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HEALTH ADVISORY FOR 2,4- AND 2,6-DINITROTOLUENE (DNT)

Health and Ecological Criteria Division
Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
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PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environment Safety and Occupational Health (OASA(IL&E)), and the U.S. Environmental Protection Agency (EPA), Office of Water (OW), Office of Science and Technology for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants 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. A Health Advisory provides health effects guidelines and analytical methods, and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, Ten-day, Longer-term, and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water, nor are they legally enforceable standards. They are not issued as official regulations and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

This report is the product of the foregoing process. Available toxicological data, as provided by the Army and as found in open literature sources, on the munitions chemicals 2,4- and 2,6-dinitrotoluene (DNT) have been reviewed, and relevant findings are presented in this report in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted for critical internal and external review by the EPA.

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EXECUTIVE SUMMARY

Dinitrotoluene, commonly known as DNT, is a white- to buff-colored solid at room temperature and exists as a mixture of two or more of its six isomers; the 2,4- and 2,6-DNT isomers are the most predominant and have been used in military munitions, dye manufacture, and the synthesis of toluenediamine (the organic intermediate used in the production of polyurethane). The toxicity of 2,4-DNT, 2,6-DNT, and technical grade DNT (tg-DNT) is reviewed in this HA. Where appropriate, data for each pure chemical isomer (~98%) or tg-DNT is discussed separately in subsections. The technical grade DNT is a mixture composed of approximately 76.5% 2,4-DNT, 18.8% 2,6-DNT, and 5% other DNT isomers (2.4% 3,4-DNT, 1.5% 2,3-DNT, 0.7% 2,5-DNT, and 0.4% 3,5-DNT).

The toxicokinetics of primarily the 2,4-DNT isomer has been studied extensively in laboratory animals. Data indicate that approximately 70 to 90% of orally administered [^{14}C]2,4- or [^{14}C]2,6-DNT is eliminated in the urine of CD (Sprague-Dawley) and Fischer rats, New Zealand white rabbits, beagle dogs, rhesus monkeys, and A/J mice. Fecal excretion generally accounts for less than 10%. In contrast, urinary excretion of orally administered 2,4-DNT by two common strains of mice (CD-1 and B6C3F$_1$) accounted for only 8 to 12% of the dose and fecal excretion accounted for 81 to 84%. Results from one study indicated that gastrointestinal absorption of 2,6-DNT in the rat is somewhat slower than that of 2,4-DNT. Percent absorption of either isomer is difficult to estimate since biliary excretion and enterohepatic cycling are significant biological processes in most animals. Metabolites that are eliminated in the bile and into the gut are hydrolyzed and reduced by intestinal microflora. Many of these compounds, in turn, are reabsorbed from the gut into the systemic circulation and then oxidized in the liver. Biliary excretion of these metabolites into the gut results in additional reduction by intestinal bacteria. Male rats usually excrete less of the administered dose of 2,4-DNT in the urine and more in the feces than females, a difference most likely due to higher biliary excretion in males. Similar sex-related differences in the absorption or elimination of 2,6-DNT have not been observed in animals or humans. Elimination of either compound via the lungs is minimal ($\leq$0.35%).

Workers exposed to tg-DNT absorb the munitions chemicals via the inhalation and dermal routes, but the extent of absorption has not been determined conclusively.

The data on distribution of 2,4- and 2,6-DNT suggest that accumulation of these compounds in the body following a single exposure is, at most, minimal. Repeated oral exposure in CD rats indicates preferential uptake of 2,4-DNT and/or its metabolites by the liver, kidneys, brain, lungs, and skeletal muscle, although total retention in the body is low. Limited data suggest that placental transfer of 2,4-DNT occurs in rats.

Significant portions of 2,4-DNT administered orally or intraperitoneally to most animals (A/J mice, CD and Fischer 344 rats, New Zealand rabbits, beagle dogs, and rhesus monkeys), and in humans exposed occupationally by inhalation and dermal routes, are converted to 2,4-dinitrobenzyl alcohol (2,4-DNBalc) and/or its glucuronide (i.e., 2,4-DNBalcG). Other primary metabolites excreted by these animals include 2-amino-4-nitrobenzyl alcohol (2A4NBalc) and 4-amino-2-nitrobenzyl alcohol (4A2NBalc); humans excrete only the former in the urine in large quantities. For Fischer rats and humans, another major product of 2,4-DNT metabolism is 2,4-dinitrobenzoic acid (2,4-DNBacid). A significant portion (4 to 9%) of a single oral dose of 2,4-DNT is reduced <u>in vivo</u> to 2,4-diaminotoluene (2,4-DAT) by rats, rabbits, dogs, and monkeys, but none is found in the urine of occupationally exposed humans. In contrast, metabolism of 2,4-DNT by CD-1 mice and Wistar rats is not extensive, and only small amounts of an oral dose of 2,4-DNT given to these animals are oxidized to one of the benzyl alcohol metabolites; reduction of 2,4-DNT to 2,4-DAT is negligible in mice. Two mutagenic metabolites of 2,4-DNT (i.e., 2-nitroso-4-nitrotoluene and 2-amino-4-nitrotoluene) have been recovered <u>in vitro</u> from cultures containing cecal or ileal material from male Swiss-Webster mice, male and female Fischer 344 rats, and male humans. It has been suggested that these intermediates may bind covalently to hepatic macromolecules.

Sex-related differences in the metabolism of 2,4-DNT have been reported only in Fischer rats and humans. Exposure in rats was by the oral route,

whereas human exposure was via the inhalation and dermal routes. For both species, females produce up to three times more 2,4-DNBalc and/or 2,4-DNBalcG than males, and in humans only, men excrete nearly twice as much 2,4-DNBacid as females.

The metabolism of 2,6-DNT has not been studied extensively. As with 2,4-DNT, Fischer rats convert the 2,6-DNT isomer to the corresponding dinitrobenzyl alcohol glucuronide and dinitrobenzoic acid; however, only one metabolite (2-amino-6-nitrobenzoic acid, 2A6NBacid) results from the in vivo reduction of 2,6-DNT. Similarly, only three major metabolites of 2,6-DNT (2,6-DNBalc, 2,6-DNBalcG, and 2,6-DNBacid) have been recovered from the urine of men and women; women appear to excrete more 2,6-DNBalcG than men. No other sex-related differences in the metabolism of 2,6-DNT have been reported.

Occupational studies of DNT characterized concurrent exposures to 2,4-DNT and tg-DNT; 2,6-DNT has not been studied epidemiologically. In humans, the toxic effects of DNT are on the heart, the circulatory system, and the central nervous system. Chronic DNT exposure, primarily via the inhalation route (although percutaneous absorption and ingestion are considered to be secondary and tertiary routes of exposure), has been characterized in munitions workers by nausea, vertigo, methemoglobinemia, cyanosis, pain or paresthesia in extremities, tremors, paralysis, chest pain, and unconsciousness. A retrospective epidemiology study suggested that following a latency period of 15 years, workers exposed to 2,4-DNT and tg-DNT exhibited excess mortality from ischemic heart disease and residual diseases of the circulatory system. However, most of the workers had taken other jobs during the 15-year latency period and were not continually exposed to either 2,4-DNT or tg-DNT. A more recent study failed to demonstrate excess mortality from cardiovascular disease in DNT-exposed workers and suggested a possible increase in mortality from liver and biliary tract cancer. Limited evidence suggests that DNT does not result in adverse effects on human reproductive performance.

Acute oral toxicity studies indicate that rats are more susceptible to 2,4-DNT than mice. The LD_{50} values ranged from 1,340 to 1,954 mg/kg in mice and from 270 to 650 mg/kg in rats. Both species exhibited ataxia and

cyanosis. When rats were administered doses of 2,4-DNT in the diet ranging from 45 to 143 mg/kg/day for 14 days, body weight gains and food consumption were decreased and testicular lesions were found in a dose-related manner. When mice (dose range, 47 to 468 mg/kg/day), rats (dose range, 34 to 266 mg/kg/day), and dogs (dose range, 1 to 25 mg/kg/day) were administered 2,4-DNT in the diet for 4 weeks, dogs were found to be most sensitive, exhibiting incoordination and paralysis at doses of 25 mg/kg/day; central nervous system (CNS) effects were not observed in rodents. Similar CNS effects were seen when dogs were orally administered 20 mg 2,6-DNT/kg/day. Methemoglobinemia, reticulocytosis, and Heinz body formation resulted from oral administration of 37.5 mg tg-DNT/kg/day to F344 rats for 4 weeks.

Subchronic toxicity was assessed in mice and rats administered 2,4-DNT via the diet and in dogs administered 2,4-DNT via capsules for 13 weeks (same doses tested as reported previously for 4-week studies). In mice of both sexes, anemia and reticulocytosis were observed at dose levels of 413 (males) and 468 (females) mg/kg/day. In rats, mortality occurred prior to study termination in all females fed 145 mg/kg/day and 75% of males fed 266 mg/kg/day. Anemia and reticulocytosis were observed at dose levels as low as 93 (males) and 108 (females) mg/kg/day; splenic hemosiderosis and depressed spermatogenesis were found in these animals as well as those dosed at 266 (males) and 145 (females) mg/kg/day. In dogs, neuromuscular incoordination and paralysis, methemoglobinemia, aspermatogenesis, hemosiderosis of the spleen and liver, cloudy swelling of the kidneys, and lesions of the brain were observed in males and females at a dose level of 25 mg 2,4-DNT/kg/day. Similar effects were seen when mice (dose range, 11 to 299 mg/kg/day), rats (dose range, 7 to 155 mg/kg/day), and dogs (dose range, 4 to 100 mg/kg/day) were orally administered 2,6-DNT for 13 weeks. All species exhibited methemoglobinemia, anemia, bile duct hyperplasia sometimes accompanied by hepatic degeneration, and depressed spermatogenesis. Incoordination, rigid paralysis, and renal degeneration occurred in dogs at a dose level of 20 mg 2,6-DNT/kg/day.

In a lifetime feeding study in male and female CD (Sprague-Dawley) rats fed 2,4-DNT in the diet at a level giving a daily intake of 34 and 45 mg/kg/day, respectively, lifespan was shortened, and the incidence of hepatocellular carcinomas was increased (6/30 and 19/35 high-dose males and females, respectively, as compared with 1/25 and 0/23 concurrent controls). Seminiferous tubules atrophied resulting in almost complete cessation of spermatogenesis, and excessive hemosiderin accumulated in the spleen. An increased incidence of benign mammary gland tumors (33/35 as compared with 10/23 in controls) was exhibited in females. Anemia (partially compensated) and testicular atrophy were evident at 3.9 mg/kg/day, and hepatocellular alterations were observed at 0.57 mg/kg/day, the lowest dose tested. In a study with CD-1 mice, all the animals fed 898 mg/kg/day died by month 18 (males) or month 21 (females). Effects at 14 mg/kg/day, the lowest dose tested, included testicular atrophy, decreased body weight in males, and hemosiderosis of many organs, primarily the liver and spleen. The incidence of malignant renal tumors was elevated in males fed 95 mg/kg/day (15/17 as compared with 0/20 concurrent controls). In a 2-year oral toxicity study in dogs, 2,4-DNT administered daily in capsules caused neurotoxicity (incoordination and paralysis, often leading to death) in all dogs at 10 mg/kg/day, and anemia (partially compensated) and biliary tract hyperplasia at 1.5 mg/kg/day. No adverse effects were observed at 0.2 mg/kg/day.

Male and female CDF (F344) rats fed tg-DNT in the diet at a daily intake of 35 mg/kg/day were sacrificed in extremis after 55 weeks. At 14 mg/kg/day, 96% of the males and 59% of the females developed hepatocellular carcinomas. After 78 weeks, decreased body weight, increased liver weight, hepatotoxicity, increased incidence of hepatocellular carcinomas in males (19/20, as compared to 0/20 in concurrent controls), renal toxicity (chronic interstitial nephritis), and parathyroid hyperplasia were reported at 3.5 mg/kg/day, the lowest dose tested.

In a 1-year study, 47% of male CDF (F344) rats given tg-DNT at dose levels of 35 mg/kg/day (the only dose tested) developed hepatocellular carcinomas (0/20 control, 9/19 treated). Under similar conditions, 2,6-DNT induced hepatocellular tumors in 100% of the high-dose (14 mg/kg/day) and 85%

of the low-dose (7 mg/kg/day) males; no lesions were found in the control group. Rats exposed to 2,4-DNT (35 mg/kg/day) did not develop liver tumors. Although limited in duration and in the number of animals tested, the data may indicate that the 2,6-DNT isomer caused much of the carcinogenic activity in the previous tg-DNT bioassays.

The 2,4- and 2,6-DNT isomers are weak mutagens in Salmonella test systems. However, metabolites of 2,4-DNT (in particular the 2,4-nitrobenzyl alcohol and the 2-amino- and 2-nitroso-4-nitrotoluenes) are mutagenic without metabolic activation. The dinitrotoluenes are nongenotoxic in mammalian cells in vitro, in the dominant lethal test in mice and rats, and in Drosophila systems. Technical grade DNT gave negative responses for unscheduled DNA synthesis (UDS) except when an in vivo/in vitro testing system was used. It has been concluded that biliary excretion, metabolism by gut microflora, and resorption from the intestine are prerequisites for genotoxic activity. Metabolites of 2,4-DNT can bind to liver DNA; 2,4-DNT appears to act as a promoter of hepatocarcinogenesis, inducing gamma glutamyl transferase positive foci in the livers of rats initiated with dimethylnitrosamine. On the other hand, 2,6-DNT had both initiating and promoting activity in the GGT^+ focus assay.

The 2,4-dinitrotoluene isomer has been shown to cause severe reproductive effects in both sexes of rats, mice, and dogs. Oral exposure to 2,4-DNT results in testicular atrophy and degeneration, reductions in spermatogenesis, cessation of follicular function, and reduction in the number of corpora lutea. Consequently, fertility is reduced in both sexes. 2,4-DNT has also been shown to cause reduced viability as well as decreases in the body weight of offspring at birth and weaning. No data on the reproductive effects of 2,6-DNT or tg-DNT were found in the available literature.

Limited data suggest that 2,4-DNT is not teratogenic in mice following ingestion. Technical grade-DNT was not teratogenic to rats administered oral doses, although embryotoxicity was observed at maternally toxic levels. In addition, the administration of tg-DNT to pregnant rats also resulted in changes in relative organ weights and hematologic parameters in their fetuses.

No data on the developmental effects of 2,6-DNT were found in the available literature.

Based on decreased body weight gain, decreased food consumption, changes in serum chemistry levels in male and female Sprague-Dawley rats, and testicular lesions in male rats administered 2,4-DNT in the diet for 14 days, the Ten-Day Health Advisory (HA) for 2,4-DNT for a 10-kg child is 0.5 mg/L (500 μg/L). In the absence of available animal data to determine a One-day HA, the Ten-day HA is considered acceptable (0.5 mg/L or 500 μg/L). Based on dose-related decreases in body weight gain and food consumption in rats administered 2,4-DNT in the diet for 13 weeks, the Longer-term HA for exposure in a 10-kg child is 0.3 mg/L (300 μg/L); the Longer-term HA for exposure in an adult is 1.0 mg/L (1,000 μg/L). The Drinking Water Equivalent Level (DWEL) for 2,4-DNT is 0.1 mg/L (100 μg/L), derived from a Reference Dose (RfD) of 0.002 mg/kg/day, which was based on neurotoxicity, Heinz bodies, and biliary tract hyperplasia in dogs dosed orally with 2,4-DNT for 2 years.

All HAs for 2,6-DNT are based on a 13-week study with dogs administered 2,6-DNT orally. The critical effects were neurotoxicity, Heinz bodies, bile duct hyperplasia, liver and kidney histopathology, and death. The Longer-term HA for exposure in a 10-kg child is 0.4 mg/L. In the absence of available animal data to determine One- and Ten-day HAs, the Longer-term HA for a 10-kg child is considered acceptable (0.4 mg/L or 400 μg/L). The Longer-term HA for exposure in an adult is 1.0 mg/L (1,000 μg/L). The Drinking Water Equivalent Level (DWEL) for 2,6-DNT is 0.04 mg/L (40 μg/L), derived from a Reference Dose (RfD) of 0.001 mg/kg/day.

DNT is classified B2: Probable Human Carcinogen; thus a Lifetime HA is not recommended. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,4-DNT at the level of the DWEL (100 μg/L) is 2×10^{-3}. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,6-DNT at the level of the DWEL (40 μg/L) is 1×10^{-3}. The cancer risk assessment is for the 2,4-/2,6-DNT mixture. The data for 2,6-DNT are limited, and 2,6-DNT is usually found in the presence of 2,4-DNT with 2,4-DNT being the more

predominant component of the mixture by volume. Therefore, the cancer risk assessment for the 2,4-/2,6-DNT mixture is used for the cancer risk assessment for both 2,4- and 2,6-DNT. Derived by the linearized multistage extrapolation method, the oral slope factor (q_1*) for the mixture is 6.8×10^{-1} $(mg/kg/day)^{-1}$ and the drinking water unit risk is 2×10^{-5} $\mu g/L$. At drinking water concentrations of 5 $\mu g/L$, 0.5 $\mu g/L$, and 0.05 μg DNT/L, excess cancer risks are estimated to be 10^{-4}, 10^{-5} and 10^{-6}, respectively. For comparison, drinking water concentrations associated with a 10^{-6} cancer risk are 8×10^{-8} $\mu g/L$, 5×10^{-2} $\mu g/L$, 2×10^{1} $\mu g/L$, 2×10^{-1} $\mu g/L$, and 2×10^{-5} $\mu g/L$ for the one-hit, multihit, probit, logit, and Weibull models, respectively.

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Water (OW), provides information on the health effects, analytical methodology, and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State, and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. Health Advisories are subject to change as new information becomes available.

Health Advisories are developed for One-day, Ten-day, Longer-term (approximately 7 years, or 10% of an individual's lifetime), and Lifetime exposures based on data describing noncarcinogenic endpoints of toxicity. For those substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime Health Advisories are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the upper 95% confidence limits of the linear multistage model. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated values. Excess cancer risk estimates may also be calculated using the one-hit, Weibull, logit, and probit models. There is no current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

II. GENERAL INFORMATION

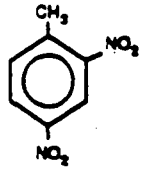
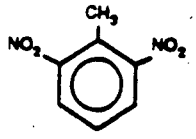
Dinitrotoluene (DNT) appears as a white- to buff-colored solid at room temperature and exists as a mixture of two or more of its six isomers; the 2,4- and 2,6-DNT isomers are the most significant by volume (Beard and Noe, 1981; Small and Rosenblatt, 1974). The isomers 2,4-DNT (CAS No. 121-14-2) and 2,6-DNT (CAS No. 606-20-2) are combustible, nitroaromatic compounds that compose technical grade DNT (tg-DNT) (ATSDR, 1989; NIOSH, 1985). When heated, DNT forms an oily liquid that turns yellow when exposed to sunlight. The general physical and chemical properties of 2,4- and 2,6-DNT are presented in Table II-1.

Dinitrotoluene is used in the manufacture of dyes, in munitions (as a smokeless propellant powder), and as a gelatinizing and plasticizing agent in both commercial and military explosive compositions, However, its major use (99%) is in the synthesis of toluenediamine, the organic intermediate used in the production of polyurethane (ATSDR, 1989; Beard and Noe, 1981; NIOSH/OSHA, 1985; Santodonato et al., 1985; Small and Rosenblatt, 1974).

Both 2,4- and 2,6-DNT are produced through the dinitration of toluene with nitric acid in the presence of concentrated sulfuric acid. This reaction produces a mixture that consists of approximately 75% 2,4-DNT isomer, 20% 2,6-DNT isomer, and small quantities of other DNT isomers. If the single 2,4-DNT isomer is required, the nitration is stopped at the mono- stage, and pure p-nitrotoluene is obtained by crystallization. Subsequent nitration of the p-nitrotoluene yields only 2,4-DNT. Small concentrations of DNT isomers also occur as byproducts in the production of trinitrotoluene (TNT) (ATSDR, 1989; Santodonato et al., 1985).

The military requirement for DNT specifies a minimum melting point of 65.5°C, which corresponds to a 2,4-DNT purity of 92%. The current approach is to purchase commercial DNT and to purify it by a process known as sweating. The commercial material is about 75% 2,4-DNT isomer, 20% 2,6-DNT isomer, and 5% other isomers. The mixture is melted and subjected to a controlled cooling-heating process. In the cooling step, DNT containing mostly the

Table II-1. Physical and Chemical Properties of 2,4- and 2,6-DNT

Property	2,4-DNT	2,6-DNT	Reference
CAS No.	121-14-2	606-20-2	
Synonyms	2,4-Dinitrotoluol; 1-methyl-2,4-dinitrobenzene	2,6-Dinitrotoluol; 1-methyl-2,6-dinitrobenzene	NIOSH/OSHA (1985)
Chemical formula	$CH_3C_6H_3(NO_2)_2$	$CH_3C_6H_3(NO_2)_2$	NIOSH/OSHA (1985)
Chemical structure			ATSDR (1989)
Molecular weight	182.14	182.14	NIOSH/OSHA (1985)
Melting point	70.5°C	66°C	NIOSH/OSHA (1985)
Boiling point	Decomposes (300°C)	Decomposes (260°C)	NIOSH/OSHA (1985)
Density	1.521 @15°C	1.28 @111°C	Sax (1975); Small and Rosenblatt (1974)
Vapor pressure, mmHg (20°C)	0.0051	0.018	ATSDR (1989)
Solubility			
Water, mg/L	270 (22°C)	180 (20°C)	ATSDR (1989); Verschueren (1977)
Organic solvents	Soluble in alcohol, ether, acetone, benzene	Soluble in alcohol	ATSDR (1989); Hawley (1981)
Partition coefficients			
Log octanol/water (K_{ow})	2.0	2.3	ATSDR (1989)
Log organic carbon-soil (K_{oc})	1.65-364.0	1.96-77.6	ATSDR (1989) Spanggord et al. (1985) Burrows (1989) Mabey et al. (1982)
Conversion factors			
ppm (v/v) to mg/m^3 in air (20°C)	1 ppm ≈ 7.5 mg/m^3	1 ppm ≈ 7.5 mg/m^3	ATSDR (1989)
mg/m^3 to ppm (v/v)	1 mg/m^3 ≈ 0.13 ppm	1 mg/m^3 ≈ 0.13 ppm	ATSDR (1989)

2,4-DNT isomer crystallizes, while the liquid impurity-rich fraction is drained off (or sweated) (Small and Rosenblatt, 1974). One procurement specification requires that the DNT used in the production of military munitions be composed of at least 98.5% of the 2,4-isomer (Department of the Army, 1990). The composition of the supplied product is approximately 99.5% 2,4-DNT and 0.5% 2,6-DNT.

As of 1986, a small number of domestic chemical companies were manufacturing DNT; these are listed in Table II-2. Du Pont and Air Products and Chemicals are the major U.S. producers of DNT. In 1982, U.S. production of tg-DNT (2,4- and 2,6-DNT isomer mixture) was approximately 720 million pounds. No data describing import or export activities for 2,4- or 2,6-DNT were found in the available literature (ATSDR, 1989; Santodonato et al., 1985).

Table II-2. U.S. Manufacturers of 2,4- and 2,6-DNT

Isomer	Manufacturer	Location
2,4-DNT	Air Products and Chemicals, Inc.	Pasadena, TX
	E.I. Du Pont de Nemours	Deepwater, NJ
	ICI Americas (Rubicon)	Geismar, LA
	Mobay Chemicals	Cedar Bayou, TX New Martinsville, WV
	Allied Chemical Corporation	Moundsville, WV
	Uniroyal, Inc.	Joliet, IL
2,6-DNT	Allied Chemical Corporation	Moundsville, WV
	Air Products and Chemicals, Inc.	Pasadena, TX
	E.I. Du Pont de Nemours	Deepwater, NJ

Source: Santodonato et al. (1985); U.S. EPA (1986).

III. SOURCES OF EXPOSURE

According to McGee et al. (1942), the body may be exposed to DNT via four routes: (1) inhalation of DNT vapors; (2) inhalation of DNT dust particles; (3) ingestion of DNT-contaminated food; and (4) absorption through the skin. Exposure to DNT may occur in the environment, or more likely, in occupational settings.

A. ENVIRONMENTAL EXPOSURE

Information concerning environmental levels of DNT is limited. Significant amounts of DNT-containing wastewaters arise from the preparation and production of smokeless powder and trinitrotoluene (TNT) at U.S. Army ammunition plants (AAPs) (Small and Rosenblatt, 1974). The major consistent discharges of DNT occur during dry solvent and water recovery processes. Total estimated rates of DNT discharged into local waters by AAPs in Virginia and Tennessee were 40 and 550 pounds per day, respectively.

STORET (Storage and Retrieval computerized database maintained by EPA) data show that five raw water observations of 2,4-DNT, each at 5 μg/L, and seven raw water observations of 2,6-DNT, from 5 to 10 μg/L with a mean of 6.4 μg/L, were made. The Chemical Manufacturers Association (CMA, 1990), on behalf of most U.S. manufacturers of DNT (Table II-2), reported median concentrations of 2,4- and 2,6-DNT in surface and groundwater in the vicinity of plant production sites. The 2,4-DNT isomer ranged from 360 μg/L to 20,000 μg/L (median 3,500 μg/L) in groundwater and up to 32 μg/L (median 9.1 μg/L) in surface water. The 2,6-DNT isomer ranged from 7 μg/L to 20,000 μg/L (median 3,700 μg/L) in groundwater and up to 49 μg/L (median 14.9 μg/L) in surface water.

During a study of 17 military installations, DNT was found to leach to groundwater at six sites where open burning directly on the ground was used as a method for munitions disposal (Murnyak, 1991). The concentrations detected in monitoring wells near five of the sites were 1 to 265 μg/L for 2,4-DNT and

2 μg/L for 2,6-DNT. Concentrations were higher at the sixth site, ranging from 32 to 1,788 μg/L for 2,4-DNT and from 11 to 651 μg/L for 2,6-DNT.

In a review of analytical methods for the determination of groundwater contamination, Garman et al. (1987) gave Practical Quantification Limits (PQLs) for a number of priority pollutants. These limits are estimates of the lowest concentrations that may be expected to be routinely measured. The PQLs for 2,4- and 2,6-DNT ranged from 0.1 to 10 μg/L depending upon the analytical method used.

Hashimoto et al. (1979) reported the occurrence of 2,4- and 2,6-DNT in the seawater of Dokai Bay, Japan. Concentrations of the isomers ranged from 0.890 to 206 μg/L for the 2,4-DNT isomer and from 0.072 to 14.8 μg/L for the 2,6-DNT isomer. This area was found to be heavily polluted by industrial waste. Hashimoto et al. (1982) estimated that the main source of waste discharged an average of 76 kg of DNT (mixed isomers) per day with a maximum of 150 kg discharged per day.

The 2,4-DNT isomer was detected in the soil of 2.2% of 800 to 900 hazardous waste sites at a geometric mean concentration of 1 mg/kg. 2,6-DNT was detected at 1.3% of the sites at a mean concentration of 0.140 mg/kg (140 μg/kg) (ATSDR, 1989).

Dinitrotoluene volatilizes from water at a relatively insignificant level. Environmental exposure to DNT in the air may occur if contaminated surface soils are eroded and re-entrained. DNT may also be released or transported in the air as dusts, aerosols, or other suspended solids. Matsushita and Iida (1986) collected and analyzed airborne samples (from unspecified sources) for the presence of nitroaromatics. Sampling was performed using a quartz filter, and analyses involved gas chromatography and flame thermoionic detection (GC/FTD). Detected concentrations of 2,4- and 2,6-DNT were 0.024 and 0.006 ng/m^3, respectively.

Toxic Release Inventory (a computerized database maintained by U.S. EPA) reports for the year 1989 show that 169,291 lbs of 2,4-DNT were released: 52%

to air, 41% to underground injection, 7% (12,657 lbs) through surface water, and 0.2% to landfills. In the same year, 102,977 lbs of 2,6-DNT were released: 81% to air, 18% to underground injection, and 1% (1,083 lbs) through surface water discharge.

B. OCCUPATIONAL EXPOSURE

It is estimated that approximately 500 workers are potentially exposed to 2,4- and 2,6-DNT in the production of munitions and explosives. Highest exposures occur in DNT unloading procedures, as a result of fugitive emissions from leaky pumps and loose pipe connections, during certain maintenance operations, and during cleanup operations (Santodonato et al., 1985).

Hamill et al. (1982) conducted occupational sampling at two plants of the Olin Corporation, a toluene diamine/DNT production facility. Air concentrations (not reported) at the plants (Lake Charles, LA, and Bradenburg, KY) were reported to be well within acceptable OSHA, ACGIH, or Olin internal standard levels.

Levine et al. (1985a) evaluated worker exposure at a tg-DNT manufacturing plant. Sampling included breathing zone air, skin, environmental surfaces, and urine to determine absorption. Concentrations obtained from personal air sampling ranged from 0.1 to 4.4 mg/m^3 for pure 2,4-DNT and from 0.6 to 5.9 mg/m^3 for tg-DNT. The lowest levels were found in stripper operators and maintenance mechanics, and the highest levels were found in "operators" (type of operator not specified) and loaders. Skin testing revealed contamination ranging from <2 to 179.5 g. The lowest concentration was found on the hands of an operator performing DNT analysis, and the highest concentration was found on the hands and face of an operator following sample collection and analysis. Environmental surface samples ranged from <2 (door handles, desktop, knobs, and switches) to 433.2 g (handrails and valve handles). Results of the wipe survey and urinalyses showed that frequent skin contamination with small quantities could add up to considerable exposure.

Two biological monitoring studies among workers exposed to tg-DNT in an explosives factory were carried out by Woollen et al. (1985). Routine personal sampling revealed atmospheric levels ranging from undetectable to 0.1 mg/m^3. Static samples positioned near potentially dusty areas revealed atmospheric concentrations ranging from 0.02 to 2.68 mg/m^3 (mean, 0.40 mg/m^3). Urinalyses were also performed. Concentrations of DNT metabolites as well as unmetabolized DNT in the urine were much higher than routine environmental sampling concentrations.

Difficulties in detecting metabolites and frequent occurrence of samples with values near the detection limits hampered interpretation of the actual exposure and disposition of DNT in both the Levine et al. (1985a) and Woollen et al. (1985) studies. The results indicated that dermal contact and inadvertent ingestion were contributing routes of exposure for workers.

IV. ENVIRONMENTAL FATE

Spanggord et al. (1980a) conducted a literature review to identify and estimate the magnitude of the transport and transformation processes believed to be important in the environmental fate of 11 munitions wastewater constituents (including 2,4- and 2,6-DNT) in the aquatic environment. Possible fate routes of DNT are biodegradation, photolysis, volatilization, sorption on soils and sediments, and hydrolysis.

A. BIODEGRADATION

Dinitrotoluene degradation occurs under aerobic and anaerobic conditions. Biotransformation occurs mainly through the reduction of the nitro group (Spanggord et al., 1981). Local (Searsville Lake in Woodside, CA, and Coyote Creek in San Jose, CA) eutrophic waters did not contain microorganisms that degrade these compounds, but they did contain microorganisms that transformed DNT when other metabolizable organic compounds were present for their growth (co-metabolism). However, Spanggord et al. (1980b) found that natural surface water microorganisms could use 2,4-DNT as a sole carbon source. After a 2- to 3-day lag period, >90% of the added 2,4-DNT (10 mg/L) was transformed in aerated water in 6 days of incubation. Bausum et al. (1990) found that both 2,4- and 2,6-DNT served as sole carbon sources for microorganisms in water samples taken downstream from wastewater outflows from the Radford (VA) Army Ammunition Plant. Up to 60% of substrate carbon appeared as CO_2. Compared to the 2,6-isomer, the 2,4-isomer was degraded at a higher rate, had a shorter lag time, and had a larger population of degrading organisms. In contrast, degradation was not observed in samples taken from Maryland surface freshwater sources. Using respiratory techniques, Chambers et al. (1963, as cited in Spanggord et al., 1980a) reported evidence of 2,4-DNT (100 mg/L) degradation by phenol-adapted bacteria.

Spanggord et al. (1985) estimated the environmental persistence of 2,4-DNT in Waconda Bay, TN. They determined that photolysis (k_p = 0.017 days^{-1}), biotransformation (k_b = 0.113 days^{-1}), and volatilization (k_v = 0.004 hours^{-1}) control the loss and movement of 2,4-DNT in Waconda Bay.

The estimated half-life was 5.2 days. Based on the data, persistence is water-body dependent and the length of time any particular nitroaromatic compound will reside in the environment will ultimately depend on the compound's unique interaction with the natural organics and biota in its surroundings. Since microorganisms readily metabolize DNT to CO_2 as the final product, it is not expected to persist in the environment.

Tabak et al. (1981) determined the acclimation periods and extent of microbial degradation of 2,4- and 2,6-DNT via the static-screening-flask test. A significant bio-oxidative activity with gradual adaptation and induced enzyme formation was noted initially. This was followed, however, by a deadaptive process from the loss of the metabolically efficient microbial population and the gradual buildup of toxicity of either the parent substrate or the metabolic products to the microbiota.

Davis et al. (1981) found that 2,4-DNT (50 mg/L) degraded rapidly in 2 days in a batch culture containing glucose and inoculated with an industrial seed containing four bacterial genera (Acinobacter, Alcaligenes, Flavobacterium, and Pseudomonas) and one yeast (Rhodotorula). Municipal seed (activated sludge organisms), however, was inhibited by concentrations as low as 10 mg 2,4-DNT/L. Under the same conditions, 2,6-DNT inhibited municipal seed at $\geq$50 mg/L and appeared to react differently in wastewater treatment operations.

Parrish (1977) screened 190 fungi, in shake cultures of basal medium with glucose, for the ability to transform 2,4-DNT. Only five organisms were found to effectively transform at an initial concentration of 100 mg 2,4-DNT/L. McCormick et al. (1978, as cited in Spanggord et al., 1980a) identified the products of transformation by a fungus Microsporum species grown in DNT, glucose, and basal medium as 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,2'-dinitro-4,4'-azoxytoluene, 4,4'-dinitro-2,2'-azoxytoluene, and 4-acetamido-2-nitrotoluene.

A study to identify and quantify the routes taken by 2,6-DNT in a model waste stabilization pond (12-hour detention time) was conducted by Davis

et al. (1983). The percentages of 2,6-DNT lost by degradation, volatilization, sedimentation, water column residuals, and effluent were 92.2, 0.3, 3.6, 1.2, and 2.7, respectively, while the half-life was 8.3 days and the bioconcentration factor was 5225.

Other sources report transformation occurring only under anaerobic conditions. Liu et al. (1984) conducted a study to identify the intermediates of the microbial transformation of 2,4-DNT (5-25 mg/L) by a mixed bacterial culture derived from activated sludge. The biotransformation took place only under anaerobic conditions with an exogenous carbon source and appeared to be energy dependent. Intermediates identified were the aminonitrotoluenes, and 2-nitroso-4-nitrotoluene and 4-nitroso-2-nitrotoluene. The two nitroso compounds were very unstable and could be found only at early stages of biotransformation.

Hallas and Alexander (1983) studied the microbial transformation of 2,6-DNT in sewage effluent. Under anaerobiosis, 2,6-DNT disappeared slowly, but no loss was evident in aerated sewage. The transformation product detected via gas chromatography-mass spectrometry was aminotoluene.

B. PHOTOLYSIS

Because nitroaromatic compounds absorb sunlight strongly in the ultraviolet and blue spectral region, they are generally susceptible to photochemical transformation in aquatic systems. Photolysis of DNT isomers was rapid in natural waters with the 2,6-isomer photolyzing more rapidly than the 2,4-isomer. Zepp et al. (1984) calculated a half-life of 17 minutes for 2,6-DNT in river water exposed to sunlight; the photoreaction was determined to be indirect. However, data suggest that direct photolysis is a major environmental fate of 2,4 DNT. Spanggord et al. (1978, as cited in Spanggord et al., 1980a) reported that 50% of the 2,4-DNT added (amount not specified) was lost after exposure to sunlight for about 5 days. A control solution maintained in the shade showed that the same amount of material was lost in

about 11 days. Spanggord et al. (1980b) studied sunlight photolysis of 2,4-DNT in distilled and natural waters. Half-lives were 43, 2.7, 9.6, and 3.7 hours in distilled, river, bay, and pond water, respectively.

Burlinson and Glover (1977, as cited in Spanggord et al., 1980a) reported that 2,4-DNT photolyzes more rapidly at a higher pH, while Wettermark et al. (1965, as cited in Spanggord et al., 1980a) reported that the first-order rate constant for decay was steady from pH 8.4 to pH 13 (1.0 sec^{-1}); below pH 5, the decay rate increased with lowered pH. Permanent visible absorptions appeared in the range 380 to 600 nm with the extent of coloration depending on pH, buffer concentrations, and the presence of oxygen.

Simmons and Zepp (1986) studied the influence of various humic substances on the photoreactions of 19 nitroaromatic substances including 2,4- and 2,6-DNT. The results observed indicate that dissolved humic substances in natural waters enhance the sunlight-induced photodegradation rates compared with rates observed in distilled water and were, by far, most pronounced in six compounds including 2,4- and 2,6-DNT. Additional experiments with 2,6-DNT to determine the effects of varying concentrations of humic substances on the sunlight photolysis rate constant showed a linear relationship between the photolysis rate constant and the total organic carbon concentration.

C. VOLATILIZATION

An estimated value of Henry's Law constant (H_c, 3.4 torr M^{-1}) suggests that the volatilization rate of 2,4-DNT is limited by gas-phase mass transport resistance. The estimated environmental half-life (using laboratory half-life information) is 410 days. Consequently, volatilization would not be an important environmental fate owing to the rapid biodegradation and photolysis of the 2,4- and 2,6- isomers (Spanggord et al., 1980a).

D. SORPTION ON SOILS AND SEDIMENTS

Spanggord et al. (1985) measured the organic carbon soil (K_{oc}), octanol-water (log K_{ow}), and microorganism (K_s) partition coefficients for 2,4-DNT and

found them to be 364, 2.0, and 64, respectively. Since the partitioning of organics to the sediment from the aqueous phase does not become a major loss until the K_{oc} values exceed 10^4, the relatively low K_{oc} values for 2,4-DNT indicate that this compound would have only a slight tendency to sorb to sediments, suspended solids, and biota. In general, nitroaromatics in soil are reduced to amino compounds and covalently bound to soil organic matter (ATSDR, 1989). It is concluded that adsorption on soils and sediments is not a significant environmental fate.

