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# ECOLOGICAL CRITERIA DOCUMENT FOR 2,4,6-TRINITROTOLUENE
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9649

TABLE OF CONTENTS

EXECUTIVE SUMMARY ..... iv

1. INTRODUCTION ..... 1

2. ENVIRONMENTAL FATE ..... 1

2.1. SOURCES AND OCCURRENCES ..... 1

2.1.1. Air ..... 1
2.1.2. Surface and Groundwater ..... 1
2.1.3. Soils ..... 2

2.2. TRANSPORT AND TRANSFORMATION PROCESSES ..... 2

2.2.1. Abiotic Processes ..... 2
2.2.2. Biotransformation ..... 3

3. AQUATIC TOXICOLOGY ..... 4

3.1. ACUTE EFFECTS - INVERTEBRATES AND FISH ..... 4
3.2. CHRONIC EFFECTS - INVERTEBRATES AND FISH ..... 4
3.3. PLANTS ..... 9
3.4. METABOLISM AND BIOCONCENTRATION ..... 11
3.5. AQUATIC CRITERIA AND SCREENING BENCHMARKS ..... 12

3.5.1. Aquatic Organisms ..... 12
3.5.2. Sediment-Associated Organisms ..... 13

4. TERRESTRIAL TOXICOLOGY ..... 13

4.1. MAMMALS ..... 13
4.2. BIRDS ..... 14
4.3. INVERTEBRATES ..... 14
4.4. PLANTS ..... 14
4.5. METABOLISM AND BIOACCUMULATION ..... 15

4.5.1. Animals ..... 15
4.5.2. Plants ..... 15

4.6. TERRESTRIAL CRITERIA AND SCREENING BENCHMARKS ..... 16

4.6.1. Animals ..... 16
4.6.2. Plants ..... 16
4.6.3. Soil Invertebrates ..... 16

5. REFERENCES ..... 19

APPENDIX A: DERIVATION OF AQUATIC BENCHMARKS ..... A-1

APPENDIX B: DERIVATION OF WILDLIFE BENCHMARKS ..... B-1

LIST OF TABLES

1. RESULTS OF ACUTE TOXICITY STUDIES WITH AQUATIC INVERTEBRATES AND FISH ..... 5

2. RESULTS OF CHRONIC AQUATIC TOXICITY STUDIES ..... 6

3. RESULTS OF TOXICITY TESTS WITH SPECIES OF ALGAE AND A VASCULAR PLANT ..... 10

4. BIOCONCENTRATION FACTORS (BCF) IN AQUATIC SPECIES ..... 11

5. WATER QUALITY CRITERIA/SCREENING BENCHMARKS FOR AQUATIC ORGANISMS ..... 13

6. SCREENING BENCHMARKS FOR SELECTED MAMMALIAN WILDLIFE SPECIES ..... 17

7. SCREENING BENCHMARKS FOR TERRESTRIAL PLANTS AND INVERTEBRATES ..... 18

## EXECUTIVE SUMMARY

2,4,6-Trinitrotoluene (TNT) is a munitions chemical released to the environment from munitions production and processing facilities and through field use and disposal practices such as open burning. Concentrations monitored in effluents at Army Ammunition and load, assemble, and pack plants ranged up to 178 mg/L, with most concentrations considerably lower. Concentrations in sludge below settling ponds ranged up to 50,000 mg/kg and concentrations in soil ranged up to 87,000 mg/kg. Concentrations in groundwater at these sites ranged up to 350 $\mu$g/L. In surface water downstream of discharge points, concentrations rapidly decreased due to dilution and photolysis.

Photolysis is the primary transformation process in surface water or exposed soil. In water, TNT is rapidly transformed by photolysis, resulting in the development of red or pink water which is rich in nitroaromatic compounds. Photolytic half-lives of 0.5 to several hours have been measured; the process is accelerated by the presence of degradation products and natural organic compounds in the water. Photolysis would not be an important transformation mechanism in groundwater and below the surface of soils. A soil organic carbon adsorption coefficient ($K_{oc}$) of 1600 and a soil adsorption coefficient ($K_p$) as low as 4 indicate limited partition or sorption to soils or sediments.

2,4,6-Trinitrotoluene is biodegraded in water, soil, sediment, and sludge by bacterial and fungal species under both aerobic and anaerobic conditions; the process occurs more slowly than photolysis, with the estimated half-life ranging from 1-6 months in surface waters. Reduction of a nitro group is the first step in transformation. Compounds formed include aminodinitrotoluenes, diaminonitrotoluenes, hydroxyaminodinitrotoluenes, 2,4,6-triaminotoluene, and tetranitroazotoluenes. Cleavage of the benzene ring has been reported in several studies.

Studies of the acute toxicity of TNT to aquatic animals were conducted on five species of invertebrates and four species of fish. Forty-eight hour $LC_{50}$ values for invertebrates ranged from >4.4 mg/L to >29 mg/L; for fish, 96-hr $LC_{50}$ values ranged from 0.8 mg/L to 3.7 mg/L. Acute studies were complicated by the photochemical transformation of TNT and the resulting presence of degradation products. In chronic studies with early life stages of fish, one of the lowest concentrations tested (0.04 mg/L), decreased reproductive parameters of fathead minnows exposed from the egg stage through reproduction, but did not affect egg hatching and fry survival of fathead minnows, channel catfish, or rainbow trout in 30-day tests. A concentration of 1.0 mg/L was the lowest concentration that affected the growth of algae and a vascular plant. A calculated log $K_{ow}$ of approximately 2 indicates a low potential for bioconcentration in aquatic organisms.

Criteria and screening benchmarks were calculated for aquatic organisms. An acute freshwater Water Quality Criterion of 0.57 mg/L was calculated according to U.S. EPA guidelines. A chronic Water Quality Criterion of 0.13 mg/L was calculated. The lowest chronic effects values for fish, daphnids, and aquatic plants were 0.04, 1.03, and 1.0 mg/L, respectively. Data were insufficient for calculation of a Final Residue Value. A screening Sediment Quality Benchmark of 0.52 mg/kg sediment was calculated based on the Equilibrium Partitioning approach.

Few data on the toxicity of TNT to terrestrial animals and plants were located in the available literature. Studies with laboratory animals were utilized to calculate screening benchmarks for mammalian wildlife. Using a reproductive endpoint, a NOAEL value of 1.6 mg/kg/day from a feeding study with the laboratory rat was used to calculate NOAEL values for food and water intake for the short-tailed shrew, white-footed mouse, meadow vole, cottontail rabbit, mink, red fox, and whitetail

deer. No studies were located for avian species. The following screening benchmarks were identified for plants and invertebrates. In a single study, growth of yellow nutsedge was reported at the lowest solution concentration tested, 5 mg/L. No effects on the growth of yellow nutsedge were reported at a soil concentration of 80 mg/kg TNT (dry weight) in one study, but a second study reported phytotoxic effects at 30 mg/kg. A soil no-effect level of 100 mg/kg TNT and a lowest-observed-effect level of 200 mg/kg were reported for numbers of soil nematodes and microarthropods. Very little confidence can be placed in these screening benchmarks derived from one to two studies; thus, additional data are needed to calculate screening benchmarks for terrestrial plants and invertebrates with a high degree of confidence.

# 1. INTRODUCTION

This ecotoxicological profile on 2,4,6-trinitrotoluene summarizes the available data on concentrations in environmental media, environmental transport and fate, and ecotoxicity and bioaccumulation data for aquatic and terrestrial species. Where sufficient data were available, toxicological criteria and screening benchmarks, based on population growth and survival effects, were derived.

# 2. ENVIRONMENTAL FATE

## 2.1 SOURCES AND OCCURRENCES

2,4,6-Trinitrotoluene (TNT) is released to the environment from munitions production and processing facilities. TNT has been identified at 19 National Priorities List sites across the U.S. (ATSDR, 1993); these include Army Ammunition Plants (AAPs) as well as load, assemble, and pack (LAP) plants. During World War II, five AAPs and 25 LAP plants were active. TNT is also present in the environment as a result of decommissioning activities and through field use and disposal practices such as open burning. TNT has been found in environmental media only in the vicinity of munitions plants.

