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EMERGENCY RESPONSE

Prepared by

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PREFACE

Health and Environmental Effects Documents (HEEDs) are prepared for the Office of Solid Waste and Emergency Response (OSWER). This document series is intended to support listings under the Resource Conservation and Recovery Act (RCRA) as well as to provide health-related limits and goals for emergency and remedial actions under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained for Agency Program Office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched for in this document and the dates searched are included in "Appendix: Literature Searched." Literature search material is current up to 8 months previous to the final draft date listed on the front cover. Final draft document dates (front cover) reflect the date the document is sent to the Program Officer (OSWER).

Several quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic and subchronic exposures for both the inhalation and oral exposures. The subchronic or partial lifetime RfD is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval i.e., for an interval that does not constitute a significant portion of the lifespan. This type of exposure estimate has not been extensively used, or rigorously defined as previous risk assessment efforts have focused primarily on lifetime exposure scenarios. Animal data used for subchronic estimates generally reflect exposure durations of 30-90 days. The general methodology for estimating subchronic RfDs is the same as traditionally employed for chronic estimates, except that subchronic data are utilized when available.

In the case of suspected carcinogens, a carcinogenic potency factor, or q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively. An RfD may also be derived for the noncarcinogneic health effects of compounds that are also carcinogenic.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity, and acute mammalian toxicity). Chemical-specific RQs reflect the lowest of these six primary criteria. The methodology for chronic toxicity and cancer based RQs are defined in U.S. EPA, 1984 and 1986a, respectively.

EXECUTIVE SUMMARY

Trinitrotoluene, commonly referred to as TNT, is a yellow crystalline solid at room temperature. It is soluble in alcohol, ether, acetone, benzene and carbon disulfide and slightly soluble in water (Sax and Lewis, 1987; Windholz et al., 1983). Between 3.1 and 31.1 million pounds of trinitrotoluene was produced or imported in the United States in 1977 at six different facilities (TSCAPP, 1989). Earlier production data list the monthly production of trinitrotoluene in the United States at ~45 million pounds during 1969-1971 (Ryon et al., 1984). Trinitrotoluene is produced by the nitration of toluene in a concentrated mixture of sulfuric and nitric acids (Ryon et al., 1984). It is used chiefly as a high or bursting explosive and as an intermediate in dyestuffs and photographic chemicals (Sax and Lewis, 1987).

The dominant fate process for trinitrotoluene in water is expected to be destruction by direct photolysis. The half-life for the sunlight photolysis of trinitrotoluene in pure water is 14 hours over the continental United States during the summer months (Mabey et al., 1983). The sunlight photolysis of trinitrotoluene in distilled water at room temperature occurred at a similar rate with a half-life of 15 hours (Burlinson et al., 1973). The rate was nearly pH-independent, increased with the presence of humic material, and decreased in the presence of oxygen and other triplet quenchers (Mabey et al., 1983). The biological degradation of trinitrotoluene in environmental waters is expected to occur under both aerobic and anaerobic conditions, although the presence of additional nutrients may be required (Carpenter et al., 1978; Osmon and Klausmeier, 1973; Chambers et al., 1963; Tabak et al., 1964; Enzinger, 1970; Spanggord et al., 1981, 1983;

Fewson, 1981; Hoffsommer et al., 1978). Products from both the aerobic and anaerobic degradation of trinitrotoluene are believed to come from initial reduction of one or more of the nitro groups, which are then capable of undergoing nonenzymatic reactions, which may result in the formation of products that are degradation-resistant. Complete degradation of trinitrotoluene to CO_2 has not been observed. Neither hydrolysis nor volatilization to the atmosphere are expected to be significant in water. Adsorption to sediment and suspended organic matter may occur, although it is not expected to be a significant process (Ryon et al., 1984). Bioconcentration in fish and aquatic organisms is not expected to be a significant process. If released to soil, microbial degradation is expected to occur (Kaplan and Kaplan, 1982a; Chambers et al., 1963; Tabak et al., 1964; Osmon and Klausmeier, 1973). The potential for strong adsorption and, thus, low mobility in soil has been demonstrated by Kayser and Burlinson (1988). Volatilization from the soil surface to the atmosphere is not expected to be significant. In the atmosphere, trinitro- toluene is expected to exist in both the vapor phase and the particulate form (Jones, 1960; Pella, 1977; Eisenreich et al., 1981). No experimental data on the direct photolytic degradation of atmospheric trinitrotoluene were located in the available literature; however, this compound reportedly undergoes light-induced decomposition in the solid state (Burlinson et al., 1973). Therefore, direct photolysis of gaseous and particulate trinitrotoluene may be a significant process in the atmosphere. No experimental data on the physical removal of trinitrotoluene were located; however, it may be deposited on the earth's surface by rain and particulate settling (Ryon et al., 1984).

Limited data on exposure to trinitrotoluene were located in the available literature cited in Appendix A. Dermal exposure for those working in areas related to the production of armaments is reportedly more significant

than exposure by inhalation (Woollen et al., 1986). Trinitrotoluene was detected in groundwater and surface water samples near sites of its production or manipulation (Jenkins et al., 1986; Nay et al., 1972; Spanggord et al., 1982; Pereira et al., 1979; Spalding and Fulton, 1988). Thus, the potential for ingestion of this compound exists for a small group of the population, although quantitative estimations of human exposure for the group cannot be made because of the lack of drinking water and food monitoring data.

Static LC_{50} values for 2,4,6-trinitrotoluene varied from 5.2-27.0 mg/ℓ among invertebrates and 0.8-3.4 mg/ℓ among fish (Liu et al., 1983; Pederson, 1970). In flowthrough tests, LC_{50} values among fish ranged from 2.0-3.7 mg/ℓ (Liu et al., 1983; Smock et al., 1976). Daphnia magna was the most sensitive species in flowthrough tests, with a 96-hour LC_{50} of 1.2 mg/ℓ and an incipient LC_{50} of 0.19 mg/ℓ at 192 hours (Liu et al., 1983). In a 21-day static model ecosystem, the LOEC values for reduced population size and individual growth were 0.6 mg/ℓ in Daphnia and 5.6 mg/ℓ in the oligochaete, Lumbriculus variegatus (Bailey, 1982). Decreased algal density may have been responsible for the reduction in Daphnia population. The population density of the green alga, Selenastrum capricornutum, fell immediately following exposure to 5.6 mg/ℓ in this study. Algal density also decreased in microcosms exposed to lower concentrations, but this was a delayed effect that may have been due to growth of the Daphnia population. Other studies on algae reported LOEC values of 4.1-5 mg/ℓ in Scenedesmus capricornutum, 1.6 mg/ℓ in S. quadricauda, 0.32-25 mg/ℓ in Microcystis aeruginosa, 4.1 mg/ℓ in Anabaena flosaquae and 18 mg/ℓ in the diatom Navicula pelliculosa (Bringmann and Kuhn, 1978; Fitzgerald et

al., 1952; Liu et al., 1983; Smock et al., 1976). Photolysis of 2,4,6-trinitrotoluene was reported in two of these studies and may have affected the results in others. The LOEC for reduced growth was 1 mg/ℓ in an aquatic flowering plant, the duckweed, Lemna perpusilla, and 5 mg/ℓ in a terrestrial plant, the yellow nutsedge, Cyperus esculentus (Palazzo and Leggett, 1986; Schott and Worthley, 1974). Concentrations ≤100 mg/ℓ had no effect on cell multiplication in the bacterium, Pseudomonas putida. Several studies included assays designed to determine the influence of water quality variables upon the toxicity of 2,4,6-trinitrotoluene in various species. None reported more than slight changes attributable to these variables (Liu et al., 1983; Pederson, 1970; Schott and Worthley, 1974). Exploratory 4-day static bioconcentration studies reported BCFs ranging from 202-453 in several representative invertebrates, fish and algae.

In mice, rats, dogs and rabbits, trinitrotoluene administered orally, dermally or intratracheally (only to rats) is readily absorbed, distributed, metabolized and excreted in the urine and, to a lesser extent, in feces (El-hawari et al., 1981). Generally, the rate of absorption by the three routes tested was intratracheal > oral > dermal. The extent of absorption in the four species tested was not significantly different when trinitrotoluene was administered by the oral route. After dermal exposure, however, the highest absorption occurred in rabbits, followed by mice, rats and dogs. Radioactivity was mainly distributed to the liver and kidney of the animals after oral dosing, but fat contained appreciable amounts of radioactivity following dermal treatment. Trinitrotoluene was extensively metabolized in all species examined regardless of the route of administration. Identification of products in the urine and bile showed that most metabolites were nitroreduction derivatives. Oxidation of the methyl group had also

occurred. Unchanged trinitrotoluene could not be identified in the urine of rabbits. The metabolic profiles of urine from rats, mice and dogs and different routes of exposure differed only quantitatively and no significant sex differences were observed. Urine of rabbits was unique because it contained larger amounts of monoamines and hydroxylamines. Urinary and biliary excretion appear to play nearly equivalent roles in the elimination of trinitrotoluene.

Reported oral LD_{50}s for trinitrotoluene administered by gavage were 660 mg/kg in male and female mice and 1320 and 795 mg/kg in male and female rats, respectively. LD_{50} data for other species were not available. Data were not available regarding the toxicity of trinitrotoluene to animals by inhalation exposure.

Data regarding the toxicity of trinitrotoluene in humans indicated that workers exposed to air levels between 0.01 and 4.0 mg/m^3 may develop skin irritation, liver damage and anemia (Hathaway, 1977; Morton et al., 1976). Although there are numerous reports of occupational exposure to trinitrotoluene, the duration and levels of exposure were usually not sufficiently defined to permit use of these studies in risk assessment.

Trinitrotoluene yielded evidence of carcinogenicity in a 24-month dietary exposure study using rats (Furedi et al., 1984a). In that study, female rats had a significantly increased incidence of urinary bladder papillomas and carcinomas. Trinitrotoluene was not carcinogenic when tested in mice (Furedi et al., 1984b). Trinitrotoluene was mutagenic in reverse mutation assays in Salmonella typhimurium in the absence of activating systems (Kaplan and Kaplan, 1982b; Whong and Edwards, 1984; Won et al., 1976). The presence of activating systems reduced the mutagenic potency. Trinitrotoluene did not induce DNA damage in mammalian test systems in vivo (Ashby et al., 1985).

Subchronic studies using animals suggest that dogs are the most sensitive species since a dose of 0.5 mg/kg/day for 26 weeks induced signs of anemia and liver alterations (Levine et al., 1983). Increasing doses increased the severity of the effects. Death occurred with a dose of 32 mg/kg/day before week 17. In contrast, death from anemia occurred in rats with doses of 300 mg/kg/day (~10 times higher than in dogs) administered for 13 weeks (Levine et al., 1984a). Mice appeared to be less sensitive. A dose of 190 mg/kg/day for 13 weeks produced liver effects, but a dose of 36 mg/kg/day was without adverse effects; it is possible that doses between 36 and 190 mg/kg/day could have been toxic. Chronic studies have been performed only on rats and mice. In rats, a dose of 2.0 mg/kg/day in the diet for 24 months caused kidney hypertrophy, spleen congestion and bone marrow fibrosis in females. A dose of 10 mg/kg/day induced signs of anemia, changes in organ weights and urinary bladder lesions in females. In contrast, a dose of 10 mg/kg/day administered in the diet for 24 months to mice was without adverse effects. The most sensitive endpoints for assessing toxicological effects of trinitrotoluene seem to be the liver and elements in the blood, such as the RBCs.

Data regarding the developmental or reproductive toxicity of trinitroluene were not available in the literature cited in Appendix A.

Based on the weight of evidence, trinitrotoluene has been assigned to U.S. EPA Group C: possible human carcinogen. A q_1^* value of 3×10^{-2} $(mg/kg/day)^{-1}$ was previously derived (U.S. EPA, 1988b, 1989) from the dose-response data for increased incidences of urinary bladder papillomas and carcinomas in female rats treated with trinitrotoluene in the diet (Furedi et al., 1984a). The concentration of trinitrotoluene in drinking water associated with a risk level of 1E-5 is 10 µg/ℓ. A carcinogenicity-based RQ of 100 was assigned.

An RfD of 5E-4 mg/kg/day was derived for subchronic and chronic oral exposure to trinitrotoluene based on the LOAEL of 0.5 mg/kg/day for liver effects in dogs in the 26-week oral study by Levine et al. (1983). An RQ of 100 for chronic (noncancer) toxicity was derived based on liver effects in dogs (Levine et al., 1983).

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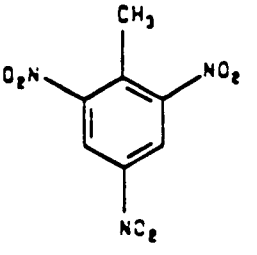
LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
BUN	Blood urea nitrogen
CAS	Chemical Abstract Service
CS	Composite score
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC_{50}	Concentration effective to 50% of recipients
FEL	Frank effect level
F344	Fischer 344
HA	Health advisory
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LDH	Lactate dehydrogenase
LOAEL	Lowest-observed-adverse-effect level
LOEC	Lowest-observed-effect concentration
MED	Minimum effective dose
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse-effect level
NOEC	No-observed-effect concentration
NOEL	No-observed-effect level
PEL	Permissible exposure level
ppm	Parts per million
ppt	Parts per trillion
RBC	Red blood cell
RfD	Reference dose
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
SGPT	Serum glutamic pyruvic transaminase
TLC	Thin layer chromatography
TLV	Threshold limit value
TWA	Time-weighted average

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Trinitrotoluene is the common name for 2,4,6-trinitrotoluene. It is also known as α-trinitrotoluene, sym or s-trinitrotoluene, 2-methyl-1, 3,5-trinitrobenzene, trilit, tolit, tritol and trinitrotoluene (Chemline, 1989; SANSS, 1989). The structure, CAS Registry number, empirical formula and molecular weight are as follows:



CAS number: 118-96-7

Empirical formula: $C_7H_5N_3O_6$

Molecular weight: 227.13

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Trinitrotoluene is a yellow crystalline solid at room temperature. It is soluble in alcohol, ether, acetone, benzene and carbon disulfide and slightly soluble in water (Sax and Lewis, 1987; Windholz et al., 1983). Selected chemical and physical properties are given below.

Melting point:	80.2-81.3°C	Pella, 1977
Boiling point:	186.6°C at 7.5 mm Hg	Boublik et al., 1984
Density at 20°C:	1.654 g/mℓ	Windholz et al., 1983
Water solubility:	104-113 mg/ℓ at 20°C	Spanggord et al., 1983
Log K_{OW}:	1.60	Hansch and Leo, 1985
Vapor pressure:	8.02×10^{-6} mm Hg at 25°C 4.09×10^{-6} mm Hg at 25°C	Pella, 1977 Jones, 1960
Conversion factor: (air at 25°C)	1 mg/m³ = 0.108 ppm 1 ppm = 9.259 mg/m³	

1.3. PRODUCTION DATA

During 1977, six U.S. plants manufactured or imported between ~3.1 and 31.1 million pounds of trinitrotoluene: IMC Plaza in Libertyville, IL; Uniroyal, Inc. in Joliet, IL; E. I. Dupont de Neumours and Co. in Wilmington, DE; Chemical Systems Division in San Jose, CA; Volunteer Army Ammunition Plant in Chattanooga, TN; and one plant with production information listed as confidential (TSCAPP, 1989). Earlier data list the production of trinitrotoluene at ~45 million pounds/month for 1969-1971 (Ryon et al., 1984). Government policy dictates that all munitions plants be government-owned, although most of the plants are operated by contractors who are usually major chemical companies (Ryon et al., 1984).

Trinitrotoluene is manufactured by the stepwise nitration of toluene in either a batch or continuous operation. Toluene, nitric acid and sulfuric acid are mixed together in the first step of a six-step process. The nitration products from each stage are passed on to the next, where progressively higher temperatures and acid concentrations are used to maximize the yield of the desired trinitrated product. The crude trinitrotoluene is purified by treatment with sodium sulfite, followed by recrystalization (Ryon et al., 1984).

1.4. USE DATA

Trinitrotoluene is used as a high explosive in military armaments and as an intermediate in dyestuffs and photographic chemicals (Sax and Lewis, 1987).

1.5. SUMMARY

Trinitrotoluene, commonly referred to as TNT, is a yellow crystalline solid at room temperature. It is soluble in alcohol, ether, acetone, benzene and carbon disulfide and slightly soluble in water (Sax and Lewis,

1987; Windholz et al., 1983). Between 3.1 and 31.1 million pounds of trinitrotoluene was produced or imported in the United States in 1977 at six different facilities (TSCAPP, 1989). Earlier production data list the monthly production of trinitrotoluene in the United States at ~45 million pounds during 1969-1971 (Ryon et al., 1984). Trinitrotoluene is produced by the nitration of toluene in a concentrated mixture of sulfuric and nitric acids (Ryon et al., 1984). It is used chiefly as a high, or bursting, explosive and as an intermediate in dyestuffs and photographic chemicals (Sax and Lewis, 1987).

2. ENVIRONMENTAL FATE AND TRANSPORT

2.1. AIR

Based on its reported vapor pressures of 4.09×10^{-6} and 8.02×10^{-6} mm Hg at 25°C (Jones, 1960; Pella, 1977), trinitrotoluene is expected to exist partially in the vapor phase but predominantly in particulate form in the atmosphere (Eisenreich et al., 1981).

2.1.1. Reaction with Hydroxyl Radicals. Using the estimation method of Atkinson (1985), a rate constant of 1.46×10^{-13} cm^3/molecule-sec can be obtained for the vapor phase reaction of photochemically produced HO• with trinitrotoluene. If the average atmospheric HO• concentration is $5 \times 10^{+5}$ molecules/cm^3 (Atkinson, 1985), then the half-life for this reaction is ~110 days. Since trinitrotoluene is not expected to exist entirely in the vapor phase in the atmosphere (Eisenreich et al., 1981; Jones, 1960; Pella, 1977), the actual rate of destruction by this process is expected to be considerably slower. Therefore, removal of atmospheric trinitrotoluene by the gas-phase destruction by photochemically produced HO• is not expected to be significant.

2.1.2. Reaction with Ozone. The gas-phase destruction of atmospheric trinitrotoluene through the reaction with ozone is not expected to be significant (Atkinson, 1985).

2.1.3. Photolysis. Pertinent quantitative data regarding the photolysis of trinitrotoluene in the atmosphere were not located in the available literature cited in Appendix A. Trinitrotoluene, however, is susceptible to photochemical degradation (Section 2.2.3.), even in the solid state (Burlinson et al., 1973). Therefore, direct photochemical degradation of both vapor-phase and particulate trinitrotoluene in the atmosphere may occur.

2.1.4. Physical Removal Processes. Pertinent data regarding the physical removal of trinitrotoluene from the atmosphere were not located in the available literature cited in Appendix A. Ryon et al. (1984) postulated that it may be deposited on the earth's surface by particulate settling or rain deposition.

2.2. WATER

2.2.1. Hydrolysis. Limited experimental data were located in the literature concerning the hydrolysis of trinitrotoluene in the environment. In the laboratory, trinitrotoluene underwent no observable hydrolysis in sea water after 108 days at 25°C at a pH of ~8.1 (Hoffsommer and Rosen, 1973). Hydrolysis is not expected to be significant for trinitrotoluene, since it contains no readily hydrolyzable functional groups (Harris, 1982).

2.2.2. Oxidation. Pertinent data regarding the chemical oxidation of trinitrotoluene in water were not located in the available literature cited in Appendix A. It is not, however, expected to be a significant fate process.

2.2.3. Photolysis. The degradation of trinitrotoluene by direct photolysis in water is well documented qualitatively and quantitatively. This process is described as the source of the pink water problem associated with the wastewater of armament production (Burlinson et al., 1973).

The half-life for the sunlight photolysis of trinitrotoluene in pure water, as estimated by experiments in natural sunlight, was 14 hours at a latitude of 20, 40 or 50° over the continental United States during the summer months. In the winter months, the respective half-lives were estimated to be 22, 45 and 85 hours. In these studies, pH had a minor effect on the rate of photolysis. The products produced in this reaction accelerated the rate of trinitrotoluene photolysis, and humic acid in

natural waters increased the rate of photolysis from one to two orders of magnitude. Oxygen and other triplet quenchers decreased the rate (Mabey et al., 1983).

The laboratory test-tube photolysis of 4.97 ppm trinitrotoluene in water obtained from a waste lagoon on an Army ammunition plant resulted in final concentrations of 3.29 and 2.34 ppm after 110 minutes (at 313 nm) and 240 minutes (at 366 nm), respectively. Photolysis of the same solution using natural sunlight resulted in a concentration decrease to 2.49 ppm after 80 minutes (Spanggord et al., 1983).

