottles. The stability of the NG stock solutions were periodically checked during storage to assure that no decomposition occurred. Standard solutions of NG were prepared by dilution of the stock solutions freshly prepared each day of analysis. Standard solutions with concentrations of 10.0, 5.0, 2.5, and 1.0 µg/L were used.

Precision and accuracy analyses of the HPLC methods were conducted prior to the start of the bioassays. The analyses were performed with all compounds dissolved in well water which was used for the bioassays. Precision was evaluated by injecting a sample three times on three separate days. The mean, standard deviation, and relative standard deviation were calculated for a low and high concentration. The accuracy of the method was assessed by calculating the percent deviation (percent recovery) of the measurement from the actual quantity injected. The data are given in Table 5.

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 h 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.

3.2 Test Solution Preparation

3.2.1 NQ, NG, RDX, and TNT

Saturated stock solutions of NQ and RDX for the fish and invertebrate tests were prepared by dissolving appropriate amounts of the materials in aerated diluent water (see Section 3.3). The stock solutions were stirred in the dark for 24 h at room temperature; occasionally the materials were heated to

≈30 °C. All stock solutions were filtered before use to remove particles >0.45 μm as well as excess reagent crystals in saturated solutions. All stocks were prepared in amber glass containers. The highest toxicant concentration tested in any test was the solubility limit of the material in JHU/APL well water at the test temperature; no solvents were used to disperse the materials. In the algal test with RDX, a stock solution of RDX was prepared by adding RDX to algal assay medium, stirred at room temperature for 24 h, and filter sterilized prior to test initiation.

NG stock solutions were prepared by adding appropriate amounts of the material to aerated JHU/APL well water and stirred for 4 to 8 h. No NG stock solutions were heated during preparation or filtering at 0.45 μm. All stock solutions were prepared in amber glass containers. In the algal test with NG, a stock solution of NG was prepared by adding NG to algal assay medium, stirred at room temperature for 4 h, and filter sterilized prior to test initiation. TNT stock solutions were prepared by dissolving appropriate amounts of the compound in aerated JHU/APL well water and stirring for 24 h; no TNT stock solutions were heated during preparation. The TNT stock solutions were filtered before use to remove particles >0.45 μm.

3.2.2 Photolyzed NQ and RDX

Photolyzed NQ and RDX were prepared by exposing the compounds in JHU/APL well water to sunlight (38° 51' N latitude; 76° 31' W longitude) until the parent compounds reached non-detectable levels as determined by periodic HPLC analysis. A 5 L stock solution of 100 mg/L NQ (measured concentration) was photolyzed in direct sunlight in mid September for a total of 80 h until NQ could not be detected. A 5 L stock solution of 10 mg/L RDX (measured concentration) was photolyzed for a total of 57 h in sunlight in early October until RDX could not be measured from background. The stock solutions of both NQ and RDX, which were photolyzed in rectangular glass containers (40 x 58 x 82 cm) with no tops, were covered each evening with black plastic so that the actual number of hours of exposure to sunlight could be calculated. Light intensity was not estimated by the use of a chemical actinometer. Both the NQ and RDX photolyzed stocks were used for the entire 7-d cladoceran static renewal tests.

3.3 Dilution Water Quality

The dilution water used in the fish and invertebrate tests was obtained from a non-chlorinated deep well located at The Johns Hopkins University Applied Physics Laboratory (JHU/APL) in Shady Side, MD. A comprehensive chemical analysis of the well water was conducted two times during the study. The analyses were separated by approximately 18 months. The average of the two analyses is provided in Table 6. Dissolved oxygen, pH,

temperature, conductivity, alkalinity, and total hardness were measured during all toxicity tests as discussed below.

3.4 Test Organisms and Holding Conditions

3.4.1 Algae and Duckweed

A green alga (S. capricornutum) starter culture was obtained from the culture collection at North Texas State University, Denton, TX. Stock algal cultures were reared in 2.5 L Pyrex culture flasks containing 1 L of sterilized double strength "AAP" algal assay medium, with sufficient P added to achieve a 20:1 N:P ratio as described in Miller et al. (1978). Cultures were maintained in a constant temperature incubator under constant cool-white fluorescent lights (≈300 foot candles) at a temperature of 20 (± 1) °C on a shaker table oscillating at 100 rpm (± 10 %). Log growth cells were used to start all tests (Table 7).

Duckweed (L. minor) was obtained from a local pond. Taxonomic identification was confirmed via Hellquist and Crow (1982). Stock colonies of duckweed were reared in 19 L glass aquaria in nutrient media described in Wang (1986). The colonies were maintained under constant cool-white fluorescent lights (≈300 foot candles) at a temperature of 25 (± 1) °C. Two frond colonies were used to initiate the bioassay with TNT.

3.4.2 Invertebrates

Hydra (H. littoralis) which were originally obtained from Carolina Biological Supply Company (Burlington, NC), were reared at 22 (± 1) °C in static glass culture dishes 6 cm deep x 20 cm in diameter partially filled with a 50:50 mixture of JHU/APL well water and deionized water. Hydra were fed freshly hatched brine shrimp (Artemia sp.) three times per week as recommended by Sabourin et al. (1985). Excess food and debris were removed from the cultures daily; 50% of the medium was renewed daily. Adult polyps were used in all toxicity tests.

The cladoceran, C. dubia, was cultured at 25 (± 1) °C in 600 mL glass beakers filled with 400 mL JHU/APL well water amended with selenium (2 μg Se/L as Na_2SeO_3) as recommended by Winner (1987 and 1989). The diet consisted of a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, MO) and the green alga, S. capricornutum, added to the daphnid culture 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.

The midge (P. parthenogenticus) was obtained for culture from the Center for Lake Superior Environmental Studies,

University of Wisconsin - Superior. The organism was cultured at 22 (± 1) °C in 38 L glass aquaria filled with JHU/APL well water to depths of 5 to 10 cm. The chironomid was reared on a diet of dried grass and trout chow (0.5 g Cerophyl® + 10 g trout chow/300 mL well water). The mixture was blended at high speed for 5 min, allowed to stand at 4 °C for 24 h, filtered to remove large particles, and stored at 4 °C prior to use.

Midge 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.

Hydra, cladocerans, and midges were all cultured under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels).

3.4.3 Fish

Rainbow trout (O. mykiss) fingerlings for the NG and NQ acute toxicity tests and eyed embryos for the NQ early life stage (ELS) test were obtained from the Pennsylvania Fish Commission, Reynoldsdale Fish Culture Station, New Paris, PA. Eyed embryos for the NG ELS test were obtained from the Pennsylvania Fish Commission, Benner Spring Fish Research Station, State College, PA. In the acute tests, fingerlings were held in aerated JHU/APL well water at 12 (± 1) °C for 14 to 16 d prior to testing. During the acclimation period, the fish were fed ground Salmon Starter Meal (Zielger Brothers, Gardners, PA) three times daily on weekdays and twice on weekends. In the ELS tests, eyed embryos were gradually adjusted to JHU/APL well water at 12 (± 1) °C over a 24-h period and held an additional 24 h prior to testing. The eyed embryos were held in reduced light of <25 foot candles at the surface of the water.

Fathead minnow (P. promelas) embryos and juveniles were obtained from the JHU/APL culture maintained at 25 (± 1) °C in JHU/APL well water. The JHU/APL culture procedures were similar to those recommend by Peltier and Weber (1985). The JHU/APL culture was initiated with mature fathead minnows obtained from the U.S. EPA Environmental Monitoring and Support Laboratory - Cincinnati, Ohio. Briefly, spawning fish were cultured in fiberglass tanks (2.4 x 0.8 x 0.5 m) containing 0.2 m JHU/APL 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 approximately 3-month intervals. 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 h old) in 19 L aquaria at 25 (± 1) °C in JHU/APL 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 h preceding the start of the test. All stages of both species were reared under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels).

3.5 Toxicity Test Methods

The specific test methods for the acute and chronic tests are given below. Deviations from the test methods are discussed where appropriate. A geometric series of at least five toxicant concentrations (plus controls), which had a dilution factor of 60%, was used for all definitive tests with the exception of 1) the acute NQ test with hydra in which only three concentrations were run and 2) the acute RDX invertebrate tests. Preliminary tests showed that RDX was not toxic to the invertebrates at the solubility limits of the compound in diluent water. Therefore, the organisms where exposed to two concentrations of RDX at the 100% solubility limit and 60% solubility limit. The photoperiod for all tests with the exception of the algal, duckweed, and fathead minnow TNT life cycle test was 16-h light:8-h dark (fluorescent lights; 60-85 foot candles at the surface of the test chambers). The alga and duckweed were tested under constant cool-white fluorescent lights (≈300 foot candles). The fathead minnow life cycle photoperiod was set to that of Evansville, IN (see below).

Flow-through exposures were conducted for the 48-h RDX midge test, all 96-h rainbow trout and fathead minnow tests, and all fish chronic studies. The test solutions in the flow-through studies were delivered by solenoid-activated proportional dilutor systems which were calibrated 24 h prior to the start of a test and checked and/or recalibrated at a minimum twice daily during a test. The dilutors were constructed of glass; polyethylene fittings and Tygon® tubing were also used. Control and toxicant solutions, which were held at the test temperatures, were aerated in their respective headboxes (polyethylene or fiberglass tanks) with air supplied by an oil-free compressor. All stock concentrations were quantified prior to the start of a test, and each time a new stock solution was prepared during a test. All dilutors were equipped with counters to monitor the cycling rate as well as to ensure proper function of the dilutor. Saturated

solutions of NG and RDX were prepared by physically mixing and aerating the compounds in dilution water for several hours at the test temperature before the stocks were used. All stock solutions were kept in the dark to avoid photolysis.

3.5.1 Acute Toxicity Methods

The toxicity (96-h EC50 for growth) of NG and RDX to S. capricornutum was determined by the procedures given in EPA TSCA Method 797.1060 (U.S. EPA, 1985 and 1986). The 96-h EC50 algal test for growth is considered to be an acute test by EPA's TSCA office. U.S. EPA's Office of Research and Development considers the test to be a chronic test for determining the toxicity of effluents (Horning and Weber, 1985; Weber et al., 1989) as do other investigators for evaluating single chemicals (for ex., see Hughes et al., 1988 and Suter, 1993). Because we used the TSCA Method, we will refer to the test as an acute test; however, we also analyzed and reported the results as chronic data so comparisons could be made with other chronic studies where appropriate. The nutrient media used for the bioassays was sterilized double strength "AAP" algal assay medium, with sufficient P added to achieve a 20:1 N:P ratio as described in Miller et al. (1978) rather that the media recommended in the test method. The alga used in the test was obtained from the culture collection at North Texas State University, Denton, TX, rather than No. 22662 from the American Type Culture Collection (Rockville, MD).

Algal test solutions were prepared by dilution of the stock solutions with filtered sterilized assay media within a sterile transfer room. Test solutions (100 mL total volume) were dispensed into 250 mL Delong flasks and inoculated with S. capricornutum cells in log growth to achieve a density of ≈4,000 and 10,000 cells/mL, respectively, for NG and RDX. Triplicates were prepared for each treatment. The flasks were placed on a shaker table in an incubator set at the culturing conditions described in Section 3.4.1. Growth measurements (cell density) were made from all replicates in each treatment at 0, 24, 48, 72, and 96 h. Algal cell density was determined from a 1 mL sample with a Model ZBI Coulter Counter (Coulter Electronics Inc., Hialeah, FL). The instrument was calibrated with each use via hemocytometer counts. Toxicant concentrations of each treatment were measured at the beginning and end of the test (i.e., 0 and 96 h).

The acute toxicity (96-h EC50 for growth) of TNT to duckweed was determined by the method given in Draft No. 6 of the ASTM proposed guide for conducting static toxicity tests with duckweed (Wang, 1986). Briefly, 10 two-frond colonies per replicate were exposed to each treatment in 250 mL glass beakers containing 150 mL nutrient medium described in Wang (1986). Triplicates were run for each treatment and control. All test solutions were

renewed at 24-h intervals. Observations on frond production were made at each renewal period and at the end of the test. The colonies were maintained under constant cool-white fluorescent lights (≈300 foot candles) at a temperature of 25 (± 0.5) °C in a constant temperature incubator. TNT concentrations were quantified in all solutions at the beginning of the test and every 24 h when the test materials were renewed.

The acute toxicity of the munitions to invertebrates and fish was determined by the procedures given in ASTM Designation E 729-80 (ASTM, 1980). 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. In the cases of NQ and RDX, the highest concentrations tested were frequently at the solubility limit of the compounds in diluent water which did not produce 100% mortality. All treatments including the controls and NG ethanol controls were replicated. A minimum of 10 organisms was run in each replicate. All organism and test chamber assignments were random.

Forty-eight-h static renewal tests (test solutions renewed at 24 h) were conducted for all invertebrates with the exception of the midge RDX test which was a 48-h flow-through test. Flow-through exposures were conducted for all 96-h fathead minnow and rainbow trout tests. The initial age of the organisms at the start of each test, the mean size of the fish at the end of the test, and the test temperatures for all species are given in Table 7. The size of the test chambers, test solution volumes, volume additions per day, and loading per test chamber are summarized in Table 8. Feeding was discontinued 24 h prior to the start of all tests with the exception of the cladoceran and midge. Ceriodaphnia were fed the diet given in Section 3.4.2 up to the initiation of each test, at the beginning of the test and at the 24-h renewal period during the test. Midge larvae were placed in testing chambers 24 h preceding the introduction of the test material and supplied with 1.2 mL midge food as described above in order to construct larval tubes before the exposure was started. The midges were not fed while exposed to the toxicants.

Test temperatures for hydra and cladocerans were held constant by placing the test chambers in an environmental chamber set at the appropriate temperature. Test temperatures for the midge and fish were maintained by placing the exposure vessels in constant temperature water baths. Temperatures in the water baths and environmental chambers were monitored and recorded continuously on strip charts. Temperature probes were placed in one of the control chambers. Dissolved oxygen and pH were measured in the static renewal tests in one replicate of all treatments at time 0 h and 24 h on day 1 and again at time 0 h and 24 h of day 2 when the test solutions were renewed. Dissolved oxygen and pH in the flow-through tests were measured

at the beginning of the test and every 24 h in one replicate of all treatments. Conductivity, alkalinity, and total hardness were measured at the same frequency given above for the static and flow-through tests in one control replicate and one replicate at the highest test concentration. Measurements were made in alternating replicates throughout all tests. Routine water quality was determined by procedures given in Standard Methods (APHA et al., 1985).

Water samples for HPLC analyses were taken in the static renewal tests in both replicates of all treatments at time 0 h and 24 h on day 1 and at time 0 h and 24 h when the test solutions were renewed. HPLC water samples for the flow-through tests were taken at time 0 h and every 24 in all treatments of one replicate. Samples were taken from alternating replicates.

3.5.2 Chronic Toxicity Methods

The following test conditions were used for all chronic studies with the exception of the Ceriodaphnia tests which are described below. The tests were conducted under flow-through conditions using proportional dilutor systems under the same conditions described in Section 3.5.1. Test temperatures were maintained by placing the exposure vessels in constant temperature water baths as described above. Dissolved oxygen and pH measurements were taken in one replicate of each treatment at the beginning and end of each test and at least weekly during a 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 each test and at least weekly during a test. Water chemistry measurements and water samples for HPLC analyses were taken in alternating replicates throughout all tests.

The chronic toxicity of all the munitions including photolyzed NQ and 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 tests were produced by daphnids in culture that had released at least three broods. The initial age of the neonates in each test is shown in Table 7. The test chambers, test volumes, etc. are given in Table 8. All tests were conducted in an environmental chamber at 25 (±1)°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels). All test organisms were fed daily as described in Section 3.4.2 after each 24-h renewal. Water quality and samples for HPLC measurements were taken daily over the 7-d period as described above for the organisms studied under 48-h static renewal conditions.

The RDX life cycle test with P. parthenogenticus was conducted by the method of Anderson (1980 and 1984). Briefly, the test was conducted under flow-through conditions in glass crystallizing dishes using the volume shown in Table 8. Five toxicant concentrations and a control were delivered to replicate experimental chambers from a proportional dilutor as described above. In addition to aerating the toxicant stock solution and diluent water, air lines were fitted with Pasteur pipettes to aerate test solutions in the test chambers when the delivery of the test solution was suspended.

At the initiation of the midge life cycle test, the flow of test solution was stopped and 10 eggs <12 h old were randomly placed in two replicate 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 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 the test chambers via overflow tubes. Food was administered at a rate governed by larval consumption (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/L).

P. parthenogenticus larvae were measured on days 6, 8, and 10 by placing the test chambers under a dissecting microscope equipped with a calibrated 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 the same 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.

The early life stage studies with rainbow trout and fathead minnow were 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 duration of the NQ rainbow trout ELS test was less than that recommended by Goodman (1986). The post-hatch exposure period was run for 28 d rather than 60 d

in order to compare the results of this study conducted under flow-through conditions with those of van der Schalie (1985) for rainbow trout exposed to NQ under static renewal conditions. All ELS tests were conducted under flow-through conditions in 10 L glass test chambers containing 6.4 L of test material (Table 8). Toxicant concentrations were established in the 10 L test chambers 24 h prior to the introduction of embryos. During the embryo incubation periods, 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.

The initial ages of the rainbow trout eyed embryos and fathead minnow embryos used in the ELS studies are given in Table 7. At the start of a 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 (30-40 embryos per cup), two embryo incubation cups were randomly placed in each replicate test chamber. The photoperiod during the rainbow trout embryo exposure phase was 16h light:8 h dark; however, the intensity at the surface of the aquaria was reduced to <25 foot candles until swim-up.

Embryos were observed at least three times per day during the hatching period. Recently hatched larvae were released immediately to the test chambers by lowing the embryo incubation cups below the surface of the test solution allowing the fish to swim free. Any embryos with fungus were removed from the test systems and counted. Embryos removed with fungus were not included in the calculations of hatching success.

Post-hatch fish were observed daily for developmental abnormalities and mortality. Rainbow trout were fed ground Salmon Starter Meal three times daily on weekdays and twice daily on weekends once swim-up occurred in the controls. The amount of ration per feeding was approximately 4% dry food/wet weight fish. Fathead minnow were fed live brine shrimp (<24 h old) beginning at day 2 post-hatch at similar time intervals. Excess food and feces were siphoned from the test chambers at least daily. At the conclusion of a test, total length, blotted wet weight, and dry weight were determined for all fish from each treatment with the exception of the rainbow trout NQ test where only a random sample of 20 fish from each treatment was measured. Dry weight was determine by drying at 60°C for a minimum of 24 h or longer until a constant weight occurred. Morphometric data were not taken on fish that died while a test was in progress.

The TNT life cycle test with fathead minnows was conducted by the procedures outlined in Benoit (1981). Deviations from the procedures recommended by Benoit (1981) are discussed where appropriate. The 9-month study was conducted under flow-through conditions as described above at 25 (±1) °C. The photoperiod (fluorescent lights; 60-85 foot candles at the surface of the test chambers) was set to simulate the dawn to dusk periods of Evansville, IN, as recommended by Benoit (1981). The Evansville photoperiod is the average light condition for the middle of the continental United States. Adjustments in day length were made on approximately the first and fifteenth day of each month during the dusk cycle. Glass aquaria (19 L) containing 15 L of test material were initially used for exposure chambers (Table 8). All fish were moved to 38-L test chambers which contained 30 L of test material when they were 60 d old. At 22 weeks when the fish showed secondary sex characteristics, three 38-L tanks containing 30 L of test material per treatment were placed in the system. The tanks were divided in half by fiberglass screen; one half of the tank served as a spawning chamber and one half served as an area for exposing the embryos and larvae.

The life cycle test was started with embryos <12 h old. 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 incubation cups were loaded with 35 embryos per cup, one embryo incubation cup was randomly placed in one of two replicate test chambers at each treatment. The embryo incubation cups, which were the same as those described above for the ELS test, were suspended in 19-L test chambers containing 15 L of test material on a rocker arm apparatus and reciprocated vertically at 2 rpm to insure good mixing within the embryo incubation cups. Embryos were observed two times per day during the hatching period. Upon hatching the larvae were released to the test chambers by lowing the embryo incubation cups below the surface of the test solution allowing the fish to swim free. All embryos with fungus were removed and recorded. The embryos with fungus were not included in the calculations of hatching success.

Larvae were fed 24 h after hatching. For the first three weeks after hatching, the fish were fed newly hatched brine shrimp nauplii three times per day during the weekdays and at least two times per day on the weekend. After three weeks, the fish were fed frozen brine shrimp pellets of known weight. The amount of food given to the fish was proportional to the number of fish present in the test chambers. Each tank was checked daily for mortalities, deformities, and abnormal behavior of the fish. All tanks were brushed when algal or fungal growth became noticeable. Debris was siphoned from the tanks at least three times per week.

The total length of all juveniles was estimated at 30 and 60 d post-hatch by the photographic method of McKim and Benoit (1971). At 8 weeks, all fish were moved from the 19-L growth chambers to 38-L growth chambers containing 30 L of test material. A dilutor malfunction occurred during week 16 (day 117) which caused a number of fish to die at all treatments because of low dissolved oxygen concentrations. Sufficient fish survived at all treatments to continue the study with the exception of the highest concentration (0.191 mg/L). Because most of the fish died at the highest test concentration, this treatment was terminated. At 22 weeks when all of the remaining fish showed secondary sexual characteristics, the fish from both replicates at each treatment were combined. Three breeding groups from each treatment, which consisted of 1 male:4 females per breeding group, were randomly selected from each combined group and placed in three spawning chambers (one group per spawning chamber). A single PVC spawning substrate, described in Section 3.4.3, was placed in each spawning chamber. All adults which were not used in the spawning studies at 22 weeks were sexually identified, measured (total length), and weighed (both wet and dry weight).

Spawning substrates were checked for embryos each afternoon including weekends. The embryos were removed from the spawning substrates and counted. Spawning substrates were replaced in the spawning chambers immediately after the embryos were removed. Fifty embryos from the first spawn, and 50 embryos from approximately every third spawn thereafter were randomly selected and transferred to embryo incubation cups for hatching success observations. The second generation (F_2) embryos were treated as described above for the first generation (F_1) embryos. The adult exposures were terminated when no spawning occurred for 7 d. At the termination of spawning, the adults were sexually identified, measured (total length), and weighed (both wet and dry weight).

One group of 50 larvae from each of the three replicates at each treatment were released from the embryo incubation cups for a 30-d ELS exposure. The newly hatched F_2 larvae were fed, observed, etc., exactly as the F_1 larvae described above. At the end of the 30-d post-hatch exposure, total length, wet weight, and dry weight were taken for all fish.

3.6 Test End Points and Data Analyses

All concentration data used in the statistical analyses were mean measured concentrations with the exception of the NQ and RDX photolyses studies where nominal concentrations were used. The nominal concentrations were based on measured concentrations of the stock solutions which were photolyzed until the parent compounds reached non-detectable levels. The dilutions used in the toxicity tests were made from the completely photolyzed stock solutions and are labeled as photolyzed (*) concentrations. For

example, a 100 mg/L stock solution of NQ completely photolyzed and diluted 1:10 would be labeled 10.0 mg/L ϕ-NQ.

The test end point for all acute toxicity tests with invertebrates and fish was mortality. The 48- and 96-h LC50s and their 95% fiducial limits were determined by the probit method. In all cases, the goodness of fit probability of the data to the probit model was >0.05. When the probit method could not be used because the goodness of fit probability was <0.05 or there were less than two concentrations at which the percent mortality was between 0 and 100, the moving average angle method was used. Both the probit LC50s and their 95% fiducial and the moving average angle LC50s and their 95% confidence limits were determined by an EPA statistical program (Stephan, 1978).

The test end point for the toxicity of NG and RDX to the green alga was growth, measured as density (cells/mL). Likewise, the test end point for duckweed exposed to TNT was growth which was measured as new frond production. The 96-h EC50s for growth were estimated for both species by using the "inhibition proportion" technique recommended by Horning and Weber (1985). The technique uses the probit analysis to estimate EC50s and their 95% fiducial limits. Since the assumptions of the probit analysis are not met in the classical sense because of the very nature of the growth data, the count data at each treatment were averaged and subsequently converted to "inhibition proportions" using the formula below before the probit analysis was performed.

$$I = C - T / C * 100$$

where: C = the mean growth of the controls
T = the mean growth at a given treatment

In addition to the EC50s for growth, 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 is then used in a multiple comparison method for comparing 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).

The end points for the 7-d survival and reproduction tests with Ceriodaphnia were survival and young production. The end points for the midge life cycle test with RDX were survival, growth (length), emergence success, egg production, and hatching success. The end points for the ELS tests were hatching success,

growth, and survival. The end points for the fathead minnow TNT life cycle test F_1 generation were hatching success, growth, number of embryos per spawn, total number of spawns, and survival. The end points for the F_2 generation were hatching success, growth, and survival.

The statistical analyses of the 7-d short-term chronic, ELS, and life cycle data were conducted as follows. Raw daphnid survival data sets were analyzed by Fisher's Exact test. Arc-sine square root transformations were made on all other survival and hatching success raw data before further data analyses were performed. With the exception of the daphnid survival data, all data (both transformed and raw data) were then subjected to a chi-square test of normality and Bartlett's test for homogeneity of variance.

When the data sets met the assumptions of normality and homogeneity of variance, a parametric statistic was used. Dunnett's test was used when the number of replicates was constant among treatments. Duncan's new multiple range test was also occasionally used. A t-test with Bonferroni adjustment of error rate was performed when the number of replicates was not constant among treatments. When a data set failed to meet the assumptions of normality or homogeneity of variance, a nonparametric statistic was used. Steel's Many-One Rank test was performed when equal number of replicates were used and at least four replicates per treatment were employed in the test. The Kruskal-Wallis procedure was used to compare differences between sample means when less than four replicates per treatment were used. The Wilcoxon Rank Sum test with Bonferroni adjustment was used when unequal number of replicates occurred. The statistical tests were performed using SAS (1979) and Toxstat (Gulley et al., 1989). A minimum probability level of 0.05 was used for all tests.

SECTION 4

RESULTS AND DISCUSSION

The results and discussion section is organized as follows. The acute and/or chronic toxicity results for all species exposed to a particular compound are presented in separate sections for each compound. The appropriate LC50/EC50s, lowest-observed-effect concentrations (LOEC), and no-observed- effect concentrations (NOEC) for each species exposed to NQ, NG, RDX, and TNT are summarized in Tables 12, 13, 14, and 15, respectively. It should be pointed out that most chronic tests have more that one test end point; however, only the specific end points for the LOEC and NOEC are given in Tables 12-15. Summaries of the data for all study end points are presented in various tables which are referred to where appropriate. The statistical analyses of all test end points for NQ, NG, RDX, and TNT are given in a separate Appendix for each compound.

