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DATA SUMMARY FOR TRINITROTOLUENE

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The US Environmental Protection Agency (USEPA) and the Department of the Army (DA) established a cooperative agreement to develop Health Advisories (HA) on chemical substances associated with munitions that may be found as drinking water contaminants. This text summarizes information about 2,4,6-trinitrotoluene (TNT) that was provided by DA to the USEPA.

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20. Abstract (continued)

TNT is produced in a batch or continuous mode by reacting toluene with nitric acid in the presence of sulfuric acid. Manufacture and load, assembly, and pack (LAP) operations provide several opportunities for TNT and its products to enter the environment. Wastewater discharged from Army ammunition plants (AAP) is the primary way that TNT enters the aquatic environment. Up to 60 mg/L of TNT have been found in stream and river waters that receive waste effluent from AAPs, and associated sediments have contained up to 617 mg/kg. TNT does not transport very well through soil into groundwater, and atmospheric transport is not environmentally significant. It is persistent in soils, but is rapidly degraded in aquatic environments.

TNT is readily absorbed and metabolized by mammals. It distributes throughout the body; however, most will go to the kidneys and liver, the latter being the site of extensive metabolism. TNT metabolites are excreted primarily by the kidneys. Bioaccumulation does not appear to be significant. Hepatotoxicity, leading to yellow atrophy of the liver, and aplastic anemia associated with bone marrow hypoplasia are the major causes of death to TNT-exposed animals. Cats, dogs, and humans appear to be more sensitive to TNT than are rabbits and rats.

LD50s reported for male and female rats are 1,320 and 794 mg/kg TNT, respectively, and for mice, 660 mg/kg for both males and females. When applied to rabbits, TNT was classified as a mild skin irritant and did not produce eye irritation. It is a moderate dermal sensitizing agent as determined by applying it to the skin of guinea pigs. Reproductive and teratogenic effects are not reported in the available scientific literature. TNT is not considered to be a carcinogen; however, urinary bladder carcinoma, malignant lymphoma, leukemia, and hepatocellular, renal, and urinary bladder hyperplasias have occurred in exposed rats and mice. These observations, reported chromosomal effects in rats, and positive results from Ames testing suggest that further study of TNT's potential carcinogenicity is merited.

In the aquatic environment, TNT causes changes in water quality and associated sediments and stresses biotic communities. The effects generally occur in areas immediately at or downstream from the wastewater outfall and decrease as distance from the outfall increases. LC50 values from static 96-hour fish assays, as reported for bluegills, fathead minnows, channel catfish, and rainbow trout, range from 1.2 to 4.2 mg/L TNT. Flow-through acute toxicity testing of Daphnia magna resulted in LC50 values of 0.19 to 11.9 mg/L TNT. The no-effect level for D. magna, from a full life-cycle study noting effects on reproduction and progeny growth and survival, is 1.13 mg/L TNT.

A 24-month study of Fisher 344 rats, given up to 50 mg/kg/day TNT in the diet, was considered appropriate for deriving a longer term health advisory (i.e., for periods beyond a few weeks up to 1 year, not to reflect lifetime chronic exposure levels). Splenic congestion, increased pigment deposition in the kidneys, and bone marrow fibrosis occurred with doses as low as 2 mg/kg/day. Based upon this study it is recommended that TNT levels in drinking water sources not exceed 0.040 mg/L (40 µg/L).

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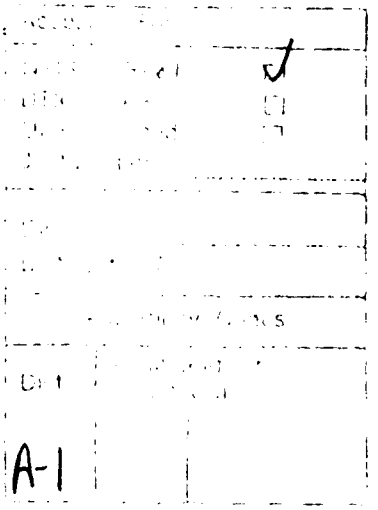

INTRODUCTION

In April 1985 the U.S. Environmental Protection Agency (USEPA) and the Department of the Army (DA) established a cooperative agreement to develop Health Advisories on chemical substances associated with munitions that may be found as drinking water contaminants within the United States and its territories.[1] Health Advisories are discretionary under the authority of the Safe Drinking Water Act [§1442(b)(1)], and normally are provided for 1-day, 10-day, and longer-term exposure periods where available toxicological data exist. The advisories provide specific advice on the levels of selected munition chemicals in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. Under the provisions of the cooperative agreement, the Department of the Army provides the USEPA relevant information, e.g., toxicological, environmental, and operational data, as required for the development of advisories for selected munitions.

This text summarizes information about trinitrotoluene as provided by the Department of the Army to the USEPA under the provisions of the cooperative agreement. The sections titled HEALTH ADVISORY DEVELOPMENT and CONCLUSIONS are based solely on the references listed in this publication.

GENERAL INFORMATION AND PROPERTIES

Trinitrotoluene (TNT) (CAS No. 118-96-7) occurs in six isomeric forms: 2,4,6- or α; 2,3,4- or R; 2,4,5- or γ; 3,4,5- or δ; 2,3,5- or ϵ; and 2,3,6- or η. The α or 2,4,6- isomer is the one of primary military interest and commonly designated TNT.[2-4] The other five, "meta", isomers are formed as by-products during TNT manufacture. (NOTE: Unless noted otherwise, throughout the text TNT will refer to the 2,4,6- isomer.)

At 20°C TNT exists as colorless to yellow monoclinic needles or orthorhombic crystals.[5] Castorina[3] provides a detailed review of the physical and chemical properties of TNT. Pal and Ryon,[6] Ryon et al.,[2] and Zakhari et al.,[5] list the following:

- Molecular formula: $C_7H_5N_3O_6$
- Molecular weight: 227.13
- Melting point: 80.1°C
- Density: 1.654 g/cm^3
- Vapor pressure: 0.046 mm Hg at 82°C
- Viscosity: 8 cP at 99°C
- Heats of: - formation, 0.293 KJ/g
  - combustion, 15.02 KJ/g

- detonation, 4.23 KJ/g
- fusion, 98.3 J/g
- vaporization, 339 J/g
- sublimation, 447 J/g

- Specific heat: 1.38 J/g^{o}K
- Thermal conductivity: 0.54 W/m^{o}K
- Coefficient of linear expansion: 6.7 x 10^{-3}/^{o}C
- Solubility, at 20^{o}C in g/100g:

- water, 0.01	- 1,2-dichloroethane, 18.7
- pyridine, 137	- diethyl ether, 3.29
- acetone, 109	- ethanol (95%), 1.23
- methyl acetate, 72.11	- carbon tetrachloride, 0.65
- benzene, 67	- carbon disulfide, 0.48
- toluene, 55	- trichloroethylene, 3.04
- chlorobenzene, 33.9	- sulfuric acid, 4.0
- chloroform, 19	- butyl carbitol acetate, 24

Elemental analysis of TNT results in 37.01%C, 2.22%H, 18.50%N, and 42.27%O.[2,5,6] Upon decomposition at temperatures around 200^{o}C, TNT produces at least 25 identified compounds and large amounts of undefined polymeric material. When one mole of TNT is detonated, e.g., in a bomb calorimeter, 3.65 moles C, 1.98 moles CO, 1.60 moles H_2O, 1.32 moles N_2, 0.46 mole H_2, 0.16 mole NH_3, and 0.10 mole CH_4 are recovered in addition to other products.[2]

Synonyms for TNT include, 2,4,6-trinitrotoluene, α-TNT, 2-methyl-1,3,5-trinitrobenzene, α-trinitrotoluol, sym-trinitrotoluene, 1-methyl-2,4,6-trinitrobenzene, tolit, trilit, trotyl, tritol, sym-trinitrotoluol, triton, trilite, TNT-tolite, 2,4,6-trinitrotolueen, 2,4,6-trinitrotoluol, and trojnitrotoluene.[2,5]

Descriptions of TNT manufacture and production, including current and past technologies, are found in Pal and Ryon,[6] Ryon et al.,[2] Castorina,[3] Small and Rosenblatt,[7] Rosenblatt et al.,[8,9] and Department of the Army.[4] TNT is produced by reacting toluene with nitric acid in the presence of sulfuric acid. The nitration is done stepwise in either batch or continuous modes of operation. Up until 1968, and the introduction of continuous nitration at Radford Army Ammunition Plant (AAP), VA, TNT was produced in the United States exclusively by the batch mode.