### 2.1.1 Air

A low vapor pressure, $1.99 \times 10^{-4}$ mm Hg at 20°C (HSDB, 1995), indicates a low potential to enter the atmosphere. Nitroaromatics associated with munitions manufacture and processing have generally not been detected in atmospheric monitoring studies (Howard et al., 1976).

### 2.1.2 Surface and Groundwater

Reported levels in production and processing facility effluents vary. Because of color changes when exposed to sunlight, effluents from production and processing facilities are referred to as red or pink water. Spanggord and coworkers (1982) characterized wastewaters resulting from the production and purification of TNT. They reported that concentrations of 0.10-3.40 mg/L occurred in 20% of condensate wastewater from plants using the sellite manufacturing process. Concentrations in a lagoon receiving pink water ranged from 774-998 μg/L (Triegel et al., 1983); concentrations in pink water effluents from LAP plants ranged from 1-178 mg/L (Patterson et al., 1977). Concentrations in untreated waste water at the Radford AAP, Virginia, ranged from 101 to 143 mg/L (Nay et al., 1972) and up to 120 mg/L at several manufacturing plants (Freeman and Colitti, 1982; Andren et al., 1977). Concentrations in aqueous samples collected onsite at the Joliet AAP ranged from <0.2 μg/L (the detection limit) to 1020 μg/L, the latter in an open wastewater channel (Jerger et al., 1976). Sediment concentrations ranged from <1 mg/kg to 44,200 mg/kg (air dry weight) in a red water pond at the same site. Respective concentrations were lower at the Iowa AAP. At the Lone Star AAP, Texas, sludge from below pink water settling ponds contained up to 50,000 mg/kg (Phung and Bulot, 1981). Concentrations in onsite streams at the Iowa AAP ranged up 29 μg/L; average concentrations in stream sediment cores ranged up to 111 mg/kg (Sanocki et al., 1976).

Runoff and leaching from lagoons and storage and disposal areas may contaminate surface and groundwater. However, concentrations downstream of discharge points rapidly decrease due to

dilution and photolysis (Spanggord et al., 1980b). TNT has been found in groundwater collected at several munitions facilities. Concentrations up to 350 μg/L were measured in groundwater offsite at the Cornhusker AAP near Grand Island, Nebraska (Spalding and Fulton, 1988). TNT concentrations of 320 μg/L (200 feet downgradient) and 1 μg/L (1070 feet downgradient) were measured in groundwater samples at the Hawthorne Naval Ammunition Depot, Nevada (Goerlitz and Franks, 1989). TNT was not detected in ocean waters near ocean dumping sites for waste munitions (Hoffsommer and Rosen, 1972) nor in ocean floor sediments near dumping sites (Hoffsommer et al., 1972).

### 2.1.3 Soils

At the Joliet AAP, soil concentrations ranged from less than the limit of detection to 87,000 mg/kg, with highest concentrations next to a LAP area (Phillips et al., 1994; Simini et al., 1995). At the Department of Energy's Weldon Springs site in St. Charles County Missouri, TNT was detected in surface soil samples at an average concentration of 13,000 mg/kg (Haroun et al., 1990). At the West Virginia Ordinance Works, TNT and other nitroaromatics have been detected in soil at concentrations up to 40,000 mg/kg at burning sites (Kraus et al., 1985). At the Lone Star AAP, Texas, surface soil samples at 0.2-0.6 m depth contained 19 mg/kg (Phung and Bulot, 1981).

## 2.2 TRANSPORT AND TRANSFORMATION PROCESSES

### 2.2.1 Abiotic Processes

Air. No information on the transformation of TNT in air was found. TNT in the atmosphere should undergo direct photolysis as it does in surface water. Based on the rate of photolysis in distilled water, the estimated photolytic half-life in the atmosphere would range from 3.7 to 11.3 hours (Howard et al., 1991). Based on the estimated rate constant for reaction with hydroxyl radicals in the atmosphere, the photooxidation half-life would range from 18.4 to 184 days (Howard et al., 1991).

Water. Photolysis is the primary transformation process in the environment. Laboratory studies of the photodecomposition of TNT in water indicate that decomposition occurs much more rapidly in sunlight than in darkness and the rate is accelerated by the presence of degradation products. In natural waters, the rate of photolysis is accelerated by the presence of natural substances (Spanggord et al., 1980b; Mabey et al., 1983). In a laboratory study, TNT photolyzed rapidly in natural water, with a half-life of 3 to 22 hr; in field studies, the compound rapidly declined within a short distance of discharge points (Spanggord et al., 1980b).

Photolysis results in the formation of pink water which is rich in nitro compounds. Numerous degradation products of TNT have been identified in pink water. Burlinson (1980) proposed a mechanism for photodecomposition of TNT; the major transformation products in natural water are 1,3,5-trinitrobenzene, 4,6-dinitroanthranil, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitrobenzonitrile, and 2,4,6-trinitrobenzoic acid; in addition, a number of azo and azoxy derivatives formed by the coupling of nitroso and hydroxylamine products have been identified (Jerger et al., 1976; Burlinson, 1980; Spanggord et al., 1980a; Mabey et al., 1983; Pennington, 1988). TNT may be more persistent in deep, quiescent waters where sunlight is attenuated. Hydrolysis and volatilization from water are not expected under environmental conditions (HSDB, 1995). TNT was stable in seawater for 108 days under neutral conditions, indicating stability against hydrolysis (Howard et al., 1976). A calculated

Henry's Law constant of 4.57 x $10^{-7}$ atm-$m^3$/mole at 20°C indicates extremely slow volatilization from water or moist soils (HSDB, 1995). Water solubility of TNT is 130 mg/L (Ryon, 1987).

Soil and Sediment. Spanggord et al. (1980a) reviewed studies on sorption of TNT on soils and sediments and found values of 5.5 to 19.3. Partition coefficients or $K_p$ ([μg chemical in soil/g of soil]/[μg chemical in water/g of water]) in the following studies range from 4 to 53, indicating little sorption (strongly adsorbed chemicals have $K_p$ values greater than 100).

Soils from 13 AAPs were tested for adsorption and desorption of TNT (Pennington, 1988). The soils were primarily silt loams, low in organic carbon content (0.5-3.5%). Both adsorption and desorption reached steady states within 2 hours. With the addition of 320 μg TNT/g of soil, adsorption percentages ranged from 7.5 to 32% (24-102 μg/g). Sequential desorption with water indicated that most of the TNT was desorbed after three sequential desorption cycles. The average percent of TNT desorbed was 88%, i.e. remaining adsorbed TNT averaged 12% (7.7-23%, 2-24 μg/g). Adsorption was highest in a clay soil (32% initially and 23% following sequential desorption). Adsorption coefficients ($K_p$) (soil concentration/solution concentration) values averaged 4 but varied with soil type. In another study, $K_p$ values on four aquatic sediments measured 5.5, 14.3, 16.5, and 22.2 after 24 hours equilibrium time, also suggesting low sorption (Sikka et al., 1980). Slightly higher values were measured by Spanggord et al. (1980b); the average $K_p$ of 5 sediments (average organic carbon content, 3.3%) from water bodies receiving munition wastewater discharges was 53 and the average $K_{oc}$ [(μg adsorbed/g organic carbon)/(μg/mL solution)] was 1600. Cataldo et al. (1989) found that the fraction of soil-sorbed $C^{14}$-labeled TNT/TNT metabolites increases with time. The amount not readily removed by exhaustive extraction increased from <6% at time 0 to 50% after 60 days of incubation.

In a field study, TNT was mixed with soil at a ratio of 1/1000, placed in open-ended tubes buried in the ground, and allowed to weather for 20 years (DuBois and Baytos, 1991). *Pseudomonas aeruginosa* added to the tubes survived less than 6 months. The half-life of TNT under these conditions was estimated at 1 year.