E. HYDROLYSIS

No hydrolysis is expected under environmental conditions (Spanggord et al., 1980a).

F. OTHER FATE ROUTES

Other fate routes to be considered are transport, partitioning, and plant uptake. Since the water solubilities of DNTs are moderate and octanol-water partition coefficients are low, there is potential for transport of DNT by surface or groundwater (ATSDR, 1989).

Sediment-water partitioning coefficients calculated for 2,4- and 2,6-DNT were 45 and 92, respectively. Depending on the nature of the sediment load, the total concentration of DNT carried in the soil and sediment water column could be high. However, no data are available to give support to this theory (ATSDR, 1989).

Limited information is available on the direct measurement of plant uptake of DNT. The structural analogy with 1,3-dinitrobenzene and paranitrobenzene suggests that 2,4- and 2,6-DNT would be readily taken up by plants (ATSDR, 1989). This suggestion is consistent with findings by several investigators who have shown that plants grown in media containing nitrotoluenes have a tendency to take up and translocate the chemical (Cataldo et al., 1989; Folsom et al., 1988, 1985; Pennington, 1988; Palazzo and Leggett, 1986a,b; Pallazzo et al., 1984).

V. TOXICOKINETICS

A. ABSORPTION AND EXCRETION

Absorption of DNT and excretion from the body occur rapidly and are usually complete within 24 to 72 hours postdosing. The urine is the primary route of elimination for both DNT isomers in most animals, including rodents, rabbits, dogs, and monkeys; in CD-1 and $B6C3F_1$ mice and Wistar rats, however, the major route of elimination of 2,4-DNT is the feces. Percent absorption of the DNT isomers is difficult to estimate since biliary excretion is significant in most animals; males excrete more of the 2,4-DNT isomer and its metabolites in the bile and less in the urine than females. The amount of DNT reabsorbed also varies among species. Humans absorb both DNT isomers following dermal or inhalation exposure; the urine provides at least one route of elimination. Elimination half-lives of about 1 to 5 hours have been reported for occupationally exposed individuals; one study indicates that urinary elimination of DNT may be biphasic. The comparative percentages of excretion and distribution of radioactivity 24 hours following administration of single oral doses of [^{14}C]2,4-DNT to mice, rats, rabbits, dogs, and monkeys are presented in Table V-1. Comparable data were not available for 2,6-DNT.

1. Humans

Absorption of DNT following oral exposure in humans has not been determined. However, occupational studies indicate that technical grade DNT (tg-DNT) is absorbed by humans after inhalation or dermal exposure and excreted in the urine. Elimination via the feces or lungs has not been examined in humans.

Woollen et al. (1985) conducted two studies in which a total of 33 workers in an explosives factory were exposed to tg-DNT (76% 2,4-DNT, 20% 2,6-DNT). Routine atmospheric sampling indicated DNT levels of 0.03 to 0.1 mg/m^3 (Threshold Limit Value (TLV) 1.5 mg/m^3); static air in dusty areas of the plant contained 0.02 to 2.68 mg/m^3 (mean 0.40 mg/m^3). The authors suggested that the skin was probably the primary route of exposure for these

Table V-1. Distribution and Excretion of Radioactivity 24 Hours After Oral Administration of a Single Dose of [ring-UL-^{14}C]2,4-DNT to Various Species[a]

Fraction	% of Administered Dose						
	Mouse						
	CD-1	B6C3F$_1$	A/J	Rat	Rabbit	Dog	Monkey
Urine	11.3±3.7[d]	7.2±1.9[e]	66	75.9±2.6[g]	75.2[h]	76.3[h]	81.3[h]
Feces	81.0±4.3	84.0±2.9	2.1	9.1±3.0	3.1	8.7	4.8
Spleen	<0.1	<0.1	--[f]	--	<0.1	<0.1	<0.1
Liver	0.2±0.0	0.1±0.0	--	0.3±0.01	<0.3	1.1	0.7
Kidney	<0.1	<0.1	--	<0.1	<0.1	0.2	<0.1
Brain	<0.1	<0.1	--	<0.1	<0.1	<0.1	<0.1
Lung	<0.1	<0.1	--	<0.1	<0.1	<0.1	<0.1
Skeletal muscle[b]	0.1±0.0	<0.1	--	0.3±0.1	0.2	1.6	1.2
Gastrointestinal tract plus contents	2.1±1.3	0.6±0.2	--	2.8±1.5	10.9	8.6	4.7
Whole blood[c]	0.1±0.0	<0.1	--	0.1±0.0	0.1	0.6	0.3
Total recovery	94.8±2.3	92.0±3.2	--	88.5±2.6	89.8	97.3	93.0

[a]CD-1 and B6C3F$_1$ mice, rats, rabbits, dogs, and monkeys received 1/10 of the acute LD_{50} (see text for additional details) (Lee et al., 1978); male A/J mice were given 100 mg 2,4-DNT/kg (Schut et al., 1985). Values are for 24 hours postdosing except those of A/J mice, which were measured 8 hours postdosing.
[b]Based on 40% of the body weight.
[c]Based on 7% of the body weight.
[d]Mean ± SE of four female mice.
[e]Mean ± SE of five female mice.
[f]Data not provided.
[g]Mean ± SE of three female CD rats.
[h]Average of two animals (female New Zealand white rabbits, female beagle dogs, and female rhesus monkeys).

SOURCE: Lee et al. (1978); Schut et al. (1985).

workers, and the lungs constituted the secondary route because of low atmospheric levels of DNT. In the first group of individuals (20 males, 8 females), urine was collected during 2 consecutive workweeks; in the second group (2 males, 3 females), 24-hour urine samples were collected during a 2-day exposure period and the subsequent 2-day nonworking period. Excretion of 2,4-dinitrobenzoic acid (2,4-DNBacid), a metabolite of DNT, was highest at the end of the work shift and generally was much greater at the end of each workweek than at the beginning of the week (i.e., mean urinary levels of 3.4 to 41 mg/mL versus <1 mg/mL); urinary concentrations of 2,4-DNBacid were comparable between the 2 weeks. Blood levels of DNT (2,4- and 2,6- combined) were low before the workday began (<10 ng/mL), gradually increased during the exposure period (20 to 90 ng/mL), and peaked at the end of the work shift (70 to 250 ng/mL). These data suggest that DNT is absorbed, is readily cleared from the body, and does not tend to accumulate. However, the extent of DNT absorption by humans cannot be determined from this study.

Elimination half-lives for all five DNT metabolites excreted in the urine of three workers exposed to 0.05 to 0.59 mg tg-DNT/m^3 were between 0.88 and 2.76 hours (Turner, 1986; Turner et al., 1985). Values for individual metabolites were between 0.80 and 4.26 hours. In the study by Woollen et al. (1985), a half-life of 2 to 5 hours was calculated for the urinary elimination of 2,4-DNT by workers exposed to 0.02 to 2.68 mg tg-DNT/m^3. These authors suggested that since low but detectable levels of 2,4-DNBacid were found in the urine 3 days after exposure was terminated, urinary elimination of DNT is biphasic.

2. Animals

a. 2,4-DNT

Lee et al. (1978) reported that approximately 81 to 84% of a single oral dose of [ring-UL-^{14}C]2,4-DNT (80 mg/kg) given to female CD-1 and female B6C3F$_1$ mice was excreted in the feces within 24 hours postdosing; only 7 to 11% was recovered from the urine during the same period (Table V-1). In contrast, A/J mice given single oral doses of 100 mg [3-^{3}H]2,4-DNT/kg eliminated 66% of the

dose in the urine and 2.1% in the feces within the first 8 hours postdosing (Schut et al., 1985). Similarly, male A/J mice given a single intraperitoneal (ip) dose of 1, 10, or 100 mg/kg of the ^{3}H-labeled compound excreted about 52.5, 60.1, and 70% of the administered radioactivity, respectively, in the urine within 4 hours (Schut et al., 1982, 1985). Fecal levels of radioactivity were not measured, but the concentrations of ^{3}H in the large intestine at the corresponding doses were 10.1, 3.9, and 2.1% at 4 hours. Elimination of radioactivity via the lungs did not exceed 0.20%. These data suggest that A/J mice either absorb DNT more readily from the gastrointestinal tract or preferentially eliminate absorbed DNT in the urine rather than in the bile (see below).

Following oral administration of [ring-UL-^{14}C]2,4-DNT to male and female F344 and CD rats, most of the radioactivity was eliminated in the urine (Rickert, 1984; Rickert et al., 1981; Ellis et al., 1985, 1979; Lee et al., 1975, 1978). Rats receiving less than 100 mg/kg excreted between 60 and 90% of the radioactivity in the urine and between 3 and 32% in the feces within 72 hours postdosing. Urinary excretion generally was higher in females (61 to 90%) than in males (55 to 80%), and ^{14}C levels in the feces usually were lower in females (7.5 to 20%) than in males (3 to 32%). At 100 mg/kg, elimination via the urine and feces was comparable between the sexes, accounting for about 52 and 16%, respectively, for both males and females. This dose therefore may indicate a saturation point in the elimination of 2,4-DNT. In a study by Mori et al. (1977), male Wistar rats given a single oral dose of [3-^{3}H]2,4-DNT (50 mg/kg) excreted only 16 and 30% of the administered ^{3}H in the urine and feces, respectively, at 7 days postdosing. Similarly, only 12.5% of a 75-mg/kg oral dose of ^{3}H-labeled test material was recovered from the urine of male Wistar rats within 7 days (Shoji et al., 1985). Low total recovery of ^{3}H may have been a problem with the latter two studies, however.

Approximately 75, 76, and 81% of a single oral dose of [ring-UL-^{14}C]-2,4-DNT was eliminated in the urine by female New Zealand rabbits, female beagle dogs, and female rhesus monkeys, respectively, within 24 hours after compound administration (Lee et al., 1978). Between 3 and 9% was excreted in the feces (see Table V-1). The authors stated that each animal received

approximately 1/10 of the LD_{50}, but the dose administered to these animals was reported only as 10 µCi [ring-UL-^{14}C]2,4-DNT (specific activity 3.55 mCi/mM).

Elimination of 2,4-DNT following repeated oral dosing also has been examined. Consumption of unlabeled 2,4-DNT in the diet for 1, 3, 9, or 20 months caused only a slight reduction in the total and urinary elimination of 2,4-DNT in male and female F344 (Rickert et al., 1981) or CD (Ellis et al., 1979) rats when compared with animals administered DNT only once. In the study by Rickert et al. (1981), animals were fed unlabeled 2,4-DNT at a level of 35 mg/kg/day in the diet for 30 days before receiving a single oral dose of [^{14}C]2,4-DNT (35 mg/kg). Ellis et al. (1979) gave male and female CD rats a single oral dose of 57 or 65 mg [ring-UL-^{14}C]2,4-DNT/kg, respectively, after animals had consumed a diet containing 0.01 or 0.07% unlabeled 2,4-DNT for 3, 9, or 20 months. In a study by Mori et al. (1980), male Wistar rats were given a single oral dose of [3-^{3}H]2,4-DNT (50 mg/kg) after being fed a diet containing 0.5% unlabeled 2,4-DNT for 4 months. A total of 59% of the administered ^{3}H was excreted within 7 days postdosing; approximately 33% was found in the urine, and about 26% was found in the feces.

Biliary excretion of 2,4-DNT appears to be significant, particularly in male rats, and most likely accounts for sex-related differences in the rate of fecal elimination of 2,4-DNT and/or its metabolites. Medinsky and Dent (1983) demonstrated that biliary excretion of radioactivity following a single oral dose of [ring-UL-^{14}C]2,4-DNT (35, 63, or 100 mg/kg for males; 35 mg/kg for females) was higher in male than in female Fischer 344 rats. About 25 and 18% of the administered dose was eliminated in the bile of males and females, respectively, at 36 hours postdosing. Biliary excretion of radiocarbon was essentially complete within 24 hours for males (excretion half-times 4.1 to 5.3 hours) and 12 hours for females (half-time 3.3 hours). Medinsky and Dent (1983) also collected more radioactivity (i.e., 60 to 90% versus 20 to 60% of the administered dose) in the urine of rats from which bile was not collected than in bile-duct cannulated rats from which bile was collected. These data suggest that biliary metabolites were absorbed from the intestines via enterohepatic cycling. In all cases, females excreted more ^{14}C in the urine than males. In another study, biliary excretion of ^{14}C in female CD rats

given a single oral dose of [ring-UL-^{14}C]2,4-DNT accounted for about 10% of the administered radioactivity at 24 hours postdosing (Ellis et al., 1980; Lee et al., 1978). The rate of excretion in the bile peaked at 2 hours. Bond et al. (1981) attributed the sex-related differences in elimination of 2,4-DNT in Fischer 344 rats to three factors: a faster rate of transport of 2,4-dinitrobenzyl alcohol glucuronide (2,4-DNBalcG) from the liver to the bile, a larger amount of 2,4-DNBalcG excreted by the liver into the bile, and a lower concentration of 2,4-DNT required to saturate the rates of metabolism of 2,4-DNT by the liver in males when compared with females.

Metabolism by gut microflora may be required before reabsorption and elimination of DNT can occur. One group of investigators (Rickert et al., 1981) demonstrated that axenic male F344 rats (i.e., those devoid of intestinal bacteria) eliminated significantly less ($p < 0.05$) ^{14}C in the urine and feces than did conventional male rats; corresponding values for the bacteria-free animals were 33.8 and 15.7%, and levels for normal rats were 54.7 and 24.2%. Recovery of ^{14}C from the urine of axenic female rats was also lower than that from conventional females (39.8 versus 60.5%), but fecal levels were comparable (17.3 and 13.8%). Sex-related differences in elimination of 2,4-DNT following oral exposure in axenic rats were not remarkable.

b. <u>2,6-DNT</u>

Similar to the 2,4-DNT isomer, orally administered 2,6-DNT was primarily eliminated via the urine. Male A/J mice excreted about 54, 54, and 49% of a single oral dose of 1, 10, or 100 mg [3-^{3}H]2,6-DNT/kg, respectively, in the urine within 8 hours postdosing (Schut et al., 1983). Feces contained no more than 2.1% of the ^{3}H dose, and levels in the small intestine accounted for about 3 to 3.6%. Elimination via the lungs was negligible (≤0.35%).

Twenty-four hours following administration of a single oral dose of [ring-UL-^{14}C]2,6-DNT (80 mg/kg) to female CD rats, about 60% of the ^{14}C was recovered from the urine and 40% was recovered from the feces and gastrointestinal contents (Ellis et al., 1980; Lee et al., 1975). Long and

Rickert (1982) reported that male and female Fischer 344 rats eliminated about 54% of a single oral dose of ^{14}C-labeled 2,6-DNT (10 mg/kg, 2 mCi/mmol) in the urine within 72 hours after dosing; feces contained about 20%. No sex-related differences were noted. The authors reported that urinary excretion of ^{14}C was complete within 24 hours, but that fecal elimination was still evident at 72 hours.

As with 2,4-DNT, a major route of elimination of 2,6-DNT is biliary excretion. For example, female CD rats excreted about 25% of a single oral dose of [ring-UL-^{14}C]2,6-DNT in the bile 24 hours after dosing (Ellis et al., 1980; Lee et al., 1978). The rate of biliary excretion of ^{14}C peaked at 6 hours after dosing as compared with 2 hours in female CD rats administered 2,4-DNT (Lee et al., 1978). Long and Rickert (1982) reported that total recovery of ^{14}C-labeled 2,6-DNBalcG from liver perfusate and bile was comparable for both male and female CD rats when livers were incubated with 20 M [ring-UL-^{14}C]2,6-DNT. At 70 μM, however, total recovery of this metabolite in the bile was significantly less ($p < 0.05$) in females than in males. These data indicate a possible saturation point in the metabolism and excretion of 2,6-DNT in female rats. Biliary flow rates were similar for both sexes, and disappearance of parent compound (20 or 70 μM) from the perfusate was biphasic: half-times of elimination were 7.5 and 8.4 minutes for males and females, respectively, for the initial phase, and 53.9 and 52.7 minutes, respectively, for the second phase.

c. tg-DNT

No information on the absorption and excretion of tg-DNT was found in the available literature.

B. DISTRIBUTION

1. Humans

No information was found on the distribution of DNT in human tissues, although Woollen et al. (1985) detected trace amounts of 2,4- and 2,6-DNT in the blood of workers exposed to this mixture.

2. Animals

a. 2,4-DNT

As shown in Table V-1, very little radioactivity was retained by mice, rats, rabbits, and monkeys 24 hours after administration of a single oral dose of [ring-UL-^{14}C]2,4-DNT (10 μCi; dose equivalent to 1/10 the acute LD_{50}) (Lee et al., 1978). For each animal, essentially all of the ^{14}C was recovered in the urine, feces, and gastrointestinal tract. Most tissues contained no more than 0.3% of the radioactive dose, although the whole blood of dogs and the liver and skeletal muscle of both dogs and monkeys contained 0.6 to 1.6%. Tissue/plasma radioactivity ratios showed a preferential initial uptake of ^{14}C by the liver and kidneys of all animals within 24 hours postdosing; the ratio for lung tissue in rats was also relatively high (Table V-2) (Lee et al., 1978). After 5 days of dosing, tissue/plasma ratios in rats were 30.3, 19.9, 5.1, 12.5, and 5.4 for the liver, kidneys, brain, lungs, and skeletal muscle, respectively (Lee et al., 1975). These data suggest that small amounts of 2,4-DNT and/or its metabolites may accumulate in animal tissue, particularly the liver, kidneys, and lungs of rats.

Additional studies on the distribution of 2,4-DNT in mice and rats are described below. Schut et al. (1981, 1982) examined the distribution of a single intraperitoneal (ip) dose (1, 10, or 100 mg/kg) of [3-^{3}H]2,4-DNT (10 μCi/mL) in male A/J mice. Total recovery of ^{3}H in body fluids and tissues was 69.4, 74.8, and 81.7% for the low, middle, and high doses, respectively. Concentrations of ^{3}H in the blood were proportional to dose. Levels peaked at 2 to 3 and 15 nmol equivalents/mL blood for the 1- and 10-mg/kg groups, respectively, within 30 minutes postdosing; for the 100-mg/kg group, blood levels of radioactivity (150 nmol equivalent/mL) peaked at 1 hour. Levels of ^{3}H in the liver were initially about two to three times higher than levels in the plasma, but after 2 hours, the two values were comparable. The authors

Table V-2. Tissue/Plasma Radioactivity Ratios in Various Species of Animals 24 Hours After Administration of a Single Oral Dose of [ring-UL-^{14}C]2,4-DNT[a]

Tissue	Mouse	Rat	Rabbit	Dog	Monkey
Spleen	1.7±0.3[b]	--[c]	1.3[e]	1.1[e]	2.6[e]
Liver	6.3±0.8	18.1±0.7[d]	8.7	6.9	17.8
Kidneys	3.4±0.4	7.4±0.5	7.0	4.9	6.4
Brain	0.6±0.1	1.5±0.1	0.6	0.5	1.3
Lungs	1.9±0.3	6.1±3.2	2.2	1.7	1.9
Skeletal muscle	0.6±0.1	1.8±0.2	0.5	0.5	1.5

[a]Ratios represent the amount of radioactivity in 1 g of wet tissue divided by the amount of radioactivity in 1 mL of plasma.
[b]Mean ± SE of four mice.
[c]Not reported.
[d]Mean ± SE of three rats.
[e]Mean ± SE of two animals.

SOURCE: Lee et al. (1978).

described similar results for the kidney, but they did not present supporting data. Terminal half-lives for ^3H in the liver and kidney were less than 2 hours. In all cases, the lungs contained no more than 0.2% of the radioactive dose. Maximum levels of ^3H in the adipose tissue (3.9 to 4.7%) were reached within 30 minutes but dropped to <0.1% within 4 hours. Uptake of radioactivity by the small intestine was highest (9 to 14%) between 0.75 and 2 hours. Levels in the brain, heart, and spleen were negligible. Preferential uptake or retention of radioactivity was not apparent for any tissue.

In male F344 rats, maximum ^{14}C levels in the liver and kidneys were proportional to dose (10, 35, or 100 mg [ring-UL-^{14}C]2,4-DNT/kg) and were about 5 to 10 times higher than levels in the plasma and red blood cells (Rickert and Long, 1980). No sex-related differences were observed in the concentration of ^{14}C in plasma or in the elimination of ^{14}C from the plasma (2 to 2.5 hours), liver (terminal half-lives of 36 and 40 hours for males and females, respectively), or kidney (30 and 41 hours, respectively). In contrast, hepatic levels of radioactivity were about two times greater in males than in females during the 72-hour postdosing period. Elimination of ^{14}C from red blood cells was significantly ($p < 0.05$) slower in female than in male rats (half-lives of 123 versus 27 hours). No parent compound was detected in the plasma at 12 hours postdosing.

Mori et al. (1978) gave male Wistar rats a single oral dose of 22 mg ^3H-labeled compound/kg. Blood levels of radioactivity peaked at 6 hours and dropped steadily during the subsequent 9 hours. Hepatic ^3H levels also peaked at 6 hours and correlated with levels in the blood. The highest concentrations of radioactivity in the stomach and small intestine were detected before 6 hours. A whole-body half-life of 22 hours was reported.

In another study, low levels of radioactivity were recovered from 16 tissues 7 days after male Wistar rats were given 50 mg [^3H]2,4-DNT/kg (2.14 µCi/mg) (Mori et al., 1977). All tissues combined (including the stomach and small and large intestine) contained only 2.95% of the administered ^3H. The highest levels of radioactivity were found in the

adipose tissue (1.6%), skin (0.60%), and liver (0.40%). Total recovery of ^{3}H in this study, however, accounted for only 49% of the radioactive dose.

Irons and Gross (1981) examined autoradiographs of female F344 rats administered up to 100 µCi [ring-UL-^{14}C]2,4-DNT (35 mg/kg). For same-sized tissue sections of animals sacrificed 3 hours after dosing, ^{14}C concentrations increased in the following order: muscle (lowest), lung, blood, liver, and kidney. Brain, heart, and adipose tissue contained negligible amounts of radioactivity.

Radioactivity was detected in the placenta and amniotic fluid of rats (strain not given) administered a single oral dose of 35 mg [ring-UL-^{14}C]-2,4-DNT/kg on gestation day 20 (Rickert et al., 1980). (The time of sacrifice was not reported.) Approximately 10 to 50% of the ^{14}C dose was recovered from these two fractions. Clearance of radioactivity from the plasma was similar for fetuses and dams (half-lives, 8.8 and 9.5 hours, respectively) but was slower from the liver (39 and 17 hours) and kidney (27 and 13 hours) of dams. The authors stated that concentrations of ^{14}C in fetal tissues were similar to those in maternal tissues, but supporting data were not provided.

The effects of repeated dosing on the tissue uptake of 2,4-DNT have been examined. As discussed above, the tissue/plasma ^{14}C ratios for the liver, kidney, brain, lungs, and skeletal muscle of rats given a single oral dose of radiolabeled 2,4-DNT for 5 consecutive days were about two to four times greater than ratios of single-dose animals, indicating that 2,4-DNT and/or its metabolites can accumulate in the body (Lee et al., 1978). In another study, Ellis et al. (1979) gave male and female CD rats a single oral dose of 57 or 65 mg [ring-UL-^{14}C]2,4-DNT/kg, respectively, after animals had consumed a diet containing 0.01% or 0.07% unlabeled 2,4-DNT for 3, 9, or 20 months. Rats in the 20-month experiment had relatively high levels of radioactivity (i.e., 4.4 and 5.7%) in the muscle tissue 24 hours after administration of the ^{14}C-labeled compound. However, the level of radioactivity in the muscle tissue of nonpretreated female controls also was high and contained about 2.3% of the ^{14}C dose. No other trends were noted in any other group. Mori et al. (1980) found that tissue levels of radioactivity generally were lower in male

Wistar rats fed unlabeled 2,4-DNT in the diet for 4 months before receiving a single oral dose of 50 mg [^{3}H]2,4-DNT/kg (47.6 μCi/kg) than in those fed a standard diet. At 7 days after dosing, the highest ^{3}H residues were in the liver, and with the exception of the kidney, heart, and small intestine, levels of radioactivity in 12 other tissues were lowest in the pretreated animals when both groups were compared.

b. 2,6-DNT

Schut et al. (1983) examined the disposition of a single oral or ip dose of [3-^{3}H]2,6-DNT in male A/J mice. Animals were given 1-, 10-, or 100-mg/kg doses of the radiolabeled compound (2.5 μCi/mouse). Blood and liver levels of ^{3}H were similar in orally dosed mice and remained fairly constant for the first 8 hours after dosing. In contrast, hepatic concentrations of radioactivity in ip-dosed mice peaked during the first hour postdosing and decreased steadily thereafter. The amount of ^{3}H in the blood was two to four times lower than that in the liver through the first 2 hours after compound administration. Kidney ^{3}H residue levels were reportedly equivalent to those in the liver and showed no treatment-related differences (data not provided). Uptake of 2,6-DNT in the small intestine peaked between 1 and 3 hours, but the maximum concentration was higher in orally dosed mice (9.5 to 16.9% of the administered ^{3}H) than in ip-dosed animals (5.2 to 8.9%). Lungs contained no more than 0.35% of the administered dose for either group, and the levels of radioactivity in the brain, heart, and spleen remained low throughout the experiment. Preferential tissue uptake was not apparent, but total recovery data suggest that the 100-mg/kg dose may have been saturating for both routes of exposure.

In a study by Ellis et al. (1980), only a small amount (<5%) of the radioactivity administered to female CD rats was recovered from the carcass 24 hours after dosing. Each animal received, by gavage, 10 μCi of [ring-UL-^{14}C]2,6-DNT at a dose equivalent to 1/10 of the LD_{50} (about 80 mg/kg).

No other studies on the distribution of 2,6-DNT were found.

c. tg-DNT

No information on the distribution of tg-DNT was found in the available literature.

3. Covalent Binding of DNT Isomers

Covalent binding of DNT to hepatic macromolecules has been examined extensively and may be particularly important in light of data showing a higher incidence of hepatic carcinomas and hepatic neoplastic nodules in DNT-exposed male rats when compared with treated female rats. Results of several studies indicate that conjugation, biliary excretion, microbial metabolism in the gut, and intestinal reabsorption may be prerequisites to hepatic binding of DNT. Hepatic binding may be greater for 2,6-DNT than for 2,4-DNT, and binding of DNT isomers appears to be lower in females than in males. Diet (i.e., as it affects microbial activity and number) may also influence the degree to which binding of DNT metabolites occurs.

Medinsky and Dent (1983) demonstrated differences in the binding of ^{14}C to hepatic macromolecules following oral administration of [ring-UL-^{14}C]-2,4-DNT to male and female Fischer 344 rats. Binding was lower in females than in males. In addition, covalent binding in bile duct-cannulated rats from which bile was collected was lower than in animals from which bile was not collected.

Swenberg et al. (1983) reported sex-related differences in the covalent binding of [^{3}H]2,6-DNT following oral dosing in Fischer 344 rats. DNA from hepatocytes of males given a single oral dose of the compound contained at least 15 to 20% more radioactivity than DNA from hepatocytes of females. Binding of 2,6-DNT to DNA in liver sinusoidal lining cells was considerably lower than binding to hepatocyte DNA through the first 4 days after compound administration, particularly in cells isolated from female rats. The amount of binding to hepatocyte DNA dropped throughout the entire 7-day postdosing period, while binding in sinusoidal cells declined during the first 4 days and then increased during the next 3 days. In another experiment, essentially all

covalently bound radioactivity was recovered from the hepatocellular DNA of male Fischer 344 rats given weekly doses of [^{3}H]2,6-DNT for 1, 2, 3, or 4 weeks. These data indicate that covalent binding of 2,6-DNT is specific for hepatocytes.

Covalent binding of ^{14}C to hepatic DNA, RNA, and protein was evident in male Fischer 344 rats at 12 through 96 hours after animals were given a single oral dose of [ring-UL-^{14}C]2,4- or 2,6-DNT (Rickert et al., 1983). The authors reported that hepatic binding of 2,6-DNT was about two to five times greater than that of 2,4-DNT. Neither isomer had an affinity for a particular macromolecule.

Kedderis et al. (1984) used sulfotransferase inhibitors to determine the effect of sulfate ester formation on the in vivo bioactivation of 2,4- and 2,6-DNT to electrophilic species, which in turn bind covalently to DNA. In this study, male Fischer 344 rats were given an oral gavage dose (28 mg/kg) of either isomer after administration of pentachlorophenol (PCP) or 2,6-dichloro-4-nitrophenol (DCNP). Total hepatic macromolecular binding of 2,4- and 2,6-DNT were reduced by 33 and 69%, respectively, following treatment with the inhibitors. Each inhibitor caused binding of 2,6-DNT to hepatic DNA to decrease by more than 95%. In contrast, DNA binding of 2,4-DNT was reduced by about 84 and 33% after administration of DCNP or PCP, respectively. These data suggest that sulfation may be involved in the hepatic covalent binding of reactive metabolites of DNT isomers.

As mentioned above, metabolism by intestinal bacteria appears to play a role in hepatic binding of DNT. In a study by Rickert et al. (1981), hepatic covalent binding was significantly greater ($p \leq 0.05$) in conventional male Fischer 344 rats than in axenic male rats following oral administration of ^{14}C-labeled 2,4-DNT. The amount of ^{14}C bound to hepatic material also was higher ($p \leq 0.05$) in both conventional and axenic males than in corresponding females. In contrast, hepatic binding was comparable in both groups of female rats. Swenberg et al. (1983) reported that at 12 hours postdosing, the level of 2,6-DNT bound to hepatocyte DNA in axenic male rats was about 10% of that

bound to the DNA of conventional rats; female axenic rats were not included in the experiment.

The effects of dietary pectin on intestinal microflora and on the hepatic binding of 2,6-DNT were investigated by DeBethizy et al. (1983) and Rickert et al. (1986b). Male F-344 rats in these studies were fed a purified diet containing 0, 5, or 10% pectin or one of two cereal-based diets containing <1 or 8.4% pectin. No pectin-related changes in the hepatic covalent binding of 2,6-DNT were observed in rats given a single low dose (10 mg/kg) of [^{3}H]2,6-DNT on day 28. In animals given a 75-mg/kg dose of the tritium-labeled 2,6-DNT, however, total covalent binding was increased by 40 and 90% after consumption of 5 or 10% pectin, respectively, when compared with animals given pectin-free diets. Binding in hepatic tissue of rats fed the cereal-based diets and dosed with 75 mg [^{3}H]2,6-DNT/kg was 135 and 150% higher than in controls. Activities of cecal glucuronidase and nitroreductase were about two to three times greater in pectin-fed rats than in controls; the total number of anaerobic microbes in the cecum of treated animals was similarly increased. The authors suggested that through the changes described above, dietary pectin could increase the potential toxicity of high doses of 2,6-DNT.

C. METABOLISM

Following gastrointestinal absorption, DNT isomers undergo oxidation in the liver. Metabolites generated from this reaction are often conjugated with sulfate or glucuronate and subsequently excreted in the urine or into the bile (section V.A.1). Metabolites that are transported from the bile to the gut are hydrolyzed and reduced by intestinal microflora (section V.C.3). Many of these compounds, in turn, are reabsorbed from the gut into the systemic circulation and then oxidized in the liver. Urinary elimination may occur next, but biliary excretion of these metabolites into the gut results in additional reduction by intestinal bacteria prior to elimination from the body.

For most animals (i.e., A/J mice, CD and Fischer 344 rats, New Zealand rabbits, beagle dogs, and rhesus monkeys) and humans, significant portions of orally or intraperitoneally administered 2,4-DNT are converted to 2,4-DNBalc and/or its glucuronide (i.e., 2,4-DNBalcG). Other primary urinary metabolites excreted by these animals include 2-amino-4-nitrobenzyl alcohol (2A4NBalc) and 4-amino-2-nitrobenzyl alcohol (4A2NBalc); humans excrete only the former in large quantities. For Fischer rats and humans, another major product of 2,4-DNT metabolism is 2,4-DNBacid. A significant portion (4 to 9%) of a single oral dose of 2,4-DNT is reduced in vivo to 2,4-diaminotoluene (2,4-DAT) and excreted in the urine by rats, rabbits, dogs, and monkeys, but none is found in the urine of occupationally exposed humans. In contrast, metabolism of 2,4-DNT by CD-1 mice and Wistar rats is not extensive, and only small amounts of an oral dose of 2,4-DNT given to these animals are oxidized to one of the benzyl alcohol metabolites; reduction of 2,4-DNT to 2,4-DAT is negligible in mice. Sex-related differences in the metabolism of 2,4-DNT have been observed in only Fischer rats and humans. For both species, females produce up to three times more 2,4-DNBalc and/or 2,4-DNBalcG than males, and in humans only, men excrete nearly twice as much 2,4-DNBacid as females.

The metabolism of 2,6-DNT has not been studied extensively. As with 2,4-DNT, Fischer rats convert the 2,6-DNT isomer to the corresponding dinitrobenzyl alcohol glucuronide and dinitrobenzoic acid; however, only one metabolite (2-amino-6-nitrobenzoic acid, 2A6NBacid) results from the in vivo reduction of 2,6-DNT. Similarly, only three major metabolites of 2,6-DNT (2,6-DNBalc, 2,6-DNBalcG, and 2,6-DNBacid) have been recovered from the urine of men and women; women appear to excrete more 2,6-DNBalcG than men. No other sex-related differences in the metabolism of 2,6-DNT have been observed.

1. Humans

Metabolism of DNT in humans is similar to that in Fischer rats. The primary urinary metabolites of 2,4- and 2,6-DNT excreted by individuals (14 men, 3 women) exposed occupationally to tg-DNT (0.05 to 0.59 mg/m^3; 76% 2,4-DNT, 19% 2,6-DNT) were the corresponding dinitrobenzyl alcohols and their glucuronides, the corresponding dinitrobenzoic acids, and 2A4NBacid

(Table V-3) (Levine et al., 1985a; Turner, 1986; Turner et al., 1985). Males excreted more DNBacid (2,4- and 2,6-DNT combined) than females (52.5 versus 28.8% of all urinary metabolites, respectively); most of the DNBacid eliminated was 2,4-DNBacid (50.5% for men and 28.9% for women). In contrast, men excreted less of the combined DNBalcG isomers than females (9.5 versus 33.3%, respectively). Approximately 29.1 and 73.9% of the urinary DNBalcG metabolites in females were the 2,4- and 2,6- isomeric forms, respectively; corresponding values for males were 5.0 and 35.6%. Urinary excretion of 2,6-DNBacid was slightly higher in males (2.2 to 14.3% of all metabolites) than in females (2.5%), and levels of 2,6-dinitrobenzyl alcohol were comparable between the sexes (4.8 to 6.6%). Concentrations of 2A4NBacid also were similar for males and females (37.2 to 37.6%). Small amounts (0.3 to 0.8%) of the metabolites in the urine of men and women were 2Ac4NBacid. Unchanged parent compounds (i.e., 2,4- and 2,6-DNT) were also recovered from the urine, and the hydrolyzed urine of one individual contained trace amounts of 4A2NBacid and 4Ac2NBacid. Reduction of both nitro groups was not evident. Wide variations in urinary metabolite profiles were attributed to differences in exposure and in the way an individual may metabolize DNT.

Woollen et al. (1985) found that the primary urinary metabolite of workers exposed to tg-DNT was 2,4-DNBacid. Other urinary metabolites isolated were 2-amino-4-nitro-, 4-amino-2-nitro-, and 2-amino-6-nitrobenzoic acids and 4Ac2NBacid. The urine of these workers contained no 2,4- or 2,6-DNBalc.

2. Animals

a. 2,4-DNT

The primary metabolites identified in the urine of male A/J mice after oral or ip dosing with [3-^{3}H]2,4-DNT were 2,4-DNBalc and 2,4-DNBalcG (Schut et al., 1985). In ip-dosed mice, the unconjugated form accounted for about 1.5 to 6% of the administered ^{3}H; the urine of these mice was not analyzed for conjugates. Between 5.45 and 16% of the oral dose given to mice was 2,4-DNBalcG; less than 0.6% was unconjugated. Small amounts (≤1.5%) of the

Table V-3. Urinary Metabolites Excreted by Humans Exposed Occupationally to Technical Grade DNT[a,b]

Metabolite	Study:	Percent of all urinary metabolites excreted by Levine et al., 1985a) Men	Women	(Turner et al., 1985) Men	Women
2,4-Dinitrotoluene		D[c]	D	D	D
2,6-Dinitrotoluene		D	D	D	D
2-Amino-4-nitrobenzoic acid		37.2	37.6	31.1	34.3
4-Amino-2-nitrobenzoic acid		tr[d]	tr	tr	tr
2,4-Dinitrobenzyl alcohol		--	--	4.9[e]	27.3[e]
2,6-Dinitrobenzyl alcohol		--	--	5.3[e]	6.6[e]
Dinitrobenzyl alcohol glucuronide (DNBalcG)		9.5	33.3	--	--
2,4-DNBalcG		5.0[g]	29.1[g]	--	--
2,6-DNBalcG		35.6[g]	73.9[g]	--	--
Dinitrobenzoic acid (DNBacid)[f]		52.5	28.8	--	--
2,4-DNBacid		--	--	50.5[e]	28.9[e]
2,6-DNBacid		--	--	7.4[e]	2.5[e]
2-Acetylamino-4-nitrobenzoic acid		0.8	0.3	--	--
4-Acetylamino-2-nitrobenzoic acid		--	--	2.1	0.4

[a]76% 2,4-DNT, 19% 2,6-DNT.
[b]Values are averages for 14 men or 3 women, except where noted (see footnote e).
[c]Detected but concentration not quantified.
[d]Trace amount detected.
[e]Average of three men or value for one woman.
[f]2,4- and 2,6- isomers combined.
[g]As percent of the total isomeric forms.