4.1 Water Quality and Measured Concentrations of the Compounds

The mean and range of the routine water quality for all tests are summarized in Tables 9 and 10. The mean and range of dissolved oxygen, pH, and temperature for all tests fell within the limits specified in the study guidelines with the exception of dissolved oxygen on day 115 in the fathead minnow TNT life cycle tests (see below). It should be noted that the ranges include the highest and lowest concentrations that occurred in the static renewal tests at the beginning and end of the 24-h renewal periods.

As mentioned in Section 3.5.2, a dilutor malfunction occurred on day 117 of the fathead minnow TNT life cycle test which caused a number of fish to die at all treatments because of low dissolved oxygen concentrations. The transient (18-24 h) low dissolved oxygen concentrations were not included in the mean and range given in Table 9 because measurements were not taken from at least one replicate at each treatment as they would have been during a regularly scheduled water quality measurement period.

The nominal concentrations and the mean and range of all measured concentrations for each compound at each test treatment are summarized in Table 11. As was the case for the water quality measurements, the ranges include the highest and lowest measured concentrations that occurred in the static renewal tests at the beginning and end of the 24-h renewal periods. The measured concentrations averaged 89.3 (±8.9; n=32)%, 91.0 (±7.0, n=45)%, 96.2 (±9.8, n=31)% and 97.5 (±8.0, n=36)%, of the nominal concentrations for NQ, NG, RDX, and TNT, respectively.

Several experiments were conducted at concentrations at the solubility limits of NQ and RDX in JHU/APL diluent water. The following saturated concentrations were obtained for NQ: 1,550 mg/L at 12°C, 2,750 at 22°C, and 3,320-4,020 mg/L at 25°C. van der Schalie (1985) reported a range of 1,638-1,787 mg/L at 12°C and a range of 2,634-3,395 mg/L at 22°C for saturated solutions of NQ in well water which are similar to the values in this study at the same temperatures. A range of 2,600 to 4,400 mg/L has been reported in the literature for the aqueous solubility of NQ at 25°C (Kenyon, 1982 and Haag et al., 1990).

The following saturated concentrations of RDX were obtained in well water after stirring for 24 h at room temperature: 36.7 mg/L at 20 °C and 29.2 to 32.7 mg/L at 22 °C. The solubility limits obtained in our study are slightly lower than those reported in the literature for aqueous solutions. Sikka et al. (1980) and the U.S Army (1984) reported saturated concentration values at 20 °C of 42.3 and 50 mg/L, respectively. Banerjee et al. (1980) reported a value of 59.5 mg/L at 25 °C. Patterson et al. (1976), as cited by Etnier (1986), reported a very low solubility value of only 7.6 mg/L at 25 °C.

4.2 NQ

4.2.1 Acute Toxicity

4.2.1.1 Invertebrates

The LC50s for the acute toxicity of NQ to the hydra and cladoceran are summarized in Table 12. The toxicity data for the hydra and cladoceran are given in Tables 16 and 17, respectively. The 48-h LC50s for the hydra and cladoceran were 2,061 and 2,698 mg/L, respectively. The statistical analyses for the 48-h LC50s for the hydra and cladoceran are given in Appendix NQ, Tables NQ1 and NQ2.

The acute toxicity of NQ to several invertebrates has been studied by van der Schalie (1985). No 48-h LC50s (<50% mortality occurred) were obtained by van der Schalie for the following invertebrates exposed to saturated solutions of NQ: water flea (D. magna) at 2,838 mg/L; amphipods Gammarus minus and Hyalella azteca at 2,720 and 2,730 mg/L, respectively; midge larva (P. dissimilis) at 3,395 mg/L; and aquatic oligochaete (L. variegatus) at 2,868 mg/L. Warner (1978) found that NQ was not acutely toxic to D. magna (20 °C) at a nominal concentration of 1,175 mg/L.

4.2.1.2 Fish

No mortality occurred to the rainbow trout or fathead minnow in 96 h at the solubility limits of the compound which were 1,550 mg/L for rainbow trout at 12° and 3,320 mg/L for fathead minnow

at 25°C (Tables 19 and 22). Since LC50s could not be determined for the rainbow trout and fathead minnow, toxicity is expressed as a value greater than the highest concentration tested for 96 h (Table 12).

No 96-h LC50s were obtained by van der Schalie (1985) for the following fish: rainbow trout at 1,638 mg/L; fathead minnow at 2,714 mg/L; bluegill at 2,634 mg/L and channel catfish at 2,636 mg/L. Warner (1978) found that NQ was not acutely toxic to fathead minnow (22 °C) at a nominal concentration of 2,000 mg/L. In an American Cyanamid Company (1955) study, 3 of 10 fathead minnow (age not given) exposed to 3,650 mg/L (nominal concentration) died during the course of a 10-d flow-through study at 25 °C; no fish died in 10 d at 3,100 mg/L.

Similar results were obtained with rainbow trout and fathead minnow in the current study with those observed by van der Schalie (1985). For example, no mortality was found in 96 h for rainbow trout (37 d old; 12°C) in the current study at 1,550 mg/L; 20% mortality occurred for rainbow trout (28 d old; 12°C) at 1,638 mg/L in the van der Schalie (1985) study. No mortality was found in 96 h for fathead minnow (16-17 d old; 25 °C) at 3,320 mg/L in the current study or in the van der Schalie (185) study (63 d old; 22 °C) at 2,634 mg/L.

It can be concluded that the acute toxicity of NQ to freshwater invertebrates and fish is quite low. With the exception of the hydra and cladoceran in this study in which the 48-h LC50s were 2,061 and 2,698 mg/L, respectively, the toxicity of NQ does appear to be an acute hazard to freshwater invertebrates and fish except at concentrations approaching the solubility limit of the compound in water.

4.2.2 Chronic Toxicity

4.2.2.1 Invertebrates

The NQ toxicity data for cladoceran survival and neonate production are summarized in Table 18. The statistical analyses of the data are given in Appendix NQ, Tables NQ3-NQ6. Significant mortality (α= 0.05) to the adults occurred at 1,400 mg/L (Tables NQ3 and NQ4). Neonate production was reduced (α=0.05) at all concentration down to 440 mg/L (Tables NQ5 and NQ6); no effect occurred at 260 mg/L. The LOEC and NOEC for the cladoceran, based on reduction in neonate production, are 440 and 260 mg/L, respectively (Table 12). To our knowledge, no other data are available on the chronic toxicity of NQ to freshwater invertebrates.

4.2.2.2 Fish

NQ was not toxic to rainbow trout during the early life

stage (ELS) test at the solubility limit of the compound in water (Table 12). A 7-d exposure to NQ at concentrations up to 1,520 mg/L did not affect hatching success of the embryos (Tables 20 and NQ7). A 28-d post-hatch exposure to concentrations up to 1,520 mg/L did not affect fry survival (Tables 20 and NQ8), total length, wet weight, or dry weight (Tables 21 and NQ9-NQ11). A total of 5 deformed fish (4 with spinal curvature and 1 with poor pigmentation) was observed at the two highest test concentrations; no other deformities were observed at the lower test concentrations or in the controls.

van der Schalie (1985) conducted an ELS study with rainbow trout under static renewal test conditions rather than flow-through conditions. As in the present study, van der Schalie found that NQ concentrations up to the solubility of the compound in water (1,703 mg/L) did not affect hatching success of the embryos or 30-d post-hatch fry survival. In contrast to the present study where no effects to the fry occurred at saturation (1,520 mg/L), a statistically significant (α=0.05) reduction in standard length and wet weight occurred at 1,703 mg/L in the van der Schalie (1985) study. van der Schalie (1985) also found significant fry deformities at 1,703 mg/L only in contrast to the present study where a total of 5 deformities at the highest two concentrations were observed. No detrimental effects occurred in the van der Schalie study at concentrations at or below 857 mg/L.

The only apparent difference between the van der Schalie (1985) rainbow trout ELS study and the current study is the the higher test concentration in the van der Schalie study where the toxicological effects occurred. van der Schalie (1985) observed significant growth effects at a mean concentration of 1,703 (1,642-1,787) mg/L in contrast to no growth effects at a mean concentration of 1,520 (1,300-1,780) mg/L in the current study. Based on the van der Schalie (1985) study, the LOEC and NOEC would be 1,703 and 857 mg/L, respectively. It should be noted that in ELS studies, the NOEC is always dependent on the choice of exposure concentrations (usually a dilution factor of 50-60%) since the calculations involved are not based on a point estimate from a response curve but on "hypothesis" tests of specific test concentrations. The actual "safe" or "no effect concentration" falls somewhere between the NOEC and LOEC determined in any given study. The further the test concentrations are apart, the less exact is the answer. Based on the two studies, the NOEC would be 857 mg/L in the van der Schalie (1985) study and 1,520 mg/L in the current study.

The toxicity data for fathead minnow during the ELS test are summarized in Tables 23 and 24. The statistical analyses of the data are given in Tables NQ12-NQ17. A 4-d exposure to NQ at the solubility limit of the compound (4,040 mg/L) did not affect the hatching success of the embryos (Tables 23 and NQ12). A 28-d post-hatch exposure at 4,040 mg/L killed all fish (Table 23);

significant mortality (α=0.05) did not occurred at a concentration of 2,030 mg/L or lower (Table NQ13). A significant reduction (α=0.05) in mean total length occurred at 2,030 mg/L (Tables NQ14 and NQ15). A statistically significant (α=0.05) effect did not occur for wet weight and dry weight at 2,030 mg/L (Tables NQ16 and NQ17); although, the data indicate that wet weight and dry weight were reduced at 2,030 mg/L. The LOEC and NOEC for the fathead minnow, based on a reduction in total length are 2,030 and 1,050 mg/L, respectively (Table 12). A total of four fish with curved spines were observed to occur randomly in the test treatments during the 28-d exposure.

With regard to chronic toxicity, NQ produced toxic effects to the cladoceran at much lower concentrations than those of rainbow trout or fathead minnow. The NOEC for the cladoceran was 260 mg/L in contrast to 1,050 mg/L for fathead minnow and >1,520 mg/L for rainbow trout.

4.2.3 Toxicity of Photolyzed NQ

The Φ-NQ chronic toxicity data for cladoceran survival and neonate production are summarized in Table 25. The statistical analyses of the data are given in Appendix NQ, Tables NQ18-NQ20. Significant mortality (α=0.05) occurred to the adults at all nominal test concentrations down to 3.6 mg/L Φ-NQ (Tables NQ18 and NQ19). No difference (α=0.05) in neonate production relative to the controls occurred at 1.3 and 2.2 mg/L Φ-NQ (Table NQ20). Neonate production from 3.6 to 10.0 mg/L Φ-NQ were excluded from the statistical analysis because 100% mortality occurred to the adults at these concentrations. The LOEC and NOEC for the cladoceran, based on survival of the adults are 3.6 mg/L Φ-NQ and 2.2 mg/L Φ-NQ, respectively (Table 12).

Photolyzed NQ was approximately two orders of magnitude more toxic to the cladoceran than the parent compound under the same test conditions. van der Schalie (1985) exposed a green alga (S. capricornutum), water flea (D. magna), and fathead minnow to Φ-NQ and also found Φ-NQ to be more toxic. The 120-h EC50 (based on dry weight standing crop) of the alga exposed to the parent compound was 2,146 mg/L versus a 120-h EC50 of 32.3 mg/L Φ-NQ when the alga was exposed to the photolyzed compound. The 48-h and 96-h LC50s of the water flea and fathead minnow were >2,838 mg/L and >2,714 mg/L, respectively, when the organisms were exposed to the parent compound. The 48-h and 96-h LC50s of the water flea and fathead minnow exposed to Φ-NQ were 24.6 and 34.5 mg/L Φ-NQ, respectively. The relative toxicity ratios of Φ-NQ:NQ for the alga, water flea, and fathead minnow were 66, >115, and >78, respectively, in the van der Schalie (1985) acute studies. The relative toxicity ratio of Φ-NQ:NQ for the cladoceran based on the chronic NOECs was 118 in the current study.

A number of products are formed in aqueous solution when NQ is photolyzed in distilled water or surface waters by UV radiation or natural sunlight. Studies of the UV photolysis of NQ in unbuffered distilled water have shown that the products are primarily guanidine, urea, and nitrite, with lesser quantities of cyanoguanidine, ammonia, and nitrate (Noss and Chyrek, 1984; Burrows et al., 1988; and Burrows et al., 1989). Nitrosoguanidine has been observed as a transient intermediate, which appears to be photolyzed to guanidinium nitrite (Burrow et al., 1988 and Burrow et al., 1989).

Several of the above products have also been observed in surface waters photolyzed by sunlight although the yields are different in most cases (Spanggord et al., 1987 and Haag et al., 1990). In contrast to the studies by Burrows et al. (1988 and 1989), Spanggord et al. (1985) and Haag et al. (1990) did not find guanidine in either distilled water or natural surface waters photolyzed by UV or sunlight. Two NQ photolysis products were detected by HPLC in the current study (Table 26). Although the products were not identified by appropriate standards, the chromatograms of Burrows et al. (1984a,b) suggest that they may be nitrosoguanidine and cyanoguanidine. No attempt was made to identify other products which must be quantified by different analytical procedures.

Burrows et al. (1988) conducted a literature review of the toxicity of photolyzed NQ products to aquatic organisms. They concluded that of five photolysis products for which data were available, four are more toxic to aquatic organisms than the parent compound. They pointed out, however, that only nitrite ion is present at a concentration high enough to account for the greatly enhanced toxicity of photolyzed NQ observed in the van der Schalie (1985) study. Burrows et al. (1988) also made the point that synergistic effects may be involved in the observed toxicity. The cladoceran in the current study was approximately an order of magnitude more sensitive to photolyzed NQ than the organisms in the van der Schalie study. Similar data on the chronic toxicity of the photolyzed products to the cladoceran are not available. Clearly, one or more of the photolyzed products is quite toxic to the cladoceran.

The environmental fate of NQ in surface waters has been shown to be dominated by photolysis (Spanggord et al., 1985 and Haag et al., 1990). The half-life of NQ at 40° N latitude ranges from 0.6 d in summer to 2.3 d in winter. The half-life of NQ in the present study was ≈1.2 d at 39° N latitude in September. Although NQ is very soluble in water, it is not toxic to several aquatic organisms at its solubility limit. However, photolyzed NQ is about two orders of magnitude more toxic than the parent compound. It is clear that photolysis should be considered in an hazard evaluation of NQ discharged to the aquatic environment.

4.3 NG

4.3.1 Acute Toxicity

4.3.1.1 Algae

The 96-h EC50 (cell density) for the green alga was 1.15 mg/L (Tables 13, 27, and Appendix NG, Table NG1). The acute toxicity of NG to S. capricornutum and three other species of freshwater algae has been studied by Bentley et al. (1978). The 96-h EC50s based on a reduction in number of cells were 0.4, 3.3, >10, and >10 mg/L (nominal concentration), respectively, for the green alga (S. capricornutum), diatom (Navicula pelliculosa), and the blue-green algae Microcystis aeruginosa and Anabaena flos-aquae. The 96-h EC50s based on chlorophyll a concentrations were 1.0, 1.0, >10, and >10 mg/L (nominal), respectively, for the green alga, diatom, and two blue-green algae. As pointed out by Smith (1986), Sullivan et al. (1979) criticized the Bentley et al. (1978) work with S. capricornutum because they did not adequately correct for a lactose vehicle used in the study. Based on this, Sullivan et al. (1979) stated that they believed the cell density and chlorophyll a EC50s values for the green alga were questionable.

4.3.1.2 Invertebrates

The LC50s for the acute toxicity of NG to the hydra, cladoceran, and midge are summarized in Table 13. The acute toxicity data for the hydra, cladoceran, and midge are given in Tables 28, 29, and 31, respectively. The 48-h LC50 for the hydra, cladoceran, and midge were 17.43, 17.83, and 34.93 mg/L, respectively. The statistical analyses of the 48-h LC50s for the hydra, cladoceran, and midge are given in Tables NG4, NG5, and NG10.

NG toxicity for the invertebrates in this study ranged from 17.43 to 34.93 mg/L (48-h LC50s). With the exception of a study by Versar (1989) discussed below, similar values have been observed for other invertebrates. Bentley et al. (1978) obtained 48-h EC50s (immobilization) for four invertebrates (water flea, D. magna; amphipod, G. fasciatus, sowbug, Asellus militaris; and midge, C. tentans) which ranged from 20 to 55 mg/L (nominal). All but one of the studies conducted by Bentley et al. (1978) were performed with nominal test concentrations which raises the obvious question about the validity of the toxicological results. Bentley et al. (1978) examined the stability of NG over time (0-96 h) under a variety of water quality conditions and concluded that the nominal concentrations were essentially the same as the mean measured concentrations. Thus, we will use the Bentley et al. (1978) data for comparative purposes but state where necessary that the concentrations were nominal.

EA (1988) obtained a 48-h LC50 for the water flea of 32.8 mg/L (nominal). The only study which has reported an acute value outside the range of 17 to 55 mg/L is the study of Versar (1989). Versar reported a 48-h LC50 of 0.92 mg/L (nominal) for the water flea. The value reported by Versar for the water flea appears questionable when one considers that Bentley et al. (1978) reported two 48-h EC50s of 32 and 46 mg/L for the water flea and EA (1988) obtained a 48-h LC50 for the water flea of 32.8 mg/L.

4.3.1.3 Fish

The 96-h LC50 for rainbow trout was 1.90 mg/L in this study (Tables 32 and NG11) while the 96-h LC50 for the fathead minnow was 3.58 mg/L (Tables 35 and NG19). Bentley et al. (1978) obtained 96-h LC50s of 2.8 mg/L (nominal) for the rainbow trout; 1.7 and 2.7 mg/L for the bluegill (L. macrochirus); >1.9 and 3.2 mg/L for the channel catfish (I. punctatus); and 1.9 and 2.5 mg/L for the fathead minnow. Versar (1989) reported a 96-h LC50 for the fathead minnow of 1.44 mg/L (nominal). A 48-h LC50 of 5.5 mg/L (nominal) was reported for the fathead minnow by EA (1988).

The potential effects of water quality on the acute toxicity of NG to the bluegill were examined by Bentley et al. (1978). They found that variations in water hardness (35 to 250 mg/L as $CaCo_3$) and pH (6.0 to 8.0) had no effect on acute toxicity. As would be expected, temperature had a slight influence on toxicity. The 96-h LC50s were 3.55, 1.92, and 1.99 mg/L (nominal) at 15, 20, and 25 °C, respectively. The 96-h LC50s obtained by Bentley et al. (1978) under all water quality conditions for bluegill ranged from 1.39 to 3.55 mg/L (nominal). Bentley et al. (1978) also examined the acute effect of NG on various life stages of fathead minnow. They found 96-h LC50s of >18 mg/L (nominal) for embryos; 5.5 mg/L for tests started with 1-h old larvae; 2.1 for 7-d old larvae; 2.1 mg/L for 30-d old juveniles; and 3.4 mg/L for 60-d old fish. Bentley et al. (1978) obtained a 96-h LC50 for head minnow embryos of >18 mg/L nominal); however, they reported a 144-h LC50 for embryos of 1.2 mg/L (nominal).

In summary, NG is more toxic to fish than invertebrates. The acute toxicity (48-h LC50/EC50s) for invertebrates ranges from 17 to 55 mg/L, while the acute toxicity (96-h LC50s) for fish ranges from 1.9 mg/L for rainbow trout to 5.5 mg/L (nominal) for fathead minnow larvae. The only exception for fish is the Bentley et al. (1978) 96-h LC50 for fathead minnow embryos which exceeded 18 mg/L (nominal); although, the 144-h was 1.2 mg/L (nominal). With the exception of the 96-h LC50 for fathead minnow embryos, the acute toxicity of NG to fish is approximately an order of magnitude more toxic than for invertebrates. The acute toxicity (96-h EC50) of NG to the alga S. capricornutum in this study was 1.15 mg/L which shows that S. capricornutum is as sensitive to NG as fish. Bentley et al. (1978) also found S.

capricornutum to be equally sensitive as fish; however, their data for three other species of algae indicate that NG is much more variable with 96-h EC50s ranging from 3.3 to >10.0 mg/L (nominal).

4.3.2 Chronic Toxicity

4.3.2.1 Algae

The 96-h exposure data for the green alga S. capricornutum are given in Table 27. When the data are treated as chronic data rather than acute data (Hughes et al., 1988), the LOEC and NOEC for growth are 0.59 and 0.37 mg/L, respectively (Tables NG2 and NG3). With regard to the 96-h S. capricornutum data in the Bentley et al. (1978) study, Sullivan et al. (1979) corrected the algal data in the Bentley et al. (1978) study for a lactose vehicle (see Section 4.3.1), reanalyzed the data, and determined that the LOEC based on chlorophyll a concentration was ≈0.1 mg/L (nominal). Sullivan et al. (1979) also reanalyzed the data for the diatom N. pelliculosa and estimated the LOEC to be 0.32 mg/L (nominal) based on both cell density and chlorophyll a concentration. The LOECs estimated by Sullivan et al. (1979) for S. capricornutum and N. pelliculosa are similar to the LOEC found in the present study for S. capricornutum. No LOEC or NOEC estimates were given by Sullivan et al. (1979) for the blue-green algae because NG was not toxic at the highest concentration tested (10 mg/L nominal).

4.3.2.2 Invertebrates

The NG chronic toxicity data for cladoceran survival and neonate production are summarized in Table 30. The statistical analyses of the data are given in Tables NG6-NG9. Significant mortality (α=0.05) to the adults occurred at 16.05 mg/L (Tables NG6 and NG7). Neonate production was significantly reduced (α=0.05) at all concentrations down to 5.48 mg/L (Tables NG8 and NG9); no effect occurred at 3.23 or 1.88 mg/L. The LOEC and NOEC for the cladoceran, based on reduction in neonate production, are 5.48 and 3.23 mg/L, respectively (Table 13).

Bentley et al. (1978) conducted a two generation life cycle test with the water flea and obtained a LOEC and NOEC of 12.5 and 6.2 mg/L (nominal), respectively based on neonate production. Bentley et al. (1978) also conducted a two generation life cycle test with the midge (C. tentans) and obtained a LOEC and NOEC for larval survival of 3.1 and 1.5 mg/L (nominal).

4.3.2.3 Fish

The toxicity data for rainbow trout exposed to NG during the ELS study are summarized in Tables 33 and 34. NG did not affect percent hatch after 8 d of exposure or fry survival after 60 d of

post-hatch exposure at concentrations up to 0.29 mg/L (Tables NG12 and NG13). The total length of fry after 60 d of post-hatch exposure was not affected at concentrations up to 0.29 mg/L (Table NG14). Wet weight was significantly (α=0.05) reduced at a concentration of 0.11 mg/L; no effect occurred at 0.06 mg/L (Tables NG15 and NG16). Dry weight was significantly (α=0.05) reduced at a concentration of 0.06 mg/L; no effect occurred at 0.03 mg/L (Tables NG17 and NG18). A total of 9 fry had obvious deformities at the end of the 60-d exposure. Seven fry (1 died before the end of the 60-d exposure) had curved spinal cords and two had extremely dark pigmentation. The deformed fry were observed at the three highest test concentrations. The LOEC and NOEC based on dry weight are 0.06 and 0.03 mg/L, respectively (Table 13).

The fathead minnow ELS data are given in Tables 36 and 37. NG caused a significant (α=0.05) reduction in hatching success of the embryos after 4 d of exposure (Tables NG20 and NG21) at a concentration of 0.20 mg/L; 0.12 mg/L did not affect hatching success. Larval survival after 28 d of exposure was reduced (α=0.05) at 0.33 mg/L, while no effect occurred at 0.20 mg/L (Tables NG22 and NG23). Total length, wet weight, and dry weight were all reduced at 0.33 mg/L, but not at 0.20 mg/L (Tables NG24-NG29). A total of 10 fish with spinal deformities were observed during the study. Five of the 10 fish died before the 28-d exposure was completed. The deformities occurred at 0.33 and 0.20 mg/L; one of the 10 deformed fish was a control fish. The LOEC and NOEC based on hatching success are 0.20 and 0.12 mg/L, respectively (Table 13).

Bentley et al. (1978) conducted an ELS on the fathead minnow with essentially the same experimental design as the present study. In contrast to most of their work with nominal concentrations, NG concentrations were measured in the ELS study. They found that NG did not affect hatching success at concentrations up to 0.48 mg/L. Survival of the fry after exposure for 30 d was significantly affected (α=0.05) at concentrations down to 0.06 mg/L; no effect occurred at 0.03 mg/L. Total length was reduced at 0.23 mg/L, but not at 0.12 mg/L. The LOEC and NOEC based on larval survival are 0.06 and 0.03 mg/L, respectively.

In a similar ELS with channel catfish using nominal test concentrations, Bentley et al. (1978), found that concentrations up to 1.25 mg/L did not affect hatching success. Larval survival after a 30-d exposure was significantly (α=0.05) affected at 0.31 mg/L but not at 0.15 mg/L. Total length was reduced (α=0.05) at 0.62 mg/L but not at 0.15 mg/L. The LOEC and NOEC based on larval survival are 0.31 and 0.15 mg/L (nominal).

The LOECs and NOECs determined during the current ELS studies and the ELS studies conducted by Bentley et al. (1978)

are quite similar. The LOECs for three species of fish in both studies ranged from a low of 0.06 mg/L for the rainbow trout and fathead minnow to a high of 0.31 mg/L for the channel catfish. The NOECs for the same species ranged from 0.03 mg/L for the rainbow trout and fathead minnow to 0.15 mg/L for the channel catfish.

In summary, NG is more toxic to fish than invertebrates under chronic exposure conditions. The LOECs for invertebrates range from 5.48 mg/L for the cladoceran to 12.5 mg/L (nominal) for the water flea; the NOECs range from 3.23 mg/L for the cladoceran to 6.2 mg/l (nominal) for the water flea. The LOECs for fish range from 0.06 mg/L for rainbow trout to 0.31 mg/L (nominal) for the channel catfish; the NOECs range from 0.03 mg/L for the rainbow trout and fathead minnow to 0.15 mg/L (nominal) for the channel catfish. The chronic toxicity of NG is approximately 1.5 to 2 orders of magnitude more toxic to fish than invertebrates.

