

**Drinking Water Health Advisory
for 2,4-Dinitrotoluene and
2,6-Dinitrotoluene**

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LIST OF ABBREVIATIONS AND ACRONYMS

4Ac2NBacid	4-(N-Acetyl)amino-2-nitrobenzoic acid
4A2NBacid	4-amino-2-nitrobenzoic acid
3,4-DNT	3,4-dinitrotoluene
3,5-DNT	3,5-dinitrotoluene
2,4-DNBacid	2,4-dinitrobenzoic acid
2,4-DNBalc	2,4-dinitrobenzyl alcohol
2,4-DNBalcG	2,4-dinitrobenzyl alcohol glucuronide
2,4-DNT	2,4-dinitrotoluene
2,6-DAT	2,6-diaminotoluene
2,6-DNBacid	2,6-dinitrobenzoic acid
2,6-DNBalc	2,6-dinitrobenzyl alcohol
2,6-DNBalcG	2,6-dinitrobenzyl alcohol glucuronide
2,6-DNT	2,6-dinitrotoluene
2,5-DNT	2,5-dinitrotoluene
2,4-DAT	2,4-diaminotoulene
2,3-DNT	2,3-dinitrotoluene
2Ac6NT	2-acetylamino-6-nitrotoluene
2A4NBacid	2-amino-4-nitrobenzoic acid
2A6NBacid	2-amino-6-nitrobenzoic acid
2A6NT	2-amino-6-nitrotoluene
AK	Alaska
AR	Arkansas
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BMD	benchmark dose
BMDL	benchmark dose level
BMR	benchmark risk
BW	body weight
CA	California
CAS	Chemical Abstracts Service
CAS No. 121-14-2	2,4-dinitrotoluene
CAS No. 606-20-2	2,6-dinitrotoluene
CIIT	Chemical Industry Institute of Toxicology
CNS	central nervous system
CO ₂	carbon dioxide
CWS	community water system
DNA	deoxyribonucleic acid
DNT	dinitrotoluene
DWEL	drinking water equivalent level
DWI	drinking water ingestion
ECD	electron capture detection
EPA, U.S. EPA	U.S. Environmental Protection Agency
FL	Florida

GC	gas chromatography
GDR	German Democratic Republic (formerly East Germany)
g/L	grams per liter
G6PD	glucose-6-phosphate dehydrogenase
HA	health advisory
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Data Bank
IA	Iowa
IARC	International Agency for Research on Cancer
IL	Illinois
IN	Indiana
ip	intraperitoneal
log K _{OC}	organic carbon soil partition coefficient
log K _{OW}	octanol/water partition coefficient
KY	Kentucky
LA	Louisiana
lbs/yr	pounds per year
LD ₅₀	50% of the lethal dose
Lifetime HA	Lifetime health advisory
LOAEL	lowest observed adverse effect level
log K _{OC}	organic carbon soil partition coefficient
log K _{OW}	octanol/water partition coefficient
mg/kg/day	milligrams per kilograms (of body weight) per day
mg/L	milligrams per liter
mg/m ³	milligrams per cubic meter
mL	milliliter
MI	Michigan
mL	milliliter
mm Hg	millimeters of mercury
MO	Missouri
MRL	minimum reporting level
MS	Mississippi, mass spectrometry
MTD	maximum tolerated dose
NAWQA	National Water-Quality Assessment (Program) (USGS)
NCEA	National Center for Environmental Assessment (U.S. EPA)
NCI	National Cancer Institute
NE	Nebraska
NJ	New Jersey
NOAEL	no observed adverse effect level
NPL	National Priorities List
NTNCWS	non-transient non-community water system
NV	Nevada
OH	Ohio
OW	Office of Water (U.S. EPA)
p value	probability value

POD	point of departure
ppm	parts per million
ppt	parts per trillion
PWS	public water system
RfD	reference dose
RL	reporting level
RSC	relative source contribution
SC	South Carolina
SVOC	semivolatile organic compound
Tg-DNT	technical grade DNT
TN	Tennessee
TNT	trinitrotoluene
TRI	Toxics Release Inventory, the TRI Program
TWA	time-weighted average
TX	Texas
UF	uncertainty factor
µg/L	micrograms per liter
U.S. EPA	Environmental Protection Agency
USGS	U.S. Geological Survey
UT	Utah
UV	ultraviolet
VA	Virginia
vs.	not v, vs, or versus
WV	West Virginia

EXECUTIVE SUMMARY

The 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) isomers, Chemical Abstracts Service Nos. 121-14-2 and 606-20-2, respectively, are yellow to reddish crystal solids at room temperature. Both isomers are moderately soluble in water (0.30 g/L and 0.18 g/L, respectively) and have a low affinity for organic particulate matter (K_{oc} 1.65 and K_{oc} 1.96, respectively). A mixture of DNTs is sold as an explosive and is a starting material for the production of 2,4,6-trinitrotoluene (TNT). The mixture is also used as a modifier for smokeless powders in the munitions industry, in airbags of automobiles, and as an intermediate in the production of urethane foams.