### 2.2.2 Biotransformation

2,4,6-Trinitrotoluene is biodegraded in water, soil, sediment, and sludge by bacterial and fungal species under both aerobic and anaerobic conditions (Spanggord et al., 1980a); the process occurs more slowly than photolysis, with the estimated half-life ranging from 1-6 months in surface waters (ATSDR, 1993). Reduction of a nitro group is the first step in transformation.

Spanggord et al. (1980b) demonstrated the degradation of TNT in natural waters collected below several AAPs. Degradation in water alone was slow, with half-lives of 19 to 25 days. Addition of organic nutrients or sediment accelerated the process. Studies with $^{14}C$-ring labeled TNT demonstrated that while the nitro groups were reduced, ring cleavage did not occur. The metabolites formed in water, soil, or sediments include 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, hydroxyaminodinitrotoluenes, 2,4-diamino-6-nitrotoluene, 2,6-diamino-4-nitrotoluene, 2,4,6-triaminotoluene, and tetranitroazotoluene compounds (Won et al., 1974; Weitzel et al., 1975; Jerger et al., 1976; McCormick et al., 1976; Parrish, 1977; Carpenter et al., 1978; Hoffsommer et al., 1978; Burlinson, 1980; Spanggord et al., 1980b). The formation of azoxy compounds probably results from the condensation of hydroxylamino and C-nitroso compounds. Although complete mineralization did not occur in most of the above studies, ring degradation of $^{14}C$-ring-labeled TNT was reported for

tests using the white-rot fungus, *Phanerochaete chrysosporium* (Fernando et al., 1990), under carefully controlled anaerobic conditions (Funk et al., 1993), and following removal of the nitro groups (Marvin-Sikkema and de Bout, 1994).

## 3. AQUATIC TOXICOLOGY

### 3.1 ACUTE EFFECTS - INVERTEBRATES AND FISH

Studies of the acute toxicity of TNT were conducted on five species of invertebrates and four species of fish (Table 1). These tests typically lasted 48 hours for invertebrates (except the rotifer) and 96 hours for fish; the reported endpoint was mortality ($LC_{50}$ values). Test temperatures ranged from 20 to 25°C except for studies with rainbow trout (10°C) or where the effect of temperature was determined. Lethal concentrations ($LC_{50}$ values) for invertebrates ranged from >4.4 mg/L to >29 mg/L; for fish, $LC_{50}$ values ranged from 0.8 mg/L to 3.7 mg/L. Pederson (1970) and Liu et al. (1983b) found that the acute toxicity of TNT was only minimally affected by water temperature, hardness, and pH. Both acute and some chronic studies were complicated by the photochemical transformation of TNT and the resulting presence of degradation products. According to Liu et al. (1983b), extensive photolysis of TNT reduced the toxicity of the resulting solutions to aquatic organisms.

Won et al. (1976) evaluated the acute toxicity of TNT to two saltwater species, a copepod, *Tigriopus californicus*, and oyster larvae, *Crassostrea gigas*. Groups of 100 copepods and 200 oyster larvae (average age 20 days) were incubated at 20°C for 72 and 96 hours, respectively, and evaluated for mortality. The lowest concentration resulting in death of copepods was 2.5 mg/L (18% mortality); mortality was 44% at 5 mg/L. For oyster larvae, the 5 mg/L concentration resulted in no deaths, but the 10 mg/L concentration was toxic, resulting in a mortality of 82%. $LC_{50}$ values were not computed.

Pederson (1970) exposed groups of 10 bluegill sunfish to TNT solutions for 96 hours under different water temperature and hardness conditions; solutions were renewed every 24 hours. Toxicity was slightly less at 10°C than 25°C, but water hardness did not influence toxicity.

Smock et al. (1976) reported a 96-hr $EC_{50}$ of 0.46 mg/L for behavioral response of bluegill sunfish. Responses consisted of shock (gasping at the surface and lethargy) followed by loss of motor control. A concentration of 0.05 mg/L did not produce a behavioral response.

### 3.2 CHRONIC EFFECTS - INVERTEBRATES AND FISH

Chronic tests were conducted with two species of invertebrates and three species of fish (Table 2). The 48-hr test with rotifers (Snell and Moffat, 1992) can be considered a chronic test because several generations were produced within the time period. Although EPA-recommended flow-through conditions were not used in the study with rotifers, incubation occurred in the dark, limiting the photolysis of TNT. The lowest concentration that statistically significantly affected reproduction of the rotifer over a period of 48 hours was 5.0 mg/L. However, the $EC_{50}$ for reproduction estimated from a linear regression was 4.0 mg/L.

In a 28-day study, *Daphnia magna* was tolerant of TNT concentrations of $\leq$0.48 mg/L. Using flow-through conditions, Bailey et al. (1985) tested the chronic effects of TNT at concentrations of

TABLE 1. RESULTS OF ACUTE TOXICITY STUDIES WITH AQUATIC INVERTEBRATES AND FISH

| Test Species | Test Duration | $LC_{50}$ (mg/L) | Reference |
|---|---|---|---|
| *Brachionus calyciflorus* (rotifer) | 24-hr | 9.1[a] | Snell and Moffat, 1992 |
| *Brachionus calyciflorus* (rotifer) | 24-hr | 5.6[a] | Toussaint et al., 1995 |
| *Lumbricus variegatus* (oligochaete) | 48-hr<br>48-hr | 5.2[a]<br>>29.0[b] | Liu et al., 1983b |
| *Daphnia magna* (water flea) | 48-hr<br>48-hr | 11.7[a]<br>>4.4[b] | Liu et al., 1983a;<br>Liu et al., 1983b |
| *Hyallela azteca* (scud) | 48-hr | 6.5[a] | Liu et al., 1983b |
| *Tanytarsus dissimilis* (midge) | 48-hr | 27.0[a] | Liu et al., 1983b |
| *Ictalurus punctatus* (channel catfish) | 96-hr | 2.4[a]<br>3.3[b] | Liu et al., 1983a<br>Liu et al., 1983b |
| *Lepomis macrochirus* (bluegill sunfish) | 96-hr<br>96-hr<br>96-hr<br>96-hr | 2.3[a,c]<br>2.3[a,d]<br>2.7[a,e]<br>2.8[a,f] | Pederson, 1970<br>Pederson, 1970<br>Pederson, 1970<br>Pederson, 1970 |
| *Lepomis macrochirus* (bluegill sunfish) | 96-hr | 2.6[a] | Nay et al., 1974 |
| *Lepomis macrochirus* (bluegill sunfish) | 96-hr<br>96-hr<br>96-hr | 2.6[a]<br>3.4[a]<br>2.5[b] | Liu et al., 1983a<br>Liu et al., 1983a<br>Liu et al., 1983b |
| *Pimephales promelas* (fathead minnow) | 96-hr | 2.58[b] | Smock et al., 1976 |
| *Pimephales promelas* (fathead minnow) | 96-hr<br>96-hr<br>96-hr | 2.9[a]<br>3.0[a]<br>3.7[b] | Liu et al., 1983a<br>Liu et al., 1983b |
| *Pimephales promelas* (fathead minnow) | 96-hr | 3.0[a] | Bailey and Spanggord, 1983 |
| *Oncorhynchus mykiss* (rainbow trout) | 96-hr<br>96-hr<br>96-hr | 1.5[a]<br>0.8[a]<br>2.0[b] | Liu et al., 1983b |

[a] Static tests
[b] Flow-through tests
[c] 25°C, 60 ppm $CaCO_3$
[d] 25°C, 180 ppm $CaCO_3$
[e] 10°C, 60 ppm $CaCO_3$
[f] 10°C, 180 ppm $CaCO_3$