The chronic toxicity of NG to algae relative to fish and invertebrates is variable. The LOEC and NOEC for the green alga S. capricornutum in the present study were 0.59 and 0.37 mg/L, respectively. The LOECs (NOECs are not available) estimated by Sullivan et al. (1979) for the Bentley et al. (1978) study for S. capricornutum and the diatom N. pelliculosa were 0.1 and 0.32 mg/L (nominal), respectively. The LOECs for the blue-green algae M. aeruginosa and A. flos-aquae in the Bentley et al. study were >10 mg/L (nominal). Thus, the chronic toxicity for the green alga S. capricornutum and diatom N. pelliculosa varies from concentrations similar to those of fish in contrast to the blue-green algae M. aeruginosa and A. flos-aquae which are more resistant than the invertebrates.

4.4 RDX

4.4.1 Acute Toxicity

4.4.1.1 Algae

RDX was not acutely toxic to S. capricornutum when tested at the solubility limit of the compound in algal assay media (Table 38). A maximum reduction of 38% in cell density occurred after a 96-h exposure to 36.69 mg/L; thus, an EC50 could not be determined. Bentley et al. (1977) studied the acute toxicity of RDX to the green alga S. capricornutum, the diatom N. pelliculosa, and the blue-green algae M. aeruginosa and A. flos-aquae. No 96-h EC50s for cell density or chlorophyll a could be determined for the four species exposed to concentrations up 32 mg/L (nominal). As was the case in the present study, however, significant reductions in cell density and chlorophyll a concentrations occurred for various species (see Section 4.4.2). Harvey et al. (1991) found that concentrations of RDX up to 10

mg/L (highest concentration tested) do not appear to affect the growth of the dicotyledon Phaseolus vulgaris (bush bean) when grown for 7 d under hydroponic conditions; a 10-fold uptake of RDX did occur in the leaves.

4.4.1.2 Invertebrates

RDX was not acutely toxic to the hydra and midge which were tested at the solubility limit of the compound in water. RDX was not acutely toxic to the cladoceran tested at a concentration of 17.04 mg/L. We originally thought from a range finding bioassay that the solubility limit of RDX at 25 °C was ≈17 mg/L. However, based on our own findings at other temperatures and various aqueous solubility concentrations published in the literature (see Section 4.1), we do not believe 17.04 mg/L is the saturation limit of RDX at 25 °C. Thus, we must qualify our acute toxicity findings for the cladoceran and state that when RDX is tested at a concentration of 17.04 mg/L the compound is not toxic. RDX may be toxic to the cladoceran at concentrations approaching the aqueous solubility of the compound.

RDX did not cause any mortality in 48 h to the hydra, cladoceran, or midge at 32.67, 17.04, and 29.22 mg/L, respectively (see Tables 39, 40, and 42 and Peters et al., 1991). Bentley et al. (1977) examined the acute toxicity of RDX dispersed in DMSO to the water flea (D. magna), amphipod (G. fasciatus), sowbug, (A. militaris), and midge C. tentans). They reported 48-h EC50s based on immobilization to be >100 mg/L (nominal) for all four species.

4.4.1.3 Fish

In contrast to algae and invertebrates discussed above, RDX is acutely toxic to fish. A 96-h LC50 of 12.73 mg/L was obtained for 15- to 17-d old fathead minnow in the current study (Tables 47 and RDX12). Bentley et al. (1977) obtained 96-h LC50s of 5.8 and 6.6 mg/L (nominal) for adult fathead minnow. Liu et. al. (1984) reported a 96-h LC50 of 4.5 mg/L for 2- to 7-d old fathead minnow. Bentley et al. (1977) also reported 96-h LC50s for rainbow trout, channel catfish, and bluegill of 6.4, 4.1 and 13, and 6.0 and 7.6 mg/L (nominal), respectively. Bentley et al. (1977) presented data comparing nominal concentrations of RDX to measured concentrations up to 5.0 mg/L and indicated that the nominal concentrations were essentially the same as the measured concentrations. However, deviations of 25% or greater are apparent between nominal concentrations at 15 mg/L and measured concentrations of 20-21 mg/L. No comparisons between nominal and measured concentrations were made at nominal test concentrations of 100 mg/L used in some tests. Thus, one must be aware that comparisons of the Bentley et al. (1977) data with other studies at nominal concentrations at 15 mg/L or higher may be in considerable error.

Bentley et al. (1977) also examined the acute toxicity of RDX (nominal concentrations in DMSO) to various life stages of the fathead minnow. The 96-h LC50s varied as follows: >100 mg/L for embryos; 43 mg/L for 1-h post-hatch larvae; 3.8 mg/L for 7-d old larvae; 16 mg/L for 30-d old juveniles; and 11 mg/L for 60-d 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-h old post-hatch LC50 which is high relative to the other post-hatch stages, the LC50s for the 7-d, 30-d, and 60-d 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. Water hardness did not affect toxicity. The 96-h LC50s for water hardness of 35, 100, and 250 mg/L as $CaCO_3$ were 3.8, 5.3, and 3.0 mg/L (nominal), respectively. Likewise, pH had no affect on toxicity. The 96-h LC50s 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, however, the data were not analyzed statistically. Toxicity appeared to be slightly less 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.

In conclusion, RDX is not acutely toxic to a number of algae or invertebrates at the solubility limit of the compound in aqueous solution. As shown below, however, RDX is toxic to algae when evaluated using chronic test end points. RDX is acutely toxic to fish at concentrations well below the solubility limit of the material in water. The 96-h LC50s range from 3.6 mg/L for bluegill to 16 mg/L for post-hatch fathead minnow. The only exception is the 96-h LC50 of 43 mg/L (nominal) obtained by Bentley et al. (1977) for 1-h old post-hatch larval fathead minnow which is high relative to the other post-hatch stages of the same species.

4.4.2 Chronic Toxicity

4.4.2.1 Algae

The 96-h RDX exposure data for the alga S. capricornutum are given in Table 38. The LOEC and NOEC for reduction in growth are 4.81 and 0.47 mg/L, respectively, when the data are treated as chronic data rather than acute data (Appendix RDX, Tables RDX1 and RDX2). Bentley et al. (1977) studied the toxicity of RDX to green alga (S. capricornutum), the diatom (N. pelliculosa), and the blue-green algae M. aeruginosa and A. flos-aquae. Using the probit analysis (which is not appropriate for the analysis), they reported that there was no significant (α=0.05) difference in cell density or chlorophyll a for any of the species. As discussed by Etnier (1986), Sullivan et al. (1979) reanalyzed the

nominal concentration data of Bentley et al. and found that RDX significantly (α=0.05) inhibited growth and chlorophyll a concentrations to a lesser extent in all four species. The NOEC for reduction in growth for the green alga was <0.32 mg/L; 3.2 mg/L for the diatom; 3.2 mg/L for M. aeruginosa, and 10 mg/L for A. flos-aquae.

4.4.2.2 Invertebrates

The chronic toxicity data for the cladoceran and midge exposed to RDX are summarized in Table 14 and Peters et al. (1991). With regard to the cladoceran, 7-d exposures to RDX concentrations up to 16.41 mg/L did not affected survival of the organisms (Table 41). Neonate production was significantly (α=0.05) reduced at concentrations down to 6.01 mg/L (Tables RDX3 and RDX4). The LOEC and NOEC for the cladoceran were 6.01 and 3.64 mg/L, respectively.

Bentley et al. (1977) conducted a chronic study of the effect of RDX on two generations of the daphnid (D. magna) and found that RDX concentrations ranging from 1.4 to 20 mg/L (measured concentrations in DMSO) had no effect on survival. However, the number of neonates produced by first generation adults was significantly (α=0.05) reduced at 4.8, 9.5, and 20 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 4.8 mg/L, Bentley et al. (1977) 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 for a limited time.

RDX was not toxic to the midge during the life cycle test conducted at concentrations up to 20.82 mg/L (Table 14). Hatching success in the first generation was not determined because the eggs quickly decomposed and their remains were easily lost in the food and detritus that accumulated on the bottoms of the test chambers. Because 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 larval growth after 6, 8, and 10 d of exposure at each treatment is summarized in Table 43. No significant difference (α=0.05) in midge length occurred on days 6, 8, or 10 between the control group and any treatment group (Tables RDX5-RDX7). Adult emergence success is given in Table 44. Despite an apparent concentration-response relationship, where percent emergence was reduced at RDX concentrations as low as 6.78 mg/L, no difference (α=0.05) occurred in total emergence success between the controls

and the RDX treatment groups using the non-parametric Kruskal-Wallis statistical test (Table RDX8).

Midge egg production, expressed as the mean number of eggs produced per adult in each replicate, ranged from 58 to 226 (Table 45). 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. A significant (α=0.05) reduction in egg production occurred at 6.78 mg/L only (Tables RDX9 and RDX10). Since no statistically significant effect occurred at any of the other concentrations including the higher concentrations of 12.67 and 20.82 mg/L, the reduction in egg production at 6.78 mg/L can most likely be attributed to statistical chance.

Second generation midge egg hatching success was calculated for each replicate as the total number of eggs that hatched successfully divided by the total number of eggs produced because egg strands could not be associated with individual adults (Table 46). RDX concentrations up to 20.82 mg/L had no affect on the hatching success of the second generation eggs (Table RDX11).

Bentley et al. (1977) conducted a life cycle test with the midge C. tentans similar to the life cycle test conducted with the midge P. parthenogenticus in this study. With one random exception for larvae, RDX did not have a significant (α=0.05) effect on percent survival of larvae, pupae, adults, or adult emergence at concentrations up 21 mg/L (measured) during the first generation exposure. The average number of eggs produced per adult, however, was greatly reduced from the controls. No fertile eggs were produced at 1.3 and 4.0 mg/L, and no eggs were produced at all at 10 mg/L. Eggs produced by the controls in the Bentley et al. (1977) test were exposed to the same RDX concentrations for a second test. In this exposure, percent survival was significantly (α=0.05) lower in all treatments relative to the controls. Percent survival of the adults was reduced at 1.3 and 4.0 mg/L only. Percent adult emergence was reduced at 2.2 mg/L only. The authors questioned the second test results and concluded that if the concentrations in the second test were indeed toxic, the response should have been evident in the first test. Thus, the findings of the Bentley et al. (1977) midge test are questionable.

In contrast to the findings that RDX is not acutely toxic to freshwater invertebrates when tested at the solubility limit of the compound in water, RDX was toxic to the cladoceran when

evaluated in a chronic test. The LOEC and NOEC based on reduced neonate production were 6.01 and 3.64 mg/L, respectively. Although the survival, growth, emergence success, egg production, and hatching success of the midge were not significantly affected by RDX, a trend toward reduced emergence success occurred at concentrations as low as 6.78 mg/L.

4.4.2.2 Fish

The toxicity data for fathead minnow exposed to RDX during the ELS study are summarized in Tables 48 and 49. RDX did not affect the hatching success of embryos after 4 d of exposure to concentrations as high as 9.83 mg/L (Tables 48 and RDX13). A significant (α=0.05) reduction in percent survival of the fish occurred at 9.83 mg/L relative to the controls after 28 days of exposure (Tables 48 and RDX15). With regard to the morphometric data (Tables 49 and RDX16-20), a significant (α=0.05) reduction was observed for both wet and dry weight at all concentrations down to 2.36 mg/L. No reduction was observed for wet and dry weight relative to the controls at 1.35 mg/L. The LOEC and NOEC for fathead minnow based on both wet and dry weight are 2.36 and 1.35 mg/L, respectively (Table 14). A total of 3 fish with spinal curvature was randomly observed in RDX concentrations up to 9.83 mg/L.

Bentley et al (1977) conducted a 30-d ELS test with channel catfish and fathead minnow. Hatching success of the channel catfish embryos was not affected at concentrations up to 2.3 mg/L; fry survival was affected at 1.2 mg/L (measured). Because a dilutor malfunction occurred at day 10, the authors speculated that the concentrations in the treatments may have increased significantly as a result of the dilutor malfunction. Thus, the fry results appear to be suspect during the 30-d post-hatch exposure. Hatching success of the fathead minnow embryos and survival of the fish after 30 d of exposure were not affected at concentrations up to 5.8 mg/L. A significant (α=0.05) reduction in total length occurred at 5.8 mg/L after 30-d of exposure. Thus, the NOEC and LOEC for fathead minnow based on total length were 5.8 and 3.0 mg/L (measured), respectively.

A partial life cycle and a complete life cycle test at 25 °C with fathead minnow using measured RDX concentrations in DMSO were also conducted 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 (α=0.05) effect was observed for percent hatch, fry survival, and total length at 30 d at a maximum test concentration of 4.9 mg/L. At 60 d, survival of the juveniles exposed to 4.9 mg/L was significantly (α=0.05) lower than the controls. Total length of the fathead minnow exposed to concentrations up to 4.9 mg/L was not affected at 60 d.

According to the authors, no mortality was observed in any of the treatments between days 63 and 140. Likewise, the fish appeared to develop normally between days 63 and 140. The LOEC and NOEC based on survival at were 4.9 mg/L and 2.7 mg/L, respectively, during the partial life cycle test.

During the complete life cycle test, Bentley et al. (1977) found that hatching success was not affected at concentrations up to 6.3 mg/L (measured). Survival of the fish was significantly (α=0.05) affected at day 30 and 60. Total lengths of the fish were not affected at 30 or 60 d at concentrations up to 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 up to 6.3 mg/L. First generation egg production and hatching success was not affected at concentrations up to 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 up to 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 LOEC and NOEC concentrations for the fathead minnow in the ELS, partial life cycle, and complete life cycle tests are quite similar. In the 30-d ELS tests, we obtained a LOEC and NOEC for wet and dry weight of 2.6 and 1.4 mg/L, respectively. Bentley et al (1977) obtained a LOEC and NOEC for total length of 5.8 and 3.0 mg/L. Bentley et al. (1977) obtained values of 4.9 and 2.7 mg/L based on survival for the LOEC and NOEC during a 140 d partial life cycle test. A LOEC and NOEC (based on survival) of 6.3 and 3.0 mg/L were obtained during the Bentley et al. (1977) complete life cycle test. The LOECs obtained in all chronic tests with the fathead minnow ranged from 2.6 to 6.3 mg/L (measured). The NOECs obtained in all tests ranged from 1.4 to 3.0 mg/L (measured).

A comparison of invertebrates and fathead minnow chronically exposed to RDX show that similar toxicity may occur. The cladoceran LOEC and NOEC based on reduced neonate production were 6.0 and 3.6 mg/L, respectively. Although the survival, growth, emergence success, egg production, and hatching success of the midge were not significantly affected by RDX, a trend toward reduced emergence success occurred at concentrations as low as 6.8 mg/L. The LOECs for the fathead minnow ranged from 2.6 to 6.3 mg/L while the NOECs ranged from 1.4 to 3.0 mg/L.

4.3.3 Toxicity of Photolyzed RDX

The ϕ-RDX chronic toxicity data for cladoceran survival and neonate production are summarized in Table 50. Photolyzed RDX did not affect survival of the cladoceran up to a nominal concentration of 10 mg/l ϕ-RDX (Table 50). Likewise, ϕ-RDX up to a nominal concentration of 10 mg/l ϕ-RDX did not affect neonate production (Table RDX21). In contrast, cladocerans exposed to

the parent compound were affected at concentrations below 10 mg/L. The LOEC and NOEC for the cladoceran exposed to RDX were 6.01 and 3.64 mg/L, respectively. Thus, photolysis reduced the toxicity of RDX.

Indirect evidence by Liu et al. (1984) also suggests that photolysis reduces the toxicity of RDX to aquatic organisms. Liu et al. (1984) found that exposure of composition B type LAP wastewater (1.6:1 mixture of TNT and RDX) and a laboratory-prepared TNT-RDX mixture (1.6:1) to simulated sunlight (filtered UV light) reduced toxicity to several aquatic organisms. Photolyzed TNT (no RDX present) was also less toxic. Although the photolysis of RDX alone was not studied by Liu et al. (1984), the data indicate that photolysis does reduce RDX toxicity.

RDX has been reported to photolyze in aqueous solution by several investigators (for ex., Kubose and Hoffsomer, 1977; Glover and Hoffsomer, 1979; and Spanggord et al. 1980). No photoproducts have been observed in aqueous solution by HPLC using UV detection at 243-254 nm (Spanggord et al. 1980; Harvey et al., 1991). We observed no photoproducts via HPLC using UV detection at 240 nm (Table 51). Spanggord et al. (1980) have shown in laboratory studies that photolysis of RDX in aqueous solution will produce formaldehyde, nitrate, and nitrite as photoproducts using analytical procedures other than HPLC UV detection at 254 nm. Burrows et al. (1989), who reviewed the literature on RDX photoproducts, show other photoproducts can be formed by a number of different pathways depending on the reaction system.

Spanggord et al. (1980) reported that photolysis of RDX in distilled water and in natural water samples followed first order kinetics when photolyzed in sunlight or at 313 nm. The calculated half-lives for RDX at 40° N latitude in summer, fall, winter, and spring in distilled water were 1.2, 2.6, 5.0, and 1.5 d, respectivaly. The half-life of RDX in well water exposed to natural sunlight (38° 51' N latitude) in early October during this study was ≈24.5 h (Table 51).

Although the primary physical mechanism that degrades RDX in aqueous solution is photolysis (Burrow et al. (1989), the role of photolysis in the environmental fate of RDX is not clear. Spanggord et al. (1980) state that RDX may be a persistent chemical in the aquatic environment for the following reasons: 1) the authors estimated half-lives up to 13 d in the Holston River at Kingsport, TN 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. Spanggord et al. (1980) concluded that the major environmental fate of RDX in natural waters will 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.

4.5 TNT

4.5.1 Acute Toxicity

4.5.1.1 Duckweed

The 96-h EC50 (reduction in frond production) of TNT to the duckweed L. minor is 1.59 mg/L (Table 52 and Appendix TNT, Table TNT1). To our knowledge, no other acute toxicity data are available for flowering plants exposed to TNT. As discussed below in Section 4.5.2.1, chronic phytotoxicity data are available for the duckweeds L. minor and L. perpusilla, and the yellow nutsedge (Cyperus esculentus), a terrestrial plant grown under hydroponic conditions.

Several acute algal tests have been conducted with various end points; however, few EC50s are available. For example, Fitzgerald et al. (1952) exposed M. aeruginosa to TNT for 24 h and found that 8 mg/L resulted in 100% mortality of the cultures. According to Dacre and Rosenblatt (1974), Gring (1971) exposed Chlamydomonas reinhardi to TNT and found that a concentration of 3.0 mg/L was "quite toxic". Bringman and Kuehn (1980) determined that Scenedesmus quadricauda exposed to TNT for 16 h had a toxicity threshold of 1.6 mg/L when growth inhibition was used as the end point.

4.5.1.2 Invertebrates

The 48-h LC50s for the cladoceran (Tables 53 and TNT4) and midge (Tables 55 and TNT7) exposed to TNT were 4.03 and 42.90 mg/L, respectively. To our knowledge, no other study has address the cladoceran C. dubia. Several acute studies have been conducted with the water flea D. magna exposed to TNT. Liu et al. (1983 and 1984) found a range of 48-h LC50s from >4.4 to 11.9 mg/L for the water flea. A 48-h LC50 of 27.0 mg/L has been found by Liu et al. (1984) for the midge Tanytarsus dissimilis which is lower than the 48-h LC50 of 42.90 mg/L found for midge (P. parthenogenticus) in this study. In addition to the above invertebrates, Liu et al. (1983) obtained 48-h LC50s of 6.5 mg/L and 5.2 to >29.0 mg/L, respectively, for the scud H. azteca and the aquatic oligochaete L. variegatus.

4.5.1.2 Fish

A 96-h LC50 of 2.66 mg/L was obtained for the fathead minnow in this study (Tables 56 and TNT8). Several 96-h LC50s have been reported for various life stages of the fathead minnow which range from 2.0 to 4.2 mg/L (Liu et al., 1976, 1983, and 1984 and Smock et al., 1976). A 96-h EC50 of 0.46 mg/L for behavioral

changes in fathead minnow was reported by Smock et al. (1976). A range of 96-h LC50s from 2.3 to 3.4 mg/L have been reported for the bluegill (Liu et al., 1983 and 1984; Nay et al., 1974; and Pederson, 1970). Rainbow trout 96-h LC50s range from 0.8 to 2.0 mg/L while those for channel catfish range from 2.4 to 3.3 mg/L (Liu et al., 1984).

The effect of water quality on the acute toxicity of TNT has been evaluated. Liu et al. (1976) studied the effect of pH on the fathead minnow. They obtained 96-h LC50s of 1.2, 2.0, and 2.4 mg/L at pH 5, 7, and 9.4, respectively. Pederson (1970) examined the effect of water hardness and temperature on acute TNT toxicity to the bluegill. A 96-h LC50 of 2.3 mg/L at both 60 and 180 mg/L as $CaCo_3$ was obtained at 25 °C. Pederson (1970) obtained 96-h LC50s of 2.7 and 2.8 mg/L at 60 and 180 mg/L as $CaCo_3$, respectively, when tested at 10 °C.

In summary, the acute toxicity of TNT appears to be about the same for the duckweed 96-h EC50 (frond production) which was 1.59 mg/L and fish which ranges from a low 96-h LC50 of 0.8 mg/L for the rainbow trout up to a high of 4.2 mg/L for the fathead minnow. The 48-h LC50s for the invertebrates range from 4.03 for the cladoceran (C. dubia) up to 42.90 mg/L for the midge (P. parthenogenticus). pH and water hardness do not appear to affect the toxicity of TNT to fish. TNT is slightly less toxic to fish at lower temperatures than at higher temperatures.

4.5.2 Chronic Toxicity

4.5.2.1 Duckweed

The 96-h exposure data for the vascular plant L. minor exposed to TNT are given in Table 27. When the data are treated as chronic data rather than acute data (Wang, 1990; Bishop and Perry, 1981; Hughes et al., 1988), the LOEC and NOEC for reduction in frond production are 1.21 and 0.59 mg/L, respectively (Table TNT3). The chronic toxicity of TNT to the duckweed L. perpusilla has been studied by Schott and Worthley (1974).. They exposed duckweed to a series of TNT concentrations for 11 d at a pH of 6.3 and 8.5 and made observations of death and frond reduction. The LOEC and NOEC at pH 6.3 were 1.0 and 0.5 mg/L, respectively, for reduced frond production . The LOEC and NOEC at pH 8.5 were 1.0 and 0.1 mg/L for depressed colony growth. The chronic values obtained by Schott and Worthley (1974) for L. perpusilla are similar to those found for L. minor in this study. We are aware of Huebert and Shay's (1993) argument concerning relative growth rate versus yield when comparisons of duckweed toxicity are made between different laboratories; however, the LOECs and NOECs between the two studies are similar even though the exposure times were different (4 vs. 11 d).

The effect of TNT on the yellow nutsedge (C. esculentus), a terrestrial plant, was studied by Palezzo and Leggett (1986) under hydroponic growth conditions. The yellow nutsedge was exposed to TNT concentrations of 5, 10, and 20 mg/L for 42 days. Root growth was most affected, followed by leaves and rhizomes at all concentrations tested. The lowest concentration tested was 5 mg/L; thus, no LOEC or NOEC values can be determined.

The chronic effect of TNT on algae has been investigated by Smock et al. (1976). They studied the growth response of S. capricornutum and M. aeruginosa for periods of 15 to 17 d under static test conditions. No growth inhibition occurred to S. capricornutum at concentrations up to 3 mg/L during a 17-d exposure. Concentrations as low as 5 mg/L inhibited S. capricornutum by day 3 of exposure while 7 and 9 mg/L inhibited growth as long as 13 d. However, by day 17 no significant (α=0.05) difference was observed between the controls and all test concentrations. TNT was less toxic to M. aeruginosa than S. capricornutum; however, similar effects were observed for both algae. No growth inhibition occurred to M. aeruginosa at concentrations up to 15 mg/L during a 15-d exposure. Growth at 25 mg/L was initially inhibited; but, by day 7 no difference existed between the 25 mg/L exposure and the control. Growth at 50 mg/L was significantly (α=0.05) inhibited throughout the 15-d exposure. The transient inhibition of growth for both algal species was attributed to photolysis of the parent compound. That is, the solution became less toxic as the parent compound was photolyzed.

Liu et al. (1984) studied the chronic effects of 4- and 14-d TNT exposures on the green alga S. capricornutum, blue-green algae M. aeruginosa and A. flos-aquae, and the diatom N. pelliculosa. They found that 4- and 14-d exposures of S. capricornutum and M. aeruginosa to TNT caused significant (α=0.05) reductions in growth (cell density) at 4.1 mg/L (nominal); no effects occurred at 0.82 mg/L (nominal) for either species at 4 or 14 d. Significant reductions in growth occurred during 14-d exposures to A. flos-aquae and N. pelliculosa at 4.1 and 18.0 mg/L, while no effect occurred at 3.6 and 8.2 mg/L, respectively. Observations were not made for A. flos-aquae and N. pelliculosa at 4 d. Liu et al. (1984) found that noticeable photolysis of TNT occurred during the algal assays; thus, they concluded that the toxicity data are questionable. Indeed, no difference was found between days 4 and 14 in the case of S. capricornutum and M. aeruginosa.

The algal toxicity estimates for TNT are in general questionable because most of the tests performed to date have been conducted using continuous light, static, non-renewal tests where photolysis of the parent compound can occur. As discussed in Section 2.5, the primary physical mechanism that degrades TNT munitions in dilute aqueous solution is photolysis (Burrows et

al., 1989). Smock et al. (1976) and Liu et al. (1984) were aware that photolysis occurred in their studies and stated that caution should be used when interrupting their data. The phytotoxicity test with duckweed (L. minor) was performed in this study to provide data from a test system where solutions could be renewed daily during the test in order to reduce the potential for photolysis. During the duckweed bioassay, we found the following differences between the maximum concentrations at time 0 h and the concentration 24 h later before renewal of test solutions: a lost of 16.7% occurred between time 0 h and 24 h at 0.28 mg/L; 11.1% occurred at 0.59 mg/L; and <1% occurred at 1.21, 2.43, and 4.92 mg/L (see Table 11).

4.5.2.1 Invertebrates

The chronic toxicity data for the cladoceran are given in Table 54. TNT did not cause any mortality to the organism at concentrations up to 2.71 mg/L. A significant (α=0.05) reduction in neonate production occurred at 2.71 mg/L (Tables TNT5 and TNT6). The LOEC and NOEC for the cladoceran are 2.71 and 1.64 mg/L, respectively (Table 15). Bailey et al. (1985) conducted a 28-d life cycle study with the water flea D. magna. They found no effect of TNT on mortality, number of young produced, time to first brood, number of young produced, or average length after 28 d of exposure to a maximum test concentration of 1.03 mg/L.