SOURCE: Levine et al. (1985a); Turner et al. (1985).

following metabolites were also identified in both orally and intraperitoneally dosed mice: 2,4-DAT, 4-amino-2-nitrotoluene (4A2NT), and 2-amino-4-nitrotoluene (2A4NT). Trace amounts of parent compound were detected in the urine of both groups of mice. Unknown metabolites accounted for approximately 0.2 to 5% of either dose. Only orally treated mice excreted 2A4NBalc and 2-acetylamino-4-nitrotoluene (2AcA4NT). Female CD-1 mice administered a single oral dose of [ring-UL-^{14}C]2,4-DNT eliminated only 11.3% of the total radioactive dose in the urine, with the largest amounts (2.6 and 2.8%, respectively) recovered as 2,4-DNBalc and 2-amino-4-nitrobenzyl- and 4-amino-2-nitrobenzyl alcohol combined (Table V-4) (Lee et al., 1978). These metabolites accounted for about 23 and 25% of the total urinary radioactivity recovered 24 hours after dosing, respectively (Table V-5); a third metabolite, 4A2NT, represented only 1.4% of the ^{14}C dose but 12.5% of the urinary radioactivity.

Shoji et al. (1985) detected several metabolites in the urine of male Wistar rats given a single oral dose of [^{3}H]2,4-DNT. The most abundant urinary metabolites were 2,4-DNBacid, 2,4-DNBalcG, and 2-amino-4-acetylaminobenzoic acid (2A4AcABacid), which accounted for 5.91, 3.15, and 1.85%, respectively, of the ^{3}H administered. The other metabolites identified were 2,4-DNBalc (0.83%), 4A2NBalcG and 2A4NBalcG combined (0.45%), 4A2NBalc and 2A4NBalc combined (0.16%), and 4A2NT (0.03%). Repeated oral dosing (i.e., administration of a single oral dose of ^{3}H-labeled material for 6 consecutive days) in male Wistar rats appeared to cause an increase in the reductive metabolism of 2,4-DNT (Mori et al., 1981b). For example, although no 2,4-DNBacid or 2,4-DNBalcG was recovered from the urine of these animals during the 7 days after the first dose was given, 2A4NT, 2,4-DAT, and 2-nitro-4-acetylamino toluene (2N4AcAT) were excreted. (Percent-recovery data were not provided.) Formation of 2N4AcAT, 2A4AcAT, and 2A4AcABacid may be unique to Wistar rats.

Male and female Fischer rats convert 2,4-DNT to two major oxidative metabolites (2,4-DNBalcG and 2,4-DNBacid) and two major reductive products (4-acetylamino-2-nitrobenzoic acid (4Ac2NBacid) and 2-amino-4-nitrobenzoic acid (2A4NBacid)) (Table V-6) (Medinsky and Dent, 1983; Rickert et al., 1981;

Table V-4. Radiolabeled Metabolites Found in the Urine of Various Species 24 Hours After Administration of a Single Oral Dose of ^{14}C-2,4-DNT

Metabolite	Percent of Administered Dose[a]				
	Mouse	Rat	Rabbit	Dog	Monkey
2,4-Dinitrotoluene	0[b]	0[c]	0.2[d]	0.6[d]	2.1[d]
4-Amino-2-nitrotoluene	1.4	1.9	6.6	6.3	1.8
2-Amino-4-nitrotoluene	1.1	0.6	5.0	2.7	7.1
2,4-Diaminotoluene	0.6	8.8	4.2	4.0	3.8
2,4-Dinitrobenzyl alcohol	2.6	25.2	32.9	26.6	20.2
2-Amino-4-nitrobenzyl alcohol and 4-amino-2-nitrobenzyl alcohol	2.8	19.2	7.6	14.2	14.8
2,4-Diaminobenzyl alcohol	0.2	4.6	5.6	2.6	2.5
2,4-Dinitrobenzoic acid	0.4	6.2	7.2	5.1	4.0
Unidentified	2.2	9.5	5.9	14.2	25.0
Total	11.3	76.0	75.2	76.3	81.3

[a]Includes free compound and glucuronide and sulfate conjugates.
[b]Mean of four CD-1 female animals.
[c]Mean of three CD female animals.
[d]Mean of two females (New Zealand white rabbits, beagle dogs, and rhesus monkeys).

SOURCE: Adapted from Lee et al. (1978).

Table V-5. Radiolabeled Metabolites Found in the Urine of Various Species 24 Hours After Administration of a Single Oral Dose of ^{14}C-2,4-DNT

Metabolite	Percent of all Urinary Metabolites[a]				
	Mouse	Rat	Rabbit	Dog	Monkey
2,4-Dinitrotoluene	0[b]	0[c]	0[d]	0.7[d]	2.6[d]
4-Amino-2-nitrotoluene	12.5(10.3[e])	4.8	8.8(7.9[e])	8.4(4.6[f];3.7[e])	2.1
2-Amino-4-nitrotoluene	9.5	0.7	6.6	3.5	8.7
2,4-Diaminotoluene	5.2	11.1	5.6	5.4	4.8
2,4-Dinitrobenzyl alcohol	22.6(19.6[f])	30.3(27.4[f])	43.5(40.3[f])	34.8(33.1[f])	25.3(21.5[f])
2-Amino-4-nitrobenzyl alcohol and 4-amino-2-nitrobenzyl alcohol	25.1(24.5[f])	31.7(22.5[f])	10.2(9.4[f])	18.5(17.9[f])	18.1(17.9[f])
2,4-Diaminobenzyl alcohol	1.6	3.1	7.5	3.4	3.2
2,4-Dinitrobenzoic acid	3.9	4.2	9.5	6.7	4.9
Unidentified	19.6	12.5	7.9	18.6	30.7

[a]Includes free compound and glucuronide and sulfate conjugates.
[b]Mean of four female CD-1 animals.
[c]Mean of three female CD animals. Values are based on available data and do not add up to 100% in all cases.
[d]Mean of two female animals (New Zealand white rabbits, beagle dogs, and rhesus monkeys).
[e]Sulfate conjugate.
[f]Glucuronide conjugate.

SOURCE: Adapted from Lee et al. (1978).

Table V-6. Urinary Metabolites of 2,4-DNT Excreted by Orally Dosed Fischer Rats

Metabolite	F344 Rat	
	Males	Females
2,4-Dinitrotoluene	--[a]	--
2-Amino-4-nitrotoluene	<4[b,c]	<4[b,c]
2-Amino-4-nitrobenzoic acid	2.2-4.2[b]	1.8-4[b]
2-Amino-2-nitrobenzyl alcohol	<4[b,c]	<4[b,c]
2,4-Dinitrobenzyl alcohol glucuronide	11.4-15.8[b]	15.9-36.6[b]
2,4-Dinitrobenzoic acid	6.3-18.8[b]	6.9-21.3[b]
4-Acetylamino-2-nitrobenzoic acid	8.4-13.5[b]	9.9-14.0[b]
4-Acetylamino-2-nitrotoluene	<4[b,c]	<4[b,c]

[a]Not detected.
[b]As percent of dose.
[c]Exact amount not reported.

SOURCE: Medinsky and Dent (1983); Rickert et al. (1981); Rickert and Long (1981).

Rickert and Long, 1981). These compounds accounted for about 11 to 37, 6 to 21, 8 to 14, and 2 to 4% of a single oral dose of [ring-UL-^{14}C]2,4-DNT, respectively. Other metabolites of 2,4-DNT recovered from the urine of Fischer rats included 2-amino-4-nitrotoluene (2A4NT), 2-amino-4-nitrobenzyl alcohol (2A4NBalc), and 4-acetylamino-2-nitrotoluene (4Ac2NT) (Medinsky and Dent, 1983). Females eliminated up to about two times more 2,4-DNBalcG in the urine than males; no other sex-related differences in the metabolism of 2,4-DNT were observed.

Lee et al. (1978) demonstrated that in female CD rats, female New Zealand white rabbits, female beagle dogs, and female rhesus monkeys, the two major urinary metabolites of orally administered [ring-UL-^{14}C]2,4-DNT were the glucuronide conjugates of 2,4-DNBalc (20 to 33% of the dose at 24 hours after compound administration) and 2A4NBalc (8 to 19%) (values for free and conjugated forms are given in Tables V-4 and V-5). Smaller amounts (about 2 to 7% of the ^{14}C dose) of 2,4-DAT, 2,4-DABalc, 2A4NT, 4A2NT, and 2,4-DNBacid were also recovered from each species. The percent distribution of urinary metabolites was fairly comparable to the amount of ^{14}C dose metabolized by these animals. In contrast, CD-1 mice excreted disproportionate amounts of 2,4-DNBalc, 2A4NBalc plus 4-amino-2-nitrobenzyl alcohol (4A2NBalc) combined, and 4A2NT in the urine (22.6, 25.1, and 12.5% of all metabolites, respectively) when compared with the corresponding amounts of administered 2,4-DNT metabolized (2.6, 2.8, and 1.4%). Unchanged parent compound was found in the urine of rabbits (0.2% of the administered radiocarbon), dogs (0.6%), and monkeys (2.1%); most 2,4-DNT in the urine of monkeys was present as a glucuronide conjugate. No 2,4-DNT was found in the urine of mice or rats. Between 7.9 and 30.7% of the urinary ^{14}C (2.2 to 25% of the dose) was unidentified. Figure V-1 shows the relative proportions of free and glucuronide- and sulfate-conjugated urinary metabolites excreted by the various animals in this study.

The placenta and amniotic fluid of pregnant Fischer 344 rats contained only unchanged parent compound after administration of a single oral dose of [ring-UL-^{14}C]2,4-DNT on gestation day 20 (Rickert et al., 1980). Small

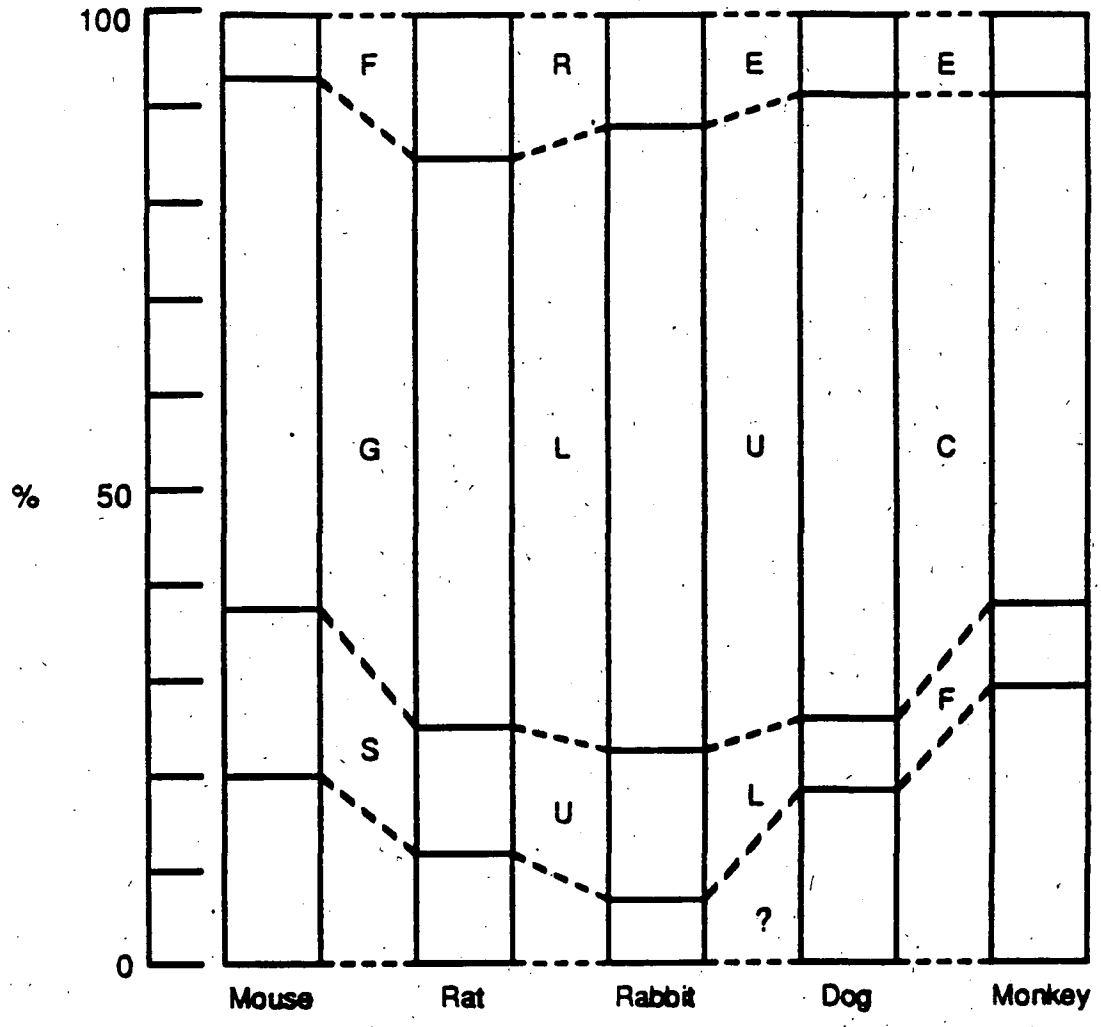


Figure V-1. Distribution of free and conjugated metabolites of 2,4-DNT in the urine of orally dosed animals. Urinary products of an oral dose of 2,4-DNT given to mice, rats, rabbits, dogs, and monkeys and expressed as free, glucuronide (GLUC) conjugates, sulfate (SULF) conjugates, and unknown (?) metabolites.

SOURCE: Ellis et al. (1980).

amounts of 2A4NT, 2,4-DNBacid, and 2A4NBacid were recovered from maternal plasma. No other data were provided.

The metabolism of 2,4-DNT in CD and Fischer 344 rats is minimally affected following repeated oral dosing. For example, male and female rats fed 2,4-DNT in the diet for 30 days and then given a single oral dose of ^{14}C-labeled 2,4-DNT excreted amounts of 4Ac2NBacid, 2,4-DNBacid, 2A4-NBacid, and 2,4-DNBalcG similar to those of nonpretreated animals (Rickert et al., 1981). Males excreted less 2,4-DNBacid than females (9.8 versus 15.8% of the ^{14}C dose), and females excreted slightly more 2,4-DNBalcG than males (24.3 versus 19.1%), but these differences were not statistically significant. In a study by Ellis et al. (1979), male and female CD rats were given a single oral dose of [ring-UL-^{14}C]2,4-DNT after consuming unlabeled test material in the diet (at a level of 0.01 or 0.07%) for 3, 9, or 20 months. Metabolism of 2,4-DNT generally was minimally affected by dosing regimen, and nonpretreated and pretreated males and females appeared to metabolize the test compound in a similar fashion. As discussed earlier, repeated oral dosing over a 6-day period appeared to cause a shift toward reductive metabolism in male Wistar rats (Mori et al., 1981b).

b. <u>2,6-DNT</u>

Very limited data on the metabolism of 2,6-DNT in mice were available. In a study by Schut et al. (1983), male A/J mice were given a single oral or ip dose (1, 10, or 100 mg/kg) of ^{3}H-labeled compound. No unchanged 2,6-DNT was recovered from the blood, liver, lungs, or small intestine of animals given a 1-mg/kg dose by either route, and less than 2% of the ^{3}H recovered from the urine of these animals during the 8-hour postdosing period was unchanged parent compound. In contrast, unchanged 2,6-DNT was isolated in the tissues of animals in all other groups, with the highest levels in high-dose mice and the slowest rate of disappearance of unchanged 2,6-DNT in orally dosed animals. No more than 11% of the ^{3}H in the liver or lungs of animals given a 10-mg/kg ip dose was unchanged parent compound; for animals administered 100 mg/kg ip, 2,6-DNT accounted for up to 20% of the total liver or lung radioactivity. Levels of unchanged parent compound in the small

intestine of ip-dosed mice initially were high (>50% of the total intestinal ^3H) but dropped to low or trace amounts within 2 hours after dosing. Unchanged 2,6-DNT in the liver and lungs of mid- and high-dose mice accounted for up to 15% of the total corresponding tissue radioactivity through 4 hours postdosing. Concentrations of 2,6-DNT in the small intestine of orally dosed animals were high, representing approximately 23 and 50% of the total intestinal ^3H within 4 hours after animals were given 10 or 100 mg/kg, respectively. Eight hours after oral administration of the high dose to mice, about 35% of the tritium in the small intestine was recovered as unchanged parent compound. The large intestine of orally dosed mice contained small amounts of 2,6-DNT (i.e., <2% of total tissue ^3H). About 50 to 90% of the ^3H administered to animals was excreted in the urine within 8 hours after dosing, but less than 2% was recovered as unchanged 2,6-DNT. The data indicate that 2,6-DNT is rapidly and extensively metabolized by mice following oral or ip dosing. The liver and intestines appear to be the primary sites for the metabolism of 2,6-DNT in mice.

Three metabolites accounted for about 95% of the urinary ^{14}C excreted by male and female Fischer 344 rats given a single oral dose of [ring-UL-^{14}C]-2,6-DNT: 2,6-dinitrobenzoic acid (2,6-DNBacid), 2,6-dinitrobenzyl alcohol glucuronide (2,6-DNBalcG), and 2-amino-6-nitrobenzoic acid (2A6NBacid) (Long and Rickert, 1982). In males, these metabolites accounted for about 21, 22, and 14% of the ^{14}C dose, respectively; in females, the corresponding values were 20, 19, and 11%.

c. tg-DNT

No information on the metabolism of tg-DNT was found in the available literature.

3. Microbial and in vitro Metabolism of 2,4- and 2,6-DNT

The role of gut microflora in the metabolism of 2,4- and 2,6-DNT has been examined *in vivo* and *in vitro*. In a study by Rickert et al. (1981), urinary concentrations of oxidized metabolites of orally administered 2,4-DNT (i.e.,

2,4-DNBacid and 2,4-DNBalcG) were comparable between conventional and axenic rats. In contrast, axenic rats excreted significantly smaller amounts (i.e., about 80 to 90% less) of metabolites requiring both oxidation and reduction (i.e., 4Ac2NBacid and 2A4NBacid) than conventional rats.

Guest et al. (1982) investigated the metabolism of 2,4-DNT incubated with cecal or ileal material from male Swiss-Webster mice, male and female Fischer 344 rats, and male humans. No remarkable sex- or species-related differences in the metabolism of 2,4-DNT were observed, although the rate of disappearance of test material was highest for mouse samples, and no 2,4-diaminotoluene (2,4-DAT) was produced in human samples for at least 3 hours. Anaerobic conversion of parent compound was complete within 20 minutes for cecal material from rats. In contrast, no 2,4-DNT was metabolized aerobically within 3 hours. The first metabolites recovered from all incubations were 2-nitro-4-nitrosotoluene and 4-nitro-2-nitrosotoluene; decreases in the concentration of these compounds were accompanied by increased amounts of 2A4NT and 4A2NT. Thus, both nitro groups were reduced to amino groups via nitroso intermediates. The authors suggested that these intermediates may bind covalently to microflora or hepatic macromolecules.

In a study by Mori et al. (1985), 2,4-DNT incubated with cecal microflora from male Wistar rats was reduced anaerobically to 2A4NT, 4A2NT, and 2,4-DAT via 2-hydroxylamino-4-nitrotoluene and 4-hydroxylamino-2-nitrotoluene. Similarly, Escherichia coli isolated from human intestines metabolized 2,4-DNT to 2A4NT and 4A2NT and converted 2,6-DNT to 2A6NT via the corresponding hydroxylaminonitrotoluenes (Mori et al., 1984b). The amount of parent compound converted by human bacteria was 10.2, 18.8, and 45.7%, respectively. The hydroxylamino intermediates were detected in vitro within 8 hours, and production of the final metabolites peaked within 24 hours. Nitrosotoluenes were not recovered in any human samples (Mori et al., 1984b). Microflora in the cecal contents of male A/J mice and male Fischer 344 rats anaerobically converted [^{3}H]2,6-DNT (240 M, 20 Ci) to 2A6NT (6.4 to 9.6% of the total ^{3}H), 2-acetylamino-6-nitrotoluene (2Ac6NT) (1.3 to 4.6%), and 2,6-diaminotoluene (2,6-DAT) (0.4 to 0.7%) (Dixit et al., 1986). About 5.3 to 6.5% of the total metabolites in mouse preparations were not identified; only trace amounts

(0.7% or less) in rat samples remained unidentified. Most parent compound (82.1 to 88.7%) was recovered as unchanged 2,6-DNT at the end of the 30-minute incubation period. Schut et al. (1985) reported that under aerobic conditions, most 2,4-DNT incubated with intestinal explants or cecal material from mice was metabolized to 2,4-DNBalc. Overall, the data suggest that gut microflora are responsible for reductive metabolism of DNT.

Figure V-2 shows the proposed pathway for the anaerobic metabolism of 2,4-DNT in rat intestinal microflora. The primary metabolites of anaerobic degradation of 2,4-DNT by rat hepatocytes are 2A4NT and 4A2NT (Lee et al., 1978; Kozuka et al., 1978; Mori et al., 1980, 1981a; Decad et al., 1982); Lee et al. (1978) reported that concentrations of these aminonitrotoluenes were highest in male liver tissue. Little or no 2,4-DNBalc was recovered from anaerobic liver homogenate preparations containing 2,4-DNT (Lee at al., 1978; Decad et al., 1982), and no 2,4-DAT was isolated following prolonged anaerobic incubation of rat hepatocytes with 2A4NT or 4A2NT (Bond and Rickert, 1981; Mori et al., 1984a). Kozuka et al. (1978) demonstrated that the 4-nitro group of 2,4-DNT is more readily reduced than the 2-nitro group.

Aerobic metabolism of 2,4-DNT by liver tissue of male and female mice, rats, rabbits, dogs, and monkeys leads to the formation of 2,4-DNBalc, the major oxidative product of this munitions chemical (Lee et al., 1978; Bond and Rickert, 1981; Decad et al., 1982; Schut et al., 1985). Similarly, the primary metabolite of 2,6-DNT produced by hepatocytes from male A/J mice and male Fischer 344 rats was 2,6-DNBalc, which constituted 57.5 to 83.8% of all metabolites; most (61.3 to 70.9%) was conjugated (Dixit et al., 1986). Small amounts (1.2 to 5.3%) of unconjugated 2-amino-6-nitrotoluene (2A6NT) also were recovered. The amount of unchanged parent compound varied considerably, from 4.8 to 28.8% of all metabolites. In another study, three metabolites of 2,6-DNT (2,6-DNBacid, 2,6-DNBalcG, and 2A6NBacid) were recovered from the bile and liver perfusate of male and female Fischer 344 rats (Long and Rickert, 1982). Although equivalent amounts of each metabolite were found in the urine of orally dosed males and females (i.e., 11 to 22% of the ^{14}C administered), approximately 41 to 48% and 8.5 to 11% of the radioactivity in the bile of males and females, respectively, were associated with 2,6-DNBalcG. Amounts of

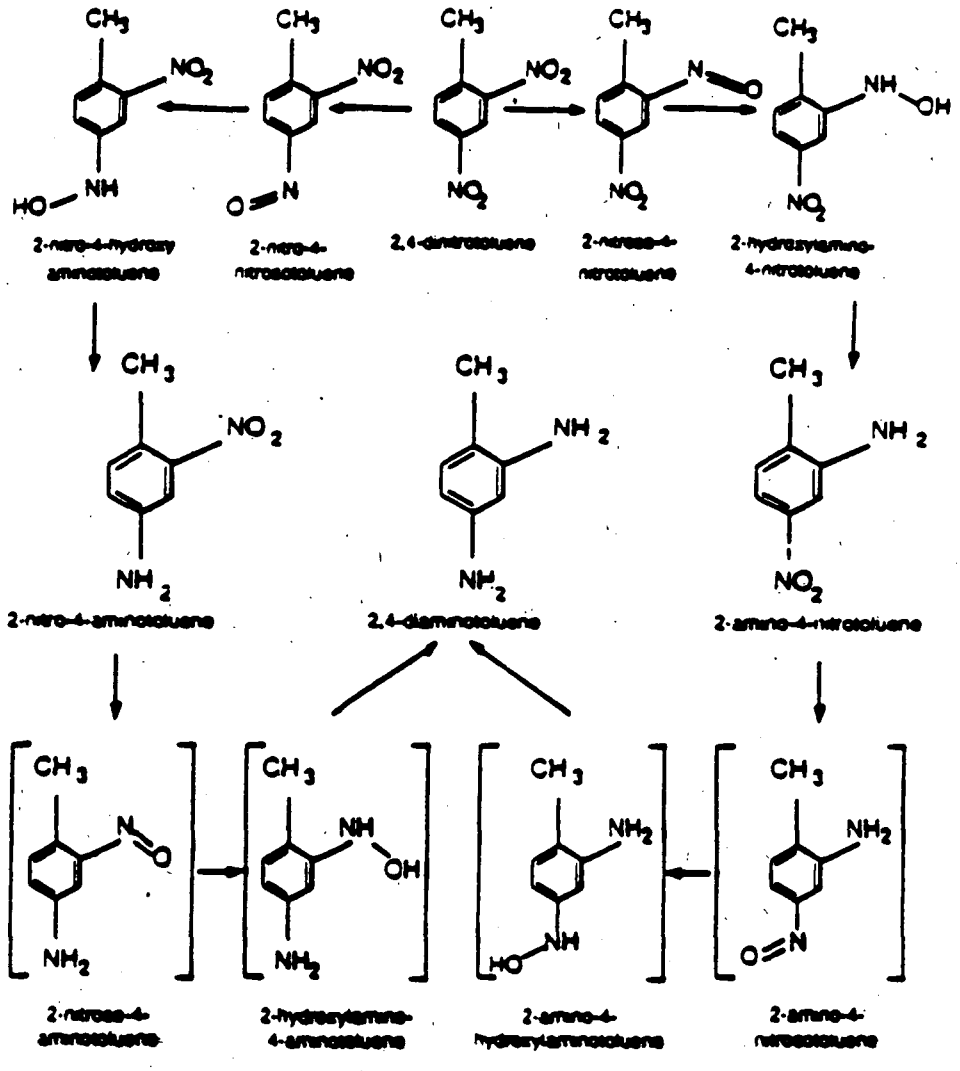


Figure V-2. Proposed pathway for the anaerobic metabolism of 2,4-DNT by rat intestinal microflora.

SOURCE: ATSDR (1989).

other metabolites in the bile were not quantitated, and the bile and liver were not further analyzed. Shoji et al. (1985) proposed that 2,4-dinitrobenzylaldehyde might be an intermediate in the conversion of 2,4-DNBalc to 2,4-DNBacid in vivo; however, the aldehyde was not isolated in the urine of dosed male Wistar rats. A subsequent study by Shoji et al. (1987) showed that 2,4-DNBalc can be converted in vitro to 2,4-dinitrobenzaldehyde in the presence of microsomal and cytosolic preparations.

Bond et al. (1981) tracked the oxidation, conjugation, and acetylation of 2,4-DNT in perfused rat liver and found that the primary route of metabolism of 2,4-DNT in both male and female tissue was through oxidation to 2,4-DNBalc and subsequent glucuronidation to 2,4-DNBalcG. Reduction of 2,4-DNT was minimal. Bond and Rickert (1981) and Lee et al. (1978) reported that as the concentration of oxygen in hepatocyte incubations was reduced, the levels of oxidized metabolites of 2,4-DNT (e.g., 2,4-DNBalc) decreased, and the amount of reduced metabolites (e.g., 2A4NT and 4A2NT) increased but remained low. For all five species studied by Lee et al. (1978), reductive hepatic metabolism of 2,4-DNT was greater in males than in females. However, the data indicate that hepatic reduction of 2,4-DNT most likely represents a minor metabolic pathway.

In a study by Mori et al. (1981a), the microsomal metabolism of 2,4-DNT was blocked by carbon monoxide and primary amines, indicating an involvement of cytochrome P-450. In contrast, cytosolic fractions metabolized 2,4-DNT to 2,4-DAT through the formation of aminonitrotoluenes. It was suggested that since allopurinol blocked this reaction, cytosolic xanthine oxidase most likely mediated the conversion of 2,4-DNT to 2,4-DAT. Bond and Rickert (1981) reported that administration of Aroclor 1254, phenobarbital, or 3-methylcholanthrene to rats induced the production of 2,4-DNBalc in hepatocytes, but that in vitro addition of SKF 525-A or 7,8-benzoflavone inhibited its production. These data together indicate that hydroxylation of the methyl group of 2,4-DNT requires both cytochrome P-450 and P-448 monoxygenases.

Sayama et al. (1989), using HPLC, showed that 2,4 DNT is metabolized to 2,4-dinitrobenzaldehyde, a potent mutagen, in the male Wistar rat. This metabolite constituted 35% of the total metabolites of 2,4-DNT; 2,4-dinitrobenzyl alcohol constituted 29%. A hypothesis was developed to explain the enterohepatic circulation of 2,4-dinitrobenzaldehyde. The 2,4-DNT is metabolized by the liver to 2,4-dinitrobenzyl alcohol glucuronide and to 2,4-dinitrobenzaldehyde and other minor metabolites. The former metabolite is excreted either in the urine or in the bile; in the bile, it is metabolized by the intestinal flora to 2,4-dinitrobenzaldehyde through the enterohepatic circulation. The 2,4-dinitrobenzladehyde formed by direct hepatic metabolism of 2,4-DNT is then excreted in the bile. The authors cautioned that the formation of 2,4-dinitrobenzaldehyde is strain dependent and, therefore, may not be found in other rat strains.

Mori et al. (1989) supported the finding of Sayama et al. (1989) that dinitrobenzaldehyde is a metabolite of DNT and that the metabolism of DNT isomers is strain dependent. Using two rat strains, Wistar and Sprague-Dawley, the authors investigated the metabolism of 2,4- and 2,6-DNT, 2,4- and 2,6-dinitrobenzyl alcohol, and 2,4- and 2,6-dinitrobenzaldehyde in the microsomal and cytosol fractions of the corresponding livers. It was concluded that dinitrobenzaldehydes are intermediate metabolites formed from the oxidation of dinitrobenzyl alcohols to dinitrobenzoic acids, and that the conversion of dinitrobenzaldehydes to dinitrobenzyl alcohols and vice versa is a reversible process. The oxidation of DNT to dinitrobenzyl alcohol occurred in the presence of microsomal cytochrome P-450, and the oxidation of the latter to the dinitrobenzaldehydes was mediated by P-450 and NAD-dependent alcohol dehydrogenase; oxidation of dinitrobenzaldehyde to benzoic acid was mediated by NAD-dependent aldehyde oxidase. Differences in oxidation and reduction rates were clearly seen between the two rat strains.

Figure V-3 provides a summary of the proposed metabolic pathway for the hepatic metabolism of 2,4-DNT.

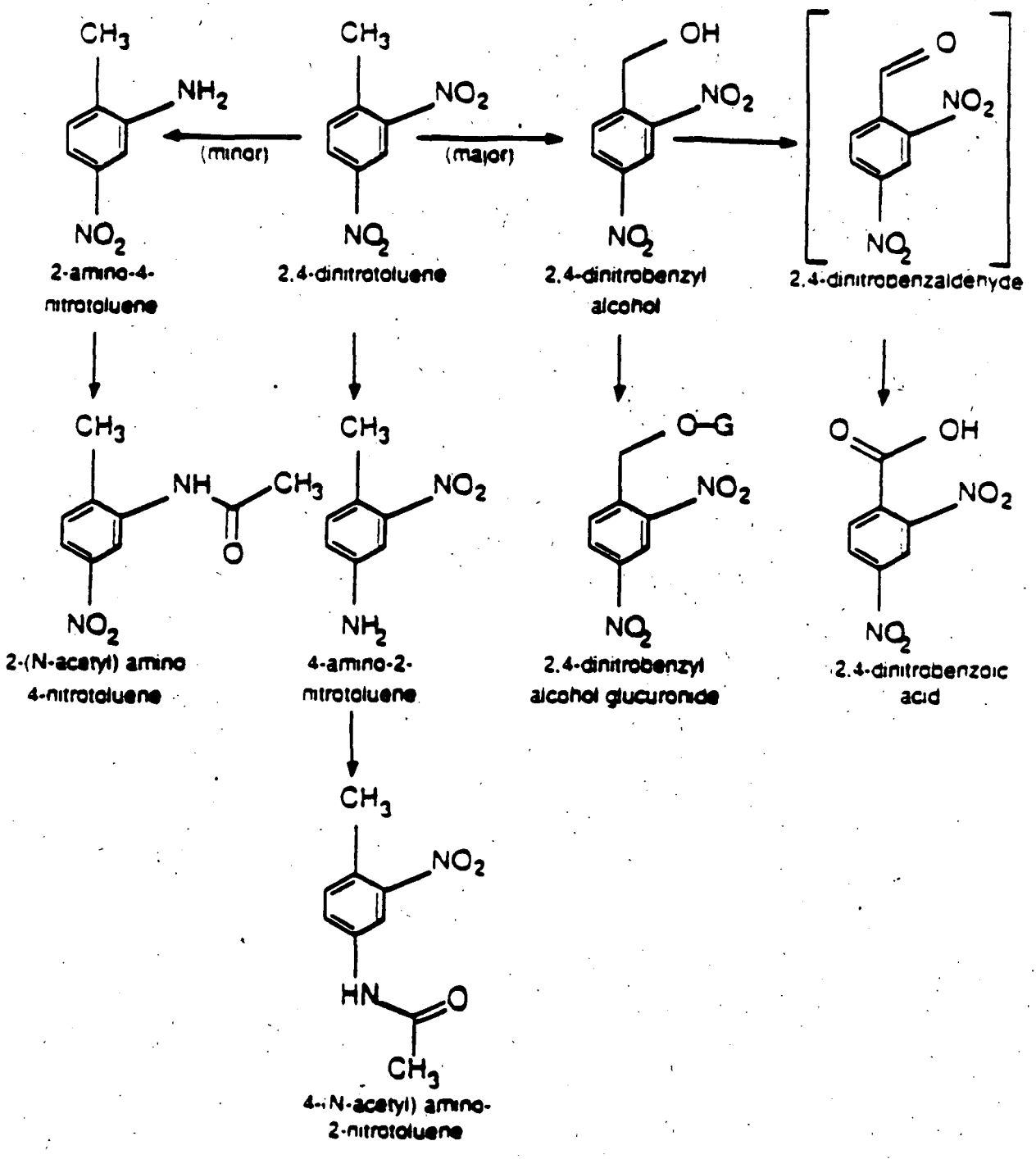


Figure V-3. Proposed metabolic pathway for the hepatic metabolism of 2,4-DNT.

SOURCE: ATSDR (1989).

VI. HEALTH EFFECTS

A. HUMANS

Since data on isomeric composition, purity and, in many cases, concentration of DNT exposures were not reported for the epidemiologic studies, these reviews will not be subdivided by DNT isomer. The studies of occupational exposure to DNT are limited by the small groups of workers studied, by exposure to more than one chemical, and by the lack of individual exposure monitoring. As a result, many of the conclusions found in these human studies are not definitive and require further investigation.

Chronic DNT exposure, primarily via the inhalation route (particulate or vapor), has been characterized in munitions workers by cyanosis, vertigo, headache, metallic taste, dyspnea, weakness and lassitude, loss of appetite, and nausea and vomiting (Etnier, 1987; Ellis et al., 1979; McGee et al., 1942; Perkins, 1919). Other symptoms have included pain or paresthesia in extremities, abdominal discomfort, tremors, paralysis, chest pain, and unconsciousness (U.S. EPA, 1986). Many of these symptoms may indicate neurotoxicity. Recovery from moderate intoxication generally has occurred 2 to 3 days following exposure; pain in extremities has persisted several months in some cases.

The initial clinical sign of DNT toxicity is methemoglobinemia (U.S. EPA, 1980, 1986). The presence of methemoglobin in erythrocytes leads to the formation of aggregates of hemoglobin degradation products, called Heinz bodies, which are readily detected and have been found following exposure to 2,4-DNT and technical grade DNT (tg-DNT) (Ellis et al., 1979; Hughes and Treon, 1954). A hemolytic hypochromic anemia may result, depending on the severity of exposure. When the methemoglobin concentration of the blood is greater than 15%, cyanosis occurs. Headache increases in intensity as the methemoglobinemia increases in severity. Weakness and dizziness occur when the concentration reaches 40%; at 70%, ataxia, dyspnea, tachycardia, nausea, vomiting, and drowsiness may occur (U.S. EPA, 1980, 1986).

Percutaneous absorption is considered to be the second most likely route of exposure to DNT for the industrial worker (U.S. EPA, 1980). Skin contamination may result from exposure to airborne vapors and particulates as well as from splashes and direct contact with contaminated surfaces (Levine et al., 1985a).

The U.S. Department of Health and Human Services (1978) Occupational Health Guideline for DNT indicates that hematological (e.g., methemoglobin) and liver function testing should be conducted annually for industrial workers exposed to DNT. In addition, the level of DNT or 2,4-dinitrobenzoic acid (2,4-DNBA, a principal metabolite of DNT) in the urine of industrial workers should be measured on a routine basis (U.S. Department of Health and Human Services, 1978; Woollen et al., 1985; Levine et al., 1985a). However, since it is not currently possible to estimate the absorbed dose of DNT from 2,4-DNBA excretion, it had been suggested that a human dosing study provide these data. However, such a dosing study would be difficult to complete. Ellis et al. (1979) indicated that the most objective hematologic parameters for detecting exposure to toxic doses of 2,4-DNT consist of Heinz body formation and reticulocyte counts. Heinz bodies persist and are easily detected, and increased reticulocytes indicate a compensatory response to erythrocyte destruction prior to frank anemia. Ellis et al. (1979) considered the measurement of methemoglobinemia to be too transient, especially for low levels of exposure.

McGee et al. (1942) reported 36 (23% of the study cohort) cases of anemia and 52 and 55 cases (34 and 36% of the study cohort, respectively) of cyanosis and pallor among 154 employees of a munitions manufacturing facility processing primarily 2,4-DNT (Tables VI-1 and VI-2). Levels of exposure and purity of the 2,4-DNT were not reported. After removal from exposure, recovery from anemia required more than 2 months, although cyanosis and pallor disappeared with 2 days. Two individuals developed jaundice, acute liver and kidney damage, and hyperchromic macrocytic anemia within 3 to 4 weeks following exposure; no instances of permanent physical impairment were identified. Workers were exposed to DNT dust, and absorption was reported

Table VI-1. Symptoms Exhibited by 2,4-DNT-Exposed Workers[a]

Symptom	Number	Percentage
Unpleasant taste in mouth	96	62
Weakness	78	51
Headache	76	49
Loss of appetite	72	47
Dizziness	68	44
Nausea	57	37
Insomnia	57	37
Pain in extremities	40	26
Vomiting	29	19
Loss of weight (5 lbs or more)	10	6.5
Diarrhea	8	5.2

[a]Data based on 154 exposed workers.