TABLE 2. RESULTS OF CHRONIC AQUATIC TOXICITY STUDIES

| Species | Stage/age | Parameter Measured | Parameter Response[a] | Concentration (mg/L) | Reference |
|---|---|---|---|---|---|
| *Brachionus calyciflorus* (rotifer) | Newly hatched | Reproduction (r) (48-hr life cycle test) | NOEC<br>LOEC<br>$EC_{50}$ | 2.3<br>5.0<br>4.0 | Snell and Moffat, 1992 |
| *Daphnia magna* (water flea) | Young | Mortality (28 days)<br>Total reproduction<br><br>Young/female<br>Young/day/female<br>Length<br>Days - first brood | No effect<br>No effect<br>Effect - 14, 21 days<br>No effect<br>No effect<br>No effect<br>No effect | 0.03 - 1.03<br>0.48<br>1.03<br>0.03 - 1.03<br>0.03 - 1.03<br>0.03 - 1.03<br>0.03 - 1.03 | Bailey et al., 1985 |
| *Ictalurus punctatus* (channel catfish) | Eggs, fry | Percent hatch<br>Fry survival (30 days) | No effect<br>No effect | 0.11 - 1.35<br>0.11 - 1.35 | Bailey et al., 1985 |
| *Oncorhynchus mykiss* (rainbow trout) | Eggs, fry | Test 1:<br>Number hatched<br>Fry survival (30 days)<br>Average length<br>Average weight<br>Test 2:<br>Number hatched<br>Fry survival (60 days)<br>Average length<br>Average weight<br>Deformities | <br>Decreased hatch<br>Decreased<br>Decreased<br>Decreased<br><br>No effect<br>Reduced<br>Decreased<br>Decreased<br>No effect | <br>0.93<br>0.93<br>0.49<br>0.49<br><br>0.02 - 1.69<br>0.24<br>0.50<br>0.87<br>0.02 - 1.69 | Bailey et al., 1985 |
| *Pimephales promelas* (fathead minnow) | Eggs, fry | Percent hatch<br>Fry survival (30 days)<br>Average length | No effect<br>Reduced<br>Inconclusive | 0.07 - 0.84<br>0.84<br>0.84 | Bailey et al., 1985 |

TABLE 2. RESULTS OF CHRONIC AQUATIC TOXICITY STUDIES

| | | | | | |
|---|---|---|---|---|---|
| *Pimephales promelas* (fathead minnow) | $F_0$ and $F_1$ generations | $F_0$ eggs: | | | Bailey et al., 1985 |
| | | Number hatched | Reduced | 1.21 (in one series) | |
| | | Number fry deformed | No effect | 0.04 - 1.21 | |
| | | Fry survival | Reduced, 30-178 d | 0.25 (in one series) | |
| | | Fry length | Inconclusive | | |
| | | $F_0$ reproduction | | | |
| | | Pair survival | Decreased | 0.04 - 1.21 | |
| | | No. of spawns/pair | Decreased | 0.04 - 1.21 | |
| | | Eggs/pair | Decreased | 0.04 - 1.21 | |
| | | Eggs/spawn | Decreased | 1.21 | |
| | | Eggs/pair/day | Decreased | 0.56 | |
| | | $F_1$ eggs: | | | |
| | | Number hatched | Decreased | 0.04 - 1.21 | |
| | | Number deformed | Increased | 0.10 - 1.21 | |
| | | Fry survival (30 days) | Decreased | 0.04 - 1.21 | |
| | | Fry length (30 days) | No effect | 0.04 - 0.25[b] | |
| | | Fry weight (30 days) | No effect | 0.04 - 0.25[b] | |
| | | Fry survival (60 days) | No effect | 0.04 - 0.25[b] | |
| | | Fry length (60 days) | Decreased | 0.04 - 0.56[b] | |
| | | Fry weight (60 days) | Decreased | 0.04 - 0.56[b] | |

[a]Statistically significant responses reported
[b]No survival above this concentration

0, 0.03, 0.08, 0.24, 0.48, and 1.03 mg/L on *D. magna*. Daphnids were observed for mortality; reproductive success at 14, 21, and 28 days; onset of reproduction; and body length of young. These concentrations had no effect on cumulative mortality, time to first brood, or length. Significant decreases occurred in the number of young produced at days 14 and 21 at the 1.03 mg/L concentration in one series, but not by day 28. The authors considered this a transitory effect, but because average daphnid survival in the environment is less than 28 days, they felt that the impaired reproduction could have biological significance and thus considered this an effect level. The no-effect level was 0.48 mg/L.

Bailey et al. (1985) investigated the effects of TNT on early life stages of channel catfish, rainbow trout, and fathead minnows. In 30- or 60-day studies, the lowest effect concentration was 0.24 mg/L, which resulted in decreased survival of rainbow trout fry at 60 days. However, in a two-generation study, a concentration as low as 0.04 mg/L decreased several reproductive parameters of $F_0$ fathead minnows (See Table 2).

Groups of channel catfish eggs were exposed in duplicate flow-through chambers to concentrations of 0.0, 0.11, 0.15, 0.30, 0.66, or 1.35 mg/L; the temperature was 25°C and the photoperiod was 16 hr light:8 hr dark. Following hatch, the fry were exposed in the chambers for a 30-day period. Groups of 30 eggs of fathead minnows were exposed under similar conditions (with a variable photoperiod) to concentrations of 0.0, 0.07, 0.10, 0.16, 0.42, or 0.84 mg/L for a total of 30 days. Fry length was additionally measured. Groups of 60 eggs of rainbow trout were exposed at 12°C to concentrations of 0.0, 0.07, 0.12, 0.21, 0.49, or 0.93 mg/L (through 30 days post hatch) or concentrations of 0.0, 0.02, 0.04, 0.13, 0.24, 0.50, 0.87, or 1.69 mg/L (through 60 days post hatch). Fry were weighed and observed for deformities in the second test.

The results are summarized in Table 2. Concentrations of $\leq$ 1.35 mg/L had no effect on percent hatch or survival of channel catfish fry over a 30-day period. In tests with rainbow trout, hatch was decreased at 0.93 mg/L in the first test, but in the second test, hatch was not affected by concentrations of $\leq$ 1.69 mg/L. Concentrations resulting in reduced survival and decreased length and weight were 0.24 and 0.50 mg/L, respectively. There was no increase in deformities at the tested concentrations.

Although percent hatch of fathead minnow eggs was not affected by concentrations $\leq$ 0.84 mg/L, fry survival was reduced at 30 days for the 0.84 mg/L treatment concentration (50% for controls vs 32% for treated group) and fry length was reduced in one of the two duplicate chambers at 0.84 mg/L. Statistical analyses were not performed. However, in comparing the 30-day studies, rainbow trout and fathead minnows appear more sensitive to TNT exposure than channel catfish.

Bailey et al. (1985) also studied effects of chronic exposure of fathead minnows to concentrations of 0.00, 0.04, 0.10, 0.25, 0.56, or 1.21 mg/L of TNT over two generations. The $F_1$ generation was observed for 30 or 60 days post hatch under test conditions. $F_0$ and $F_1$ egg and fry survival, length, deformities and $F_0$ fertility indices were determined. Most of the reproductive parameters of breeding pairs that developed from the $F_0$ eggs were adversely affected at all concentrations. The overall effect on reproduction was due to reductions in the survival of the breeding pairs and frequency of spawns per pair. Total survivability and total productivity indices indicated that TNT had a deleterious effect on fathead minnows at all tested concentrations.

Incipient $LC_{50}$ values, the concentration above which 50% of organisms cannot survive indefinitely, were determined for several aquatic species (Liu et al., 1983b). The tests were conducted under flow-through conditions for various periods of time. Incipient $LC_{50}$ values and exposure time in hours (in parentheses) were: *D. magna*, 0.19 mg/L (192); *L. variegatus*, 13.9 mg/L (336); *L. macrochirus*, 1.4 mg/L (312); *O. mykiss*, 1.9 mg/L (240); *I. punctatus*, 1.6 mg/L (288), and *P. promelas*, 1.5 mg/L (384).