The incipient LC50 of the water flea (D. magna) exposed to TNT has been determined by Liu et al. (1984). The incipient LC50 is defined as the concentration above which 50 percent of the test organisms cannot survive indefinitely. The incipient LC50 is determined by conducting a time independent acute toxicity test under flow-through conditions. The test is usually terminated when there is no additional mortality in any treatment group for 48 h after a 96-h exposure. The incipient LC50 was originally described as an acute test in the 1960s (Sprague, 1969); however, the period of exposure of most incipient LC50 tests with invertebrates is similar to that of the more recent C. dubia 7-d short-term chronic test. Thus, we will use the Liu et al. (1984) invertebrate data for comparative purposes with the cladoceran. Liu et al. (1984) obtained an incipient LC50 for the water flea of 0.19 mg/L after a total exposure of 8 d, while the 48-h LC50 was >4.4 mg/L. It is not clear how meaningful the incipient LC50 value is when one considers that a 28-d life cycle study of the same species conducted in the same laboratory was not affected at concentrations up to 1.03 mg/L (Bailey et al., 1985). The incipient LC50 for the aquatic oligochaete (L. variegatus) in the Liu et al. (1984) was 13.9 mg/L after a total exposure of 14 d in contrast to a 48-h LC50 >29.0 mg/L.

4.5.2.2 Fish

The data for the fathead minnow life cycle test are given in

Tables 57 to 65. The statistical analyses of the data are given in Tables TNT9-TNT37. The LOEC and NOEC are 0.014 and 0.005 mg/L, respectively, for total length of the first generation parental females (Table 15).

TNT concentrations up to 1.91 mg/L had no effect on the hatching success of the first generation embryos after four days of exposure (Tables 57 and TNT9). A statistically significant (α=0.05) effect of TNT was found for percent survival of the fish after 30 d of exposure (Tables 58, TNT10 and TNT11). Percent survival was higher in the fish exposed to 0.005, 0.032, and 0.077 mg/L than to the control fish. No difference was found for the fish exposed to 0.014 and 0.191 mg/L relative to the controls. Mean percent survival for the controls was 80.4% which is well within the limits given in the test protocol (Benoit, 1981). The statistically significant differences found for percent survival at 30 d do not appear to be important biologically, especially when the random nature of the difference between treatment concentrations is considered. TNT concentrations up to 0.191 mg/L had no effect on mean total length of the fish after 30 d of post-hatch exposure (Tables 58 and TNT12).

Juvenile survival and total length after 60 d of exposure are given in Table 59. TNT concentrations up to 0.191 mg/L had no effect on survival or total length after 60 d of exposure (Tables TNT13 and TNT14). The mean total lengths, wet weights, and dry weights of the first generation females and males not selected for use in the spawning tests at 22 weeks are given in Table 60. The females and males were treated separately because of the large difference between sexes. TNT concentrations up to 0.077 mg/L had no effect on the total length, wet weight, or dry weight of the females or males (Tables TNT15-TNT20). With regard to skeletal deformities, a total of six females had stunted growth with enlarged stomachs at 22 weeks; no malformed males were observed. The malformed females occurred randomly in the three highest test concentrations. As discussed in Section 3.5.2, the TNT concentration of 0.191 mg/L was eliminated from the study at week 16 because most fish were lost at 0.191 mg/L as a result of a dilutor malfunction.

The mean total length, wet weight, and dry weight of the first generation parental females at the end of the spawning period is given in Table 61. A significant (α=0.05) reduction in total length occurred in females at all concentrations down to 0.014 mg/L relative to the controls (Tables TNT21 and TNT22). No difference in total length was found between the females at 0.005 mg/L and the control treatment. No statistically significant (α=0.05) difference in dry weight or wet weight relative to the controls was found for the same group of females exposed to TNT concentrations up to 0.077 mg/L (Tables TNT23 and TNT 24). Similarly, TNT concentrations up to 0.077 mg/L had no effect on

the first generation parental male total length, wet weight, or dry weight (Tables 62 and TNT25-TNT27).

The number of broods, number of embryos hatched, and hatching success during the spawning phase of the study are given in Table 63. TNT concentrations up to 0.077 mg/L had no effect on the number of broods, number of embryos hatched, or hatching success (Tables TNT28-TNT30). Likewise, TNT concentrations up to 0.077 mg/L did not affect the hatching success of the embryos selected for the second generation 30-d ELS test (Tables 64 and TNT31). Fish survival during the second generation 30-d ELS test was not affected at TNT concentrations up to 0.077 mg/L (Tables 64 and TNT32). The total length, wet weight, and dry weight of the 30-d post-hatch fish exposed to TNT during the second generation test are given in Table 65. TNT concentrations up to 0.077 mg/L did not affect the mean length of the fish (Tables 65 and TNT34). Significant (α=0.05) reductions in wet weight and dry weight occurred in the second generation 30-d ELS test. A significant reduction in wet weight occurred at 0.077 mg/L; no effect was observed at the lower concentrations (Tables 65 and TNT34-TNT35). Dry weight was significantly (α=0.05) reduced at concentrations down to 0.032 mg/L; no effect occurred at concentrations below 0.014 mg/L (Tables 65 and TNT36-TNT37).

The most sensitive end point in the life cycle test was a reduction in total length of the spawning females. Suter et al. (1987) evaluated 176 life cycle tests on 93 chemicals with 18 species of freshwater fish. The end points examined were reductions in parental survival, fecundity, hatching success, larval survival, and weight of early juveniles. Based on the MATCs (maximum acceptable toxicant concentration which is the geometric mean of the LOEC and NOEC) of the studies, they found that the most sensitive end point was reduction in fecundity (42% of the studies) followed by reduction in weight of early juveniles (35% of the studies). Changes in growth of the parental fish were not evaluated by Suter et al. (1987); therefore, it is difficult to compare the most sensitive end point found in the current study with the findings of Suter et al. (1987). In contrast to the findings of Suter et al. (1987), TNT had no effect on fecundity at the highest concentration tested in this study. The second most sensitive end point in the current study was a reduction of dry weight of the second generation fish after 30 d of exposure. A reduction in weight of early juveniles was also found by Suter et al. (1987) to be the second most important end point.

Woltering (1984) evaluated the growth response of fish in chronic and early life cycle toxicity tests. He evaluated a total of 173 partial and complete life cycle tests. Fry survival was the most important end point. Fry survival was reduced in 57% of all studies at the lowest effect concentration, while fry growth was reduced in 36% and egg hatching in 19% of the tests.

Of the 173 tests evaluated, only 60% of the tests had exposure data on adult organisms. Of the tests with adults, reproduction was reduced in 30% of the tests. Adult survival and growth were reduced only 13% and 5%, respectively. Fry growth was affected in only 13% of the cases.

As stated above, a reduction in adult growth was the most sensitive end point in the current study followed by fry growth. Adult growth was found to be the most sensitive end point in only 5% of the cases evaluated by Woltering (1984) for tests which included adults. In the present study, a significant effect was found for total length only; TNT did not affect wet weight or dry weight. It is not clear why a significant (α=0.05) reduction in total length occurred in the present study when no statistically significant (α=0.05) effect occurred for dry or wet weight of the same fish. In contrast to Woltering's (1984) evaluation in which fry survival was the most important end point, first and second generation fry survival was not affected by TNT at the highest concentrations tested.

Bailey et al. (1985) conducted a complete life cycle study with the fathead minnow similar to that conducted in the present study. However, a NOEC was not obtained during the study. The lowest concentration used in the Bailey et al. (1985) was 0.04 mg/L. Briefly, they found no effect on hatching success of the first generation embryos at concentrations up to 1.21 mg/L which was the highest concentration used in the study. First generation fish survival between 30 and 178 d post-exposure was affected at concentrations down to 0.25 mg/L. Fry growth at 30 d, but not at 60 and 90 d (growth was not measured beyond 90 d), was affected at concentrations down to 0.56 mg/L. The concentrations found by Bailey et al. (1985) to have an effect on the first generation fish were considerably higher than the concentrations used in the present study.

Bailey et al. (1985) found a significant (α=0.05) reduction in survival of the spawning adults down to 0.04 mg/L (lowest concentration tested). A significant effect was found in the present study for a reduction in total length of spawning females down to 0.014 mg/L; survival was not affected at concentrations up to 0.077 mg/L. Bailey et al. (1985) also found a significant reduction in the number of broods produced and number of eggs hatched during spawning at concentrations down to 0.04 mg/L. We found no effect of TNT on these two reproductive parameters at concentrations up to 0.077 mg/L.

Second generation fathead minnow fry survival after 30 d post-hatch exposure was affected at concentrations down to 0.04 mg/L in the Bailey et al. (1985) study. Fry survival after a 30-d post-hatch exposure was not affected in the present study at concentrations up to 0.077 mg/L. Total length and wet weight were not affected after a 30-d exposure in the Bailey et al.

(1985) study at concentrations up to 0.25 mg/L (highest second generation concentration tested). Length and weight were affected after 60 d of exposure at concentrations down to 0.04 mg/L. A significant reduction in wet weight at 0.077 mg/L for 30-d post-hatch exposure fish and a significant reduction in dry weight at concentrations down to 0.032 mg/L after 30 d of post-hatch exposure were found in the present study.

Bailey et al. (1985) also conducted two 60-d ELS tests with rainbow trout, a 30-d ELS test with the channel catfish, and a range-finding 30-d ELS test with the fathead minnow. The results of the two rainbow trout ELS tests were highly variable. Based on the discussion of the data by Bailey et al. (1985), the LOEC and NOEC for rainbow trout fry were 0.49 and 0.24 mg/L, respectively, for both total length and wet weight. TNT concentrations up to 1.35 mg/L (highest concentration tested) had no effect on channel catfish embryo hatching success or fry survival after 30 d of exposure. The preliminary 30-d ELS fathead minnow test suggested that fry survival may be affected at 0.84 mg/L but not at concentrations below 0.42 mg/L. Because the data were not analyzed statistically, no firm conclusions can be drawn from the study.

In summary, TNT is generally more toxic to fish than duckweed and invertebrates. The LOEC and NOEC for the full life cycle test with the fathead minnow were 0.014 and 0.005 mg/L, respectively, for total length of parental fish. Bailey et al. (1985) obtained a LOEC and NOEC for growth of rainbow trout during a 60-d ELS test of 0.49 and 0.24 mg/L; TNT was not toxicity at concentrations up to 1.35 mg/L during a 30-d ELS test with channel catfish. The LOEC and NOEC for duckweed, using reduction in frond production as the end point, were 1.21 and 0.59 mg/L, respectively. The LOEC and NOEC for the cladoceran (C. dubia) using neonate production as the end point were 2.71 and 1.64 mg/L.

SECTION 5

CONCLUSIONS AND RECOMMENDATIONS

The primary objective of the study was to conduct the necessary toxicity tests to provide sufficient toxicity data to complete the existing data bases for deriving U.S. EPA numerical water quality criteria for freshwater organisms exposed to NQ, NG, RDX, and TNT. The toxicity tests required for each compound were completed.

A secondary objective of the study was to determine what effect photolysis may have on the toxicity of RDX and NQ. Photolyzed RDX was found to be less toxic than the parent compound to the cladoceran C. dubia during a chronic survival and reproduction study. Indirect evidence by Liu et al. (1984) also suggests that photolysis reduces the toxicity of RDX to aquatic organisms.

In contrast to RDX, photolyzed NQ (natural sunlight) was found to be about two orders of magnitude more toxic than the parent compound to the cladoceran (C. dubia) under chronic exposure conditions. NQ photolyzed by ultraviolet light in the laboratory has also been shown by van der Schalie (1985) to be ≈1.5 to 2 orders of magnitude more toxic in acute studies than the parent compound to the green alga (S. capricornutum), water flea (D. magna), and fathead minnow (P. promelas).

Several investigators have provided evidence which suggests that the environmental fate of NQ in surface waters may be dominated by photolysis. Based on a limited number of toxicological and environmental fate studies of photolyzed NQ, it appears that photolysis should be considered in ecological risk assessments of NQ discharged to the aquatic environment. Thus, it is recommended that additional studies be conducted to provide an adequate data base to fully access the toxicity of photolyzed NQ. If additional studies confirm the current findings that photolyzed NQ may be 1.5 to 2 orders of magnitude more toxic than the parent compound, it may be necessary to derive numerical water quality criteria for photolyzed NQ.

SECTION 6

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TABLE 1. SPECIES AND TYPE OF TEST USED FOR THE COMPLETION OF THE FINAL ACUTE VALUE DATA BASE

Compound	Species	Type of Test
NQ	Hydra (Hydra littoralis)	48-h LC50
NG	Hydra (Hydra littoralis)	48-h LC50
	Midge (Paratanytarsus parthenogenticus)	48-h LC50
RDX	Hydra (Hydra littoralis)	48-h LC50
	Midge (Paratanytarsus parthenogenticus)	48-h LC50[a]
TNT	Midge (Paratanytarsus parthenogenticus)	48-h LC50

[a] The midge is shown in both Tables 1 and 3 in order to summarize all the organisms needed to complete the data base for the Final Acute Value and the Final Acute-Chronic Ratio. The acute toxicity of RDX to the midge was determined only one time.

TABLE 2. SPECIES AND TYPE OF CHRONIC TOXICITY TEST USED FOR THE COMPLETION OF THE FINAL CHRONIC VALUE DATA BASE

Compound	Species	Type of Test
NQ	Cladoceran (*Ceriodaphnia dubia*)	7-d survival & reproduction
	Rainbow trout (*Oncorhynchus mykiss*)	ELS (28 d)[a]
	Fathead minnow (*Pimephales promelas*)	ELS (28 d)
NG	Cladoceran (*Ceriodaphnia dubia*)	7-d survival & reproduction
	Rainbow trout (*Oncorhynchus mykiss*)	ELS (60 d)
	Fathead minnow (*Pimephales promelas*)	ELS (28 d)
RDX	Cladoceran (*Ceriodaphnia dubia*)	7-d survival & reproduction
	Midge (*Paratanytarsus parthenogenticus*)	Egg to egg life cycle
	Fathead minnow (*Pimephales promelas*)	ELS (28 d)
TNT	Cladoceran (*Ceriodaphnia dubia*)	7-d survival & reproduction
	Fathead minnow (*Pimephales promelas*)	F_1 Life cycle (≈8 months)
		F_2 ELS (30 d)

[a] ELS duration is the mean time from post-hatch in all studies.

TABLE 3. SPECIES AND TYPE OF ACUTE TEST USED FOR THE COMPLETION OF THE FINAL CHRONIC VALUE DATA BASE

Compound	Species	Type of Test
NQ	Cladoceran (*Ceriodaphnia dubia*)	48-h LC50
	Rainbow trout (*Oncorhynchus mykiss*)	96-h LC50
	Fathead minnow (*Pimephales promelas*)	96-h LC50
NG	Cladoceran (*Ceriodaphnia dubia*)	48-h LC50
	Rainbow trout (*Oncorhynchus mykiss*)	96-h LC50
	Fathead minnow (*Pimephales promelas*)	96-h LC50
RDX	Cladoceran (*Ceriodaphnia dubia*)	48-h LC50
	Midge (*Paratanytarsus parthenogenticus*)	48-h LC50[a]
	Fathead minnow (*Pimephales promelas*)	96-h LC50
TNT	Cladoceran (*Ceriodaphnia dubia*)	48-h LC50
	Fathead minnow (*Pimephales promelas*)	96-h LC50

[a] The midge is shown in both Tables 1 and 3 in order to summarize all the organisms needed to complete the data base for the Final Acute Value and the Final Acute-Chronic Ratio. The acute toxicity of RDX to the midge was determined only one time.

TABLE 4. SPECIES AND TYPE OF TEST USED FOR THE COMPLETION OF THE FINAL PLANT VALUE DATA BASE AND PHOTOLYSIS STUDIES

Compound	Species	Type of Test
	Final Plant Value	
NG	Green alga (Selenastrum capricornutum)	96-h EC50[a]
RDX	Green alga (Selenastrum capricornutum)	96-h EC50[a]
TNT	Duckweed (Lemna minor)	96-h EC50[a]
	Photolysis	
ϕ-NQ	ϕ-Cladoceran (Ceriodaphnia dubia)	7-d survival & reproduction
ϕ-RDX	ϕ-Cladoceran (Ceriodaphnia dubia)	7-d survival & reproduction

[a] 96-h EC50 for growth.

TABLE 5. PRECISION AND ACCURACY OF THE HPLC MEASUREMENTS OF NQ, NG, RDX, AND TNT

Date	Quantity Injected	Mean (n=3)	Percent Deviation	S.D. (±)	Relative S.D. (%)
			NQ		
12/09/87	1.000 µg	1.014	+1.40	0.002	0.20
12/15/87		0.999	-0.10	0.000	0.00
12/16/87		0.993	-0.70	0.019	1.91
Grand Means		1.002	0.20	0.007	0.70
12/09/87	5.000 µg	5.024	+0.48	0.014	0.28
12/15/87		4.999	-0.02	0.002	0.04
12/16/87		4.994	-0.12	0.016	0.32
Grand Means		5.006	0.11	0.011	0.21
			NG		
06/23/88	89.41 ng	77.35	-13.49	5.64	7.29
06/24/88		81.37	-8.99	8.27	10.16
06/27/88		131.30	+46.85	7.46	5.68
Grand Means		96.67	8.12	7.21	7.71
06/23/88	4471 ng	4773	+6.75	23	0.48
06/24/88		4461	-0.22	46	1.03
06/27/88		4527	+1.25	50	1.10
Grand Means		4587	2.59	40	0.87

TABLE 5. (CONTINUED)

Date	Quantity Injected	Mean (n=3)	Percent Deviation	S.D. (±)	Relative S.D. (%)
			RDX		
11/05/87	54.90 ng	57.46	+4.66	1.37	2.38
11/06/87		54.99	+0.16	0.67	1.22
11/09/87		57.55	+4.83	1.40	2.43
Grand Means		56.70	3.22	1.15	2.01
11/05/87	1098 ng	1100	+0.18	1	0.09
11/06/87		1097	-0.09	3	0.27
11/09/87		1100	+0.18	10	0.91
Grand Means		1099	0.09	5	0.42
			TNT		
11/05/87	51.50 ng	28.33	-44.99	0.47	1.66
11/06/87		30.50	-40.78	4.20	13.77
11/11/87		51.50	0.00	0.79	1.53
Grand Means		36.86	-28.59	1.82	5.65
11/05/87	1030 ng	1019	-1.07	10	0.98
11/06/87		1017	-1.26	25	2.45
11/11/87		1030	+0.00	88	8.54
Grand Means		4587	-0.78	41	3.99

TABLE 6. COMPREHENSIVE JHU/APL WELL WATER (DILUTION WATER) ANALYSIS

Base/Neutrals

Compound	µg/L[a]	Compound	µg/L[a]
bis(2-Chloroethyl)Ether		Di-n-butylphthalate	
1,3-Dichlorobenzene		Flouranthene	
1,4-Dichlorobenzene		Pyrene	
1,2-Dichlorobenzene		Butylbenzylphthalate	
bis(2-Chloroisopropyl)Ether		3,3'-Dichlorobenzidine	
N-Nitroso-di-n-propylamine		Benzo(a)Anthracene	
Hexachloroethane		bis(2-Ethylhexyl)Phthalate	
Nitrobenzene		Chrysene	
Isophorone		Di-n-octylphthalate	
bis(2-Chloroethoxy)Methane		Benzo(b)Fluoranthene	
1,2,4-Trichlorobenzene		Benzo(k)Fluoranthene	
Naphthalene		Benzo(a)Pyrene	
Hexachlorobutadiene		Indeno(1,2,3-cd)Pyrene	
Hexachlorocyclopentadiene		Dibenzo(a,h)Anthracene	
2-Chloronaphthalene		Benzo(g,hi,i)Perylene	
Dimethylphthalate			
Acenaphthylene		The following are non-priority pollutant hazardous substance list compounds:	
Acenaphthene			
2,4-Dinitrotoluene			
2,6-Dinitrotoluene		Aniline	
Diethylphthalate		Benzyl Alcohol	
4-Chlorophenyl-phenylether		4-Chloroaniline	
Fluorene		2-Methylnaphthalene	
N-Nitrosodiphenylamine		2-Nitroaniline	
4-Bromophenyl-phenylether		3-Nitroaniline	
Hexachlorobenzene		Dibenzofuran	
Phenanthrene		4-Nitroaniline	
Anthracene			
		DETECTION LIMIT	2

TABLE 6. (CONTINUED)

Pesticides		Metals	
Compound	µg/L[a]	Metal (Total)	mg/L
alpha-BHC		Antimony	<0.005
beta-BHC		Arsenic	<0.005
delta-BHC		Beryllium	<0.005
gamma-BHC (Lindane)		Cadmium	<0.001
Heptachlor		Chromium	<0.05
Aldrin		Copper	<0.02
Heptachlor Epoxide		Mercury	<0.0002
alpha-Endosulfan		Lead	<0.005
Dieldrin		Nickel	<0.20
4,4'-DDE		Selenium	<0.005
Endrin		Silver	<0.01
beta-Endosulfan		Thallium	<0.005
4,4'-DDD		Zinc	<0.04
Endrin Aldehyde			
Endosulfan Sulfate		General Water Quality	mg/L
4,4'-DDT			
Methoxychlor		Alkalinity (as $CaCO_3$)	156
Chlordane		Ammonia (as N)	0.15
Toxaphene		Hardness (as $CaCO_3$)	190
Aroclor 1016		Nitrate	<0.10
Arcolor 1221		Nitrite	<0.10
Aroclor 1232		Total Kjeldahl Nitrogen	0.15
Aroclor 1242		Total Organic Carbon	19
Aroclor 1248			
Aroclor 1254		pH (standard units)	7.8
Aroclor 1260			
DETECTION LIMIT	0.1		

[a] Concentrations less than the detection limit are left blank.

TABLE 7. GENERAL INFORMATION ON THE ORGANISMS USED IN THE TOXICITY TESTS

Comp	Species	Test	Initial Age at Start of Test	Mean Size at End of Test[a] Total Length (mm)	Mean Size at End of Test[a] Dry Weight (mg)	Test Temp (°C)
NQ	Hydra	Acute	Adult polyp	NA	NA	22 (±1)
	Cladoceran	Acute	Neonate (<12 h)	NA	NA	25 (±1)
		Chronic	Neonate (<6 h)	NA	NA	25 (±1)
	Rainbow trout	Acute	Fingerling (37 d)	24.7 (±0.8) (n=10)	120 (±19)[b] (n=10)	12 (±1)
		ELS	Eyed embryo (14-15 d)	29.3 (±1.9) (n=59)	31 (±8) (n=59)	12 (±1)
	Fathead minnow	Acute	Juvenile (16-17 d)	8.4 (±2.7) (n=10)	3.9 (±1.2) (n=10)	25 (±1)
		ELS	Embryo (<12 h)	17.8 (±3.2) (n=26)	8.7 (±5.7) (n=26)	25 (±1)
ϕ-NQ	Cladoceran	Chronic	Neonate (<6 h)	NA	NA	25 (±1)
NG	Green alga	Acute	Log growth	NA	NA	20 (±1)
	Hydra	Acute	Adult polyp	NA	NA	22 (±1)
	Cladoceran	Acute	Neonate (<6 h)	NA	NA	25 (±1)
		Chronic	Neonate (<6 h)	NA	NA	25 (±1)
	Midge	Acute	Larva (6 d)	NA	NA	22 (±1)
	Rainbow trout	Acute	Fingerling (48-49 d)	29.5 (±1.4) (n=10)	314 (±50)[b] (n=10)	12 (±1)
		ELS	Eyed embryo (12-14 d)	47.2 (±2.7) (n=38)	161 (±31) (n=38)	12 (±1)
	Fathead minnow	Acute	Juvenile (20 d)	10.7 (±1.9) (n=10)	3.8 (±2.5) (n=10)	25 (±1)
		ELS	Embryo (<12 h)	19.2 (±3.7) (n=64)	14.8 (±7.2) (n=64)	25 (±1)
RDX	Green alga	Acute	Log growth	NA	NA	20 (±1)
	Hydra	Acute	Adult polyp	NA	NA	22 (±1)

TABLE 7. CONTINUED

Comp	Species	Test	Initial Age at Start of Test	Mean Size at End of Test[a] Total Length (mm)	Mean Size at End of Test[a] Dry Weight (mg)	Test Temp (°C)
RDX	Cladoceran	Acute	Neonate (<12 h)	NA	NA	25 (±1)
		Chronic	Neonate (<6 h)	NA	NA	25 (±1)
	Midge	Acute	Larva (6 d)	NA	NA	22 (±1)
		Life cycle	Egg (<12 h)	NA	NA	22 (±1)
	Fathead minnow	Acute	Juvenile (15-17 d)	8.3 (±0.9) (n=10)	2.2 (±1.3) (n=10)	25 (±1)
		ELS	Embryo (<12 h)	24.1 (±2.2) (n=23)	32.2 (±8.9) (n=23)	25 (±1)
ε-RDX	Cladoceran	Chronic	Neonate (<6 h)	NA	NA	25 (±1)
TNT	Duckweed	Acute	Two frond colony	NA	NA	25 (±1)
	Cladoceran	Acute	Neonate (<12 h)	NA	NA	25 (±1)
		Chronic	Neonate (<12 h)	NA	NA	25 (±1)
	Midge	Acute	Larva (7 d)	NA	NA	22 (±1)
	Fathead minnow	Acute	Juvenile (23 d)	28.8 (±1.2) (n=10)	32.0 (±0.9) (n=10)	25 (±1)
		Life cycle[c]				
		F_1	Embryo (<12 h)	53.3 (±3.2)♀ (n=12)	334 (±75)♀ (n=12)	25 (±2)
				71.0 (±6.0)♂ (n=3)	905 (±398)♂ (n=3)	
		F_2	Embryo (<12 h)	20.6 (±1.9) (n=93)	24.5 (±1.6) (n=93)	

[a] Mean size of control organisms.
[b] Mean wet weight (± S.D.); dry weight was not determined.
[c] F_1 is embryo to spawning adult; F_2 is embryo to 30 d juvenile (ELS test).