SOURCE: McGee et al. (1942).

Table VI-2. Clinical Signs in 2,4-DNT-Exposed Workers[a]

Symptom	Number	Percentage
Pallor	55	36
Cyanosis	52	34
Anemia	36	23
Leukocytosis	19	12
Hypotension	9	5.8
Dermatitis	6	3.9
Leukopenia	5	3.2
Acute toxic hepatitis/ jaundice	2	1.4

[a]Data based on 154 exposed workers.

SOURCE: McGee et al. (1942).

to be primarily via inhalation; however, six cases of compound-related dermatitis were reported. When hygienic measures were followed, the incidence of DNT intoxication decreased.

Levine et al. (1986a,b) reported excessive mortality from ischemic heart disease and residual diseases of the circulatory system. Ischemic heart disease was characterized by myocardial infarction, coronary thrombosis, or occlusion or acute coronary or myocardial insufficiency. Residual circulatory system diseases included congestive heart failure, cardiac arrest, and arteriosclerosis. A Standard Mortality Ratio (SMR) of 141 ($p < 0.01$) was reported for workers exposed to 2,4-DNT (90% pure) and tg-DNT (76% 2,4-DNT, 19% 2,6-DNT, 5% other isomers) at two separate manufacturing facilities following a 15-year latency period. Evidence of a relationship between duration (>5 months) and intensity of exposure to DNT and increased mortality from ischemic heart disease was suggested. Mortality from ischemic heart disease, reported to be related to coronary atherosclerosis, was less than expected prior to the 15-year latency period. Since the heart disease mortality risk of workers is usually lower than that of the general population because of the "healthy worker" effect, it is considered unlikely that risk factors independent of DNT exposure could explain the observed excess of heart disease. Although worker exposure included other chemical agents (nitric and sulfuric acid, toluene, mononitrotoluene), DNT was the only exposure common to both plant cohorts. In addition, duration and concentration of DNT exposure, although estimated, correlated with the extent of mortality from ischemic heart disease. Levine et al. (1986b) stated that compound-related cardiovascular lesions were not reported in bioassays with rats or mice; however, these species were considered to be resistant to naturally occurring or experimentally induced atherosclerosis. This study was conducted to determine whether the carcinogenicity of DNT in rodent bioassays had relevance for humans. No evidence of a carcinogenic effect was exhibited in these cohorts. However, the adequacy of DNT exposure and latency for the development of carcinogenic lesions was questioned by the study author; it was concluded that while the results do not exclude the possibility that DNT may cause human cancer, the risk of this occurrence is small.

A more recent retrospective cohort mortality study of ammunition plant workers exposed to DNT was not consistent with the finding of Levine et al. (1986a,b). Stayner et al. (1989) found that mortality from ischemic heart disease (SMR = 0.95) and cerebrovascular diseases (SMR = 0.98) in DNT-exposed workers was similar to that of nonexposed populations. The study site was one of the facilities included in the Levine et al. studies; however, the workers were from different time periods. Levine et al. (1986a,b) studied workers employed during 1940 to 1950, and Stayner et al. (1989) studied employees who were exposed during 1949 to 1980. Stayner et al. (1989) also analyzed workers employed during a period time that overlapped Levine's studies and did not observe an excess of ischemic heart disease. During the same study, and also contrary to the results reported by Levine et al. (1986a,b), Stayner (1989) detected a greater than twofold increase (SMR = 2.67) in cancer of the liver and biliary passages in DNT-exposed workers. Even though the excess cancers were based only on six cases and were not statistically significant, they were nearly significant ($p = 0.054$) and consistent with increased liver and bile duct cancers in DNT-exposed rodents.

Woollen et al. (1985) indicated that Gillis et al. had conducted an epidemiologic study of the carcinogenic potential of explosives workers using a cohort that included persons exposed to DNT (unpublished). The results did not indicate an excess of cancer at any site in the body; no further data were provided.

Alcohol consumption has a synergistic effect on the toxicity of 2,4-DNT, increasing susceptibility to DNT-induced cyanosis. Exposed workers who ingested alcohol shortly after exposure experienced chest pain, palpitations, head "fullness," and severe, acute illness (U.S. EPA, 1980).

Floret (1929, as cited in U.S. EPA, 1986) reported a case of 2,4-DNT intoxication in which an industrial worker exhibited tremors of the extremities, head, and tongue; visual disturbances; and impaired reflexes. No further information was reported.

Ahrenholz (1980) studied the reproductive effects of exposure to mixed isomers of DNT and toluene diamine (TDA) in 30 male workers employed at a chemical manufacturing facility. Individuals were divided into three groups: those with the highest current potential exposure to DNT (n = 9, Group 1); those with intermittent exposure over several years but none in the preceding 2 years (n = 12, Group 2); and the control group with no known exposure to DNT (n = 9, Group 3). The DNT and TDA concentrations in workroom air ranged from 0.013 to 0.42 mg/m^3 and from 0.008 to 0.39 mg/m^3, respectively, during the initial survey, and from undetectable levels to 0.10 mg/m^3 and from undetectable levels to 0.38 mg/m^3, respectively, during the followup survey. No differences were detected between groups with respect to renal and hepatic profiles or selected endocrine assays. Results indicated a significant ($p < 0.05$) reduction in the sperm counts of exposed workers (Group 1) (55.9×10^6) when compared with controls (156.8×10^6); however, this significant reduction in sperm counts has been questioned by Hamill et al. (1982), who considered the sperm counts of the control group to be abnormally high. Control sperm counts would be expected to be in the range of 80-100 x 10^6. A slight increase in the number of spontaneous abortions was also observed among the wives of exposed workers (Groups 1 and 2). However, Ahrenholz (1980) did not consider this effect to be due to chemical exposure alone.

In response to the Ahrenholz (1980) study, Hamill et al. (1982) conducted a more extensive survey (84 exposed workers, 119 controls) of male workers exposed to DNT and TDA in a similar chemical manufacturing facility. Specific exposure concentrations were not reported, although the authors indicated that exposure levels were below the Occupational Safety and Health Administration (OSHA) threshold limit value for DNT (1.5 mg/m^3). Individuals were divided into groups according to intensity, frequency, and recency of chemical exposure. No differences in sperm counts or morphology, serum follicle-stimulating hormone (FSH) concentration, testicular volume, or fertility were observed among control or exposed workers of any group.

Ahrenholz and Meyer (1982) conducted a followup study of the reproductive effects of exposure to DNT and TDA in 52 male workers employed at a chemical

factory. Exposure categories were based on quantitation and duration of exposure. Area sampling of DNT and TDA ranged from 0.026 to 0.890 mg/m^3 (mean, 0.207 mg/m^3) and below detection limits to 0.687 mg (mean, 0.221 mg/m^3), respectively; personal sampling data were found to be invalid. No significant differences in sperm counts or morphology, liver function, or renal function tests were observed among control or exposed DNT workers of any category. These results disagree with those reported by Ahrenholz (1980).

No significant reductions in fertility were observed in workers from three separate factories exposed to DNT, TDA, sulfuric acid, nitric acid, and nitrobenzene (Levine et al., 1985b). Exposure concentrations, duration of exposure, and chemical purity were not reported. In general, small exposure populations and lack of individual exposure monitoring limit the power of these studies to detect adverse effects on human reproduction.

Urinary metabolites of dinitrotoluenes have been monitored in occupationally exposed workers (Woollen et al., 1985; Levine et al., 1985a; Turner et al., 1985; Turner, 1986). (A more complete review is found in Chapter V, Pharmacokinetics.)

Woollen et al. (1985) monitored urinary levels of 2,4-DNBA in 33 explosives factory workers exposed to tg-DNT. Workers were generally exposed to atmospheric levels of 0.03 to 0.1 mg/m^3, although levels between 0.2 and 2.68 (0.04 ± 0.65) mg/m^3 were measured in more concentrated processing areas. Exposure levels were below the OSHA 8-hour Time-Weighted Average (TWA) exposure of 1.5 mg/m^3. These atmospheric levels of DNT were not considered to be sufficient to account for the observed excretion of 2,4-DNBA. Dermal absorption of DNT was considered to be a major route of uptake secondary to that of inhalation. A concurrent study of five individuals indicated that the highest urinary 2,4-DNBA levels were measured in end-of-shift specimens. Blood levels of DNT peaked at the end of the work shift at 250 ng/mL. Although Woollen et al. (1985) reported that 2,4-dinitrobenzyl alcohol, a major urinary metabolite and precursor of the proximal carcinogen in the rat, could not be detected in the urine of exposed workers, this urinary metabolite

was detected and documented in the studies conducted by Levine et al. (1985a), Turner et al. (1985), and Turner (1986) in DNT manufacturing plant workers (see Chapter V, Pharmacokinetics). These latter studies also indicated that the metabolic pathway for bioactivation of 2,6-DNT occurs to a much lesser extent in humans as compared with rats. The presence of unmetabolized 2,6-DNT in the urine of these workers indicates that not all absorbed 2,6-DNT is available for bioactivation. Since 2,6-DNT is genotoxic and active as a carcinogenic initiator, the absence of evidence of reduced metabolites of 2,6-DNT in the urine of exposed humans may be an important difference concerning the risk of DNT-exposed humans.

Levine et al. (1985a) estimated the atmospheric concentration of 2,4- and 2,6-DNT combined to be between 0.05 and 0.59 mg/m^3 in a DNT manufacturing plant. Seventy-six percent of the DNT in the breathing zone was the 2,4-DNT isomer. Wipe samples of surfaces in the work area contained up to 433 µg 2,4-DNT, and the largest quantity of DNT recovered from the skin was 180 µg (isomer not specified). Clinical effects of exposure for the 17 persons (13 workers, 4 office employees) undergoing analysis of urinary metabolites were not reported. Urinary excretion of DNT metabolites appeared to increase during the workday and to decline to nearly undetectable levels by the start of the following workday, suggesting rapid absorption of DNT following inhalation (Turner, 1986). The proportion of metabolites excreted varied considerably between all workers and within individuals on any one day (Levine et al., 1985a). This was attributed to differences in exposure or to technical error or differences in absorption, metabolic transformation, and excretion.

B. ANIMAL EXPERIMENTS

1. Short-term Exposure (<4-week studies)

a. Acute

The acute toxicity data are summarized in Table VI-3. When administered via the oral route, 2,4-DNT was moderately toxic to mice and highly toxic to

Table VI-3. Acute LD_{50} Values for 2,4- and 2,6-DNT in Mice and Rats

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg) 2,4 DNT	LD_{50} (mg/kg) 2,6 DNT	Reference
Mouse	Swiss	Male	Oral	Peanut oil	1,954 ± 68 (1,848 - 2,178)[a]	621 ± 51 (488 - 721)	Lee et al. (1975)
		Female	Oral	Peanut oil	1,340 ± 67 (1,205 - 1,500)	807 ± 35 (735 - 893)	Lee et al. (1975)
Mouse	CF-1	--[b]	Oral	--	1,630 (1,180 - 2,240)	1,000 (590 - 1,700)	Vernot et al. (1977)
Rat	CD	Male	Oral	Peanut oil	568 ± 59 (434 - 705)	535 ± 58 (397 - 646)	Lee et al. (1975)
		Female	Oral	Peanut oil	650 ± 49 (520 - 743)	795 ± 22 (744 - 844)	Lee et al. (1975)
Rat	Sprague-Dawley	Male	Oral	--	270 (180 - 400)	180 (130 - 240)	Vernot et al. (1977)

[a] The values in parentheses are 95% confidence limits.

[b] Data not reported.

rats. The oral LD_{50} values for 2,4-DNT range from 1,340 to 1,954 mg/kg for mice and from 270 to 650 mg/kg for rats. Toxic signs observed in both species were ataxia and cyanosis; death usually occurred within the first 24 hours or not at all. Surviving animals recovered completely within 48 hours. No treatment-related gross pathology was seen in animals found dead (Lee et al., 1975; Vernot et al., 1977).

The acute toxicity of 2,6-DNT appears to be less species-specific, with oral LD_{50} values ranging from 621 to 1,000 mg/kg in mice and from 180 to 795 mg/kg in rats; male rats were slightly less tolerant than females. Times to death, recovery, and gross pathology were similar for 2,6-DNT and 2,4-DNT (Lee et al., 1975; Vernot et al., 1977).

b. Primary eye and skin irritation and skin sensitization

Neither 2,4- nor 2,6-DNT produced ocular irritation when instilled as a 50% paste in peanut oil (volume not reported) into the eyes of groups of six New Zealand white rabbits (Lee et al., 1975).

In a primary skin irritation test, groups of six New Zealand White rabbits (sex not reported) received a single dermal application of 2,4-DNT (98% pure) or 2,6-DNT (>99% pure) as a 50% paste with peanut oil. Test sites were scored at 24 and 72 hours. Both isomers were classified as very mild skin irritants (mean primary irritation score was 0.21 and 0.25 for 2,4- and 2,6-DNT, respectively) (Lee et al., 1975).

In a skin sensitization test, groups of 10 guinea pigs (sex and strain not reported) were exposed to either 2,4- or 2,6-DNT as a 5% solution; 2,4-DNT was not a skin sensitizer, and 2,6-DNT was a mild sensitizer producing a response in two animals (Lee et al., 1975).

c. Subacute

1) 2,4-DNT

Lane et al. (1985) orally administered to groups of 10 male Sprague-Dawley rats, by gavage, 2,4-DNT (purity not specified) in corn oil at doses of 0, 60, 180, or 240 mg/kg/day for 5 consecutive days. Because of high mortality at the high dose, another group of 15 rats was started at 240 mg/kg/day on the same schedule; 8 of the 15 animals in this group died within 2 weeks after receiving the first dose. No other deaths were seen. Rats at the mid- and high-dose levels exhibited cyanosis; body weight loss was seen at the high dose. This study is discussed in Section VI.B.3, Reproductive Effects.

Groups of 10 female CD-1 mice were orally administered, by gavage, 2,4-DNT (purity not specified) in corn oil daily for 8 consecutive days at 0, 310, 525, 1,250, 2,500, or 3,500 mg/kg body weight (bw). No treatment-related mortality was seen at the low dose. Mortality occurred in six animals dosed at 525 mg/kg. All mice dosed at the higher levels (1,250 to 3,500 mg/kg) died by study day 3. The mean body weight of the surviving rats dosed at 525 mg/kg was significantly ($p < 0.007$) lower on day 8 of treatment and also on day 8 posttreatment. Toxic signs observed were lethargy, dyspnea, rough hair coat, hunched posture, tilted head, tremors, ataxia, and prostration. The NOAEL was 310 mg/kg (Smith, 1983).

McGown et al. (1983) fed groups of five male and five female Sprague-Dawley rats diets containing 0, 900, 1,200, 1,900, or 3,000 mg 2,4-DNT/kg (97% 2-4 DNT, 2% 2,6-DNT, 1% unspecified) for 14 days. This represents an intake of 0, 45, 60, 94, or 143 mg/kg/day, based on an average body weight of 0.25 kg and an average total dietary intake of 0.0175 kg/day (U.S. EPA, 1986). Hematology, clinical chemistry, urinary parameters, and histopathology were evaluated. No treatment-related clinical signs of toxicity were observed. Both the control and treated groups gained weight consistently during the dosing period; however, the rate of weight gain was decreased in a dose-related manner in males and females fed 2,4-DNT. Food consumption was also

depressed in the same manner. No treatment-related effects were seen in hematology or urinary parameters. Serum cholesterol levels were significantly ($p < 0.05$) higher than in the controls in all dosed males and females. Serum glucose levels increased in a dose-related manner in both sexes fed DNT; however, the increase was significant ($p < 0.05$) only in females fed the 143-mg/kg/day diet. The albumin/globulin ratio was significantly ($p < 0.05$) higher than controls in females fed diets containing 60 or 143 mg/kg/day. Alanine aminotransferase levels were significantly ($p < 0.05$) higher than control groups at all levels in males; no significant differences were seen in females. Histopathology revealed hyaline droplet formation (not dose-dependent) in the epithelium of the proximal convoluted tubules of the kidneys in both sexes, with males being more susceptible than females. Oligospermatism with degenerative changes in the testes of male rats occurred in a dose-dependent manner. Based on decreased body weight gain, decreased food consumption, and changes in serum chemistry levels in males and females and testicular lesions in males, the LOAEL was 45 mg/kg/day, the lowest dose tested.

Groups of 10 male Sprague-Dawley rats were maintained on a diet containing 0, 0.1, or 0.2% 2,4-DNT (50 or 100 mg/kg/day, based on Lehman, 1959) for 3 weeks. The final body weights were significantly ($p < 0.01$) lower in treated animals at both dietary levels when compared with controls. No systemic toxicity was seen. This study is discussed in Section VI.B.3, Reproductive Effects (Bloch et al., 1988).

Lee et al. (1978) studied the toxic effects of the subacute (4-week) dietary administration of 2,4-DNT in Swiss mice, CD rats, and beagle dogs. The study in rats was also published by Lee et al. (1985), and the study in dogs was also published by Ellis et al. (1985).

In the study with mice and rats, groups of eight animals/sex/species were fed diets containing 0, 0.07, 0.20, or 0.70% 2,4-DNT (98% pure) for 4 weeks. This represents a corresponding daily intake of 0, 47, 137, or 413 mg/kg/day for male mice; 0, 52, 147, or 468 mg/kg/day for female mice; 0, 34, 93, or 266 mg/kg/day for male rats; and 0, 38, 108, or 145 mg/kg/day for female rats

(U.S. EPA, 1986). At the end of 4 weeks, four animals/group/species were sacrificed. Treatment was discontinued and the surviving animals were observed for 4 more weeks and then sacrificed to study the reversibility of the adverse effects. Hematology and clinical chemistry tests were conducted at 0, 4, and 8 weeks. At necropsy, terminal body weights were measured; the liver, spleen, heart, and kidneys were weighed and organ-to-body weight ratios were calculated. Selected organs were examined histologically. No mortality occurred in mice. The low- and mid-dose levels were nontoxic, and mice at the high-dose level showed slight body weight loss. No abnormalities were seen in blood parameters, organ weights, or gross pathology. Histopathology revealed a mild depression of spermatogenesis in two males at the high dose. After 4 weeks, the mice recovered. The NOAEL was 137 mg/kg/day in males and 147 mg/kg/day in females. Based on body weight loss in males and females and depression of spermatogenesis in males, the LOAEL was 413 mg/kg/day in males and 468 mg/kg/day in females.

2,4-DNT was toxic to both sexes of rats at all levels. At the high dose, two males and two females died; the surviving rats showed body weight loss and decreased food consumption. Rats dosed at the lower levels exhibited a slight depression in body weight gain and food consumption. No consistent changes were observed in hematology or clinical chemistry parameters. A significant ($p < 0.05$) increase was seen in both the absolute and relative liver weights of males fed 93 mg/kg/day and females fed 38 or 145 mg/kg/day. Histopathology revealed mild to moderate hemosiderosis in the spleen of males fed 93 or 266 mg/kg/day and females fed 108 or 145 mg/kg/day. Males at the high dose (266 mg/kg/day) showed aspermatogenesis and testicular atrophy. After 4 weeks, there was only partial recovery; rats regained the weight lost, but the hemosiderosis and testicular lesions were not reversible. Based on body weight loss and decreased food consumption in both sexes, the LOAEL was 34 mg/kg/day in male rats and 38 mg/kg/day in female rats; a NOAEL was not established (Lee et al., 1978).

In the study with dogs, groups of two males and two females were given 2,4-DNT in capsules at doses of 0, 1, 5, or 25 mg/kg/day for 4 weeks. Dogs were observed daily, and body weight and food consumption were measured

weekly; blood samples collected prior to initiation and at termination were analyzed for hematology and clinical chemistry parameters. After 4 weeks of exposure, one male and one female from each group were sacrificed; the remaining animals were maintained on a standard diet and sacrificed following an additional 4 weeks. At termination, the liver, spleen, kidneys, adrenals, and gonads were weighed; organ-to-body weight ratios were calculated; and a bromosulfophthalein (BSP) retention test was performed. All major tissues underwent histopathological examination. No treatment-related toxicity was observed in dogs given 1 or 5 mg 2,4-DNT/kg/day. At the high dose (25 mg/kg/day), dogs showed signs of toxicity after 12 days of treatment. Toxic signs included decreased food consumption, weight loss, yellow stain on and near hind legs, pale gums, neuromuscular incoordination, and paralysis. No significant changes were observed in the hematology or clinical chemistry parameters, and 2,4-DNT did not cause any retention of BSP. Gross necropsy showed that the high-dose dogs were in fair to poor nutritional condition with little or no body fat. Treatment had no effect on organ weights. Histopathology of the high-dose dogs revealed hemosiderosis in the liver, cloudy swelling and tubular degeneration of the kidneys, and lesions of the brain (gliosis, edema, and demyelination of the cerebellum, brain stem, and spinal cord) in both sexes and aspermatogenesis in males. After 4 weeks, dogs showed partial recovery. Two dogs kept for 8 months completely recovered. Based on decreased weight gain, decreased food consumption, neurotoxic signs, and histopathology, the LOAEL was 25 mg/kg/day for males and females, and the NOAEL was 5 mg/kg/day (Lee et al., 1978).

In summary, dogs were the most sensitive species tested; a dose of 5 mg/kg/day had no effect, and a dose of 25 mg/kg/day was toxic. In rats, dietary administration of 34 mg/kg/day to males and 38 mg/kg/day to females, the lowest dose tested, was slightly toxic. Mice were much less affected, since administration of 137 mg/kg/day to males and 147 mg/kg/day to females had no adverse effects, and administration of high doses (413 mg/kg/day to males and 468 mg/kg/day to females) was not as toxic as much lower doses given to rats or dogs. Typical toxic effects observed in all species were decreased body weight gain and food consumption in both sexes and decreased spermatogenesis in males. Dogs showed neuromuscular symptoms of

incoordination and paralysis. After 4 weeks, mice recovered completely, and rats and dogs recovered only partially. Based on the results of this study, the NOAELs and LOAELS shown in Table VI-4 can be established for 2,4-DNT following subacute exposure.

2) 2,6-DNT

Lee et al. (1976) assessed the subacute (4-week) toxicity of 2,6-DNT (>99% pure) in Swiss mice, CD rats, and beagle dogs. The basic design and procedure for these experiments were similar to those for studies conducted with 2,4-DNT (discussed above). 2,6-DNT was administered in the diet to mice and rats at levels of 0.01, 0.05, or 0.25% for 4 weeks. This represents a corresponding daily intake of 0, 11, 51, or 289 mg/kg/day for male mice; 0, 11, 55, or 299 mg/kg/day for female mice; 0, 7, 35, or 145 mg/kg/day for male rats; and 0, 7, 37, or 155 mg/kg/day for female rats. Dogs were given doses of 0, 4, 20, or 100 mg/kg/day in capsules for 4 weeks.

No treatment-related effects were observed in mice (eight/sex/dose) fed the 11-mg/kg/day diet. No treatment-related deaths occurred; however, all male mice dosed at 51 mg/kg/day died by week 3 as a result of fighting. The mid- and high-dose levels caused decreased body weight gain and food consumption. Extramedullary hematopoiesis in the spleen and the liver was increased in both sexes at the high-dose level; males fed the high dose showed aspermatogenesis and testicular atrophy. After a 4-week recovery period following discontinuation of treatment, the testicular lesions were not seen; however, the extramedullary hematopoiesis in the liver or spleen continued to appear in treated mice. The NOAEL was 11 mg/kg/day for males and females. Based on decreased body weight gain and food consumption, the LOAEL was 51 mg/kg/day in males and 55 mg/kg/day in females.

No treatment-related deaths were observed in rats (eight/sex/dose) fed 2,6-DNT. Treated and control rats gained weight during the dosing period. However, the rate of weight gain was decreased in a dose-related manner in treated males and females. Food consumption was also depressed in a dose-related manner. Histopathology revealed hematopoiesis in the spleen and liver

Table VI-4. Adverse Effect Levels for 2,4-DNT Following Subacute Exposure

	Parameter (mg/kg/day)				
	NOAEL		LOAEL		
Species	Males	Females	Males	Females	Basis for LOAEL
Mouse	137	147	413	468	Weight loss.
Rat	--	--	34	38	Decreased body weight gain and food consumption.
Dog	5	5	25	25	Decreased body weight gain and food consumption; neurotoxic signs; and lesions in the brain, liver, kidneys, and testes.

of both sexes and degeneration of spermatogenesis in males. Allowed to recover for 4 weeks, most rats regained their weight, but only partial recovery of the spleen, liver, and testicular lesions was seen. Based on decreased body weight gain and decreased food consumption at the lowest dose tested, the LOAEL was 7 mg/kg/day for males and females; a NOAEL was not established.

Dogs given 4 mg 2,6-DNT/kg/day showed no signs of toxicity. At doses of 20 or 100 mg/kg/day, 2,6-DNT induced body weight loss and reduced food consumption. Dogs exhibited listlessness, incoordination, lack of balance, pale gums, dark urine, and weakness (particularly of the hind limbs). Laboratory tests showed anemia in these dogs with decreased hematocrit, hemoglobin concentration, and compensatory reticulocytosis. Histopathology revealed extramedullary hematopoiesis in the liver and spleen and bile duct hyperplasia in both sexes, and degeneration of spermatogenesis in males. After 4 weeks of recovery, dogs showed some improvement, with lesser amounts of extramedullary hematopoiesis and testicular lesions. Two dogs given 100 mg 2,6-DNT/kg/day and allowed to recover for 19 weeks showed complete recovery. The NOAEL was 4 mg/kg/day. Based on decreased body weight gain and food consumption, the LOAEL was 20 mg/kg/day for both males and females.

In summary, the effects produced by 2,6-DNT were very similar to those seen in parallel studies with the 2,4-DNT isomer. Dogs appeared to be the most sensitive, and mice were the least affected; rats were somewhat less sensitive to 2,6-DNT with a NOAEL of 7 mg/kg/day. Treatment-related effects observed in all species included decreased body weight gain and food consumption, hematopoiesis in the spleen and liver, and degeneration of spermatogenesis. Dogs exhibited clinical signs of central nervous system toxicity that were not seen in rodents. Based on the results of this study, the NOAELs and LOAELs shown in Table VI-5 can be established for 2,6-DNT following subacute exposure.

The Chemical Industry Institute of Toxicology (CIIT, 1977) conducted a 30-day toxicity study in Fischer 344 rats with technical grade DNT (76.4%

Table VI-5. Adverse Effect Levels for 2,6-DNT Following Subacute Exposure

	Parameter (mg/kg/day)				
	NOAEL		LOAEL		
Species	Males	Females	Males	Females	Basis for LOAEL
Mouse	11	11	51	55	Decreased body weight gain and food consumption.
Rat	--	--	7	7	Decreased body weight gain and food consumption.
Dog	4	4	20	20	Clinical signs, weight loss, and lesions in the spleen, liver, and testes.

2,4-DNT, 18.83% 2,6-DNT, 4.6% other isomers). Groups of 10 male rats and 10 female rats were fed diets containing 0, 37.5, 75, or 150 mg DNT/kg/day for 30 days. Animals were observed daily, and body weight and food consumption were measured weekly. Blood samples collected from females on day 27 and from males on day 28 were analyzed for methemoglobin, reticulocytosis, and Heinz body formation. At termination, gross pathology was evaluated, but no histopathology was conducted. All animals survived the 30 days of treatment. Urine stains on the fur were seen in six rats in the high-dose group. Two females in the mid-dose group developed alopecia around the eyes; no clinical signs were observed at the low dose. The mean body weights of females in the high-dose group and of males in all groups were significantly ($p < 0.05$) lower than those of the controls. Suppression of body weights occurred earliest and to the greatest extent in rats fed the high-dose diet. The mean food consumption was slightly lower for rats in the low-dose group, moderately lower for rats in the mid-dose group, and markedly lower for rats in the high-dose group. A significant ($p < 0.05$), dose-related increase in mean values was observed for methemoglobin, reticulocyte counts, and Heinz body formation, with the exception of mean percent methemoglobin for females in the mid-dose group. Methemoglobin values for females in the low-dose group and for males and females in the high-dose group were significantly ($p < 0.05$) higher than the control values. Treatment-related gross pathological alterations seen in rats in the high-dose group included discoloration, enlargement, and irregular surfaces of the spleen in both sexes, and discoloration of the kidneys in males. In addition, males at all dietary levels had livers with discolorations and/or surface irregularities. Based on decreased body weight gain and food consumption, blood effects, and gross pathological changes in male rats, the LOAEL was 37.5 mg/kg/day, the lowest dose tested.

3. <u>tg-DNT</u>

No subacute studies on tg-DNT were found in the available literature.

2. Longer-term Exposure

a. 13-Week studies

1) 2,4-DNT

The subchronic toxicity of 2,4-DNT was evaluated in 13-week studies with mice (Hong et al., 1985), rats (Lee et al., 1985), and dogs (Ellis et al., 1985). These studies also were reported by Lee et al. (1978).

Hong et al. (1985) fed groups of 16 male and 16 female CD-1 mice diets containing 0, 0.07, 0.20, or 0.70% 2,4-DNT (98% pure) for 13 weeks. This represents a corresponding daily intake of 0, 47, 137, or 413 mg/kg/day for males and 0, 52, 147, or 468 mg/kg/day for females. Mice were observed daily, and body weight and food consumption were recorded weekly. At the end of 13 weeks, four males and four females were sacrificed; treatment was discontinued for the remaining animals. These animals (recovery group) were observed for an additional 4 weeks and were then sacrificed. At termination, blood was collected for a standard battery of hematology and clinical chemistry tests. The liver, spleen, kidneys, heart, and brain were weighed, and organ-to-body weight ratios were calculated. Major tissues from all animals underwent histopathological examination. The results of this study are summarized in Table VI-6. A total of five mice died during the study. A dose-dependent decrease in body weight gain was observed for males, and decreased food consumption was seen in males in the high-dose group. Females in the high-dose group exhibited decreased body weight gain and food consumption. Both sexes of mice fed the high-dose diet showed anemia characterized by a decrease in erythrocyte count, hematocrit, and hemoglobin concentrations, concurrent with reticulocytosis. Histopathology revealed mild hepatocellular dysplasia and pigmentation in Kupffer cells of the liver in both males and females, and mild degeneration of seminiferous tubules in males in the high-dose group. Mild hepatocellular dysplasia was also seen in some males (number not specified) in the mid-dose group. After 4 weeks, mice recovered completely. The LOAEL was 47 mg/kg/day, based on body weight loss in males, and 52 mg/kg/day in females; a NOAEL was not established.

Table VI-6. Summary of Subchronic Toxicity of 2,4-DNT in Mice

Parameter	Males				Females			
2,4-DNT in feed (%):	0	0.07	0.2	0.7	0	0.07	0.2	0.7
(mg/kg per day):	0	47	137	413	0	52	147	468
Average feed intake (g/day/mouse)	2.3	2.2	2.2	1.9	2.2	2.1	2.1	1.7
Total weight gain (g/mouse)	5.0	3.3	1.9	-2.9	2.9	4.1	3.5	-0.3
Number of deaths/total number of mice	0/8	1/8	0/8	2/8	0/8	0/8	0/8	2/8
Hematology								
Anemia[a]				+				+
Lesions								
Testicular degeneration[a]				+				
Hepatocellular dysplasia[a]			+	+				+
Pigment deposition[a]				+				+

[a]+ indicates positive finding of hematologic change or lesion; these changes were not exhibited in control or low-dose males or control, low-, or mid-dose females.

SOURCE: Hong et al. (1985).

Lee et al. (1985) reported the results of a subchronic toxicity study with CD rats fed diets containing 0, 0.07, 0.20, or 0.70% 2,4-DNT (98% pure) for 13 weeks. This represents a corresponding daily intake of 0, 34, 93, or 266 mg/kg/day for males and 0, 38, 108, or 145 mg/kg/day for females. The basic design and procedure for these experiments with rats were similar to those described for mice (Hong et al., 1985). The results of the study with rats are summarized in Table VI-7. 2,4-DNT administered at lower levels produced minimal adverse effects; administration at higher levels caused severe adverse effects. All high-dose females died within 3 weeks; one male at the mid-dose level and six at the high-dose level died between weeks 4 and 13. Dose-dependent decreases in body weight gain and food consumption were observed in both sexes. Orange to yellow colored urine stains were observed on the fur of high-dose rats, and one male rat had widespread and stiff hind legs. Hematologic indices (erythrocyte count, hematocrit, hemoglobin) indicated anemia with concurrent reticulocytosis in mid- and high-dose males and females. Absolute liver and kidney weights were slightly increased in mid-dose males; relative liver, kidney, and brain weights of these animals were significantly increased statistically. Relative increases in organ weights of other dosed animals were consistent with body weight reduction in these animals. Histopathology revealed splenic hemosiderosis in mid- and high-dose males and females. Spermatogenesis was decreased in mid-dose males and was completely arrested in high-dose males. One high-dose male showed some signs of neuromuscular effects with demyelination in the cerebellum and brain stem. The LOAEL was 34 mg/kg/day in males and 38 mg/kg/day in females based on decreased body weight gain and food consumption; a NOAEL was not established. Hemosiderosis and testicular lesions of mid- and high-dose animals were not reversible following the 4-week recovery period. Gliosis and demyelination of the cerebellum of one mid- and one high-dose male were also found in these recovery animals.

Ellis et al. (1985) gave 2,4-DNT in capsules to groups of two male and two female dogs at doses of 0, 1, 5, or 25 mg/kg/day for 13 weeks. At the end of 13 weeks, one pair from each group was sacrificed. The remaining pair was maintained on a control diet and observed for an additional 4 weeks, and was

Table VI-7. Summary of Subchronic Toxicity of 2,4-DNT in Rats

Parameter	Males				Females			
2,4-DNT in feed (%):	0	0.07	0.2	0.7	0	0.07	0.2	0.7
(mg/kg per day):	0	34	93	266	0	38	108	145
Average feed intake (g/day per rat)	24.3	21.6	19.6	10.6	17.0	14.8	14.4	5.0
Total weight gain (g/rat)	138	35	-11	-157	35	2	-10	
Number of deaths/total number of rats	0/8	0/8	1/8	6/8	0/8	0/8	0/8	8/8
Hematology								
Reticulocytosis[a]			+	+		+	+	
Anemia[a]				+				+
Lesions								
Organ weight changes[a]			+	+			+	
Abnormal pigments in spleen[a]			+	+			+	+
Spermatogenesis[b]			+	++				
Demyelination (cerebellum, brain stem)[a]			+	+				

[a]+ indicates positive finding of hematologic change or lesion; these changes were not exhibited in control or low-dose males or females.
[b]+ indicates decreased spermatogenesis; ++ indicates aspermatogenesis.

SOURCE: Lee et al. (1985).

then sacrificed. Dogs were observed daily, and body weight was measured weekly. Hematology and clinical chemistry tests were conducted prior to treatment and at termination. A BSP retention test was conducted at termination. 2,4-DNT did not induce toxicity in the low- and mid-dose groups. In the high-dose groups, 2,4-DNT was toxic after 12 to 22 days and was lethal after 22 or more days. The primary target organs were the neuromuscular system, erythrocytes, and testes. Dogs showed great variation in individual susceptibility. All affected dogs exhibited decreased food consumption, weight loss, urine stains on the fur, pale gums, neuromuscular incoordination, and paralysis. Hematologic indices indicated the presence of methemoglobinemia, anemia, and Heinz bodies. Treatment did not cause any retention of BSP. Gross pathology indicated that the dogs were in fair to poor nutritional condition with little or no body fat. Histopathology revealed hemosiderosis in the liver and spleen and cloudy swelling of the kidneys of males and females and aspermatogenesis in males. Lesions of the brain observed in high-dose dogs sacrificed during weeks 6 and 7 were gliosis, edema, and demyelination of the cerebellum, spinal cord, and brain stem. After 4 weeks, dogs recovered partially from the various effects. Based on mortality, body weight loss, hematological abnormalities, neurological signs, and histopathological alterations, the LOAEL was 25 mg/kg/day and the NOAEL was 5 mg/kg/day.

Based on the results of these studies, the NOAELs and LOAELs shown in Table VI-8 can be established for 2,4-DNT following subchronic (13 weeks) exposure.

2) <u>2,6-DNT</u>

Lee et al. (1976) conducted subchronic toxicity studies of 2,6-DNT (>99% pure) in CD-1 mice, CD rats, and beagle dogs. The basic experimental design and procedures were similar to those described above in studies conducted with 2,4-DNT. Mice and rats were fed diets containing 0, 0.01, 0.05, or 0.25% 2,6-DNT for 13 weeks. This represents a corresponding daily intake of 0, 11, 51, or 289 mg/kg/day for male mice; 0, 11, 55, or 299 mg/kg/day for female

Table VI-8. Adverse Effect Levels for 2,4-DNT Following Subchronic Exposure

	Parameter (mg/kg/day)					
	NOAEL		LOAEL			
Species	Males	Females	Males	Females	Basis for LOAEL	Reference
Mouse	--	--	47	52	Decreased body weight gain.	Hong et al. (1985)
Rat	--	--	34	38	Decreased body weight gain and food consumption.	Lee et al. (1985)
Dog	5	5	25	25	Weight loss, clinical signs, hematological effects, and lesions in the spleen, liver, kidneys, testes, and brain.	Ellis et al. (1985)

mice; 0, 7, 35, or 145 mg/kg/day for male rats; and 0, 7, 37, or 155 mg/kg/day for female rats. Dogs were given 0, 4, 20, or 100 mg 2,6-DNT/kg/day in capsules for 13 weeks.

No treatment-related effects were observed in mice in the low-dose group, and only a few mice in the mid-dose group showed weight loss. 2,6-DNT administered at high levels produced decreased body weight gain and food consumption, hematopoiesis in the liver and/or spleen, bile duct hyperplasia in both sexes, and depression of spermatogenesis and atrophy of the testes in males. After 4 weeks, mice showed partial recovery as indicated by weight gain and the disappearance of testicular lesions; however, hematopoiesis continued in the liver or spleen. Based on decreased body weight and food consumption and histopathologic changes in the spleen, liver, bile duct, and testes observed at the mid-dose level, the LOAEL was 51 mg/kg/day in males and 55 mg/kg/day in females. The NOAEL was 11 mg/kg/day for both males and females (Lee et al., 1976).