The photolysis (>280 nm) of trinitrotoluene in pure water at 60°C in a continuous flow apparatus produced 1,3,5-trinitrobenzene, 2,6-dinitroanthranil, 2,4,6-trinitrobenzaldehyde and 2,4,6-trinitrobenzonitrile, however; 80% of the mass balance could not be accounted for. The sunlight photolysis of trinitrotoluene in pure water at room temperature in a quartz vessel was 75% complete in ~30 hours. In addition to the products listed above, four tetranitroazoxytoluenes were isolated reportedly because of the condensation of the initial products (Burlinson et al., 1973).

2.2.4. Microbial Degradation. No initial carbon-14 labeled trinitrotoluene could be detected after 3-5 days in aerated reactors using a sewage sludge inoculum. Very little radiolabeled CO_2 (<0.5%) was found in these experiments, indicating that complete mineralization did not occur. The authors concluded that one or more of the nitro groups in trinitrotoluene was converted to the corresponding amine, which then reacted with a carboxylate group from the cellular matter of the medium, forming polyamides. The formation of polyamides, thought to resist degradation, accounts for the lack of CO_2 production and is consistent with the observed lack of destruction for the aromatic skeleton of trinitrotoluene (Carpenter et al., 1978).

Trinitrotoluene at an initial concentration of 100 mg/ℓ completely disappeared under aerobic conditions in 6 days using inocula obtained from sewage treatment plants, wastewater from an ordinance loading facility and soil, pond or aquarium water, all of which had been exposed to trinitrotoluene previously. Yeast extract was added to this experiment to serve as a source of nutrients. When no yeast extract was added, microbial degradation did not occur (Osmon and Klausmeier, 1973).

Microbes obtained from a sewage treatment plant can be acclimated and grown successfully in the presence of high concentrations (29-100 ppm) of trinitrotoluene, such that the normal respiration of other organic compounds could occur. However, no evidence for the biodegradation of trinitrotoluene was presented in this experiment. Pure cultures of bacteria, Zoogloea ramigera 115, grown on trinitrotoluene degraded it under aerobic conditions (Enzinger, 1970).

Trinitrotoluene undergoes reductive biotransformation under both aerobic and anaerobic conditions. 4-Amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene are metabolites. Trinitrotoluene at an initial concentration of 10 ppm did not degrade after 6 weeks under aerobic conditions in water containing sediment obtained from the Searsville Pond, CA, or with eutrophic water obtained from the Coyote Creek, CA. When the experiment was repeated with the addition of 500 ppm yeast extract, the amount of trinitrotoluene was reduced to a level below the limit of detection within 5 days. Mixed cultures obtained from Waconda Bay, TN, and raised on 2,4-dinitrotoluene did not degrade trinitrotoluene under aerobic conditions. In the presence of other nutrients such as yeast extract, degradation proceeded (Spanggord et al., 1981).

Trinitrotoluene at an initial concentration of 5 ppm degraded to 2 ppm over a 90-day period under aerobic conditions in lagoon water obtained from an Army ammunition plant. The addition of yeast extract increased the rate of biodegradation. In lagoon sediment and water samples, an initial trinitrotoluene concentration of 74 ppm was reduced to 59 ppm over the course of 90 days under aerobic conditions. Under anaerobic conditions in the lagoon water alone, trinitrotoluene underwent biotransformation, although at a slower rate than seen for the aerobic degradation in this sample. Yeast extract, again, increased the rate of biodegradation (Spanggord et al., 1983).

Trinitrotoluene at a concentration between 10 and 50 ppm underwent 97% biodegradation when continuously fed into an aerated oxidation ditch containing activated sludge and a cornsteep nutrient. Metabolites from this process, in addition to the amino-nitrotoluenes mentioned above, were postulated to be 2- or 2,4-hydroxylaminonitrotoluenes, although they were not isolated (Hoffsommer et al., 1978). These hydroxyaminoninitrotoluenes reportedly dimerize nonenzymatically to azoxy compounds (Fewson, 1981). Complete degradation of the aromatic ring of trinitrotoluene to carbon dioxide does not occur, possibly because of the formation of these azoxy dimers, which are thought to resist degradation. The formation of the degradation-resistant polyamides described earlier may also be responsible (Fewson, 1981).

2.2.5. **Bioconcentration.** The BCF, useful in estimating the potential for uptake in fish and aquatic organisms, can be calculated based on chemical properties if it is not available experimentally. The linear regression equation log BCF = 2.791 - 0.564 log S, where S is the water solubility in ppm, can be used for this purpose (Bysshe, 1982). For trinitrotoluene, a

BCF of ~45 can be obtained based on its water solubility of 104-113 mg/ℓ at 20°C (Spanggord et al., 1983). This value suggests that bioaccumulation in fish and aquatic organisms is not significant.

2.2.6. Adsorption. Pertinent data regarding the adsorption of trinitrotoluene to sediment and suspended organic matter were not located in the available literature cited in Appendix A. The potential for the strong adsorption of trinitrotoluene to soil (Section 2.3.2.) suggests that adsorption to sediment may occur. However, Ryon et al. (1984) reported that sorption to sediment was of minor importance compared with its photolysis and biodegradation in natural waters. Under cloudy skies in winter, when both photolysis and biodegradation are at their minimum, sorption to sediment may become significant.

2.2.7. Volatilization. Using the estimation method of Hine and Mookerjee (1975), a Henry's Law constant of 3.30×10^{-9} atm/m³-mol at 25°C can be obtained for trinitrotoluene. An estimated volatilization half-life of >16,000 days from a model river 1 m deep, flowing 1 m/sec, with a wind velocity of 3 m/sec can be obtained using the above Henry's Law constant (Thomas, 1982). This value suggests that volatilization of trinitrotoluene from the water to the atmosphere will not be an important process.

2.3. SOIL

2.3.1. Microbial Degradation. Trinitrotoluene underwent degradation in an active compost system under aerobic conditions at 55°C. Although the rate was not given, some material remained after 91 days. The metabolites identified were the same as those seen in the microbial degradation of trinitrotoluene in water (Kaplan and Kaplan, 1982a). Soil not previously exposed to trinitrotoluene degraded trinitrotoluene, but at a slower rate than that in experiments with water (Osmon and Klausmeier, 1973). Microbes

obtained from soil, compost or waste lagoon sediments and adapted to phenol biodegraded trinitrotoluene at a slow but steady rate under aerobic conditions, with maximum respiration reaching 21.2 times the endogenous level (Chambers et al., 1963; Tabak et al., 1964).

2.3.2. Adsorption. In a lysimeter column study using four different kinds of soil (clay, silt, loam and sandy loam), no trinitrotoluene was found in water samples taken from the bottom of the column over a period of 6 months. None of the known metabolites of trinitrotoluene biodegradation were found in the leachate. After the termination of the dynamic portion of the experiment, trinitrotoluene was adsorbed to the soil of all four columns (Kayser and Burlinson, 1988). These data suggest that trinitrotoluene and its metabolites have the potential for adsorbing strongly to soil.

2.3.3. Volatilization. Reported vapor pressures for trinitrotoluene in the range 4.09×10^{-6} to 8.02×10^{-6} mm Hg at 25°C (Jones, 1960; Pella, 1977) suggest that volatilization from dry soil to the atmosphere will not be a significant process. Similarly, the estimated Henry's Law constant of 3.3×10^{-9} atm/m^3-mol (Hine and Mookerjee, 1975) suggests also that this process will not be significant in moist soil.

2.4. SUMMARY

The dominant fate process for trinitrotoluene is expected to be destruction by direct photolysis. The half-life for the sunlight photolysis of trinitrotoluene in pure water is 14 hours over the continental United States during the summer months (Mabey et al., 1983). The sunlight photolysis of trinitrotoluene in distilled water at room temperature occurred at a rate equal to 75% removal after 30 hours (Burlinson et al., 1973). The rate was nearly pH-independent, increased with the presence of humic material, and

decreased in the presence of oxygen and other triplet quenchers (Mabey et al., 1983). The biological degradation of trinitrotoluene in environmental waters is expected to occur under both aerobic and anaerobic conditions, although the presence of additional nutrients may be required (Carpenter et al., 1978; Osmon and Klausmeier, 1973; Chambers et al., 1963; Tabak et al., 1964; Enzinger, 1970; Spanggord et al., 1981, 1983; Fewson, 1981; Hoffsommer et al., 1978). Products from both the aerobic and anaerobic degradation of trinitrotoluene are believed to come from initial reduction of one or more of the nitro groups, which are then capable of undergoing nonenzymatic reactions, which may result in the formation of products that are degradation-resistant. Complete degradation of trinitrotoluene to CO_2 has not been observed. Neither hydrolysis nor volatilization to the atmosphere are expected to be significant in water. Adsorption to sediment and suspended organic matter may occur, although it is not expected to be a significant process (Ryon et al., 1984). Bioconcentration in fish and aquatic organisms is not expected to be a significant process. If released to soil, microbial degradation is expected to occur (Kaplan and Kaplan, 1982a; Chambers et al., 1963; Tabak et al., 1964; Osmon and Klausmeier, 1973). The potential for strong adsorption and, thus, low mobility in soil has been demonstrated by Kayser and Burlinson (1988). Volatilization from the soil surface to the atmosphere is not expected to be significant. In the atmosphere, trinitrotoluene is expected to exist in both the vapor phase and the particulate form (Jones, 1960; Pella, 1977; Eisenreich et al., 1981). No experimental data on the direct photolytic degradation of trinitrotoluene were located in the available literature; however, this compound reportedly undergoes light-induced decomposition in the solid state (Burlinson et al., 1973).

Therefore, direct photolysis of gaseous and particulate trinitrotoluene may be a significant process in the atmosphere. No experimental data on the physical removal of trinitrotoluene were located; however, it may be deposited on the earth's surface by rain and particulate settling (Ryon et al., 1984).

3. EXPOSURE

3.1. WATER

Trinitrotoluene was detected in an on-site waste lagoon and in the wastewater at an unspecified Army ammunition plant at 1314 and 19 µg/ℓ, respectively (Jenkins et al., 1986). The wastewater from a modern, counter-current, continuous flow manufacturing process contained trinitrotoluene at a concentration ranging from 101-142.9 mg/ℓ (Nay et al., 1972). It was also found in 20.3% of the ether extracts of condensate water obtained from trinitrotoluene production and purification at a concentration range of 0.10-3.40 mg/ℓ (Spanggord et al., 1982).

Trinitrotoluene was detected in shallow groundwaters obtained near disposal beds on a Naval ammunition depot in Colorado at a maximum concentration of 620 µg/ℓ (Pereira et al., 1979). Also identified in the groundwater were known metabolites of the microbial degradation of trinitrotoluene: 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene. Trinitrotoluene was also identified in groundwater wells downgradient from the Cornhusker Army Ammunition Plant, Nebraska (Spalding and Fulton, 1988).

Trinitrotoluene was not found at two sites, one 200 miles off the coast of Florida and the other 45 miles west of San Francisco, where old liberty ships loaded with antiquated munitions were scuttled at sea (detection limit 2 ppt) (Hoffsommer et al., 1972; Hoffsommer and Rosen, 1972).

3.2. FOOD

Pertinent data regarding exposure to trinitrotoluene by ingestion of food were not located in the available literature cited in Appendix A.

3.3. INHALATION

Little data on the concentration of trinitrotoluene in the atmosphere were located in the available literature cited in Appendix A. The atmospheric concentration of trinitrotoluene at an explosives manufacturing plant in the United Kingdom ranged from <0.01-2.53 mg/m³; however, it was not clear whether this was a measurement of trinitrotoluene vapor or particulates. Metabolites of trinitrotoluene excreted in urine were determined in exposed workers. There was no correlation between urinary excretion and atmospheric levels of trinitrotoluene. Oral uptake was considered to be negligible. The levels excreted in urine were higher than the theoretical maximum uptake by inhalation. Because urine is not the only route of excretion, uptake of trinitrotoluene must occur, to a large extent, by routes other than oral and inhalation. The authors concluded that occupational exposure to trinitrotoluene by inhalation is not expected to be significant when compared with dermal exposure (Woollen et al., 1986).

3.4. DERMAL

Trinitrotoluene exposure by workers involved in the manufacture of explosives is thought to occur predominantly through dermal contact (Woollen et al., 1986). Extensive tests of dermal uptake, however, have not been reported in the available literature.

3.5. OTHER

Trinitrotoluene was found in the urine of munitions workers in Israel at a concentration of 11-278 ng/mℓ. Also found in this study were the products of trinitrotoluene metabolism (Yinon and Hwang, 1986).

3.6. SUMMARY

Limited data on exposure to trinitrotoluene were located in the available literature cited in Appendix A. Dermal exposure for those working in

areas related to the production of armaments is reportedly more significant than exposure by inhalation (Woollen et al., 1986). Trinitrotoluene was detected in groundwater and surface water samples near sites of its production or manipulation (Jenkins et al., 1986; Nay et al., 1972; Spanggord et al., 1982; Pereira et al., 1979; Spalding and Fulton, 1988). Thus, the potential for ingestion of this compound exists for a small group of the population, although quantitative estimations of human exposure for the group cannot cannot be made because of the lack of drinking water and food monitoring data.

4. ENVIRONMENTAL TOXICOLOGY

4.1. AQUATIC TOXICOLOGY

4.1.1. Acute Toxic Effects on Fauna. A series of tests on the acute toxicity of 2,4,6-trinitrotoluene to freshwater invertebrates and fish was performed by Liu et al. (1983). Static 48-hour assays in which members of the test species (in groups of 10 or 20) were exposed to five nominal concentrations of 2,4,6-trinitrotoluene and a control were done on invertebrates. The 48-hour LC_{50} values recorded were 5.2 mg/ℓ in the oligochaete worm, Lumbriculus variegatus, 6.5 mg/ℓ in the scud, Hyalella azteca, 11.7 mg/ℓ in the water flea, Daphnia magna, and 27.0 in the midge, Tanytarsus dissimilis. Static 96-hour tests were done on fish using six nominal concentrations (including control), group sizes of 10 and duplicate tests. All fish tested were juveniles and all tests were conducted at 20°C except for those using trout, which were conducted at 12°C. The 96-hour LC_{50} values were 0.8-1.5 mg/ℓ in rainbow trout, Salmo gairdneri, 2.4 mg/ℓ in channel catfish, Ictalurus punctatus, 2.6-3.4 mg/ℓ in bluegill sunfish, Lepomis macrochirus, and 2.9 mg/ℓ in fathead minnows, Pimephales promelas. In a separate series of nonreplicated tests on the fathead minnow, it was found that pH had a minimal effect on 2,4,6-trinitrotoluene toxicity, with the 96-hour LC_{50} increasing from 1.2 mg/ℓ at pH=5-2.1 mg/ℓ at pH=7 and 2.4 mg/ℓ at pH=9.4. These authors also conducted flow-through acute toxicity tests using measured concentrations of 2,4,6-trinitrotoluene. The tests were conducted in duplicate using 10 worms, 15 water fleas or 20 fish per replicate. The 96-hour LC_{50} value for L. variegatus was >29.0 mg/ℓ and the incipient LC_{50} was 13.9 mg/ℓ and was reached after 336 hours. The 96-hour and incipient LC_{50} values in Daphnia were 1.2 and 0.19 mg/ℓ (after 192 hours), respectively. Among fish, the

96-hour LC_{50} values were slightly higher than in the static tests, with values of 2.0 mg/ℓ in rainbow trout, 3.3 mg/ℓ in channel catfish, 2.5 mg/ℓ in bluegill sunfish and 3.7 mg/ℓ in fathead minnows. The incipient LC_{50} values and times they were reached in these species were 1.9 mg/ℓ (240 hours), 1.6 mg/ℓ (288 hours), 1.4 mg/ℓ (312 hours) and 1.5 mg/ℓ (384 hours), respectively.

Pederson (1970) reported the results of acute toxicity bioassays on bluegill sunfish, L. macrochirus. These were 96-hour static tests and the water was renewed every 24 hours. The tests were run at either 10 or 25°C and in either soft (60 ppm as $CaCO_3$) or hard water (180 ppm as $CaCO_3$). For each of the four test series, one group of 10 fish was exposed at each measured concentration. The 96-hour LC_{50} values varied from 2.3-2.8 mg/ℓ. This range is similar to the values reported by Liu et al. (1983). The LC_{50} values were significantly lower at 10°C (2.3 mg/P) than at 25°C (2.7-2.8 mg/ℓ), indicating that 2,4,6-trinitrotoluene was more toxic at the lower temperature. Toxicity was not affected by water hardness in this study.

Fathead minnows, P. promelas, were exposed to measured concentrations of 2,4,6-trinitrotoluene ranging from 0.05-44.9 mg/ℓ under flowthrough conditions (Smock et al., 1976). One group of 15 fish was tested at each concentration. The 96-hour LC_{50} was 2.58 ± 0.1 mg/ℓ. This is similar to the result reported by Liu et al. (1983); the concentration below which no deaths were reported was 1.78 mg/ℓ. Behavioral responses before death were also noted. Gasping at the surface was the initial reaction by the fish to 2,4,6-trinitrotoluene. This reaction was followed by lethargy, loss of motor control (exhibited by the fish swimming jerkily at the surface, rapidly opening and closing their gills) and finally, lethargic swimming,

responding only to tactile stimuli. The 96-hour EC_{50} for a behavioral response to 2,4,6-trinitrotoluene was 0.46±0.1 mg/ℓ. No behavioral responses were seen at concentrations of ≤0.05 mg/ℓ.

4.1.2. Chronic Effects on Fauna.

4.1.2.1. TOXICITY -- Bailey (1982) used a model ecosystem to study the chronic toxicity of 2,4,6-trinitrotoluene to invertebrates. Five chemical concentrations and a control, each tested in duplicate, were included. Each microcosm was started with the green alga, Selenastrum capricornutum, at an initial density of 10,000 to 15,000 cells/mℓ, the benthic oligochaete, L. variegatus, at an initial population of 30 and the water flea, D. magna, at an initial population of 15. The study was continued for 21 days, with young Daphnia periodically counted and removed. Measurement of 2,4,6-trinitrotoluene concentrations revealed that the chemical was steadily lost from the water in this static system and the rate of loss was proportional to the initial test concentration. The total number of Daphnia produced was reduced at concentrations of ≥0.6 mg/ℓ. This could result from a direct effect of the chemical on daphnid reproduction, or alternatively, the daphnid population may have been limited by decreased algal density. The total number of worms was reduced at 5.6 mg/ℓ and the length of these worms was also reduced. No animals of either species survived 21-day exposure to ≥10 mg/ℓ.

4.1.2.2. BIOACCUMULATION/BIOCONCENTRATION -- Exploratory 4-day static bioconcentration studies using 0.5 mg/ℓ of ^{14}C-labeled 2,4,6-trinitrotoluene in DMSO were performed on invertebrates and fish by Liu et al. (1983). The 4-day BCFs were 202.0 in the oligochaete worm, L. variegatus, (50 tested) and 209.0 in the water flea, D. magna (100 tested). BCFs of 338.0 and 9.5 were reported in the viscera and muscles, respectively, of the

bluegill sunfish, L. macrochirus (three tested). The difference between viscera and muscle BCF is unknown, but the authors speculate that 2,4,6-trinitrotoluene is metabolized mostly in the liver and that radioactivity in the viscera was concentrated in the liver. This chemical did not bioconcentrate significantly in this preliminary study.

4.1.3. Effects on Flora.

4.1.3.1. TOXICITY -- The effect of 2,4,6-trinitrotoluene on algal growth was studied by Smock et al. (1976). One series of tests was conducted using the green alga, S. capricornutum. Algal cultures with an initial concentration of 10^3 cells/mℓ were exposed to 2,4,6-trinitrotoluene concentrations ranging from 1-9 mg/ℓ (three replicates per concentration) under static conditions for 17 days. Cell counts were made throughout the experiment. Concentrations $\leq$3 mg/ℓ had no effect on algal growth when compared with untreated controls. Growth was initially inhibited at concentrations of $\geq$5 mg/ℓ. Although growth later recovered and no difference from controls was noted at the end of the experiment, chemical analysis showed that recovery coincided with transformation of 2,4,6-trinitrotoluene to other compounds. A similar study was conducted using the blue-green alga, Microcystis aeruginosa (initial concentration ~60 mg/ℓ biomass). The results had a pattern similar to those described above except that the NOEC was 15 mg/ℓ and the LOEC was 25 mg/ℓ. The blue-green algae colonies exposed to 2,4,6-trinitrotoluene produced gas vacuoles and gelatinous sheaths. This led to the formation of mats on the water surface. The degree of matting was proportional to the concentration of 2,4,6-trinitrotoluene (it was not seen in controls) and may have been a reaction to the toxic environment.