TABLE 8. TEST CHAMBERS, TEST SOLUTION VOLUMES, VOLUME ADDITIONS, AND LOADING OF TEST CHAMBERS

Comp	Species	Test	Test Chamber	Test Solution Vol (L)	Volume Additions per day[a]	Loading (g/L)[b]
NQ	Hydra	Acute	0.6-L Glass beaker	0.2	1	NA
	Cladoceran	Acute	0.05-L Glass beaker	0.03	1	NA
		Chronic	0.05-L Glass beaker	0.03	1	NA
	Rainbow trout	Acute	10-L Glass aquarium	6.4	5.2	0.189
		ELS-embryo	0.2-L Glass cylinder	≈0.14	6.4	NA
		ELS -fry	10-L Glass aquarium	6.4	6.4	0.940
	Fathead minnow	Acute	10-L Glass aquarium	6.4	5.6	0.030
		ELS-embryo	0.2-L Glass cylinder	≈0.14	5.1	NA
		ELS-juv	10-L Glass aquarium	6.4	5.1	0.099
₱-NQ	Cladoceran	Chronic	0.05-L Glass beaker	0.03	1	NA
NG	Green alga	Acute	0.25-L Delong flask	0.1	Static	NA
	Hydra	Acute	0.6-L Glass beaker	0.2	1	NA
	Cladoceran	Acute	0.05-L Glass beaker	0.03	1	NA
		Chronic	0.05-L Glass beaker	0.03	1	NA
	Midge	Acute	0.6-L Glass beaker	0.2	1	NA
	Rainbow trout	Acute	10-L Glass aquarium	6.4	6.0	0.490
		ELS-embryo	0.2-L Glass cylinder	≈0.14	4.5	NA
		ELS-fry	10-L Glass aquarium	6.4	4.5	2.532
	Fathead minnow	Acute	10-L Glass aquarium	6.4	4.8	0.030
		ELS-embryo	0.2-L Glass cylinder	≈0.14	5.3	NA
		ELS-juv	10-L Glass aquarium	6.4	5.3	0.365
RDX	Green Alga	Acute	0.25-L Delong flask	0.1	Static	NA
	Hydra	Acute	0.6-L Glass beaker	0.2	1	NA
	Cladoceran	Acute	0.04-L Glass beaker	0.01	1	NA
		Chronic	0.05-L Glass beaker	0.03	1	NA
	Midge	Acute	1.325-L Glass dish	0.6	17.0	NA
		Life cycle	1.325-L Glass dish	0.6	17.6	NA

TABLES 8. CONTINUED

Comp	Species	Test	Test Chamber	Test Solution Vol (L)	Volume Additions per day[a]	Loading (g/L)[b]
RDX	Fathead minnow	Acute	2-L Glass aquarium	1.3	18.2	0.085
		ELS-embryo	0.2-L Glass cylinder	≈0.14	4.9	NA
		ELS-juv	10-L Glass aquarium	6.4	4.9	0.279
ε-RDX	Cladoceran	Chronic	0.05-L Glass beaker	0.03	1	NA
TNT	Duckweed	Acute	0.25-L Glass beaker	0.15	1	NA
	Cladoceran	Acute	0.05-L Glass beaker	0.03	1	NA
		Chronic	0.05-L Glass beaker	0.03	1	NA
	Midge	Acute	0.6-L Glass beaker	0.2	1	NA
	Fathead minnow	Acute	2-L Glass aquarium	1.3	16.3	0.123
		Life cycle[c]				
		F_1 Embryo	0.2-L Glass cylinder	≈0.14	5.1	NA
		Juv[d]	19-L Glass aquarium	15	5.1	NA
		Adult[e]	38-L Glass aquarium	30	2.5	0.488
		Adult[f]	38-L Glass aquarium	15	2.5	0.623
		F_2 Embryo	2-L Glass cylinder	≈0.14	2.5	NA
		Juv	38-L Glass aquarium	15	2.5	0.239

[a] Mean volume addition per day for the complete exposure period.
[b] Mean loading (g wet weight/L) at the end of the exposure period for control organisms.
[c] F_1 is embryo to spawning adult; F_2 is embryo to 30 d juvenile (ELS test).
[d] Juveniles up to 60 post-hatch held in 19 L aquaria; no weight measurements were taken.
[e] Juveniles 60 days post-hatch to sexually mature adults at 22 weeks; the mean wet weight of the fish at 22 weeks was used to estimate the weight of the fish removed for the spawning study.
[f] Spawning adults until the end of the F_1 study were placed in 38-L aquaria divided one half for spawning and one half for F_2 embryo/larval ELS study.

TABLE 9. MEAN (RANGE) WATER QUALITY OF TOXICITY TESTS - DISSOLVED OXYGEN, pH, AND TEMPERATURE

Comp	Species	Test	Dissolved Oxygen (mg/L)	pH (Std. Units)	Temperature (°C)
NQ	Hydra	Acute	8.0 (6.7-8.7)	7.6 (7.4-7.9)	22.2 (22.0-22.5)
	Cladoceran	Acute	7.9 (7.5-9.1)	7.9 (7.7-8.2)	24.6 (24.5-24.9)
		Chronic	7.9 (7.4-8.1)	7.9 (7.4-8.1)	24.4 (24.0-25.0)
	Rainbow trout	Acute	8.8 (8.4-9.7)	7.6 (7.4-7.7)	12.1 (10.4-13.8)
		ELS	9.8 (8.9-10.4)	7.8 (7.5-8.1)	12.4 (11.0-13.9)
	Fathead minnow	Acute	7.5 (7.4-7.6)	8.3 (7.8-8.5)	25.5 (25.0-26.0)
		ELS	8.2 (7.3-8.8)	8.1 (7.8-8.3)	25.3 (24.9-26.0)
ϕ-NQ	Cladoceran	Chronic	7.0 (6.5-7.6)	7.5 (6.7-8.0)	25.2 (25.1-25.4)
NG	Green alga	Acute	*	*	20.1 (19.7-20.4)
	Hydra	Acute	8.1 (8.0-8.3)	7.4 (7.3-7.5)	22.2 (22.1-22.3)
	Cladoceran	Acute	8.1 (7.4-8.5)	8.5 (7.9-8.7)	24.8 (24.6-25.0)
		Chronic	7.9 (7.6-8.3)	8.1 (7.6-8.4)	24.7 (24.2-25.1)
	Midge	Acute	8.1 (7.9-8.6)	7.9 (7.6-8.4)	22.1 (21.5-22.6)
	Rainbow trout	Acute	9.7 (9.0-10.2)	8.3 (7.8-8.9)	11.5 (10.7-11.9)
		ELS	7.6 (6.0-8.7)	7.9 (7.5-8.5)	12.2 (11.6-13.0)
	Fathead minnow	Acute	7.4 (7.0-7.9)	7.4 (7.0-7.9)	25.3 (24.7-25.7)
		ELS	7.5 (7.1-7.8)	7.5 (6.8-8.1)	24.9 (24.1-25.5)
RDX	Green alga	Acute	*	*	20.0 (19.9-20.1)
	Hydra	Acute	8.1 (7.9-8.4)	7.5 (7.2-7.9)	22.0 (21.5-22.5)
	Cladoceran	Acute	8.3 (8.1-8.8)	8.3 (8.0-8.4)	25.0 (24.5-25.5)
		Chronic	7.3 (6.6-7.9)	8.1 (7.6-8.4)	25.0 (24.4-25.4)
	Midge	Acute	7.6 (7.1-8.2)	7.9 (7.4-8.1)	22.3 (21.4-22.9)
		Life cycle	7.7 (6.9-8.4)	7.8 (7.4-8.2)	22.0 (20.9-24.0)
	Fathead minnow	Acute	7.6 (7.0-8.1)	7.9 (7.4-8.1)	25.3 (24.6-25.9)
		ELS	7.6 (7.0-8.1)	7.6 (7.1-8.1)	25.1 (24.7-25.3)
ϕ-RDX	Cladoceran	Chronic	6.1 (5.0-7.9)	7.8 (7.5-8.1)	25.1 (24.9-25.3)

TABLE 9. CONTINUED

Comp	Species	Test	Dissolved Oxygen (mg/L)	pH (Std. Units)	Temperature (°C)
TNT	Duckweed	Acute	7.9 (7.6-8.3)	7.7 (7.3-8.1)	24.9 (24.5-25.5)
	Cladoceran	Acute	7.6 (7.4-8.0)	8.1 (8.0-8.2)	24.9 (24.6-25.2)
		Chronic	8.1 (7.9-8.5)	8.1 (7.8-8.5)	25.0 (24.5-26.0)
	Midge	Acute	7.3 (6.2-8.7)	7.4 (7.2-7.7)	22.2 (21.8-22.7)
	Fathead minnow	Acute	8.0 (7.6-8.3)	7.9 (7.7-8.2)	24.9 (24.2-25.7)
		Life cycle			
		F_1	7.5 (6.6-8.2)[b]	8.0 (6.9-8.6)	24.4 (23.5-25.9)
		F_2	7.7 (7.0-8.3)	8.1 (7.8-8.4)	24.8 (24.4-25.3)

[a] Parameter not measured.

[b] Reported mean and range do not include the low dissolved oxygen event that occurred on day 115 because of a dilutor malfunction; see Sections 3.5.2 and 4.1 for further explanation.

TABLE 10. MEAN (RANGE) WATER QUALITY OF TOXICITY TESTS - CONDUCTIVITY, ALKALINITY, AND TOTAL HARDNESS

Comp	Species	Test	Conductivity (μmhos/cm)	Alkalinity (mg $CaCO_3$/L)	Hardness (mg/L as $CaCO_3$)
NQ	Hydra	Acute	273 (225-295)	149 (135-160)	169 (132-192)
	Cladoceran	Acute	214 (195-250)	109 (105-110)	149 (146-152)
		Chronic	335 (320-354)	126 (118-132)	161 (150-172)
	Rainbow trout	Acute	297 (255-320)	115 (90-133)	167 (160-174)
		ELS	242 (230-262)	122 (100-170)	184 (164-198)
	Fathead minnow	Acute	329 (320-350)	129 (115-142)	193 (180-216)
		ELS	325 (260-370)	104 (90-137)	183 (164-212)
♦-NQ	Cladoceran	Chronic	347 (300-400)	63 (45-80)	167 (128-198)
NG	Green alga	Acute	•	•	•
	Hydra	Acute	103 (100-110)	80 (70-88)	103 (100-110)
	Cladoceran	Acute	409 (370-425)	159 (120-204)	164 (145-186)
		Chronic	392 (360-450)	116 (90-135)	165 (140-200)
	Midge	Acute	338 (320-360)	132 (128-140)	161 (158-164)
	Rainbow trout	Acute	225 (210-239)	109 (110-135)	181 (176-189)
		ELS	334 (300-370)	98 (72-140)	165 (132-192)
	Fathead minnow	Acute	345 (300-390)	100 (80-130)	199 (120-240)
		ELS	342 (300-390)	110 (75-140)	225 (190-250)
RDX	Green alga	Acute	•	•	•
	Hydra	Acute	294 (285-310)	150 (136-162)	161 (148-170)
	Cladoceran	Acute	307 (260-340)	114 (110-120)	168 (156-176)
		Chronic	322 (280-390)	92 (70-130)	154 (144-200)
	Midge	Acute	241 (210-275)	106 (105-110)	165 (156-170)
		Life cycle	281 (244-317)	134 (110-145)	186 (164-216)
	Fathead minnow	Acute	290 (270-310)	121 (115-128)	165 (156-176)
		ELS	324 (290-370)	100 (65-125)	221 (192-240)
♦-RDX	Cladoceran	Chronic	376 (320-420)	68 (35-110)	196 (152-230)

TABLE 10. CONTINUED

Comp	Species	Test	Conductivity (μmhos/cm)	Alkalinity (mg $CaCO_3$/L)	Hardness (mg/L as $CaCO_3$)
TNT	Duckweed	Acute	221 (200-230)	32 (25-43)	35 (14-46)
	Cladoceran	Acute	278 (260-310)	128 (120-130)	167 (146-188)
		Chronic	545 (370-685)	104 (84-130)	226 (186-278)
	Midge	Acute	296 (287-310)	134 (100-162)	158 (128-186)
	Fathead minnow	Acute	311 (302-321)	138 (121-145)	164 (140-178)
		Life cycle			
		F_1	357 (310-400)	126 (80-190)	185 (148-240)
		F_2	325 (260-370)	104 (90-137)	183 (164-212)

* Parameter not measured.

TABLE 11. MEAN AND RANGE OF ALL HPLC MEASUREMENTS (MG/L) AT EACH TEST CONCENTRATION OF THE TOXICITY TESTS

Comp	Species	Test	Nominal Conc	Measured Conc Mean (Range)
NQ	Hydra	Acute	Control	0
			1,100	990 (930-1,050)
			1,800	1,650 (1,560-1,760)
			3,000	2,750 (2,590-2,950)
	Cladoceran	Acute	Control	0
			570	500 (460-540)
			950	920 (830-950)
			1,580	1,440 (1,330-1,500)
			2,640	2,540 (2,240-2,790)
			4,400	4,020 (3,900-4,100)
		Chronic	Control	0
			260	260 (240-280)
			430	440 (410-490)
			720	730 (680-760)
			1,200	1,180 (990-1,420)
			2,000	1,400 (1,080-1,760)
	Rainbow trout	Acute	Control	0
			230	200 (140-230)
			390	370 (280-440)
			650	570 (520-600)
			1,080	950 (8201,030)
			1,800	1,550 (1,430-1,630)
		ELS	Control	0
			200	200 (130-260)
			400	320 (210-410)
			600	540 (370-690)
			1,000	880 (600-1,110)
			1,700	1,520 (1,300-1,780)
	Fathead minnow	Acute	Control	0
			510	470 (420-550)
			950	830 (720-1,000)
			1,580	1,360 (1,220-1,550)
			2,650	2,010 (1,770-3,050)
			4,400	3,320 (3,050-3,640)

TABLE 11. CONTINUED

Comp	Species	Test	Nominal Conc	Measured Conc Mean (Range)
NQ	Fathead minnow	ELS	Control	0
			480	380 (270-470)
			810	610 (460-820)
			1,330	1,050 (900-1,410)
			2,200	2,030 (1,490-2,370)
			3,700	4,040 (3,900-4,220)
ϕ-NQ	Cladoceran	Chronic	Control	
			1.3	•
			2.2	•
			3.6	•
			6.0	•
			10.0	•
NG	Green alga	Acute	Control	0.0
			0.22	0.18 (0.14-0.21)
			0.44	0.37 (0.30-0.40)
			0.72	0.59 (0.51-0.67)
			1.20	1.14 (1.11-1.20)
			2.00	1.89 (1.81-2.00)
	Hydra	Acute	Control	0.0
			Ethanol	0.0
			5.8	5.06 (4.41-5.70)
			9.7	7.66 (6.37-9.28)
			16.0	15.17 (15.00-15.35)
			27.0	23.82 (23.19-24.67)
			45.0	40.18 (37.59-42.82)
	Cladoceran	Acute	Control	0.0
			5.8	5.48 (5.20-5.70)
			9.7	9.45 (9.30-9.60)
			16.0	15.53 (15.20-15.80)
			27.0	26.98 (26.80-27.10)
			45.0	44.80 (44.20-45.00)
		Chronic	Control	0.0
			2.1	1.88 (1.80-2.00)
			3.5	3.23 (3.10-3.40)
			5.8	5.48 (5.19-5.70)
			9.7	9.65 (9.49-9.81)
			16.2	16.05 (15.01-16.09)

TABLE 11. CONTINUED

Comp	Species	Test	Nominal Conc	Measured Conc Mean (Range)
NG	Midge	Acute	Control	0.0
			Ethanol	0.0
			7.8	5.36 (4.64-6.36)
			13.0	10.69 (9.61-11.21)
			21.6	18.07 (16.09-18.86)
			36.0	31.21 (29.98-31.89)
			60.0	52.78 (52.22-54.31)
	Rainbow trout	Acute	Control	0.0
			Ethanol	0.0
			1.00	0.91 (0.84-0.97)
			1.67	1.46 (1.21-1.60)
			2.78	2.47 (2.10-2.72)
			4.63	3.89 (3.33-4.44)
			7.72	6.25 (5.97-6.76)
		ELS	Control	0.0
			0.03	0.03 (0.03-0.04)
			0.07	0.06 (0.05-0.06)
			0.11	0.11 (0.10-0.13)
			0.18	0.19 (0.17-0.21)
			0.30	0.29 (0.27-0.33)
	Fathead minnow	Acute	Control	0.0
			1.04	0.94 (0.90-0.98)
			1.76	1.63 (1.37-1.82)
			3.68	3.53 (3.31-3.81)
			4.80	4.51 (4.27-4.71)
			8.00	7.71 (7.42-8.13)
		ELS	Control	0.0
			0.13	0.12 (0.11-0.13)
			0.22	0.20 (0.20-0.21)
			0.36	0.33 (0.30-0.37)
			0.60	0.57 (0.56-0.60)
			1.00	0.94 (0.91-0.96)
RDX	Green alga	Acute	Control	0.0
			0.52	0.47 (0.37-0.57)
			5.18	4.81 (4.76-4.90)
			8.64	7.95 (7.86-8.07)
			14.40	13.02 (12.86-13.21)
			24.00	21.80 (20.97-22.61)
			40.00	36.69 (35.43-38.49)

TABLE 11. CONTINUED

Comp	Species	Test	Nominal Conc	Measured Conc Mean (Range)
RDX	Hydra	Acute	Control	0.0
			21.0	19.03 (17.40-21.22)
			35.0	32.67 (31.49-35.69)
	Cladoceran	Acute	Control	0.0
			12.0	10.44 (9.43-11.19)
			20.0	17.04 (14.24-18.51)
		Chronic	Control	0.0
			2.6	2.15 (1.77-2.55)
			4.3	3.64 (3.09-4.21)
			7.2	6.01 (5.26-6.96)
			12.0	9.74 (8.79-11.14)
			20.0	16.41 (15.30 -17.80)
	Midge	Acute	Control	0.0
			18.0	17.51 (16.69-18.12)
			30.0	29.22 (27.12-30.92)
		Life cycle	Control	0.0
			3.11	2.42 (2.01-2.96)
			5.18	3.91 (3.28-4.51)
			8.64	6.78 (5.77-7.74)
			14.40	12.67 (11.49-13.68)
			24.00	20.82 (16.75-23.44)
	Fathead minnow	Acute	Control	0.0
			2.59	2.81 (2.51-3.53)
			4.32	4.84 (4.39-5.20)
			7.20	7.66 (6.85-8.26)
			12.00	12.73 (11.46-14.11)
			20.00	18.50 (18.41-18.60)
		ELS	Control	0.0
			1.3	1.35 (1.20-1.49)
			2.2	2.36 (2.19-2.49)
			3.6	3.42 (3.23-3.68)
			6.0	6.42 (6.23-6.80)
			10.0	9.83 (9.48-10.18)

TABLE 11. CONTINUED

Comp	Species	Test	Nominal Conc	Measured Conc Mean (Range)
Φ-RDX	Cladoceran	Chronic	Control	
			1.3	•
			2.2	•
			3.6	•
			6.0	•
			10.0	•
TNT	Duckweed	Acute	Control	0.0
			0.30	0.28 (0.25-0.30)
			0.60	0.59 (0.56-0.63)
			1.25	1.21 (1.18-1.23)
			2.50	2.43 (2.39-2.47)
			5.00	4.92 (4.85-4.97)
	Cladoceran	Acute	Control	0.0
			3.11	3.20 (2.82-3.42)
			5.18	5.08 (4.90-5.39)
			8.64	7.99 (7.51-8.28)
			14.40	13.40 (12.96-14.18)
			24.00	21.28 (21.11-21.52)
		Chronic	Control	0.0
			0.39	0.48 (0.00-0.81)
			0.65	0.66 (0.17-0.95)
			1.08	1.04 (0.42-1.63)
			1.80	1.64 (1.00-2.31)
			3.00	2.71 (1.76-3.53)
	Midge	Acute	Control	0.0
			7.78	6.82 (5.00-8.20)
			12.96	11.47 (8.8413.09)
			21.60	19.90 (17.20-21.61)
			36.00	32.36 (27.57-34.36)
			60.00	53.48 (48.04-58.23)
	Fathead minnow	Acute	Control	0.0
			0.97	1.17 (0.98-1.35)
			1.62	1.65 (1.41-1.82)
			2.70	2.83 (2.65-3.16)
			4.50	4.42 (4.38-4.45)
			7.50	7.19 (7.08-7.29)

TABLE 11. CONTINUED

Comp	Species	Test	Nominal Conc	Measured Conc Mean (Range)
TNT	Fathead minnow	Life cycle F_1	Control	0.0
			0.005	0.005 (0.003-0.010)
			0.013	0.014 (0.009-0.020)
			0.032	0.032 (0.024-0.040)
			0.080	0.077 (0.067-0.092)
			0.200	0.191 (0.179-0.211)
		F_2	Control	0.0
			0.005	0.005 (0.003-0.006)
			0.013	0.014 (0.009-0.017)
			0.032	0.032 (0.027-0.037)
			0.080	0.077 (0.068-0.089)
			0.200	0.190 (.0179-0.207)

[a] Only nominal concentrations were used in the photolysis studies (see Section 3.2.2 for further details).

TABLE 12. ACUTE AND/OR CHRONIC TOXICITY OF NQ AND PHOTOLYZED NQ (Φ-NQ) TO THE TEST ORGANISMS[a]

Comp	Species	Test	LC50/EC50	Lowest Observed Effect Conc	No Observed Effect Conc
NQ	Hydra	Acute	2,061[b]	NA	NA
	Cladoceran	Acute	2,698[b]	NA	NA
		Chronic	NA	440[c]	260[c]
	Rainbow trout	Acute	>1,550[d]	NA	NA
		ELS	NA	>1,520[e]	>1,520[e]
	Fathead minnow	Acute	>3,320[d]	NA	NA
		ELS	NA	2,030[f]	1,050[f]
Φ-NQ	Cladoceran	Chronic	NA	3.6 Φ-NQ[g]	2.2 Φ-NQ[g]

[a] All toxicity values are based on measured concentrations (mg/L) with the exception of the photolyzed NQ (Φ-NQ) nominal concentrations.
[b] 48-h LC50.
[c] Test endpoint- neonate production.
[d] No mortality occurred in 96 h at the concentration shown which was the solubility limit of the compound in JHU/APL diluent water at the temperature shown in Table 7.
[e] No statistically significant effect occurred at any of the endpoints measured during the test.
[f] Test endpoint- total length.
[g] Test endpoint- adult survival.

TABLE 13. ACUTE AND/OR CHRONIC TOXICITY OF NG TO THE TEST ORGANISMS[a]

Comp	Species	Test	LC50/EC50	Lowest Observed Effect Conc	No Observed Effect Conc
NG	Green alga	Acute	1.15[b]	NA	NA
		Chronic		0.59[c]	0.37[c]
	Hydra	Acute	17.43[d]	NA	NA
	Cladoceran	Acute	17.83[d]	NA	NA
		Chronic	NA	5.48[e]	3.23[e]
	Midge	Acute	34.93[d]	NA	NA
	Rainbow trout	Acute	1.90[f]	NA	NA
		ELS	NA	0.06[g]	0.03[g]
	Fathead minnow	Acute	3.58[f]	NA	NA
		ELS	NA	0.20[h]	0.12[h]

[a] All toxicity values are based on measured concentrations (mg/L).
[b] 96-h EC50- cell density.
[c] Test endpoint- cell density.
[d] 48-h LC50.
[e] Test endpoint- neonate production.
[f] 96-h LC50.
[g] Test endpoint- dry weight.
[h] Test endpoint- percent hatch.

TABLE 14. ACUTE AND/OR CHRONIC TOXICITY OF RDX AND PHOTOLYZED RDX (ϕ-RDX) TO THE TEST ORGANISMS[a]

Comp	Species	Test	LC50/EC50	Lowest Observed Effect Conc	No Observed Effect Conc
RDX	Green alga	Acute	>36.69[b]	NA	NA
		Chronic		4.81[c]	0.47[c]
	Hydra	Acute	>32.67[d]	NA	NA
	Cladoceran	Acute	>17.04[e]	NA	NA
		Chronic	NA	6.01[f]	3.64[f]
	Midge	Acute	>29.22[d]	NA	NA
		Life cycle	NA	>20.82	>20.82[g]
	Fathead minnow	Acute	12.73[h]	NA	NA
		ELS	NA	2.36[i]	1.35[i]
ϕ-RDX	Cladoceran	Chronic	NA	>10.0 ϕ-RDX	>10.0 ϕ-RDX

[a] All toxicity values are based on measured concentrations (mg/L) with the exception of the photolyzed RDX (ϕ-RDX) nominal concentrations.
[b] 96-h EC50 for reduction in cell density not be calculated because a maximum reduction of 38% occurred at the solubility limit of the compound.
[c] Test endpoint- cell density.
[d] No mortality occurred in 48 h at the concentration shown which was the solubility limit of the compound in JHU/APL diluent water at the temperature shown in Table 7.
[e] No mortality occurred at the highest concentration tested.
[f] Test endpoint- neonate production.
[g] No statistically significant effects occurred at the highest concentration tested.
[h] 96-h LC50.
[i] Test endpoint- wet weight and dry weight.

TABLE 15. ACUTE AND/OR CHRONIC TOXICITY OF TNT TO THE TEST ORGANISMS[a]

Comp	Species	Test	LC50/EC50	Lowest Observed Effect Conc	No Observed Effect Conc
TNT	Duckweed	Acute	1.59[b]	NA	NA
		Chronic	NA	1.21[c]	0.59[c]
	Cladoceran	Acute	4.03[d]	NA	NA
		Chronic	NA	2.71[e]	1.64[e]
	Midge	Acute	42.90[d]	NA	NA
	Fathead minnow	Acute	2.66[f]	NA	NA
		Life cycle	NA	0.014[g]	0.005[g]

[a] All toxicity values are based on measured concentrations (mg/L).
[b] 96-h EC50- frond production.
[c] Test endpoint- frond production.
[d] 48-h LC50.
[e] Test endpoint- neonate production.
[f] 96-h LC50.
[g] Test endpoint- total length of parental females.

TABLE 16. HYDRA NQ ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a,b]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	[c]	0
	2	[c]	0
990	1	[c]	0
	2	[c]	0
1,650	1	[c]	2
	2	[c]	1
2,750	1	[c]	6
	2	[c]	10

[a] Only three test concentrations were used in the study.
[b] See Appendix NQ, Table NQ1 for statistical analysis.
[c] Observations were not made at 24 h in order to avoid disturbing the organisms during the test.