No adverse effects were seen in rats in the low-dose groups. Mid-dose males and females exhibited decreased body weight gain and food consumption, extramedullary hematopoietic activity in the liver and spleen, and bile duct hyperplasia; males also exhibited depressed spermatogenesis and testicular atrophy. Rats in the high-dose groups were severely affected. The effects included decreased body weight, body weight gain, and food consumption, methemoglobinemia, Heinz body formation, anemia, compensatory reticulocytosis, severe extramedullary hematopoiesis in the spleen and liver, bile duct hyperplasia, and renal degeneration in both sexes. In males, by the end of 13 weeks, the testicular lesions had progressed to encompass virtually all connective tissues. After 4 weeks, only partial recovery was observed; rats regained the weight lost, but lesions continued to occur in the spleen, liver, and testes of treated rats. Based on weight loss, blood effects, and histopathology, the LOAEL was 35 mg/kg/day in male rats and 37 mg/kg/day in female rats. The NOAEL was 7 mg/kg/day for both sexes (Lee et al., 1976).

In the study with dogs, Lee et al. (1976) did not observe adverse effects in the low-dose group (4 mg/kg/day); however, 2,6-DNT was toxic at higher

levels. All high-dose (100 mg/kg/day) males (4/4) and females (4/4) died between study weeks 2 and 8; 2 of 4 mid-dose (20 mg/kg/day) females died during study week 9. Toxic effects included decreased food consumption leading to weight loss; listlessness; incoordination leading to rigid paralysis with occasional tremors; elevation in serum alkaline phosphatase, serum glutamic-pyruvic transaminase (SGPT, currently referred to as "alanine aminotransferase" or "ALT"), and/or blood-urea-nitrogen (BUN); methemoglobinemia leading to Heinz body formation; anemia with compensatory reticulocytosis and extramedullary hematopoiesis; and lymphoid depression leading to peripheral lymphocytopenia. Histopathology revealed bile duct hyperplasia and degenerative and inflammatory changes in the liver and kidneys in both sexes, and degeneration and atrophy of the spermatogenic cells of the testes. Effects in the high-dose animals were more pronounced and appeared earlier than those in the mid-dose animals. A great variation in the onset of symptoms was seen among dogs given the same dose. The effects were partially reversed in 4 weeks and completely reversed in 19 weeks following cessation of treatment. The NOAEL was 4 mg/kg/day. Based on mortality, weight loss, blood effects, neurological signs, and histopathology, the LOAEL was 20 mg/kg/day.

In summary, dogs were the most sensitive species; doses of 4 mg/kg/day had no effect, and doses of 20 mg/kg/day were toxic. Rats were somewhat less sensitive, since 7 mg/kg/day was nontoxic. Higher doses (35 mg/kg/day in males and 37 mg/kg/day in females) produced a variety of toxic effects; more severe, earlier effects were seen in the high-dose group. The effects observed in rats and dogs exposed to 2,6-DNT were quantitatively and qualitatively similar to those reported for 2,4-DNT. The main effects on the erythrocytes and testes were virtually identical. In addition, 2,6-DNT induced bile duct hyperplasia. Effects in mice were similar to those in rats: the NOAEL was 11 mg/kg/day, and 51 mg/kg/day in males and 55 mg/kg/day in females caused dose-related toxicity. In all three species, the primary effect was methemoglobinemia; numerous sequelae included Heinz body formation, reticulocytosis, anemia, and extramedullary hematopoiesis. Bile duct hyperplasia, sometimes accompanied by hepatic degeneration or elevated serum enzymes, was seen in all species. Males of all species had depressed spermatogenesis and, in some cases, the effect was severe leading to

testicular atrophy. The principal effects seen in dogs, but not in mice or rats, were incoordination leading to rigid paralysis. Dogs also showed renal degeneration and elevated BUN levels. Based on the results of this study, the NOAELs and LOAELs shown in Table VI-9 can be established for 2,6-DNT following subchronic (13 weeks) exposure.

3) tg-DNT

No 13-week studies on tg-DNT were found in the available literature.

b. 6-Month study

Kozuka et al. (1979) fed a group of 20 male Wistar rats diets containing 0.5% 2,4-DNT (purity not reported) for a period of 6 months. The estimated ingestion of 2,4-DNT was 66 mg/day for the first 3 months and 75 mg/day for the last 3 months. Based on an average weight of 0.35 kg for an adult rat, this represents a daily intake of 190 and 214 mg/kg/day for the first and the last 3 months, respectively. A group of 23 rats served as controls. Rats were observed daily, and body weight and food consumption were measured weekly. Blood samples were collected for the determination of methemoglobin and for biochemical tests. Mortality occurred in two treated rats during week 2; 10 rats died between weeks 14 and 22, and 1 control rat died at week 14. Toxic signs observed in treated rats were piloerection, whitened skin color, humpback, jerky movements, decreased spontaneous movement, and general weakness. Treated animals showed a slower growth rate, and body weights were lower during the study. At the end of study, treated animal body weights were approximately 58% lower than controls. A significant ($p < 0.01$) increase was observed for relative liver, spleen, and kidney weights, and relative testicular weights were significantly lower. A significant ($p < 0.01$) sevenfold increase was seen in methemoglobin formation after 6 months based on measurements in five animals. After 1 month of treatment, significant ($p < 0.01$) differences were observed in serum components (triglycerides, glucose, albumin, and albumin/globulin ratios) and in serum enzymes (serum

Table VI-9. Adverse Effect Levels for 2,6-DNT Following Subchronic Exposure

	Parameter (mg/kg/day)				
	NOAEL		LOAEL		
Species	Males	Females	Males	Females	Basis for LOAEL
Mouse	11	11	51	55	Body weight loss; lesions in spleen, liver, and testes.
Rat	7	7	35	37	Body weight loss; hematological effects; lesions in the liver, spleen, and kidneys.
Dog	4	4	20	20	Body weight loss; hematological effects; neuromuscular incoordination; lesions in the liver, spleen, kidneys, and testes.

glutamic-oxaloacetic transaminase [SGOT, now referred to as "aspartate aminotransaminase" or "AST"], serum glutamate pyruvate transaminase [SGPT, now referred to as "alanine aminotransferase" or "ALT"], alkaline phosphatase [AlP], and acid-phosphatase) of treated rats as compared with controls. Gross pathology revealed the formation of puruloid matter and hypertrophy in the liver, and atrophy of the testes. No histopathology was conducted.

c. Lifetime/chronic studies

1) 2,4-DNT

Leonard et al. (1987) observed significant reductions in body weight gain ($p < 0.05$) and significant increases in liver weight (approximately 150% of control) of 24 male CDF (F344)/CrlBR rats following administration of purified 2,4-DNT (>99.4% pure) in the diet for 12 months (6-month interim sacrifice of 4 rats/group) at concentrations that resulted in doses of 27 mg/kg/day. Hepatocytic degeneration and vacuolation were apparent in the majority of treated animals. Acidophilic foci were present in 70% of the animals, whereas basophilic foci were encountered in only 10% of the livers. Neither type of focus was apparent in the controls. Bile duct hyperplasia was noted in the majority of treated animals, and a highly variable incidence of cholangiofibrosis was observed. Based on changes in the liver and biliary tract in male rats, the LOAEL is 27 mg/kg/day, the only dose tested.

Ellis et al. (1979) evaluated the chronic toxicity of 2,4-DNT (≥98.5% 2,4-DNT; ≤1.5% 2,6-DNT) in dogs, rats, and mice. The study in dogs was also reported by Ellis et al. (1985); the study in rats was also reported by Lee et al. (1985); and the study in mice was also reported by Hong et al. (1985).

Groups of six male and six female beagle dogs were fed 2,4-DNT daily in gelatin capsules containing 0, 0.2, 1.5, or 10 mg/kg. Thorough clinical and histopathological examinations were performed on each dog following sacrifice. According to the protocol, one pair of dogs was to be killed for necropsy after 1 year and another pair was allowed a 4-week recovery period to assess reversibility. A similar procedure was to be used for the remaining four

pairs of dogs at 24 months. However, three of the six high-dose males were killed for necropsy (at weeks 8, 18, and 19) following the development of progressive paralysis starting in the hind legs and progressing to the forelimbs, head, and neck. Initial symptoms included rapid weight loss, intermittent tremors or convulsions, and vomiting. The death of another high-dose dog (week 5) was unrelated to treatment. The two remaining males in the high-dose group were continued to the end of 24 months, when one was necropsied and the other was placed on the recovery study.

One high-dose female dog showed uncoordinated movements especially in the hind limbs during week 10. Symptoms were most apparent 1 to 2 hours after dosing; none were apparent by week 13. Intermittent symptoms indicative of neurotoxicity observed in the surviving high-dose dogs lasted a few days or, at most, weeks, and recurred weeks or months later. Recovery was facilitated by supplying affected dogs with a soft diet when incoordination prevented them from consuming the standard food.

In the mid-dose group (1.5 mg/kg/day), the first toxic signs were observed in one male dog during week 66. Symptoms, including loss of muscular control of the hindquarters and occasional convulsive tremors, lasted about a week and recurred occasionally during the remainder of the study. No other mid-dose dog displayed such signs. One mid-dose female dog was found dead during week 98, but the cause of death was not apparent.

The primary hematological effects were on erythrocytes; female dogs in the high-dose group developed anemia with the erythrocyte count depressed by 20% after 3 months of treatment. Adaptive compensation was indicated by increases in the mean cell volume (MCV) and mean cell hemoglobin (MCHB) and decreases in the total hemoglobin as the proportion of reticulocytes increased. The cause of anemia was reflected in the small, inconsistent methemoglobinemia and the more considerable, more consistent presence of Heinz bodies. After 18 or 24 months of dosing, only a slight anemia or none at all was evident with reticulocyte levels near normal, no Heinz bodies, and minimal methemoglobin. Minimal amounts of methemoglobin were found in most of the high-dose dogs, in half of the mid-dose dogs, and in very few low-dose dogs.

Although not statistically significant, these findings appear to be toxicologically significant.

Histopathologic examination after 24 months revealed several lesions that were related to treatment with 2,4-DNT. Central nervous system lesions in the three high-dose dogs that developed paralysis and were killed for necropsy during weeks 8, 18, and 19 included vacuolization, endothelial proliferation, gliosis of the cerebellum, and perivascular hemorrhage in the cerebellum and the brain stem. A mild bile duct hyperplasia was seen, and clusters of brown pigment-laden Kupffer cells were found in the livers of all surviving high-dose dogs and in one low-dose dog. Cystic hyperplasia of the epithelium occurred in the gallbladder of all high-dose dogs, one mid-dose dog, and one control dog. Brown epithelial pigmentation was seen in the gallbladder, kidneys, and spleen of two high-dose dogs, and in the gallbladder of three mid-dose dogs.

In summary, the primary targets affected by chronic oral dietary administration of 2,4-DNT to dogs were the nervous system, erythrocytes, and biliary tract (Ellis et al., 1979, 1985). The major adverse effect was neurotoxicity, characterized by incoordination and paralysis. Effects were observed within 2 months in all dogs given 25 mg/kg/day (see Section VI.B.2.a, Subchronic Toxicity), within 6 months in all dogs receiving 10 mg/kg/day, and during month 16 in one dog given 1.5 mg/kg/day. A wide range of variability was observed among individuals as to the onset and severity of the neuropathy. Central nervous system lesions including vacuolization, endothelial proliferation, and gliosis of the cerebellum were present in three of the high-dose dogs. The primary erythrocyte effect, methemoglobinemia, was rarely seen in the chronic study, but sequelae, including the presence of Heinz bodies and reticulocytosis, were evident in the mid- and high-dose groups within 3 months. During the second year, these erythrocyte effects were minimal, presumably because of an adaptive response by the dogs. Methemoglobinemia and its sequelae were the only reversible effects in dogs allowed a 4-week recovery period. Mild biliary hyperplasia was seen in most dogs receiving 10 mg/kg/day and in some dogs given 1.5 mg/kg/day. Based on neurotoxicity, hematologic changes, and effects on biliary tract

histopathology in dogs, the LOAEL is 1.5 mg/kg/day and the NOAEL is 0.2 mg/kg/day.

In a chronic study with rats, diets containing 0, 0.0015, 0.01, or 0.07% 2,4-DNT (0, 15, 100, or 700 ppm) were administered to groups of 38 male and 38 female CD (Sprague-Dawley) rats, yielding an average intake of 0.57, 3.9, or 34 mg/kg/day, respectively, for males and 0.71, 5.1, or 45 mg/kg/day, respectively, for females (Ellis et al., 1979; Lee et al., 1985). After 12 months, eight males and eight females from each group were killed for necropsy; the remaining rats were sacrificed after 24 months. Cumulative deaths in high-dose males and females were significantly higher than in controls; 50% mortality occurred in high-dose rats by month 20 and in controls by month 23. Weight gains in high-dose animals were markedly reduced, reaching a plateau considerably below control levels after 2 months in females and after 4 months in males. Growth in mid-dose males and females began to decrease during month 9, while low-dose rats exhibited growth rates comparable to those of controls. Anemia, partially compensated as evidenced by increased reticulocyte counts, was prominent in mid- and high-dose males and high-dose females after 12 months. Histopathological examination revealed the progressive development of hyperplastic foci in the liver, the severity of which was increased in high-dose males and mid-dose females (Table VI-10). There was marked atrophy of the testes with severe atrophy of the seminiferous tubules and almost complete lack of spermatogenesis in 86% (6/7) high-dose males at 12 months. Beyond 12 months, severe atrophy of the seminiferous tubules occurred in 16% of the control males and in 25% of the low-dose, 32% of the mid-dose, and 83% of the high-dose males. Based on the incidence of changes in the seminiferous tubules of male rats and in the liver of female rats, the LOAEL is 0.57 mg/kg/day, the lowest dose tested.

In a chronic study with mice, diets containing 0, 0.01, 0.07, or 0.5% 2,4-DNT (0, 100, 700, or 5,000 ppm) were administered to groups of 38 male and 38 female CD-1 mice, yielding an average intake of 0, 14, 95, or 898 mg/kg/day (Ellis et al., 1979; Hong et al., 1985) using procedures similar to those described for rats by Lee et al. (1985). Unscheduled deaths occurred considerably sooner in the high-dose mice; all males and females in this group died by months 18 and 21, respectively. Weight gain was drastically reduced

Table VI-10. Incidence of Histopathological Lesions in CD Rats Fed 2,4-DNT for 24 Months

Lesion	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.57	3.9	34	0	0.71	5.1	45
Testicular atrophy	4/25 (16)[a]	7/28 (25)	6/19 (32)	25/30 (83)				
Splenic hemosiderosis	4/25 (16)	5/28 (18)	1/19 (5.3)	5/30 (17)	3/23 (13)	5/35 (14)	3/27 (11)	7/35 (20)
Liver hyperplastic foci or cellular alteration	9/25 (36)	10/28 (36)	9/19 (47)	16/29 (55)	7/23 (30)	18/35 (51)	19/27 (70)	13/34 (38)

[a]Number of rats with lesions/number of rats examined for lesions (percent incidence).

SOURCE: Ellis et al. (1979).

in all high-dose mice with effects more pronounced in males than in females. Males consistently weighed less than controls after months 11 (low-dose) and 3 (mid-dose), whereas females in these groups weighed about the same as controls. Toxic anemia and concurrent reticulocytosis occurred in high-dose males and females after 12 months. In addition, all high-dose mice displayed increases in relative spleen and liver weights after 12 months of treatment. Mice at all dietary levels exhibited an increased, dose-related incidence of an unidentified, brown pigment in many tissues; the intensity increased with length of treatment. This pigment was most prominent in the liver and spleen but was also present in the lungs, kidneys, adrenals, bone marrow, heart, gonads, and central nervous system (CNS). Hepatocellular dysplasia, characterized by metabolic, degenerative, and hyperplastic alterations of cells, was observed in all treated males (17/25, 11/20, and 31/32 low-, mid-, and high-dose males, respectively) and in high-dose females (24/25) (Table VI-11). Renal tumors in males and testicular or ovarian atrophy in mid- and high-dose males and high-dose females, respectively, were also prominent lesions (see Carcinogenicity, Section VI.B.5, and Reproductive Effects, Section VI.B.3). Based on liver changes in male mice, the LOAEL is 14 mg/kg/day, the lowest dose tested.

In an NCI (1978) bioassay, groups of 50 male and 50 female Fischer 344 rats were fed diets containing 0.008 or 0.02% 2,4-DNT (80 or 200 ppm; >95% purity) for 78 weeks and observed for another 26 weeks. Assuming that rats consume approximately 5% of their body weight in food each day, the diets were equivalent to 4 and 10 mg/kg/day of DNT, respectively (Lehman, 1959). Corresponding control groups containing 25 (low-dose control) or 50 (high-dose control) rats of each sex were assigned because the low- and high-dose groups were tested at different times. Necropsies were performed on each rat that died, that was killed when moribund, or that was sacrificed at the end of the bioassay. Gross and microscopic histopathological examinations were conducted on major tissues. The only significant clinical observation was a mean body weight depression that was evident as early as week 16 in high-dose males. At the end of the assay, this group exhibited a mean average body weight about 25% less than controls, and high-dose females exhibited an 18% decrease in mean body weight. The incidence and variety of nonneoplastic lesions in the major organs were similar in control and treated rats.

Table VI-11. Incidence of Histopathological Lesions in Mice Fed 2,4-DNT for 12 or 24 Months

Finding	Dosage (mg/kg/day)							
	Males				Females			
	0	14	95	898	0	14	95	898
Generalized abnormal pigmentation								
12 months	0/8	0/8	0/8	0/8	0/8	0/8	0/8	8/8
24 months	0/25	19/25	6/21	32/32	0/23	13/21	12/23	23/25
Hepatocellular dysplasia								
12 months	1/8	1/8	1/8	8/8	0/8	1/8	0/8	5/8
24 months	1/25	17/25	11/20	31/32	5/23	3/21	5/23	24/25
Testicular/ovarian atrophy								
12 months	0/8	1/8	1/8	7/8	1/8	0/8	0/8	6/8
24 months	8/24	3/25	13/20	26/30	1/29	2/23	0/27	15/24

SOURCE: Hong et al. (1985).

A similar protocol was used for B6C3F, mice (NCI, 1978). Fifty mice of each sex were exposed to 2,4-DNT at dietary concentrations of 0, 0.008, or 0.04% (0, 80, or 400 ppm, respectively) for 78 weeks followed by 13 weeks without treatment. Because the low- and high-dose groups were tested at different times, each was assigned its own control group. Mean body weight depression was observed in all treated groups by week 30. At study termination, the approximate weight gain for low-dose males was 91% of control; for high-dose males, 82%; for low-dose females, 89%; and for high-dose females, 76%. No clinical observations were reported, and no increase was reported for the incidence of nonneoplastic lesions in treated mice as compared with controls.

Based on studies by Leonard et al. (1987), Ellis et al. (1979), and NCI (1978), the LOAELs and NOAELs shown in Table VI-12 can be established following chronic or lifetime exposure.

2) <u>2,6-DNT</u>

Leonard et al. (1987) observed significant reductions in body weight gain and significant increases in liver weight following dietary administration of purified 2,6-DNT (>99.4% pure) to 24 male CDF (F344)/CrlBR rats for 12 months (6-month interim sacrifice of 4 rats/group) at concentrations that resulted in doses of 7 or 14 mg/kg/day. Serum alanine aminotransferase was elevated following administration of both doses of 2,6-DNT for 1 year, and serum gamma-glutamyl transferase was increased after 26 and 52 weeks at the high dose. Hepatocytic degeneration and vacuolization were apparent in the majority of treated animals, although these effects were not shown to be dose-dependent. Acidophilic and basophilic foci were observed in over 90% of the animals fed 2,6-DNT. Neither type of focus was apparent in the controls. Bile duct hyperplasia was noted in the majority of treated animals, and a highly variable incidence of cholangiofibrosis was observed. Based on changes in the serum enzyme levels, the liver, and the biliary tract histopathology in male rats, the LOAEL is 7 mg/kg/day, the lowest dose tested.

Table VI-12. Adverse Effect Levels for 2,4-DNT Following Chronic or Lifetime Exposure

Species	Isomer (% Purity)	Exposure Duration/ Route	Effects	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference(s)
Dog						
Beagle	2,4-DNT (98%)[a]	24 months/ diet	Biliary hyperplasia; anemia; Heinz bodies; paralysis; death	0.2	1.5	Ellis et al., 1985; Ellis et al., 1979
Rat						
SD	2,4-DNT (98%)	24 months/ diet	Anemia; hepatic foci; testicular atrophy	3.9	34	Lee et al., 1985; Ellis et al., 1979
F344	2,4-DNT (>99%)	12 months/ diet	Decreased body weight; increased liver weight; hepatocytic degeneration; bile duct hyperplasia	None	27[b]	Leonard et al., 1987
F344	2,4-DNT (>95%)	78 weeks/ diet	Decreased body weight	None	200 ppm	NCI, 1978
Mouse						
CD1	2,4-DNT (98%)	24 months/ diet	Decreased body weight; increased liver and spleen weights; organ and tissue pigmentation; testicular and ovarian atrophy	None	14	Hong et al., 1985; Ellis et al., 1979

[a]Other 2% consists of other isomers, but primarily 2,6-DNT.

[b]Only one dose tested.

Dinitrotoluene-induced hepatotoxicity was investigated in relationship to structure-activity parameters using six DNT isomers (Spanggord et al., 1990). Sprague-Dawley rat hepatocyte suspensions were used as the biological test system. All isomers produced a concentration-dependent inhibition of protein synthesis and lactate dehydrogenase (LDH) release during 4-hour incubations. This finding suggested that DNT isomers as well as their metabolites are directly hepatotoxic without the need for metabolic activation. Protein synthesis inhibition occurred at lower DNT concentrations than those affecting LDH release; therefore, protein synthesis inhibition was the most sensitive indicator for hepatotoxicity. None of the isomers affected lipid peroxidation. The results also showed that the ortho- and the para-isomers were more toxic at the same concentration than the meta-isomers regardless of the indicator. The authors concluded that based on the present metabolite analysis (HPLC) and other reports, the reduction of the nitro groups of the parent compounds plays the primary role in determining the potential of hepatotoxicity of these compounds.

3) <u>tg-DNT</u>

Leonard et al. (1987) administered a technical grade mixture of dinitrotoluenes in the diet to 24 male CDF (F344)/CrlBR rats for 12 months (6-month interim sacrifice of 4 rats/group) at concentrations that resulted in a dose of 35 mg/kg/day. The tg-DNT was prepared by mixing purified DNT isomers in a ratio representative of a standard technical grade, with a final composition of 76.5% 2,4-DNT, 18.8% 2,6-DNT, 2.43% 3,4-DNT, 1.54% 2,3-DNT, 0.69% 2,5-DNT, and 0.04% 3,5-DNT. Feeding tg-DNT for 6 months or 1 year significantly reduced body weight gain and significantly increased liver weights at 1 year. No effects were seen on serum alanine aminotransferase or serum gamma-glutamyl transferase activities. Hepatocytic degeneration and vacuolization were apparent in the majority of treated animals, although these effects were not shown to be dose-dependent. Acidophilic and basophilic foci were observed in over 90% of the animals fed tg-DNT. Neither type of focus was apparent in the controls. Bile duct hyperplasia was noted in the majority of treated animals, and a highly variable incidence of cholangiofibrosis was

observed. Based on changes in the body weight, liver, and biliary tract in male rats, the LOAEL for tg-DNT is 35 mg/kg/day, the only dose tested.

The toxicologic effects of tg-DNT (composition not reported) in Charles River CDF (F344) rats were evaluated following administration at dietary levels of 0, 3.5, 14.0 and 35.0 mg/kg/day (130 rats/sex/group) in a study conducted by CIIT (1982). Sacrifice and necropsy of 10 rats/sex/treatment group occurred at weeks 26 and 52; 20 rats/sex/group were sacrificed at week 78, all surviving high-dose rats at week 55, and all surviving animals at termination after 104 weeks. A significant dose-related decrease in body weight gain was seen in all treated groups throughout the first year of the study. After 54 weeks, a dose-related decrease in mean body weight was observed at all dose levels in both sexes. Analysis of hematological data suggested a low-grade regenerative anemia in high-dose males and females. Anemia at this dose level was also indicated by the increased hemosiderin and extramedullary hematopoiesis in the spleen, which was indicative of enhanced red cell turnover. Analysis of organ weight data revealed alterations in several organs. Consistent significant increases were observed in relative liver and brain weights in all treated rats, relative kidney weights in all but low-dose males, and relative ovarian weights in low- and mid-dose females. At 104 weeks, absolute organ weight increases were reported for liver and kidney in both sexes at the mid-dose level and for liver in both sexes at the low-dose level. Absolute testicular weight was increased in low-dose males at 104 weeks. Gross necropsy findings revealed alterations in the liver characterized by white or yellow foci in all treated males and females as well as nodules and tissue masses in mid-dose males and females.

At the end of 1 year, histopathological examination showed hepatotoxicity in all mid- and high-dose animals and in low-dose males, characterized by areas of cell alteration, hyperbasophilia and megalocytosis of hepatocytes, and vacuolization and necrosis of individual hepatocytes. Other treatment-related lesions in the high-dose males were observed in the kidney, pancreas, testes, spleen, and bone marrow. Following 78 weeks of treatment, primary histologic findings pertained to the increased incidence of neoplasms in the liver (see Section VI.B.5, Carcinogenicity). Hepatotoxicity was observed in

mid-dose males, as was an exacerbation of chronic interstitial nephritis in mid-dose males and females. At the end of 2 years, low- and mid-dose animals exhibited a high incidence of hepatoxicity as well as chronic interstitial nephritis accompanied by parathyroid hyperplasia and adenoma. In addition, an enhancement of degenerative changes in the adrenal glands was noted in mid-dose rats. In general, treatment-related histologic tissue alterations were characterized by the presence of hepatic neoplasms in all treatment groups, an increased incidence of mammary fibroadenomas in all mid-dose rats, and an increased incidence of subcutaneous fibromas and sarcomas primarily in mid-dose males (see Section VI.B.5, Carcinogenicity). Based on histopathological changes in the liver, kidney, and parathyroid gland in rats, the LOAEL for tg-DNT is 3.5 mg/kg/day, the lowest dose tested.

3. Reproductive Effects

a. 2,4-DNT

The dominant lethal effects of 2,4-DNT in rats were studied by Lane et al. (1985). Groups of 10 male Sprague-Dawley rats were administered, by oral gavage, 2,4-DNT (purity not reported) in corn oil at 0, 60, 180, or 240 mg/kg/day for 5 days. Significant reductions in the mating index were observed in rats administered 240 mg/kg/day; a sharp decrease in sperm-positive and pregnant females was observed at this dose level. Because of this finding, statistical evaluation of the results was difficult. However, no dominant lethal effects, characterized by early fetal deaths, were observed. Dose levels at or below 180 mg/kg/day did not result in changes in fertility or fetal death. The authors concluded that exposure to 2,4-DNT did not result in dominant lethal mutations but did adversely affect reproductive performance.

In a study by Bloch et al. (1988), groups of 9 to 10 male Sprague-Dawley rats were fed 2,4-DNT (purity 97%) at dietary levels of 0, 0.1, or 0.2% (0, 1,000, or 2,000 ppm, respectively) for 3 weeks, and the effects of 2,4-DNT on the testes were evaluated. Body weights were significantly reduced ($p < 0.05$) at 2,000 ppm when compared with controls. Significant increases ($p < 0.05$) in

serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were observed in rats ingesting 2,000 ppm. Total sperm count was also significantly reduced ($p < 0.01$) at 2,000 ppm. No significant reductions were found at 1,000 ppm. Rats fed 2,000 ppm demonstrated extensive disruption of spermatogenesis with vacuolization and other cytoplasmic alterations of Sertoli cells. These included varying sized vesicles, swollen mitochondria, and distended endoplasmic reticulum. Degeneration of spermatocytes and spermatids was present, and irregularity in the basal lamina and distortion of the peritubular tissue were observed. No microscopic changes were observed in rats fed 1,000 ppm when compared with controls. The NOAEL is 1,000 ppm, which corresponds to a daily intake of approximately 80 mg/kg/day (assuming that rats consume approximately 5% of their body weight in food daily [Lehman, 1959], and body weights were approximately 325 g).

Ellis et al. (1979) evaluated the reproductive effects of 2,4-DNT in rats following dietary exposure for three generations. Groups of 10 to 24 Sprague-Dawley rats of each sex were fed diets containing 0, 15, 100, or 700 ppm (approximately 0, 0.75, 5, or 35 mg/kg/day) 2,4-DNT (purity 98%) for up to 6 months prior to mating. Each parental generation produced two sets of offspring (F_a and F_b litters); the study was terminated during the third generation after weaning of the second litter (F_b).

Parental body weights were significantly reduced ($p < 0.05$) at the high-dose level during the first two generations. Fertility was low for all animals from the control and test groups during the first (F_0) generation (Table VI-13). This probably was due to the age of the animals at mating (8 months). The survival of high-dose pups (F_{1b}) from the first generation was greatly reduced (Table VI-14). Four of five litters died, and subsequently, only three males and three females (F_1) were available for mating during the second generation. The fertility of the high-dose F_1 animals was reduced, and consequently, no litter data were available from the second mating of this generation, and a third generation was not produced. Mean litter size and pup weight at weaning were slightly, but not significantly, lower for high-dose pups when compared with controls from the

Table VI-13. Effects on the Fertility and Gestation Length of Parental Rats Fed 2,4-DNT in the Diet for Three Generations

Dietary level (ppm)	Mating index (%)	Pregnancy rate (%)	Gestation length (days)
F_0 generation			
0	75	57	23
15	84	62	23
100	76	59	23
700	87	48	23
F_1 generation			
0	100	100	22
15	91	83	22
100	97	92	22
700	67	75	22
F_2 generation			
0	97	97	22
15	97	97	22
100	95	100	22
700	--[a]	--	--

[a]No pups produced.

SOURCE: Ellis et al. (1979).

Table VI-14. Summary of Reproductive Effects in Rats Fed 2,4-DNT in the Diet for Three Generations

Litter generation	Dietary level (ppm)	Mean litter size	Live birth index (%)	Mean pup birth weight (g)	Viability index (%)	Lactation index (%)	Mean pup weight at weaning (g)	Sex ratio
F_{1a}	0	6.9±1.1	85	7.2±0.3	70	61	45±8	60
	15	6.8±1.0	83	7.1±0.2	69	91	53±2	50
	100	4.9±1.1	89	7.4±0.1	60	61	38±10	63
	700	4.5±1.1	94	7.5±0.4	64	90	53±6	48
F_{1b}	0	12.7±1.8	91	7.0±0.5	100	91	55±7	37
	15	8.2±1.9	100	7.2±0.8	99	70	45±9	50
	100	9.7±1.1	88	7.5±0.4	83	83	44±13	54
	700	7.4±1.2	90	7.0±0.3	20*	78	44	57
F_{2a}	0	12.7±0.6	98	7.0±0.2	98	96	43±2	50
	15	13.9±0.4	97	6.6±0.1	99	95	41±1	52
	100	13.8±0.4	99	6.9±0.2	94	95	42±1	48
	700	11.0±1.0	100	6.4±0.2	89	100	35±1	66
F_{2b}	0	14.0±0.3	96	7.2±0.1	98	92	42±1	48
	15	15.4±0.6	95	6.8±0.3	96	83	41±2	41
	100	14.2±0.8	97	7.1±0.2	94	97	41±2	45
	700	--[a]	--	--	--	--	--	--
F_{3a}	0	11.3±0.4	97	7.1±0.1	96	95	39±1	49
	15	12.2±0.6	95	7.1±0.2	96	93	36±1	38
	100	12.6±0.5	98	6.9±0.1	94	87	35±1	43
	700	--	--	--	--	--	--	--
F_{3b}	0	12.2±0.6	99	6.4±0.1	94	94	40±1	43
	15	13.2±0.4	98	6.4±0.2	95	87	35±1*	38
	100	13.6±0.5	98	6.5±0.1	97	91	35±2*	46
	700	--	--	--	--	--	--	--

[a]No pups produced.
*Significantly different from controls ($p < 0.05$).

SOURCE: Ellis et al. (1979).

first generation. Slight reductions in body weight at weaning were also observed in low- and mid-dose pups from the first and third generations. However, parental fertility and offspring viability were not affected at the low- and mid-dose levels. The parental NOAEL and LOAEL for this study were 100 and 700 ppm, respectively, based on consistent significant reductions in body weight at 700 ppm. The NOAEL and LOAEL for reproductive effects were 100 and 700 ppm (approximately 5 and 35 mg/kg/day), respectively, based on severe reductions in fertility at 700 ppm.

Although testicular weights were not recorded in the reproduction study discussed above, significant reductions ($p < 0.05$) in relative (to brain) testicular weight were observed in Sprague-Dawley rats (38 animals/group) from a concurrent study fed 700 ppm (approximately 35 mg/kg/day) 2,4-DNT for 12 months or longer (Lee et al., 1985). These rats developed severe testicular atrophy and reduced spermatogenesis. However, no testicular effects were observed in rats similarly fed approximately 100 ppm (3.9 mg/kg/day).

In a 13-week subchronic study in which male CD-1 mice (16 animals/group) were fed 2,4-DNT, mild testicular degeneration was observed at approximately 1,000 ppm (137 mg/kg/day) but not at 350 ppm (47 mg/kg/day) (Hong et al., 1985). Testicular degeneration and atrophy and decreased spermatogenesis were observed in male mice (58 mice/sex/group were initially used) fed 700 ppm (approximately 95 mg DNT/kg/day) for up to 24 months (Ellis et al., 1979). In the same study, a lack of follicular function and reductions in the number of corpora lutea were observed in female mice fed 5,000 ppm (898 mg/kg/day) for 24 months. Similar effects were observed in male beagles (six dogs/group) administered 2,4-DNT in gelatin capsules at 25 mg/kg/day for up to 24 months; the dogs exhibited mild to severe testicular degeneration and reduced spermatogenesis (Ellis et al., 1985). No testicular effects were observed in dogs administered 10 mg/kg/day. (See Sections VI.B.2.a, 13-Week Studies, and VI.B.2.c, Lifetime Studies, for further discussion.) No data on the reproductive toxicity of 2,6-DNT or tg-DNT were found in the available literature.

b. 2,6-DNT

No studies of reproductive toxicity were found in the available literature.

c. tg-DNT

No studies of reproductive toxicity of the technical grade mixture of dinitrotoluene were found in the available literature.

4. Developmental Toxicity

a. 2,4-DNT

In a series of screening tests, 60 chemicals were evaluated for developmental toxicity potential in CD-1 mice (Hardin et al., 1987). Fifty time-mated females were administered, by oral gavage, 2,4-DNT in corn oil at 390 mg/kg/day on gestation days 6-13. The dams were observed twice daily during treatment and once daily before and after treatment for mortality and clinical signs of toxicity. The dams were allowed to deliver their pups, and on postnatal day 1, the number of live pups and litter weight were recorded. On postnatal day 3, the number of pups, litter weight, and maternal body weight were recorded. External, visceral, and skeletal examinations of pups were not conducted.

Maternal toxicity as evidenced by a 30% mortality rate (15/50 dams died during the study) was observed. However, 2,4-DNT had no effect on maternal weight gain, the number of viable litters, the number of live pups born, birth weight and weight gain of the pups, or the survival of offspring. Although the pups were not examined for abnormalities, the authors stated that malformations observed in a conventional developmental toxicity study would be expressed in this system as reduced litter size, decreased birth weight, and impaired survival. Therefore, 2,4-DNT was not considered to be teratogenic in this study. However, the authors recommended the use of this screening test

as a tool for prioritizing conventional testing rather than as a predictive test for teratogenicity.

b. 2,6-DNT

No data on the developmental toxicity of 2,6-DNT were found in the available literature.

c. tg-DNT

In a study by Price et al. (1985), the teratogenic potential of technical grade DNT was investigated in Fischer 344 rats. The test material was a mixture of 2,4-DNT (76%), 2,6-DNT (19%), 3,4-DNT (2.4%), 2,3-DNT (1.5%), 2,5-DNT (<1%), and 3,5-DNT (<1%). This study was conducted in two phases to evaluate the possible teratogenicity of DNT as well as DNT effects on postnatal development. For the teratogenic phase, groups of 5 to 20 time-mated rats were administered, by gavage, DNT in corn oil at 0, 14, 35, 37.5, 75, 100, or 150 mg/kg/day on gestation days 7-20. A group of 20 time-mated females was administered 200 mg hydroxyurea/kg/day and served as positive controls. In addition to collection of other maternal and fetal data at terminal sacrifice on gestation day 20, blood was collected from dams and fetuses (pooled by litter) in the vehicle and positive control groups and in the 100-mg/kg/day group. Methemoglobin content and reticulocyte, red blood cell (RBC), and platelet counts were the hematologic parameters measured. Mean corpuscular volume (MCV) and red blood cell distribution width (RDW) were calculated.

Mortality was high at the 150-mg/kg/day level; 46% of the dams died prior to gestation day 18. Mortality was similar among controls and other treatment groups. Clinical signs of toxicity, including lethargy, rough hair coat, and weakness of the hind legs, were observed in dams given 150 mg/kg/day beginning on gestation day 11. Body weight and corrected body weight gain (minus gravid uterine weight) were significantly decreased ($p < 0.01$) in dams administered 150 mg/kg/day. Corrected body weight gain was also significantly reduced in dams receiving 14 ($p < 0.05$) or 100 ($p < 0.01$) mg/kg/day. A significant

increase ($p < 0.01$) in relative (to body weight) liver weight was observed in dams administered 75 or 100 mg/kg/day. An increase in relative liver weight was also observed at 150 mg/kg/day, but the difference was not significant. Relative spleen weights were significantly increased in dams receiving 35, 37.5, 75, 100, and 150 mg/kg/day when compared with controls. Dams administered 100 mg/kg/day exhibited significant increases in methemoglobin ($p < 0.01$), reticulocyte count ($p < 0.05$), MCV ($p < 0.05$), RDW ($p < 0.05$), and platelet count ($p < 0.05$). In addition, significant decreases ($p < 0.05$) in RBC count and hematocrit were observed in the DNT-dosed dams when compared with controls. No differences in the number of corpora lutea, implantations, or live and dead fetuses were observed among the vehicle control and test groups. An increase in the percent resorptions was observed at the high-dose level; 16.8, 2.3, 4.1, 14.6, 11.0, 12.7, and 46.0% resorptions were noted in dams administered the vehicle or DNT at levels of 0, 14, 35, 37.5, 75, 100, and 150 mg/kg/day, respectively. Although the difference was not statistically significant, the increase was indicative of a compound-related embryotoxic effect. No differences in litter size, sex ratio, fetal weight, crown-rump length, or placental weight were observed among vehicle control and treatment groups. The incidences of malformations and variations were similar among vehicle control and test groups. All abnormalities observed were spontaneous in nature. Therefore, DNT was not teratogenic in rats. However, some developmental effects were noted. Relative liver weight was significantly reduced ($p < 0.01$) for fetuses from the 14-mg/kg/day group and significantly increased for fetuses from the 35-mg/kg/day group. Significant increases in relative spleen weight were observed in fetuses from the 35- and 75-mg/kg/day groups. Reticulocyte count ($p < 0.05$) and RBC count ($p < 0.01$) were significantly increased ($p < 0.05$) in the fetuses of dams administered 100 mg/kg/day when compared with the vehicle control group.