Standards for exposure to TNT include occupational exposure limits, environmental criteria for protection of aquatic organisms, and interim criteria for levels in drinking water sources. The current recommended American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for respiratory exposure to TNT is 0.5 mg/m^3 for an 8-hour time-weighted average (TWA) with a short-term exposure limit (STEL) of 3 mg/m^3. The TLV is based upon a medical survey[10] that demonstrated significant physiological changes in workers exposed to TNT dust ranging from 0.3 to 0.8 mg/m^3. The ACGIH TLV recommendations for 1985-86 in its "Notice of Intended Changes" deletes the STEL for TNT.11 The Occupational Safety and Health Act (OSHA) lists a respiratory exposure limit of 1.5 mg/m^3 based upon the edition of the ACGIH TLV listing current when the law was enacted (Code of Federal Regulations, Part 1910). Both the ACGIH and OSHA exposure limits include a "skin" notation indicating a potential contribution to exposure by percutaneous absorption from either airborne or direct contact with TNT. The U.S. Army Materiel Command (AMC), the Department of Defense agency responsible for TNT manufacture and production, adopted a respiratory exposure limit of 0.5 mg/m^3,12 referencing, among other sources, several US Army occupational health studies.[13-15] Rosenblatt[16] reports that the USSR has a maximum permissible inhalation level of 1.0 mg/m^3. Dacre[17] recommended an interim ambient water quality criterion for Army TNT production, for protection of human health, of 0.04424 mg/L, based upon mammalian studies by Dilley et al.[18] Sufficient data were not available to calculate an interim criterion for protection of aquatic organisms; however, Dacre[17] did cite 0.060 mg/L for assessing the effectiveness of waste treatment technology. An emergency TNT drinking water limit (EDL) of 0.75 mg/kg body weight, for 3 days duration, was established by an ad hoc committee of the National Academy of Sciences (NAS), based upon a formula for converting air inhalation data to water ingestion estimates.[19] In 1982, the NAS supported a Department of the Navy established drinking water criterion of 0.05 mg/L.[20]

SOURCES OF EXPOSURE

Most of the high explosives and propellants, including TNT, are manufactured for all the United States armed services in Army Ammunition Plants (AAPs).[2] Currently there are six AAPs involved with TNT manufacture and/or load, assembly, and pack (LAP) operations. One and nine-tenths millions of pounds of TNT are produced currently per month, and the maximum monthly production capacity is approximately 15 to 18 times that quantity. Manufacture and LAP operations provide several opportunities for TNT and its products to enter the environment by water, soil, and air.

Palazzo and Leggett[21,22] addressed the issue of TNT uptake by terrestrial plants that grow near AAPs and subsequent environmental fate of the munition. A literature review by Palazzo and Leggett[21] discussed unpublished data and suggested that TNT is taken up and translocated by tall fescue grass, ryegrass, orchard grass, and alfalfa. In 1986, Palazzo and Leggett published the results of growing yellow nutsedge (Cyperus esculentus) in hydroponic cultures containing TNT concentrations of 0, 5, 10, and 20 mg/L.[22] TNT and its aminodinitrotoluene metabolites were found throughout the plant. In the 20 mg/L cultures, 4-amino-2,6-dinitrotoluene ranged up to 2,200 mg/kg in roots and exceeded the levels of TNT and the other noted metabolite, 2-amino-4,6-

dinitrotoluene. The quantities of all three compounds increased in the plants as the concentrations of TNT were increased.

Discharge of TNT from Army Ammunition Plants

Ryon et al.[2] described direct waste discharge from AAPs as the primary way by which TNT enters the aquatic environment, with levels ranging from 0.05 to 178 mg/L. One type of discharge, "pink water" is descriptive of the LAP wastewater's characteristic color caused by photolytic decomposition, a process associated with reduction of TNT. Another type of discharge, "red water," is formed during a purification process of TNT manufacture. TNT is not the sole constituent of these discharges; other explosives and/or TNT by-products also are present. Many of these other wastewater constituents also have significant biologic and toxicologic actions, and in assessing the environmental impact of TNT wastewater, its total composition should be considered. This text addresses only the impacts attributable to TNT.

The following subparagraphs describe the presence of TNT in aquatic environments surrounding AAPs. When reviewing and comparing the concentrations of TNT found in water and sediment, be aware that differences in extraction and analytical techniques resulted in detection limits that varied from study to study. Detection limits were determined by the minimum amount of TNT that could be distinguished from indigenous oils present in each sample extract with adequate confidence.

• Longhorn AAP, 16 miles northeast of Marshall, TX, has sumps that collect munition wastes in the manufacturing areas. The sumps are pumped out and the water trucked to evaporation-percolation ponds. There are no direct discharges into streams; however, overflow may enter small streams. Samples from one stream, which originated in an area that was formerly used to manufacture TNT but had been abandoned for several years, showed traces of TNT, less than 0.01 mg/L, in the water.[23]

• Water from evaporation ponds and streams that received munition waste from Louisiana AAP, 20 miles east of Shreveport-Bossier City, contained TNT concentrations ranging from a trace, less than 0.1 mg/L, to 60 mg/L.[24] Munition waste from this AAP was collected in sumps and periodically pumped out and trucked to a complex of 10 evaporation-percolation ponds. Reportedly, small amounts of waste would get into streams at the loading areas. Core sediment samples from one of the ponds were reported to contain TNT ranging from 9.34 percent (dry weight) within 5 cm of the surface to 0.04 percent at depths between 46 and 51 cm.[25] The author considered TNT content in sediment exceeding 0.60 percent as saturated; therefore, this study suggested that TNT exceeded absorption capacity at depths less than 25 cm.

• Joliet AAP, IL (now in stand-by status), discharged effluents from TNT manufacture and LAP operations into three creeks and a small man-made lake. All of the waters flow into the region of the confluence of the Kankakee and Des Plaines Rivers. Cooper et al.[26] measured TNT in water samples from the lake and creeks and found concentrations ranging from less than 0.0005 to 1.140 mg/L. Stillwell et al.[27] also sampled for TNT at the lake and one creek and included sediment analysis. The TNT was greater at the effluent outfalls,

(0.075 mg/L in the lake and 0.024 to 0.043 mg/L in the creek), than at the downstream sample stations (0.009 mg/L in the lake and less than 0.0006 mg/L in the creek). Sediment samples ranged from less than 0.0002 to 0.205 mg/kg also decreasing as distance from the outfall increased.

• Radford AAP, VA, discharges TNT manufacturing waste products into the New River or its tributary, Stroubles Creek. Huff et al.[28] measured TNT in water and sediment sampled from the river and creek and found that concentrations were below detection limits, 0.1 mg/L, in water and 10 mg/kg in sediment. At the time of the survey the plant was not in operation; however, the authors did predict that, based upon the discharge history and typical river flow rates, the total nitrobody content in the water, i.e. TNT and TNT products, would be less than 0.2 mg/L.

• Water samples from a survey of McAlester Naval Ammunition Depot in Pittsburgh County, OK,[29] did not show traces of TNT. During the survey, the depot was a TNT loading facility, and there was a concern that TNT could reach Brown Lake, a major water source for the depot and Savanna, OK.

• Volunteer AAP, TN, has a drainage system consisting of two large ditches fed by many small tributaries. Storm water runoff and waste effluent from the plant flow through a series of treatment ponds that discharge into the Waconda Bay. Waconda Bay is part of the Lake Chickamauga Reservoir on the Tennessee River. Huff et al.[30] found that TNT levels in water and sediment were below the detection limits of 0.1 mg/L and 10 mg/kg, respectively. Sullivan et al.[31] reported data collected in 1974 showing TNT levels in the upper bay above 1 mg/L and 1975 data ranging up to 0.102 mg/L. Ryon et al.[2] considered the Sullivan et al. study to be the best environmental survey of stream transportation of wastes generated from an AAP and stated that the data demonstrated the decline in munition concentrations in the water column with distance from the plant. At distances of 0.4 to 1.0 mile from the AAP, TNT in water was at the detection limit. TNT in sediment decreased down to the detection limit after 1.0 mile. Sullivan et al.[32] similarly demonstrated decreases in TNT as distance from the outfall increased.

• The wastewaters from the Milan AAP, TN, flow into the Rutherford Fork of the Obion River. Huff et al.[33] found that TNT concentrations in water and sediment were below detection limits (0.1 mg/L and 10 mg/kg, respectively).

• During an assessment of the impact of the Holston AAP effluents on aquatic life in the Holston River, TN, Huff et al.[34] did not find detectable levels of TNT in the water or sediment. Sullivan et al.[35] indicated that munition waste impact on the river was influenced by a wide variation in flow due to intermittent releases from a dam and found TNT in sediment ranging up to 4.2 mg/kg (detection limit was 0.1 mg/kg), increasing in concentrations downstream along the north bank, with none detected along the south bank. The investigators attributed the difference to the confluence of the rivers' north and south forks, which resulted in two contiguous streams occupying the same riverbed. Wide divergence in the chemical characteristics of the water along the north and south banks was the evidence for this observation. TNT results

for water samples generally were negative; however, occasionally it was found at levels ranging from less than 0.002 to 0.089 mg/L (detection limit was 0.0001 mg/L).