TABLE 17. CLADOCERAN NQ ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	0	0
	2	0	0
500	1	0	0
	2	0	0
920	1	0	1
	2	0	0
1,440	1	0	0
	2	0	0
2,540	1	0	3
	2	0	7
4,020	1	0	10
	2	0	6

[a] See Appendix NQ, Table NQ2 for statistical analysis.

TABLE 18. CLADOCERAN NQ CHRONIC TOXICITY DATA - SURVIVAL OF ADULTS, NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD AFTER 7 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
Control	1	4	0	10	14	7.0
	2	3	6	10	19	6.3
	3	4	8	12	24	8.0
	4	4	8	9	21	7.0
	5	3	9	8	20	6.7
	6	2	6	12	20	6.7
	7	4	7	10	21	7.0
	8	3	8	13	24	8.0
	9	2	6	11	19	6.3
	10	4	6	9	19	6.3
260	1	4	4	10	18	6.0
	2	3	6	12	21	7.0
	3	4	9	9	22	7.3
	4	3	10	8	21	7.0
	5	4	0	13	17	8.5
	6	0	3	14	17	8.5
	7	4	6	11	21	7.0
	8	DEAD				
	9	4	2	14	20	6.7
	10	4	4	16	24	8.0
440	1	0	2	6	8	4.0
	2	0	2	7	9	4.5
	3	0	0	4	4	4.0
	4	0	3	4	7	3.5
	5	0	1	6	7	3.5
	6	0	2	6	8	4.0
	7	0	4	5	9	4.5
	8	0	4	0	4	4.0
	9	0	3	6	9	4.5
	10	0	1	4	5	2.5

TABLE 18. CONTINUED

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
730	1	0	0	2	2	2.0
	2	0	0	4	4	4.0
	3	0	0	3	3	6.0
	4	0	0	5	5	5.0
	5	0	0	2	2	2.0
	6	0	0	3	3	3.0
	7	0	0	3	3	3.0
	8	0	0	1	1	1.0
	9	0	0	3	3	3.0
	10	0	0	1	1	1.0
1,180	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
	7	0	0	0	0	0
	8	0	0	0	0	0
	9	0	0	0	0	0
	10	0	0	0	0	0
1,400	1	DEAD				
	2	DEAD				
	3	DEAD				
	4	DEAD				
	5	DEAD				
	6	DEAD				
	7	DEAD				
	8	DEAD				
	9	DEAD				
	10	DEAD				

* See Appendix NQ, Tables NQ3 to NQ6 for statistical analyses.

TABLE 19. RAINBOW TROUT NQ ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality			
		24h	48h	72h	96h
Control	1	0	0	0	0
	2	0	0	0	0
200	1	0	0	0	0
	2	0	0	0	0
370	1	0	0	0	0
	2	0	0	0	0
570	1	0	0	0	0
	2	0	0	0	0
950	1	0	0	0	0
	2	0	0	0	0
1,550	1	0	0	0	0
	2	0	0	0	0

[a] No mortality occurred at the solubility limit of the compound in JHU/APL diluent water (1,550 mg/L); thus, no LC50 could be obtained.

TABLE 20. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY DATA - PERCENT HATCH OF EYED EMBRYOS AFTER 7 DAYS OF EXPOSURE AND SURVIVAL OF FRY AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched and Fry Exposed	Hatching Success (%)	No. of Fry that Survived	Fry Survival (%)
Control	1	30	0	30	100.0	30	100.0
	2	30	0	29	96.7	29	100.0
200	1	30	0	30	100.0	29	96.7
	2	40	0	27	90.0	25	92.6
320	1	30	0	26	86.7	24	92.3
	2	30	0	28	93.3	25	89.3
540	1	30	0	27	90.0	27	100.0
	2	30	0	30	100.0	30	100.0
880	1	30	0	29	96.7	27	93.1
	2	30	0	29	96.7	28	96.6
1,520	1	30	0	29	96.7	29	100.0
	2	30	0	26	86.7	26	100.0

[a] See Appendix NQ, Tables NQ7 and NQ8 for statistical analyses.

TABLE 21. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF FRY AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	30	29.3 (27.0-32.0)	206.3 (139.8-284.2)	30.8 (19.3-42.4)
	2	29	29.4 (27.0-33.5)	202.3 (155.6-316.8)	30.5 (21.3-48.3)
200	1	29	29.2 (27.0-32.0)	189.6 (153.0-256.9)	30.0 (21.9-40.5)
	2	25	29.0 (26.0-31.5)	196.7 (126.3-246.9)	30.0 (18.2-38.0)
320	1	24	27.6 (25.0-31.5)	169.8 (98.4-224.3)	26.4 (15.2-36.6)
	2	25	30.1 (28.0-33.5)	224.0 (187.9-282.0)	34.7 (27.3-44.4)
540	1	27	28.8 (26.0-32.0)	191.2 (137.4-320.3)	30.0 (19.6-51.7)
	2	30	28.5 (25.0-30.5)	193.2 (163.5-215.8)	28.6 (9.8-35.7)
880	1	27	28.8 (25.5-31.5)	196.2 (114.6-266.6)	29.1 (16.1-39.7)
	2	28	29.0 (27.5-31.0)	217.5 (172.8-257.2)	32.7 (27.0-38.1)
1,520	1	29	27.4 (25.5-29.0)	185.2 (117.5-224.0)	28.7 (18.6-32.5)
	2	26	27.0 (23.0-29.5)	176.5 (110.4-222.8)	27.1 (15.9-33.9)

[a] See Appendix NQ, Tables NQ9 to NQ11 for statistical analyses.

TABLE 22. FATHEAD MINNOW NQ ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality			
		24h	48h	72h	96h
Control	1	0	0	0	0
	2	0	0	0	0
470	1	0	0	0	0
	2	0	0	0	0
830	1	0	0	0	0
	2	0	0	0	0
1,360	1	0	0	0	0
	2	0	0	0	0
2,010	1	0	0	0	0
	2	0	0	0	0
3,320	1	0	0	0	0
	2	0	0	0	0

[a] No mortality occurred at the solubility limit of the compound in JHU/APL diluent water (3,320 mg/L); thus, no LC50 could be obtained.

TABLE 23. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY DATA - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE AND SURVIVAL OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched	Hatching Success (%)	No. of Larvae that Survived	Larval Survival (%)
Control	1	30	13	17	100.0	15	88.2
	2	30	13	17	100.0	11	64.7
380	1	30	0	29	96.7	27	93.1
	2	30	7	23	100.0	20	87.0
610	1	30	6	22	91.3	22	100.0
	2	30	10	20	100.0	17	85.0
1,050	1	30	11	19	100.0	15	78.9
	2	30	11	19	100.0	18	94.7
2,030	1	30	10	18	90.0	15	83.3
	2	30	11	19	100.0	14	73.7
4,040	1	30	3	27	100.0	0	N/A
	2	30	11	19	100.0	0	N/A

[a] See Appendix NQ, Tables NQ12 and NQ13 for statistical analyses.

TABLE 24. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	15	16.9 (12.0-22.0)	37.7 (10.0-87.5)	6.7 (2.3-14.5)
	2	11	19.0 (12.0-22.0	58.6 (11.8-88.9)	11.5 (2.5-18.5)
380	1	27	16.5 (12.5-21.0)	37.4 (12.9-82.6)	6.9 (1.6-17.3)
	2	20	17.7 (12.5-22.0)	45.4 (11.8-84.1)	8.9 (2.5-18.1)
610	1	22	15.8 (11.0-21.0)	30.7 (6.8-85.6)	5.5 (1.3-17.4)
	2	17	17.6 (12.0-22.0)	47.5 (36.8-90.7)	9.9 (1.9-19.9)
1,050	1	15	16.5 (12.5-22.0)	36.1 (11.6-90.4)	6.9 (3.2-20.4)
	2	18	16.0 (11.0-24.0)	36.5 (8.8-118.8)	7.2 (2.5-26.1)
2,030	1	15	13.4 (10.0-18.0)	17.8 (3.9-51.6)	2.7 (0.2-9.9)
	2	14	12.4 (9.0-16.0)	13.9 (3.5-30.6)	1.9 (0.1-5.8)
4,040[b]					

[a] See Appendix NQ, Tables NQ14 to NQ17 for statistical analyses.
[b] 100% mortality occurred in this treatment group; therefore, the data could not used for further analysis.

TABLE 25. CLADOCERAN PHOTOLYZED NQ (Φ-NQ) CHRONIC TOXICITY DATA - SURVIVAL OF ADULTS, NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD AFTER 7 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
Control	1	6	12	13	29	9.7
	2	6	13	17	36	12.0
	3	5	14	17	36	12.0
	4	6	16	15	37	12.3
	5	6	11	15	32	10.7
	6	5	12	17	34	11.3
	7	5	13	13	31	10.3
	8	5	10	10	25	8.3
	9	5	12	16	33	11.0
	10	6	15	15	36	12.0
1.3 Φ-NQ	1	4	12	14	30	10.0
	2	3	9	17	29	9.7
	3	5	14	15	34	11.3
	4	4	12	18	34	11.3
	5	7	15	10	32	10.7
	6	6	14	13	33	11.0
	7	6	12	16	34	11.3
	8	4	10	18	32	10.7
	9	4	14	14	32	10.7
	10	6	13	15	35	11.7
2.2 Φ-NQ	1	5	12	14	31	10.3
	2	4	11	16	31	10.3
	3	4	10	15	29	9.7
	4	4	13	14	31	10.3
	5	5	11	12	28	9.3
	6	4	11	16	31	10.3
	7	5	DEAD		5	5.0
	8	4	12	15	31	10.3
	9	6	12	15	33	11.0
	10	6	10	16	32	10.7

TABLE 25. CONTINUED

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
3.6 ♦-NQ	1	0	3	DEAD	3	3.0
	2	5	DEAD		5	5.0
	3	4	DEAD		4	4.0
	4	4	DEAD		4	4.0
	5	0	DEAD			
	6	0	DEAD			
	7	3	DEAD		3	3.0
	8	0	0	DEAD		
	9	DEAD				
	10	0	0	DEAD		
6.0 ♦-NQ	1	DEAD				
	2	DEAD				
	3	DEAD				
	4	DEAD				
	5	DEAD				
	6	DEAD				
	7	DEAD				
	8	DEAD				
	9	DEAD				
	10	DEAD				
10.0 ♦-NQ	1	DEAD				
	2	DEAD				
	3	DEAD				
	4	DEAD				
	5	DEAD				
	6	DEAD				
	7	DEAD				
	8	DEAD				
	9	DEAD				
	10	DEAD				

♦ See Appendix NQ, Tables NQ18 to NQ20 for statistical analyses.

TABLE 26. HPLC MEASUREMENTS (RELATIVE PEAK AREA) OF PHOTOLYZED NQ (Φ-NQ) AND TWO UNKNOWN COMPOUNDS AS A FUNCTION OF TIME

Hours Exposed to Sunlight	Unknown No. 1 2.074)[a]	Unknown No. 2 (3.031)[a]	Nitroguanidine (4.091)[a]
0	-	-	2505285
4	205433	27558	2259767
7	157832	90190	1848900
22.5	243013	75158	1676036
24	265560	25053	1555782
28	353245	112738	1245127
31	400845	132780	954514
47	516088	55116	726533
51	703985	65137	465983
57	591247	152822	230486
69	453457	50106	185391
73	305644	-	65137
77	222970	-	7766
80	370782	32569	5762

[a] Average peak retention time (minutes).

TABLE 27. GREEN ALGA NG TOXICITY DATA - MEAN CELL DENSITY (CELLS/ML) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Control	1	4772	39752	345080	796250	1190000
	2	4596	38440	364140	800000	1180340
	3	5200	40048	359680	757280	1201340
0.18	1	4960	38792	355620	770459	1167360
	2	5192	39160	359480	747520	1159420
	3	5016	39960	364870	759080	1151420
0.37	1	4712	37040	353400	742600	1163400
	2	5104	36032	355060	760980	1151600
	3	5032	35984	338680	746700	1146600
0.59	1	4232	29192	262740	611280	841260
	2	5068	27240	256140	598980	812400
	3	4784	29320	258900	663100	802580
1.14	1	5172	27152	237380	498480	659920
	2	4540	28192	227360	502000	615620
	3	4960	26208	241160	471500	576800
1.89	1	4856	21240	148140	295380	372000
	2	4704	18664	145140	255180	337840
	3	5076	20352	172280	262080	343080

[a] See Appendix NG, Tables NG1 to NG3 for statistical analyses.

TABLE 28. HYDRA NG ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	[b]	0
	2	[b]	0
Ethanol Control	1	[b]	0
	2	[b]	0
5.06	1	[b]	1
	2	[b]	0
7.66	1	[b]	1
	2	[b]	1
15.17	1	[b]	3
	2	[b]	5
23.82	1	[b]	4
	2	[b]	7
40.18	1	[b]	10
	2	[b]	10

[a] See Appendix NG, Table NG4 for statistical analysis.
[b] Observations were not made at 24 h in order to avoid disturbing the organisms during the test.

TABLE 29. CLADOCERAN NG ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	0	0
	2	0	0
5.48	1	0	0
	2	0	0
9.45	1	0	0
	2	0	0
15.53	1	0	0
	2	0	0
26.98	1	2	10
	2	0	10
44.80	1	3	10
	2	5	10

[a] See Appendix NG, Table NG5 for statistical analysis.

TABLE 30. CLADOCERAN NG CHRONIC TOXICITY DATA - SURVIVAL OF ADULTS, NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD AFTER 7 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
Control	1	5	11	14	30	10.0
	2	DEAD				
	3	5	7	17	29	9.7
	4	4	9	12	25	8.3
	5	4	9	16	29	9.7
	6	5	11	16	32	10.7
	7	4	10	17	31	10.3
	8	0	10	11	21	10.5
	9	7	9	15	31	10.3
	10	4	9	18	31	10.3
1.88	1	3	9	12	24	8.0
	2	4	10	15	29	9.7
	3	DEAD				
	4	4	8	13	25	8.3
	5	3	9	15	27	9.0
	6	2	10	14	26	8.7
	7	0	4	9	13	6.5
	8	4	10	13	27	9.0
	9	5	8	14	27	9.0
	10	DEAD				
3.23	1	3	9	11	23	7.7
	2	4	9	12	25	8.3
	3	4	8	11	23	7.7
	4	4	11	15	30	10.0
	5	3	7	12	22	7.3
	6	4	8	11	23	7.7
	7	4	9	15	28	9.3
	8	3	10	11	24	8.0
	9	0	0	DEAD		
	10	3	8	15	26	8.7

TABLE 30. CONTINUED

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
5.48	1	3	7	13	23	7.7
	2	0	0	0	0	0
	3	3	8	11	22	7.3
	4	4	8	13	25	8.3
	5	0	0	0	0	0
	6	2	8	3	13	4.3
	7	0	0	0	0	0
	8	3	9	10	22	7.3
	9	3	9	10	22	7.3
	10	4	7	11	22	7.3
9.65	1	0	7	10	17	8.5
	2	3	8	7	18	6.0
	3	4	7	10	21	7.0
	4	0	0	0	0	0
	5	3	8	9	20	6.7
	6	3	7	7	17	5.7
	7	0	0	0	0	0
	8	3	6	7	16	5.3
	9	2	7	11	20	6.7
	10	4	7	9	20	6.7
16.05	1	0	DEAD			
	2	0	DEAD			
	3	0	DEAD			
	4	0	DEAD			
	5	0	DEAD			
	6	0	0	DEAD		
	7	DEAD				
	8	0	0	DEAD		
	9	0	0	DEAD		
	10	DEAD				

* See Appendix NG, Tables NG6 to NG9 for statistical analyses.

TABLE 31. MIDGE NG ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	[b]	0
	2	[b]	0
Ethanol Control	1	[b]	0
	2	[b]	0
5.36	1	[b]	0
	2	[b]	0
10.69	1	[b]	0
	2	[b]	1
18.07	1	[b]	0
	2	[b]	0
31.21	1	[b]	3
	2	[b]	4
52.78	1	[b]	9
	2	[b]	9

[a] See Appendix NG, Table NG10 for statistical analysis.
[b] Observations were not made at 24 h in order to avoid disturbing the organisms during the test.

TABLE 32. RAINBOW TROUT NG ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality			
		24h	48h	72h	96h
Control	1	0	0	0	0
	2	0	0	0	0
Ethanol	1	0	0	0	0
	2	0	0	0	0
0.91	1	0	0	0	0
	2	0	0	0	0
1.46	1	0	0	0	1
	2	0	0	0	1
2.47	1	0	0	0	9
	2	0	0	3	9
3.89	1	5	7	10	10
	2	6	9	10	10
6.25	1	10	10	10	10
	2	10	10	10	10

[a] See Appendix NG, Table NG11 for statistical analysis.

TABLE 33. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY DATA - PERCENT HATCH OF EYED EMBRYOS AFTER 8-DAY EXPOSURE AND SURVIVAL OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched and Fry Exposed	Hatching Success (%)	No. of Fry that Survived	Fry Survival (%)
Control	1	35	8	24	83.9	22	91.7
	2	35	12	23	91.3	16	76.2
0.03	1	40	4	32	88.9	21	65.6
	2	40	8	27	84.4	22	81.5
0.06	1	35	13	18	81.8	17	94.4
	2	35	10	21	84.0	20	95.2
0.11	1	35	4	27	87.1	25	92.6
	2	35	3	29	90.6	26	89.7
0.19	1	40	4	30	83.3	23	76.7
	2	35	5	26	86.7	18	69.2
0.29	1	35	9	21	80.8	18	85.7
	2	35	5	26	86.7	15	57.7

[a] See Appendix NG, Tables NG12 and NG13 for statistical analyses.

TABLE 34. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	22	47 (42-52)	830.7 (585.7-1,097)	156.1 (102.2-222.5)
	2	16	48 (42-54)	883.0 (662.3-1,154)	166.9 (110.0-221.5)
0.03	1	21	45 (35-54)	851.0 (324.2-1,397)	156.4 (51.9-261.6)
	2	22	45 (36-50)	787.4 (343.7-1,098)	145.3 (59.1-200.9)
0.06	1	17	40 (26-45)	793.5 (137.2-1,248)	139.3 (18.0-230.7)
	2	20	40 (29-45)	747.6 (189.0-1,056)	125.5 (23.5-183.0)
0.11	1	25	42 (37-47)	711.8 (445.0-1,048)	127.8 (76.0-208.0)
	2	26	39 (31-47)	641.1 (222.5-1,097)	116.6 (32.7-182.4)
0.19	1	23	41 (30-45)	611.0 (299.0-833.6)	106.4 (44.3-158.1)
	2	18	42 (35-49)	709.2 (318.4-1,072)	121.6 (48.2-179.8)
0.29	1	18	39 (36-47)	579.0 (332.7-1,486)	81.8 (64.6-151.8)
	2	15	40 (32-47)	615.8 (284.1-1,204)	93.6 (36.6-211.0)

[a] See Appendix NG, Tables NG14 to NG18 for statistical analyses.

TABLE 35. FATHEAD MINNOW NG ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality			
		24h	48h	72h	96h
Control	1	0	0	0	0
	2	0	0	0	0
0.94	1	0	0	0	0
	2	0	0	0	1
1.63	1	0	0	1	2
	2	0	0	0	1
3.53	1	0	0	1	3
	2	0	0	1	3
4.51	1	0	1	2	5
	2	0	1	2	7
7.71	1	0	1	8	10
	2	1	5	7	10

[a] See Appendix NG, Table NG19 for statistical analysis.

TABLE 36. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY DATA - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE AND SURVIVAL OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched	Hatching Success (%)	No. of Larvae that Survived	Larval Survival (%)
Control	1	40	8	29	90.6	28	96.7
	2	40	2	36	94.7	36	100.0
0.12	1	40	0	37	92.5	36	97.3
	2	40	5	32	91.4	30	93.8
0.20	1	40	2	29	76.3	27	93.1
	2	40	7	23	69.7	16	69.6
0.33	1	40	5	20	57.1	5	25.0
	2	40	0	22	55.0	4	18.2
0.57	1	40	3	14	37.8	0	N/A
	2	40	4	16	44.4	0	N/A
0.94	1	40	2	11	28.9	0	N/A
	2	40	0	9	22.5	0	N/A

[a] See Appendix NG, Tables NG20 to NG23 for statistical analyses.

TABLE 37. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	28	19 (14-29)	72.3 (45.6-134.7)	15.0 (4.7-36.0)
	2	36	20 (14-24)	73.7 (20.8-134.8)	14.6 (4.2-28.3)
0.12	1	36	18 (14-25)	73.6 (22.3-163.7)	14.4 (4.4-30.9)
	2	30	20 (15-25)	82.8 (32.2-183.5)	16.4 (5.4-34.0)
0.20	1	27	17 (9-25)	56.4 (7.2-144.1)	11.0 (0.8-30.2)
	2	16	18 (12-25)	75.2 (17.3-184.8)	14.4 (2.7-38.4)
0.33	1	5	13 (11-16)	25.0 (13.3-42.7)	3.9 (2.1-6.3)
	2	4	12 (8-16)	19.6 (7.0-35.9)	2.7 (1.0-4.2)
0.57[b]					
0.94[b]					

[a] See Appendix NG, Tables NG26 to NG29 for statistical analyses.
[b] 100% mortality occurred in this treatment group; therefore, the data could not used for further analysis.

TABLE 38. GREEN ALGA RDX TOXICITY DATA - MEAN CELL DENSITY (CELLS/ML) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Control	1[b]	10699	16256	26597	83213	269507
	2	9093	13395	50195	220883	1044928
	3	9568	14571	63989	261008	1186997
0.47	1	8477	11656	91688	220040	989500
	2	9523	13419	48744	215171	1003753
	3	8848	12037	47064	237661	1033027
4.81	1	10600	13269	56651	259448	901000
	2	10317	15328	46224	176947	802913
	3	10203	16043	46181	160723	787680
7.95	1	10227	22896	46475	215797	928147
	2	10531	17216	59595	270280	1017213
	3	10219	13389	49837	232611	915880
13.02	1	10707	16339	56029	229739	895780
	2	11493	12736	53909	252917	847627
	3	9643	13104	49304	232821	840587
21.80	1	12144	12024	55877	189949	670067
	2	11163	15091	48888	181339	683847
	3	10651	17109	46363	204784	769940
36.69	1	13624	14709	52251	214413	767220
	2	10693	15664	49152	187171	638587
	3	9520	14197	48056	183629	662040

[a] An acute 96-h EC50 for reduction in growth could not be calculated because a maximum reduction of 38% occurred in cell density at the solubility limit of the compound (36.69 mg/L) in algal assay media. The statistical analyses of mean cell density when the data are treated as a chronic 96-h exposure are given in Appendix RDX, Tables RDX1 and RDX2.

[b] Replicate 1 was treated as an outlier (decision based on a regression analysis and comparison of residuals) and was not included in the growth calculations and subsequent statistical analyses.

TABLE 39. HYDRA RDX ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a,b]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	[c]	0
	2	[c]	0
19.03	1	[c]	0
	2	[c]	0
32.67	1	[c]	0
	2	[c]	0

[a] Only two concentrations were run because the range finding test showed that RDX was not toxic at the solubility limit of the compound in the diluent water.

[b] No mortality occurred at the solubility limit of the compound (32.67 mg/L) in JHU/APL diluent water; thus, no LC50 could be obtained.

[c] Observations were not made at 24 h in order to avoid disturbing the organisms during the test.

TABLE 40. CLADOCERAN RDX ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a,b]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	0	0
	2	0	0
10.44	1	0	0
	2	0	0
17.04	1	0	0
	2	0	0

[a] Only two concentrations were run because the range finding test showed that RDX was not toxic at a concentration originally believed to be at the solubility limit of the compound in JHU/APL diluent water (see Section 4.4.1.2).

[b] No mortality occurred at the highest concentration tested; thus, no LC50 could be obtained.

TABLE 41. CLADOCERAN RDX CHRONIC TOXICITY DATA - SURVIVAL OF ADULTS, NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD AFTER 7 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
Control	1	4	10	17	31	10.3
	2	3	12	21	36	12.0
	3	5	11	19	35	11.6
	4	5	11	18	34	11.3
	5	5	10	18	33	11.0
	6	4	12	21	37	12.3
	7	6	14	24	44	14.7
	8	7	10	24	41	13.7
	9	4	11	25	40	13.3
	10	6	10	21	37	12.3
2.15	1	4	11	20	35	11.7
	2	6	11	18	35	11.7
	3	4	12	19	35	11.7
	4	2	12	34	48	16.0
	5	5	12	37	54	18.0
	6	4	14	16	34	11.3
	7	3	10	20	33	11.0
	8	4	11	18	38	12.7
	9	5	10	18	33	11.0
	10	4	10	18	32	10.7
3.64	1	4	10	21	35	11.7
	2	3	11	18	32	10.7
	3	3	11	21	35	11.7
	4	3	9	20	32	10.7
	5	3	9	25	37	12.3
	6	4	11	17	32	10.7
	7	4	11	21	36	12.0
	8	6	12	20	38	12.7
	9	5	12	23	40	13.3
	10	4	12	19	35	11.7

TABLE 41. CONTINUED

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
6.01	1	4	11	15	20	10.0
	2	4	14	16	34	11.3
	3	3	11	11	25	8.3
	4	4	12	15	31	10.3
	5	3	12	10	25	8.3
	6	4	3	DEAD	7	3.5
	7	6	11	19	36	12.0
	8	4	13	15	32	10.7
	9	4	9	16	29	9.7
	10	3	10	13	26	8.7
9.74	1	4	11	19	34	11.3
	2	5	12	14	31	10.3
	3	3	12	17	32	10.7
	4	4	11	10	25	8.3
	5	3	15	12	30	10.0
	6	3	11	17	31	10.3
	7	4	11	16	31	10.3
	8	3	11	14	28	9.3
	9	6	10	17	33	11.0
	10	3	13	15	31	10.3
16.41	1	1	7	7	15	5.0
	2	4	9	10	23	7.7
	3	3	6	9	18	6.0
	4	3	10	6	19	6.3
	5	3	6	11	20	6.7
	6	4	6	9	19	6.3
	7	4	8	10	22	7.3
	8	3	9	8	20	6.7
	9	4	4	9	17	5.7
	10	4	6	5	15	5.0

[a] No statistical analysis was conducted for the effect of RDX on survival since only one adult died during the experiment. The statistical analyses for the effect of RDX on neonate production are given in Appendix RDX, Tables RDX3 and RDX4.