Static toxicity bioassays were performed on the green alga, S. capricornutum, the blue-green algae, M. aeruginosa and Anabaena flos-aquae, and the diatom, Navicula pelliculosa, using 2,4,6-trinitrotoluene (Liu et al., 1983). Initial algae concentrations in culture were 10^4 cells for S. capricornutum and $5x10^4$ cells for the other species. Each species was exposed to six nominal treatment levels (including control) and three replicates were used for each level. Temperature was maintained at 24°C for the 14 days of the study. Population growth was significantly reduced in the green and blue-green algae at 2,4,6-trinitrotoluene concentrations of $\geq$4.1 mg/ℓ. In the diatom, concentrations of $\geq$18.0 mg/ℓ had this effect; however, photolysis of 2,4,6-trinitrotoluene occurred during this study and these results cannot be considered reliable.

Bailey (1982) used a model ecosystem to study the toxicity of 2,4,6-trinitrotoluene to the green alga, S. capricornutum. Five chemical concentrations and a control, each tested in duplicate, were included. Each microcosm was started at an initial algal density of 10,000-15,000 cells/mℓ. Other species included in the microcosm were the benthic oligochaete, L. variegatus, and the water flea, D. magna. The study was continued for 21 days. Measurement of 2,4,6-trinitrotoluene concentrations revealed that the chemical was steadily lost from the water in this static system and that the rate of loss was related to the initial test concentration. Algal density decreased soon after exposure to 2,4,6-trinitrotoluene at concentrations $\geq$5.6 mg/ℓ. Algal density later decreased in the 0, 0.6 and 1.0 mg/ℓ groups. This was attributed to growth of the daphnid population.

Bringmann and Kuhn (1978) reported the results of cell multiplication inhibition tests on the green alga, Scenedesmus quadricauda, and the blue-green alga, M. aeruginosa. Test cultures were maintained for 8 days

following addition of 2,4,6-trinitrotoluene at various concentrations. At the end of the experiment, algal concentrations were measured turbidimetrically. The toxicity threshold (lowest concentration of 2,4,6-trinitrotoluene that produced inhibition of cell multiplication) was 1.6 mg/ℓ in S. quadricauda and 0.32 mg/ℓ in M. aeruginosa. In a screening-type study, a concentration of 8 ppm (mg/ℓ) of trinitrotoluene (isomer not specified) was reported to kill 100% of the blue-green alga, M. aeruginosa in culture (initial concentration ~10^6 cells/mℓ) (Fitzgerald et al., 1952).

One study was conducted on a flowering aquatic plant, the duckweed. Lemna perpusilla (Schott and Worthley, 1974). Each test was started with two fronds, and growth results were tabulated 11 days later. The plants were exposed to 2,4,6-trinitrotoluene concentrations ranging from 0.01-50 ppm (mg/ℓ). Two replicates were used at each concentration and the study was repeated under both acidic (pH=6.3) and basic (pH=8.5) conditions. No effect on growth was seen at concentrations ≤0.5 ppm, but growth (number of fronds in colony) was depressed compared with controls at 1 ppm; higher concentrations were lethal to the plants. The results were not affected by pH of the dilution water.

4.1.3.2. BIOCONCENTRATION -- Liu et al. (1983) conducted an exploratory bioconcentration test using the green alga, S. capricornutum. A concentration of 0.5 mg/ℓ of 2,4,6-trinitrotoluene in DMSO was added to a culture containing 10^4 cells and maintained under static conditions for 4 days at 24°C. A 4-day BCF of 453 was reported. This preliminary result does not indicate significant bioconcentration of 2,4,6-trinitrotoluene.

4.1.4. Effects on Bacteria. Concentrations of 2,4,6-trinitrotoluene ≤100 mg/ℓ had no effect on the results of a cell multiplication inhibition test conducted using Pseudomonas putida. The toxicity threshold for 2,4,6-trinitrotoluene in this test was >100 mg/ℓ (Bringmann and Kuhn, 1980).

4.2. TERRESTRIAL TOXICOLOGY

4.2.1. Effects on Fauna. Pertinent data regarding the effects of exposure of terrestrial fauna to 2,4,6-trinitrotoluene were not located in the available literature cited in Appendix A.

4.2.2. Effects on Flora. Yellow nutsedge plants, Cyperus esculentus, were exposed to 2,4,6-trinitrotoluene under static conditions for 42 days (Palazzo and Leggett, 1986). Four groups, consisting of three plants each, were exposed at each concentration (0, 5, 10 and 20 mg/ℓ). Solutions were renewed after 21 days when measurements revealed loss of 2,4,6-trinitrotoluene from the test solutions. Plant growth was significantly reduced compared with untreated controls at concentrations of ≥5 mg/ℓ. Total plant yields were reduced 54-74% in treated plants. Most affected were the roots and leaves whose weights were reduced 95-97% and 51-74%, respectively. The effects did not increase with dose; however, no difference was seen between effects at 5 and 20 mg/ℓ. This was true even though the concentration of 2,4,6-trinitrotoluene (and metabolites) in various parts of the plant increased with exposure concentration.

4.3. FIELD STUDIES

Pertinent data regarding the effects of 2,4,6-trinitrotoluene on flora and fauna in the field were not located in the available literature cited in Appendix A.

4.4. AQUATIC RISK ASSESSMENT

The lack of pertinent data regarding the effects of exposure of aquatic fauna and flora to 2,4,6-trinitrotoluene prevented the development of a freshwater criterion by the method of U.S. EPA/OWRS (1986). Available data are displayed in Figure 4-1. Additional data required for the development of a freshwater criterion include the results of acute 4-day assays with a

Family	TEST TYPE		
	GMAV[a] (mg/L)	GMCV[a] (mg/L)	BCF[a]
#1 Chordate (Salmonid-fish)	2.0	NA	NA
#2 Chordate (warmwater fish)	2.5	NA	NA
#3 Chordate (fish or amphibian)	3.1	NA	NA
#4 Crustacean (planktonic)	1.2	NA	NA
#5 Crustacean (benthic)	NA	NA	NA
#6 Insectan	27.0	NA	NA
#7 non-Arthropod/-Chordate	29.0	NA	NA
#8 New Insectan or phylum representative	NA	NA	NA
#9 Algae	NA	3.9	NA
#10 Vascular plant	NA	0.71	NA

[a]NA = Not available

FIGURE 4-1

Organization Chart for Listing GMAVs, GMCVs and BCFs Required to Derive Numerical Water Quality Criteria by the Method of U.S. EPA/OWRS (1986) for the Protection of Freshwater Aquatic Life from Exposure to 2,4,6-Trinitrotoluene.

benthic crustacean and an insect or species from a phylum not previously represented. The development of a freshwater criterion will also require data from chronic toxicity tests with two species of fauna and at least one bioconcentration study. The chronic and bioconcentration studies reported above did not meet the standards necessary for inclusion in calculation of a criterion.

Pertinent data regarding the effects of exposure of marine fauna and flora to 2,4,6-trinitrotoluene were not located in the available literature cited in Appendix A. Acute studies with representatives from eight families of marine fauna and at least three chronic studies and one bioconcentration study with marine fauna and flora are needed to develop a saltwater criterion by the method of U.S. EPA/OWRS (1986).

4.5. SUMMARY

Static LC_{50} values for 2,4,6-trinitrotoluene varied from 5.2-27.0 mg/ℓ among invertebrates and 0.8-3.4 mg/ℓ among fish (Liu et al., 1983; Pederson, 1970). In flowthrough tests, LC_{50} values among fish ranged from 2.0-3.7 mg/ℓ (Liu et al., 1983; Smock et al., 1976). D. magna was the most sensitive species in flowthrough tests, with a 96-hour LC_{50} of 1.2 mg/ℓ and an incipient LC_{50} of 0.19 mg/ℓ at 192 hours (Liu et al., 1983). In a 21-day static model ecosystem, the LOEC values for reduced population size and individual growth were 0.6 mg/ℓ in Daphnia and 5.6 mg/ℓ in the oligochaete L. variegatus (Bailey, 1982). Decreased algal density may have been responsible for the reduction in Daphnia population. The population density of the green alga S. capricornutum fell immediately following exposure to 5.6 mg/ℓ in this study. Algal density also decreased in microcosms exposed to lower concentrations, but this was a delayed effect that may have been due to growth of the Daphnia population.

Other studies on algae reported LOEC values of 4.1-5 mg/ℓ in S. capricornutum, 1.6 mg/ℓ in S. quadricauda, 0.32-25 mg/ℓ in M. aeruginosa, 4.1 mg/ℓ in A. flos-aquae and 18 mg/ℓ in the diatom, N. pelliculosa (Bringmann and Kuhn, 1978; Fitzgerald et al., 1952; Liu et al., 1983; Smock et al., 1976). Photolysis of 2,4,6-trinitrotoluene was reported in two of these studies and may have affected the results in others. The LOEC for reduced growth was 1 mg/ℓ in an aquatic flowering plant, the duckweed, L. perpusilla, and 5 mg/ℓ in a terrestrial plant, the yellow nutsedge, C. esculentus (Palazzo and Leggett, 1986; Schott and Worthley, 1974). Concentrations ≤100 mg/ℓ had no effect on cell multiplication in the bacterium, P. putida. Several studies included assays designed to determine the influence of water quality variables upon the toxicity of 2,4,6-trinitrotoluene in various species. None reported more than slight changes attributable to these variables (Liu et al., 1983; Pederson, 1970; Schott and Worthley, 1974). Exploratory 4-day static bioconcentration studies reported BCFs ranging from 202-453 in several representative invertebrates, fish and algae.

5. PHARMACOKINETICS

A comprehensive study regarding absorption, distribution, metabolism and excretion of 2,4,6-trinitrotoluene in rats, mice, rabbits and dogs using oral, dermal and intratracheal routes of exposure was conducted (El-hawari et al., 1981).

Male and female Sprague-Dawley rats and Swiss albino mice received single gavage doses of 100 mg/kg body weight ^{14}C-trinitrotoluene (ring-labeled) and male and female New Zealand rabbits and beagle dogs were given a single gavage dose of 5 mg/kg bw of labeled trinitrotoluene. The compound was dissolved in peanut oil. Urine and feces were collected separately. After 24 hours, the animals were sacrificed; blood was collected and tissues and organs were removed and analyzed for radioactivity.

Male and female rats and male mice received a single application of 50 mg/kg body weight of radioactive trinitrotoluene in peanut oil on a clipped area of their backs. Male rabbits and dogs were treated with either 5 or 50 mg/kg trinitrotoluene in peanut oil. Precautions were taken to prevent the animals from grooming their fur. Concurrent experiments were conducted with animals treated orally with the same dose of trinitrotoluene. Urine and feces were collected separately for 24 hours. Blood samples were taken from the tail vein of rats at 4, 8 and 24 hours after dosing. After 24 hours, the animals were sacrificed and organs and tissues were removed for analysis of radioactivity. Skin, including that from the site of application, was not retained for analysis.

A dose of 50 mg/kg body weight of radioactive trinitrotoluene suspended in 0.5% methylcellulose was administered orally or intratracheally to anesthetized and tracheotomized male rats. The trinitrotoluene particle size

was 1-3 μm. Serial blood samples were taken from the femoral artery over a period of 4 hours; the rats were then sacrificed for tissue sampling. In addition, bladder urine was collected for radioactivity analysis. In an additional group of rats, bile samples were collected from the cannulated common bile duct at different times after dosing and analyzed for radioactivity. Blood samples were also collected, and the rats were sacrificed after 4 hours for tissue sampling.

5.1. ABSORPTION

According to El-hawari et al. (1981), trinitrotoluene was readily absorbed in the treated animals after oral exposure. The latter can be inferred by the recovery of radioactivity in the urine 24 hours after dosing. The amount of radioactivity, expressed as percentage of the dose, recovered in the urine is presented in Table 5-1. The rate of absorption was estimated only in rats. Following dermal exposure to a 50 mg/kg dose of trinitrotoluene in this species, the radioactivity in the blood increased with time until $\geq$24 hours after dosing. In contrast, a 50 mg/kg oral dose produced a peak of radioactivity in the blood at 8 hours. Although the extent of oral absorption can only be approximated since the extent of biliary excretion and enterohepatic circulation was not studied, several generalizations regarding absorption can be made based on urinary excretion: there is more trinitrotoluene absorbed after oral dosing than after dermal administration; dogs and rabbits appear to absorb more trinitrotoluene after oral administration than rats and mice; in decreasing order, dermal absorption is greater in rabbits than in mice, rats and dogs. Based on blood levels of radioactivity, oral absorption of trinitrotoluene by rats was greater after 4 hours when the compound was suspended in methylcellulose than when it was dissolved in peanut oil. Based on urinary excretion and

TABLE 5-1

Excretion of Radioactivity 24 Hours After Administration of ^{14}C-Trinitrotoluene[a,b,c]

Species	Route	Sex	Dose/Vehicle (mg/kg)	Urine	Feces	Gastrointestinal Tract and Contents	Bile	Recovery
Rat	oral	M	50/peanut oil	59.5	10.7	20.2	ND	92.9
		F	50/penut oil	42.5	2.1	35.3	ND	81.3
Rat	oral	M	100/peanut oil	52.7	8.1	39.8	ND	91.6
		F	100/peanut oil	64.5	2.1	33.9	ND	102.4
Rat	oral[d]	M	50/methylcellulose	14.6	ND	73.7	ND[e]	93.3
		M	50/methylcellulose	10.7	ND	68.3	11.6[f]	95.5
		F	50/methylcellulose	10.0	ND	79.0	ND[e]	97.9
		F	50/methylcellulose	8.4	ND	64.2	9.7[f]	91.2
Rat	dermal	M	50/peanut oil	17.4	1.3	3.1	ND	22.8
		F	50/peanut oil	14.6	2.5	6.4	ND	24.9
Rat	intratracheal[d]	M	50/methylcellulose	19.3	ND	18.2	ND[e]	45.6
		M	50/methylcellulose	17.5	ND	1.8	19.8[f]	47.1
		F	50/methylcellulose	13.2	ND	12.1	ND[e]	40.2
		F	50/methylcellulose	12.7	ND	2.9	14.5[f]	45.0
Mice	oral	M	100/peanut oil	41.9	22.0	13.4	ND	80.0
		F	100/peanut oil	42.8	8.9	7.4	ND	60.4
Mice	oral	M	50/peanut oil	59.1	24.1	10.2	ND	94.4
Mice	dermal	M	50/peanut oil	22.7	14.2	3.6	ND	41.7
Rabbit	oral	M	5/peanut oil	68.1	5.4	19.7	ND	95.6

TABLE 5-1 (cont.)

Species	Route	Sex	Dose/Vehicle (mg/kg)	Urine	Feces	Gastrointestinal Tract and Contents	Bile	Recovery
Rabbit	dermal	M	5/peanut oil	52.8	7.8	5.7	ND	68.3
Rabbit	oral	M	50/peanut oil	74.3	5.1	22.7	ND	103.7
Rabbit	dermal	M	50/peanut oil	47.2	2.8	5.8	ND	56.9
Dog	oral	M	5/peanut oil	70.5	8.9	14.6	ND	99.4
Dog	dermal	M	5/peanut oil	11.7	1.7	1.6	ND	16.8
Dog	oral	M	50/peanut oil	61.0	22.2	1.7	ND	94.2
Dog	dermal	M	50/peanut oil	11.8	0.8	1.7	ND	15.9

[a]Source: El-hawari et al., 1981

[b]Mean values of 3-6 rats, 6-8 mice, 2-4 rabbits and 1-3 dogs

[c]Expressed as percent of administered dose

[d]Samples were collected 4 hours after dosing

[e]No bile cannulated

[f]Bile cannulated

ND = Not determined

amounts in the gastrointestinal tract and contents (see Table 5-1), intratracheal instillation of trinitrotoluene (conducted with only rats) resulted in faster and greater absorption than after oral administration. In rabbits and dogs, the extent of oral or dermal absorption seemed independent of the amount administered over a dose ranging from 5-50 mg/kg.

5.2. DISTRIBUTION

The distribution of radioactivity in tissues of the rat and rabbit following different routes of administration of radioactive trinitrotoluene is shown in Table 5-2. In rats, the distribution of radioactivity was similar after both oral and dermal administration of trinitrotoluene in peanut oil. Intratrachael instillation of a dose of 50 mg/kg resulted in high accumulation of radioactivity at 4 hours in the liver, kidney, lung and fat and (not shown in Table 5-2) blood and the gastrointestinal tract. Levels of radioactivity in lungs and fat were markedly higher at 4 hours than at 24 hours. In male rabbits, radioactivity in blood and residual bile was higher after oral administration of a dose of 50 mg/kg of trinitrotoluene (not shown in Table 5-2). Increasing the oral or dermal dose by 10-fold resulted in a similar distribution as with the lower dose.

In other data (El-hawari et al., 1981), radioactivity in the blood, liver, kidney, spleen, muscle and residual bile of dogs was higher after oral administration of a 5 mg/kg dose of trinitrotoluene than after dermal application of the same dose. The radioactivity in fat was higher after dermal dosing. The distribution pattern of radioactivity after a dose of 50 mg/kg was similar to that seen after dosing with 5 mg/kg. Oral and dermal administration of trinitrotoluene to rabbits and dogs resulted in greater levels of radioactivity in the residual bile than in liver and blood.

TABLE 5-2

Tissue Distribution of Radioactivity 24 Hours After Administration of ^{14}C-Trinitrotoluene Using Rats[a,b,c]

Tissue	Sex	Oral 100 mg/kg in Peanut Oil	Oral 50 mg/kg in Peanut Oil	Dermal 50 mg/kg in Peanut Oil	Oral[d] 50 mg/kg in Methyl-cellulose	Ultra[d] Tracheal 50 mg/kg in Methylcellulose
Liver	M	10.7	7.3	2.8	12.2	13.5
	F	13.9	5.5	3.1	9.6	14.3
Kidney	M	3.5	5.8	3.1	11.7	17.5
		2.6	4.5	4.0	19.1	23.2
Lungs	M	0.3	2.1	1.4	44.0	35.7
	F	0.4	2.1	1.7	21.4	23.6
Spleen	M	1.8	1.0	0.6	3.4	3.2
	F	4.7	1.0	0.5	2.0	5.8
Brain	M	0.2	0.6	0.9	4.4	6.5
	F	0.2	0.5	1.2	9.4	16.2
Muscle	M	0.8	0.9	0.6	2.4	4.9
	F	2.2	0.7	1.1	7.0	11.3
Fat	M	ND	1.1	2.4	30.8	82.4
	F	ND	0.8	3.8	96.3	154.7

[a]Source: El-Hawari et al., 1981

[b]Mean values of three to six rats

[c]µg/g tissue

[d]Tissue samples were collected 4 hours after dosing.

ND = Not determined

TABLE 5-3

Tissue Distribution of Radioactivity 24 Hours After Administration of ^{14}C-Trinitrotoluene Using Rabbits[a,b,c]

Tissue	Sex	Oral 5 mg/kg in Peanut Oil	Dermal 5 mg/kg in Peanut Oil	Oral 50 mg/kg in Peanut Oil	Dermal 50 mg/kg in Peanut Oil
Liver	M	1.5	1.0	8.7	7.3
	F	1.7	ND	ND	ND
Kidney	M	0.5	0.6	3.7	6.9
		0.9	ND	ND	ND
Lungs	M	1.7	0.6	2.4	4.2
	F	3.8	ND	ND	ND
Spleen	M	0.2	0.1	1.2	1.0
	F	0.3	ND	ND	ND
Brain	M	0.09	0.09	0.5	0.5
	F	0.1	ND	ND	ND
Muscle	M	0.1	0.1	0.7	0.6
	F	0.2	ND	ND	ND
Fat	M	0.1	0.2	1.8	2.8
	F	ND	ND	ND	ND

[a]Source: El-Hawari et al., 1981

[b]Mean values of two to four rabbits

[c]µg/g tissue

[d]Tissue samples were collected 4 hours after dosing.