2,4-DNT and 2,6-DNT are released into the environment primarily from facilities that manufacture or process DNT. 2,4-DNT and 2,6-DNT may subsequently contaminate surface and groundwaters during runoff events. However, monitoring data from sampling conducted under the U.S. Environmental Protection Agency's (U.S. EPA) first Unregulated Contaminant Monitoring Regulation (UCMR1) program indicate that the frequency of detection of 2,4-DNT and 2,6-DNT in public water systems is low.

Human exposure to 2,4-DNT and 2,6-DNT occurs through inhalation, dermal contact, and incidental ingestion, usually in occupational settings. 2,4-DNT and 2,6-DNT are readily and rapidly absorbed following oral or inhalation exposure and are eliminated through urinary and fecal excretion.

Human toxicity has been evaluated in DNT factory workers, munitions handlers, and underground mining workers. DNT-related effects have been noted in the central nervous system, heart, and circulatory system. Other effects that are possibly due to 2,4-DNT and 2,6-DNT exposure include increased mortality from ischemic heart disease, hepatobiliary cancer, and urothelial and renal cell cancers. No apparent reproductive or developmental effects have been evaluated in humans exposed to DNT.

In animal studies with rats, mice, and dogs, 2,4-DNT and 2,6-DNT isomers have similar effects and have been shown to cause adverse neurological, hematological, reproductive, hepatic, and renal effects. Dogs generally are the most sensitive of the three species. Oral studies of 50% of the lethal dose in rats and mice indicate that both 2,4-DNT and 2,6-DNT are moderately to highly toxic.

Short-term (5 days to 4 weeks) oral exposures of 2,4-DNT were both lethal and toxic to experimental animals. Chemical-related mortality at doses as low as 145 milligrams per kilogram of body weight (BW) per day (mg/kg/day) was observed in rats and mice at doses $\geq 1,250$ mg/kg/day. At sublethal doses in rats (≥ 45 mg/kg/day), toxicity was characterized by decreased food consumption and decreased BW gain, BW loss, cyanosis, changes in serum chemistry levels, increased absolute and relative liver weights, splenic hemosiderosis, testicular lesions and atrophy, and aspermatogenesis. Treatment-related toxicity was observed in dogs at 25 mg/kg/day and manifested as decreased food consumption, BW loss, neurological effects, aspermatogenesis, and histopathology of the liver, brain, and spinal cord. Short-term (4 weeks)

oral exposures to 2,6-DNT were toxic, but not lethal, to experimental animals. The Lowest Observed Adverse Effect Level (LOAEL) was 35 mg/kg/day for rats, 55 mg/kg/day for mice and 20 mg/kg/day for dogs. Toxic effects were similar to those found for 2,4-DNT. Rats fed diets with technical grade-DNT (Tg-DNT) for 4 weeks exhibited toxicity at doses ≥ 75 mg/kg/day, experiencing similar symptoms to that listed above for isomeric components.

Subchronic (13 weeks) oral exposures to 2,4-DNT were both lethal and toxic to all species tested. In rats there was chemical-related mortality at doses as low as 145 mg/kg/day, in mice at doses ≥ 413 mg/kg/day and in dogs at 25 mg/kg/day. Toxic effects observed in rats included decreased BW gain, urine-stained fur, neurological effects, anemia, increased liver and kidney weights, and decreased spermatogenesis. Subchronic oral exposures to 2,6-DNT were lethal only to dogs at doses ≥ 20 mg/kg/day; however, the toxic effects observed in rats, mice, and dogs were very similar to the effects noted above for 2,4-DNT. There were no subchronic animal exposures to Tg-DNT found in the available literature.

In chronic studies, oral doses of 2,4-DNT given to rats, mice, and dogs for 1-2 years were lethal and toxic. 2,4-DNT was lethal to rats at doses ≥ 34 mg/kg/day and to mice at 898 mg/kg/day; dogs given 10 mg/kg/day 2,4-DNT were sacrificed after 19 weeks due to their poor condition. Toxic effects in rats included reduced BW gain, liver histopathology, bile duct hyperplasia, cholangiofibrosis, seminiferous tubule atrophy, almost complete aspermatogenesis, pigmentation of the spleen, anemia, and reticulocytosis. Toxicity in mice was reported to include testicular atrophy, decreased food consumption, decreased BW, hemosiderosis of many organs (primarily the liver and spleen), and an elevated incidence of malignant renal tumors. Toxicity in dogs was reported as neuropathology, methemoglobinemia with associated reticulocytosis and Heinz body formation, biliary tract hyperplasia, and pigmentation of the gallbladder, kidneys, and spleen. When 2,6-DNT was administered in the diet of rats for 12 months, the observed effects of hepatic histopathology included acidophilic and basophilic foci, elevated serum alanine aminotransferase and gamma-glutamyl transferase, and bile duct hyperplasia. Tg-DNT administered in the diets of rats for 6 months to 2 years did not cause any mortality, but toxic effects were notable.