• Three streams - Long Creek, Brush Creek, and Spring Creek - flow through Iowa AAP and receive treated TNT wastewater, sewage treatment plant effluent, and/or surface runoff. All three ultimately discharge into the Mississippi River approximately 10 miles south of Burlington. The pattern of declining munition waste concentrations in the water column and sediments with distance was noted by Ryon et al.[2] Surveys by Weitzel et al.,[36] reporting means of daily sampling, and Sanocki et al.[37] demonstrated detectable amounts of TNT in water and sediment from various sample locations along each stream. Data from the two surveys showed water concentrations that ranged up to 3.4 mg/L in Spring and Brush Creeks and up to 3 mg/L in Long Creek. TNT levels in the sediments ranged up to 0.1 mg/kg in Spring Creek, up to 617 mg/kg in Brush Creek, and from 0.1 to 1 mg/kg in Long Creek. Sanocki et al.[37] sampled six representative potential point and non-point sources of munition related substances on tributaries associated with Brush Creek. Means of daily sampling ranged from less than the detectable limit to 0.0167 mg/L TNT. Sediments from an inactive lagoon previously used to treat TNT process water contained more than 3000 mg/kg TNT.

• Ryon et al.,[2] describing a study of the Alabama AAP, indicated that in surface waters there was no evidence for migration of munition wastes. However, detectable levels of munitions in associated sediments were greater in the upper portion of the streams than in the lower.

Transfer to Drinking Water and Air

Rosenblatt et al.[8] discussed the theoretical flow of TNT from wastewater outfalls of several AAPs to municipal drinking water sources. The concentrations of TNT in the drinking water sources (i.e., untreated water) were predicted based upon AAP discharge levels and rates and mean flows of the water channels that carry the waste from the outfall to the drinking water source. These predictions represent upper limits because some factors, e.g., settling and degradation, were not considered. TNT from Holston AAP was estimated to result in a maximum concentration of 0.038 mg/L at the water supply intake for the city of Morristown, TN. Discharge from Volunteer AAP was estimated to result in drinking water concentrations of 0.014 mg/L for Chattanooga, TN. TNT concentrations in the Kanawha River were estimated to range from 0.025 to 0.040 mg/L and were predicted to be such in the drinking water sources of the West Virginia towns of Gauley Bridge, Charlton Heights, Boomer, Montgomery, Pratt, Cedar Grove, and Belle. The munition waste of Joliet AAP was predicted to result in 0.006 mg/L TNT occurring in the drinking water of Peoria, IL. The corresponding prediction for Keokuk, IA, downstream from Iowa AAP, was less than 0.001 mg/L. TNT in the Bossier City, LA, drinking water source was predicted to reach a yearly mean of 0.0006 mg/L assuming a complete washout of holding ponds at Lone Star AAP, TX. Cornhusker, Milan, and Louisiana AAP effluents were discharged into bodies of water that did not eventually lead to municipal drinking water sources.

Ryon et al.[2] noted the relative unimportance of sorption to sediments in determining the environmental fate of TNT and cited Spanggord et al.[38] as laboratory and field confirmation. They indicated that the higher levels of TNT found in sediments as compared to the water column are due to long-term rather than short-term deposition.

Ryon et al.[2] described several laboratory studies and field surveys conducted to evaluate the transportation of TNT through soils and into groundwater. In the laboratory, investigators used various sized cylinders filled with a variety of soil types. TNT and/or ^{14}C-TNT was applied to the top of the soil, and leachate was collected at various distances down the column and at the end of the column for periods of up to 6 months. TNT migration was minimal, with variations due to soil composition noted. Sikka et al.[39] noted that sorption of TNT to soil was affected by pH and temperature. Field surveys reviewed by Ryon et al.[2] detected measurable amounts of TNT in groundwater, suggesting migration through soil from AAP surface soils and from one landfill. Surface soil TNT levels ranging from less than 0.002 to 3 x 10^3 mg/kg were associated with groundwater TNT concentrations ranging from less than 10 and up to 10.27 mg/L. One of the studies suggested for TNT a maximum rate of 2 cm per year for horizontal migration and 25 cm per year for vertical recharge to an aquifer. Tucker et al.[40] conducted TNT adsorption experiments on 12 soils that had a wide range of organic carbon content, cation exchange capacity, pH, and clay content. TNT was moderately mobile, and its adsorption coefficient correlated with organic carbon content and cation exchange capacity.

Ryon et al.[2] did not find any information in the literature concerning atmospheric transport of TNT. Carpenter et al.[41] indicated its presence in atmospheric emissions from two AAPs, but did not quantitate its emission rates, reporting that they were believed to be very small. Albeit the atmospheric emission rates for TNT were undefined, Carpenter, et al.[41] cautioned that their significance should not be dismissed. Ryon et al.[2] suggested that TNT particulates released in the air from incineration would undergo photolysis prior to or after settling on the ground. Spanggord et al.[42] and Ryon et al.[2] considered the atmospheric transport of TNT due to volatilization from surface water as environmentally insignificant.

Biological and Physical Degradation

Summarizing the investigations of Spanggord et al.,[38] Jerger et al.,[43] Small,[44] and Carpenter et al.,[41] Ryon et al.[2] concluded that TNT has little persistance in aquatic environments, due to photolysis (half life less than 24 hours) and biological degradation (half life less than 65 days). Degradation in soil is not as effective as in aquatic environments. TNT has been detected in soils 20 to 35 years after deposition.[2,37]

Biological and physical degradation of TNT has been studied by numerous investigators and is reviewed in detail by Pal and Ryon[6] and Ryon et al.[2] Numerous species of bacteria, fungi, and yeast degrade TNT into various classes of chemical products, including azoxy compounds and isomers of hydroxylaminodinitroluene, aminodinitrotoluene, and diaminonitrotoluene. Physical degradation is caused by photolysis resulting from exposure of TNT

to ultraviolet or sunlight. Photolysis experiments with TNT in distilled and natural waters have produced single ring aromatic compounds and azo- and azoxy derivatives formed by the coupling of nitroso- and hydroxylamine products.[2,38,45,46] Spanggord et al.[25,47] observed that photolysis was promoted when TNT complexed with natural organic and humic substances and that the photochemical rate was inversely proportional to water depth.

METABOLISM/PHARMACOKINETICS

TNT may enter the body through the respiratory system or GI tract, or by absorption through the skin. In a review of occupational safety and health aspects of TNT, Zakhari et al.[5] characterized the various routes of entry. The skin is the primary route, with oily, sweaty skin favoring absorption and the palms being the site where most absorption occurs. TNT also is readily absorbed when administered orally or subcutaneously, or when introduced into respiratory passages as dust.[5,48] Administering ^{14}C ring labeled TNT orally to rats, Lee et al.[51] determined that 60 to 90 percent of the dose was absorbed in 24 hours. Ellis et al.[50] determined that 50 to 75 percent of the dose was absorbed. Each investigator indicated that absorption probably was greater than reported because the radioactivity measured in the carcass and urine did not include that in the bile. Ellis et al.[52] found 10 to 28 percent of the TNT from an oral dose in bile. TNT is soluble in various body fluids including saliva, stomach juice, cow milk, human bile, human serum, and urine.[5]

Studies with rats, mice, rabbits, and dogs show that TNT distributes throughout the body to include the blood, liver, kidneys, lungs, spleen, brain, fat, muscle, and GI tract. TNT is taken readily into most tissues, especially liver and kidney; however, only trace amounts are found distributed in all tissues.[48,49,51,52] El-hawari et al.[48] compared tissue distribution in animals subsequent to single dose administration of ^{14}C ring-labeled TNT by intratracheal, dermal, or oral routes and found that storage and retention differed by species and route of administration.

In view of the absence of unchanged TNT in urine and the presence of several classes of TNT derived metabolites, Lee et al.[49] and Ellis et al.[51] concluded that mammals extensively metabolize TNT. The primary site of detoxication is the liver, involving either reduction or oxidation processes.[5,19] Ryon et al.[2] described TNT metabolism as occurring primarily by oxidation of the methyl group and, to a lesser extent, hydroxylation of the ring. Hodgson et al.[52] explained that the number of possible metabolites is due to the presence of the methyl, nitro, and benzene nucleus functional groups of the TNT molecule. These groups can be oxidized to form alcohols, acids, aldehydes, phenols, and hydroxylamino compounds, or the nitro can be reduced to form amino metabolites. These metabolites are capable of further coupling with other compounds or groups resulting in products that can further conjugate to form glucuronides, ethereal sulfates, and conjugates of hippuric acids and glutathione. Ryon et al.[2] listed the TNT metabolites identified in human studies including: 4-amino-2,6-dinitrotoluene; 6-amino-2,4-dinitrotoluene; 2,4-diamino-6-nitrotoluene; 4-hydroxylamino-2,6-dinitrotoluene; and an amino-nitrocresol. These metabolites, additional isomers, and other compounds derived from TNT metabolism have been identified in animals,

microbes, and tissue/enzyme extracts.[2,53] El-hawari et al.[48] occasionally found trace quantities of unmetabolized TNT in the urine of rats, mice, rabbits and dogs. Hodgson et al.[52] suggested species differences in TNT metabolism, with the rabbit exhibiting a different metabolic profile from the rat, mouse, and dog, all of which had similar profiles.