### 3.3 PLANTS

Short-term and chronic tests using growth parameters as the endpoint were conducted with several species of algae and an aquatic vascular plant (Table 3). As noted by some of the authors, the light necessary for plant growth in these static tests resulted in photolysis of the TNT. Fitzgerald et al. (1952) exposed cultures of the bluegreen algae, *Microcystis aeruginosa*, grown in 125-mL flasks at 22°C to TNT for 24 hrs. A concentration of 8 ppm (mg/L) resulted in 100% mortality. Bringmann and Kuehn (1980) tested *Scenedesmus quadricauda* colonies for effects using the cell multiplication inhibition test. Colonies were exposed to a series of TNT concentrations for 16 hr at 27°C; effects were measured by comparing turbidimetric values expressed by the extinction of primary light of monochromatic radiation at 578 nm for a 10-mm thick layer. The "toxicity threshold" was 1.6 mg/L.

In longer-term studies, Won et al. (1976) evaluated the toxicity of TNT to the green alga, *Selenastrum capricornutum*. Incubation was for seven days and toxicity was evaluated in terms of dry weight and morphological alterations. A concentration of 2.5 mg/L significantly depressed growth (44% of controls) and produced a population of ballooned, extensively granulated cells. A chronic $EC_{50}$ was not calculated.

Smock et al. (1976) exposed cultures of *S. capricornutum* to concentrations of 0, 1, 3, 5, 7, or 9 mg/L for 17 days and cultures of *M. aeruginosa* to 0, 5, 10, 15, 25, or 50 mg/L for 15 days under static test conditions. Concentrations up to 3 mg/L had no effect on the growth of *S. capricornutum*. An initial decrease in growth occurred at concentrations $\geq$5 mg/L, but the effect disappeared by day 17. For *M. aeruginosa*, no effects on growth occurred up to a concentration of 15 mg/L, temporary decreases occurred at 25 mg/L, and growth was permanently retarded throughout the 15-day assay at 50 mg/L. This result contrasts with that of Fitzgerald et al. (1952) who reported 100% mortality for *M. aeruginosa* at 8 mg/L. Smock et al. (1976) reported that TNT could no longer be measured in the test solutions after 7 days.

Liu et al. (1983b) studied the effects of TNT on several species of algae: *S. capricornutum*, *M. aeruginosa*, *Anabaena flos-aquae* (bluegreen algae), and *Navicula pelliculosa* (diatom) using the U.S. EPA bottle technique (static test). Effects were detected by measuring cell concentrations following exposure to a series of TNT concentrations for 14 days. Growth stimulation occurred at low concentrations. The lowest concentrations that inhibited growth was 4.1 mg/L for *S. capricornutum* and *M. aeruginosa*, 8.2 mg/L for *A. flos-aquae*, and 18 mg/L for *N. pelliculosa*. However, photolysis of the TNT, as indicated by the reddish-brown color of the solutions, made the results of these tests unreliable.

Schott and Worthley (1974) exposed the aquatic vascular plant, *Lemna perpusilla*, to concentrations of TNT ranging from 0.01 to 50 mg/L for 11 days. A concentration of 0.5 mg/L had

TABLE 3. RESULTS OF TOXICITY TESTS WITH SPECIES OF ALGAE AND A VASCULAR PLANT[a]

| Test Species | Test Duration | Effect | Concentration (mg/L) | Reference |
|---|---|---|---|---|
| *Microcystis aeruginosa* (bluegreen algae) | 24 hr | 100% mortality | 8 | Fitzgerald et al., 1952 |
| *Microcystis aeruginosa* (bluegreen algae) | 14 days | Growth inhibition (LOEC) | 4.1 | Liu et al., 1983b |
| *Microcystis aeruginosa* (bluegreen algae) | 15 days | No effect on growth<br>Initial decrease in growth<br>Permanent decrease in growth | 15<br>25<br>50 | Smock et al., 1976 |
| *Anabaena flos-aquae* (bluegreen algae) | 14 days | Growth inhibition (LOEC) | 8.2 | Liu et al., 1983b |
| *Scenedesmus quadricauda* (green algae) | 16 hr | "Toxicity threshold" | 1.6 | Bringmann and Kuehn, 1980 |
| *Selenastrum capricornutum* (green algae) | 7 days | Growth depression (12%)<br>Growth depression (56%); changes in morphology | 1.0<br>2.5 | Won et al., 1976 |
| *Selenastrum capricornutum* (green algae) | 14 days | $EC_{50}$ for growth | 1.5 | Bailey, 1982 |
| *Selenastrum capricornutum* (green algae) | 14 days | Growth inhibition (LOEC) | 4.1 | Liu et al., 1983b |
| *Selenastrum capricornutum* (green algae) | 17 days | No effect on growth<br>Initial decrease in growth<br>Significant decrease in growth | 3<br>5<br>$\geq$7 | Smock et al., 1976 |
| *Navicula pelliculosa* (diatom) | 14 days | Growth inhibition (LOEC) | 18 | Liu et al., 1983b |
| *Lemna perpusilla* (vascular plant) | 11 days | No effect on colony growth<br>Growth depression ($\geq$10%)<br>Mortality | 0.5<br>1.0<br>$\geq$5 | Schott and Worthley, 1974 |

[a] All tests were conducted under static conditions.

no effect on colony growth, whereas growth was depressed at 1.0 mg/L and death occurred at concentrations $\geq$5.0 mg/L. A change in pH (6.3 or 8.5) did not affect toxicity.

## 3.4 METABOLISM AND BIOCONCENTRATION

Studies on the metabolism of TNT by aquatic organisms were not located in the available literature. Calculated and measured log octanol-water partition coefficients ($K_{ow}$) values of 1.6-2.7 (ATSDR, 1993) indicate a low potential for bioconcentration.

The potential for TNT to bioconcentrate in aquatic species as determined by uptake of $^{14}C$-labeled-TNT was evaluated by Liu et al. (1983a; 1983b). The tests were performed for 96 hours under static exposure conditions. The four-day bioconcentration factor (BCF) was calculated by dividing the average amount of radioactivity in the biological samples by the amount in the test water; no corrections were made for metabolites. For the intact organisms, the BCF ranged from 200 to 450 (Table 4). The authors felt that steady-state conditions were not achieved in this experiment; thus, the values represent one point on the uptake curve. Because steady-state conditions were not reached and metabolic products were not considered, Liu et al. (1983a; 1983b) also calculated the potential to bioconcentrate in fish using a calculated $K_{ow}$ and the relationship between log $K_{ow}$ and the steady-state BCF developed by Veith et al. (1980). The calculated value of 20.5 represents a low potential for bioconcentration. Although the implication of not reaching steady-state conditions in the laboratory study would be empirical BCF values that are too low, it should be noted that the measured values are much higher than the modeled value.

TABLE 4. BIOCONCENTRATION FACTORS (BCF) IN AQUATIC SPECIES

| Species | Value | Comments | Reference |
|---|---|---|---|
| Fish | 20.5 | steady-state BCF computed using calculated log $K_{ow}$ of 2.03 and equation for organism lipid content of 8% | Liu et al., 1983a<br>Liu et al., 1983b |
| *Selenastrum capricornutum* (green algae) | 453 | four-day BCF, static test | Liu et al., 1983b |
| *Daphnia magna* (water flea) | 209 | four-day BCF, static test | Liu et al., 1983b |
| *Lumbricus variegatus* (oligochaete) | 202 | four-day BCF, static test | Liu et al., 1983b |
| *Lepomis macrochirus* (bluegill)<br>viscera<br>muscle | <br>338<br>9.5 | four-day BCF, static test | Liu et al., 1983b |

## 3.5 AQUATIC CRITERIA AND SCREENING BENCHMARKS

### 3.5.1 Aquatic Organisms

Although the U.S. EPA has not calculated numerical national Water Quality Criteria (WQC) for TNT, sufficient data are available to do so according to the guidelines (Stephan et al., 1985). Data are sufficient for deriving both an acute and a chronic criterion. In the absence of chronic criteria, U.S. EPA lists lowest chronic values for fish and invertebrates. Although a chronic criterion was derived, lowest chronic values are also listed in Table 5 as they can be used as screening benchmarks. From the data in Tables 1-4, the following were derived or identified (calculations are contained in Appendix A), and values are listed in Table 5.