ND = Not determined

TABLE 5-4

Tissue Distribution of Radioactivity 24 Hours After Oral Administration of ^{14}C-Trinitrotoluene Using Dogs[a,b,c]

Tissue	Sex	5 mg/kg in Peanut Oil	50 mg/kg in Peanut Oil
Liver	M	4.0	22.6
	F	2.6	ND
Kidney	M	1.1	9.9
	F	1.6	ND
Lungs	M	0.7	8.7
	F	1.5	ND
Spleen	M	1.0	19.8
	F	1.3	ND
Brain	M	0.3	2.2
	F	0.4	ND
Muscle	M	0.2	1.6
	F	0.3	ND
Fat	M	ND	5.2
	F	ND	ND

[a]Source: El-Hawari et al., 1981

[b]Mean values of one to three dogs

[c]µg/g tissue

ND = Not determined

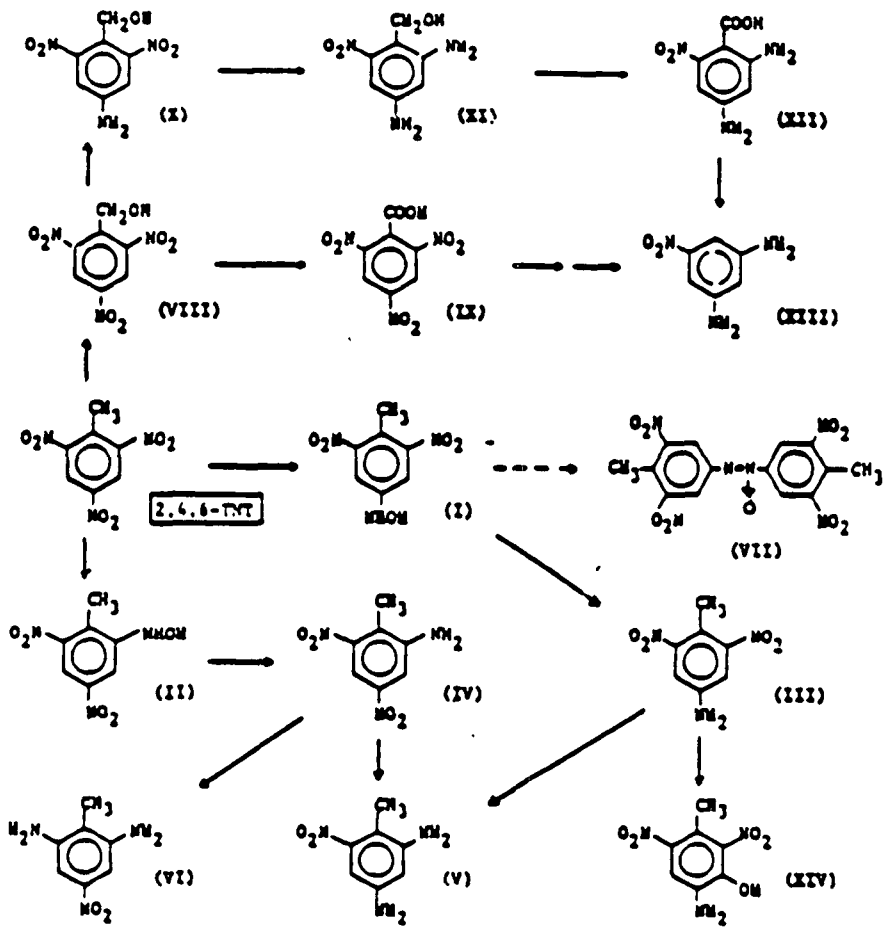
In the four animal species tested, tissue/blood concentration ratios 24 hours after oral or dermal administration of trinitrotoluene were >1.0 in liver, kidney, lung and occasionally spleen and <1 in muscle and brain. Fat tissue had tissue/blood ratios <1.0 after oral dosing and >1.0 after dermal treatment. The tissues accumulated more radioactivity after intratracheal instillation than after oral dosing.

5.3. METABOLISM

Analysis of trinitrotoluene metabolites in urine and bile was conducted using TLC. Because of the large number of metabolites identified, detailed quantitative determinations were not attempted. A schematic presentation of possible metabolic products and pathways is shown in Figure 5-1.

The results of El-hawari et al. (1981) indicate that trinitrotoluene was metabolized extensively in the four species studied, regardless of the route of exposure. Large portions of the products were conjugated with glucuronic acid. No conjugation with sulfuric acid was detected. The urine from bile duct-cannulated rats contained lesser amounts of glucuronides than urine from noncannulated rats. Most of the metabolic products found were nitro-reduction derivatives, such as the 2- and 4-hydroxylamines, the 2- and 4-monoaminodinitro and 2,6- and 4,6-diaminomononitro compounds. There was also evidence for oxidation of the methyl group. The parent compound, trinitrotoluene, was present in small amounts in the urine of rats, mice and dogs, but could not be demonstrated in rabbits.

Only quantitative differences were noted between the metabolic profiles of rats, mice and dogs and between different routes of exposure. The urine of rats contained large amounts of the 4,6-diamine and lesser amounts of the 2,6-diamine and either or both of the 2- or 6-monoamines. The 2- and



(I) 4-Hydroxylamino-2,6-dinitrotoluene
(II) 2-Hydroxylamino-4,6-dinitrotoluene
(III) 4-Amino-2,6-dinitrotoluene
(IV) 2-Amino-4,6-dinitrotoluene
(V) 4,6-Diamino-2-nitrotoluene
(VI) 2,6-Diamino-4-nitrotoluene
(VII) 2,6,2',6'-Tetranitro-4,4'-azoxytoluene
(VIII) 2,4,6-Trinitrobenzylalcohol
(IX) Trinitrobenzoic acid
(X) 4-Amino-2,6-dinitrobenzylalcohol
(XI) 2,4-Diamino-6-nitrobenzylalcohol
(XII) 2,4-Diamino-6-nitrobenzoic acid
(XIII) 5-Nitro-m-phenylenediamine
(XIV) 4-Amino-2,6-dinitro-m-cresol

FIGURE 5-1

Schematic Presentation for Some Possible Biotransformation Products of 2,4,6-Trinitrotoluene

Source: El-hawari et al., 1981

4-hydroxylamines were found in small quantities. The formation of trinitrobenzyl alcohol was also postulated. No significant differences in metabolic profiles were detected between male and female rats. Also, the 4-hour urine contained more of the polar metabolites and more parent compound than did 24-hour urine. Mouse urine contained smaller quantities of the polar metabolites and the diamines and more of the monoamines and hydroxylamines. It also contained considerable amounts of trinitrobenzyl alcohol and trinitrobenzoic acid. The metabolic profiles of dog urine contained appreciable amounts of diamines and monoamines and possibly trinitrobenzyl alcohol and trinitrobenzoic acid. Only trace amounts of the 4-hydroxylamine, the 2-hydroxylamaine and some azoxytoluene were found. Rabbit urine was unique in that the presence of larger quantities of monoamines and hydroxylamines was revealed. Furthermore, it contained either or both of the diamines, trinitrobenzyl alcohol and trinitrobenzoic acid. The only significant difference between urine profiles from orally and dermally dosed rats was that the latter excreted larger amounts of the parent compound.

After ß-glucuronidase hydrolysis of the urine from the different species, the extractable radioactivity increased considerably. No major changes in the profiles were noticed. The amount of glucuronides varied among species, with the least amounts observed in the urine of mice. Urine from dermally exposed animals contained smaller amounts of glucuronide conjugates than did urine from orally exposed animals. The bile was found to contain considerable amounts of low molecular weight glucuronide conjugates.

5.4. EXCRETION

The comparative excretion of radioactivity in the urine, feces and bile in the four species examined (El-hawari et al., 1981) is presented in Table

5-1. No attempt was made to determine elimination of radioactivity through the exhaled air; however, the investigators reported that previous experiments in the same laboratory showed that ~0.1% of an oral dose is eliminated by this route. No significant differences in excretion were noted between males and females. As seen in Table 5-1, large percentages of the orally administered doses were excreted in the urine. The urine of rats and mice had a bright red color. This seems to correspond to a partial reduction product of 2,4,6-trinitrobenzyl alcohol. Rabbits excreted a larger amount of radioactivity in the urine 24 hours after dermal exposure compared with the other species tested.

Biliary excretion seemed to play an important role in the elimination of trinitrotoluene in the four species examined, as indicated by levels of radioactivity located in residual bile. Bile duct-cannulation experiments in rats indicated that biliary and urinary excretion may be equivalent in the elimination of trinitrotoluene.

5.5. SUMMARY

In mice, rats, dogs and rabbits, trinitrotoluene administered orally, dermally or intratracheally (only to rats) is readily absorbed, distributed, metabolized and excreted in the urine and, to a lesser extent, in feces (El-hawari et al., 1981). Generally, the rate of absorption by the three routes tested was intratracheal > oral > dermal. The extent of absorption in the four species tested was not significantly different when trinitrotoluene was administered by the oral route. After dermal exposure, however, the highest absorption occurred in rabbits, followed by mice, rats and dogs. Radioactivity was mainly distributed to the liver and kidney of the animals after oral dosing, but fat contained appreciable amounts of radioactivity following dermal treatment. Trinitrotoluene was extensively metabolized in

all species examined regardless of the route of administration. Identification of products in the urine and bile showed that most metabolites were nitroreduction derivatives. Oxidation of the methyl group had also occurred. Unchanged trinitrotoluene could not be identified in the urine of rabbits. The metabolic profiles of urine from rats, mice and dogs and different routes of exposure differed only quantitatively and no significant sex differences were observed. Urine of rabbits was unique because it contained larger amounts of monoamines and hydroxylamines. Urinary and biliary excretion appear to play nearly equivalent roles in the elimination of trinitrotoluene.

6. EFFECTS

6.1. SYSTEMIC TOXICITY

6.1.1. Inhalation Exposure. Pertinent data regarding inhalation exposure to 2,4,6-trinitrotoluene were not located in the available literature cited in Appendix A.

6.1.2. Oral Exposure.

6.1.2.1. SUBCHRONIC -- In a study conducted by Levine et al. (1983), which is the basis for the currently verified oral RfD (Chapter 7), beagle dogs (6/sex/dose) were administered daily gelatin capsules containg trinitrotoluene (99.1% pure) at doses of 0 (controls), 0.5, 2.0, 8.0 or 32.0 mg/kg/day for 26 weeks. All animals received a blank gelatin capsule for 1 week before testing. Physical examinations, including body weights and food consumption monitoring, were conducted 1 day/week, 3 weeks before trinitrotoluene dosing. A complete hematological profile, clinical chemistry analyses and urinalyses were performed on samples collected several times during the pretest and test periods. Ophthalmic examinations and electrocardiograms were also performed on all test animals during the pretest and test periods. All surviving animals were sacrificed and necropsied during test week 27 following a 16- to 18-hour fast. All major organs and tissues were fixed for microscopic examination.

Clinical signs of toxicity attributed to trinitrotoluene treatment included transient ataxia, darkening of the tongue and gums, evidence of jaundice in animals receiving the 32 mg/kg/day dose, orange/brown urine and orange/red feces in the two highest dose groups. Histological examination of masses, developed in two females in the two highest dose groups, was unremarkable. Two treatment-related deaths occurred in the 32 mg/kg/day female group before week 17. Both animals showed signs of malnutrition.

Significant body weight reduction was reported at the 8 (males only) and 32 mg/kg/day dose levels. Food consumption was significantly reduced for animals at the highest dose level during most of the treatment period. Dose-related anemia (decreased hematocrit, hemoglobin and erythrocyte counts) developed in all trinitrotoluene-treated dogs starting at week 3 and lasted throughout the testing period. These changes were significant at the two highest dose levels. Methemoglobinemia occurred at the 8 and 32 mg/kg/day dose levels. Physiologic compensatory responses to anemia at these doses included reticulocytosis, microcytosis and increased numbers of nucleated RBCs.

Clinical chemistry effects included significant increases in serum globulin levels (at all doses) and serum LDH (in males and possibly females in the 32 mg/kg group) and decreases in SGPT in both sexes at all doses. Total and direct bilirubin levels were elevated at the highest dose tested in males and females and males only, respectively. Urinary bilirubin levels were significantly increased at the two highest dose levels from test week 17 and thereafter. Also, trace levels of urobilinogen were seen during this time in the two highest dosage groups. Opthalmologic and electrocardiographic testing revealed no conclusive effects attributable to trinitrotoluene treatment.

Male (8 and 32 mg/kg/day) and female (32 mg/kg/day) dogs had significant ($p<0.05$) increases in relative and absolute liver weight. A slight but statistically significant ($p<0.05$) increase in relative kidney weights was observed for high-dose females but not males. Relative and absolute increases ($p<0.05$) in spleen weight occurred in females at 8 and 32 mg/kg/day, but only at the latter dose in males. Hepatocytic cloudy swelling and hepatocytomegaly were present in all trinitrotoluene-treated groups;

however, the incidence and severity of the lesion was dose-related. Microscopic evidence of cirrhosis, observed only in treated animals, was reported in one male at the 8 mg/kg/day dose level, six males at the 32 mg/kg/day dose level and one female at the latter dose level. One female dog at the 2 mg/kg/day dose level and all animals at the 8 and 32 mg/kg/day dose levels showed hemosiderosis in histocytes of the liver. The lesion was not reported in controls. In addition, enlargement of the spleen with marked to severe generalized congestion was attributed to trinitrotoluene treatment, particularly at the two highest dose levels tested. None of the microscopic lesions described were observed in the females necropsied before termination of the study. No NOAEL was identified in this study and the dose level of 0.5 mg/kg/day was identified as a LOAEL for liver effects.

Levine et al. (1984a) also conducted a subchronic feeding study in rats. In this study, F344 rats (10/sex/group) were fed a commercial diet containing trinitrotoluene (99.1% pure) at levels providing doses of 1, 5, 25, 125 or 300 mg/kg/day for 13 weeks. A group of 30 rats/sex served as untreated controls. Test animals were monitored daily for toxicological signs. Physical examinations, including body weights and palpations for masses, were performed weekly. Clinical biochemistry and hematological tests were performed on all survivors on week 13. Gross necropsy and histopathological examinations were conducted on all animals. Lethargy and ataxia were observed in some animals receiving doses of $\geq$125 mg/kg/day throughout the testing period. The investigators attributed two deaths at the 300 mg/kg/day dose level on week 13 to severe anemia. Doses of $\geq$125 mg/kg/day resulted in decreased food intake with concommitant reduction in body weight gain. Dose-related anemia (decreased hematocrit, hemoglobin and erythrocyte counts) was observed in treated rats. These changes were statistically

significant ($p<0.05$) in males at $\geq$25 mg/kg/day, but only at $\geq$125 mg/kg/day in females. Methemoglobinemia occurred in both males and females administered doses of 300 mg/kg/day. Elevated liver weights were reported in male and female rats $\geq$25 mg/kg/day. Serum cholesterol levels and relative spleen weights increased significantly in both males and females at trinitrotoluene dose levels $\geq$125 mg/kg/day. Dose-dependent congestive lesions were observed in the spleen, whereas hyperplasia was noticed in the liver of animals receiving doses $\geq$125 mg/kg/day. Degenerative lesions in tracts of the cerebellar folia were seen at 300 mg/kg/day. Histological examination of the testes revealed dose-related degeneration of the germinal epithelium lining the seminiferous tubules of males at 125 and 300 mg/kg/day. The investigators concluded that the liver, testes and blood are the main targets of trinitrotoluene toxicity and that the splenic lesions were secondary to the hemolytic effect.

Dilley et al. (1982) examined the toxicity of trinitrotoluene in subchronic studies in mice, rats and dogs. Beagle dogs (5/sex/group) received daily gelatin capsules containing equal weights of trinitrotoluene (>99% pure) and lactose, which provided doses of 0 (control), 0.2, 2.0 or 20 mg/kg/day of trinitrotoluene. One male and one female were treated for 1 week and sacrificed. A second male and female were treated for the same period and then allowed to recover for 4 weeks. The remaining dogs were treated for 13 weeks and then two males and two females from each group were sacrificed. The remaining dogs were allowed to recover for 4 weeks and then sacrificed. Sprague-Dawley rats (20/sex/group) were fed trinitrotoluene in a commercial diet at levels of 0 (control), 0.002, 0.01, 0.05 or 0.25%. Based on food consumption and body weight data, the investigators estimated that the intake of trinitrotoluene by treated rats was 1.40, 6.97, 34.7 and 160 mg/kg/day for males, and 1.45, 7.41, 36.4 and 164 mg/kg/day for females.

Five rats/sex/group were treated for 4 weeks and then sacrificed; 5/sex/group were treated for 13 weeks and sacrificed; the remaining 5/sex/group were treated for 13 weeks and were then allowed to recover for 4 weeks, at which time they were sacrificed. The mice (Swiss-Webster strain) were fed a commercial diet containg trinitrotoluene at levels of 0 (control), 0.001, 0.005, 0.025 or 0.125%. Based on food consumption and body weight data, the investigators estimated that the intake of trinitrotoluene was 1.56, 7.46, 35.7 or 193 mg/kg/day for males, and 1.57, 8.06, 37.8 or 188 mg/kg/day for females. The group sizes, group compositions and treatment schedule were the same as for rats. All animals were observed at least once daily and weighed once weekly. Food consumption was recorded 5 days/week for dogs and once weekly for rodents. Blood samples were taken periodically from dogs, but only at terminal sacrifice from mice and rats. Hematological parameters were determined in the three species; clinical chemistry tests were performed in dogs and rats only. Bladder urine was collected from dogs, mice and rats at the time of sacrifice. All major organs and tissues were examined grossly and then fixed for microscopic examination.

The resulting changes in hematological parameters and in gross and microscopical appearance of the spleen are suggestive of the development of hemolytic anemia at the highest dose used. Because the number of dogs used was small, the significance of the results is questionable but compatible with the Levine et al. (1983) study upon which the RfD was based.

Red-colored urine at the 0.05 and 0.25% levels was the only sign of toxicity displayed by rats during treatment. However, urine color returned to normal after treatment was discontinued. Hematologic changes seen in rats included low RBC counts, hemoglobin and hematocrit and increased mean corpuscular volume and leukocyte counts, particularly at the 0.25% dose

level. Other effects in rats were confined to the 0.25% group. A significant ($p<0.05$) reduction in food consumption was seen, but food consumption increased when treatment was discontinued. Body weight gain was significantly ($p<0.01$) reduced but recovered when treatment ceased. Spleen weight and the spleen/brain weight ratios (both sexes) were significantly increased, and absolute and relative testes weight were significantly decreased. At weeks 4 and 13, rats had a significant ($p<0.01$) increase in serum cholesterol. Also at week 13, SGPT and serum iron levels were markedly ($p<0.01$) reduced in males but not in females. Hemosiderosis of the spleen (both sexes) and testicular atrophy (males) accompanied by hyperplasia were seen in animals sacrificed immediately after treatment. Males allowed to recover for 4 weeks showed atrophy of the epididymis.

Mice also had a red coloration in their urine, which disappeared after cessation of trinitrotoluene treatment. No other overt signs of toxicity attributable to treatment were noticed. Food consumption and body weight decreased initially in the groups at the highest dose and remained slightly low for females given the 0.125% trinitrotoluene diet. Changes in body weight were sporadic and inconsistent. Treatment with trinitrotoluene induced an increase in absolute and relative spleen weight, but a dose-response relationship was not always obvious. Increased liver weight and occasional necrosis was also noticed in high-group male mice sacrificed at week 17. Mild hematological changes indicative of hemolytic anemia were seen in mice at the 0.125% dose level. No histopathological signs were noticed in mice sacrificed after 4 weeks of treatment, with or without a recovery period. Hemosiderosis of the spleen was observed in mice (3/5 males, 5/5 females) after 13 weeks of treatment at the 0.125% trinitrotoluene level and in 1/5 male and 4/5 females at the 0.025% level at week 17.

Martin (1974) conducted a study using cynomolgus monkeys (3/sex/group) in which trinitrotoluene (purity not reported) suspended in methylcellulose was administered by gavage at doses of 0 (solvent alone), 0.02, 0.1 or 1.0 mg/kg/day for 90 days. Hematology, clinical chemistry tests, urinalysis and liver function tests revealed no alterations attributable to trinitrotoluene treatment. Gross lesions, observed only with the two higher doses, included two cases of subcapsular renal hemorrhage and one monkey with mucosal reddening and focal thickening of the large intestine. Histological examination revealed some increases in the numbers of necrotic megakaryocytes in bone marrow and increased amounts of iron-positive material in the liver. According to the investigators, the significance of the histological findings is uncertain.

Hart (1974) also conducted a study in which beagle dogs (3/sex/dose) were fed a commercial diet containing trinitrotoluene (purity not reported) for 90 days. This diet provided trinitrotoluene doses of 0 (control), 0.02, 0.1 or 1.0 mg/kg/day. Hematology, clinical chemistry tests, urinalysis and gross and microscopic appearance of organs and tissues were not affected by administration of trinitrotoluene. Temporary episodes of emesis occurred but tolerance appeared to develop.

6.1.2.2. CHRONIC -- Furedi et al. (1984a) evaluated the chronic toxicity of trinitrotoluene in rats (Section 6.2.2.). In this study, 6- to 7-week-old F344 rats (75/sex/dose) were administered trinitrotoluene (>99% pure) mixed in a commercial diet for $\leq$24 months. According to the investigators, this diet provided doses of 0 (control), 0.4, 2, 10 or 50 mg/kg/day of trinitrotoluene. Ten rats/sex/dose were sacrificed following 27 and 53 weeks on test and the remaining animals were sacrificed after 24 months of treatment. All animals were observed once daily for pharmacological and

toxicological signs. Physical examinations were conducted weekly until test week 13 and biweekly thereafter. Food consumption and body weights were also monitored weekly until test week 13 and biweekly thereafter until termination of the study. Ophthalmic examinations were performed before testing commenced and during weeks 25, 51, 76 and 103. Complete hematological and clinical chemistry tests were performed on blood samples drawn from the same 10 rats/sex/dose during weeks 14, 26, 52, 78 and 104. The brain, gonads, heart, liver, kidneys, spleen, spinal cord, pituitary gland, urinary bladder (females) and sternal bone marrow (females) from rats receiving doses of 0.4, 2.0 and 10 mg/kg/day were examined microscopically. Additional tissues and organs from control and 50 mg/kg/day dose groups were also examined.