TNT metabolites are excreted primarily by the kidneys.[2,5,19,48,49,51] After administering a single oral dose of ^{14}C ring-labeled TNT to rats, Lee et al.[49] recovered 53.3 percent of the labeled carbon from urine. In a similar study of rats, mice, dogs and rabbits, Hodgson et al.[52] recovered from 45 percent to 79 percent from urine. Lesser amounts of TNT are excreted in feces than in urine following oral administration. El-hawari[48] reported from 1.8 percent to 22 percent of administered TNT in the feces of rats, mice, rabbits, and dogs; and Lee et al.[49] reported 5.5 percent in the feces of rats. TNT undergoes biliary excretion, and negligible amounts, less than or equal to 0.1 percent, are recovered in expired air.[5,48,49] From 10 percent to 15 percent of orally-administered ^{14}C ring-labeled TNT in rats has been measured in the bile.[51,52]

The significance of TNT bioaccumulation has not been investigated extensively; however, published studies indicate that the levels of accumulation are insignificantly small.[2,54] Spanggord et al.[38] studied biosorption using heat killed bacterial cells at concentrations of 0.49 and 0.74 mg bacteria/mL in initial TNT concentrations of 10 and 20 mg/L (each bacterial cell concentration was studied at both TNT concentrations). The resulting biosorption coefficient was very small, 93 $\pm$ 18, and was not considered to be environmentally significant. Rosenblatt and Small[55] calculated a bioconcentration factor (BF) of 2.24×10^{-2} for TNT, which Ryon et al.[2] described as a low value that would not indicate an environmental problem. Cooper et al.[26] showed a possibility of TNT bioaccumulation, ranging from 0.280 mg/kg to 1 mg/kg, in fish and crayfish collected from an AAP. The data were not conclusive, due to difficulties in sampling and analytical methods.

HEALTH EFFECTS (MAMMALIAN DATA)

Several literature reviews[2,5,16,19] indicate that hepatotoxicity leading to yellow atrophy of the liver and aplastic anemia associated with bone marrow hypoplasia are the major causes of death from TNT exposure. These reviews and several US Army studies[13-15] describe a number of effects on other organs and organ systems. Hematological changes include decreases in hemoglobin, red blood cells (RBC) and platelets; Heinz body formation; RBC dyscrasias (polychromasia, poikilocytosis, anisocytosis, reticulocytosis, eosinophilia); white blood cell changes (leukocytosis, lymphocytosis); capillary fragility leading to nosebleeds and skin/mucosa hemorrhages; and nitric oxide hemoglobin. At exposures to high concentrations of TNT, methemoglobinemia and cyanosis may occur. Hemolytic anemia has been reported to occur in TNT workers with glucose-6-phosphate dehydrogenase (G6PD) deficiency.[48] Other TNT effects include: cardiac effects (e.g., dull heart sounds, systolic murmur, bradycardia, hypotension, EKG abnormalities, contraction abnormalities, and myocardial dystrophy); nephrotoxicity, causing an increase in renal filtration rates; neurotoxicity, indicated by neurasthenia and polyneuritis; pancreatic

toxicity associated with a decrease in enzyme secretion; gastrointestional tract effects, evidenced by gastroenteritis; dermatitis; dental toxicities leading to carious and noncarious tooth injury and periodontal and oral cavity mucous membrane disease; cataract formation; and biochemical changes, e.g., decreased plasma proteins, increased plasma bilirubin (icterus), and increases in lactic dehydrogenase (LDH) and serum glutamic oxaloacetic transaminase (SGOT).

Short-Term Exposure

Several reviews[2,5,16,19] describe studies and observations dating from the 1940s of TNT effects to humans subjected to short exposures. One study involved 62 volunteers, exposing themselves to air concentrations of TNT from 0.3 to 13 mg/m^3 for an average of 33 days, 8 hours daily. Decreased hemoglobin and red blood cells were the primary effects, and most subjects developed rashes. Other observations in some volunteers included increases in reticulocytes, plasma bilirubin, and plasma phosphatase and decreases in plasma proteins and hippuric acid. Jaffe, et al.[19] and Rosenblatt[16] describe an experiment where two subjects were given oral doses of 1 mg TNT/per kg body weight (mg/kg) daily for 4 days. With the exception of an increase in the turbidity of hemolyzed blood samples, symptoms and chemical/physical changes were absent. In another experiment, two subjects rubbed 500 mg of TNT (approx. 7.1 mg/kg) into the palms of their hands and wore rubber gloves for 8 hours. The only apparent effect was a moderately bitter taste in the mouth. Zakhari et al.[5] described the development of pronounced jaundice in a 35 year old woman subsequent to 1-month's exposure to TNT. Zakhari et al.[5] also indicated that dermatitis may develop as early as 5 days after exposure.

Jaffe et al.[19] and Zakhari et al.[5] reviewed a number of animal lethality studies reported between 1918 and 1976. In cats, death occurred subsequent to: oral administration of TNT, 480 and 1,850 mg/kg; subcutaneous administration, 20 mg to 200 mg/kg, though sometimes not until 23 days after dosing; cutaneous administration, two doses of 2,000 mg/kg each, in 8 hours; and intraperitoneal administration, 100 to 150 mg/kg. Symptoms were not observed in cats given a 300 mg/kg dose cutaneously or a 750 mg total dose, route unspecified. Zakhari et al.[5] cited a study where death occurred in both cats and rabbits 49 hours after dosing initially with 750 mg and 24 hours later with 500 mg of TNT. The route of administration was not specified. In other rabbit studies death occurred upon oral administration of 500 mg/kg, and subcutaneous administration of 500 to 700 mg/kg, and, in one study, when 300 mg/kg TNT was given to rabbits every 2 days for 18 days the animals died. Symptoms were not observed in rabbits given either 125 to 300 mg/kg cutaneously or orally or a 750 mg dose, route unspecified. Death in rats occurred subsequent to oral dosing of 700 mg/kg TNT and subcutaneous administration of 500 to 1,000 mg/kg. In a guinea pig study described by Zakhari et al.,[5] 70 percent of the animals given 20 mg of TNT orally died within 6 weeks.

Acute oral LD50s (lethal dose to 50 percent of the test population) were determined in rats and mice.[49] Males and females of both species were given TNT dissolved in peanut oil orally by intragastric intubation and observed for 14 days. Convulsions occurred within 15 minutes after dosing and persisted

for 1 to 2 hours. Toxic doses produced ataxia, respiratory depression, and transient cyanosis lasting for 2 to 4 hours. LD50s (and 95 percent confidence limits) for male and female rats were 1,010 (922 to 1,108) and 820 (747 to 889) mg/kg, respectively. Those for male and female mice were 1,014 (905 to 1,163) and 1,009 (880 to 1,117) mg/kg, respectively. Dilley et al.[18] also reported acute oral LD50s in rats and mice. Using 10 male and 10 female animals per dose level per species and a dosing/observation regimen similar to that of Lee et al.,[49] Dilley et al.[18] observed that the animals became inactive after dosing. Tremors occurred within 1 to 2 hours followed by petit mal convulsions. Some animals died within 4 hours. LD50s for male and female rats were 1,320 (95 to 1,824) and 794 (602 to 1,047) mg/kg, respectively and 660 mg/kg for both male and female mice (95 percent confidence limits 524 mg/kg to 831 mg/kg and 574 mg/kg to 758 mg/kg, respectively).