TABLE 42. MIDGE RDX ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a,b]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	[c]	0
	2	[c]	0
17.51	1	[c]	0
	2	[c]	0
29.22	1	[c]	0
	2	[c]	0

[a] Only two concentrations were run because the range finding test showed that RDX was not toxic at the solubility limit of the compound in the diluent water.

[b] No mortality occurred at the solubility limit of the compound (29.22 mg/L) in JHU/APL diluent water; thus, no LC50 could be obtained.

[c] Observations were not made at 24 h in order to avoid disturbing the organisms during the test.

TABLE 43. MIDGE RDX LIFE CYCLE TOXICITY DATA - GROWTH OF LARVAE AFTER 6, 8, AND 10 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mean Length (Range) (mm) Day 6	Day 8	Day 10
Control	1	1.41 (1.19-1.55)	2.56 (1.55-3.00)	4.65 (4.00-5.00)
	2	1.67 (1.43-1.90)	2.94 (2.50-3.25)	4.13 (3.75-4.50)
2.42	1	1.65 (0.95-1.79)	3.05 (1.67-3.25)	5.42 (5.25-5.50)
	2	0.83 (0.71-0.95)	1.03 (0.83-1.19)	2.57 (2.00-3.50)
3.91	1	1.65 (1.43-1.79)	2.96 (2.75-3.25)	5.18 (4.75-6.00)
	2	1.77 (1.19-2.02)	3.13 (2.00-3.50)	5.33 (3.75-6.00)
6.78	1	1.55 (1.07-1.79)	2.97 (1.19-4.50)	4.92 (4.25-5.50)
	2	1.55 (1.31-1.67)	3.00 (2.75-3.25)	3.63 (3.50-3.75)
12.67	1	1.55 (1.31-1.79)	2.82 (2.50-3.25)	4.56 (3.75-5.25)
	2	1.86 (1.07-3.75)	3.16 (1.75-5.50)	4.35 (2.75-5.00)
20.82	1	1.31 (1.07-1.55)	2.15 (1.75-2.75)	3.31 (2.00-4.00)
	2	1.71 (1.19-2.38)	2.31 (2.00-2.75)	3.39 (2.25-4.25)

[a] See Appendix RDX, Tables RDX5 to RDX7 for statistical analyses.

TABLE 44. MIDGE RDX LIFE CYCLE TOXICITY DATA - ADULT EMERGENCE SUCCESS AFTER 13 TO 17 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	No. of Adults that Emerged					Total No. Emerged	No. of Larvae Observed	Percent Emerged
		Day 13	Day 14	Day 15	Day 16	Day 17			
Control	1	0	4	1	0	0	5	5	100
	2	0	1	0	4	1	6	6	100
2.42	1	6	1	0	2	2	11	11	100
	2	0	0	0	1	4	5	5	100
3.91	1	1	5	0	1	0	7	7	100
	2	6	1	0	0	0	7	10	70
6.78	1	3	4	1	0	0	8	10	80
	2	0	0	2	0	1	3	6	60
12.67	1	1	2	1	0	0	4	8	50
	2	0	0	0	0	1	1	8	12.5
20.82	1	0	0	1	2	1	4	8	50
	2	0	1	0	0	0	1	6	16.7

[a] See Appendix RDX, Table RDX8 for statistical analysis.

TABLE 45. MIDGE RDX LIFE CYCLE TOXICITY DATA - EGG PRODUCTION[a]

Conc (mg/L)	Rep	No. of Adults Captured for Egg Production	No. of Eggs Produced per Adult	No. of Eggs per Adult
Control	1	5	941	188
	2	4	823	206
2.42	1	11	2,484	226
	2	5	1,120	224
3.91	1	7	981	140
	2	7	1,357	194
6.78	1	8	1,077	135
	2	3	173	58
12.67	1	4	677	169
	2	1	205	205
20.82	1	4	461	115
	2	1	149	149

[a] See Appendix RDX, Tables RDX9 and RDX10 for statistical analyses.

TABLE 46. MIDGE RDX LIFE CYCLE TOXICITY DATA - SECOND GENERATION HATCHING SUCCESS AFTER 2 TO 3 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	No. of Eggs Produced	No. of Eggs Hatched	Hatching Success (%)
Control	1	941	909	96.6
	2	823	814	98.9
2.42	1	2,484	2,378	95.7
	2	1,120	1,113	99.4
3.91	1	981	905	92.3
	2	1,357	1,297	95.6
6.78	1	1,077	1,029	95.5
	2	173	171	98.8
12.67	1	677	580	85.7
	2	205	194	94.6
20.82	1	461	442	95.9
	2	149	143	96.0

[a] See Appendix RDX, Table RDX11 for statistical analysis.

TABLE 47. FATHEAD MINNOW RDX ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality			
		24h	48h	72h	96h
Control	1	0	0	0	0
	2	0	0	0	0
2.81	1	0	0	0	0
	2	0	0	0	0
4.84	1	0	0	0	0
	2	0	0	0	0
7.66	1	0	0	0	0
	2	0	0	0	0
12.73	1	1	2	4	7
	2	0	0	1	3
18.50	1	10	10	10	10
	2	8	10	10	10

[a] See Appendix RDX, Table RDX12 for statistical analysis.

TABLE 48. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY DATA - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE AND SURVIVAL OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched	Hatching Success (%)	No. of Larvae that Survived	Larval Survival (%)
Control	1	20	8	12	100.0	11	91.7
	2	20	2	12	66.7	12	100.0
1.35	1	30	9	20	95.2	18	90.0
	2	30	8	19	86.4	18	94.7
2.36	1	30	3	24	88.9	23	95.8
	2	30	6	21	87.5	20	95.2
3.42	1	30	4	23	88.4	20	87.0
	2	30	5	20	80.0	16	80.0
6.42	1	40	1	31	79.5	31	100.0
	2	40	5	29	82.9	25	86.2
9.83	1	30	4	24	92.3	11	45.8
	2	30	2	19	67.9	7	36.8

[a] See Appendix RDX, Tables RDX13 to RDX15 for statistical analyses.

TABLE 49. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	11	24 (22-26)	145.1 (96.0-186.6)	30.2 (18.3-41.8)
	2	12	24 (17-27)	163.2 (62.0-224.9)	34.1 (10.5-46.5)
1.35	1	18	23 (18-27)	146.1 (92.5-210.0)	30.2 (18.7-42.5)
	2	18	22 (18-27)	138.7 (92.7-208.3)	28.9 (18.0-43.3)
2.36	1	23	22 (20-25)	104.9 (59.4-174.2)	18.8 (9.5-32.5)
	2	20	23 (18-26)	121.3 (67.9-195.8)	22.7 (12.2-37.2)
3.42	1	20	22 (19-25)	107.8 (65.6-161.1)	20.2 (10.5-32.0)
	2	16	22 (17-25)	100.6 (47.3-148.6)	19.9 (9.1-34.6)
6.42	1	31	21 (16-25)	83.5 (27.5-165.4)	15.3 (7.3-33.7)
	2	25	21 (14-25)	84.3 (25.7-140.2)	15.1 (5.3-28.3)
9.83[b]					

[a] See Appendix RDX, Tables RDX16 to RDX20 for statistical analyses.
[b] Lost replicate while conducting morphometric analyses; therefore, no statistical analyses were performed on this data set.

TABLE 50. CLADOCERAN PHOTOLYZED RDX (Φ-RDX) CHRONIC TOXICITY DATA - SURVIVAL OF ADULTS, NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD AFTER 7 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
Control	1	4	11	15	30	10.0
	2	5	13	13	31	10.3
	3	4	14	13	30	10.0
	4	4	13	12	33	11.0
	5	4	11	16	29	9.7
	6	5	10	14	29	9.7
	7	3	13	15	31	10.3
	8	4	11	15	30	10.0
	9	3	11	16	30	10.0
	10	4	11	14	29	9.7
1.3 Φ-RDX	1	5	10	13	28	9.3
	2	4	9	14	27	9.0
	3	4	9	LOST[b]	13	6.5
	4	5	11	14	30	10.0
	5	4	12	16	32	10.7
	6	3	10	16	29	9.7
	7	4	11	16	31	10.3
	8	3	13	15	31	10.3
	9	3	13	14	30	10.0
	10	4	11	14	29	9.7
2.2 Φ-RDX	1	4	13	15	32	10.7
	2	3	9	17	29	9.7
	3	3	12	15	30	10.0
	4	3	11	15	29	9.7
	5	3	10	11	24	8.0
	6	3	9	14	26	8.7
	7	3	9	16	28	9.3
	8	4	9	15	28	9.3
	9	4	10	16	30	10.0
	10	5	10	15	30	10.0

TABLE 50. CONTINUED

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
3.6 ϕ-RDX	1	4	10	15	29	9.7
	2	4	11	17	32	10.7
	3	3	12	18	33	11.0
	4	4	10	17	31	10.0
	5	2	9	16	27	9.0
	6	4	10	17	28	9.3
	7	2	8	15	25	8.3
	8	4	9	15	28	9.3
	9	4	11	15	30	10.0
	10	4	9	18	31	10.3
6.0 ϕ-RDX	1	4	13	16	33	11.0
	2	4	10	15	29	9.7
	3	3	9	15	27	9.0
	4	3	9	19	31	10.3
	5	4	8	16	28	9.3
	6	3	11	15	29	9.7
	7	3	9	12	24	8.0
	8	3	11	16	30	10.0
	9	4	11	13	28	9.3
	10	4	8	16	28	9.3
10.0 ϕ-RDX	1	4	13	13	30	10.0
	2	DEAD				
	3	3	7	15	25	8.3
	4	4	11	15	30	10.0
	5	3	9	15	27	9.0
	6	3	8	14	25	8.3
	7	3	9	17	29	9.7
	8	3	7	18	28	9.3
	9	3	10	14	27	9.0
	10	4	8	16	28	9.3

[a] No statistical analysis was conducted for the effect of ϕ-RDX on survival since only one adult died during the experiment. The statistical analysis for the effect of ϕ-RDX on neonate production is given in Appendix RDX, Table RDX21.

[b] Organism lost during renewal of test solution.

TABLE 51. 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
21	6.38
23	5.42
26	4.21
29	3.26
31	2.43
47	2.07
49	1.16
51	0.42
53	0.11
55	0.06
57	0.03

TABLE 52. DUCKWEED TNT TOXICITY DATA - MEAN FROND PRODUCTION AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mean New Frond Production				
		0H	24H	48H	72H	96H
Control	1	0	0	10	21	28
	2	0	0	9	24	31
	3	0	0	7	22	33
0.28	1	0	0	11	27	36
	2	0	0	12	22	34
	3	0	0	12	26	38
0.59	1	0	0	9	20	29
	2	0	0	5	13	22
	3	0	0	11	14	24
1.21	1	0	0	1	4	10
	2	0	0	7	13	21
	3	0	0	9	16	22
2.43	1	0	0	8	13	14
	2	0	0	3	9	10
	3	0	0	4	9	13
4.92	1	0	0	5	7	7
	2	0	0	1	3	-3
	3	0	0	6	6	4

[a] See Appendix TNT, Tables TNT1 to TNT3 for statistical analysis.

TABLE 53. CLADOCERAN TNT ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	0	0
	2	0	1
3.20	1	0	1
	2	0	0
5.08	1	0	9
	2	0	10
7.99	1	0	10
	2	4	10
13.40	1	5	10
	2	9	10
21.28	1	10	10
	2	10	10

[a] See Appendix TNT, Table TNT4 for statistical analysis.

TABLE 54. CLADOCERAN TNT CHRONIC TOXICITY DATA - SURVIVAL OF ADULTS, NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD AFTER 7 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
Control	1	6	7	9	22	7.3
	2	4	3	10	17	5.7
	3	5	4	8	17	5.7
	4	5	2	8	15	5.0
	5	5	6	13	24	8.0
	6	5	4	13	22	7.3
	7	5	7	12	24	8.0
	8	5	7	10	22	7.3
	9	5	8	10	23	7.7
	10	6	7	9	22	7.3
0.48	1	4	6	8	18	6.0
	2	4	6	8	18	6.0
	3	5	7	10	22	7.3
	4	4	6	9	19	6.3
	5	4	8	9	21	7.0
	6	3	6	8	17	5.7
	7	4	8	8	20	6.7
	8	5	6	11	22	7.3
	9	5	6	10	21	7.0
	10	6	8	10	24	8.0
0.66	1	6	11	16	33	11.0
	2	5	9	9	23	7.7
	3	6	9	10	25	8.3
	4	6	9	12	27	9.0
	5	3	10	13	26	8.7
	6	6	10	14	30	10.0
	7	6	10	14	30	10.0
	8	5	12	15	32	10.7
	9	6	7	15	28	9.3
	10	5	10	11	26	8.7

TABLE 54. CONTINUED

Conc (mg/L)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
1.04	1	6	10	15	31	10.3
	2	5	10	13	28	9.3
	3	6	11	14	31	10.3
	4	5	11	15	31	10.3
	5	6	10	14	30	10.0
	6	6	10	15	31	10.3
	7	6	10	15	31	10.3
	8	6	8	11	25	8.3
	9	6	10	13	29	9.7
	10	5	8	14	27	9.0
1.64	1	5	10	13	28	9.3
	2	5	10	12	27	9.0
	3	5	9	12	26	8.7
	4	6	10	14	30	10.0
	5	4	9	10	23	7.7
	6	5	10	11	26	8.7
	7	4	8	8	20	6.7
	8	6	10	11	27	9.0
	9	6	9	12	27	9.0
	10	4	7	11	22	7.3
2.71	1	3	4	9	16	5.3
	2	3	5	8	16	5.3
	3	2	4	2	8	2.7
	4	2	6	6	14	4.7
	5	0	0	3	3	3.0
	6	0	0	1	1	1.0
	7	0	5	2	7	3.5
	8	3	8	2	13	4.3
	9	0	0	2	2	2.0
	10	0	1	1	2	1.0

* No statistical analysis was conducted for the effect of TNT on survival since no adults died during the experiment. The statistical analyses for the effect of TNT on neonate production are given in Appendix TNT, Tables TNT5 and TNT6.

TABLE 55. MIDGE TNT ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 48 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality	
		24h	48h
Control	1	[b]	1
	2	[b]	0
6.82	1	[b]	0
	2	[b]	0
11.47	1	[b]	2
	2	[b]	0
19.90	1	[b]	0
	2	[b]	0
32.36	1	[b]	4
	2	[b]	0
53.48	1	[b]	10
	2	[b]	4

[a] See Appendix TNT, Table TNT7 for statistical analysis.
[b] Observations were not made at 24 h in order to avoid disturbing the organisms during the test.

TABLE 56. FATHEAD MINNOW TNT ACUTE TOXICITY DATA - CUMULATIVE MORTALITY (NUMBER DEAD) AFTER 96 HOURS OF EXPOSURE[a]

Conc (mg/L)	Rep	Mortality			
		24h	48h	72h	96h
Control	1	0	0	0	0
	2	0	0	0	0
1.17	1	0	0	0	0
	2	0	0	0	0
1.65	1	0	0	0	0
	2	0	0	0	0
2.83	1	0	0	5	7
	2	0	0	4	6
4.42	1	10	10	10	10
	2	6	10	10	10
7.19	1	10	10	10	10
	2	10	10	10	10

[a] See Appendix TNT, Table TNT8 for statistical analysis.

TABLE 57. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - PERCENT HATCH OF FIRST GENERATION EMBRYOS AFTER 4 DAYS OF EXPOSURE[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched	Hatching Success (%)
Control	1	35	7	26	92.9
	2	35	9	25	96.2
0.005	1	35	10	24	96.0
	2	35	6	24	82.8
0.014	1	35	4	30	96.8
	2	35	4	27	87.1
0.032	1	35	4	27	87.1
	2	35	4	29	93.6
0.077	1	35	2	26	78.8
	2	35	4	28	90.3
0.191	1	35	8	23	85.2
	2	35	6	26	89.7

[a] See Appendix TNT, Table TNT9 for statistical analysis.

TABLE 58. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - SURVIVAL AND TOTAL LENGTH (RANGE) OF FIRST GENERATION LARVAE AFTER 30 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Larvae at Day 0	No. of Larvae at Day 30	Larval Survival at Day 30 (%)	Mean Total Length (mm) at Day 30
Control	1	26	21	80.8	20.0 (17.6-22.4)
	2	25	20	80.0	21.0 (19.0-23.4)
0.005	1	24	24	100.0	19.5 (16.4-23.3)
	2	24	23	95.8	20.6 (16.5-23.5)
0.014	1	30	25	83.3	19.9 (15.6-25.8)
	2	27	24	88.9	20.4 (16.7-25.1)
0.032	1	27	25	92.3	20.1 (15.9-22.7)
	2	29	27	93.1	19.7 (16.2-22.5)
0.077	1	26	23	88.5	18.5 (11.1-22.5)
	2	28	26	92.9	19.6 (17.3-23.0)
0.191	1	23	18	78.3	18.5 (11.4-25.7)
	2	26	21	80.8	20.7 (14.8-23.6)

[a] See Appendix TNT, Tables TNT10 to TNT12 for statistical analyses.

TABLE 59. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - SURVIVAL AND TOTAL LENGTH (RANGE) OF FIRST GENERATION JUVENILES AFTER 60 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	No. of Larvae at Day 30	No. of Juveniles at Day 60	Juvenile Survival at Day 60 (%)	Mean Total Length (mm) at Day 60
Control	1	21	21	100.0	30.8 (28.1-36.2)
	2	20	20	100.0	30.3 (22.3-36.7)
0.005	1	24	17	70.8	29.4 (23.6-36.7)
	2	23	23	100.0	29.4 (21.0-34.1)
0.014	1	25	20	80.8	29.2 (20.3-36.7)
	2	24	24	100.0	29.3 (23.7-36.8)
0.032	1	25	25	100.0	29.5 (19.9-36.9)
	2	27	27	100.0	28.9 (16.8-35.6)
0.077	1	23	23	100.0	27.9 (23.8-36.1)
	2	26	26	100.0	28.2 (21.9-33.5)
0.191	1	18	16	88.9	28.4 (21.5-35.4)
	2	21	21	100.0	28.7 (19.0-36.0)

[a] See Appendix TNT, Tables TNT13 and TNT14 for statistical analysis.

TABLE 60. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF FIRST GENERATION FEMALES AND MALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST[a]

Conc (mg/L)	Rep	N	Sex	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	4	F	53 (49-57)	649 (370-928)	125 (66-141)
	2	4	F	53 (56-56)	728 (523-805)	145 (100-163)
	1	3	M	64 (61-67)	1,642 (1,344-1,632)	382 (310-439)
	2	3	M	66 (63-68)	1,810 (1,537-2,069)	355 (310-439)
0.005	1	6	F	55 (50-62)	944 (492-1,480)	186 (99-277)
	2	10	F	51 (48-59)	553 (299-937)	112 (69-175)
	1	5	M	65 (62-67)	1,551 (1,371-1,830)	353 (287-404)
	2	3	M	63 (62-64)	1,214 (1,001-1,431)	276 (229-307)
0.014	1	6	F	51 (48-54)	533 (388-710)	112 (86-147)
	2	4	F	54 (52-57)	677 (564-825)	134 (105-153)
	1	2	M	64 (62-66)	1,436 (1,061-1,811)	332 (273-391)
	2	4	M	63 (60-65)	1,211 (1,046-1,528)	307 (274-345)
0.032	1	4	F	50 (47-55)	538 (269-767)	117 (55-184)
	2	5	F	49 (46-53)	502 (332-702)	105 (68-147)
	1	6	M	66 (63-69)	1,862 (1,449-2,105)	375 (170-279)
	2	5	M	61 (56-63)	1,117 (730-1,424)	240 (170-279)
0.077	1	3	F	53 (51-57)	506 (463-586)	108 (98-126)
	2	4	F	53 (50-58)	543 (425-701)	121 (93-154)
	1	7	M	65 (58-72)	1,446 (848-2,131)	310 (189-406)
	2	6	M	65 (60-70)	1,449 (996-1,884)	309 (222-399)
0.191[b]						

[a] See Appendix TNT, Tables TNT15 to TNT20 for statistical analyses.
[b] Most fish were lost at this treatment during week 16 because of a dilutor malfunction; therefore, this treatment was eliminated from the study (See Section 3.5.2).

TABLE 61. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF FIRST GENERATION PARENTAL FEMALES AT THE END OF THE SPAWNING TEST PERIOD[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	4	54 (49-56)	1,363 (1,008-1,616)	331 (219-406)
	2	4	53 (52-54)	1,414 (1,389-1,429)	336 (294-367)
	3	4	52 (47-55)	1,277 (1,034-1,515)	307 (246-368)
0.005	1	4	52 (49-54)	1,390 (1,284-1,485)	325 (283-354)
	2	4	50 (48-52)	1,219 (1,064-1,325)	277 (215-338)
	3	4	49 (42-52)	1,221 (827-1,510)	298 (182-371)
0.014	1	4	48 (45-50)	1,105 (908-1,315)	239 (202-307)
	2	4	47 (43-49)	1,255 (1,026-1,457)	286 (231-307)
	3	3	49 (45-52)	1,424 (1,163-1,570)	318 (284-372)
0.032	1	3	50 (49-50)	1,443 (1,414-1,468)	350 (304-392)
	2	3	47 (45-48)	1,278 (1,032-1,569)	282 (208-337)
	3	3	45 (44-46)	1,064 (909-1,175)	231 (195-281)
0.077	1	4	48 (46-50)	1,218 (949-1,377)	278 (234-304)
	2	4	48 (46-49)	1,314 (1,151-1,577)	287 (246-362)
	3	4	46 (40-48)	1,240 (748-1,583)	267 (111-330)

[a] See Appendix TNT, Tables TNT21 to TNT24 for statistical analyses.

TABLE 62. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF FIRST GENERATION PARENTAL MALES AT THE END OF THE SPAWNING TEST PERIOD[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	1	77	4,918	1,346
	2	1	65	3,288	574
	3	1	71	3,741	794
0.005	1	1	68	4,145	948
	2	1	62	3,118	623
	3	1	70	4,270	847
0.014	1	1	62	2,894	1,119
	2	1	69	4,261	520
	3	1	68	3,339	682
0.032	1	1	64	3,132	699
	2	1	62	2,937	602
	3	1	68	4,114	906
0.077	1	1	61	2,735	540
	2	1	64	3,444	741
	3	1	64	3,342	662

[a] See Appendix TNT, Tables TNT25 to TNT27 for statistical analyses.

TABLE 63. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - NUMBER OF BROODS, TOTAL NUMBER OF EMBRYOS, AND HATCHING SUCCESS DURING THE SPAWNING TEST[a]

Conc (mg/L)	Rep	No. of Broods	No. of Embryos Produced	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched	Hatching Success (%)
Control	1	15	3292	300	48	232	92.1
	2	9	2333	250	88	150	92.6
	3	13	2567	250	80	149	87.6
0.005							
	1	18	3558	300	110	157	82.6
	2	17	3122	300	80	195	88.6
	3	11	2007	250	111	114	82.0
0.014	1	5	684	100	52	42	87.5
	2	11	2207	200	93	89	83.2
	3	19	3190	300	100	174	87.0
0.032	1	14	2399	250	124	101	80.2
	2	12	2017	150	64	71	82.6
	3	15	2076	200	85	98	85.2
0.077	1	11	1862	250	112	107	77.5
	2	16	2548	200	110	65	72.2
	3	6	1183	125	40	79	92.9

[a] See Appendix TNT, Tables TNT28 to TNT30 for statistical analyses.

TABLE 64. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE AND SURVIVAL OF LARVAE AFTER 30 DAYS OF POST-HATCH EXPOSURE WHICH WERE SELECTED FOR THE SECOND GENERATION EARLY LIFE STAGE (ELS) TEST[a]

Conc (mg/L)	Rep	No. of Embryos Exposed	No. of Embryos Lost Because of Fungus	No. of Embryos Hatched	Hatching Success (%)	No. of Larvae that Survived	Larval Survival (%)
Control	1	50	3	44	93.6	43	97.7
	2	50	24	26	100.0	26	100.0
	3	50	20	26	86.7	24	92.3
0.005	1	50	20	24	80.0	22	91.7
	2	50	30	17	85.0	17	100.0
	3	50	23	22	81.5	19	86.4
0.014	1	50	26	21	87.5	21	100.0
	2	50	23	24	88.9	23	95.8
	3	50	10	37	92.5	35	94.6
0.032	1	50	23	26	96.3	24	92.3
	2	50	24	22	84.6	21	95.5
	3	50	26	20	83.3	18	90.0
0.077	1	50	25	21	84.0	17	81.0
	2	50	26	18	69.2	15	83.3
	3	50	16	31	91.2	29	93.6

[a] See Appendix TNT, Tables TNT31 and TNT32 for statistical analyses.

TABLE 65. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY DATA - TOTAL LENGTH (RANGE), WET WEIGHT (RANGE), AND DRY WEIGHT (RANGE) OF SECOND GENERATION LARVAE AFTER 30 DAYS OF POST-HATCH EXPOSURE[a]

Conc (mg/L)	Rep	N	Mean Length (mm)	Mean Wet Weight (mg)	Mean Dry Weight (mg)
Control	1	43	20 (18-23)	113.2 (96.4-132.0)	23.9 (13.2-44.0)
	2	26	24 (19-28)	122.4 (57.4-236.7)	26.3 (11.7-53.6)
	3	24	21 (18-23)	111.1 (87.6-140.1)	23.4 (16.9-41.1)
0.005	1	22	20 (18-25)	115.1 (69.9-184.1)	21.9 (11.6-32.6)
	2	17	20 (16-22)	115.5 (52.7-182.2)	21.8 (6.8-29.2)
	3	19	20 (18-23)	113.5 (78.2-147.0)	20.3 (12.3-37.4)
0.014	1	21	21 (14-25)	122.3 (30.4-225.7)	23.9 (12.0-47.8)
	2	23	20 (17-24)	115.6 (78.3-150.1)	22.0 (13.8-32.9)
	3	35	20 (18-25)	108.0 (61.6-128.4)	21.9 (9.9-28.4)
0.032	1	24	20 (16-23)	114.9 (76.1-178.1)	20.8 (12.6-34.1)
	2	21	19 (15-23)	110.8 (69.7-173.0)	18.6 (3.6-34.8)
	3	18	19 (15-24)	68.4 (28.9-147.4)	14.0 (5.8-29.8)
0.077	1	17	19 (16-22)	76.2 (34.2-178.6)	15.9 (6.5-30.2)
	2	15	19 (15-21)	93.1 (51.0-122.6)	20.0 (7.5-37.5)
	3	29	20 (16-23)	75.8 (29.4-128.7)	14.7 (6.0-29.0)

[a] See Appendix TNT, Tables TNT33 to TNT37 for statistical analyses.