Administration of trinitrotoluene did not affect survival rate nor did it induce signs of toxicity other than an apparent increase in ocular discharge in high-dose males during the second year of the study. Doses of trinitrotoluene $\geq$10 mg/kg/day induced dose-related reduction in body weight gain and in food consumption. A dose-related reduction in hematocrit, hemoglobin and RBC count was observed in males throughout the study and in females during the first year at the 10 and 50 mg/kg/day dose level. Compensatory responses to the anemic state were minimal. The only other hematological effects seen, considered related to trinitrotoluene-treatment, were methemoglobinemia in males at 10 and 50 mg/kg/day and thrombocytosis in male and female rats at 50 mg/kg/day during the second year of the study.

Clinical chemistry tests showed an increase in serum cholesterol in males with doses $\geq$2.0 mg/kg/day and in females at 50 mg/kg/day. At week 104, females in the highest dose group had a decrease in serum triglyceride levels; males showed hypertriglyceridemia. In general, serum total protein, albumin and globulin levels were increased in male and female rats given

doses of trinitrotoluene of 50 mg/kg/day. In addition, high-dose rats had slightly increased BUN levels during the second year of the study. Other changes in clinical chemistry parameters were sporadic and not considered related to trinitrotoluene treatment.

Administration of trinitrotoluene did not induce ophthalmologic abnormalities. Dose-related hepatomegaly and increased kidney weights were seen during interim sacrifices at weeks 27, 53 and at the end of the study period at dose levels of 10 and 50 mg/kg/day. Absolute spleen weight (both sexes) was increased at weeks 27 and 53 in animals administered 50 mg/kg/day. Relative heart weight increased in females at weeks 27 and 53 and in both sexes at week 105 at trinitrotoluene dosage levels of 10 and 50 mg/kg/day. Treatment-related lesions were seen in the spleen and kidneys of rats sacrificed after 27 and 53 weeks of treatment. The histological changes were seen primarily at doses $\geq$2.0 mg/kg/day. Increased pigmentation, sinusoidal congestion and extramedullary hematopoiesis were seen in the spleen. Changes in the kidney included hypertrophy of proximal convoluted tubules, increased pigmentation and chronic nephropathy.

After 24 months of treatment, lesions were present in the liver of males, urinary bladder and bone marrow of females and the spleen and kidney of both sexes. Male rats in the 10 and 50 mg/kg/day dose levels had a dose-related increased incidence of hepatocellular hyperplasia associated with peliosis and cystic degeneration. Urinary bladder lesions in females included hyperplasia of the mucosal epithelium at $\geq$10 mg/kg/day. A significant increase in incidence of sternal bone marrow fibrosis was observed in females with doses $\geq$2.0 mg/kg/day. The latter effect was also seen in males at 50 mg/kg/day, but lower dose males were not examined. Based on the occurrence of splenic, renal and bone marrow effects at doses $\geq$2.0 mg/kg/day, a NOEL of 0.4 mg/kg/day can be identified from this study.

Furedi et al. (1984b) also investigated the chronic toxicity of trinitrotoluene in mice (Section 6.2.2.). In this study, B6C3F1 hybrid mice (75/sex/dose) were administered trinitrotoluene (>98.8% pure) mixed in a commercial diet for ≤24 months. According to the investigators, this diet provided doses of trinitrotoluene of 0 (control), 1.5, 10 or 70 mg/kg/day. The protocol used and the endpoints examined were identical to the ones used in the rat study (Furedi et al., 1984a). Trinitrotoluene administration did not affect mortality rate. Reductions in body weight gain for both sexes were seen in animals receiving 10 and 70 mg/kg/day, but this effect was significant only at the highest dose tested. Sporadic and mild episodes of anemia (reduced hematocrit, hemoglobin and RBC count) were observed in males and females at 70 mg/kg/day. No compensatory responses were apparent. Furthermore, splenic lesions indicative of hemolytic anemia were not observed in this study. Hepatomegaly was present at 70 mg/kg/day, but without histological alterations. Weight changes in other organs were described as sporadic and not supported histologically.

6.1.3. Other Relevant Information. Dilley et al. (1982) administered single gavage doses of trinitrotoluene dissolved in corn oil to Swiss-Webster mice and Sprague-Dawley rats. Oral LD_{50}s of 660 mg/kg in male and female mice and 1320 and 795 mg/kg in male and female rats, respectively, were reported. The mice and rats were observed for ≤14 days. Signs of toxicity included inactivity, development of tremors and mild convulsions and death. Animals that survived convulsions were still alive after 14 days.

Levine et al. (1984b) studied the acute effects of trinitrotoluene in hybrid B6C3F1 mice (10/sex/dose). Trinitrotoluene (>99% pure) was mixed in the diet to provide doses of 0, 0.3, 2, 14, 100 or 700 mg/kg/day for 28 days. Trinitrotoluene did not affect survival rate at any dose level.

Weight loss (700 mg/kg/day) and weight gain reduction (100 mg/kg/day) were the only clinical signs observed. Treatment-related morphologic alterations (hemosiderosis) were seen in the spleens at 100 and 700 mg/kg/day. Further toxic effects of trinitrotolueune seen primarily at the 700 mg/kg/day dose level were leukopenia, thrombocytosis, slight hepatomegaly, marginal decrease in testes weight and increased kidney weight. Organ weight changes, however, were not accompanied by histological alterations. A NOAEL of 14 mg/kg/day was identified.

Single intraperitoneal injections of 100 mg/kg of trinitrotoluene in olive oil to adult male Wistar rats caused damage in cerebral, hepatic and renal biomembranes (Zitting et al., 1982). According to the investigators, intracellular damage observed, predominantly in brain and kidney, is consistent with the formation of superoxides in aerobic conditions during nitroreduction of trinitrotoluene.

Cases of human exposure to trinitrotoluene are numerous but involved multiple routes of exposure. Details regarding levels and exposure durations were usually incomplete, rendering these studies unsuitable for quantitative risk assessment. A review of occupational exposure studies (Hathaway, 1977) indicated that workers exposed to air levels between 0.01 and 4.0 mg/m^3 may develop skin irritation, liver damage and anemia. Morton et al. (1976) reported that workers in an ammunition plant producing trinitrotoluene had significant increases in serum LDH ($p<0.005$) and SGOT ($p<0.01$) when the concentration of trinitrotoluene in the air increased from 0.3 to 0.8 mg/m^3 in ~30 days because of an increase in the trinitrotoluene production rate. Hemoglobin values were not significantly different from values before the production increase. When affected individuals were removed from exposure, it took 1-3 weeks for LDH values to return to normal range. Goodwin (1972) reported a mean of 1.80 MacLagen units in the thymol

turbidity test in 1537 workers exposed to trinitrotoluene in an ammunition shell loading plant. The author considered the test to be an indication of hepatic irritation. Mean preemployment levels were 0.93 MacLagen units. Levels of trinitrotoluene in the workroom atmosphere ranged from 0.2-4.7 mg/m³ with a mean of 2.38 mg/m³. Reportedly, 36 workers (2.0%) had "classical symptoms of liver damage."

Cone (1944) reported that transient leukocytosis and moderate eosinophilia developed in 17 workers exposed to trinitrotoluene levels between 0.5 and 2.0 mg/m³ of trinitrotoluene in the air (duration not specified) as compared with preexposure levels. Stewart et al. (1945) reported the case of 62 employees of a munitions loading plant exposed to a presumed average trinitrotoluene level between 0.3 and 1.3 mg/m³ for 4-11 weeks. Skin exposure was inferred by the appearance of skin rashes and 85% of the individuals had considerable reduction in their hemoglobin levels. RBC counts decreased and bilirubin levels increased. Friedlander et al. (1974) reported that anemia developed in workers exposed to trinitrotoluene levels between <0.03 and 4.0 mg/m³. No differences, however, were noticed in clinical parameters between test individuals and unexposed controls in a follow-up study performed at the same facility after reducing the exposure levels to 0.08-0.59 mg/m³ for 8 hours/day. Buck and Wilson (1975) reported that in a case of 533 employees (865 controls) exposed to trinitrotoluene levels ranging from <0.01-1.84 mg/m³ (only 12.2% were exposed to >0.5 mg/m³), the only significant finding was an inverse relationship between levels of exposure and hemoglobin concentration. Harkonen et al. (1983) reported that 6/12 workers exposed to trinitrotoluene levels in the air between 0.14 and 0.58 mg/m³ for a mean duration of 6.8 years developed equatorial lens opacities. There was no effect on visual acuity or visual fields. Blood chemistry and hematological tests were unremarkable. These

findings suggest that the eye may be the critical target organ for chronic exposure to low levels of trinitrotoluene in the air.

6.2. CARCINOGENICITY

6.2.1. Inhalation. Pertinent data regarding the inhalation carcinogenicity of trinitrotoluene were not located in the available literature cited in Appendix A.

6.2.2. Oral. The carcinogenic properties of trinitrotoluene in rats were studied by Furedi et al. (1984a). In this study, 6- to 7-week-old F344 rats (75/sex/dose) were administered trinitrotoluene (>99% pure) mixed in a commercial diet for ≤24 months. According to the investigators, this diet provided doses of 0 (control), 0.4, 2, 10 or 50 mg/kg/day of trinitrotoluene. A complete description of the protocol and of the noncarcinogenic effects was provided in Section 6.1.2.2. Observations regarding systemic toxicity indicated that the MTD had been achieved (U.S. EPA, 1988a). A significant increase in the combined incidence of urinary bladder papilloma and carcinoma was observed at terminal sacrifice in female rats administered the 50 mg/kg/day dose of trinitrotoluene (Table 6-1). No such lesions were noticed at interim sacrifices after 6 or 12 months of treatment. The investigators indicated that the fact that incidences of hepatocellular (male rats) and renal and urinary bladder hyperplasia (female rats) also significantly increased at the 50 mg/kg/day dose level support the conclusion that trinitrotoluene is carcinogenic to F344 rats under these experimental conditions (Table 6-2).

The carcinogenic potential of trinitrotoluene was also investigated in mice (Furedi et al., 1984b). In this study, 4- to 5-week-old B6C3F1 hybrid mice (75/sex/dose) were administered trinitrotoluene (>98.8% pure) mixed in a commercial diet for ≤24 months. According to the investigators, this diet provided doses of trinitrotoluene of 0 (control), 1.5, 10, or 70 mg/kg/day.

TABLE 6-1

Incidence of Urinary Bladder Tumors in Female F344 Rats fed Diets Containing Trinitrotoluene (>99% pure) for 24 Months[a]

Dose[b] (mg/kg/day)	Tumor Type	Incidence
0	papilloma	0/54
	carcinoma	0/54
	combined	0/54
0.4	papilloma	0/54
	carcinoma	0/54
	combined	0/54
2	papilloma	0/55
	carcinoma	0/55
	combined	0/55
10	papilloma	1/55
	carcinoma	0/55
	combined	1/55
50	papilloma	5/55[c]
	carcinoma	12/55[d]
	combined	17/55[d]

[a]Source: Furedi et al., 1984a

[b]Provided by investigators

[c]$p<0.05$

[d]$p<0.01$

TABLE 6-2

Incidence of Hyperplastic Lesions in F344 Rats Fed Diets Containing Trinitrotoluene (>99% pure) for 24 months[a]

Dose[b] (mg/kg/day)	Hepatocellulor Hyperplasia (males)	Urinary Bladder Hyperplasia (females)
0	6/26	0/37
0.4	7/22	0/40
2	6/20	0/40
10	16/14[d]	2/46
50	27/12[d]	12/47[d]

[a]Source: Furedi et al., 1984a

[b]Provided by investigators

[c]$p<0.05$

[d]$p<0.01$

The use of these doses is based on the results of a range-finding study by the same investigators (Levine et al., 1984b) in which an MTD between 14 and 100 mg/kg/day was estimated. The protocol used and the endpoints examined were identical to the ones used in the rat study (Furedi et al., 1984a). A complete description of the noncarcinogenic effects was provided in Section 6.1.2.2. Neoplastic lesions observed after 6 and 12 months of treatment were considered incidental and not treatment-related. A significant ($p<0.05$) increase in the combined incidence of leukemia/malignant lymphoma in the spleen was observed in females at the 70 mg/kg/day dose level. The incidences were 9/45, 15/39, 17/37 and 21/33 in the 0, 1.5, 10 and 70 mg/kg/day dietary levels, respectively. U.S. EPA (1988a), however, concluded that these tumors were not chemical-related because when malignant lymphomas and lymphocytic leukemia in all tissues were combined rather than considered separately, there was neither a significantly increased incidence nor a significant trend.

Trinitrotoluene has not been scheduled for carcinogenicity testing by NTP (1989).

6.2.3. Other Relevant Information. The carcinogenic potential of 2,4,6-trinitrotoluene is supported by the carcinogenicity of the structurally related 2,4- and 2,6-dinitrotoluene. Both isomers are considered to be class B2 probable human carcinogens based upon the existence of sufficient evidence of carcinogenicity in two species of animals (U.S. EPA, 1988c).

The possibility that one or more of the metabolites of 2,4,6-trinitrotoluene was carcinogenic was also considered. No information on the carcinogenicity or mutagenicity of the proposed metabolites was found in the available literature but an examination of the structure of the compounds reveals several that are likely to form DNA adducts. The

N-hydroxylated species (II), as well as aminobenzene derivatives (III, IV, XIII) seem likely to be mutagenic and to have potential as carcinogens (Figure 5-1). Many of the remaining compounds are highly polar. Those compounds would be unlikely to form DNA adducts and would be rapidly excreted (El-hawari et al., 1981).

6.3. MUTAGENICITY

Trinitrotoluene gave positive mutagenic responses when tested by the reverse mutation assay in several strains of Salmonella typhimurium in the absence of activating systems (Table 6-3). In the presence of activating systems, trinitrotoluene was nonmutagenic (Won et al., 1976; Whong and Edwards, 1984). Furthermore, the mutagenicity of trinitrotoluene in S. typhimurium was heavily dependent on the presence of nitroreductases (Whong and Edwards, 1984). The latter indicates that reduction of the nitro groups, possibly to hydroxylamino intermediates, may be an essential step for the in vitro mutagenic activity. When tested in mammalian systems in vivo, trinitrotoluene did not induce chromosome damage in mice bone marrow cells or unscheduled DNA synthesis in rat hepatocytes (Ashby et al., 1985). However, trinitrotoluene was mutagenic in mouse lymphoma cells in culture in the absence of an activating system (Styles and Cross, 1983).

6.4. DEVELOPMENTAL TOXICITY

Pertinent data regarding the developmental effects of trinitrotoluene were not located in the available literature cited in Appendix A.

6.5. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding other reproductive effects of trinitrotoluene were not located in the available literature cited in Appendix A.

6.6. SUMMARY

Reported oral LD_{50}s for trinitrotoluene administered by gavage were 660 mg/kg in male and female mice and 1320 and 795 mg/kg in male and female

TABLE 6-3

Mutagenicity Testing of 2,4,6-Trinitrotoluene

Assay	Indicator Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
MICROBIOLOGICAL ASSAY								
Reverse mutation	Salmonella typhimurium TA1535, TA100, TA1537, TA1538, TA98	commercial grade	plate incorporation	11-176 nmol/ plate	-S-9	+	Mutagenicity was lost in the presence of activating system or when a nitroreductase-deficient strain was used	Whong and Edwards, 1984
Reverse mutation	S. typhimurium TA98, TA1538, TA100	commercial grade	plate incorporation	5-500 µg/plate	±S-9	+ +	NC	Kaplan and Kaplan, 1982a
Reverse mutation	S. typhimurium TA98	explosive grade	plate incorporation	0.5-10 µg/mL	-S-9	+	Frameshift mutagen. No mutagenicity was seen when tested in cultures inoculated with base substitution tester strains	Won et al., 1976
Reverse mutation	S. typhimurium TA98	explosive grade	plate incorporation	0.5-10 mg/mL	+S-9	-	NC	Won et al., 1976
MAMMALIAN SYSTEMS								
Bone marrow micronucleus assay (chromosome damage)	male mice (CBAxBalbC)F1	>99.0	intraperitoneal	40 mg/kg	NA	-	Mice received a single intraperitoneal injection of test compound. Sampling was done at 24, 48 and 72 hours after injection	Ashby et al., 1985
DNA repair (UDS)	male rats Alderley Park and/or F344	>99.0	gavage	100-1000 mg/kg	NA	-	Hepatocytes were isolated 12 hours after dosing	Ashby et al., 1985
Forward mutation	P388 mouse lymphoma cells	NR	cell culture	0-1000 µg/mL	-S-9	+	In the presence of activating system TNT was not mutagenic	Styles and Cross, 1983

NR = Not reported; NA = not applicable; NC = no comment

rats, respectively. LD_{50} data for other species were not available. Data were not available regarding the toxicity of trinitrotoluene to animals by inhalation exposure.

Data regarding the toxicity of trinitrotoluene in humans indicated that workers exposed to air levels between 0.01 and 4.0 mg/m³ may develop skin irritation, liver damage and anemia (Hathaway, 1977; Morton et al., 1976). Although there are numerous reports of occupational exposure to trinitrotoluene, the duration and levels of exposure were usually not sufficiently defined to permit use of these studies in risk assessment.

Trinitrotoluene yielded evidence of carcinogenicity in a 24-month dietary exposure study using rats (Furedi et al., 1984a). In that study, female rats had a significantly increased incidence of urinary bladder papillomas and carcinomas. Trinitrotoluene was not carcinogenic when tested in mice (Furedi et al., 1984b). Trinitrotoluene was mutagenic in reverse mutation assays in S. typhimurium in the absence of activating systems (Kaplan and Kaplan, 1982b; Whong and Edwards, 1984; Won et al., 1976). The presence of activating systems reduced the mutagenic potency. Trinitrotoluene did not induce DNA damage in mammalian test systems in vivo (Ashby et al., 1985).

Subchronic studies using animals suggest that dogs are the most sensitive species since a dose of 0.5 mg/kg/day for 26 weeks induced signs of anemia and liver alterations (Levine et al., 1983). Increasing doses increased the severity of the effects. Death occurred with a dose of 32 mg/kg/day before week 17. In contrast, death from anemia occurred in rats with doses of 300 mg/kg/day (~10 times higher than in dogs) administered for 13 weeks (Levine et al., 1984a). Mice appeared to be less sensitive. A dose of 190 mg/kg/day for 13 weeks produced liver effects, but 36 mg/kg/day

was without adverse effects; it is possible that doses between 36 and 190 mg/kg/day could have been toxic. Chronic studies have been performed only on rats and mice. In rats, a dose of 2.0 mg/kg/day in the diet for 24 months caused kidney hypertrophy, spleen congestion and bone marrow fibrosis in females. A dose of 10 mg/kg/day induced signs of anemia, changes in organ weights and urinary bladder lesions in females. In contrast, a dose of 10 mg/kg/day administered in the diet for 24 months to mice was without adverse effects. In general, the most sensitive endpoints for assessing toxicological effects of trinitrotoluene seem to be the liver and elements in the blood, such as the RBCs.

Data regarding the developmental and reproductive toxicity of trinitroluene were not available in the literature cited in Appendix A.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

The U.S. EPA (1988b) verified a chronic oral RfD of 5×10^{-4} mg/kg/day for 2,4,6-trinitrotoluene based on a LOAEL of 0.5 mg/kg/day for liver effects in a 26-week feeding study using beagle dogs conducted by Levine et al. (1983). An uncertainty factor of 1000 was used.

ACGIH (1988) recommended a TLV-TWA of 0.5 mg/m³ for trinitrotoluene. This recommendation is based largely on the conclusions of Goodwin (1972) and Morton et al. (1976) who reported liver damage and alterations in biochemical parameters in workers at munitions plants (ACGIH, 1986). Trinitrotoluene was also identified as a methemoglobin inducer (ACGIH, 1988). In addition, ACGIH (1988) indicates that dermal contact may contribute to overall exposure. OSHA (1989) established a PEL for trinitrotoluene of 0.5 mg/m³ TWA.

7.2. AQUATIC

U.S. Army limits of 1 mg/ℓ in potable water and 5 mg/ℓ in waters used by fish and wildlife were reported by Smock et al. (1976).

8. RISK ASSESSMENT

8.1. CARCINOGENICITY

8.1.1. Inhalation. Pertinent data regarding the inhalation carcinogenicity of 2,4,6-trinitrotoluene were not located in the available literature cited in Appendix A.