Animal studies of TNT exposure reported between 1921 and 1961, as reviewed by Jaffe et al.[19] and Zakhari et al.,[5] reveal effects to numerous organs and biological systems. Dogs given doses of TNT ranging from 1.5 to 100 mg/kg by various routes of administration for up to 4 weeks duration, exhibited varying degrees and combinations of cyanosis, incoordination, ataxia, diarrhea, asynergia, weakness, hind, quarter paresis, nystagmus, bowel inflammation, darkened urine, and involuntary urination. Jaffe et al.[19] noted that studies in dogs demonstrated individual variation in susceptibility to TNT toxicity. Cats given 20 mg/kg of TNT, orally or subcutaneously, daily for up to 30 days developed hemosiderosis in the spleen pulp, reticuloendothelium, and hepatic Kupffer cells. Intraperitoneal doses of less than 40 mg/kg caused nervous manifestations in cats. Daily oral doses of 30 mg/kg TNT given to rats caused a progressive decrease of leukocytic phagocytosis (which increased with niacin, 1 mg/kg administered simultaneously by subcutaneous injection), and less than 400 mg/kg, given subcutaneously or with diet, increased porphyrin excretion. Guinea pigs given total doses of 600 to 2,000 mg of TNT orally or 800 mg/kg (the route was not specified) developed hematological disorders including severe anemia, increased reticulocytes, decreased platelets, and leukocytosis. A study described by Zakhari et al.[5] that did not specify the dosing regimen summarized the effects of TNT given to guinea pigs and rabbits orally, subcutaneously, intravenously, and percutaneously. Stools became brownish and liquid in 2 to 3 days, moderate anemia developed in 4 to 8 days, and other effects included cyanosis, myocardial depression, and hypothermia. Rabbits given 650 mg TNT/kg body weight developed hypochromic anemia, acute leukocytopenia, and acute thrombocytopenia. A decrease of 50 percent of total plasma proteins was reported to occur in rabbits given TNT; however, the dose was not specified.[5] Lee et al.[49] classified TNT as a mild skin irritant after applying a 50 percent paste in peanut oil to rabbits (modified Draize test) and as a moderate sensitizing agent following dermal sensitization testing in guinea pigs using 4.12 percent solution in peanut oil. TNT did not produce eye irritation in rabbits.

Longer-Term Exposure

Evaluations of health effects due to human occupational exposures, usually several years in duration, have provided the evidence for concluding that TNT affects numerous biological systems and organs. Jaffe et al.,[19] Zakhari et al.,[5] and Ryon et al.[2] summarized the literature covering reports of

occupational exposures from 1917 through 1976. When reported, the mode of exposure generally was due to atmospheric concentrations of TNT dust and vapor. The exposure route is assumed to have been by inhalation and/or skin absorption, with maybe some contribution from ingestion. The air concentrations reported ranged from 0.3 to 2.0 mg TNT per cubic meter of air (mg/m^3). Some exposures were reported simply as exceeding the current threshold limit value, 1.5 mg/m^3. The exposed persons, both male and female, generally were TNT workers involved with its manufacture and use in munition production. However, some individuals suffered effects from handling the finished products, e.g., a 70-year old man who worked as a tunpeler for 15 years and enlisted men who transferred stored cast TNT charges.[5] Frequently the exposure duration was not specified and was described only as chronic or occupational, which may have been 6, 8, or 12 hours daily for 5, 6, or 7 days a week. When specified, durations were daily 8-hour exposures ranging from 6 months to 32 years.

Toxicity (4,000 cases) and deaths (580) from occupational TNT exposures were greatest during World War I and decreased during World War II (only 22 fatalities) due to the introduction of industrial hygiene measures. Death most frequently resulted from either toxic hepatitis, aplastic anemia, or both. The clinical manifestations of TNT exposure resulted from liver, spleen, kidney, and other organopathies as well as toxic effects to the hematological, hematopoietic, and nervous systems. The affected systems and resultant pathologies have been addressed in the introduction of this summary's Health Effects section and will not be repeated here. Even though dose-response relations have not been elucidated in studies of occupational exposure, several studies of AAPs[13-15] concluded that atmospheric TNT levels between 0.5 and 1.88 mg/m^3 caused significant hematological and liver enzyme responses that were evidence of developing pathology. These studies helped to bring about the lowering of the TLV from 1.5 to 0.5 mg/m^3.[2,5,10]

TNT effects in animals are similar to, and confirm, those observed in humans. Jaffe et al.[19] reviewed the reported effects of TNT to various animal species and observed that cats, dogs, and humans appeared to be more sensitive than rabbits and rats. This review and the one by Zakhari et al.[5] discussed the effects of longer-term exposure, i.e., greater than 30 days, to TNT observed in studies of dogs, rats, guinea pigs, cats, rabbits, and monkeys between 1918 and 1972. Dogs given oral, subcutaneous, or inhaled doses of TNT ranging from 0.02 to 50 mg/kg of body weight daily for periods ranging from 84 days up to 2 ? years showed a variety of effects. With the exception of emesis, dogs given 0.02 to 1.0 mg/kg (study by Hart[56]) did not show any apparent signs of toxicity; however, larger doses, 5.0 to 50 mg/kg resulted in hematological, hepatic, neurological, splenic, pancreatic, and biochemical dysfunctions similar to those previously described. Death occurred in some animals only at the highest dose. In the review of effects in rats,[19] the animals were dosed orally with 30 to 150 mg/kg or given TNT at concentrations of 150 or 300 mg percent in the diet. Observed effects included weight and appetite changes, hematologic alterations, hair loss, alterations in neuromuscular junction excitability and, only at the higher dose (150 mg/kg) in some animals, death.

Guinea pigs given 500 mg of TNT percutaneously for 11 months developed cerebral and brain stem damage, and those given 200 mg/kg for 16 days followed by 400 mg/kg for an additional 43 days developed liver and splenic toxicities. Seventy percent of guinea pigs given oral doses of 20 mg TNT per week for 6 weeks died. All given 2 mg orally every 48 hours for 60 days remained alive. A cat study lasting up to 15 weeks resulted in wasting, cyanosis, and death upon oral or subcutaneous administration of 20 to 130 mg/kg given every 1 to 3 days. A 1920 study indicated that rabbits were not affected by subcutaneous doses of TNT, 34 to 446 mg/kg, administered for 2 months; however, later studies demonstrated a range of toxicities including hematotoxicity, hepatotoxicity, splenopathy, neurotoxicity, renal toxicity, and hematopoietic and biochemical alterations. Effects were subsequent to various routes of administration, i.e., intragastric, oral, and subcutaneous, with dosages ranging from 0.05 to 450 mg/kg given for periods of 17 to 60 days. A study of TNT effects in Rhesus monkeys was reported by Hart.[57] Three monkeys of each sex per dose were given daily oral doses of TNT, either 0.02, 0.1, or 1.0 mg/kg, for 13 weeks. The highest dose produced increased hemosiderin in liver and cell cytoplasm, and there were no normal megakaryocytes in bone marrow. The significance of these observations was uncertain.

Dilley et al.[18] studied the effects that TNT produced in beagle dogs, Sprague-Dawley rats, and Swiss Webster mice. Five male and five female dogs per dose were given TNT orally in capsules at daily doses of 0.2, 2.0, or 20 mg/kg body weight for up to 90 days. One dog of each sex per dose was killed after 4 weeks of treatment and an additional dog of each sex was held for 4 weeks of recovery without further treatment. After 13 weeks all survivors were killed except one of each sex per dose, which was held for 4 weeks of recovery. Twenty rats of each sex per dose were subjected to a study protocol similar to that of the dogs except that doses were 1.45, 7.19, 35.6, and 161 mg/kg and given orally with the diet. At 4 and 13 weeks five rats of each sex and dosage group were killed and five removed from treatment for the recovery study. Twenty mice of each sex per dose were treated similarly except that the doses were 1.56, 7.76, 36.8, and 180 mg/kg. The TNT dosages for rats and mice are average daily intakes for males and females using quantities reported by Ryon et al.[2] At doses of 2.0 mg/kg some toxic effects were noted in the dogs at the 4th and 13th week of study. These included decreased serum iron, focal lymphocyte deposition in the kidneys of females, and enlarged kidneys in males. Twenty mg/kg caused dogs to temporarily lose weight and decrease feed intake. Fourth- and 13th-week observations included increases in liver, spleen, and adrenal weights and increases in bilirubin, mean corpuscular volume (MCV), cholesterol, and lymphocytes. Decreases were noted in hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), polymorphonuclear neutrophiles (PMN), serum glutamic pyruvic transaminase (SGPT), iron, and red blood cells (thus causing anemia). The 20 mg/kg dose also caused amber to red colored urine, neurological symptoms, e.g., inactivity and nystagmus, and enlarged kidneys and smaller hearts in males. Effects in rats included the formation of red urine after 50 days and decreased blood iron and anemia after 13 weeks at doses of 7.19 mg/kg. TNT at 35.6 mg/kg in rats resulted in decreases in body weight, feed intake, and serum iron in females, and anemia occurred in 13 weeks. Other observations included enlarged spleens in males, splenic hemosiderosis, and increased liver weights in females. At 161 mg/kg, additional effects included atrophied

testes, and at 13 weeks decreased kidney sizes, with leukocytosis and lymphocytosis, decreased SGPT, and increases in uric acid and cholesterol. Bilirubin increased in females with 4 weeks of treatment. Doses of 36.8 and 18 mg/kg caused temporary weight loss, decreased feed intake, and splenic hemosiderosis in mice. Additional effects from the 180 mg/kg dose were a permanent decrease in body weight, spleen enlargement, increased heart to brain weight ratio, mild anemia, and increases in the PMN to lymphocyte ratio with corresponding white blood cell damage.