**Final Acute Value.** Although not all tests for the species of the eight required families were conducted under flow-through conditions, the guidelines make provisions for the use of static tests if performed under acceptable standards. In addition, data on an additional, unrequired genus were available.

**Acute Water Quality Criterion.** The acute WQC, also called the Criterion Maximum Concentration, is equal to one-half the Final Acute Value.

**Final Chronic Value.** According to U.S. EPA guidelines (Stephan et al., 1985), the chronic WQC can be calculated from acute/chronic ratios. Acute tests and chronic tests conducted under flow-through conditions are available for *D. magna* and three species of fish (Table 2). The static tests with *Brachionus calyciflorus* may be considered in this series as they were of a short duration and conducted in the dark, inhibiting the decomposition of TNT. The Final Chronic Value is calculated by dividing the Final Acute Value by the Final Acute/Chronic Ratio. The chronic WQC, also called the Criterion Continuous Concentration, is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value.

**Lowest Chronic Values** for invertebrates and fish, used by EPA in the absence of chronic data, are also listed in Table 5. These values are lowest observed effect levels.

Although data from tests of longer than 96 hours duration were available for five species of algae and one aquatic vascular plant (Table 3), the tests were conducted under static conditions, resulting in decomposition of the TNT and color changes in the solutions as noted by the investigators. In addition, concentrations were not measured at the end of the test. Because of these changes and the presence of decomposition products of unknown toxicity, the tests cannot be considered reliable and a **Final Plant Value** was not calculated. Until test results using static renewal or flow-through conditions become available, the 1.0 mg/L concentration for growth depression measured by Won et al. (1976) for *Selenastrum capricornutum* and Schott and Worthley (1974) for *Lemna perpusilla* can be considered protective of aquatic plants (lowest chronic value). This value is further confirmed as conservative by the absence of an effect on the growth of *Selenastrum capricornutum* during the first three days when tested at 3 mg/L (Smock et al., 1976). Since this value is higher than that calculated from chronic animal studies, it can be assumed that aquatic plants would be protected by the level determined from the chronic animal data.

**Final Residue Value.** A Final Residue Value for aquatic organisms cannot be calculated because data on maximum permissible tissue concentrations are not available. In addition, data for

| Table 5. Water Quality Criteria/Screening Benchmarks for Aquatic Organisms | |
|---|---|
| Criterion | Value (mg/L) |
| Acute Water Quality Criterion | 0.57[a] |
| Chronic Water Quality Criterion | 0.13[a] |
| Lowest Chronic Value - fish | 0.04 |
| Lowest Chronic Value - daphnids | 1.03 |
| Lowest Chronic Value - plants | 1.0 |
| Final Residue Value | insufficient data |
| Screening Sediment Quality Benchmark | 0.52[b] |

[a] Calculated by ORNL.
[b] mg/kg sediment

determining a BCF were not sufficient as the test by Liu et al. (1983a) lasted only four days versus the 28 days required by U.S. EPA guidelines.

### 3.5.2 Sediment-Associated Organisms

No laboratory studies utilizing benthic organisms and sediments were located. In a survey of periphyton and benthic macroinvertebrate communities in streams at the Iowa AAP, no correlations could be made between aqueous and sediment levels of TNT and diversity indices (Sanocki, et al., 1976).

For nonionic chemicals, the Equilibrium Partitioning (EqP) approach can be used to develop a screening Sediment Quality Benchmark (SQB) using the equation SQB = $K_p$ x WQC where $K_p$ (the partition coefficient) is the ratio of the sediment concentration of a chemical to the interstitial water concentration in units of L/kg and the chronic WQC is in units of mg/L (Di Toro et al., 1991; Hull and Suter, 1994). For this calculation the most conservative $K_p$ value of 4 (Pennington, 1988) and the chronic WQC of 0.13 mg/L were used to calculate a screening SQB. The resulting screening SQB is listed in Table 5.

## 4. TERRESTRIAL TOXICOLOGY

### 4.1 MAMMALS

No subchronic or chronic studies on the toxicity of TNT utilizing mammalian wildlife were located. Laboratory studies utilizing laboratory rats and mice were summarized in the companion document to this one, *Toxicity Summary for 2,4,6-Trinitrotoluene* (Opresko, 1995).

## 4.2 BIRDS

No subchronic or chronic studies on the toxicity of TNT to birds were located.

## 4.3 INVERTEBRATES

Earthworm survival and growth tests were conducted with munitions-contaminated soils from the Joliet AAP (Simini et al., 1995). Toxicity of soil samples was highly correlated with TNT concentrations although the results may have been influenced by the presence of other contaminants. The LOECs of TNT at two sites within the plant were 7 and 19 mg/kg of soil.

Parmelee et al. (1993) used a soil microcosm to test the effects of TNT on soil fauna communities and trophic structure. TNT was added at concentrations of 0, 25, 50, 100, or 200 mg/kg to tubes containing soil with natural populations of invertebrates. After 7 days, nematode numbers were increased in the treated soils as a result of greater numbers of hatchlings. Total microarthropod numbers at 200 mg/kg were reduced by 50% compared to the control soil, but the difference was not statistically significant. The reduction in oribatid mite numbers was statistically significant at this concentration. Only 15% of the applied TNT was extractable after 7 days.

## 4.4 PLANTS

Tubers of yellow nutsedge (*Cyperus esculentus*) were germinated in hydroponic solutions containing 0, 5, 10, or 20 mg/L TNT (Palazzo and Leggett, 1986). Solutions were replaced after three weeks and plants were harvested after 42 days. Leaf and root growth were statistically significantly reduced at all TNT concentrations compared to the control. At the 5 mg/L concentration, root growth (dry weight) was reduced by 95% compared with the control value (0.06 g compared with 1.18 g); root length (6 cm) was 26% of the control value (23 cm). Plant height was 18 cm compared with the control value of 28 cm. Rhizome dry weight was affected at concentrations $\geq$10 mg/L. Although tuber dry weights were greatly reduced compared to controls, the differences were not statistically significant.

Yellow nutsedge was grown in pots containing either Tunica silt or Sharkey clay soils and 80 $\mu$g/g (mg/kg, dry weight) of TNT (Pennington, 1988). Forty-five days after planting, plant yields (g, dry weight) of the treated plants from the two soils were higher than yields of respective control plants, but the differences were not statistically significant.

Bean, wheat, or blando brome were grown in pots amended with TNT at concentrations of 10, 30, or 60 ppm (mg/kg, dry weight) TNT (Cataldo et al., 1989). Two soils were tested, Palouse (1.7% organic matter) and Cinebar (7.2% organic matter). Plant height was reduced by >50% in all species in both soils at the 60 mg/kg concentration and a reduction of ~25% occurred in the wheat and grass at the 30 mg/kg concentration. Marked chlorosis and tip burn were also observed at the 60 mg/kg concentration. No phytotoxic symptoms were observed at the 10 mg/kg concentration.

The root elongation test was conducted with lettuce (*Lactuca sativa*) seeds placed in TNT amended agar (Toussaint et al., 1995). After 96 hours at TNT concentrations of 5 and 50 mg/L, root tip blackening and negative geotropism occurred, with roots growing away from the agar. The $EC_{50}$ was 2.34 mg/L.

Cucumber and radish seedling survival and growth tests were conducted with munitions-contaminated soils from the Joliet AAP (Simini et al., 1995). Toxicity of soil samples was highly correlated with TNT concentrations although, as noted above, the results may have been influenced by the presence of other contaminants. The LOECs of TNT at two sites within the plant were 7 and 19 mg/kg of soil.

## 4.5 METABOLISM AND BIOACCUMULATION

### 4.5.1 Animals

Metabolism. In mammalian species, TNT is absorbed by inhalation, ingestion, or skin contact. More than 50% of an orally administered dose is absorbed in most test species. Metabolism is extensive and rapid, with 50 to 70% excreted in the urine and the remainder in the feces; the majority of the ingested compound is excreted within 24 hours. Distribution to the tissues is less than 1%. Several metabolites including the dinitro-aminotoluene, diamino-nitrotoluene, hydroxy-amino-dinitrotoluene as well as glucuronide-conjugated metabolites have been recovered from the urine. The urine of some species is bright red in color (ATSDR, 1993).