8.1.2. Oral. In a study conducted by Furedi et al. (1984a), 6- to 7-week-old F344 rats (75/sex/dose) were administered trinitrotoluene in the diet at doses of 0, 0.4, 2, 10 or 50 mg/kg/day for 24 months. Administration of trinitrotoluene did not affect survival rate nor did it induce signs of toxicity throughout the study. Dose-related hematological alterations and histological examination of organs and tissues indicated that the MTD had been achieved. A significant increase ($p<0.01$) in the combined incidence of urinary bladder papilloma and carcinoma (0/54, 0/54, 1/55 and 17/55 with increasing doses of trinitrotoluene) was observed in female rats. According to the investigators, the historical incidence of these tumors is low (<1%). The incidences of hepatocellular (male rats), renal and urinary bladder hyperplasia (female rats) were also increased at the 50 mg/kg/day dose level. Thus, the conclusion that trinitrotoluene is carcinogenic in F344 rats under the experimental conditions used can be made.

In a study conducted by Furedi et al. (1984b), 4- to 5-week-old B6C3F1 hybrid mice (75/sex/dose) were administered trinitrotoluene in the diet at doses of 0, 1.5, 10 or 70 mg/kg/day for 24 months. An MTD between 14 and 100 mg/kg/day had been previously estimated. A significant ($p<0.05$) increase in the combined incidence of leukemia/malignant lymphoma in the spleen (9/45, 15/39, 17/37 and 21/33 with increasing levels of trinitrotoluene) was observed in females. However, the U.S. EPA (1988a) noted that

the incidence of malignant lymphomas and lymphocytic leukemia in all tissues combined was not significantly increased and concluded that the effect was not chemical-related.

8.1.3. Other Routes. Pertinent data regarding the carcinogenicity of trinitrotoluene administered by other routes were not located in the available literature cited in Appendix A.

8.1.4. Weight of Evidence. No data were available regarding the carcinogenicity of trinitrotoluene in humans. The animal carcinogenicity data are limited to positive results in female F344 rats (Furedi et al., 1984a) and negative results in B6C3F1 mice (Furedi et al., 1984b). Both studies were well conducted with an adequate number of animals/sex/dose. Trinitrotoluene was mutagenic when tested in S. typhimurium, but was not genotoxic in mammalian cells in vivo. Based on the evidence discussed above, trinitrotoluene has been assigned to U.S. EPA Group C: possible human carcinogen, using the U.S. EPA (1986b) guidelines (U.S. EPA, 1988a, 1989).

8.1.5. Quantitative Risk Estimates.

8.1.5.1. INHALATION -- Pertinent data regarding carcinogenicity to humans or animals of inhalation exposure to trinitrotoluene were not located in the available literature cited in Appendix A. U.S. EPA (1988a) did not estimate a slope factor for inhalation exposure or risk-specific levels in air from the oral slope factor (Section 8.1.5.2.).

8.1.5.2. ORAL -- A human slope factor (q_1^*) of 3.0×10^{-2} $(mg/kg/day)^{-1}$ was computed with the linearized multistage model by the U.S. EPA (1988a, 1989) using bladder tumor incidence data from female rats in the study by Furedi et al. (1984a). The concentration of trinitrotoluene in drinking water associated with increased lifetime risks of cancer are

0.01, 0.001 and 0.0001 mg/ℓ, equivalent to 10, 1 and 0.1 µg/ℓ, respectively, at risk levels of 10^{-5}, 10^{-6} and 10^{-7}, respectively. These concentrations were calculated by dividing the given risk level by the q_1^* to obtain a risk specific dose and then multiplying the dose by the body weight for humans (70 kg) and dividing by the reference daily water consumption of 2 ℓ for humans (U.S. EPA, 1980).

8.2. SYSTEMIC TOXICITY

8.2.1. Inhalation Exposure. Pertinent data regarding inhalation exposure to trinitrotoluene were not located in the available literature cited in Appendix A.

8.2.2. Oral Exposure.

8.2.2.1. LESS THAN LIFETIME (SUBCHRONIC) -- Several subchronic studies have been performed with trinitrotoluene using dogs, rats and mice. In the study by Levine et al. (1983), beagle dogs were treated with 0, 0.5, 2.0, 8.0 or 32 mg/kg/day in capsules for 26 weeks. Hepatocytic cloudy swelling and increased liver weight were reported at 0.5 mg/kg/day (Rec. #1, Appendix C). The severity of the lesions increased with increasing doses of trinitrotoluene. Furthermore, higher doses of trinitrotoluene induced hematological and clinical chemistry alterations and death. The lowest dose of trinitrotoluene tested, 0.5 mg/kg/day, was identified as a LOAEL.

In the study by Levine et al. (1984a), F344 rats were administered doses of 0, 1, 5, 125 or 300 mg/kg/day in the diet for 13 weeks. Rats at 25 mg/kg/day had a significant increase in liver weight (Rec. #10, Appendix C). Male rats, at this dietary level of trinitrotoluene, showed a significant decrease in the hematocrit, hemoglobin concentration and erythrocyte counts. These changes are indicative of anemia. Effects seen at higher doses included histological alterations in the testes and cerebellum (Rec. #11,

Appendix C). A dose of 5 mg/kg/day of trinitrotoluene was without adverse effects and represent a NOAEL in this study (Rec. #9, Appendix C).

Dilley et al. (1982) conducted subchronic studies using dogs, rats and mice. Dogs were treated with trinitrotoluene in capsules at 0, 0.2, 2.0 or 20 mg/kg/day for ≤13 weeks. The lowest dose was a NOEL; red colored urine and splenic effects were reported at 2.0 and 20 mg/kg/day, respectively. Because of the small number of dogs used, the results cannot be used for risk assessment. Rats were fed diets containing 0, 0.002, 0.01, 0.05 or 0.25% trinitrotoluene for ≤13 weeks. The high group showed depressed body weight gain, increased spleen weight and testicular atrophy with hyperplasia and anemia (Rec. #14, Appendix C). The 0.05% diet (equivalent to a dose of 34.7 mg/kg/day in the males and 36.4 mg/kg/day in the females) did not appear to cause adverse effects and is designated as a NOAEL (Rec. #13, Appendix C). Mice were fed diets containing 0, 0.001, 0.005, 0.025 or 0.125% trinitrotoluene for ≤13 weeks. High-group mice showed increased spleen and liver weights, occasional hepatocellular necrosis in some male mice and mild hematological changes (Rec. #15, Appendix C). No adverse effects were reported at 0.05% in the diet, equivalent to 35.7 mg/kg/day in the males and 37.8 mg/kg/day in the females (Rec. #16, Appendix C).

The three studies considered above appear comparable in quality. Examination of the these three studies clearly indicates that dogs are the most sensitive species. The study of dogs (Levine et al., 1983) has served as the basis for deriving the verified chronic oral RfD (U.S. EPA, 1988b). The LOAEL identified in dogs, 0.5 mg/kg/day, is an order of magnitude lower than the NOEL for F344 rats (Levine et al., 1984a) and remains the most appropriate basis for estimation of an RfD for subchronic oral exposure.

In an earlier analysis of these data, U.S. EPA (1989) concluded that the dog LOAEL of 0.5 mg/kg/day was the most appropriate basis from the subchronic data for the longer-term HA. Application of an uncertainty factor of 1000 (10 to estimate a NOAEL from a LOAEL, 10 to extrapolate from animals to humans and 10 to provide additional protection for more sensitive members of the population) would result in an RfD for subchronic oral exposure of 0.0005 mg/kg/day. Because this value is identical to the RfD for chronic oral exposure, U.S. EPA (1989) conservatively adopted the chronic RfD to serve as the basis for the longer-term HA. To maintain consistency with the U.S. EPA (1989) analysis, and because this current evaluation has revealed no additional data or changes in methodology to suggest that another approach would be more appropriate, the RfD of 0.0005 mg/kg/day for chronic oral exposure (Section 8.2.2.2.) is adopted as the RfD for subchronic oral exposure. Confidence in the key study, data base and RfD are medium as discussed in Section 8.2.2.2.

8.2.2.2. CHRONIC -- Two studies that examined the chronic toxicity of trinitrotoluene were identified. In the first study (Furedi et al., 1984a), F344 rats were administered trinitrotoluene in the diet at doses of 0, 0.4, 2, 10 or 50 mg/kg/day for 24 months. A dose of 2 mg/kg/day was associated with hypertrophy of the proximal convoluted tubules in the kidney and spleen congestion (Rec. #5, Appendix C). In addition, females receiving the 2 mg/kg/day dose had sternal bone marrow fibrosis. Higher doses induced reduction in body weight gain, anemia, increase in kidney and liver weights and urinary bladder lesions in females. A dose of 0.4 mg/kg/day was identified as a NOEL in this study (Rec. #4, Appendix C).

Doses of 0, 1.5, 10 or 70 mg/kg/day were provided in the diet to B6C3F1 mice for 24 months (Furedi et al., 1984b). The dose of 70 mg/kg/day caused

a significant decrease in body weight gain, mild transient anemia and increased liver weight (Rec. #8, Appendix C). A dose of 10 mg/kg/day was without significant adverse effects (Rec. #7, Appendix C).

The NOEL of 0.4 mg/kg/day from the Furedi et al. (1984a) rat study is slightly lower than the LOAEL of 0.5 mg/kg/day from the subchronic dog study (Levine et al., 1983). Other subchronic studies using rats and dogs demonstrate that dogs are the more sensitive species; therefore, it is appropriate to base the RfD for chronic oral exposure on the LOAEL of 0.5 mg/kg/day in dogs. When considered with the NOEL of 0.4 mg/kg/day in rats, U.S. EPA (1989) concluded that the LOAEL of 0.5 mg/kg/day in dogs was near the threshold for adverse effects and suggested that an uncertainty factor of 1000 would be sufficient to provide for inter- and intraspecies extrapolation, estimation of a NOAEL from a LOAEL and expansion from subchronic to chronic exposure. Applying the uncertainty factor of 1000 results in an RfD for chronic oral exposure of 0.0005 mg/kg/day. This RfD has been verified and is available on IRIS (U.S. EPA, 1988b). U.S. EPA (1988b) considered confidence in the key study to be medium, with the only criticism that administration of the test substance by capsule was not ideal. Confidence in the data base is medium because there are no data on reproductive effects. The subchronic and chronic data support the magnitude of the RfD. Confidence in the RfD is medium.

9. REPORTABLE QUANTITIES

9.1. BASED ON SYSTEMIC TOXICITY

The toxicity of 2,4,6-trinitrotoluene was discussed in Chapter 6 and dose-response data considered for CS derivation are summarized in Table 9-1. Absent from Table 9-1 are the subchronic dog studies by Dilley et al. (1982) and Hart (1974) and the study using monkeys by Martin (1974) that used too few animals for meaningful analysis. Hyperplasia of the epithelium of the urinary bladder in female rats at 10 mg/kg/day in the 24-month dietary study by Furedi et al. (1984a) is not included because of the likelihood that this was a preneoplastic lesion. All studies in Table 9-1 examined similar endpoints in the species tested, which contributes to a better comparative assessment of the overall toxicity of trinitrotoluene.

Effects attributed to subchronic and chronic exposure to trinitrotoluene are mortality (RV_e=10), lethargy and ataxia (RV_e=8), depressed body weight gain or altered organ weights (RV_e=4), anemia (RV_e=5), kidney or liver hypertrophy (RV_e=3) and splenic congestion, bone marrow fibrosis or cloudy swelling in liver hepatocytes (RV_e=5) (Furedi et. al., 1984a; Levine et al., 1983, 1984a). Based on estimated human equivalent doses calculated for these effects, the liver alterations in dogs (Levine et al., 1983) appear to be most sensitive endpoint.

CSs and the corresponding RQs are calculated in Table 9-2 for the effects identified in Table 9-1. Data selection for inclusion in Table 9-2 include the lowest human equivalent dose associated with mortality, as well as the lowest human equivalent dose associated with each of the less severe effects. In the derivation of the CSs from subchonic studies, an uncertainty factor of 10 was applied to expand from subchronic to chronic exposure. From the studies presented in Table 9-2, the highest CS of 25.5,

TABLE 9-1

Toxicity Summary for 2,4,6-Trinitrotoluene

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose[a] (mg/kg/day)	Response	Reference
Oral/ gavage	dog/beagle	M,F	6/sex	9.5[b]	gelatin capsules	99.1%	0.5 mg/kg/day for 26 weeks	0.5	0.26	Anemia; hepatocytic cloudy swelling and increased liver weight	Levine et al., 1983
Oral/ gavage	dog/beagle	M,F	6/sex	9.2[b]	gelatin capsules	99.1%	8.0 mg/kg/day for 26 weeks	8.0	4.1	Liver cirrhosis, methemoglobinemia spleen congestion and increase in spleen weight	Levine et al., 1983
Oral/ gavage	dog/beagle	F	6	8.3[b]	gelatin capsules	99.1%	32.0 mg/kg/day for 26 weeks	32.0	15.7	Death	Levine et al., 1983
Oral	rat/F344	M,F	10/sex	0.35[c]	food	99.1%	25 mg/kg/day for 13 weeks	25.0[d]	4.3	Increased liver weight; hematological signs of anemia	Levine et al., 1984a
Oral	rat/F344	M,F	10/sex	0.35[c]	food	99.1%	125 mg/kg/day for 13 weeks	125[d]	21.4	Decreased body weight gain; degeneration of testes; liver hyperplasia; spleen congestion, ataxia	Levine et al., 1984a
Oral	rat/F344	M,F	10/sex	0.35[c]	food	99.1%	300 mg/kg/day for 13 weeks	300[d]	51.3	Deaths from severe anemia	Levine et al., 1984a
Oral	rat/ Sprague-Dawley	M,F	5/sex	0.26[b]	food	>99.0%	0.25% in diet for 13 weeks	162[e]	25.1	Decreased body weight gain; increase in organ weights; testicular atrophy	Dilley et al., 1982
Oral	rat/ Swiss-Webster	M,F	5/sex	0.031[b]	food	>99.0%	0.125% in diet for 13 weeks	190[e]	14.5	Liver necrosis, hepatomegaly, splenomegaly	Dilley et al., 1982
Oral	rats/F344	M,F	75/sex	0.29[b]	food	>99%	2.0 mg/kg/day for 24 months	2.0[d]	0.32	Kidney hypertrophy; spleen congestion, bone marrow fibrosis in females	Furedi et al., 1984a
Oral	rats/F344	M,F	75/sex	0.25[b]	food	>99%	10 mg/kg/day for 24 months	10.0[d]	1.5	Decreased body weight gain; methemoglobinemia; anemia; increased liver and kidney weights; spleen congestion	Furedi et al., 1984a

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose[a] (mg/kg/day)	Response	Reference
Oral	mice/B6C3f1 hybrid	M,F	75/sex	0.03[b]	food	>98.8%	70 mg/kg/day for 24 months	70[d]	5.3	Decreased body weight gain; mild anemia; changes in blood chemistry, increased liver weight	Furedi et al., 1984b

[a]Interspecies extrapolation is performed by multiplying the animal dose expressed as mg/kg/day by a body surface area scaling factor.

[b]Calculated from data provided by the investigators

[c]Reference body weight from U.S. EPA (1980)

[d]Reference food consumption: rat (0.05 x body weight); mouse (0.13 x body weight) from U.S. EPA (1980)

[e]Estimated by the investigators (average of values for males and females)

TABLE 9-2

Composite Scores for Oral Toxicity of 2,4,6-Trinitrotoluene

Species	Animal Dose (mg/kg/day)	Chronic Human MED[a] (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
Rat	2.0	22.4	3.5	Kidney hypertrophy; spleen congestion; bone marrow fibrosis	5	17.5	1000	Furedi et al., 1984a
Dog	0.5	1.8[b]	5.1	Hepatocytic cloudy swelling and increased liver weight	5	25.5	100	Levine et al., 1983
Dog	32.0	110	2.4	Death	10	24	100	Levine et al., 1983
Rat	10	105	2.5	Increased liver weight; signs of anemia; methemoglobinemia	5	12.5	1000	Furedi et al., 1984a
Rat	125	150[b]	2.5	Ataxia	8	17.6	1000	Levine et al., 1984a

[a]Calculated by multiplying the human equivalent dose by 70 kg to present the MED in terms of mg/day for a 70 kg human.

[b]The dose was divided by an uncertainty factor of 10 to approximate chronic exposure.

which corresponds to an RQ of 100, is chosen to represent the hazard associated with chronic (noncancer) toxicity resulting from exposure to trinitrotoluene (Table 9-3).

9.2. BASED ON CARCINOGENICITY

As discussed in Chapter 6, trinitrotoluene has been shown to cause urinary bladder papillomas and carcinomas in female rats (Furedi et al., 1984a), but was not carcinogenic when tested in mice (Furedi et al., 1984b). Trinitrotoluene was appropriately assigned to U.S. EPA Group C because of limited evidence of carcinogenicity in animals and lack of human data. The data for trinitrotoluene-induced urinary bladder tumors in female F344 rats (Furedi et al., 1984a) was used by the U.S. EPA (1988a, 1989) to calculate an oral slope factor (q_1^*) of 3.0×10^{-2} $(mg/kg/day)^{-1}$. Using the data on bladder tumor incidence and the GLOBAL 82 version of the multistage model (Howe and Crump, 1982), an F factor of 0.1856 $(mg/kg/day)^{-1}$ was derived (Table 9-4). Trinitrotoluene is therefore assigned to Potency Group 3, which corresponds to a Hazard Ranking of LOW and a cancer-based RQ of 100.

TABLE 9-3

2,4,6-Trinitrotoluene
(CAS No. 118-96-7)

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral/gelatin capsules
Species/Sex:	dogs/male and female
Dose*:	1.8
Duration:	26 weeks
Effect:	increased liver weight and cloudy swelling in hepatocytes
RV_d:	5.1
RV_e:	5.0
CS:	25.5
RQ:	100
Reference:	Levine et al., 1983

*Equivalent human dose

TABLE 9-4

Derivation of Potency Factor (F) for 2,4,6-Trinitrotoluene

Reference:	Furedi et al., 1984a				
Exposure route:	oral/food				
Species:	rat				
Strain:	F344				
Sex:	female				
Vehicle or physical state:	food				
Body weight:	0.30 kg				
Duration of treatment:	24 months				
Duration of study:	24 months				
Lifespan of animal:	24 months				
Target organ:	urinary bladder				
Tumor type:	papilloma and carcinoma				
Experimental doses/exposures:	0	0.4	2.0	10.0	50.0
Transformed doses (mg/kg/day):	0	0.4	2.0	10.0	50.0
Tumor incidence:	0/54	0/54	0/55	1/55	17/55
Unadjusted $1/ED_{10}$:	NA				
Adjusted $1/ED_{10}$ (F Factor):	0.185632 $(mg/kg/day)^{-1}$				

NA = Not applicable

10. REFERENCES

ACGIH (American of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH. p. 610.

ACGIH (American Conference of Governmental Industrial Hygienists). 1988. Threshold Limit Values and Biological Exposure Indices for 1988-1989, 5th ed. Cincinnati, OH. p. 37.

Ashby, J., B. Burlinson, P.A. Lefevre and J. Topham. 1985. Non-genotoxicity of 2,4,6-trinitrotoluene (TNT) to the mouse bone marrow and the rat liver: Implications for its carcinogenicity. Arch. Toxicol. 58(1): 14-19.

Atkinson, R. 1985. Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions. Chem. Rev. 85: 69-201.

Bailey, H.C. 1982. Development and testing of a laboratory model ecosystem for use in evaluating biological effects and chemical fate pollutants. In: 5th Conf. Aquat. Toxicol. p. 221-233.

Boublik, T., V. Fried and E. Hala. 1984 The Vapour Pressures of Pure Substances. Selected Values of the Temperature Dependence of the Vapour Pressures of Some Pure Substances in the Normal and Low Pressure Region, 2nd ed. Elsevier Science Publishing Company, Inc., New York. p. 538.

Bringmann, G. and R. Kuhn. 1978. Testing of substances for their toxicity threshold: Model organisms Microcystis (diplocystis) aeruginosa and Scenedesmus quadricauda. Mitt. Int. Ver. Theor. Angew. Limnol. 21: 275-284.

Bringmann, G. and R. Kuhn. 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibition test. Water Res. 14(3): 231-241.

Buck, C.R. and S.E. Wilson. 1975. Adverse Health Effects of Selected Explosives. Occupational Health Special Study No. 32-049-75/76, U.S. EHA. (Cited in Hathaway, 1977)

Burlinson, N.E., L.A. Kaplan and C.E. Adams. 1973. Photochemistry of TNT: Investigation of the pink water problem. NTIS AD-769 670. Naval Ordinance Laboratory, Silver Spring, MD. p. 23.