Effects were not detected in the dogs given TNT doses of 0.2 mg/kg, rats given 1.45 mg/kg, and mice 1.56 or 7.76 mg/kg. Dilley et al.[18] considered these exposures to be "no effect" levels. In mice 7.76 mg/kg was described as the highest no observable effect level (NOEL). Dogs appeared to be the species most susceptible to the effects of TNT. Dacre[17] criticized Dilley's et al.[18] dog feeding study because of the small number of animals used, five sex/dose group, with the final conclusion based on only six survivors in each dosage group.

Levine et al.[58] dosed beagle dogs, six/sex/dose group, with TNT orally by capsule daily for 6 months (26 weeks). Dosage groups were 0 (control), 0.5, 2, 8, and 32 mg/kg. The major toxic effects occurred at the 8 and 32 mg/kg doses and included hemolytic anemia, hepatomegaly, splenomegaly, and, at 32 mg/kg, death. The authors observed liver injury at all dose levels and therefore did not report a NOEL.

Levine et al.[59,60] gave daily doses of TNT to Fischer 344 rats, 10/sex/dose, for 13 weeks. Doses, given orally via diet, were 1, 5, 25, 125, and 300 mg/kg. Furedi et al.[61] also dosed Fischer 344 rats, 75/sex/dose, for up to 24 months. The doses were given daily via diet at levels of 0 (control), 0.4, 2, 10, and 50 mg/kg. Both studies produced a variety of hematologic, hematopoietic, hepatic, splenic, neurologic, and urogenital toxicities. Levine et al.[59] concluded that the maximum tolerated dose (MTD, as defined by the National Cancer Institute for carcinogenicity testing) for TNT was between 5 and 25 mg/kg, and since slight anemia was observed at 25 mg/kg the MTD probably was slightly less. Furedi et al.[61] established 0.4 mg/kg as the no-effects level because splenic congestion, increased pigment deposition in kidneys, and bone marrow fibrosis occurred at the other doses studied (2, 10, 50 mg/kg).

Furedi et al.[62] dosed groups of 75 B6C3F1 hybrid mice per sex with 0, 1.5, 10, and 70 mg/kg of TNT daily for 24 months. Ten animals per sex and dosage group were killed following 6 and 12 months of study, and the remainder were killed after 24 months of treatment. The highest dose caused anemia, possible hepatotoxicity, peripheral lymphocytosis, and leukemia; lymphoma was apparent in the spleen. There was a single incidence of lymphocytosis in a male mouse at the 10 mg/kg/day dose. A 5 percent decrease in body weight gain was noted throughout the study. The investigators determined 1.5 mg/kg/day to be the no-effect level of the study.

Reproductive/Teratogenic Effects

Both Zakhari et al.[5] and Ryon et al.[2] reported that no teratogenicity studies were in the available scientific literature. Jaffe et al.[19] cited irregular menstruation as a symptom of chronic TNT intoxication in humans. Dilley et al.[18] observed testicular atrophy in rats given 0.25 percent TNT in their diet for 90 days [averaged as 160 mg/kg/day in Ryon et al.[2]] and possibly in a dog given 20 mg/kg/day for the same duration. The observation in the dog is uncertain because controls also had testicular atrophy. Levine et al.[52,60] reported the occurrence of testicular atrophy with degeneration of seminiferous tubule epithelium in rats given oral doses of TNT, 125 and 300 mg/kg/day, for 90 days.

Carcinogenic Effects

Zakhari et al.[5] and Ryon et al.[2] indicated that carcinogenicity attributable to TNT exposure had not been reported. Furedi et al.[61] suggested that TNT was a carcinogen based on the occurrence of urinary bladder carcinoma and hepatocellular, renal, and urinary bladder hyperplasia in Fischer 344 rats at 10 mg/kg/day or greater. She also found leukemia and malignant lymphoma in female mice fed 70 mg/kg/day TNT for 2 years. These were not seen in male rats. Dilley et al.[18] had earlier suggested that because of mutagenic activity in the Ames assay TNT's potential carcinogenicity merits further study. Ashby et al.[63] support this view, citing also mutagenic activity in mouse lymphoma cells, as reported by Styles and Cross.

Mutagenic Effects

Up to 1973 no studies were found in the scientific literature addressing mutagenic effects of TNT.[19] In 1974, Geshev and Kincheua (described in Ryon et al.,[2] Zakhari et al.,[5] and Rosenblatt[16]) reported that chromosomal changes occurred in tibial bone marrow cells in 10 out of 20 Wistar rats given 30 percent TNT five times a week for 6 months. Some specified changes included chromosome breaks, dislocations, and chromatid changes. Ryon et al.[2] and Zakhari et al.[5] citing a 1976 study by Won et al. describe the results of an Ames assay of various concentrations of explosive grade TNT. Histidine requiring strains of Salmonella typhimurium were growth inhibited at concentrations of TNT greater than 10 μg/mL, of overlay agar. In concentrations of 0.5 to 10 μg/mL, TNT was a frameshift mutagen characterized by a linear mutagenic response curve. Simmon et al.[64] reported that TNT did not demonstrate any mutagenic activity in the Ames assay or with mitotic recombination in Saccharomyces cerevisiae D_3. In these studies TNT was tested at a very low concentration, i.e., its water solubility limit, and the authors caution that the absence of mutagenic activity should not be construed as representing nonmutagenicity. Dilley et al.[18] evaluated higher concentrations of TNT (10, 50, 100, 500, 1,000, 5,000 μg/plate) using the Ames assay with and without metabolic activation. There was a dose-related increase in mutants with and without the metabolic activator. The activator reduced mutagenicity. The investigators concluded that the observed activity was possibly higher than the potential hazard of TNT because the strains of Salmonella used have endogenous aromatic nitro reductase enzymes. Ellis et al.[50] also reported an Ames assay with results similar to those of Dilley et al.[18] Styles and Cross

reported a significant increase in mutation frequency in P388 mouse lymphoma cells. Results were positive only in the absence of S9 activation, thus suggesting (Ryon et al.[2]) that mammalian metabolic activity deactivates the genotoxic potential of TNT. Ashby et al.[63] tested the mutagenic potential of TNT in two rodent systems: a mouse bone marrow micronucleus test and an *in vitro*/*in vivo* rat liver assay for unscheduled DNA syntheses. The results in both systems were negative.

ENVIRONMENTAL (AQUATIC)

The effects of TNT on aquatic organisms and water quality have been studied and were reviewed by Jaffe et al.,[19] Ryon et al.,[2] and Pal and Ryon.[6] Assessment of water quality effects has been limited primarily to bodies of water receiving waste discharges from AAPs. Parameters typically evaluated included alkalinity, color, sulfates, total solids, total dissolved solids, total suspended solids, total volatile solids, nitrates, nitrites, total Kjeldahl nitrogen (TKN), ammonia, phosphorus, organophosphates, chemical oxygen demand (COD), total organic carbon (TOC), specific conductance, pH, hardness, and chlorides. Pal and Ryon[6] and Ryon et al.[2] summarized the results of several investigators[27,28,30,31,34,36,37,43] indicating that TNT wastewater increased COD, dissolved and suspended solids, nitrates, nitrites, TKN, and, to a lesser degree, TOC, pH and phosphates in the water column and associated sediments. These effects generally occurred in areas immediately at or downstream from the wastewater outfall, decreased with increasing distance from the outfall, and were associated with stressed biotic communities. It should be noted that the changes in water quality could not be attributed exclusively to TNT because the AAP wastewaters also contained TNT by-products (e.g. trinitrotoluenes and dinitrotoluenes), other munition wastes at some facilities, and in one case[30] other upstream industrial discharges, all of which may have influenced water quality characteristics.