For the postnatal phase, groups of 5-7 time-mated rats were administered, by gavage, DNT in corn oil (2 mL/kg) at 14, 35, 37.5, 75, or 100 mg/kg/day on gestation days 7-20; in addition, groups of 11-14 time-mated females were similarly administered corn oil (vehicle control) or 200 mg/kg hydroxyurea (positive control). The dams were allowed to deliver their litters and were sacrificed on postnatal day 30. The following data were collected for each

pup on postnatal day 0: sex, body weight, crown-rump length, external abnormalities, clinical signs of toxicity, and mortality. The litters were then culled to eight pups (four/sex), if possible. Liver and spleen weight and blood samples were collected from randomly selected, culled pups on postnatal day 0. The postnatal physical (e.g., righting reflex, pinna unfolding, hair growth) and neurobehavioral development (e.g., open field testing) of surviving pups were evaluated during the remainder of the study. Pups were randomly sacrificed on postnatal day 10, 25, or 50, and remaining pups were sacrificed on postnatal day 60. Liver and spleen weights were collected for one pup/litter and for all dams sacrificed at the above-specified intervals during the study. In addition, testicular weights from pups sacrificed on postnatal day 60 were recorded. Blood was collected from dams and pups administered DNT at 75 or 100 mg/kg/day, hydroxyurea, or the vehicle at sacrifice, and the above-specified hematological parameters were analyzed.

Three females (one from each group) from the 14-, 35-, and 100-mg/kg/day groups died during the study. No other deaths were reported. As seen during the teratogenic phase, corrected body weight (minus gravid uterine weight) was significantly reduced at 100 mg/kg/day during gestation. Reduced body weight was still evident on postnatal day 15 in dams receiving 100 mg/kg/day, but the reduction apparently was not evident at postnatal day 30 (final sacrifice), indicating that some recovery had occurred. The only other maternal effect was a significant decrease ($p < 0.05$) in reticulocyte count at 75 mg/kg/day on postnatal day 30. Other hematologic parameters, including methemoglobin levels, were similar among dams from the vehicle control and from the 75- and 100-mg/kg/day groups. Relative liver weights were slightly, but nonsignificantly, higher in dams receiving DNT at 35 mg/kg/day or at greater levels than controls. Relative spleen weights were similar among the vehicle control and test groups.

No dose-related patterns in developmental effects were observed in pups from dams administered DNT when compared with the vehicle controls. A significant decrease ($p < 0.01$) in female pup weight was observed at 75 mg/kg/day, but this was associated with a significant increase ($p < 0.05$) in

litter size at this dose level and was not compound related. A slight, but nonsignificant, increase in postnatal death was observed in offspring at 100 mg/kg/day. However, this was apparently due to the loss of one entire litter and did not appear to be related to DNT administration. Significant increases ($p < 0.05$-0.01) in relative liver weight were observed in pups from all DNT-dosed groups on postnatal day 0 and in pups from the 37.5- and 75-mg/kg/day groups on postnatal day 50. However, relative spleen and testicular weights were comparable among the vehicle control and DNT-dosed groups. In addition, physical and neurobehavioral development were similar among offspring from the vehicle control and test groups; no significant differences were noted. Changes in hematologic parameters were observed in offspring, but they were not considered to be dose related. Reticulocyte count was significantly increased ($p < 0.01$) in pups from the 75-mg/kg/day group and significantly decreased ($p < 0.01$) in pups from the 100-mg/kg/day group. In addition, a significant increase ($p < 0.05$) in MCV was observed in pups from the 75-mg/kg/day group. Methemoglobin levels were similar among vehicle control and DNT-treated pups.

The maternal NOAEL and LOAEL for the study were 14 and 35 mg/kg/day, respectively, based on a significant increase in relative spleen weight in dams receiving DNT at levels of 35 mg/kg/day or greater (Price et al., 1985). Changes in hematologic parameters, including methemoglobin content, were observed in dams administered 100 mg/kg/day. However, analysis of blood was conducted only in dams receiving the vehicle or DNT at 100 mg/kg/day, and therefore, a definitive assessment of hematologic effects could not be accomplished. The NOAEL and LOAEL for developmental toxicity were 14 and 35 mg/kg/day, respectively, based on significant increases in relative liver and spleen weight in the fetuses of dams administered DNT at levels of 35 mg/kg/day or greater. However, the numbers of animals per group for each phase of this study were not adequate to evaluate the teratogenic potential of DNT.

5. Carcinogenicity

a. 2,4-DNT

When fed in the diet in 2-year bioassays, 2,4-DNT induced benign tumors in Fischer 344 rats (males and females), benign and malignant tumors in CD rats (males and females), and malignant tumors in CD-1 mice (males only). No treatment-related increase in tumors was observed in B6C3F, mice, in beagle dogs, or in F344 rats fed 2,4-DNT for 1 year. These studies have been reviewed in detail in Section VI.B.2.b, Lifetime Studies, and carcinogenicity findings are discussed below.

In an NCI (1978) bioassay, groups of 50 male and 50 female Fischer 344 rats were fed diets containing 0.008 or 0.02% 2,4-DNT (80 or 200 ppm; >95% purity) for 78 weeks and observed for another 26 weeks. Assuming that rats consume approximately 5% of their body weight in food each day, the diets were equivalent to 4 and 10 mg/kg/day of DNT, respectively (Lehman, 1959). Corresponding control groups containing 25 (low-dose control) and 50 (high-dose control) rats of each sex were assigned because the low- and high-dose groups were tested at different times. At termination, gross and microscopic histopathological examinations were conducted on major tissues. The survival rate for the two treatment groups did not differ significantly from their respective controls, with 52-64% of the males and 48-62% of the females surviving until the end of the study. Reduced body weight gain in the high-dose groups indicated that a Maximum Tolerated Dose (MTD) had been approached. As shown in Table VI-15, a significantly increased incidence of fibroma of the skin and subcutaneous tissue occurred in both high- and low-dose males, and a significantly increased incidence of fibroadenoma of the mammary gland was observed in high-dose females. These benign lesions were the only tumors reported to have incidences significantly greater than the respective control groups; these lesions are within the range of those reported for strain-matched historical controls (Goodman et al., 1978).

A similar protocol was used for B6C3F, mice (NCI, 1978). Fifty mice of each sex were exposed to 2,4-DNT at dietary concentrations of 0, 0.008, or

Table VI-15. Tumor Incidence in F344 Rats Fed 2,4-DNT for 78 Weeks[a]

Lesion	Sex	Control		Low dose (80 ppm)	High dose (200 ppm)
		Low dose	High dose		
Subcutaneous tissue or skin fibroma	M	0/46	0/25	7/49 (p = 0.008)[b]	13/49 (p = 0.003)[b]
Mammary gland fibroadenoma	F	9/48	4/23	12/49 NS[c]	23/50 (p = 0.016)[b]

[a]Incidence given as number of tumor-bearing animals/number of animals examined at that site.
[b]Significantly different from respective control by Fisher's Exact test.
[c]NS = Not statistically significant.

SOURCE: Adapted from NCI (1978).

0.04% (80 or 400 ppm) for 78 weeks followed by 13 weeks without treatment. Assuming that mice consume approximately 15% of their body weight in food each day, the diets were equivalent to 12 and 60 mg/kg/day DNT, respectively (Lehman, 1959). Because the low- and high-dose groups were tested at different times, each was assigned its own control group. Survival rates were similar for treated and control mice of both sexes, ranging between 74 and 90% for males and between 70 and 84% for females. Mean body weight depression was observed in all treated groups by week 30. No statistically significant increase in tumors was observed in mice of either sex.

Ellis et al. (1979) evaluated the carcinogenicity of 2,4-DNT (≥98.5% 2,4-DNT and ≤1.5% 2,6-DNT) in rats, mice, and dogs. The study in rats was also reported by Lee et al. (1985); the study in mice was also reported by Hong et al. (1985); and the study in dogs was also reported by Ellis et al. (1985).

In the study with rats (Ellis et al., 1979; Lee et al., 1985), diets containing 0, 0.0015, 0.01, or 0.07% 2,4-DNT (0, 15, 100, or 700 ppm) were administered to groups of 38 male and 38 female CD (Sprague-Dawley) rats, yielding an average intake of 0.57, 3.9, or 34 mg/kg/day, respectively, for males and 0.71, 5.1, or 45 mg/kg/day, respectively, for females. Eight males and eight females from each group were killed for necropsy after 12 months, and the surviving rats were sacrificed after 24 months. Unscheduled deaths for both sexes began after 12 months of treatment and increased until the end of the study. Cumulative deaths reached 100% (high dose), 75% (mid dose), and 55% (low dose and controls) for male rats and 97% (high dose), 65% (mid dose), and 60% (low dose and controls) for females. Histopathology data obtained from rats that died after 12 months were used in calculating tumor incidences. An increased incidence of hepatocellular carcinoma was seen in high-dose male and female rats, although statistical significance ($p < 0.05$) was reached only in females (Table VI-16). Mammary tumors were prevalent in all female dose groups; benign lesions (fibroadenoma, adenoma, and fibroma) predominated. The incidence of these mammary gland lesions reached statistical significance only for the high-dose group. The frequency of malignant tumors, specifically adenoma-carcinoma, was consistently low in all treatment groups.

Table VI-16. Tumor Incidence in CD Rats Fed 2,4-DNT for 24 Months[a]

Lesion	Males (mg/kg/day)				Females (mg/kg/day)			
	0	0.57	3.9	34	0	0.71	5.1	45
Hepatocellular neoplastic nodule	1/25 (4)	2/28 (7)	1/19 (5)	2/29 (7)	0/23	3/35 (9)	2/27 (7)	6/34 (18) ($p = 0.07$)[b]
Hepatocellular carcinoma	1/25 (4)	0/28	1/19 (5)	6/29 (21) ($p = 0.08$)[b]	0/23	0/35	1/27 (4)	18/34 (53) ($p = 4.3 \times 10^{-6}$)[b]
Neoplastic nodule or carcinoma	2/25 (8)	2/28 (7)	2/19 (10)	8/30 (27) ($p = 0.07$)[b]	0/23	3/35 (9)	3/27 (11)	23/35 (66) ($p = 9.5 \times 10^{-8}$)[b]
Mammary gland tumors[c]	0/25	0/28	0/19	2/30 (7)	11/23 (48)	12/35 (34)	17/27 (63)	33/35 (94) ($p \leq 0.0001$)[d]
Skin tumors[e]	2/25 (8)	4/28 (14)	3/19 (16)	17/30 (57)	1/22 (5)	3/35 (9)	0/27	6/35 (17)
Pituitary adenoma	9/22 (41)	14/23 (61)	7/14 (50)	2/20 (10)	18/23 (78)	24/30 (80)	20/24 (83)	7/23 (30)

[a]Based on data from animals fed 2,4-DNT for more than 12 months. Incidence given as the number of tumor-bearing rats/number of rats examined at that site (percent incidence). Rats that died before week 52 are not included in the calculation of tumor incidence.
[b]Fisher's Exact test performed by Syracuse Research Corporation (SRC), as cited in U.S. EPA (1986).
[c]Includes benign and malignant tumors from epithelial or mesenchymal cells.
[d]Fisher's Exact test performed by reviewers.
[e]Includes epidermal, epithelial, and subcutaneous mesenchymal tumors.

SOURCE: Ellis et al. (1979).

In the mouse study (Ellis et al., 1979; Hong et al., 1985), diets containing 0, 0.01, 0.07 or 0.5% 2,4-DNT (0, 100, 700, or 5,000 ppm, respectively) were administered to groups of 38 male and 38 female CD-1 mice yielding an average intake of 0, 14, 95, or 898 mg/kg/day, respectively, using procedures similar to those described for rats by Lee et al. (1985). Unscheduled deaths occurred early in the high-dose mice; all males in this group died by month 18 and all females died by month 21. Since most of the high-dose mice died before 12 months (31/38 males and 23/38 females), this group was not considered to be representative of a lifetime exposure and was excluded from further analysis. Approximately 70% of the control and low-dose and 85% of the mid-dose males and 80% of the control and low- and mid-dose females died before scheduled sacrifice. Mice that died after 12 months were used in calculating tumor incidences. There was no evidence of a treatment-related increase in tumor frequency in any tissue in female mice. Among males, a very high incidence of renal tumors, including cystic papillary adenomas, solid renal cell carcinomas, and cystic papillary carcinomas, was seen in the mid-dose (700 ppm) group (Table VI-17). Similar lesions were observed in the low-dose males, but the incidence was not significantly different from controls. Liver tumors, described as most probably hepatomas, were observed in male mice of all dose groups, but no significant difference from controls was seen.

In the study with dogs (Ellis et al., 1979; Ellis et al., 1985), groups of six male and six female beagle dogs received daily doses of gelatin capsules containing 0, 0.2, 1.5, or 10 mg 2,4-DNT/kg. The high-dose level was toxic to all dogs and proved lethal to five. The mid-dose level was toxic to some of the dogs, whereas the low-dose level had no apparent adverse effects. A thorough clinical and histopathological examination was performed on each dog following sacrifice. No evidence of carcinogenicity was seen in any of the dogs fed 2,4-DNT for 2 years.

In a 12-month study, Leonard et al. (1987) observed significant reductions in body weight gain ($p < 0.05$) and increases in liver weight (approximately 150% of control) following dietary administration of purified 2,4-DNT (>99.4% pure) to 20 male CDF (F344)/CrlBR rats at concentrations

Table VI-17. Tumor Incidence in Male CD-1 Mice Fed 2,4-DNT for 24 Months[a]

Lesion	Dose (mg/kg/day)		
	0	14	95
Liver tumor[b]	7/33	9/33	8/28
Kidney			
Benign	0/33	3/33	3/28 ($p = 0.014$)[c]
Malignant	0/33	5/33	16/28 ($p = 1.45 \times 10^{-8}$)[c]
Benign or malignant	0/33	8/33	19/28 ($p = 1.32 \times 10^{-9}$)[c]
			($p = 0.059$)[c]
All tumors (Kidney + liver)	7/33	17/33	24/28 ($p = 2.17 \times 10^{-5}$)[c]

[a]Incidence given as the number of tumor-bearing mice/number of mice examined at that site. Animals that died before week 52 are not included in the calculation of tumor incidence. Since most of the high-dose mice died before 1 year, the entire group has been excluded.
[b]Tumor type not specified.
[c]Fisher's Exact test performed by SRC, as cited in U.S. EPA (1986).

SOURCE: Ellis et al. (1979).

yielding doses of 27 mg/kg/day. One neoplastic nodule of the liver was found. Due to some limitations (i.e., the short study duration and the low number of animals), the study failed to demonstrate the carcinogenic activity of the chemical.

b. 2,6-DNT

In a 12-month study, Leonard et al. (1987) administered purified 2,6-DNT (>99.4% pure) to 20 male CDF (F344)/CrlBR rats at dietary concentrations yielding doses of 0, 7, or 14 mg/kg/day. At both dose levels, 2,6-DNT significantly reduced body weight gain and increased liver weight. Hepatocellular carcinomas were found in 17/20 (85%) low-dose and in 19/19 (100%) high-dose male rats, and pulmonary metastases were found in 3/20 (15%) low-dose and in 11/19 (58%) high-dose rats. Cholangiocarcinomas were reported in 2/20 (10%) low-dose and 0/19 high-dose males. No neoplastic lesions were found in the control group.

c. tg-DNT

In a 12-month study, Leonard et al. (1987) observed significant reductions in body weight gain and increases in liver weight following dietary administration of a technical grade mixture of dinitrotoluenes to 20 male CDF (F344)/CrlBR rats at concentrations providing a dose of 35 mg/kg/day. The tg-DNT was prepared by mixing purified DNT isomers in a ratio representative of a standard technical grade, with a final composition of 76.5% 2,4-DNT, 18.8% 2,6-DNT, 2.43% 3,4-DNT, 1.54% 2,3-DNT, 0.69% 2,5-DNT, and 0.04% 3,5-DNT. Hepatocellular carcinomas were observed in 9/19 (47%) and cholangiocarcinomas in 2/19 (11%) of the male rats fed tg-DNT for 1 year. No neoplastic lesions were found in the control group of 20 males.

The carcinogenic effects of tg-DNT (composition not reported) were evaluated in Charles River CDF (F344) rats (130 rats/sex/group) following administration at dietary levels providing 0, 3.5, 14.0, and 35.0 mg/kg/day (CIIT, 1982). Sacrifice and necropsy of 10 rats/sex/group occurred at weeks 26 and 52; 20 rats/sex/group were sacrificed at week 78, all surviving high-dose rats at week 55, and all surviving animals at termination after 104

weeks. A statistically significant dose-related decrease in body weight gain was seen in all treated groups throughout the first year of the study. After 54 weeks, a dose-related decrease in mean body weight was observed at all dose levels in both sexes.

Hepatocellular carcinomas were first evident in 2/10 high-dose males sacrificed at 26 weeks. At 52 weeks, a high incidence of hepatocellular carcinomas and hepatic neoplastic nodules was evident in the high-dose group and the mid-dose males. Two cholangiocarcinomas observed in the high-dose males were considered to be treatment-related because this neoplasm is unusual in CDF rats. Biliary hyperplasia with atypia of bile duct epithelium that occurred in two high-dose and one mid-dose male may have been precursor lesions of cholangiocarcinoma. All high-dose rats were terminated at week 55 because of a significant decrease in survival. Histological examination revealed hepatocellular carcinomas in all males (20/20) and most females (11/20), with hepatic neoplastic nodules in 5/20 males and 12/20 females. Cholangiocarcinomas were observed in three males, and biliary hyperplasia with atypia of bile duct epithelium was observed in two males. At 78 weeks, a high incidence of hepatocellular carcinomas and hepatic neoplastic nodules was observed in the mid-dose animals and to a much lesser degree in the low-dose animals. Increased incidence of cholangiocarcinomas and probable precursor lesions was seen in the mid-dose males along with some benign lesions including mammary fibroadenomas and subcutaneous fibromas. The significance of pituitary chromophobe adenomas observed in mid-dose females was equivocal. At 104 weeks, a high incidence of hepatocellular carcinomas and hepatic neoplastic nodules was seen in mid-dose animals and to a lesser degree in low-dose animals (Table VI-18). The incidence of hepatocellular carcinomas showed a dose-related increase in males, and pulmonary metastatases were found in seven mid-dose males and females. The incidence of hepatic neoplastic nodules was dose-related in both males and females. Increased incidences in the relatively common benign neoplasms were also observed at 78 weeks. These included mammary fibroadenomas in mid-dose rats and low-dose males; subcutaneous fibromas (a dose-related increase), particularly in male rats; and testicular mesothelioma in mid-dose males (equivocal). An exacerbation of chronic interstitial nephritis was observed, particularly in females, with a

Table VI-18. Incidence of Liver Tumors in CDF Rats Fed tg-DNT for up to 2 Years[a]

Duration of treatment (weeks)	Lesion[b]	Males (mg/kg/day)				Females (mg/kg/day)			
		0	3.5	14	35	0	3.5	14	35
52	NN			4/10*	3/10				8/10**
	HC			3/10	10/10**				4/10*
	NN or HC	0/10	0/10	5/10*	10/10**	0/10	0/10	0/10	9/10**
55[c]	NN			5/20					12/20**
	HC			20/20**					11/20**
	NN or HC			20/20**					19/20**
78	NN		1/20	11/20**			2/20	10/20**	
	HC			19/20**			0/20	0/20	
	NN or HC	0/20		20/20**		0/20	2/20	10/20	
104	NN	9/61	11/70	16/23**		5/57	12/61	53/68**	
	HC	1/61	9/70*	22/23**		0/57	0/61	40/68**	
	NN or HC	10/61	19/70	23/23**		5/57	12/61	66/68**	
UD[d]	NN		3/20	31/65**		1/23	1/29	12/22**	
	HC		1/20	57/65**		0/23	1/29	1/22	
	NN or HC	0/19	4/20	60/65**		1/23	2/29	13/22**	

[a]Incidence given as the number of tumor-bearing animals/number of animals examined at that site. Statistical significance determined by SRC using Fisher's Exact test, as cited in U.S. EPA (1986).
[b]NN, neoplastic nodule; HC, hepatocellular carcinoma.
[c]All high-dose animals were sacrificed at 55 weeks, and 20 rats of each sex were examined histologically. No other dose group was examined at this time. Statistics for this group are based on comparison with controls at 52 weeks.
[d]UD = Unscheduled deaths. Results include all animals found dead or sacrificed moribund throughout the study.

*$p < 0.05$; **$p < 0.001$.

SOURCE: CIIT (1982).

resultant increase in parathyroid hyperplasia and adenoma, and an exacerbation of degenerative changes in the adrenal glands was reported in mid-dose rats. Histopathologic examination of the major tissues supported the above observations and revealed, in addition, an increased incidence of neurofibromas and subcutaneous sarcomas in mid-dose males.

d. DNT isomers

Holen et al. (1990) studied the possible relationship between carcinogenicity of DNT isomers and the response in the Syrian hamster embryo cell (SHE) using two short-term *in vitro* assays. The 2,6- and 2,4-DNT isomers were 10 times less toxic than the 2,3- and 3,4-DNT isomers on the relative survival of SHE cells. Also, none of these four isomers increased morphological cell transformation of SHE cells relative to the control cells. None of the isomers possessed promoter or initiator effects in the transformation assay. Because of lack of effect, four oxidative or reductive metabolites of DNT were examined. All four metabolites were less toxic than the parent DNT, and none (together with technical grade (tg) DNT) induced an increase in the morphological transformation of SHE cells. Intercellular communication, based on a dye transfer, was inhibited by 2,4- and 2,6-DNT isomers and tg-DNT but only at toxic concentrations. It was suggested that this effect was similar to data reported *in vivo* indicating a similar promoter effect of these compounds. The 2,3- and the 3,4-DNTs did not affect this assay.

6. Genotoxicity

a. Microbial systems

Both 2,4- and 2,6-DNT are weak mutagens in *Salmonella* test systems. Because of this weak activity, some of the studies using plate incorporation assays gave equivocal and conflicting results. Hodgson et al. (1976) reported that 2,4-DNT was mutagenic in *S. typhimurium* strain TA1535. Chiu et al. (1978) reported that 2,4-DNT was not mutagenic in strain TA98 or TA100. Mori et al. (1982) found that mutation frequency was less than 3 times the control rate in *Salmonella* strains TA98 and TA100 at 2,4-DNT concentrations between 100 and 300 μg/plate. Tokiwa et al. (1981) found a modest response in strain

TA100 but negative responses in strain TA98 with 500 or 1,000 μg/plate 2,4-DNT or 2,6-DNT. Spanggord et al. (1982) reported positive dose responses with 2,4- and 2,6-DNT in Salmonella strain TA100 with or without S9 activation but no mutagenicity in strain TA1535, TA1537, TA1538, or TA98; the 2,5- and 3,5-isomers were more mutagenic in strain TA100 with peak activities at 200 and 500 μg/plate, respectively. 2,4- and 2,6-DNT were tested at 100, 500, and 1,000 μg/plate. Neither isomer showed mutagenic activity in a nitro reductase-deficient strain (TA100 NRS). The interstrain variability in these studies may have been partly accounted for by exceeding the narrow concentration range for cytotoxicity.

Couch et al. (1981) carried out a comprehensive analysis of the mutagenic potential of technical DNT and each of the six pure isomers of DNT in Salmonella. A liquid suspension assay was used, with a 3-hour incubation of bacteria with test compound with or without S9 mix prior to plating. Cytotoxicity was quantitated, and dose-response data for mutation frequency were developed. Technical grade DNT and all the pure isomers of DNT were mutagenic to one or more strains of S. typhimurium (TA1535, TA1537, TA1538, TA98, or TA100). Mutation frequency was not high compared with other mutagens, but it was reproducible and concentration related. The most mutagenic isomer, 3,5-DNT, was almost an order of magnitude more potent than any of the other isomers and was mutagenic without S9 activation in strains TA1538, TA98, and TA100. In dose-response experiments in strain TA98, 2,4- and 2,6-DNT and tg-DNT all caused about a fivefold increase in mutation frequency at concentrations of 500 μg/mL when compared with the DMSO control. When added, S9 did not appreciably affect the mutagenicity. In an 8-azaguanine forward mutation assay in S. typhimurium TM677, similar mutation frequencies were noted for tg-DNT and 2,4- and 2,6-DNT.

Because the weak mutagenic activity did not correlate with carcinogenicity of tg-DNT, studies have been performed to test the mutagenicity of DNT metabolites and possibly to identify the proximate carcinogen. Spanggord et al. (1982) initially showed that nitroreductase activity was required for mutagenic activity in S. typhimurium TA100. Couch et al. (1987) found that 2,4-dinitrobenzyl alcohol, 2-amino-4-nitrotoluene, and 2-nitroso-4-nitrotoluene were more mutagenic that 2,4-DNT in S. typhimurium strain TA98 (Table VI-19). The first compound is a major

Table VI-19. Comparative Mutagenicity of 2,4-DNT and Its Metabolites

Compound	Revertants/viable cell/mmol (x 10^7)[a]	
	-S9	+S9
2,4-Dinitrotoluene	1.7	1.8
2,4-Diaminotoluene	0.1	0.7
2,4-Dinitrobenzoic acid	1.6	1.5
2-Amino-4-nitrotoluene	12.4	2.9
2-Amino-4-nitrobenzoic acid	5.9	40.6
2-Nitroso-4-nitrotoluene	47.5	25.9
2,4-Dinitrobenzyl alcohol	13.5	27.2

[a]Calculated from the slopes of the concentration versus mutant fraction by linear regression analysis.

SOURCE: Adapted from Couch et al. (1987).

urinary metabolite in rats, and the 2-amino and 2-nitroso derivatives are formed from 2,4-DNT by anaerobic microorganisms from rat cecum (see Chapter V, Pharmacokinetics). Mori et al. (1982, 1985), in similar studies on the mutagenicity of 2,4-DNT metabolites and derivatives, found that 2,4-dinitrophenyl alcohol was a strong mutagen in strains TA98 and TA100 but that the corresponding aldehyde was 25 to 40 times more active than the alcohol; they suggested that this correlated with the carcinogenicity of 2,4-DNT. Both oxidative and reductive metabolism may be involved in the production of mutagenic metabolites.

Simmon et al. (1977) reported that 2,4- and 2,6-DNT did not produce mitotic recombination in Saccharomyces cerevisiae D3; however, insufficient data were provided to determine if the dose used was adequate.

b. Mammalian cells in vitro

Abernethy and Couch (1982) found that 2,4- and 2,6-DNT were not mutagenic in the HGPRT system in Chinese hamster ovary cells. The isomers were tested at their limit of solubility in culture medium (3.0 mM 2,4-DNT or 2.5 mM 2,6-DNT) in the presence or absence of S9 from livers of Aroclor-treated rats.

Styles and Cross (1983) tested tg-DNT and 2,4- and 2,6-DNT in the P388 lymphoma assay. Both tg-DNT and 2,6-DNT gave a negative response to +/- S9 activation. 2,4-DNT gave a weak but positive result at 200 and 1,000 µg/mL but only in the absence of S9 activation; the response was weaker than with trinitrotoluene.

2,4-DNT was also reported to be negative in the C3H10T½ transformation assay system (Rickert et al., 1984).

c. In vivo assays

Ellis et al. (1979) carried out cytogenetic examinations of primary cultures of bone marrow and kidney cells from rats fed 2,4-DNT in the diet

(100 or 700 ppm) for 2 years. No clear effects on ploidy, chromosome number, or chromosomal abnormalities were observed.

Soares and Lock (1980) found that 2,4-DNT and tg-DNT were negative in the sperm morphology test performed 8 weeks after two daily intraperitoneal doses of 250 mg/kg. Styles and Penman (1985) found that 2,4-DNT was negative in the mouse spot assay. No mutant clones of pigment cells were found in embryos after dams were intraperitoneally dosed with 100 mg/kg on gestation day 10. Soares and Lock (1980) similarly found a negative response in the mouse spot test with 100 mg/kg tg-DNT.

Negative results from tg-DNT and 2,4-DNT have been found in the dominant lethal test in mice and rats. Soares and Lock (1980) dosed male DBA/J mice intraperitoneally or by oral gavage with a single dose or two daily doses of 250 mg/kg tg-DNT or pure 2,4-DNT and mated them after 48 hours. Lane et al. (1985) dosed Sprague-Dawley rats by oral gavage for 5 days at 2,4-DNT levels between 60 and 240 mg/kg/day. Ellis et al. (1979) evaluated rats fed 200 or 2,000 ppm 2,4-DNT for 10 to 13 weeks. In all of these studies, adverse effects on fertility, but no dominant lethal mutations, were seen.

2,4-DNT was tested in the recessive lethal test and reciprocal translocation test in Drosophila (Woodruff et al., 1985). Negative results were found after feeding at 1,000 ppm or injection of 200 or 10,000 ppm.

d. Unscheduled DNA synthesis (UDS)

Bermudez et al. (1979) found that technical grade DNT (10^{-4} and 10^{-5}M) and 2,4- or 2,6-DNT (10^{-3} or 10^{-4}M) gave a negative response for UDS in primary hepatocyte cultures from Fischer 344 male rats using an assay based on autoradiography. Negative results for 2,4- and 2,6-DNT were also reported for UDS in primary cultures of human hepatocytes (Butterworth et al., 1989). Working and Butterworth (1984) reported negative results for UDS in cultured rat spermatocyte at 10 or 100 μm 2,4- or 2,6-DNT with or without S9 activation.

Mirsalis et al. (1982) utilized an in vivo/in vitro assay for UDS in which male Fischer 344 rats were dosed by oral gavage with 2,4- or 2,6-DNT (99.9% pure). Hepatocytes were isolated by liver perfusion 12 hours after dosing, and UDS was measured in primary culture by incubation with ^{3}H-thymidine (with or without hydroxyurea) by determining nuclear grain counts with autoradiography. A positive response was found 12 hours after dosing with 200 mg 2,4-DNT/kg or 20 mg 2,6-DNT/kg. Mirsalis and Butterworth (1982) assayed tg-DNT in this system. A sharp dose-response curve was seen with a marked increase in UDS between 50 and 100 mg/kg. UDS activity peaked 12 hours after dosing, decreased substantially by 24 hours, and returned to background levels by 48 hours. Hydroxyurea (10 or 20 mM) suppressed the number of cells in S-phase at 48 hours but had no effect on UDS. This confirms that the increase in grains is not due to replicative synthesis but is due to repair synthesis. The potency of tg-DNA and of 2,4- and 2,6-DNT was compared. The 2,4-DNT gave a weaker response at 10 mg/kg than the tg-DNT (33 versus 73% of cells in repair), and 2,6-DNT gave a strong response at 20 mg/kg (79% cells in repair) but was toxic at 100 mg/kg. Female rats gave a much lower level of UDS than males at 100 mg/kg. When 0.1% tg-DNT was administered in the diet for 1, 2, or 4 weeks, a modest but significant increase was seen in UDS in cultured hepatocytes. Metabolism of DNT by gut flora is necessary for UDS expression, since the effects could not be elicited in germ-free rats (Mirsalis et al., 1982). The activation of DNT probably requires P-450-dependent hepatic metabolism to the benzyl alcohol, excretion into the bile after glucurondation, metabolism in the gut, and reabsorption and further hepatic action (Popp and Leonard, 1982).

e. Initiation, promotion, and DNA binding

Dorman and Boreiko (1983) found that tg-DNT and 2,4-DNT did not inhibit metabolic cooperation between 6-thioguanine resistant and sensitive V79 Chinese hamster lung cells. This test is positive with several tumor promoters. The compounds were tested at levels of 10^{-3} to 10^{-9}M; toxicity was seen at levels between 5×10^{-4} and 10^{-3}M.

Leonard et al. (1983, 1986) studied tg-DNT and purified 2,4-DNT and 2,6-DNT in initiation-promotion protocols in which the increase in liver foci staining for gamma glutamyltranspeptidase (GGT) was the endpoint (also discussed in Popp and Leonard, 1983). In the 1983 study, initiation potential was studied in nonhepatectomized rats and at various periods after partial hepatectomy. Technical grade DNT did not prove to be a reproducible initiator of GGT^+ foci except when administered as a single oral dose of 75 mg/kg 12 hours after partial hepatectomy. Under the same conditions, 2,6-DNT was the only isomer that induced GGT^+ foci. A dose-response increase was seen between 37.5 and 150 mg/kg. However, both tg-DNT and 2,6-DNT were weak initiators in comparison with other initiators previously studied. In the 1986 study, male Fischer 344 rats were initiated with a single intraperitoneal dose of dimethylnitrosamine (a necrogenic dose) and allowed to recover for 2 weeks. The rats were then fed diets containing tg-DNT, 2,4-DNT, 2,6-DNT, or phenobarbital to test their promoting activity. The dietary levels were 14 or 35 mg/kg/day for tg-DNT, 2.8, 7 or 14 mg/kg/day for 2,6-DNT, 27 mg/kg/day for 2,4-DNT, and 0.05% of diet for phenobarbital. Treated animals and appropriate controls were killed at 3 and 6 weeks (tg-DNT) or 6 and 12 weeks (2,4- and 2,6-DNT, and phenobarbital) and liver GGT^+ foci were quantified. Technical grade DNT caused a dose-dependent increase in foci; 2,6-DNT produced a time- and dose-related increase as well as an increase in foci and noninitiated controls, but 2,4-DNT and phenobarbital produced only time-related increases. The 2,6-DNT isomer was approximately 10 times more potent than the 2,4-DNT isomer. Under the conditions of this study, 2,4-DNT appears to be a pure promoter, whereas 2,6-DNT has initiating as well as promoting activity (complete hepatocarcinogen). Although the specificity of GGT^+ foci as an early endpoint marker of hepatocarcinogenicity has not been adequately established in animal and human studies, the results of these studies on initiation-promotion using GGT^+ foci as a short-term endpoint correlate with the results of carcinogenicity bioassays of tg-DNT and 2,4-DNT.

Kedderis et al. (1984) showed that when radioactively labeled 2,4-DNT or 2,6-DNT were administered orally to rats, radioactive residues were bound to liver macromolecules. Binding of radioactivity from 2,6-DNT to liver DNA was abolished by prior administration of the sulfotransferase inhibitors

pentachlorophenol or 2,6-dichloro-4-nitrophenol. A similar finding was reported for 2-nitrotoluene but not the 3- or 4-nitrotoluene isomers (Rickert et al., 1984). It was suggested that the action of sulfotransferase in the metabolism of dinitrophenols was required for formation of an electrophilic compound capable of reacting with hepatic DNA. Metabolic pathways for 2,6-DNT and 2,4-DNT are similar (biliary excretion of glucuronide of the nitrobenzoic acid and resorption and recirculation to the liver), and both compounds are potent inducers of DNA repair in the in vivo/in vitro hepatic UDS assay (Popp and Leonard, 1982; Mirsalis et al., 1982; Doolittle et al., 1983).

7. Neurotoxicity

No neurotoxicity studies per se were found in the available literature. However, subacute (4-week) and subchronic (13-week) studies in dogs administered 2,4- or 2,6-DNT showed some evidence of neurotoxicity following oral (capsule) administration (Lee et al., 1978; Ellis et al., 1985). These studies are discussed in detail in Sections VI.B.1, Short-term Exposure, and VI.B.2, Long-term Exposure. Groups of four male dogs and four female dogs were given 0, 1, 5, or 25 mg 2,4-DNT/kg/day for 4 or 13 weeks. After 4 or 13 weeks of exposure, two dogs/sex/dose were sacrificed, and treatment was discontinued for the remaining dogs. These dogs were sacrificed following a 4-week recovery period. No neurotoxic signs were observed in dogs given 1 or 5 mg 2,4-DNT/kg/day for 4 or 13 weeks. Dogs exposed to 25 mg/kg/day showed neurotoxic symptoms after 12 weeks of treatment. Neuromuscular system effects (incoordination and paralysis) were characteristic. The first manifestation, stiffness and incoordination of the hind legs, was followed by difficulty in maintaining balance, which culminated with the animals lying on their sides; the muscles appeared to be stiffened, and the legs were extended and arched. The stiffness progressed upward from hind legs to trunk, forelegs, neck, and head. Several dogs exhibited occasional tremors, and one dog had a grand mal-type convulsion. Two dogs treated for 13 weeks developed transient blindness; one dog was functionally blind with widely dilated pupils unresponsive to light. Vertical or oval nystagmus was present when the dog's position was changed or when she was stimulated by a loud noise. Three dogs (sacrificed at weeks 6, 7, and 13) showed mild to moderate gliosis, edema, and demyelination

in the cerebellum, brain stem, or spinal cord. Demyelination of the optic nerve was observed in only one of the two dogs that suffered from transient blindness. Allowed to recover for 4 weeks, one dog developed a minor balance problem and some demyelination in the cerebrum and optic nerve. Two dogs kept for 8 months completely recovered from all effects (Lee et al., 1978).