Bysshe, S.E. 1982. Bioconcentration factor in aquatic organisms. In: Handbook of Chemical Property Estimation Methods, W.J. Lyman, W.F. Reehl and D.H. Rosenblatt, Ed. McGraw Hill Book Co., New York, NY. Chapter 5.

Carpenter, D.F., N.G. McCormick, J.H. Cornell and A.M. Kaplan. 1978. Microbial transformation of ^{14}C-Labeled 2,4,6-trinitrotoluene in an activated sludge system. Appl. Environ. Microbiol. 35: 949-954.

Chambers, C.W., H.H. Tabak and P.W. Kabler. 1963. Degradation of aromatic compounds by phenol-adapted bacteria. J. Water Pollut. Control Fed. 35: 1517-1528.

Chemline. 1989. National Library of Medicine Chemline Database. Online: 4/3/89.

Cone, T.E. 1944. A review of the effects of trinitrotoluene (TNT) on the formal elements of blood. J. Ind. Hyg. Tox. 26: 260-263. (Cited in Hathaway, 1977)

Crockett, P.W., K. Billian, K.S. Crump and R.B. Howe. 1985. Descriptive Methods for Using Data from Dissimilar Experiments to Locate a No-Adverse-Toxic-Effects Region in the Dose-Duration Plane. Prepared by K.S. Crump and Company, Inc. Under Contract No. 6807-007 for Environmental Criteria and Assessment Office, Cincinnati, OH.

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.

Durkin, P. and W. Meylan. 1988. Users Guide for D2PLOT: A Program for Dose/Duration Graphs. Prepared by Chemical Hazard Assessment Division, Syracuse Research Corporation Under Contract No. 68-C8-0004 for Environmental Criteria and Assessment Office, Cincinnati, OH.

Eisenreich, S.J., B.B. Looney and J.D. Thornton. 1981.. Airborne organic contaminants in the Great Lakes ecosystem. Environ. Sci. Technol. 15: 30-38.

El-hawari, A.M., J.R. Hodgson, J.M. Winston, M.D. Sawyer, M. Hainje and C.C. Lee. 1981. Species differences in the disposition and metabolism of 2,4,6-trinitrotoluene as a function of route of administration. Final Report. Midwest Research Institute, Project NO. 4274-B, Kansas City, MO. DAMD17-76-C-6066. AD-A114-025.

Enzinger, R.M. 1970. Special study of the effect of alpha-TNT on microbiological systems and the determination of biodegradability of alpha-TNT, No. 24-017-70/71. January-August, 1970. NTIS 728497. 22 p.

Fewson, C.A. 1981. Biodegradation of aromatics with industrial relevance. In: Fems Symp. 12(Microb. Degr. Xenobiotics Recalitrant Compds). p. 141.

Fitzgerald, G.P., G.C. Gerloff and F. Skoog. 1952. Stream pollution: Studies on chemicals with selective toxicity to blue-green algae. Sewage Ind. Wastes. 24(7): 888-896.

Friedlander, B.R., K.W. Vorphal, R.E. Glenn and P.T. Jordan. 1974. Occupational Health Special Study No. 99-020-74, APE1300 Wash-out Plant, U.S.A. E.H.A. (Cited in Hathaway, 1977)

Furedi, E.M., B.S. Levine, D.E. Gordon, V.S. Rac and P.M. Lish. 1984a. Determination of the chronic mammalian toxicological effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitroluene (TNT) in the Fischer 344 rats). Final Report - Phase III, Volume 1. ITT Research Institute, Project No. L6116 Study No. 9, Chicago, IL. DAMD17-79-C-9120. AD-A168 637.

Furedi, E.M., B.S. Levine, J.W. Sagartz, V.S. Rac and P.M. Lish. 1984b. Determination of the chronic mammalian toxicological effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the B6C3F1 hygrid mouse). Final Report - Phase IV, Volume I. IIT Research Institute, Project No. L6166-Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-1168 754.

Goodwin, J.W. 1972. Twenty years handling TNT in a shell loading plant. Am. Ind. Hyg. Assoc. J. 33(1): 41-44.

Hansch, C. and A.J. Leo. 1985. Medchem Project. Issue No. 26. Pomona College, Claremont, CA.

Harkonen, H., M. Karki, A. Lahti and H. Savolainen. 1983. Early equatorial cataracts in workers exposed to trinitrotoluene. Am. J. Ophthalmol. 95(6): 807-810.

Harris, J.C. 1982. Rate of hydrolysis. In: Handbook of Chemical Property Estimation Methods, W.J. Lyman, W.F. Reehl and D.H. Rosenblatt, Ed. McGraw Hill Book Co., New York. Chapter 7.

Hart, E.R. 1974. Subacute toxicity of RDX and TNT in dogs. Final Report. Litton Bionetics, Inc., Project No. 1399, Kensington, MD. N00014-73-C-0162. AD-A035 717.

Hathaway, J.A. 1977. Trinitrotoluene: A review of reported dose-related effects providing documentation for a workplace standard. J. Occup. Med. 19(5): 341-345.

Hine, J. and P.K. Mookerjee. 1975. The intrinsic hydrophilic xharacter of organic compounds. Correlations in terms of atructural xontributions. J. Org. Chem. 40: 292-298.

Hoffsommer, J.C. and J.M. Rosen. 1972. Analysis of explosives in sea water. Bull. Environ. Contam. Toxicol. 7: 177-181.

Hoffsommer, J.C. and J.M. Rosen. 1973. Hydrolysis of explosives in sea water. Bull. Environ. Contam. Toxicol. 10: 78-79.

Hoffsommer, J.C., D.J. Glover and J.M. Rosen. 1972. Analysis of explosives in sea water and in ocean floor sediment and fauna NOLTR-72-215.. (NTIS-AD-75778). Naval Ordinance Lab., White Oak, Silver Springs, MD. p. 16.

Hoffsommer, J.C., L.A. Kaplan, D.J. Glover, D.A. Kubose and C. Dickinson. 1978. Biodegradability of TNT: A three-year pilot plant study. NTIS AD-A061144. White Oak Lab; Nav. Surf. Weapons Cent., Silver Spring, MD. NSWC/WOL-TR-77-136. 70 p.

Howe, R.B. and K.S. Crump. 1982. GLOBAL82: A computer program to extrapolate quantel animal toxicity data to low doses. Prepared for the Office of Carcinogen Standards, OSHA, U.S. Department of Labor, Contract 41USC252C3.

Jenkins, T.F., D.C. Leggett, C.L. Grant and C.F. Bauer. 1986. Reversed-phase high-performance liquid chromatographic determination of nitroorganics in munitions wastewater. Anal. Chem. 58: 170-175.

Jones, A.H. 1960. Sublimation-pressure data for organic compounds. J. Chem. Eng. Data. 5: 196-200.

Kaplan, D.L. and A.M. Kaplan. 1982a. Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. Appl. Environ. Microbiol. 44: 757-760.

Kaplan, D.L. and A.M. Kaplan. 1982b. Mutagenicity of 2,4,6-trinitrotoluene surfactant complexes. Bull. Environ. Contam. Toxicol. 28(1): 33-38.

Kayser, E.G. and N.E. Burlinson. 1988. Migration of explosives in soil: Analysis of RDX, TNT and tetryl from a carbon-14 lysimeter study. J. Energ. Mater. 6: 45-71.

Levine, B.S., J.H. Rust, J.M. Burns and P.M. Lish. 1983. Determination of the chronic mammalian toxicological effects of TNT. Twenty-six week subchronic oral toxicity study of trinitrotoluene (TNT) in the beagle dog. Phase II, Final Report, IIT, Research Institute, Report No. L6116, Study No. 5, Chicago, IL. DAMD 17-79-C-9120, AD-A157 082.

Levine, B.S., E.M. Furedi, D.E. Gordon, P.M. Lish and J.J. Barkely. 1984a. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. Toxicology. 32: 253-265.

Levine, B.S., E.M.Furedi, J.M. Burns and P.M. Lish. 1984b. Four week subchronic (exploratory/rang-finding) oral (diet) toxicity study of trinitrotoluene (TNT) in the B6C3F1 hybrid mouse. Final Report. IIT Research Institute, Project No, L6116, Study No. 8, Chicago, IL, Contract No. DAMD-17-79-C-9120 as Appendix IV in Furedi et al., 1984d.

Liu, D.H.W, R.J. Spanggord, H.C. Bailey, H.S Javitz and D.C.L. Jones. 1983. Toxicity of TNT Wastewater (Pink Water) to Aquatic Organisms. Contract No. DAMD 17-75-C-5056, Defense Techincal Information Center, U.S. Army Med. Res. Develop. Command, Washington, DC. p. 1-82.

Mabey, W.R., D. Tse, A. Baraze and T. Mill. 1983. Photolysis of nitroaromatics in aquatic systems. I. 2,4,6-trinitrotoluene. Chemosphere. 12: 3-17.

Mantel, N. and M.A. Schneiderman. 1975. Estimating "safe" levels, a hazardous undertaking. Cancer Res. 35: 1379-1386.

Martin, D.P. 1974. Subacute toxicity of RDX and TNT in monkeys. Final Report. Litton Bionetics, Inc., Project No. 1366, Kensington, MD. N00014-73-C0162. NR 108-985. AD-A044 650/0.

Morton, A.R., M.V. Ranadive and J.A. Hathaway. 1976. Biological effects of trinitrotoluene from exposure below the threshold limit value. Am. Ind. Hyg. Assoc. J. 37(1): 56-60.

Nay, M.W., Jr., C.W. Randall and P.H. King. 1972. Factors affecting color development during treatment of TNT wastes. Ind. Wastes. 18: 20-29.

NTP (National Toxicology Program). 1989. Management Status Report. 04/07/89.

OSHA (Occupational Safety and Health Administration). 1989. Occupational Standards. Permissible Exposure Limits. 29 CFR 1910.1000.

Osmon, J.C. and R.E. Klausmeier. 1973. The microbial degradation of explosives. Dev. Ind. Microbiol. 14: 247-252.

Palazzo, A.J. and D.C. Leggett. 1986. Effect and disposition of 2,4,6-trinitrotoluene in a terrestrial plant. J. Environ. Qual. 15(1): 49-52.

Pederson, G. 1970. Sanitary Engineering Special Study No. 24-007-70/71 Evaluation of Toxicity of Selected TNT Wastes on Fish Phase I- Acute Toxicity of Aplha-TNT to Bluegills. January 1970-October 1970. Army Environ. Health Agency, Edgewood Arsenal, MD. NTIS AD-725. 525: 35.

Pella, P.A. 1977. Measurement of the vapor pressures of TNT, 2,4-DNT and EGDN. J. Chem. Thermodyn. 9: 301-305.

Pereira, W.E., D.L. Short, D.B. Manigold and P.K. Roscio. 1979. Isolation and characterization of TNT and its metabolites in groundwater by gas chromatograph-mass spectrometer-computer techniques. Bull. Environ. Contam. Toxicol. 21: 554-562.

Ryon, M.G., B.C. Pal, S.S. Talmage and R.H. Ross. 1984. Database assessment of the health and environmental effects of munition production waste products. Final Report. ORNL-6018. NTIS DE84-016512. Oak Ridge Natl. Lab., Oak Ridge, TN. p. 217.

SANSS. 1989. Structure and Nomenclature Search System (Database). Online: 4/8/89.

Sax, N.I. and R.J. Lewis. 1987. Hawley's Condensed Chemical Dictionary, 11th ed. Van Nostrand Reinhold Co., New York, NY. p. 1191.

Schott, C.D. and E.C. Worthley. 1974. The Toxicity of TNT and Related Wastes to an Aquatic Flowering Plant, Lemna perpusilla Torr. Edgewood Arsenal Technical Report EB-TR-74016, Edgewood Aresenal, Aberdeen Proving Ground, MD. U.S. NTIS, AD-778. 158: 18.

Smock, L.A., D.L. Stoneburner and J.R. Clark. 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. Water Res. 10(6): 537-543.

Spalding, R.F. and J.W. Fulton. 1988. Groundwater munition residues and nitrate near Grand Island, Nebraska, USA. J. Contam. Hydrol. 2: 139-153.

Spanggord, R.J., W.R. Mabey, T. Mill, T. Chou and J.H. Smith. 1981. Environmental fate studies on certain munition wastewater constituents. Phase 3, Part 2. Laboratory Studies. NTIS AD-A131 908. SRI International, Menlo Park, CA. 58 p.

Spanggord, R.J., B.W. Gibson, R.G. Keck, D.W. Thomas and J.J. Barkley, Jr. 1982. Effluent analysis of waste water generated in the manufacture of 2,4,6-trinitrotoluene. I. Characterization study. Environ. Sci. Technol. 16: 229-232.

Spanggord, R.J., W.R. Mabey, T. Mill, et al. 1983. Environmental fate studies on certain munitions wastewater constituents. NTIS AD A138550. SRI International, Menlo Park, CA.

Stewart, A., L.T. Witts, G. Higgins et al. 1945. Some early effects of exposure to trinitrotoluene. Br. J. Ind. Med. 2: 74-82. (Cited in Hathaway, 1977)

Styles, J.A. and M.F. Cross. 1983. Activity of 2,4,6-trinitrotoluene in an in vitro mammalian gene mutation assay. Cancer Lett. 20: 103-108.

Tabak, H.H., C.W. Chambers and P.W. Kabler. 1964. Microbial metabolism of aromatic compounds. I. Decomposition of phenolic compounds and aromatic hydrocarbons by phenol-adapted bacteria. J. Bacteriol. 87: 910-919.

Thomas, R.G. 1982. Volatilization from water. In: Handbook of Chemical Property Estimation Methods, W.J. Lyman, W.F. Reehl and D. H. Rosenblatt, Ed. McGraw Hill Book Co., New York. Chapter 15.

TSCAPP. 1989. Computer Print-out of Non-Confidential Production Data from TSCA Inventory OPTS (CID). U.S. EPA, Washington, DC. Online: 3/28/89.

U.S. EPA. 1980. Guidelines and Methodology Used in the Preparation of Health Effect Assessment Chapters of the Consent Decree Water Criteria Documents. Federal Register. 45(231): 79347-79357.

U.S. EPA. 1984. Methodology and Guidelines for Ranking Chemicals Based on Chronic Toxicity Data. Prepared by the Office of Health and Environmental Assessment, Enviornmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 1986a. Methodology for Evaluating Reportable Quantity Adjustments Pursuant to CERCLA Section 102. Prepared by Carcinogen Assessment Group, Office of Health and Environmental Assessment for the Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 1986b. Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185): 33992-34003.

U.S. EPA. 1988a. Integrated Risk Information System (IRIS). Risk Estimate for Carcinogenicity for 2,4,6-Trinitrotoluene. Online. (Verification date 09/22/88.) Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

U.S. EPA. 1988b. Integrated Risk Information System (IRIS). Reference Dose (RfD) for Oral Exposure for 2,4,6-Trinitrotoluene. Online. (Verification date 04/20/88.) Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

U.S. EPA. 1988c. Health Effects Assessment for 2,4- and 2,6-Dinitrotoluene. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. EPA/600/8-88/032. NTIS PB88-178793/AS.

U.S. EPA. 1989. Trinitrololuene. Health Advistory. Office of Drinking Water, Washington, DC.

U.S. EPA/OWRS (Environmental Protection Agency /Office of Water Regulations and Standards). 1986. Guideline for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. U.S. EPA, Washington, DC. p. 22-58, 98. NTIS PB85-227049/XAB.

Whong, W.Z. and G.S. Edwards. 1984. Genotoxic acitivity of nitroaromatic explosives and related compound in Salmonella typhimurium. Mutat. Res. 136(3): 209-216.

Windholz, M., S. Budavari, R.F. Blumetti and E.S. Otterbein. 1983. The Merck Index. Merck and Co., Inc., Rahway, NJ. p. 1390.

Won, W.D., L.H. DiSalvo and J. Ng. 1976. Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. Appl. Environ. Microbiol. 31(4): 576-580.

Woolen, B.H., M.G. Hall, R. Graig and G.T. Steel. 1986. Trinitritoluene: Assessment of occupational absorption during manufacture of explosives. Br. J. Ind. Med. 43: 465-473.

Yinon, J. and D.G. Hwang. 1986. Metabolic studies of explosives. 5. Detection and analysis of 2,4,6-trinitrotoluene and its metabolites in urine of munition workers by micro liquid chromatography/mass spectrometry. Biomed. Chromatogr. 1: 123-125.

Zitting, A., G. Szumanska, J. Nickels and H. Savolainen. 1982. Acute toxic effects of trinitrotoluene in rat brain, liver and kidney; Role of radical production. Arch. Toxicol. 51(1): 53-64.

APPENDIX A

LITERATURE SEARCHED

This HEED is based on data identified by computerized literature searches of the following:

CHEMLINE
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
TOXLINE
TOXLIT
TOXLIT 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register
CAS ONLINE (Chemistry and Aquatic)
HSDB
SCISEARCH
Federal Research in Progress

These searches were conducted in April, 1989, and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1987. TLVs: Threshold Limit Values for Chemical Substances in the Work Environment adopted by ACGIH with Intended Changes for 1987-1988. Cincinnati, OH. 114 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1984. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., Littleton, MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. IARC, WHO, Lyons, France.

Jaber, H.M., W.R. Mabey, A.T. Lieu, T.W. Chou and H.L. Johnson. 1984. Data acquisition for environmental transport and fate screening for compounds of interest to the Office of Solid Waste. EPA 600/6-84-010. NTIS P884-243906. SRI International, Menlo Park, CA.

NTP (National Toxicology Program). 1987. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, I.N. 1984. Dangerous Properties of Industrial Materials, 6th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1987. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1986. Report on Status Report in the Special Review Program, Registration Standards Program and the Data Call in Programs. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

USITC (U.S. International Trade Commission). 1986. Synthetic Organic Chemicals. U.S. Production and Sales, 1985, USITC Publ. 1892, Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. Office of Pesticide Programs, U.S. EPA, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.

APPENDIX B

Summary Table for 2,4,6-Trinitrotoluene

	Species	Exposure	Effect	RfD or q_1^*	Reference
Inhalation Exposure					
Subchronic	ID	ID	ID	ID	NA
Chronic	ID	ID	ID	ID	NA
Carcinogenicity	ID	ID	ID	ID	NA
Oral Exposure					
Subchronic	dog	0.5 mg/kg/day in gelatin capsules for 26 weeks	hepatocytic cloudy swelling and increased liver weight	0.5×10^{-3} mg/kg/day	Levine et al., 1983
Chronic	dog	0.5 mg/kg/day in gelatin capsules for 26 weeks	hepatocytic cloudy swelling and increased liver weight	0.5×10^{-3} mg/kg/day	Levine et al., 1983
Carcinogenicity	rat	0-50 mg/kg/day in the diet for 24 months	urinary bladder papillomas and carcinomas in females	3.0×10^{-2} $(mg/kg/day)^{-1}$	Furedi et al., 1984a
REPORTABLE QUANTITIES					
Based on Chronic Toxicity:	100				Levine et al., 1983
Based on Carcinogenicity:	100				Furedi et al., 1984a

ID = Insufficient data; NA = not applicable

APPENDIX C

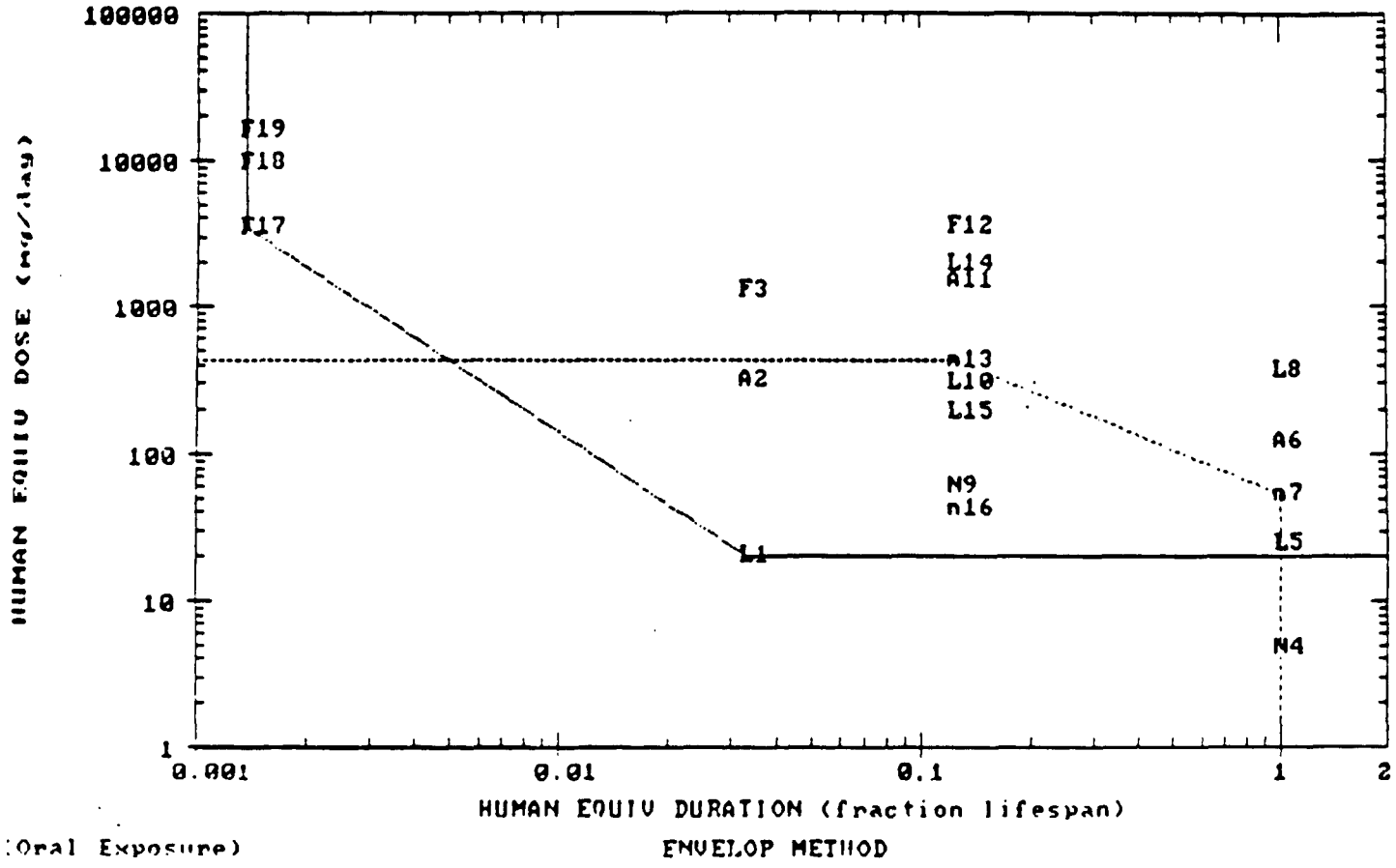
DOSE/DURATION RESPONSE GRAPHS FOR EXPOSURE TO TRINITROTOLUENE

C.1. DISCUSSION

Dose/duration-response graphs for oral exposure to trinitrotoluene generated by the method of Crockett et al. (1985) using the computer software by Durkin and Meylan (1988) developed under contract to ECAO-Cincinnati are presented in Figures C-1 and C-2. Data used to generate these graphs are presented in Section C.2. In the generation of these figures, all responses are classified as adverse (FEL, AEL or LOAEL) or nonadverse (NOEL or NOAEL) for plotting.