Bioaccumulation. Rapid metabolism and excretion in mammalian species (ATSDR, 1993), indicates a low potential for bioaccumulation.

### 4.5.2 Plants

Metabolism. TNT is metabolized by plants grown in TNT-amended soils and solutions as evidenced by the recovery of metabolites such as 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene from the plant tissues (Palazzo and Leggett, 1986; Cataldo et al., 1989).

Bioconcentration. Bioconcentration of TNT by yellow nutsedge was studied in hydroponic solutions containing 5, 10, or 20 mg/L of TNT (Palazzo and Leggett, 1986). Solutions were replaced after three weeks. After 42 days, roots, leaves, rhizomes, and tubers were analyzed for TNT and its metabolites, 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene. TNT was taken up by the plant and, along with its metabolites, was translocated throughout the plants with highest concentrations in the roots. Uptake generally increased with increasing TNT concentrations in the growth medium. At the 20 mg/L concentration, concentrations in plant roots were 714, 614, and 2180 mg/kg dry weight for TNT, 2-amino-4,6-dinitrotoluene, and 4-amino-2,6-dinitrotoluene, respectively. At the 20 mg/L concentration, leaves, rhizomes, and tubers contained 13, 95, and 69 mg/kg dry weight of TNT, respectively. Results of analyses for metabolites in the growth medium were negative.

$^{14}$C-labeled-TNT added to soil (80 mg/kg) was used to study uptake by yellow nutsedge (Pennington, 1988). Uptake of TNT was limited and the compound did not bioaccumulate. Measurement of plant radioactivity did not allow identification of specific compounds. Lack of bioavailability was attributed to loss from soil by volatilization of degradation products and adsorption of TNT and its metabolites to soil.

## 4.6. TERRESTRIAL CRITERIA AND SCREENING BENCHMARKS

### 4.6.1 Animals

Mammals. No subchronic or chronic studies on the toxicity of TNT utilizing mammalian wildlife were located. Several subchronic and chronic studies utilizing TNT and laboratory animals have been reported. A human oral Reference Dose (RfD) of 0.0005 mg/kg/day was identified by extrapolation (and multiplication by uncertainty factors) from a chronic feeding study with dogs (U.S. EPA, 1993). In the same manner that safe levels for humans are based on laboratory animals, reference doses or screening benchmarks for wildlife may also be calculated by extrapolation among mammalian or avian species. Studies on surrogate laboratory species are summarized in the companion document to this one, *Toxicity Summary for 2,4,6-Trinitrotoluene* (Opresko, 1995). In these studies NOAELs for TNT administered by the oral route were identified; endpoints selected were those that diminished population growth or survival.

The study by Dilley et al. (1982) in which Sprague-Dawley rats were administered TNT in the diet at doses of 0, 1.40, 6.97, 34.7 or 160 mg/kg/day for 13 weeks was chosen to derive screening benchmarks for representative wildlife. A NOAEL of 1.60 mg/kg/day was calculated from a subchronic LOAEL of 160 mg/kg/day with the endpoint being effects on the reproductive system (testicular atrophy). Uncertainty factors of 0.1 for conversion of the subchronic LOAEL to a subchronic NOAEL and 0.1 for conversion of the subchronic NOAEL to a chronic NOAEL were applied. Screening benchmarks for oral intake for selected wildlife species are presented in Table 6. The general methodology for deriving these benchmarks is presented in Appendix B (see also Opresko et al., 1994).

Birds. No suitable subchronic or chronic studies were found for representative avian species.

### 4.6.2 Plants

In the absence of criteria for terrestrial plants, no- or lowest-observed-effects-levels or concentrations from the literature can be used to screen for chemicals of potential concern.

Palazzo and Leggett (1986) reported reductions in growth of yellow nutsedge at the lowest solution tested, 5 mg/L. Therefore, based on this single study, the screening benchmark for soil water would be <5 mg/L. Pennington (1988) reported no effects on the growth of yellow nutsedge at a soil concentration of 80 mg/kg (dry weight) of TNT. However, Cataldo et al. (1989) reported phytotoxic effects at 30 mg/kg, but not at 10 mg/kg. Therefore, based on the second study, the lowest-observed-effect-concentration (LOEC) would be 30 mg/kg and the no-observed-effect-concentration (NOEC) would be 10 mg/kg. Very little confidence can be placed in benchmarks determined from one or two studies; thus, additional studies are needed in order to determine screening benchmarks with a high degree of confidence. Using the method Will and Suter (1994a), the phytotoxicity benchmarks in solution and soil would be 5 mg/L and 30 mg/kg, respectively. However, the benchmarks have a rating of "low confidence."

### 4.6.3 Soil Invertebrates

A single soil microcosm study, conducted over a period of 7 days (Parmelee et al., 1993), reported a NOEC on numbers of soil nematodes and microarthropods of 100 mg/kg TNT and a

## TABLE 6. SCREENING BENCHMARKS FOR SELECTED MAMMALIAN WILDLIFE SPECIES[a]

| | Wildlife | | Experimental Values | | | Extrapolated Values for Chronic Exposures | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Screening Benchmarks | | | |
| Experimental animal (body wt) | Species | Body Wt. (kg) | LOAEL (mg/kg/d) | NOAEL (mg/kg/d) | Endpoint | NOAEL (mg/kg/d) | Diet (mg/kg food) | Water (mg/L) | Aquatic feeding sp. (mg/L) | Reference (LOAEL/NOAEL) |
| Sprague-Dawley rat (0.318 kg) | | | 160 (90 d) | - | testes | 1.6 | | | | Dilley et al., 1982 |
| | short-tailed shrew | 0.015 | | | | 4.4 | 7.3 | 20.1 | | |
| | white-footed mouse | 0.022 | | | | 3.9 | 25.1 | 13.0 | | |
| | meadow vole | 0.044 | | | | 3.1 | 27.1 | 22.7 | | |
| | cottontail rabbit | 1.2 | | | | 1.0 | 5.2 | 79.1 | | |
| | mink | 1.0 | | | | 1.1 | 8.0 | 11.0 | 0.28 | |
| | red fox | 4.5 | | | | 0.66 | 6.6 | 7.9 | | |
| | whitetail deer | 56.5 | | | | 0.28 | 9.2 | 4.4 | | |

[a]For a detailed description of the methodology, see Opresko et al., 1994.

LOEC of 200 mg/kg TNT. Therefore, using the method of Will and Suter (1994b), the benchmark for other soil invertebrates is 200 mg/kg. That benchmark has a rating of "low confidence." There are no benchmarks for earthworms or for microbial processes.

TABLE 7. SCREENING BENCHMARKS FOR TERRESTRIAL PLANTS AND INVERTEBRATES[a]

| Screening Benchmark | Value |
|---|---|
| Plants - solution<br>Plants - soil | 5 mg/L<br>30 mg/kg |
| Soil invertebrates (earthworms)<br>Soil invertebrates (other) | no data<br>200 mg/kg |
| Soil microbial processes | no data |

[a]For a detailed description of the methodology, see Will and Suter, 1994a; 1994b.