Ellis et al. (1985) dosed groups of six male and six female beagle dogs with 0, 0.2, 1.5, or 10 mg 2,4-DNT/kg/day in capsules for up to 24 months. This study is discussed in Section VI.B.5, Carcinogenicity. The first neurotoxic symptoms were seen in dogs dosed at 1.5, 5, or 10 mg/kg/day after 465, >91, and 52 days of dosing, respectively. Individual variation in susceptibility to these effects was observed; some animals were moribund while others were barely affected. The minimal signs of neurotoxicity were incoordination and stiffness. The hind legs were affected to the greatest extent; abnormal gait was pronounced when running. More severely affected dogs experienced rigid paralysis of the hind legs that progressed upward affecting the forelimbs and eventually the neck; during this stage, the dogs assumed a rigid horizontal position. In some dogs (number not specified), paralysis regressed after affecting the hind limbs; the dogs were without toxic signs within days to weeks. Neurotoxic signs were also observed in the lips and tongue; the affected dogs were unable to pick up food. Histopathology revealed central nervous system lesions that included generalized vacuolation, hypertrophy and mitosis of the endothelium, focal gliosis in the cerebellum, and some perivascular hemorrhages in the cerebellum and brain stem. These lesions were most severe in dogs that developed toxic signs late in the study. During the recovery period beginning 4 weeks after treatment and continuing for 12 or 24 months, the less affected dogs recovered sooner than those severely affected.

Lee et al. (1978) gave groups of four male and four female dogs doses of 0, 4, 20, or 100 mg 2,6-DNT/kg/day in capsules for 4 or 13 weeks. The experimental design and procedures were identical to the studies with 2,4-DNT described above. No neurotoxic signs were seen in dogs dosed at 4 or 20 mg/kg/day. However, dogs dosed at 100 mg/kg/day showed listlessness, incoordination, lack of balance, and general weakness, particularly of the

hind limbs. The clinical symptoms were not substantiated by any histopathological lesions of the central nervous system.

VII. HEALTH ADVISORY DEVELOPMENT

A. SUMMARY OF HEALTH EFFECTS DATA

In humans, the toxic effects of DNT are on the central nervous system and also may be on the on the heart and circulatory system. Chronic DNT exposure, primarily via the inhalation route, is characterized in munitions workers by nausea, vertigo, methemoglobinemia, cyanosis, pain or paresthesia in extremities, tremors, paralysis, chest pain, and unconsciousness (Etnier, 1987; U.S. EPA, 1980, 1986; Levine et al., 1985a; Ellis et al., 1979). Following a latency period of 15 years, workers exposed to 2,4-DNT and tg-DNT exhibited excessive mortality from ischemic heart disease and residual diseases of the circulatory system (Levine et al., 1986a,b). Limited evidence suggests that DNT does not result in adverse effects on human reproductive performance (Hamill et al., 1982; Ahrenholz and Meyer, 1982).

Acute oral toxicity studies indicate that rats are more susceptible to 2,4-DNT than mice. The LD_{50} values ranged from 1,340 to 1,954 mg/kg in mice and from 270 to 650 mg/kg in rats (Lee et al., 1975; Vernot et al., 1977). Both species exhibited ataxia and cyanosis. When mice, rats, and dogs were administered 2,4-DNT in the diet for 4 weeks, dogs were found to be most sensitive, exhibiting incoordination and paralysis at doses of 25 mg/kg/day; CNS effects were not observed in rodents (Lee et al., 1978; Ellis et al., 1985). Similar CNS effects were seen when dogs were orally administered 20 mg 2,6-DNT/kg/day. Methemoglobinemia, reticulocytosis, and Heinz body formation resulted from oral administration of 37.5 mg tg-DNT/kg/day to F344 rats for 4 weeks (CIIT, 1977).

Subchronic toxicity was assessed in mice and rats administered 2,4-DNT via the diet and in dogs administered 2,4-DNT via capsules for 13 weeks. In mice of both sexes, anemia and reticulocytosis were observed at a dose level of 413 (males) and 468 (females) mg/kg/day (Hong et al., 1985). In rats, mortality occurred prior to study termination in 100% of females and 75% of males fed 145 (females) to 266 (males) mg/kg/day (Lee et al., 1985). Anemia and reticulocytosis were observed at dose levels as low as 93 (males) to 108

(females) mg/kg/day; splenic hemosiderosis and depressed spermatogenesis were found in these animals. In dogs, neuromuscular incoordination and paralysis, methemoglobinemia, aspermatogenesis, hemosiderosis of the spleen and liver, cloudy swelling of the kidneys, and lesions of the brain were observed in males and females at a dose level of 25 mg 2,4-DNT/kg/day (Ellis et al., 1985). Similar effects were seen when mice, rats, and dogs were orally administered 2,6-DNT for 13 weeks (Lee et al., 1975). All species exhibited methemoglobinemia, anemia, bile duct hyperplasia sometimes accompanied by hepatic degeneration, and depressed spermatogenesis. Incoordination, rigid paralysis, and renal degeneration occurred in dogs at a dose level of 20 mg 2,6-DNT/kg/day.

Lifetime feeding studies in rats and mice and a 2-year study in dogs showed increased mortality, weight loss, anemia, neurotoxicity, hepatotoxicity, renal toxicity, and testicular atrophy (Ellis et al., 1979; Ellis et al., 1985; Lee et al., 1985; Hong et al., 1985). In CD (Sprague-Dawley) rats fed 2,4-DNT in the diet at a level giving a daily intake of 34 (males) and 45 (females) mg/kg/day, respectively, lifespan was shortened; the incidence of hepatocellular carcinomas was increased (6/30 and 19/35 high-dose males and females, respectively, as compared with 1/25 and 0/23 concurrent controls); seminiferous tubules atrophied resulting in almost complete cessation of spermatogenesis; and excessive pigmentation accumulated in the spleen. An increased incidence of benign mammary gland tumors (33/35 as compared with 10/23 in controls) was exhibited in females. Anemia, partially compensated, and testicular atrophy were evident at 3.9 mg/kg/day, and hepatocellular alterations were observed at 0.57 mg/kg/day, the lowest dose tested (Lee et al., 1985). In a study in CD-I mice, all the animals fed 898 mg/kg/day died by month 18 (males) or month 21 (females). Effects at 14 mg/kg/day, the lowest dose tested, included testicular atrophy, decreased body weight in males, and hemosiderosis of many organs, primarily the liver and spleen. The incidence of malignant renal tumors was elevated in males fed 95 mg/kg/day (15/17 as compared with 0/20 concurrent controls) (Hong et al., 1985). In a study in dogs, 2,4-DNT administered daily in capsule form caused neurotoxicity (incoordination and paralysis, often leading to death) in all dogs at 10 mg/kg/day, and anemia (partially compensated) and biliary tract

hyperplasia at 1.5 mg/kg/day. No adverse effects were observed at 0.2 mg/kg/day (Ellis et al., 1985).

Male and female CDF (F344) rats fed tg-DNT in the diet at a level to give a daily intake of 35 mg/kg/day were sacrificed in extremis after 55 weeks (Leonard et al., 1987). At 14 mg/kg/day, 96% of the males and 59% of the females developed hepatocellular carcinomas. After 78 weeks, decreased body weight, increased liver weight, hepatotoxicity (increased incidence of hepatocellular carcinomas, 9/19) renal toxicity, and parathyroid hyperplasia were reported at 3.5 mg/kg/day, the lowest dose tested.

In a limited 1-year study, 47% of male CDF (F344) rats given tg-DNT at dose levels providing 35 mg/kg/day (the only dose tested) developed hepatocellular carcinomas (0/20 control, 9/19 treated) (CIIT, 1982). Under similar conditions, 2,6-DNT induced hepatocellular tumors in 100% of the high-dose (14 mg/kg/day) and 85% of the low-dose (7 mg/kg/day) males. Rats exposed to 2,4-DNT (35 mg/kg/day) did not develop tumors (Leonard et al., 1987). Although limited in duration and in the number of animals tested, these data may indicate that the 2,6-DNT isomer causes much of the carcinogenic activity in the previously tested mixed-isomer DNT bioassays. DNT is classified as Group B2: Probable Human Carcinogen.

Both 2,4- and 2,6-DNT are weak mutagens in Salmonella test systems. However, metabolites of 2,4-DNT are mutagenic without metabolic activation, particularly the 2,4-nitrobenzyl alcohol and the 2-amino- and 2,nitroso-4-nitrotoluenes (Couch et al., 1987). The dinitrotoluenes are also negative genotoxins in mammalian cells in vitro, in the dominant lethal test in mice and rats, and in Drosophila systems (Abernethy and Couch, 1982; Styles and Cross, 1983; Soares and Lock, 1980; Lane et al., 1985; Ellis et al., 1979; Woodruff et al., 1985). Technical grade DNT gave negative responses for unscheduled DNA synthesis (UDS) except when an in vivo/in vitro testing system was used (Bermudez et al., 1979; Mirsalis and Butterworth, 1982a). It was concluded that biliary excretion, metabolism by gut flora, and resorption from the intestine are prerequisites for genotoxic activity (Mirsalis et al., 1982; Popp and Leonard, 1982). Metabolites of 2,4-DNT can bind to liver DNA, and

2,4-DNT appears to act as a promoter, inducing gamma glutamyl transferase positive foci in the livers of rats initiated with dimethylnitrosamine (Leonard et al., 1983, 1986). On the other hand, 2,6-DNT has both initiation and promoting activity (Popp and Leonard, 1982; Mirsalis et al., 1982; Doolittle et al., 1983).

The 2,4-DNT isomer causes severe reproductive effects in rats, mice, and dogs (Ellis et al., 1979; Lee et al., 1985; Hong et al., 1985; Ellis et al., 1985). Oral exposure to 2,4-DNT results in testicular atrophy and degeneration as well as reductions in spermatogenesis in males. In females, oral exposure resulted in cessation of follicular function and reduction in the number of corpora lutea. Consequently, fertility is reduced in both sexes. Also, 2,4-DNT causes reduced viability as well as decreases in the body weight of offspring at birth and weaning. No data on the reproductive effects of 2,6-DNT or technical grade DNT were found in the available literature. Limited avaialable data suggest that 2,4-DNT is not teratogenic in mice following ingestion (Hardin et al., 1987). Technical grade DNT was not teratogenic to rats administered oral doses, although embryotoxicity was observed at maternally toxic levels (Price et al., 1985). In addition, the administration of technical grade DNT to pregnant rats also resulted in changes in relative organ weights and hematologic parameters in their fetuses. No data on the developmental effects of 2,6-DNT were found in the available literature.

B. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years), and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic endpoint of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(NOAEL \text{ or } LOAEL)\ (bw)}{(UF)\ (_____\ L/day)} = mg/L\ (____\ \mu g/L)$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (in mg/kg bw/day).

bw = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor (10, 100, or 1,000) chosen in accordance with NAS/OW guidelines.

L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

1. 2,4-DNT

a. One-day Health Advisory

A 5-day oral reproduction study in male Sprague-Dawley rats resulted in the deaths of 8/15 rats dosed at 240 mg/kg/day, the highest dose tested (Lane et al., 1985). Rats dosed at this same level exhibited body weight loss and cyanosis; a sharp decrease in the mating index and in the number of resulting sperm-positive and pregnant females was observed at 240 mg/kg/day. Cyanosis was also exhibited at 180 mg/kg/day. Since only one sex was tested and precedence in the use of a reproduction study for setting an HA has not been established, this study was considered to be inappropriate for deriving the One-day HA.

Since these data were not judged suitable for determining a One-day HA value for 2,4-DNT, it is recommended that the Ten-day HA for a 10-kg child (0.5 mg/L) be used as a conservative estimate for the One-day HA value.

b. Ten-day Health Advisory

The 14-day study with 2,4-DNT in Sprague-Dawley rats (McGown et al., 1983) is acceptable for derivation of the Ten-day HA. Body weight gain and food consumption were decreased in a dose-related manner in males and females. A number of serum chemistry parameters (cholesterol, glucose, alanine

aminotransferase) were significantly elevated in dosed males and/or females. Hyaline droplets were found histologically in the epithelium of the proximal convoluted tubules of the kidneys of dosed rats of both sexes, with males being more susceptible than females. Oligospermia was found in a dose-related manner in males with accompanying degenerative changes of the testes. Based on decreased body weight gain, decreased food consumption, and changes in serum chemistry levels in males and females and testicular lesions in males, the LOAEL is 45 mg/kg/day, the lowest dose tested.

The Ten-day HA for a 10-kg child is calculated as follows:

$$\frac{(45\ \text{mg/kg/day})\ (10\ \text{kg})}{(1{,}000)\ (1\ \text{L/day})} = 0.45\ \text{mg/L}\ (\text{rounded to } 0.5\ \text{mg/L or } 500\ \mu\text{g/L})$$

where:

45 mg/kg/day = LOAEL, based on decreased body weight gain, decreased food consumption, and changes in serum chemistry levels in males and females, and testicular lesions in males following 14-day dietary dosing.

10 kg = assumed weight of a child.

1,000 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a LOAEL from an animal study.

1 L/day = assumed water consumption of a 10-kg child.

c. Longer-term Health Advisory

The 13-week feeding study in CD rats by Lee et al. (1985) will be used to derive the Longer-term HA. The 2,4-DNT isomer was administered to male rats at 0, 34, 93, or 266 mg/kg/day and to female rats at 0, 38, 108, or 145 mg/kg/day. Mortality occurred in 100% of the high-dose females and 75% of the high-dose males prior to study termination. Body weight gains and food consumption decreased in a dose-related manner in males and females. Hematologic indices (erythrocyte counts, hematocrit, hemoglobin) indicated anemia with concurrent reticulocytosis in mid- and high-dose males and females. Absolute liver and kidney weights were slightly decreased in

mid-dose males; relative liver and kidney weights of these animals were significantly increased. Histopathological examination revealed splenic hemosiderosis in mid- and high-dose rats of both sexes, decreased spermatogenesis in mid-dose males, and aspermatogenesis in high-dose males. Demyelination was found in the brain stem and cerebellum of one high-dose male. Based on dose-related decreases in body weight gain and food consumption, the LOAEL was 34 mg/kg/day for male rats and 38 mg/kg/day for female rats, the lowest doses tested. The LOAEL for males, 34 mg/kg/day, will be used as the most conservative LOAEL for derivation of the Longer-term HA.

Results of the 13-week feeding study in dogs (Ellis et al., 1985) indicated the primary target organs to be the neuromuscular system, erythrocytes, and testes following dosing with 25 mg 2,4-DNT/kg/day. Dogs fed this same dose exhibited body weight loss, decreased food consumption, neuromuscular incoordination, and paralysis; dogs appeared to be in poor nutritional condition. Hematologic indices indicated the presence of methemoglobinemia, anemia, and Heinz bodies. Histopathology revealed hemosiderosis of the liver and spleen, cloudy swelling of the kidneys, and brain lesions including gliosis, edema, and demyelination of the cerebellum, brain stem, and spinal cord in high-dose males and females. In addition, aspermatogenesis was found in males of this dose group. The LOAEL is 25 mg/kg/day and the NOAEL is 5 mg/kg/day. Even though the dog appears to be the species most sensitive to DNT treatment, the study with rats will be used for derivation of the Longer-term HA. Since the number of dogs/group tested in this study (one dog/sex/group sacrificed at 13 weeks) was less than that required by EPA for subchronic dog studies, this study is considered to be unacceptable but supports the level of toxicity found in the rat study.

The Longer-term HA for the 10-kg child is calculated as follows:

$$\frac{(34\ \text{mg/kg/day})\ (10\ \text{kg})}{(1{,}000)\ (1\ \text{L/day})} = 0.34\ \text{mg/L}$$ (rounded to 0.3 mg/L or 300 μg/L)

where:

34 mg/kg/day = LOAEL, based on dose-related decreases in body weight gain and food consumption in males and females following 13-week dietary dosing.

10 kg = assumed weight of a child.

1,000 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a LOAEL from an animal study.

1 L/day = assumed water consumption of a 10-kg child.

The Longer-term HA for a 70-kg adult is calculated as follows:

$$\frac{(34\ \text{mg/kg/day})\ (70\ \text{kg})}{(1{,}000)\ (2\ \text{L/day})} = 1.19\ \text{mg/L}\ (\text{rounded to } 1.0\ \text{mg/L or } 1{,}000\ \mu\text{g/L})$$

where:

34 mg/kg/day = LOAEL, based on dose-related decreases in body weight gain and food consumption in males and females following 13-week dietary dosing.

70 kg = assumed weight of an adult.

1,000 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a LOAEL from an animal study.

2 L/day = assumed water consumption of a 70-kg adult.

d. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a

medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water may be based on actual exposure data or, if data are not available, a value of 20% is assumed. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution should be exercised in assessing the risks associated with lifetime exposure to this chemical.

Three 24-month continuous feeding studies, one in CD rats, one in CD-I mice, and one in beagle dogs, were considered for the Lifetime HA of 2,4-DNT. The LOAELs and NOAELs established are presented in Table VII-I.

In a 2-year chronic toxicity study in which 2,4-DNT was fed daily to beagle dogs in gelatin capsules, four of six males receiving 10 mg/kg died or were sacrificed moribund by study week 19 after exhibiting progressive paralysis. Neurotoxic effects, characterized by incoordination and paralysis, were exhibited in all dogs at this dose level within 6 months of study initiation and during month 16 in one dog receiving 1.5 mg/kg/day. CNS lesions included vacuolization, endothelial proliferation, and gliosis of the cerebellum. Methemoglobinemia including the presence of Heinz bodies and reticulocytosis was evident within 3 months in dogs fed 1.5 and 10 mg/kg/day; these hematologic effects were minimal during study year 2, presumably as a result of an adaptive response by the dogs. Biliary tract hyperplasia was exhibited in dogs fed 1.5 and 10 mg/kg/day; brown epithelial pigmentation was found in the gallbladder, kidneys, and spleen of two dogs receiving 10 mg/kg/day and in three dogs receiving 1.5 mg/kg/day. No adverse effects were observed at 0.2 mg/kg/day. This study is the most sensitive for derivation of the DWEL. The LOAEL in this study is 1.5 mg/kg/day based on neurotoxicity and the presence of Heinz bodies and biliary tract hyperplasia The NOAEL is 0.2 mg/kg/day.

Table VII-1. Summary of Candidate Studies for Derivation of the Drinking Water Equivalent Level (DWEL) for 2,4-DNT

Species/strain	Route	Duration	Endpoint	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Rat/CD	Oral/diet	Lifetime	Hepatocellular alteration		0.57	Ellis et al. (1979); Lee et al. (1985)
			Anemia	0.57	3.9	
			Testicular atrophy	0.57	3.9	
Mouse/CD-1	Oral/diet	Lifetime	Generalized abnormal pigmentation of organs		14	Ellis et al. (1979); Hong et al. (1985)
			Hepatocellular dysplasia		14	
			Decreased body weight in males		14	
Dog/beagle	Oral/capsule	2 years	Incoordination	0.2	1.5	Ellis et al. (1979); Ellis et al. (1985)
			Anemia	0.2	1.5	
			Bile duct hyperplasia	0.2	1.5	

In the study with rats by Ellis et al. (1979) and Lee et al. (1985), Sprague-Dawley rats were fed doses of 2,4-DNT at 0.57, 3.9, or 34 mg/kg/day or 0.71, 5.1, or 45 mg/kg/day for males and females, respectively, for 24 months. Based on a dose-related increase in the incidence of testicular atrophy (seminiferous tubules) in males and increased incidence of hyperplastic foci in the liver of females, the LOAEL is 0.57 mg/kg/day, the lowest dose tested.

In a 2-year feeding study in CD-I mice, testicular and ovarian atrophy were found in males and females receiving 898 mg/kg/day and in males receiving 95 mg/kg/day. An increased incidence of hepatocellular dysplasia and renal tumors was seen in males receiving 14, 95, or 898 mg/kg/day and in females receiving 898 mg/kg/day. A dose-dependent increase in the amount of an unidentified brown pigment was most prominent in the liver and spleen but was also found in the lungs, kidneys, adrenals, bone marrow, heart, gonads, and CNS of all dosed males and females.

The study by Ellis et al. (1985) has been selected for calculation of the DWEL, since it is considered to be well conducted and has the lowest NOAEL (0.2 mg/kg/day).

Using this study, the DWEL is derived as follows:

Step 1. Determination of the Reference Dose (RfD)

$$RfD = \frac{(0.2\ mg/kg/day)}{100} = 0.002\ mg/kg/day$$

where:

0.2 mg/kg/day = NOAEL, based on neurotoxicity, Heinz bodies, biliary tract hyperplasia, and organ pigmentation.

100 = uncertainty factor, chosen in accordance with OW/NAS guidelines using a NOAEL from a chronic animal study (10x for intraspecies variation and 10x for interspecies variation).

Step 2. Determination of the Drinking Water Equivalent Level (DWEL)

$$DWEL = \frac{(0.02\ mg/kg/day)\ (70\ kg)}{(2\ L/day)} = 0.07\ mg/L\ (\text{rounded to } 0.1\ mg/L \text{ or } 100\ \mu g/L)$$

where:

0.002 mg/kg/day = RfD.

70 kg = assumed body weight of an adult.

2 L = assumed daily water consumption of an adult.

Step 3. Determination of Lifetime HA

Dinitrotoluene is classified B2: Probable Human Carcinogen; thus, a Lifetime HA is not recommended. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,4-DNT at the level of the DWEL (100 μg/L) is 2×10^{-3}. This estimate represents the upper 95% confidence limit from extrapolations prepared by EPA's Carcinogen Assessment Group using the linearized multistage model. The actual risk is unlikely to exceed this value.

2. 2,6-DNT

a. One-day Health Advisory

No studies were suitable for determining a One-day HA value for 2,6-DNT. It is recommended that the Longer-term HA for a 10-kg child (0.4 mg/L) be used as a conservative estimate for the One-day HA value.

b. Ten-day Health Advisory

No studies were suitable for determining a Ten-day HA value for 2,6-DNT. It is recommended that the Longer-term HA for a 10-kg child (0.4 mg/L) be used as a conservative estimate for the Ten-day HA value.

c. Longer-term Health Advisory

A 13-week study (Lee et al., 1976) with several species is used to derive the Longer-term HA.

Dogs (4/sex/dose) were given 0, 4, 20, or 100 mg/kg/day 2,6-DNT in capsules for 13 weeks. Dogs in the low-dose group showed no adverse effects. All high-dose dogs (4/4 of each sex) died between weeks 2 and 8; two out of four mid-dose females died during study week 9. Toxic effects included decreased food consumption leading to weight loss, listlessness, and incoordination leading to rigid paralysis with occasional tremors. Elevations were seen in serum alkaline phosphatase, alanine aminotransferase (ALT), and/or blood-urea-nitrogen (BUN). Hematological effects included methemoglobinemia with Heinz body formation, anemia with reticulocytosis and extramedullary hematopoiesis, and lymphoid depression leading to peripheral lymphocytopenia. Both sexes developed bile duct hyperplasia and degenerative/inflammatory changes in the liver and kidneys. Males exhibited degeneration and atrophy of spermatogenic cells. Effects in high-dose animals were more pronounced and appeared earlier than those in the mid-dose animals. Effects were partially reversed in 4 weeks and completely reversed in 19 weeks following cessation of treatment. The LOAEL is 20 mg/kg/day based on weight loss, blood and neurological effects, and histopathology. The NOAEL is 4 mg/kg/day.

Groups of mice (16/sex/dose) were fed diets containing 2,6-DNT (>99%) at 0, 0.01, 0.05, or 0.25% for 13 weeks. The corresponding daily intakes were 0, 11, 51, or 289 mg/kg/day for male mice; and 0, 11, 55, or 299 mg/kg/day for female mice. Mice in the low-dose group did not exhibit any treatment-related effects, and only a few in the mid-dose group showed weight loss. At the high-dose level, 2,6-DNT produced decreased body weight gain and food consumption, hematopoiesis in the liver and/or spleen, and bile duct hyperplasia in both sexes. Depression of spermatogenesis and atrophy of the testes were seen in high-dose males. After 4 weeks, there was partial recovery as demonstrated by weight gain and the disappearance of testicular lesions; however, liver and spleen hematopoiesis continued. The LOAEL is

51 mg/kg/day based on weight, food consumption, histopathology, and testicular effects in male mice. The NOAEL is 11 mg/kg/day.

Groups of rats (16/sex/dose) were fed diets containing 2,6-DNT (>99%) at 0, 0.01, 0.05, or 0.25% for 13 weeks. The corresponding daily intakes were 0, 7, 35, or 145 mg/kg/day for male rats; and 0, 7, 37, or 155 mg/kg/day for female rats. No adverse effects were seen in rats in the low-dose groups. Mid-dose rats of both sexes had decreased body weight gain (approximately 22-26%) and food consumption, extramedullary hematopoietic activity in the liver and spleen, and bile duct hyperplasia; males also exhibited depressed spermatogenesis and testicular atrophy. High-dose rats of both sexes were affected severely with effects that included decreased body weight and body weight gain (approximately 41-46%), decreased food consumption, methemoglobinemia, Heinz body formation, anemia, reticulocytosis, splenic and liver extramedullary hematopoiesis, bile duct hyperplasia, and renal degeneration. After 4 weeks, only partial recovery was observed. The LOAEL is 35 mg/kg/day based on weight loss, blood effects, and histopathology in male rats. The NOAEL is 7 mg/kg/day.

In this case, the dog was considered to be the most sensitive species from which to derive a HA. The 20 mg/kg/day is a Frank-Effect Level (FEL) based on neurotoxicity, Heinz bodies, bile duct hyperplasia, liver and kidney histopathology, and death. A LOAEL is not identified for this study. The NOAEL is 4 mg/kg/day.

The Longer-term HA for the 10-kg child is calculated as follows:

$$\frac{(4\ \text{mg/kg/day})\ (10\ \text{kg})}{(100)\ (1\ \text{L/day})} = 0.4\ \text{mg/L}\ (400\ \mu\text{g/L})$$

where:

4 mg/kg/day = NOAEL, based on neurotoxicity, Heinz bodies, bile duct hyperplasia, liver and kidney histopathology, and death.

10 kg = assumed weight of a child.

100 = uncertainty factor, chosen in accordance with OW/NAS guidelines using a NOAEL from an animal study.

1 L/day = assumed water consumption of a 10-kg child.

The Longer-term HA for a 70-kg adult is calculated as follows:

$$\frac{(4\ \text{mg/kg/day})\ (70\ \text{kg})}{(100)\ (2\ \text{L/day})} = 1.4\ \text{mg/L}\ (\text{rounded to } 1.0\ \text{mg/L or } 1{,}000\ \mu\text{g/L})$$

where:

4 mg/kg/day = NOAEL, based on neurotoxicity, Heinz bodies, bile duct hyperplasia, liver and kidney histopathology, and death.

70 kg = assumed weight of an adult.

100 = uncertainty factor, chosen in accordance with OW/NAS guidelines using a NOAEL from an animal study.

2 L/day = assumed water consumption of a 70-kg adult.

d. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects

would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water may be based on actual exposure data or, if data are not available, a value of 20% is assumed. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution should be exercised in assessing the risks associated with lifetime exposure to this chemical.

Three 13-week continuous feeding studies, one in CD rats, one in CD-I mice, and one in beagle dogs, were considered for the Lifetime HA of 2,6-DNT. The studies are discussed in detail in section VII.B.2.c (2,6-DNT, Longer-term Health Advisory). The LOAELs and NOAELs established are presented in Table VII-2.

The dog is considered the most sensitive species to derive a HA. The 20 mg/kg/day is a Frank-Effect Level (FEL) based on neurotoxicity, Heinz bodies, bile duct hyperplasia, liver and kidney histopathology, and death. A LOAEL is not identified for this study. The NOAEL is 4 mg/kg/day.

Using this study, the DWEL is derived as follows:

Step 1. Determination of the Reference Dose (RfD)

$$RfD = \frac{(4\ mg/kg/day)}{3{,}000} = 0.001\ mg/kg/day$$

Table VII-2. Summary of Candidate Studies for Derivation of the Drinking Water Equivalent Level (DWEL) for 2,6-DNT

Species	Exposure Duration/ Route	Effects	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Dog Beagle	13 weeks/ diet	testicular atrophy; hepatic degeneration; anemia; paralysis; renal inflammation	4	20	Lee et al., 1976
Rat CD	13 weeks/ diet	decreased body weight; hepatic and splenic hemosiderosis; bile duct hyperplasia; anemia; Heinz bodies	7	35	Lee et al., 1976
Mouse Swiss-Albino	13 weeks/ diet	decreased body weight; hepatic and splenic hematopoiesis; bile duct hyperplasia; testicular atrophy	11	51	Lee et al., 1976

where:

4 mg/kg/day = NOAEL, based on neurotoxicity, Heinz bodies, bile duct hyperplasia, liver and kidney histopathology, and death.

3,000 = uncertainty factor, chosen in accordance with OW/NAS guidelines using a NOAEL from an animal study (10x for intraspecies variation, 10x for interspecies variation, and 10x for use of a less-than-lifetime study). An additional factor of 3 is used to account for the limited database.

Step 2. Determination of the Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.001\ \text{mg/kg/day})\ (70\ \text{kg})}{(2\ \text{L/day})} = 0.035\ \text{mg/L (rounded to 0.04 mg/L or 40 } \mu\text{g/L)}$$

where:

0.001 mg/kg/day = RfD.

70 kg = assumed body weight of an adult.

2 L = assumed daily water consumption of an adult.

Step 3. Determination of Lifetime HA

DNT is classified B2: Probable Human Carcinogen; thus, a Lifetime HA is not recommended. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,6-DNT at the level of the DWEL (40 μg/L) is 1×10^{-3}. This estimate represents the upper 95% confidence limit from extrapolations prepared by EPA's Carcinogen Assessment Group using the linearized multistage model. The actual risk is unlikely to exceed this value.

C. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Leonard et al. (1987) treated four groups of 20 F344 male rats with various isomers and isomer mixtures of DNT for 1 year. Technical grade DNT (76% 2,4-DNT, 19% 2,6-DNT) (35 mg/kg/day) induced hepatocellular tumors and cholangiocarcinomas in 47 and 11%, respectively, of the treated males. 2,6-DNT (99.9% purity) induced hepatocellular carcinomas in 100% of the

high-dose group and 85% of the low-dose group (14 and 7 mg/kg/day, respectively); pulmonary metastases were found in 58% of the high-dose and 15% of the low-dose male rats. No tumors were found in rats exposed to 2,4-DNT (99.9% purity) at 35 mg/kg/day in this study. Although these studies were limited in duration (1 year) and in number of animals on test, the data may indicate that the 2,6- isomer accounts for much of the carcinogenic activity in the previously tested mixed-isomer DNT bioassays.

1. Dose-Response Data (Carcinogenicity, Oral Exposure)

Based on monitoring and production data, 2,6-DNT usually occurs in the presence of 2,4-DNT, with 2,4-DNT the more significant component by volume. For example, tg-DNT is composed of approximately 76.5% 2,4-DNT and 18.8% 2,6-DNT; 2,6-DNT is unlikely to occur alone. In addition, available data on the long-term effects of 2,6-DNT and tg-DNT are limited. For these reasons, the carcinogenic assessment for lifetime exposure of DNT was determined using a chronic toxicity/oncogenicity study conducted with 2,4-DNT; the assessment is representative of the 2,4-/2,6-DNT mixture.

Tumor type -- liver: hepatocellular carcinomas, neoplastic nodules; mammary gland: adenomas, fibroadenomas, fibromas, adenocarcinomas/carcinomas

Test Animals -- rat/Sprague-Dawley, female

Route -- oral, diet

Reference -- Ellis et al., 1979

Dose		Tumor
Administered (ppm)	Human Equivalent* (mg/kg/day)	Incidence
0	0	11/23
15	0.129	12/35
100	0.927	17/27
700	7.557	34/35

*Administered dose ÷ $(70\ kg/0.425\ kg)^{0.33}$.

The human equivalent dose was determined using a standard surface area correction factor. The administered study dose (ppm) was converted to mg/kg/day (1 ppm = 0.05 mg/kg/day for the aging rat; Lehman, 1959); this dose conversion was divided by the ratio of the human weight (70 kg) to the aging rat weight raised to the 1/3 power. Transformed doses reflect the measured weight of the rats for each treatment period (0.425 kg control and low dose, 0.410 kg medium dose, 0.325 kg high dose). The tumor incidences were combined for quantitative purposes because the report by Ellis et al. (1979) provided pathology data for the individual animals. The unit risk should not be used if the water concentration exceeds 500 μg/L, since above this concentration the slope factor may differ from that stated.

2. Summary of Risk Estimates

Oral Slope Factor $(mg/kg/day)^{-1}$ -- 6.8E-1

Drinking Water Unit (μg/L) Risk -- 2.0E-5

Extrapolation Method -- Linearized multistage procedure

3. Drinking Water Concentrations at Specific Risk Levels

Risk Level	Concentration (μg/L)
E-4 (1 in 10,000)	5.0
E-5 (1 in 100,000)	0.5
E-6 (1 in 1,000,000)	0.05

The multistage model was used for high- to low-dose extrapolation (Crump and Watson, 1979; Howe and Crump, 1982). GLOBAL83 was used to fit the data in the experimental dose range and to obtain upper 95% confidence limits on risk. The multistage model conforms to a biological model of tumor initiation and promotion (Crump et al., 1977) and provided an adequate fit to the dose-response data for 2,4-DNT. The relationship of the concentration (μg/L) of a chemical in drinking water to cancer risk is expressed as follows:

$$\frac{35,000}{q_1^*} \times R = C$$

where:

35,000 = conversion factor for mg to μg and exposure assumption that a 70-kg adult consumes 2 L of water/day.

q_1^* = $(mg/kg/day)^{-1}$, human slope factor.

R = 10^{-4}, 10^{-5}, 10^{-6}, etc.

C = concentration of chemical in μg/L.

For comparison purposes, drinking water concentrations associated with an excess cancer risk of 10^{-6} are 8×10^{-8} μg/L, 5×10^{-2} μg/L, 2×10^{1} μg/L, 2×10^{-1} μg/L, and 2×10^{-5} μg/L for the one-hit, multihit, probit, logit, and Weibull models, respectively. The parameter estimates for these models were computed with RISK81 (Kovar and Krewski, 1981) and MULTI80 (Rai and VanRyzin, 1980; Lavenhar, 1986).

4. Discussion of Confidence (Carcinogenicity, Oral Exposure)

Relatively few animals were observed for a period of time approximating the lifespan of the animals. A slope factor of 3.9E-1/mg/kg/day based on renal tumors in male CD-1 mice (Ellis, 1979) is supportive of the risk estimate.

The NOAEL for the 13-week oral toxicity study in CD rats conducted by Lee et al. (1978) was 7 mg/kg/day. In a 12-month oral toxicity study in CDF (F344)/CrlBR rats conducted by Leonard et al. (1987), 7 mg 2,6-DNT/kg/day was found to produce hepatocellular carcinomas in 85% (17/20) of the rats tested; hepatocellular carcinomas were found in 100% (19/19) rats fed 14 mg/kg/day. Beagle dogs fed 20 mg 2,6-DNT for 13 weeks exhibited weight loss, hematological effects, neuromuscular incoordination, and lesions of the liver, spleen, kidneys, and testes; two of eight dogs died during study week 9. In the absence of sufficient data and because 2,6-DNT usually occurs in the

presence of 2,4-DNT, with 2,4-DNT the more significant component by volume, the cancer risk assessment for the 2,4-/2,6-DNT mixture is used as a conservative approach to the determination of a cancer risk assessment for either 2,4-DNT or 2,6-DNT, as well as the mixture (Table VII-3).

Table VII-3. Cancer Risk Data for Exposure to 2,4-DNT, 2,6-DNT, and tg-DNT

Potential Exposure	Risk Data
2,4-DNT	Health Advisory + cancer risk assessment for 2,4-/2,6-DNT mixture.
2,4-/2,6-mixture (tg)	Cancer risk assessment for 2,4-/2,6-DNT mixture.
2,6-DNT	Default to cancer risk assessment for 2,4-/2,6-DNT mixture.

VIII. OTHER CRITERIA, GUIDANCE, AND STANDARDS

A number of regulations and guideline values have been established for 2,4- and 2,6-DNT by various U.S. and State agencies. These values are listed in Table VIII-1.

Table VIII-1. Regulations and Guidelines Applicable to 2,4- and 2,6-DNT[a]

Agency	Description	Value
Regulations		
Occupational Safety and Health Administration (OSHA)	Permissible Exposure Limit (PEL)/ Time-Weighted Average (TWA) (DNTs) (skin)	1.5 mg/m^3
U.S. Environmental Protection Agency, Office of Water Regulations and Standards (EPA/OWRS)	Reportable Quantity (DNTs)	1,000 lb
U.S. Environmental Protection Agency, Office of Environmental Rules and Regulations (EPA/OERR)	Reportable Quantity (2,4-DNT)	1,000 lb
	Reportable Quantity (proposed)	
	2,4-DNT	10 lb
	2,6-DNT	100 lb
Guidelines		
American Conference of Governmental and Industrial Hygienists (ACGIH)[b]	Threshold Limit Value-Time Weighted Average (TLV-TWA)	1.5 mg/m^3
National Institute of Occupational Safety and Health (NIOSH)	Recommended Exposure Limit for Occupational Exposure (DNTs)	Potential human carcinogen. Reduce exposure to lowest feasible level.
	Immediately Dangerous to Life or Health (ILDH) (DNTs)	200 mg/m^3
EPA/OWRS	Ambient Water Quality Criteria to Protect Human Health (2,4-DNT)	
	Ingestion of Water and Organisms	1.1×10^{-5} µg/L
	Ingestion of Organisms Only	91×10^{-5} µg/L
EPA	Carcinogenic Classification	
	2,4-DNT and 2,6-DNT as a mixture	B2
State Environmental Agencies	Drinking Water Standards and Guidelines	
	2,4-DNT	
	Kansas	1.1 µg/L
	Minnesota	1.1 µg/L
	2,6-DNT	
	Kansas	0.4 µg/L

(continued)

Table VIII-1. (Continued)

Agency	Description	Value
State Environmental Agencies (cont.)	Acceptable Ambient Air Concentration Guidelines or Standards	
	2,4-DNT	
	Connecticut	15 $\mu g/m^3$ (8 hr)
	Nevada	0.036 $\mu g/m^3$ (8 hr)
	DNTs	
	Virginia	25 $\mu g/m^3$ (24 hr)
Agency for Toxic Substances and Disease Registry, U.S. Public Health Service	Minimum Risk Level (MRL)	
	2,4-DNT	
	Short-term exposure in food	1 ppm
	Long-term exposure in food	1 ppm
	Acute oral exposure	0.6 mg/kg/day
	Intermediate oral exposure	0.05 mg/kg/day
	Chronic oral exposure	0.002 mg/kg/day
	2,6-DNT	
	Long-term exposure in food	1 ppm
	Intermediate oral exposure	0.04 mg/kg/day

[a]SOURCE: ATSDR (1989).