For oral exposure, the ordinate expresses dosage as human equivalent dose. Interspecies extrapolation is performed by multiplying the animal dosage in mg/kg/day by a scaling factor (the cube root of the ratio of the animal:human body weight) to adjust for species differences in basal metabolic rate (Mantel and Schnelderman, 1975). The result is then multiplied by 70 kg, the reference human body weight, to express the human equivalent dose as mg/day for a 70 kg human.

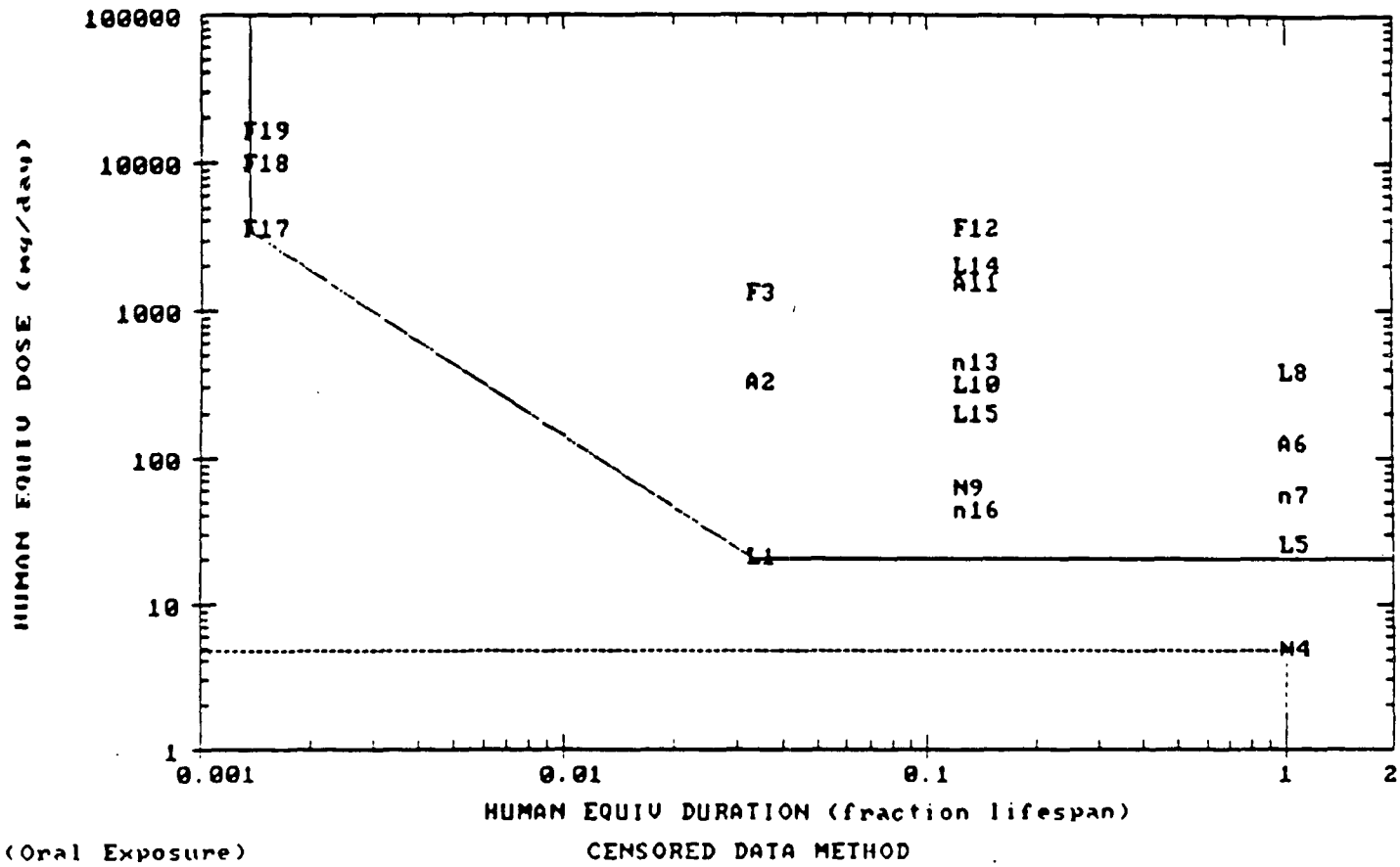
The boundary for adverse effects (solid line) is drawn by identifying the lowest adverse effect dose or concentration at the shortest duration of exposure at which an adverse effect occurred. From this point, an infinite line is extended upward parallel to the dose axis. The starting point is then connected to the lowest adverse effect dose or concentration at the next longer duration of exposure that has an adverse effect dose or concentration equal to or lower than the previous one. This process is continued to the lowest adverse effect dose or concentration. From this point a line is extended to the right parallel to the duration axis. The region of adverse effects lies above the adverse effects boundary.



N = NOEL
n = NOAEL
L = LOAEL
A = AEL
F = FEL
Solid line = Adverse Effects Boundary
Dashed line = No Adverse Effects Boundary

FIGURE C-1

Dose/Duration - Response Graph for Oral Exposure to Trinitrotoluene: Envelope Method



n = NOAEL
L = LOAEL
A = AEL
F = FEL
N = NOEL

FIGURE C-2

Dose/Duration - Response Graph for Oral Exposure to Trinitrotoluene: Censored Data Method

Using the envelope method, the boundary for no adverse effects (dashed line) is drawn by identifying the highest no adverse effects dose or concentration. From this point, a line parallel to the duration axis is extended to the dose or concentration axis. The starting point is then connected to the next lower or equal no adverse effect dose or concentration at a longer duration of exposure. When this process can no longer be continued, a line is dropped parallel to the dose or concentration axis to the duration axis. The no adverse effects region lies below the no adverse effects boundary. At either end of the graph between the adverse effects and no adverse effects boundaries are regions of ambiguity. The area (if any) resulting from intersection of the adverse effects and no adverse effects boundaries is defined as the region of contradiction.

In the censored data method, all no adverse effect points located in the region of contradiction are dropped from consideration and the no adverse effect boundary is redrawn so that it does not intersect the adverse effects boundary and no region of contradiction is generated. This method results in the most conservative definition of the no adverse effects region.

Figures C-1 and C-2 show the dose/duration-effects graphs generated by the envelope and censored data methods for oral exposure to trinitrotoluene, respectively. The boundary for adverse effects is defined by three FELs (F17, F18 and F19) corresponding to LD_{50}s in mice and rats in the Dilley et al. (1982) study and a LOAEL (L1) from a subchronic study in dogs (Levine et al., 1983). N4, which is below the line for adverse effects, and corresponds to a NOEL in rats (Furedi et al., 1984a), was not used as the basis for deriving the chronic oral RfD because dogs are clearly more sensitive than rats. The area of contradiction results from the relative insensitivity of rats and mice compared with dogs. The verified chronic RfD of 5×10^{-4} mg/kg/day is well below the boundary for adverse effects.

C.2. DATA USED TO GENERATE DOSE/DURATION-RESPONSE GRAPHS

C.2.1. Oral Exposure.

Chemical Name: 2,4,6-Trinitrotoluene
CAS Number: 118-96-7
Document Title: Health and Environmental Effects Document for 2,4,6-Trinitrotoluene
Document Number: Pending
Document Date: Pending
Document Type: HEED

RECORD #1:

Species:	Dogs		Dose:		0.500
Sex:	Both		Duration Exposure:		26.0 weeks
Effect:	LOAEL		Duration Observation:		26.0 weeks
Route:	Capsul				
Number Exposed:	12	12	12		
Number Responses:	NR	NR	NR		
Type of Effect:	WGTIN	HEMAT	DEGEN		
Site of Effect:	LIVER	BLOOD	LIVER		
Severity Effect:	4	5	5		

Comment: Doses given: 0, 0.5, 2.0, 8.0, 32.0 mg/kg/day; mild anemia, hepatocelluar cloudy swelling and increased liver weight.

Citation: Levine et al., 1983

RECORD #2:

Species:	Dogs		Dose:		8.000
Sex:	Both		Duration Exposure:		26.0 weeks
Effect:	AEL		Duration Observation:		26.0 weeks
Route:	Capsul				
Number Exposed:	12	12	12	12	
Number Responses:	NR	1	NR	NR	
Type of Effect:	HEMAT	DEGEN	WGTIN	DEGEN	
Site of Effect:	BLOOD	LIVER	SPLEN	SPLEN	
Severity Effect:	5	5	4	5	

Comment: See previous record; methemoglobinemia, 1 dog with liver cirrhosis, splenic congestion.

Citation: Levine et al., 1983

RECORD #3:

Species:	Dogs	Dose:	32.000
Sex:	Both	Duration Exposure:	26.0 weeks
Effect:	FEL	Duration Observation:	26.0 weeks
Route:	Capsul		

Number Exposed:	12
Number Responses:	2
Type of Effect:	DEATH
Site of Effect:	BODY
Severity Effect:	10

Comment: See record #1; 2 dogs died before 17 weeks of treatment.

Citation: Levine et al., 1983

RECORD #4:

Species:	Rats	Dose:	0.400
Sex:	Both	Duration Exposure:	24.0 months
Effect:	NOEL	Duration Observation:	24.0 months
Route:	Food		

Number Exposed:	150	150	75
Number Responses:	0	0	0
Type of Effect:	HYPRT	DEGEN	HISTO
Site of Effect:	KIDNY	SPLEN	BONE
Severity Effect:	3	5	5

Comment: Doses given: 0, 0.4, 2, 10, 50 mg/kg/day.

Citation: Furedi et al., 1984a

RECORD #5:

Species:	Rats	Dose:	2.000
Sex:	Both	Duration Exposure:	24.0 months
Effect:	LOAEL	Duration Observation:	24.0 months
Route:	Food		

Number Exposed:	150	150	75
Number Responses:	NR	NR	NR
Type of Effect:	HYPRT	DEGEN	HISTO
Site of Effect:	KIDNY	SPLEN	BONE
Severity Effect:	3	5	5

Comment: See previous record; kidney hypertorphy and congestion of spleen in both sexes, bone marrow fibrosis in females.

Citation: Furedi et. al., 1984a

RECORD #6:

Species:	Rats		Dose:			10.000
Sex:	Both		Duration Exposure:			24.0 months
Effect:	AEL		Duration Observation:			24.0 months
Route:	Food					
Number Exposed:		150	150	75	150	150
Number Responses:		NR	NR	NR	NR	NR
Type of Effect:		HYPRT	DEGEN	HISTO	WGTIN	HEMAT
Site of Effect:		KIDNY	SPLEN	BONE	LIVER	BLOOD
Severity Effect:		3	5	5	4	5

Comment: See previous record; additional effects at this dose include increased liver and kidney weight, anemia and methemoglobinemia.

Citation: Furedi et. al., 1984a

RECORD #7:

Species:	Mice		Dose:		10.000
Sex:	Both		Duration Exposure:		24.0 months
Effect:	NOAEL		Duration Observation:		24.0 months
Route:	Food				
Number Exposed:		150	150	150	
Number Responses:		0	NR	0	
Type of Effect:		HEMAT	WGTDC	WGTIN	
Site of Effect:		BLOOD	BODY	LIVER	
Severity Effect:		5	4	4	

Comment: Doses given: 0, 1.5, 10, 70 mg/kg/day; decreased body weight gain was significant only at higher level.

Citation: Furedi et al., 1984b

RECORD #8:

Species:	Mice		Dose:		70.000
Sex:	Both		Duration Exposure:		24.0 months
Effect:	LOAEL		Duration Observation:		24.0 months
Route:	Food				
Number Exposed:		150	150	150	
Number Responses:		0	NR	0	
Type of Effect:		HEMAT	WGTDC	WGTIN	
Site of Effect:		BLOOD	BODY	LIVER	
Severity Effect:		5	4	4	

Comment: See previous record; mild anemia and altered blood chemistry.

Citation: Furedi et al., 1984b

RECORD #9:	Species:	Rats		Dose:		5.000
	Sex:	Both		Duration Exposure:		13.0 weeks
	Effect:	NOEL		Duration Observation:		13.0 weeks
	Route:	Food				
	Number Exposed:	20	20	20	10	20
	Number Responses:	0	0	0	0	0
	Type of Effect:	WGTIN	HEMAT	FUND	DEGEN	DEATH
	Site of Effect:	LIVER	BLOOD	CNS	TESTE	BODY
	Severity Effect:	4	5	8	6	10

Comment: Doses of 0, 1, 5, 25, 125, 300 mg/kg/day.

Citation: Levine et al., 1984a

RECORD #10:	Species:	Rats		Dose:		25.000
	Sex:	Both		Duration Exposure:		13.0 weeks
	Effect:	LOAEL		Duration Observation:		13.0 weeks
	Route:	Food				
	Number Exposed:	20	10	20	10	20
	Number Responses:	NR	NR	0	0	0
	Type of Effect:	WGTIN	HEMAT	FUND	DEGEN	DEATH
	Site of Effect:	LIVER	BLOOD	CNS	TESTE	BODY
	Severity Effect:	4	5	8	6	10

Comment: See previous record; increased liver weight in both sexes, anemia only in males.

Citation: Levine et al., 1984a

RECORD #11:	Species:	Rats		Dose:		125.000
	Sex:	Both		Duration Exposure:		13.0 weeks
	Effect:	AEL		Duration Observation:		13.0 weeks
	Route:	Food				
	Number Exposed:	20	20	20	10	20
	Number Responses:	NR	NR	NR	NR	0
	Type of Effect:	WGTIN	HEMAT	FUND	DEGEN	DEATH
	Site of Effect:	LIVER	BLOOD	CNS	TESTE	BODY
	Severity Effect:	4	5	8	6	10

Comment: See previous record; anemia, ataxia in both sexes.

Citation: Levine et al., 1984a

RECORD #12:

Species:	Rats	Dose:			300.000
Sex:	Both	Duration Exposure:			13.0 weeks
Effect:	FEL	Duration Observation:			13.0 weeks
Route:	Food				

Number Exposed:	20	20	20	10	20
Number Responses:	NR	NR	NR	NR	2
Type of Effect:	WGTIN	HEMAT	FUND	DEGEN	DEATH
Site of Effect:	LIVER	BLOOD	CNS	TESTE	BODY
Severity Effect:	4	5	8	6	10

Comment: See previous record; deaths attributed to severe anemia.

Citation: Levine et al., 1984a

RECORD #13:

Species:	Rats	Dose:			35.600
Sex:	Both	Duration Exposure:			13.0 weeks
Effect:	NOAEL	Duration Observation:			13.0 weeks
Route:	Food				

Number Exposed:	10	5	10	10
Number Responses:	0	0	0	0
Type of Effect:	HEMAT	ATROP	WGTDC	WGTIN
Site of Effect:	BLOOD	TESTE	BODY	SPLEN
Severity Effect:	5	5	4	4

Comment: Dietary levels used: 0, 0.002, 0.01, 0.05, 0.25% corresponding to doses (average male and female)of 0, 1.4, 7.2, 35.6, 162 mg/kg/ day; red urine during exposure.

Citation: Dilley et al., 1982

RECORD #14:

Species:	Rats	Dose:			162.000
Sex:	Both	Duration Exposure:			13.0 weeks
Effect:	LOAEL	Duration Observation:			13.0 weeks
Route:	Food				

Number Exposed:	10	5	10	10
Number Responses:	NR	NR	NR	NR
Type of Effect:	HEMAT	ATROP	WGTDC	WGTIN
Site of Effect:	BLOOD	TESTE	BODY	SPLEN
Severity Effect:	5	5	4	4

Comment: See previous record; 0.25% in diet; decreased body weight gain accompanied by decreased food consumption.

Citation: Dilley et al., 1982

RECORD #15:

Species:	Mice		Dose:		36.800
Sex:	Both		Duration Exposure:		13.0 weeks
Effect:	LOAEL		Duration Observation:		13.0 weeks
Route:	Food				
Number Exposed:	10	10	10	10	
Number Responses:	0	0	0	5	
Type of Effect:	HEMAT	WGTIN	WGTDC	PIGMN	
Site of Effect:	BLOOD	LIVER	BODY	SPLEN	
Severity Effect:	5	4	4	5	

Comment: Dietary levels of 0, 0.001, 0.005, 0.025, 0.125% corresponding to doses (average for male and female) of 0, 1.6, 7.8, 36.8, 191 mg/kg/day; hemosiderosis of spleen.

Citation: Dilley et al., 1982

RECORD #16:

Species:	Mice		Dose:		7.800
Sex:	Both		Duration Exposure:		13.0 weeks
Effect:	NOAEL		Duration Observation:		13.0 weeks
Route:	Food				
Number Exposed:	10	10	10	10	
Number Responses:	0	0	0	0	
Type of Effect:	HEMAT	WGTIN	WGTDC	PIGMN	
Site of Effect:	BLOOD	LIVER	BODY	SPLEN	
Severity Effect:	5	4	4	5	

Comment: See previous record; red urine.

Citation: Dilley et al., 1982

RECORD #17:

Species:	Mice		Dose:		660.000
Sex:	Both		Duration Exposure:		1.0 days
Effect:	FEL		Duration Observation:		14.0 days
Route:	Gavage				
Number Exposed:	20				
Number Responses:	NR				
Type of Effect:	DEATH				
Site of Effect:	BODY				
Severity Effect:	10				

Comment: Swiss-Webster mice were used. The dose is an LD_{50} and was administered in corn oil.

Citation: Dilley et al., 1982

RECORD #18:

Species:	Rats	Dose:	795.000
Sex:	Female	Duration Exposure:	1.0 days
Effect:	FEL	Duration Observation:	14.0 days
Route:	Gavage		

Number Exposed:	20
Number Responses:	NR
Type of Effect:	DEATH
Site of Effect:	BODY
Severity Effect:	10

Comment: Sprague-Dawley rats were used. The dose was administered in corn oil and corresponds to an LD_{50}.

Citation: Dilley et al., 1982

RECORD #19:

Species:	Rats	Dose:	1320.000
Sex:	Male	Duration Exposure:	1.0 days
Effect:	FEL	Duration Observation:	14.0 days
Route:	Gavage		

Number Exposed:	20
Number Responses:	NR
Type of Effect:	DEATH
Site of Effect:	BODY
Severity Effect:	10

Comment: Sprague-Dawley rats were used. The dose is an LD_{50} and was administered in corn oil.

Citation: Dilley et al., 1982