APPENDIX NQ

STATISTICAL ANALYSES OF THE ACUTE AND/OR CHRONIC NQ AND PHOTOLYZED NQ (Φ-NQ) TOXICITY TEST DATA

TABLE NQ1. HYDRA NQ ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

Data set could not be analyzed by the probit method.

Moving Average Angle Method:

48-h LC50 = 2,061 mg/L
95% Confidence limits = 1,830-2,437 mg/L

TABLE NQ2. CLADOCERAN NQ ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

48-h LC50 = 2,698 mg/L
LC50 95% fiducial limits = 2,276-3,298 mg/L
Slope = 4.87
Slope 95% fiducial limits = 3.02-6.73

TABLE NQ3. CLADOCERAN NQ CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - ADULT SURVIVAL AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Fisher's Exact Test:

Calculated test statistic:	See Table NQ4
Alpha value:	0.05
Critical value:	See Table NQ4
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ4. CLADOCERAN NQ CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF FISHER'S EXACT TEST ON ADULT SURVIVAL AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	Number Alive	Number Dead	Critical Value	b Value	Sign
Control	10	0			
260	9	1	6	9	
440	10	0	6	10	
730	10	0	6	10	
1,180	10	0	6	10	
1,400	0	10	6	0	*

* Significantly different at alpha = 0.05.

TABLE NQ5. CLADOCERAN NQ CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	14.44
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Reject the null hypothesis that the data are normally distributed

Steel's Many-One Rank Test:

Calculated test statistic:	See Table NQ6
Alpha value:	0.05
Critical value:	76.0
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE NQ6. CLADOCERAN NQ CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF STEEL'S MANY-ONE RANK TEST ON MEAN NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	No. of Rep	Mean Neonates Produced	Rank Sum	Critical Value	Sign
Control	10	20.1			
260	10	18.1	101.0	76.0	
440	10	7.0	55.0	76.0	*
730	10	2.7	55.0	76.0	*
1,180	10	0.0	55.0	76.0	*

* Significantly different at alpha = 0.05.

TABLE NQ7. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF EYED EMBRYOS AFTER 7 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	6.09
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	3.95
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ8. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF FRY AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	2.05
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	10.52
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ9. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF FRY AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	10.09
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.11
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ10. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF FRY AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	8.47
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.60
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ11. RAINBOW TROUT NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF FRY AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	6.09
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	4.06
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ12. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	2.05
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	3.71
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ13. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.16
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.88
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ14. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.43
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	7.26
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NQ15
Alpha value:	0.05
Critical value:	2.85
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NQ15. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON TOTAL LENGTH OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE

Conc (mg/L)	No. of Reps	Mean Length (mm)	T Statistic	Significance
Control	2	18.0		
380	2	17.1	0.84	
610	2	16.7	1.23	
1,050	2	16.3	1.67	
2,030	2	12.9	4.97	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.85).

TABLE NQ16. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	6.19
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	3.59
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ17. FATHEAD MINNOW NQ EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF LARVAE AFTER 28 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.70
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.93
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ18. CLADOCERAN PHOTOLYZED NQ (♦-NQ) CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - ADULT SURVIVAL AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Fisher's Exact Test:

Calculated test statistic:	See Table NQ19
Alpha value:	0.05
Critical value:	See Table NQ19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NQ19. CLADOCERAN NQ (♦-NQ) CHRONIC TOXICITY TEST - RESULTS OF FISHER'S EXACT TEST ON ADULT SURVIVAL AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	Number Alive	Number Dead	Critical Value	b Value	Sign
Control	10	0			
1.3 ♦-NQ	10	0	6	10	
2.2 ♦-NQ	9	1	6	9	
3.6 ♦-NQ	0	10	6	0	*
6.0 ♦-NQ	0	10	6	0	*
10.0 ♦-NQ	0	10	6	0	*

* Significantly different at alpha = 0.05.

TABLE NQ20. CLADOCERAN PHOTOLYZED NQ (Φ-NQ) CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	5.16
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	8.07
Alpha value:	0.01
Critical value:	9.21
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.72
Alpha value:	0.05
Critical value:	3.37
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX NG

STATISTICAL ANALYSES OF THE ACUTE AND/OR CHRONIC NG TOXICITY TEST DATA

TABLE NG1. GREEN ALGA NG TOXICITY TEST STATISTICAL ANALYSIS - MEAN CELL DENSITY (CELLS/ML) WHEN THE DATA ARE TREATED AS AN ACUTE 96-HOUR EXPOSURE

Probit Method:

96-h EC50 for reduction in growth = 1.15 mg/L
EC50 95% fiducial limits = 0.80-2.06 mg/L
Slope = 2.61
Slope 95% fiducial limits = 1.23-3.98

TABLE NG2. GREEN ALGA NG TOXICITY TEST STATISTICAL ANALYSIS - MEAN CELL DENSITY (CELLS/ML) WHEN THE DATA ARE TREATED AS A CHRONIC 96-HOUR EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.76
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	7.12
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	796.58
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG3
Alpha value:	0.05
Critical value:	2.50
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE NG3. GREEN ALGA NG TOXICITY TEST STATISTICAL ANALYSIS-RESULTS OF DUNNETT'S TEST ON MEAN CELL DENSITY (CELLS/ML) WHEN THE DATA ARE TREATED AS A CHRONIC 96-HOUR EXPOSURE

Conc (mg/L)	No. of Reps	Mean Cell Density	T Statistic	Significance
Control	3	1,190,560		
0.18	3	1,159,400	1.79	
0.37	3	1,153,867	2.11	
0.59	3	818,747	21.38	*
1.14	3	617,447	32.96	*
1.89	3	350,973	48.29	*

* Significantly different at alpha = 0.05 (Dunnett critical value = 2.50).

TABLE NG4. HYDRA NG ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

48-h LC50 = 17.43 mg/L
LC50 95% fiducial limits = 14.25-21.52 mg/L
Slope = 3.66
Slope 95% fiducial limits = 2.48-4.84

TABLE NG5. CLADOCERAN NG ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

Data set could not be analyzed by the probit method.

Moving Average Angle Method:

48-h LC50 = 17.83 mg/L
95% Confidence limits = 16.48-19.51 mg/L

TABLE NG6. CLADOCERAN NG CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - ADULT SURVIVAL AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Fisher's Exact Test:

Calculated test statistic:	See Table NG7
Alpha value:	0.05
Critical value:	See Table NG7
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NG7. CLADOCERAN NG CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF FISHER'S EXACT TEST ON ADULT SURVIVAL AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	Number Alive	Number Dead	Critical Value	b Value	Sign
Control	9	1			
1.88	8	2	4	8	
3.23	9	1	4	9	
5.48	10	0	<0	0	
9.65	10	0	<0	0	
16.05	0	10	4	0	*

* Significantly different at alpha = 0.05.

TABLE NG8. CLADOCERAN NG CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	16.22
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Reject the null hypothesis that the data are normally distributed

Steel's Many-One Rank Test:

Calculated test statistic:	See Table NG9
Alpha value:	0.05
Critical value:	72.0
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE NG9. CLADOCERAN NG CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF STEEL'S MANY-ONE RANK TEST ON MEAN NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Neonates Produced	Rank Sum	Critical Value	Sign
Control	10	25.9			
1.88	10	19.8	77.5	72.0	
3.23	10	22.4	79.5	72.0	
5.48	10	14.9	70.0	72.0	*
9.65	10	14.9	64.5	72.0	*

* Significantly different at alpha = 0.05.

TABLE NG10. MIDGE NG ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

Probit data were not used since the probability level was <0.05.

Moving Average Angle Method:

48-h LC50 = 34.93 mg/L
95% Confidence limits = 31.14-40.14 mg/L

TABLE NG11. RAINBOW TROUT NG ACUTE TOXICITY STATISTICAL ANALYSIS - 96-H LC50

Probit Method:

96-h LC50 = 1.90 mg/L
LC50 95% fiducial limit = 1.69-2.14 mg/L
Slope = 11.28
Slope 95% fiducial limits = 6.76-15.79

TABLE NG12. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF EYED EMBRYOS AFTER 8 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.86
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.00
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NG13. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	5.06
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.38
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NG14. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	2.51
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	9.21
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE NG15. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.87
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	9.84
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG16
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG16. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON WET WEIGHT OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE

Conc (mg/L)	No. of Reps	Mean Wet Weight (mg)	T Statistic	Significance
Control	2	856.9		
0.03	2	819.2	0.83	
0.06	2	770.6	1.90	
0.11	2	676.5	3.96	*
0.19	2	660.1	4.32	*
0.29	2	579.4	5.70	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.83).

TABLE NG17. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.15
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	18.23
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG18
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG18. RAINBOW TROUT NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON DRY WEIGHT OF FRY AFTER 60 DAYS OF POST-HATCH EXPOSURE

Conc (mg/L)	No. of Reps	Mean Dry Weight (mg)	T Statistic	Significance
Control	2	161.5		
0.03	2	150.9	1.21	
0.06	2	132.4	3.31	*
0.11	2	122.2	4.47	*
0.19	2	114.0	5.41	*
0.29	2	87.7	8.40	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.83).

TABLE NG19. FATHEAD MINNOW NG ACUTE TOXICITY STATISTICAL ANALYSIS - 96-H LC50

Probit Method:

96-h LC50 = 3.58 mg/L
LC50 95% fiducial limit = 2.91-4.41 mg/L
Slope = 3.57
Slope 95% fiducial limits = 2.35-4.79

TABLE NG20. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.10
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	98.00
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG21
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG21. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Hatch (%)	T Statistic	Significance
Control	2	92.7		
0.12	2	91.9	0.35	
0.20	2	73.0	6.12	*
0.33	2	56.1	10.12	*
0.57	2	41.1	13.49	*
0.94	2	25.8	17.16	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.83).

TABLE NG22. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	8.53
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.06
Alpha value:	0.01
Critical value:	11.34
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	23.56
Alpha value:	0.05
Critical value:	6.59
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG23
Alpha value:	0.05
Critical value:	0.36
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG23. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNCAN'S MULTIPLE RANGE TEST ON PERCENT SURVIVAL OF LARVAE AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Survival[a]	Difference from Control	Significance
Control	2	1.44		
0.12	2	1.36	0.03	
0.20	2	1.15	0.17	
0.33	2	0.48	0.77	*

[a] Arc sine square root transformed means.
* Significantly different at alpha = 0.05 (Duncan's critical value = 0.36).

TABLE NG24. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	8.53
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.60
Alpha value:	0.01
Critical value:	11.34
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	23.38
Alpha value:	0.05
Critical value:	6.59
Conclusion:	Reject the null hypothesis that all groups are equal

Duncan's Multiple Range Test:

Calculated test statistics:	See Table NG25
Alpha value:	0.05
Critical value:	2.66
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG25. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNCAN'S MULTIPLE RANGE TEST ON TOTAL LENGTH OF LARVAE AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Length (mm)	Difference from Control	Significance
Control	2	19.5		
0.12	2	19.0	0.50	
0.20	2	17.5	2.00	
0.33	2	12.5	7.00	*

* Significantly different at alpha = 0.05 (Duncan's critical value = 2.66).

TABLE NG26. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	8.53
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.44
Alpha value:	0.01
Critical value:	11.34
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	29.46
Alpha value:	0.05
Critical value:	6.59
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG27
Alpha value:	0.05
Critical value:	21.77
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG27. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNCAN'S MULTIPLE RANGE TEST ON WET WEIGHT OF LARVAE AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Wet Weight (mg)	Difference from Control	Significance
Control	2	73.0		
0.12	2	78.2	-5.20	
0.20	2	65.8	7.20	
0.33	2	22.3	50.70	*

* Significantly different at alpha = 0.05 (Duncan's critical value = 21.77).

TABLE NG28. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	8.53
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.32
Alpha value:	0.01
Critical value:	11.34
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	22.22
Alpha value:	0.05
Critical value:	6.59
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table NG29
Alpha value:	0.05
Critical value:	4.16
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE NG29. FATHEAD MINNOW NG EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNCAN'S MULTIPLE RANGE TEST ON DRY WEIGHT OF LARVAE AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Dry Weight (mg)	Difference from Control	Significance
Control	2	14.8		
0.12	2	15.4	-0.60	
0.20	2	12.7	2.10	
0.33	2	3.3	11.50	*

* Significantly different at alpha = 0.05 (Duncan's critical value = 4.16).

APPENDIX RDX

STATISTICAL ANALYSES OF THE ACUTE AND/OR CHRONIC RDX AND PHOTOLYZED RDX (ϕ-RDX) TOXICITY TEST DATA

TABLE RDX1. GREEN ALGA RDX TOXICITY TEST STATISTICAL ANALYSIS - MEAN CELL DENSITY (CELLS/ML) WHEN THE DATA ARE TREATED AS A CHRONIC 96-HOUR EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	4.96
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.01
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	19.77
Alpha value:	0.05
Critical value:	2.92
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table RDX2
Alpha value:	0.05
Critical value:	2.75
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE RDX2. GREEN ALGA RDX TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF BONFERRONI T-TEST ON MEAN CELL DENSITY (CELLS/ML) WHEN THE DATA ARE TREATED AS A CHRONIC 96-HOUR EXPOSURE

Conc	No. of Reps	Mean Cell Density	T Statistic	Significance
Control	2	1,115,963		
0.47	3	1,008,760	2.07	
4.81	3	830,531	5.51	*
7.95	3	953,747	3.13	*
13.02	3	861,331	4.92	*
21.80	3	707,951	7.88	*
36.69	3	689,282	8.24	*

* Significantly different at alpha = 0.05 (Bonferroni T-test critical value = 2.75).

TABLE RDX3. CLADOCERAN RDX CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	2.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	24.30
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Reject the null hypothesis that the variances are homogenous

Steel's Many-One Rank Test:

Calculated test statistic:	See Table RDX4
Alpha value:	0.05
Critical value:	75.0
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE RDX4. CLADOCERAN RDX CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF STEEL'S MANY-ONE RANK TEST ON MEAN NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Neonates Produced	Rank Sum	Critical Value	Sign
Control	10	36.8			
2.15	10	37.7	99.0	75.0	
3.64	10	35.2	93.5	75.0	
6.01	10	27.5	63.5	75.0	*
9.74	10	30.6	62.0	75.0	*
16.41	10	18.8	55.0	75.0	*

* Significantly different at alpha = 0.05.

TABLE RDX5. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - LARVAL GROWTH AFTER 6 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	6.09
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	3.03
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX6. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - LARVAL GROWTH AFTER 8 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	10.83
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.03
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX7. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - LARVAL GROWTH AFTER 10 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	9.95
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.92
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX8. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - PERCENT ADULT EMERGENCE AFTER 13 TO 17 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	2.51
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	9.82
Alpha value:	0.05
Critical value:	11.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX9. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - MEAN EGG PRODUCTION

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	5.30
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	4.48
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test

Calculated test statistics:	See Table RDX10
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE RDX10. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON MEAN EGG PRODUCTION

Conc (mg/L)	N	Mean Eggs Produced	T Statistic	Significance
Control	9	197		
2.42	16	225	-0.90	
3.91	14	167	0.96	
6.78	11	96	3.23	*
12.67	5	187	0.32	
20.82	5	132	2.09	

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.83).

TABLE RDX11. MIDGE RDX LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - SECOND GENERATION HATCHING SUCCESS AFTER 2 TO 3 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	5.26
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.80
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX12. FATHEAD MINNOW RDX ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

Data set could not be analyzed by the probit method.

Moving Average Angle Method:

96-h LC50 = 12.73 mg/L
95% Confidence limits = 11.14-14.97 mg/L

TABLE RDX13. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF EMBRYOS AFTER 4 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	6.85
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.20
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX14. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.22
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	12.09
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test

Calculated test statistics:	See Table RDX15
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE RDX15. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON PERCENT SURVIVAL OF LARVAE AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Survival (%)	T Statistic	Significance
Control	2	95.9		
1.35	2	92.3	0.71	
2.36	2	95.5	0.11	
3.42	2	83.5	2.04	
6.42	2	93.1	0.42	
9.83	2	41.3	6.40	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.83).

TABLE RDX16. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	2.73
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	7.42
Alpha value:	0.05
Critical value:	7.93
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE RDX17. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.17
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	23.21
Alpha value:	0.05
Critical value:	3.19
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table RDX18
Alpha value:	0.05
Critical value:	2.85
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE RDX18. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON WET WEIGHT AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Wet Weight (mg)	T Statistic	Significance
Control	2	154.2		
1.35	2	142.4	1.40	
2.36	2	113.1	4.89	*
3.42	2	104.2	5.95	*
6.42	2	83.9	8.37	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.85).

TABLE RDX19. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF LARVAE AFTER 28 DAYS OF EXPOSURE

Data Transformation:	
None	
Chi-Square Test for Normality:	
Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed
Bartlett's Test for Homogeneity of Variances:	
Calculated test statistic:	6.41
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous
ANOVA:	
Calculated test statistic:	30.98
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Reject the null hypothesis that all groups are equal
Dunnett's Test:	
Calculated test statistics:	See Table RDX20
Alpha value:	0.05
Critical value:	2.85
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE RDX20. FATHEAD MINNOW RDX EARLY LIFE STAGE (ELS) TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON DRY WEIGHT AFTER 28 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Dry Weight (mg)	T Statistic	Significance
Control	2	32.2		
1.35	2	29.6	1.45	
2.36	2	20.8	6.35	*
3.42	2	20.1	6.74	*
6.42	2	15.2	9.44	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.85).

TABLE RDX21. CLADOCERAN PHOTOLYZED RDX (Φ-RDX) CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	2.73
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	5.51
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.86
Alpha value:	0.05
Critical value:	2.45
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX TNT

STATISTICAL ANALYSES OF THE ACUTE AND/OR CHRONIC TNT TOXICITY TEST DATA

TABLE TNT1. DUCKWEED TNT TOXICITY TEST STATISTICAL ANALYSIS-MEAN FROND PRODUCTION WHEN THE DATA ARE TREATED AS AN ACUTE 96-HOUR EXPOSURE

Probit Method:

96-h EC50 for reduction in growth = 1.59 mg/L
EC50 95% fiducial limits = 1.11-2.37 mg/L
Slope = 2.52
Slope 95% fiducial limits = 1.47-3.57

TABLE TNT2. DUCKWEED TNT TOXICITY TEST STATISTICAL ANALYSIS - MEAN FROND PRODUCTION WHEN THE DATA ARE TREATED AS A CHRONIC 96-HOUR EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	5.15
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.11
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	27.69
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test

Calculated test statistics:	See Table TNT3
Alpha value:	0.05
Critical value:	2.90
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE TNT3. DUCKWEED TNT TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON MEAN FROND PRODUCTION WHEN THE DATA ARE TREATED AS A CHRONIC 96-HOUR EXPOSURE

Conc (mg/L)	No. of Reps	Mean Fronds Produced	T Statistic	Significance
Control	3	30.7		
0.28	3	36.0	-1.61	
0.59	3	25.0	1.71	
1.21	3	17.7	3.93	*
2.43	3	12.3	5.55	*
4.92	3	2.7	8.47	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.90).

TABLE TNT4. CLADOCERAN TNT ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

96-h LC50 = 4.03 mg/L
LC50 95% fiducial limits = 3.65-4.46 mg/L
Slope = 16.39
Slope 95% fiducial limits = 9.87-22.91

TABLE TNT5. CLADOCERAN TNT CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.88
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	14.55
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	46.94
Alpha value:	0.05
Critical value:	2.45
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test

Calculated test statistics:	See Table TNT6
Alpha value:	0.05
Critical value:	2.31
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE TNT6. CLADOCERAN TNT CHRONIC TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON MEAN NEONATE PRODUCTION AFTER 7 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Neonates Produced	T Statistic	Significance
Control	10	20.8		
0.48	10	20.2	0.38	
0.66	10	28.0	-4.51	
1.04	10	29.4	-5.39	
1.64	10	25.6	-3.01	
2.71	10	8.2	7.90	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.31).

TABLE TNT7. MIDGE TNT ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 48-H LC50

Probit Method:

Probit data were not used since the probability level was <0.05.

Moving Average Angle Method:

48-h LC50 = 42.90 mg/L
95% Confidence limits = 37.10-54.62 mg/L

TABLE TNT8. FATHEAD MINNOW TNT ACUTE TOXICITY TEST STATISTICAL ANALYSIS - 96-H LC50

Probit Method:

Data set could not be analyzed by the probit method.

Moving Average Angle Method:

96-h LC50 = 2.66 mg/L
95% Confidence limits = 2.32-3.13 mg/L

TABLE TNT9. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF FIRST GENERATION EMBRYOS AFTER 4 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.52
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.64
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT10. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF FIRST GENERATION LARVAE AFTER 30 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.51
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	14.50
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table TNT11
Alpha value:	0.05
Critical value:	2.83
Conclusion:	Reject the null hypothesis that the controls are greater than the treatments

TABLE TNT11. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON PERCENT SURVIVAL OF FIRST GENERATION LARVAE AFTER 30 DAYS OF POST-HATCH EXPOSURE

Conc (mg/L)	No. of Reps	Mean Survival (%)	T Statistic	Significance
Control	2	80.4		
0.005	2	97.9	6.83	*
0.014	2	86.1	1.75	
0.032	2	92.7	4.14	*
0.077	2	90.7	3.37	*
0.191	2	79.6	-0.23	

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.63).

TABLE TNT12. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF FIRST GENERATION LARVAE AFTER 30 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	12.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.50
Alpha value:	0.01
Critical value:	15.09
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.70
Alpha value:	0.05
Critical value:	4.39
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT13. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF FIRST GENERATION JUVENILES AFTER 60 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	2.05
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	7.42
Alpha value:	0.05
Critical value:	7.93
Conclusion:	Fail to reject the null hypothesis that all groups are equal

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TABLE TNT14. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF FIRST GENERATION JUVENILES AFTER 60 DAYS OF POST-HATCH EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	6.09
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	7.42
Alpha value:	0.05
Critical value:	7.93
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT15. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF FEMALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	1.62
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	4.71
Alpha value:	0.05
Critical value:	7.42
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT16. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF FEMALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	5.37
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.11
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT17. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF FEMALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.82
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.73
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT18. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF MALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	4.27
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	2.08
Alpha value:	0.05
Critical value:	7.42
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT19. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF MALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	8.16
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.64
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT20. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF MALES NOT SELECTED AT 22 WEEKS FOR THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	10.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	7.97
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.50
Alpha value:	0.05
Critical value:	5.19
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT21. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF PARENTAL FEMALES AT THE END OF THE SPAWNING TEST PERIOD

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	4.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.33
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	7.54
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table TNT22
Alpha value:	0.05
Critical value:	2.47
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE TNT22. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON TOTAL LENGTH OF PARENTAL FEMALES AT THE END OF THE SPAWNING TEST PERIOD

Conc (mg/L)	No. of Reps	Mean Length (mm)	T Statistic	Significance
Control	3	53.0		
0.005	3	50.3	2.11	
0.014	3	48.0	3.95	*
0.032	3	47.3	4.48	*
0.077	3	47.3	4.80	*

* Significantly different at alpha = 0.05 (Dunnett's Test critical value = 2.47).

TABLE TNT23. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF PARENTAL FEMALES AT THE END OF THE SPAWNING TEST PERIOD

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	4.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.63
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.30
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT24. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF PARENTAL FEMALES AT THE END OF THE SPAWNING TEST PERIOD

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.50
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.21
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT25. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF PARENTAL MALES AT THE END OF THE SPAWNING TEST PERIOD

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	4.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	2.39
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.68
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT26. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF PARENTAL MALES AT THE END OF THE SPAWNING TEST PERIOD

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.96
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.77
Alpha value:	0.05
Critical value:	3.11
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT27. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF PARENTAL MALES AT THE END OF THE SPAWNING TEST PERIOD

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.79
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.42
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT28. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - NUMBER OF BROODS DURING THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.56
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.45
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT29. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - NUMBER OF EMBRYOS PRODUCED DURING THE SPAWNING TEST

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.54
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.03
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT30. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - HATCHING SUCCESS DURING THE SPAWNING TEST

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	6.39
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that che variances are homogenous

ANOVA:

Calculated test statistic:	1.33
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT31. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - PERCENT HATCH OF SECOND GENERATION EMBRYOS AFTER 4 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	4.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.67
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.67
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT32. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - PERCENT SURVIVAL OF SECOND GENERATION LARVAE AFTER 30 DAYS OF EXPOSURE

Data Transformation:

Arc sine square root

Chi-Square Test for Normality:

Calculated test statistic:	3.14
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.71
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.00
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT33. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - TOTAL LENGTH OF SECOND GENERATION LARVAE AFTER 30 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	5.70
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Data fail to meet the homogeneity of variance assumption

Kruskal-Wallis Test:

Calculated test statistic:	8.71
Alpha value:	0.05
Critical value:	8.33
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE TNT34. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - WET WEIGHT OF SECOND GENERATION LARVAE AFTER 30 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	4.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	11.60
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	3.99
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table TNT35
Alpha value:	0.05
Critical value:	2.47
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE TNT35. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON WET WEIGHT OF SECOND GENERATION LARVAE AFTER 30 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Wet Weight (mg)	T Statistic	Significance
Control	3	115.6		
0.005	3	114.7	0.08	
0.014	3	115.3	0.03	
0.032	3	98.0	1.65	
0.077	3	81.7	3.18	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.47).

TABLE TNT36. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - DRY WEIGHT OF SECOND GENERATION LARVAE AFTER 30 DAYS OF EXPOSURE

Data Transformation:

None

Chi-Square Test for Normality:

Calculated test statistic:	6.41
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.11
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	6.46
Alpha value:	0.05
Critical value:	3.48
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistics:	See Table TNT37
Alpha value:	0.05
Critical value:	2.47
Conclusion:	Reject the null hypothesis that the treatments are equal to the controls

TABLE TNT37. FATHEAD MINNOW TNT LIFE CYCLE TOXICITY TEST STATISTICAL ANALYSIS - RESULTS OF DUNNETT'S TEST ON DRY WEIGHT OF SECOND GENERATION LARVAE AFTER 30 DAYS OF EXPOSURE

Conc (mg/L)	No. of Reps	Mean Dry Weight (mg)	T Statistic	Significance
Control	3	24.5		
0.005	3	21.3	1.78	
0.014	3	22.6	1.08	
0.032	3	17.8	3.75	*
0.077	3	16.9	4.27	*

* Significantly different at alpha = 0.05 (Dunnett's critical value = 2.47).

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