Field and laboratory studies to assess the impact of TNT on fish have been reported. Ryon et al.[2] reviewed studies by Weitzel et al.[36] and by Huff et al.[28,30,33] of TNT effects on fish collected from waters that received AAP effluent. Weitzel et al.[36] noted at one AAP that species diversity increased as the distance from the wastewater outfall increased; however, at three other AAPs Huff et al.[28,30,33] could not make conclusive observations due to interfering factors including the presence of other wastes. Jaffe et al.[19] reviewed several laboratory studies, dating from 1943 to 1971, of TNT lethality in a variety of fish. These included: 24- and 96-hour median lethal doses (MLD) of 2.6 and 2.0 mg/L TNT, respectively, in bluegills; 6 hour and 87 to 110 minutes MLD of 4.0 to 5.0 and 40 mg/L, respectively, in minnows; 455 minute MLD of 40 mg/L in carp; 556 minute MLD of 50 mg/L in bullheads; 25 percent mortality in 3 days at 3 mg/L and 100 percent at 5 mg/L in goldfish; and lethality in undefined fish species at 1.5 to 2.0 and 5 mg/L TNT and at 1/400 dilutions of combined TNT wastes. Ryon et al.[2] summarized more recent laboratory studies in bluegills and fathead minnows. Static 96-hour assays with bluegills resulted in LC50 values ranging from 2.3 to 2.8 mg/L TNT over temperatures of 10 to 25°C. Similar assays with fathead minnows resulted in LC50 values ranging from 1.2 to 2.58 mg/L for 96-hour exposures and 3.0 to 4.2 mg/L TNT for 24-hour exposures. Liu et al.54 reported incipient LC50 values ranging from 1.4 to 1.8 mg/L TNT from flow-through acute toxicity testing with

fathead minnows, bluegills, channel catfish, and rainbow trout. Ryon et al.[2] noted reports of behavioral responses observed in TNT-exposed fish that included shock, loss of motor control, and loss of equilibrium. An EC50 value of 0.46 mg/L was determined for a moribund response criterion. Bailey et al.[65] noted that in a full life cycle chronic exposure to fathead minnows, the no-effect level, based on reproductive effects and progeny (F1) survival and growth effects, was approximately 0.04 mg/L TNT.

Laboratory studies of organisms representative of the benthic community, i.e., invertebrates and periphyton, have quantitated TNT toxicity. Flow-through acute toxicity tests have been done with the aquatic invertebrate Daphnia magna and have resulted in LC50 values of 0.19 to 11.9 mg/L TNT.[2,54,66] The no-effect level for D. magna was 1.13 mg/L TNT from a full life cycle chronic study which noted effects on reproduction, survival, and growth of progeny (F1). Studies of other aquatic invertebrates have included the tidepool copepod Trigriopus californicus and oyster larvae Crassostrea gigas.[2] The LC50 values reported were approximately 5 mg/L TNT for the copepod and between 5 and 10 mg/L TNT for the oyster larvae. Laboratory studies of representative periphyton have included diatoms and algae of the green and bluegreen groups. Jaffe et al.[19] reviewed toxicity effects, including death, to Microcystis aeruginosa and Chlamydomonas reinhardi upon exposure to 3 and 8 mg/L TNT, respectively. Noting questionable toxicity results due to TNT photolysis, Liu et al.[54] reported statistically significant growth reductions in Selenastrum capricornutum, M. aeruginosa, Anabaena flos-aquae, and Naviculla pelliculosa at 4.1 mg/L. The review by Ryon et al.[2] of laboratory studies indicates that 2.5 mg/L TNT caused decreased growth, chlorosis, and morphological changes in S. capricornutum and 5 mg/L inhibited the growth rate; in M. aeruginosa, growth rate was decreased, with morphological changes at 15 mg/L, and the threshold for inhibiting cell propagation was 0.32 mg/L; the threshold for inhibiting cell propagation for Scenedesmus quadricauda was 1.6 mg/L. Ryon et al.[2] also reviewed one study of an aquatic vascular plant, Lemna perpusilla (duckweed) exposed to TNT. At a pH of 6.3, TNT caused a significant reduction (10%) in growth rate. Death occurred at TNT concentrations greater than 1 mg/L.

Other information about effects on aquatic invertebrates and periphyton is derived from a number of field studies[2,26,27,31,35,37] that evaluated environmental impact of TNT waste effluents from AAPs. Ecological effects, e.g., inhibition of species diversity and decreases in the number of species, corresponded to simultaneous variations in aqueous and sediment TNT and nutrient levels. Sullivan et al.[32] quantitated the ecological response as no effect at 0.025 mg/L TNT, minimal effect at 0.050 to 0.100 mg/L, and definite/pronounced effect at 0.500 to 0.600 mg/L. Stillwell et al.[27] indicated that if the combined levels of TNT, 2,4-dinitrotoluene and 2,6-dinitrotoluene were between 0.050 and 0.100 mg/L, then ecological changes in the benthic macroinvertebrates and algae communities occurred. Sullivan et al.[31] noted that if the same mixture was less than or equal to 0.020 mg/L in water or 0.100 mg/kg in sediment then environmental impact would be minimal.

The effects of TNT on exposed microorganisms, i.e., bacteria, yeast, and fungi, were reviewed by Ryon et al.[2] and Jaffe et al.[19] Generally, initial acute exposures in laboratory studies cause either decreased metabolic

activity, growth inhibition, or death. After organisms have been adapted or acclimated, either tolerance increases or there is no effect from TNT exposure. Weitzel et al.[36] noted that bacterial growth in aquatic sediments downstream from an AAP was not affected as long as nutrients were present. Jerger et al.[43] reported that stream sediment bacteria from two AAPs were not affected by exposure of up to 44,200 mg/kg (mg of TNT per kg of sediment). Parameters evaluated included bacterial numbers, microbial inhibition, dissolved oxygen uptake, dehydrogenase activity, and adenosine triphosphate (ATP) level.

HEALTH ADVISORY (HA) DEVELOPMENT

An interim ambient water quality criterion of 0.04424 mg/L (44.24 ?g/L) TNT was developed for US Army TNT production.[17] The interim value was derived by the US Environmental Protection Agency (USEPA) method reported in Federal Register 44: 15926-81 (15 March 1979), and was based upon a bioconcentration factor and NOEL from a 90 day male rat study reported by Dilley et al.[18] In addition to daily water consumption, the method also accounts for daily ingestion of TNT absorbed into the edible portions of fish. The following discussion will address only selected studies performed since the report by Dilley et al.[18] The method for quantifying the toxicological effects of TNT will be the one currently applied by the USEPA in health advisory (HA) development. The general formula is:

$$\frac{\text{(NOAEL or LOAEL) (BW)}}{\text{(UF(s)) (\_\_\_L/day)}} = \text{\_\_\_ mg/L}$$

Where: NOAEL or LOAEL = No-Observed-Adverse-Effect-Level

or

Lowest-Observed-Adverse-Effect-Level (the exposure dose in mg/kg bw)

BW = assumed body weight of protected individual in kg (10 or 70)

UF(s) = uncertainty factors, based upon quality and nature of data

__ L/day = assumed daily water consumption (1 or 2) in liters

The results of Furedi et al.[61] are recommended for deriving a longer-term HA (i.e., for periods beyond a few weeks up to 1 year; however, not to reflect lifetime, chronic levels). In this study, Fischer 344 rats,

75/sex/dose, were given daily doses of 0 (control), 0.4, 2, 10, and 50 mg/kg/day of TNT in the diet for up to 24 months. Toxicological end points included clinical signs, body weights, food consumption, hematology, clinical chemistry, ophthalmology, organ weights, and gross and tissue morphology. Major toxic effects observed were anemia with secondary splenic lesions, urogenital lesions, hepatotoxicity, and hyperplastic/neoplastic lesions of the liver, kidneys, and urinary bladder. Splenic congestion, increased pigment deposition in the kidneys, and bone marrow fibrosis occurred with doses as low as 2 mg/kg/day. Effects were not observed at the 0.4 mg/kg/day level, which is considered to be the no-effect level dose.

Two other studies were also considered significant for determining the NOAEL for the longer-term HA. Furedi et al.[62] used B6C3F1 hybrid mice and a 24-month study design identical to the rat study described above, with the exception that TNT doses were 0, 1.5, 10, and 70 mg/kg/day. The 1.5 mg/kg/day dose was determined to be the no-effect level. The other study was reported by Levine et al.[58] who dosed beagle dogs, 6/sex/dose, orally by capsule with TNT for 6 months. The dosage groups were 0, 0.5, 2, 8, and 32 mg/kg/day of TNT, and the toxicological end points were similar to those evaluated by Furedi et al.,[61,62] described above. The major toxic effects were anemia, hepatomegaly, and splenomegaly. Because liver injury was observed at all doses, the authors did not report a NOEL. Toxic effects occurred primarily at the higher dose levels. There were only trace to mild toxic effects, i.e., hepatocytomegalia and hepatocytic cloudy swelling, at the 0.5 mg/kg/day level.

With respect to the mouse study by Furedi et al.[62] and the dog study by Levine et al.,[58] the 0.4 mg/kg/day TNT level from the rat study by Furedi et al.[61] is more appropriate as the NOAEL than the lower-exposure levels in the two other studies. The 0.4 mg/kg/day level is less than the lower-exposure levels in the mouse and dog studies, and presumably would cause lesser, or at most, equivalent toxic effects in these animals. Even though the dog is more sensitive to the toxic effects of TNT than the rat,[19] the 0.4 mg/kg/day would be expected to cause minimal, if any, effects in the dog.