[b]Listed in a notice of intended changes for 1991-92 to lower the TLV-TWA to 0.15 mg/m^3 and to classify DNT as a "Suspected Human Carcinogen" (ACGIH, 1991; Dinitrotoluene, 1992).

IX. ANALYTICAL METHODS

Published analytical methods for 2,4- and 2,6-DNT for a variety of situations refer predominantly to gas chromatography (GC) and high performance liquid chromatography (HPLC); however, other methods include electron spin resonance spectrometry, tandem mass spectrometry (MS), and cluster analysis.

A. GC ANALYSIS

Gas chromatography has been studied by a number of scientists utilizing different detection methods for various situations. Hartley et al. (1981) studied the separation and identification of DNT isomers in water by GC using an electron capture detector (ECD). Extraction was performed using hexane. Retention times for 2,4- and 2,6-DNT were 25.43 and 16.55 minutes, respectively, while corresponding detection limits were 25.0×10^{-5} and 62.5×10^{-4} µg. This method was judged to be adequate to monitor DNT in environmental samples and laboratory toxicity studies.

Belkin et al. (1985) also studied the quantitative analysis of water for 2,4- and 2,6-DNT using GC coupled with ECD. The study was designed to detect ppb levels of the compounds. The DNT isomers were extracted from water samples with toluene, analyzed by capillary GC, and detected with an ECD. The DNT isomers were found to be stable in water for 33 days at pH values below 8. Recoveries for 2,4- and 2,6-DNT ranged from 98.4 to 106.0 and from 95.1 to 97.4%, respectively, at concentration levels of 1, 10, or 100 ppb.

Eichelberger et al. (1983) utilized both packed column GC (method 1) and fused silica capillary GC (method 2) coupled with MS to determine the presence of a number of compounds in water. The extraction procedure involved methylene chloride at different pH levels for both methods. Mean recoveries for methods 1 and 2 for 2,4- and 2,6-DNT, respectively, were 35 and 70%, and 79 and 116%. The authors concluded that the levels of precision and accuracy of these methods are generally less than the levels that may be achieved with chemical-specific methods.

Capillary GC or capillary GC/MS was used in conjunction with robotics to analyze wastewater samples for a variety of compounds including 2,4- and 2,6-DNT. The sample was extracted with methylene chloride. Although the robotics reduced the analysis time by half, the detection limits provided are adequate only for routine monitoring of biological systems; they do not equal those found in EPA procedures (Hornbrook and Ode, 1987).

A procedure for the determination of munitions in water utilizes a macroreticular resin for extraction, elution with ethyl acetate, concentration of the eluate, separation by GC, and detection by ECD (Richard and Junk, 1986). Recovery values for 2,4- and 2,6-DNT ranged from 89 to 102% and from 33 to 91%, respectively. The results obtained were similar to methylene chloride extraction results, but with a savings in time and solvent/transportation costs.

Gas chromatography has also been used to analyze sludge samples. A method for the determination of 2,4- and 2,6-DNT in biosludges was described by Phillips et al. (1983). A shakeout centrifugation procedure was used to isolate DNT isomers from the sludge, and quantitation was performed using GC and a thermal energy analyzer (TEA). The detection limit was 0.05 mg/L for both isomers, and the precision was 9-14% at the detection limit. The authors stated that the use of TEA eliminates the need for further sample cleanup.

Lichtenberg et al. (1987), in a study utilizing GC in conjunction with either electron capture detection (ECD) or flame ionic detection (FID), stated that parameters in providing successful GC are the column packing, temperature conditions, and selection of a detector as specific to the analyte as possible. The use of selective detectors in conjunction with GC/MS gives a higher reliability in the identification and quantitation of toxic organic substances in complex matrices such as wastewater and sludge samples (Giabbai et al., 1983).

A method for the determination of 2,4- and 2,6-DNT in airborne particulates using GC was developed by Matsushita and Iida (1986). Samples are collected on a quartz filter and extracted with a benzene/ethanol mixture.

DNTs in the fraction are analyzed by GC in conjunction with a flame thermoionic detector (FTD). The authors stated that this method has high separation efficiency and reproducibility for both retention time and peak height.

B. HPLC ANALYSIS

Krull et al. (1981) studied the use of HPLC with electron capture detection (ECD) and HPLC with GC/ECD for the analysis of 2,4-DNT. The authors state that ECD is perhaps the optimal detection system for use with HPLC owing to its sensitivity and specificity for nitro derivatives. HPLC with ECD did not produce optimum results. However, the detection limit for 2,4-DNT (1.0×10^{-4} g) obtained with HPLC coupled with GC/ECD suggests that this method may be more suitable for real-world application.

Lloyd (1983) described a technique that was developed primarily as a basis for the screening of samples for trace amounts of explosives. 2,4-DNT was analyzed using a pendant mercury drop electrode (PMDE) in conjunction with HPLC. The detection limit with this method was 12.0×10^{-4} g and was similar to that obtained by GC/electron capture detection. Characteristics of the PMDE method are its high reproducibility, renewal of the electrode at the start of a chromatogram, and its resistance to contamination problems.

Reverse phase HPLC has been used in the analysis of munitions wastewater samples. Bauer et al. (1986) stated that this method was developed specifically to provide industrywide uniformity for quality control for compliance monitoring but was deemed applicable for the determination of munitions residues. DNT was recovered quantitatively, and the repeatability value was 7 μg/L. Jenkins et al. (1986), using the same method, conservatively estimated the detection limit to be 10 μg/L with a standard deviation of 4.6 μg/L. This method was found to perform adequately for load, pack, and manufacture wastewaters and contaminated groundwater.

The HPLC method (USATHAMA Method SM02) generally has been accepted as the standardized method for measurement of TNT and related metabolites in soils

and wastewaters. However, in certain instances aminodinitrotoluenes are present and may interfere with the quantitation of dinitrotoluene. Therefore, a modification to method SM02 has been made (Preslan et al., 1991) which involves an intermediate derivatization step that ensures separation of 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, and DNT itself. Thus, greater accuracy is assured in the measurement of these compounds.

Bongiovanni et al. (1984) used a combination of HPLC and ultraviolet (uv) light to detect and analyze explosive-bearing soils for trace amounts of 2,4-and 2,6-DNT. DNT was extracted from the soil with acetonitrile, separated by reverse phase HPLC, and detected by ultraviolet spectrometry at a wavelength of 254-nm. Estimated detection limits for 2,4- and 2,6-DNT were 0.58 and 0.87 g, respectively. Corresponding average recovery values were 100.4 and 99.6%. This method provided good accuracy and low detection limits. The authors stated that it is particularly suitable for routine short turnaround analyses with minimum operator attention.

C. OTHER METHODS

Yinon (1989) analyzed and identified a number of 2,4-DNT metabolites using electron impact and chemical ionization mass spectrometry. The metabolites were differentiated by the electron-impact ions that are specific to the position of the methyl group in relation to the nitro group.

Hable et al. (1991) detected 2,4- and 2,6-DNT in drinking water at levels below those measured by gas chromatography. Using electron-capture detection together with a DB-1301 widebore-fused silica capillary column, 2,6-DNT was detected at 0.003 g/L; 2,4-DNT was detected at 0.04 g/L. This method may be advantageous over other detection techniques.

Burns et al. (1987) studied the possibility of identification and determination of 2,6-DNT by electron spin resonance spectrometry. DNT was quantitatively converted to the corresponding anion radical form adsorbed on magnesium oxide. Linear plots indicated that determination of 2,6-DNT by electron spin resonance spectrometry is possible.

McLuckey et al. (1985) studied the use of tandem MS for the analysis of explosives. Tandem MS employs the use of two stages of MS in which the first stage serves as a separator for the second. Three detection methods (electron impact ionization, isobutane chemical ionization, and negative chemical ionization) were used to determine the most suitable method in conjunction with tandem MS. Isobutane and negative chemical ionization methods were suitable; the latter was the most sensitive for nitroaromatic compounds such as 2,4-DNT. Tandem MS facilitates rapid screening for particular types of explosives.

Spanggord and Suta (1982) studied the use of cluster analysis to characterize the distribution of waste components resulting from the production and purification of TNT. This method selectively groups samples together according to the observed concentration values of all components in the sample. The relative concentrations of 2,4- and 2,6-DNT in average cluster analysis were 49.3 and 21.7%, respectively. This approach can be used to determine a representative ratio of components in complex industrial wastewater.

X. TREATMENT TECHNOLOGIES

Treatment technologies found in the available literature include adsorption, chlorination, ozonation, ultraviolet radiation, and several lesser used techniques.

The use of activated carbon for the adsorptive displacement of 2,4- and 2,6-DNT has been investigated. Activated carbon adsorption is the technique most frequently used to clean nitroorganic-contaminated wastewater in military munitions plants. When the carbon becomes exhausted, it must be disposed of at an approved hazardous waste disposal site. Ho and Daw (1988) investigated the possibilities of regenerating spent carbons. Solvents tested for extracting the adsorbed DNT were water, acetone, methanol, and mixtures of the solvents. Both acetone and methanol were effective for the removal of DNT from activated carbon.

Thakkar and Manes (1987) also studied the adsorptive displacement of 2,4- and 2,6-DNT. After being preloaded onto activated carbon, the compounds were equilibrated with benzo[a]anthracene-7,12-dione in methylene chloride/ methanol. The 2,6-DNT isomer showed essentially complete displacement, while 2,4-DNT exhibited nonlinear displacement.

The use of resin for the adsorption of munitions components in aqueous solutions followed by desorption in acetone was studied by Maskarinec et al. (1984) and Richard and Junk (1986). Resin adsorption techniques appear to offer several advantages: specific sorptivity toward nitro groups, increased stability, and field sorption to ensure sample integrity.

Lloyd (1985) determined the distribution coefficients of DNT for the adsorption of 10 representative adsorbents used in cleanup procedures. The adsorbents were placed in solutions of methanol, and the DNT was loaded into the solution. Two of the adsorbents gave nonsignificant results, three showed negative selectivity, and the remaining five gave distribution coefficients of 0.129 to 0.356.

The effects of chlorination and ozonation on 2,4- and 2,6-DNT were studied by Lee and Hunter (1985). Concentrations of 21.3 (ozone) and 45.5 mg/L (chlorine) were added to compound concentrations of 100 mg/L and observed. Reduction recoveries of 2,4-DNT by chlorine and ozone at 1 hour were 35 and 60%, respectively. Corresponding values for 2,6-DNT were 17 and 13%.

Ho (1986) studied the synergistic effect of hydrogen peroxide and ultraviolet (uv) radiation on the decomposition of 2,4-DNT in water and found that at molar ratios of H_2O_2/DNT between 26 and 52, DNT disappeared very rapidly. The degradation rate of DNT in aqueous solution was also found to be affected by the energy of the incident light.

Other treatment methods investigated include solvent and sediment extraction and partial reduction. Hwang (1981) conducted a literature review to determine the feasibility of solvent extraction as a treatment method for separating organic materials in wastewater effluent. He found that solvent extraction can remove up to 99.9% of targeted materials. Lopez-Avila et al. (1983) used an extraction technique involving the homogenization of a sediment sample with dichloromethane at dual pH and phase separation by centrifugation to determine priority pollutants in a standard reference sediment sample. Total recoveries for 2,4- and 2,6-DNT were 95 and 93%, respectively. Ono and Kitazawa (1983) obtained a successful partial reduction of 2,4-DNT by mild reduction under controlled conditions with a metal and organic acid system. The reduction products obtained were 4-methyl-3-nitroaniline and 2,4-diaminotoluene. This method is useful for the "recycling" of 2,4-DNT. In addition, biodegradation and photolysis techniques may be considered as treatment technology alternatives because of the rapid degradation of DNT by these two processes (Liu et al., 1984; Davis et al., 1981; Hallas and Alexander, 1983).

XI. CONCLUSIONS AND RECOMMENDATIONS

Advisory values for 2,4- and 2,6-DNT are summarized in Table XI-1. Based on decreased body weight gain, decreased food consumption, and changes in serum chemistry levels in male and female Sprague-Dawley rats and testicular lesions in male rats administered 2,4-DNT in the diet for 14 days, the One-day and Ten-day HAs for exposure in a 10-kg child are 0.5 mg/L (500 μg/L). Based on dose-related decreases in body weight gain and food consumption in rats administered 2,4-DNT in the diet for 13 weeks, the Longer-term HA for exposure in a 10-kg child is 0.30 mg/L (300 μg/L); the Longer-term HA for exposure in a 70-kg adult is 1.0 mg/L (1,000 μg/L). The Drinking Water Equivalent Level (DWEL) for 2,4-DNT is 0.1 mg/L (100 μg/L), derived from a Reference Dose (RfD) of 0.002 mg/kg/day, based on neurotoxicity, Heinz bodies, and biliary tract hyperplasia in dogs dosed orally with 2,4-DNT for 2 years.

The critical effects for 2,6-DNT were neurotoxicity, Heinz bodies, biliary tract hyperplasia, liver and kidney histopathology, and death. The One- and Ten-day HAs and the Longer-term HA for exposure in a 10-kg child are 0.4 mg/L (400 μg/L). The Longer-term HA for exposure in an adult is 1.0 mg/L (1,000 μg/L). The Drinking Water Equivalent Level (DWEL) for 2,6-DNT is 0.04 mg/L (40 μg/L), derived from a Reference Dose (RfD) of 0.001 mg/kg/day.

Dinitrotoluene is classified B2: Probable Human Carcinogen; thus a Lifetime HA is not recommended. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,4-DNT at the level of the DWEL (100 μg/L) is 2×10^{-6}. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,6-DNT at the level of the DWEL (40 μg/L) is 1×10^{-6}. The cancer risk assessment is for the 2,4-/2,6-DNT mixture and, therefore, is used for the cancer risk assessment for both 2,4- and 2,6-DNT.

A companion report titled "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for 2,4- and 2,6-DNT" (Appendix) summarizes the scope of existing data reviewed for this HA. This companion report delineates the areas where additional data and/or a clarification of existing data would be appropriate for a second HA.

Table XI-1. Drinking Water Advisory Values for 2,4- and 2,6-Dinitrotoluene

Classification	2,4-DNT	2,6-DNT
One-day HA[a] (mg/L)	0.5	0.4
Ten-day HA (mg/L)	0.5	0.4
Longer-term HA:		
Child (mg/L)	0.3	0.4
Adult (mg/L)	1.0	1.0
Lifetime HA (mg/L)	NA[b]	NA
DWEL[c] (mg/L)	0.1	0.04
Cancer Unit Risk Factor[d] $(mg/kg/day)^{-1}$	0.68	0.68
Excess Cancer Risk at the DWEL	$2x10^{-3}$	$1x10^{-3}$

[a]Health Advisory.

[b]Dinitrotoluene is classified as a Group B2: Probable Human Carcinogen; therefore a Lifetime HA is not recommended.

[c]Drinking Water Equivalent Level.

[d]Also referred to as "Human Potency Factor" and "q_1*".

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APPENDIX

Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for 2,4- and 2,6-DNT

DATA BASE DEVELOPMENT

A. OBJECTIVES

The objective of this document is to provide an evaluation of data deficiencies and/or problem areas encountered in the review process for 2,4-/2,6-DNT and to provide recommendations, as appropriate, for additional data base development. This document is presented as an independent analysis of the current status of 2,4-/2,6-DNT toxicology, as related to its possible presence in drinking water, and includes a summary of the background information used in development of the Health Advisory (HA). For greater detail on the toxicology of 2,4-/2,6-DNT, the Health Advisory on 2,4-/2,6-DNT should be consulted.

B. BACKGROUND

Dinitrotoluene, commonly known as DNT, is a white- to buff-colored solid at room temperature and exists as a mixture of two or more of its six isomers; the 2,4- and 2,6-DNT isomers are the most significant and have been used in military munitions, dye manufacture, and the synthesis of toluenediamine (the organic intermediate in the production of polyurethane). Both isomers are soluble in water and are found in industrial wastewater. DNT is removed from water by rapid photochemical transformation or biodegradation. Biodegradation occurs via cometabolic transformation in local eutrophic waters; wastewater and municipal sludge utilize microbial and/or fungal populations under aerobic/anaerobic conditions (Bausum et al., 1991; Davis et al., 1981; Hallas and Alexander, 1983; Hashimoto et al., 1982; Liu et al., 1984; Small and Rosenblatt, 1974; Spanggord et al., 1981; Tabak et al., 1981). DNT is best detected at low concentrations in water media by high-performance liquid chromatography (HPLC) or gas chromatography (GC) in combination with electron-capture detection (ECD) or pendant mercury drop electrode (PMDE) detection. Detection limits range from 12.0×10^{-4} to 6.25×10^{-6} µg (Belkin et al., 1985; Hartley et al., 1981; Lloyd, 1983).

The pharmacokinetics of primarily the 2,4-DNT isomer has been studied extensively in laboratory animals. Data indicate that approximately 70 to 90% of orally administered 2,4- or 2,6-DNT is eliminated in the urine of CD and Fischer rats, New Zealand white rabbits, beagle dogs, rhesus monkeys, and A/J mice (Rickert et al., 1984; Schut et al., 1983; Ellis et al., 1985, 1979; Lee et al., 1975, 1978). Fecal excretion generally accounts for less than 10%. Results of one study indicated that absorption of 2,6-DNT by rats is somewhat slower than that of 2,4-DNT (Lee et al., 1975); a series of other studies demonstrated that urinary excretion of 2,4-DNT by two common strains of mice (CD-1 and $B6C3F_1$) is only 8 to 12% following oral exposure (Shoji et al., 1985; Lee et al., 1978; Mori et al., 1977). Percent absorption of either isomer is difficult to estimate since biliary excretion is significant in most animals. Male rats usually excrete less of the administered dose of 2,4-DNT in the urine and more in the feces than females, a difference due most likely to higher biliary excretion in males (Medinsky and Dent, 1983; Bond and Rickert, 1981; Ellis et al., 1980; Lee et al., 1978). No sex-related differences in the absorption or elimination of 2,6-DNT have been observed in animals or humans (Levine et al., 1985b; Turner et al., 1985; Schut et al., 1983; Long and Rickert, 1982; Ellis et al., 1980; Lee et al., 1978). Fecal metabolites have not been identified in either animals or humans exposed to DNT. Elimination of either compound via the lungs is minimal. Workers exposed to technical grade DNT absorb the munitions chemicals via the inhalation and dermal routes, but the extent of absorption cannot be determined (Levine et al., 1985b; Turner et al., 1985; Woollen et al., 1985).

The data on distribution of 2,4- and 2,6-DNT suggest that accumulation of these compounds in the body following a single exposure is, at most, minimal (Schut et al., 1981, 1982, 1983; Ellis et al., 1980; Rickert et al., 1980; Lee et al., 1975, 1978; Mori et al., 1977, 1980). Repeated oral exposure in CD rats indicates preferential uptake of 2,4-DNT and/or its metabolites by the liver, kidneys, brain, lungs, and skeletal muscle, although total retention in the body is low (Mori et al., 1980; Ellis et al., 1979; Lee et al., 1978). Limited data suggest that placental transfer of 2,4-DNT occurs in rats (Rickert et al., 1980).

The metabolism of DNT isomers has been studied in a variety of experimental systems and animals (Etnier, 1987; Guest et al., 1982; Bond et al., 1981; Rickert et al., 1981; Rickert and Long, 1981; Ellis et al., 1980; Lee et al., 1978). The first step in the metabolism of DNT appears to involve hepatic oxidation of the methyl group to form 2,4- or 2,6-dinitrobenzyl alcohol (2,4- or 2,6-DNBalc). Sulfate or glucuronate conjugation occurs next. Metabolites that are eliminated in the bile and into the gut are hydrolyzed and reduced by intestinal microflora to aminonitrobenzyl alcohols. Many of these compounds, in turn, are reabsorbed from the gut into the systemic circulation and then oxidized in the liver. Biliary excretion of these metabolites into the gut results in additional reduction by intestinal bacteria. Hepatic metabolism probably plays only a minor role in the reduction of either isomer. The only remarkable sex-related difference in the metabolism of DNT is the high rate of biliary excretion of 2,4-dinitrobenzyl alcohol glucuronide (2,4-DNBalcG) in male rats.

For most animals (A/J mice, CD and Fischer 344 rats, New Zealand rabbits, beagle dogs, and rhesus monkeys) and humans, significant portions of orally or intraperitoneally administered 2,4-DNT are converted to 2,4-DNBalc and/or its glucuronide (i.e., 2,4-DNBalcG) (Turner, 1986; Levine et al., 1985b; Schut et al., 1985; Woollen et al., 1985; Rickert et al., 1981). Other primary metabolites excreted by these animals include 2-amino-4-nitrobenzyl alcohol (2A4NBalc) and 4-amino-2-nitrobenzyl alcohol (4A2NBalc) (Schut et al., 1985; Lee et al., 1978); humans excrete only the former in large quantities. For Fischer rats and humans, another major product of 2,4-DNT metabolism is 2,4-dinitrobenzoic acid (2,4-DNBacid) (Levine et al., 1985b; Rickert et al., 1981; Rickert and Long, 1981). A significant portion (4 to 9%) of a single oral dose of 2,4-DNT is reduced <u>in vivo</u> to 2,4-diaminotoluene (2,4-DAT) by rats, rabbits, dogs, and monkeys, but none is found in the urine of occupationally exposed humans (Levine et al., 1985b; Woollen et al., 1985; Lee et al., 1978). In contrast, metabolism of 2,4-DNT by CD-1 mice and Wistar rats is not extensive, and only small amounts of an oral dose of 2,4-DNT given to these animals are oxidized to one of the benzyl alcohol metabolites; reduction of 2,4-DNT to 2,4-DAT is negligible in mice (Schut et al., 1985;

Shoji et al., 1985; Mori et al., 1981; Lee et al., 1978). Sex-related differences in the metabolism of 2,4-DNT have been reported in only Fischer rats and humans. Exposure in rats was by the oral route, whereas human exposure was via the inhalation and dermal routes. For both species, females produce up to three times more 2,4-DNBalc and/or 2,4-DNBalcG than males, and in humans only, men excrete nearly twice as much 2,4-DNBacid as females (Levine et al., 1985b; Turner et al., 1985; Rickert et al., 1981; Rickert and Long, 1981).

The metabolism of 2,6-DNT has not been studied extensively. As with 2,4-DNT, Fischer rats convert the 2,6-DNT isomer to the corresponding dinitrobenzyl alcohol glucuronide and dinitrobenzoic acid; however, only one metabolite (2-amino-6-nitrobenzoic acid, 2A6NBacid) results from the in vivo reduction of 2,6-DNT (Long and Rickert, 1982). Similarly, only three major metabolites of 2,6-DNT (2,6-DNBalc, 2,6-DNBalcG, and 2,6-DNBacid) have been recovered from the urine of men and women; women appear to excrete more 2,6-DNBalcG than men (Levine et al., 1985b; Turner et al., 1985). No other sex-related differences in the metabolism of 2,6-DNT have been reported.

In humans, the toxic effects of DNT are on the heart, the circulatory system, and the central nervous system. Chronic DNT exposure, primarily via the inhalation route (although percutaneous absorption and ingestion are considered to be secondary and tertiary routes of exposure) has been characterized in munitions workers by nausea, vertigo, methemoglobinemia, cyanosis, pain or paresthesia in extremities, tremors, paralysis, chest pain, and unconsciousness (Etnier, 1987; U.S. EPA, 1980, 1986; Levine et al., 1985a; Ellis et al., 1979). Following a latency period of 15 years, workers exposed to 2,4-DNT and tg-DNT exhibited excessive mortality from ischemic heart disease and residual diseases of the circulatory system (Levine et al., 1986a,b). A more recent study failed to demonstrate excess mortality from ischemic heart disease and cardiovascular diseases in DNT-exposed workers (Stayner et al., 1989); however, it did suggest a possible increase in mortality from liver and biliary tract cancer (Stayner, 1989). Limited evidence suggests that DNT does not result in adverse effects on human reproductive performance (Hamill et al., 1982; Ahrenholz and Meyer, 1982).

Acute oral toxicity studies indicate that rats are more susceptible to 2,4-DNT than mice. The LD_{50} values ranged from 1,340 to 1,954 mg/kg in mice and from 270 to 650 mg/kg in rats (Lee et al., 1975; Vernot et al., 1977). Both species exhibited ataxia and cyanosis. When mice, rats, and dogs were administered 2,4-DNT in the diet for 4 weeks, dogs were found to be most sensitive, exhibiting incoordination and paralysis at doses of 25 mg/kg/day; CNS effects were not observed in rodents (Lee et al., 1978; Ellis et al., 1985). Similar CNS effects were seen when dogs were orally administered 20 mg 2,6-DNT/kg/day. Methemoglobinemia, reticulocytosis, and Heinz body formation resulted from oral administration of 37.5 mg tg-DNT/kg/day to F344 rats for 4 weeks (CIIT, 1977).

Subchronic toxicity of mice and rats administered 2,4-DNT via the diet, and in dogs administered 2,4-DNT via capsules for 13 weeks, was assessed. In mice of both sexes, anemia and reticulocytosis were observed at a dose level of 413 (males) to 468 (females) mg/kg/day (Hong et al., 1985). In rats, mortality occurred prior to study termination in 100% of females and 75% of males fed 145 (females) to 266 (males) mg/kg/day (Lee et al., 1985). Anemia and reticulocytosis were observed at dose levels as low as 93 (males) to 108 (females) mg/kg/day; splenic hemosiderosis and depressed spermatogenesis were found in these animals. In dogs, neuromuscular incoordination and paralysis, methemoglobinemia, aspermatogenesis, hemosiderosis of the spleen and liver, cloudy swelling of the kidneys, and lesions of the brain were observed in males and females at a dose level of 25 mg 2,4-DNT/kg/day (Ellis et al., 1985). Similar effects were seen when mice, rats, and dogs were orally administered 2,6-DNT for 13 weeks (Lee et al., 1975). All species exhibited methemoglobinemia, anemia, bile duct hyperplasia sometimes accompanied by hepatic degeneration, and depressed spermatogenesis. Incoordination, rigid paralysis, and renal degeneration occurred in dogs at a dose level of 20 mg 2,6-DNT/kg/day.

Lifetime feeding studies in rats and mice and a 2-year study in dogs showed increased mortality, weight loss, anemia, neurotoxicity, hepatotoxicity, renal toxicity, and testicular atrophy (Ellis et al., 1979,

1985; Lee et al., 1985; Hong et al., 1985). In CD (Sprague-Dawley) rats fed 2,4-DNT in the diet at a level giving a daily intake of 34 (males) and 45 (females) mg/kg/day, respectively, lifespan was shortened, the incidence of hepatocellular carcinomas was increased (6/30 and 19/35 high-dose males and females, respectively, as compared with 1/25 and 0/23 concurrent controls), seminiferous tubules atrophied resulting in almost complete cessation of spermatogenesis, and excessive pigmentation accumulated in the spleen. An increased incidence of benign mammary gland tumors (33/35 as compared with 10/23 in controls) was exhibited in females. Anemia, partially compensated, and testicular atrophy were evident at 3.9 mg/kg/day, and hepatocellular alterations were observed at 0.57 mg/kg/day, the lowest dose tested (Lee et al., 1985). In a study in CD-1 mice, all the animals fed 898 mg/kg/day died by month 18 (males) or month 21 (females). Effects at 14 mg/kg/day, the lowest dose tested, included testicular atrophy, decreased body weight in males, and hemosiderosis of many organs, primarily the liver and spleen. The incidence of malignant renal tumors was elevated in males fed 95 mg/kg/day (15/17 as compared with 0/20 concurrent controls) (Hong et al., 1985). In a study in dogs, 2,4-DNT administered daily in capsule form caused neurotoxicity (incoordination and paralysis, often leading to death) in all dogs at 10 mg/kg/day, and anemia (partially compensated) and biliary tract hyperplasia at 1.5 mg/kg/day. No adverse effects were observed at 0.2 mg/kg/day (Ellis et al., 1985).

Male and female CDF (F344) rats fed tg-DNT in the diet at a level to give a daily intake of 35 mg/kg/day were sacrificed <u>in extremis</u> after 55 weeks (Leonard et al., 1987). At 14 mg/kg/day, 96% of the males and 59% of the females developed hepatocellular carcinomas. After 78 weeks, decreased body weight, increased liver weight, hepatotoxicity (increased incidence of hepatocellular carcinomas; 9/19), renal toxicity, and parathyroid hyperplasia were reported at 3.5 mg/kg/day, the lowest dose tested. In a limited 1-year study, 47% of male CDF (F344) rats given tg-DNT at dose levels providing 35 mg/kg/day (the only dose tested) developed hepatocellular carcinomas (0/20 control, 9/19 treated) (CIIT, 1982). Under similar conditions, 2,6-DNT induced hepatocellular tumors in 100% of the high-dose (14 mg/kg/day) and 85%

of the low-dose (7 mg/kg/day) males. Rats exposed to 2,4-DNT (35 mg/kg/day) did not develop tumors (Leonard et al., 1987). Although limited in duration and in the number of animals tested, these data may indicate that the 2,6-DNT isomer causes much of the carcinogenic activity in the previously tested mixed-isomer DNT bioassays. DNT is classified as Group B2: Probable Human Carcinogen.

2,4- and 2,6-DNT are weak mutagens in Salmonella test systems. However, metabolites of 2,4-DNT, particularly the 2,4-nitrobenzyl alcohol and the 2-amino- and 2-nitroso-4-nitrotoluenes, are mutagenic without metabolic activation (Couch et al., 1987). The dinitrotoluenes are also negative genotoxins in mammalian cells in vitro, in the dominant lethal test in mice and rats, and in Drosophila systems (Abernethy and Couch, 1982; Styles and Cross, 1985; Soares and Lock, 1980; Lane et al., 1985; Ellis et al., 1979; Woodruff et al., 1983). Technical grade DNT gave negative responses for unscheduled DNA synthesis (UDS) except when an in vivo/in vitro testing system was used (Bermudez et al., 1979; Mirsalis and Butterworth, 1982a). It has been concluded that biliary excretion, metabolism by gut flora, and resorption from the intestine are prerequisites for genotoxic activity (Mirsalis et al., 1982b; Popp and Leonard, 1982). Metabolites of 2,4-DNT can bind to liver DNA, and 2,4-DNT appears to act as a promoter, inducing gamma glutamyl transferase-positive foci in the livers of rats initiated with dimethylnitrosamine (Leonard et al., 1983, 1986). On the other hand, 2,6-DNT has both initiation and promoting activity (Popp and Leonard, 1982; Mirsalis et al., 1982; Doolittle et al., 1983).

2,4-Dinitrotoluene causes severe reproductive effects in rats, mice, and dogs (Ellis et al., 1979; Lee et al., 1985; Hong et al., 1985; Ellis et al., 1985). Oral exposure to 2,4-DNT results in testicular atrophy and degeneration as well as reductions in spermatogenesis in males. In females, oral exposure resulted in cessation of follicular function and reduction in the number of corpora lutea. Consequently, fertility is reduced in both sexes. Also, 2,4-DNT causes reduced viability as well as decreased body weight of offspring at birth and weaning. No data on the reproductive effects of 2,6-DNT or tg-DNT were found in the available literature. Limited

available data suggest that 2,4-DNT is not teratogenic in mice following ingestion (Hardin et al., 1987). Technical grade DNT was not teratogenic to rats administered oral doses, although embryotoxicity was observed at maternally toxic levels (Price et al., 1985). In addition, the administration of tg-DNT to pregnant rats also resulted in changes in relative organ weights and hematologic parameters in their fetuses. No data on the developmental effects of 2,6-DNT were found in the available literature.

The One-day Health Advisory (HA) and Ten-day HA for 2,4-DNT for a 10-kg child are 0.5 mg/L (500 μg/L), and the Longer-term HA for exposure in a 10-kg child is 0.30 mg/L (300 μg/L). The Longer-term HA for exposure in an adult is 1.0 mg/L (1,000 μg/L). The Drinking Water Equivalent Level (DWEL) for 2,4-DNT is 0.1 mg/L (100 μg/L), derived from a Reference Dose (RfD) of 0.002 mg/kg/day. The One- and Ten-day, and the Longer-term HAs for 2,6-DNT exposure in a 10-kg child is 0.4 mg/L (400 μg/L), and the Longer-term HA for an adult is 1.0 mg/L (1,000 μg/L). The Drinking Water Equivalent Level (DWEL) for 2,6-DNT is 0.04 mg/L (40 μg/L), derived from a Reference Dose (RfD) of 0.001 mg/kg/day. DNT is classified B2: Probable Human Carcinogen; thus a Lifetime HA is not recommended. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,4-DNT at the level of the DWEL (100 μg/L) is 2×10^{-3}. The estimated excess cancer risk associated with lifetime exposure to drinking water containing 2,6-DNT at the level of the DWEL (40 μg/L) is 1×10^{-3}.

C. DISCUSSION

Available data on the pharmacokinetics, health effects, analysis, and treatment of DNT have been reviewed.

Pharmacokinetic studies in rodents, dogs, and monkeys indicate that DNT is effectively absorbed via the oral route. Despite the significant role of gut microflora and biliary excretion in the metabolism of DNT, fecal metabolites have not been identified. Limited data indicate that 2,4-DNT is absorbed by fetal rats; further studies on placental transfer and fetal

accumulation are needed. Humans absorb tg-DNT primarily via the inhalation and dermal routes; however, studies on the pharmacokinetics of DNT in animals using these routes of exposure are not available. Data on the pharmacokinetics of 2,6-DNT are limited; additional single- and multiple-dose studies should be conducted, particularly in light of the carcinogenic and mutagenic properties of the isomer.

Available acute toxicity studies include oral LD_{50}s in mice and rats. Subacute (5-, 8-, 14-, 21-, and 30-day) studies were available for Sprague-Dawley rats, CD-1 mice, Swiss mice, and beagle dogs administered 2,4-DNT. Mouse and rat studies appeared to be adequate; dog studies were not conducted with sufficient numbers of animals (one dog/sex/dose sacrificed at dosing termination, one dog/sex/dose sacrificed following 4 weeks of recovery). Four-week subacute studies were available for Sprague-Dawley rats, Swiss mice, and beagle dogs administered 2,6-DNT, and a 4-week study was available for F344 rats administered tg-DNT; studies appeared to be adequate.

Subchronic (13-week) studies were available for Sprague-Dawley rats, CD-1 mice, and beagle dogs administered 2,4-DNT or 2,6-DNT. Mouse and rat studies appeared to be adequate; dog studies conducted with 2,4-DNT were not conducted with sufficient numbers of animals. Subchronic studies were not conducted using tg-DNT.

Chronic toxicity/oncogenicity studies were conducted in Sprague-Dawley rats, CD-1 mice, and beagle dogs administered 2,4-DNT. Further chronic studies are unlikely to yield additional data pertinent to the development of HA values for 2,4-DNT. However, chronic/oncogenicity rodent studies on 2,6-DNT and tg-DNT were limited to 1 year in duration as a result of high tumor incidence; additional lifetime rodent studies with 2,6-DNT or tg-DNT should be conducted at low-dose levels.

The data currently available are adequate to determine the reproductive effects of 2,4-DNT in animals. However, since there is sufficient evidence in animal studies to conclude that 2,4-DNT adversely affects reproductive performance and fertility and causes testicular degeneration, more information

on health effects associated with human exposure is needed to evaluate the reproductive effects on humans. No data are currently available on the reproductive toxicity potential of 2,6-DNT or tg-DNT in animals or humans. Therefore, a multigeneration reproductive toxicity study in animals, preferably rats, should be conducted. In addition, since 2,4-DNT has been shown to cause reductions in reproductive performance and fertility in animals, as well as testicular degeneration, information on the reproductive effects associated with human exposure is needed.

The data currently available are not adequate to assess the developmental toxicity of 2,4-DNT or tg-DNT in animals or humans. The only available animal study found for 2,4-DNT was a screening test using mice, and this was insufficient to adequately assess developmental effects. The only available animal study for tg-DNT tested insufficient numbers of animals and was considered to be inadequate. Therefore, two developmental toxicity studies using 2,4-DNT in two different species, preferably in rats and rabbits, are needed. Since no data are currently available on the developmental toxicity potential of 2,6-DNT in animals or humans, two developmental toxicity studies in two different species of animals, preferably rats and rabbits, should be conducted. In addition, information on the developmental effects associated with human exposure to 2,6-DNT are needed.

Further investigation of initiation/promotion activity for 2,4-DNT and 2,6-DNT should be conducted.

D. CONCLUSIONS/RECOMMENDATIONS

The following conclusions/recommendations are based upon the above discussion:

1. The available studies on 2,4-DNT toxicity are generally considered to be adequate for development of Health Advisories useful in dealing with the potential contamination of drinking water; available studies on 2,6-DNT and tg-DNT are limited.

2. It is recommended that single- and multiple-dose delayed neuropathy studies be performed on 2,4-DNT, 2,6-DNT, and tg-DNT in hens (sensitive animal model).

3. It is recommended that additional pharmacokinetics studies be conducted on 2,4-DNT, including identification of fecal metabolites, placental transfer, fetal accumulation, and metabolism of animals exposed to tg-DNT by the inhalation and dermal routes. In addition, single- and multiple-dose pharmacokinetics studies should be conducted on 2,6-DNT.

4. It is recommended that the 13-week study in which beagle dogs were administered 2,4-DNT be repeated using at least four animals/sex/group, since this species was the most sensitive species treated.

5. It is recommended that adequate lifetime chronic toxicity/oncogenicity studies be performed in rats and mice using 2,6-DNT at dose levels up to 7 mg/kg/day.

6. It is recommended that adequate reproduction studies be performed using 2,6-DNT in at least one rodent species.

7. It is recommended that adequate developmental toxicity studies be performed using 2,4-DNT and 2,6-DNT in at least one rodent species.

8. It is recommended that further investigation of initiation/promotion activity for 2,4-DNT and 2,6-DNT be conducted.

The recommended order of completion for these studies is as follows: pharmacokinetics, initiation/promotion activity, subchronic dog study, chronic toxicity/oncogenicity, developmental toxicity, reproduction, and delayed neuropathy.

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