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# APPENDICES

## APPENDIX A

## DERIVATION OF AQUATIC BENCHMARKS

### A. Calculation of Final Acute Value (FAV)[a]

Genus Mean Acute Values (GMAVs) were calculated from data in Table 1. The 9 GMAVs were ordered and the four lowest were assigned ranks (R) from the lowest to highest: rainbow trout, Rank 1; bluegill sunfish, Rank 2; channel catfish, Rank 3; and fathead minnow, Rank 4. The tests with bluegill sunfish conducted at 10°C were not included in the GMAV calculation.

| Rank (R) | GMAV[b] | ln GMAV[c] | $(\ln GMAV)^2$ | $P=R/(N+1)$[d] | $\sqrt{P}$ |
|---|---|---|---|---|---|
| 4 | 3.02 | 1.1053 | 1.2216 | 0.4 | 0.6325 |
| 3 | 2.81 | 1.0332 | 1.0675 | 0.3 | 0.5477 |
| 2 | 2.59 | 0.9517 | 0.9057 | 0.2 | 0.4472 |
| 1 | 1.34 | 0.2927 | 0.0857 | 0.1 | 0.3162 |
| Sum | | 3.3828 | 3.2804 | 1.0 | 1.9436 |

[a] Based on Stephan et al., 1985.
[b] Genus Mean Acute Value of $LC_{50}$ values in mg/L, based on data in Table 1.
[c] ln GMAV = natural log of GMAV
[d] P = comparative probability for each GMAV; N = 9 GMAVs

$$S^2 = \frac{\Sigma[(\ln GMAV)^2] - [\frac{(\Sigma \ln GMAV)^2}{4}]}{\Sigma(P) - \frac{[(\Sigma\sqrt{P})^2]}{4}} = 7.55$$

$$S = 2.75$$

$$L = \frac{[\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P}))]}{4} = -0.4893$$

$$A = S(\sqrt{0.05}) + L = 0.1250$$

$$FAV = e^{0.1250} = 1.1332\ mg/L$$

B. Calculation of Final Chronic Value (FCV)

The FCV was calculated by dividing the FAV by the Final Acute-Chronic Ratio (FACR) according to the method of Stephan et al. (1985). From data in Tables 1 and 2, acute-chronic ratios (A/C) were available for a rotifer, a daphnid, rainbow trout and the fathead minnow. A lowest effect concentration was not found for channel catfish.

rotifer A/C = 9.1/4.0 = 2.28
5.6/4.0 = 1.4
Geometric mean = 1.78

daphnid A/C = 7.17/1.03 = 6.97

rainbow trout A/C = 1.34/0.24 = 5.58

fathead minnow A/C = 3.02/0.04 = 75.5

FACR = Geometric mean of the A/C ratios = 8.51

FCV = FAV/FACR = 1.1325/8.51 = 0.1331 mg/L

## APPENDIX B

## DERIVATION OF WILDLIFE BENCHMARKS

### B.1 General Methodology

The general method used in estimating screening benchmarks for wildlife is based on EPA methodology for deriving human toxicity values (e.g., Reference Values, Reportable Quantities, and unit risks for carcinogenicity) from laboratory animal data (see Opresko et al., 1994). In this approach a NOAEL (no-observed-adverse-effect level) for population-related effects (e.g., survival, reproductive function) is identified from a study conducted with laboratory animals, and the equivalent NOAEL for a particular species of wildlife (wildlife NOAEL) is obtained by scaling the laboratory data (test NOAEL) on the basis of differences in body size according to the following equation:

$$\textit{Wildlife NOAEL} = \textit{test NOAEL} \times \left[\frac{\textit{test bw}}{\textit{wildlife bw}}\right]^{1/4}$$

where:

wildlife bw = body weight of wildlife species in kg
test bw = body weight of laboratory species in kg
test NOAEL = experimental dose in mg/kg/day

Body weights for selected wildlife species are given in Table B-1; data sources are listed in Opresko et al. (1994).

The dietary level, or concentration in food (Cf, in mg/kg food) that would result in a dose equivalent to the NOAEL (assuming no other exposure through other environmental media) was calculated from the food factor $f$, which is the amount of food consumed per unit body weight per day:

$$Cf = \frac{\textit{Wildlife NOAEL}}{f}$$

Food factors for the species of wildlife used in the calculations are shown in Table B-1; data sources are given in Opresko et al. (1994).

The drinking water concentration (Cw, in mg/L) that would result in a dose equivalent to the NOAEL (assuming no other exposure through other environmental media) was calculated from the water factor $\omega$, which is the amount of water consumed per unit body weight per day:

$$Cw = \frac{\textit{Wildlife NOAEL}}{\omega}$$

Water factors for the species of wildlife used in the calculations are shown in Table B-1.

| Table B-1. Reference Values for Selected Wildlife | | | |
|---|---|---|---|
| Species | Body Weight (kg) | Food Factor $f$ | Water Factor $\omega$ |
| short-tailed shrew | 0.015 | 0.6 | 0.22 |
| white-footed mouse | 0.022 | 0.155 | 0.3 |
| meadow vole | 0.044 | 0.114 | 0.136 |
| cottontail rabbit | 1.2 | 0.198 | 0.013 |
| mink | 1.0 | 0.137 | 0.099 |
| red fox | 4.5 | 0.1 | 0.084 |
| whitetail deer | 56.5 | 0.031 | 0.065 |

If a wildlife species (such as mink) feeds primarily on aquatic organisms, and the concentration of the contaminant in the food is proportional to the concentration in the water, then the food consumption rate (F, in mg/day), the body weight, the water consumption rate (W, in L/day), and the aquatic life bioaccumulation factor (BAF) can be used to derive an overall Cw that incorporates both water and food consumption:

$$Cw = \frac{NOAEL_w \times bw_w}{W + (F \times BAF)}$$

The BAF is the ratio of the concentration of the contaminant in tissue (mg/kg) to its concentration in water (mg/L), where the organism and the prey are exposed. BAF can be predicted by multiplying the bioconcentration factor (BCF), by the appropriate for food chain multiplying factor (FCM) (see Opresko et al., 1994). The FCM is a function of the log $K_{ow}$ of the contaminant and the prey trophic level. Because mink feed on small fish the trophic level 3 FCM was used in the calculation. The log $K_{ow}$ for TNT is 2.2 (Spanggord et al., 1985). The trophic level 3 FCM for this log $K_{ow}$ is 1.0 (see Opresko et al., 1994).

The BCF for TNT can be estimated from the log $K_{ow}$ by the following equation:

$$\log BCF = 0.76 \log K_{ow} - 0.23 = 1.4$$

$$BCF = 27.7$$

For mink, bw = 1.0 kg, F = 0.137 kg/day, and W = 0.099 L/day (see Opresko et al., 1994).

Therefore:

$$Cw = \frac{1.1 \times 1.0}{[0.099 + (0.137 \times 27.7)]} = 0.28\ mg/L$$

B.2 Reference Study for Use in Deriving Wildlife Benchmarks

| | |
|---|---|
| Compound: | 2,4,6-Trinitrotoluene |
| Reference: | Dilley, J.V., C.A. Tyson, R.J. Spanggord, D.P. Sasmore, G. W. Newell and J.C. Dacre. 1982. Short-term oral toxicity of 2,4,6-trinitrotoluene in mice, rats and dogs. J. Toxicol. Environ. Health 9(4):565-585. |
| Test species: | Sprague-Dawley rats.<br>Body weight: average weights for males in the different test groups ranged from 0.168 to 0.170 kg at the start of the study, and from 0.369 to 0.474 kg at the end of 13 weeks. An overall time-weighted average of 0.318 kg was estimated for the high-dose group, and this value was used in the calculations. |
| Exposure Duration: | 13 weeks |
| Endpoint: | Reproductive system - testicular atrophy |
| Exposure Route: | Dietary |
| Dosage: | 0, 1.40, 6.97, 34.7, and 160 mg/kg/day (calculated by the authors from food intake and dietary levels of 0.0025, 0.01, 0.05, and 0.25%, respectively |
| Calculations: | not applicable |
| Comments: | Although signs of anemia and some organ weight changes occurred at two intermediate dose levels, the testicular atrophy seen at the highest dose is considered to be more relevant for population effects. Therefore, 160 mg/kg is considered to be a subchronic LOAEL. The authors state that testicular "lesions did not occur or were far less frequent in other groups"; however, it is not clear whether the dose level of 34.7 mg/kg could be considered the NOAEL for these effects. Therefore, the NOAEL was estimated by multiplying the LOAEL by the LOAEL-NOAEL uncertainty factor of 0.1 and the chronic NOAEL was estimated by multiplying the subchronic NOAEL by the subchronic-chronic uncertainty factor of 0.1. |
| Final NOAEL: | 1.60 mg/kg/day |