The longer-term HA is calculated as follows:

$$\text{For the 10 kg child, HA} = \frac{(0.4 \text{ mg/kg/day})\ (10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.04 \text{ mg/L} = 40\ \mu\text{g/L}$$

$$\text{For the 70 kg adult, HA} = \frac{(0.4 \text{ mg/kg/day})\ (70 \text{ kg})}{(100)\ (2 \text{ L/day})} = 0.14 \text{ mg/L} = 140\ \mu\text{g/L}$$

Where: 0.4 mg/kg/day = NOAEL

10 kg = assumed weight of a protected child

70 kg = assumed weight of a protected adult

1 L/day = assumed volume of drinking water consumed daily by a 10 kg child

2 L/day = assumed volume of drinking water consumed daily by a 70 kg adult

100 = uncertainty factor appropriate for use with an animal NOAEL

The reports and studies reviewed in this text do not provide appropriate information for determining 1-day and 10-day HAs. Human studies described in several reviews (see paragraph 4a, Health Effects/Short-Term Exposure) may be considered for establishing shorter-term advisories; however, the reviews do not provide sufficient detail to determine whether the studies are appropriate for HA development.

ANALYSIS

Brueggemann[67] described high pressure liquid chromatography (HPLC) as the method of choice for separating and identifying mixtures of aromatic nitro explosives, including TNT, in water and demonstrated its efficacy. Prior to analysis the water sample was cleaned up and concentrated by passing it through a C_{18} solid phase extraction column. A 100 μL volume of the concentrated and cleaned sample was injected into a liquid chromatography (LC) system. The separation of explosives was achieved by use of a mobile phase of methanol and water on a reverse phase column. The lower detection limit was 100 ng. Quantitation was achieved by comparing the resulting peak areas with the peak area of an internal standard. TNT recovery from laboratory spiked wastewater was 75 percent. A precision and accuracy study of this technique recovered more than 90 percent TNT.

Other chromatography techniques used by investigators to characterize TNT-containing waters include gas chromatography (GC), gas chromatography/mass spectroscopy (GC/MS), capillary gas chromatography, plasma chromatography and thin-layer chromatography (TLC).[23,24,26-28,30-35,37,45,54,66,68-71] The water samples, ranging in volume from a few milliliters (20 mL) to several liters (6 L), were extracted several times with a solvent and dried with either anhydrous magnesium sulfate or sodium sulfate. The extract was concentrated by evaporation, sometimes with heat and sometimes through nitrogen gas aeration. Solvents used typically included benzene, methylene chloride, chloroform, and diethyl ether. Generally, the extract was evaporated to a reduced volume and then brought to a final volume by addition of the extracting solvent. In some studies the extract was completely dried and the residue redissolved in either the extracting solvent or a different solvent, e.g., acetone. The extract then was injected into a gas chromatograph or applied to thin layer chromatography plates. Some investigators applied such an extract to an HPLC. Zakhari et al.[5] described an extraction technique for subsequent LC analysis,

which involved adsorption of nitrocompounds on a styrene-divinylbenzene copolymer resin. Gas chromatograph columns, detectors and temperature programs varied considerably, and the references cited above can be consulted for details. Generally, nitrogen was the carrier gas, and flow rates differed from study to study. TLC plates were developed in either a benzene or a benzene-cyclohexane-ethyl acetate system. Spots were visualized either under short wave UV (254 nm) or by spraying with ethylenediamine/DMSO chromogenic reagent.

Zakhari et al.[5] and Rosenblatt et al.[8] reviewed additional analytical methods for measuring TNT in water. These included:

- Formation of a Meisenheimer complex upon addition of hydroxide solution to the water sample and measuring the treated sample colorimetrically (440 nm) or spectrophotometrically (505 μm). This method is usable up to 80 mg/L TNT, has an analytical working curve that is linear up to 20 mg/L, and has a sensitivity of 0.0001 μg/L.

- Addition of 2-diethylaminoethanol to the water sample and measuring the resulting color change with a UV spectrophotometer at 525 μm. The upper and lower detection limits are 0.5 and 50 mg/L, and sensitivity is 1 mg/L.

TREATMENT

Activated charcoal adsorption and discharge into either holding ponds, leaching ponds, or lagoons are the wastewater treatment methods at a number of TNT manufacturing or processing plants. Adsorption of TNT from effluent onto activated charcoal columns from LAP operations at two AAPs was described by Rosenblatt et al.[8] TNT breakthrough was controlled to less than 1 mg/L. Rosenblatt et al.,[8] Weitzel et al.,[36] and Spanggord et al.[25] described several AAPs that used either leaching ponds, lagoons, or holding ponds to treat TNT effluent. Rosenblatt et al.[8] indicated that the TNT discharge from a pond system at one AAP averaged 57 mg/L. Spanggord et al.[25] performed a lagoon model study at an AAP and concluded that photo- and biotransformation were the major processes causing TNT reduction.

Eskelund et al.[72] measured the Freundlich adsorption isotherm of granular activated carbon, using TNT solution as the test substance. The study also evaluated charcoal adsorption by passing TNT solution through columns packed with granular activated charcoal. The methods agreed with each other, with adsorption capacities ranging up to 0.817 g TNT/g carbon. The investigators concluded that carbon adsorption was an effective method for removing TNT and other nitrobodies from waste streams.

Carnahan et al.[73] evaluated the design of an AAP water treatment plant that consisted of anaerobic denitrification filters (anoxic filters), aerobic fixed film reactors (trickling filters), and aerobic suspended growth reactors (activated sludge). The wastewater from the activated sludge process was clarified and filtered through dual media filters with the effluent receiving aeration prior to being discharged. The reviewers concluded that the design would meet the NPDES permit criteria (for BOD_5, total suspended solids,

total dissolved solids, settleable solids, total nitrogen, ammonia, phosphorus, phenols, and heavy metals).

Ryon et al.[2] reviewed the waste treatment of two types of aqueous effluents from TNT manufacture and processing. The first is red water, which is formed during a purification process of TNT manufacture and contains sulfonates derived from TNT isomers, TNT and its Meisenheimer complexes,[9] sodium carbonate, sodium sulfate, sodium sulfite, and other more complex chemicals. Castorina[3] provides a detailed discussion of TNT purification with sodium sulfite. Treatment of red water is aimed at the recovery of sulfur and sodium which can be done by several processes. in the course of which TNT is totally decomposed. These include incineration, molten salt bath reduction, pyrolysis reduction, sulfite recovery, and a carbonate process. The second kind of effluent is pink water, which forms during LAP and demilitarization operations and contains TNT and its meta-isomers and, depending on the source, may contain RDX. Activated charcoal and diatomaceous earth filtration are described as treatments for pink water abatement by Pal and Ryon.[6]

Based on evidence that TNT would survive secondary biological treatment, several laboratory studies were conducted to evaluate tertiary treatment technologies for TNT removal. Granular activated charcoal (GAC) would readily remove TNT when it was the only munition present; however, when nitramines were present (e.g., RDX, HMX, TAX, SEX), there was competition for adsorption sites and a decrease in overall efficiency, and TNT progressively displaced the nitramines as it approached breakthrough.[74] The investigator concluded that the results did not encourage the use of GAC for nitramine control in wastewaters. Corona oxidation from electrochemical reactions of the Innova process effectively degraded TNT, but at high energy cost.[75] Burrows[76] concluded that combined UV radiation and ozone might be suitable for TNT destruction if applied to a small and relatively clean process stream. Studies of UV radiation and hydrogen peroxide by Noss and Chyrek[77] demonstrated that when TNT was the only munition present it was resistent to destruction, but it degraded when nitramines were present. Hydrogen peroxide alone did not react with TNT; however, in combination with UV radiation it increased the destruction rate over that for controls. The authors cautioned that further studies were required to evaluate UV-hydrogen peroxide treatment because TNT concentrations and other UV absorbing substances found in actual AAP effluents may affect this mode of treatment.

CONCLUSIONS

The recommended longer term health advisory for TNT levels in drinking water sources is 0.040 mg/L (40 μg/L). This summary of US Army TNT health effects research is the basis for recommending this level. At or below 0.040 mg/L TNT, adverse health effects would not be expected to occur in the most sensitive members of the US population. The surrogate for the most sensitive member is the 10 kg child, imbibing 1 liter of water per day. The reports and studies reviewed in this text do not provide sufficient detail to determine 1- and 10-day health advisories. Many of the human studies have been reviewed briefly in several of the references cited in this summary. In-depth

assessment of the original reports may provide the details required for the shorter-term advisories.

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