Studies in rats demonstrate that oral exposure to 2,4-DNT causes severe reproductive effects. Some of the toxic effects observed in parental animals include reduced BW, cyanosis, decreased mating index, reduced fertility, testicular atrophy and degeneration, reduced spermatogenesis and sperm count, increases in serum follicle stimulating hormone and luteinizing hormone, cessation of follicular function, reduced number of corpora lutea, and histopathology of Sertoli cells, spermatocytes, and spermatids. Effects observed in offspring were lower mean litter size, reduced viability, decreases in BW at birth and at weaning, changes in relative organ weights and hematologic parameters, and reduced fertility. Limited available data suggest that orally administered 2,4-DNT is not teratogenic in mice. Data on the reproductive or developmental effects of 2,6-DNT were not found in the current literature. Tg-DNT was not teratogenic to rats administered oral doses up to 150 mg/kg/day; however, embryotoxicity was observed at maternally toxic levels (≥ 14 mg/kg/day). Developmental effects noted in the fetuses were reduced liver weight and increased spleen weight.

Both 2,4-DNT and 2,6-DNT are weak mutagens in *Salmonella* test systems. Tg-DNT is negative for unscheduled DNA synthesis except when an *in vivo/in vitro* testing system was used.

Experimental studies with 2,4-DNT administered in the diet demonstrate that it is tumorigenic in rats and mice but not in dogs. In a 1-year feeding study, 2,6-DNT administered in the diet to mice induced hepatocellular carcinomas and cholangiocarcinomas (a type of liver cancer involving the bile duct). Males fed Tg-DNT in the diet for 1 year and males and females fed Tg-DNT in the diet for up to 2 years developed hepatocellular carcinomas and/or cholangiocarcinomas.

2,4,6-TNT can cause hemolysis in humans with a genetic defect in the enzyme glucose-6-phosphate dehydrogenase (G6PD). There is a lack of experimental data that indicate that the DNTs have a similar effect but based on animal data showing hemolytic anemia, G6PD deficient individuals may be a sensitive population for DNT exposure as well.

Health advisories (HAs) were determined for 1-day, 10-day, and longer term (up to 7 years) exposures. The 1-day HA for 2,4-DNT is 0.5 mg/L, and the 10-day HA is 1.0 mg/L. The longer term HA for 2,4-DNT for the 10-kg child is 0.3 mg/L; for the 70-kg adult, it is 1.0 mg/L. The reference dose (RfD) for 2,4-DNT is 0.002 mg/kg/day, and the drinking water equivalent level (DWEL) is 0.1 mg/L.

The 1-day HA for 2,6-DNT is 0.4 mg/L, and the 10-day HA is 0.4 mg/L. The longer term 2,6-DNT HA for the 10-kg child is 0.4 mg/L; for the 70-kg adult, it is 1.0 mg/L. The RfD for 2,6-DNT is 0.001 mg/kg/day, and the DWEL is 0.04 mg/L.

A mixture of 2,4-DNT and 2,6-DNT is classified as “likely to be carcinogenic to humans”; thus, Lifetime HAs for 2,4-DNT and 2,6-DNT are not recommended. The cancer risk estimate for the 2,4-DNT/2,6-DNT mixture is derived from a feeding study where female rats were the sensitive species/sex and mammary gland tumors were the critical endpoint. The point of departure selected for the quantification of cancer risk from DNT is the lower 95% confidence bound on the benchmark dose level of 0.15 mg/kg/day, derived from the cancer incidence data in female rats. The oral slope factor is 6.67×10^{-1} mg/kg/day, and the drinking water unit risk is 1.90×10^{-5} µg/L. The drinking water concentrations at specific risk levels are 5 µg/L for a risk of 10^{-4} (1 in 10,000); 0.5 µg/L for a risk of 10^{-5} (1 in 100,000); and 0.05 µg/L for a risk of 10^{-6} (1 in 1,000,000).

The only other criteria, guidance, or standard found for any of the DNT isomers was a U.S. EPA ambient water quality criteria to protect human health for 2,4-DNT at a 10^{-6} risk level. The criteria are 0.11 µg/L for ingestion of water and organisms and 9.1 µg/L for ingestion of organisms only.

Analytical methods for DNT isomers most frequently rely on gas chromatography and high-performance liquid chromatography. Other methods include electron spin resonance

spectrometry, tandem mass spectrometry, and cluster analysis. Treatment technologies include adsorption, chlorination, ozonation, and ultraviolet radiation.

1.0 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 are useful in dealing with the contamination of drinking water. Health Advisories describe non-regulatory concentrations of drinking water contaminants at which adverse health effects are not 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. The HAs are subject to change as new information becomes available. HAs usually are based on adverse health effects but HA documents may also provide information on the organoleptic or aesthetic properties (color, taste, odor) of contaminants in drinking water.

Health Advisories are developed for both short-term and long-term (Lifetime) exposure periods based on data describing non-carcinogenic end points of toxicity. Short-term exposures can include one-day to ten-day exposure periods. In many cases a longer-term value is included covering approximately 7 years, or 10 percent of an individual's lifetime. For those substances that are "known" or "likely to be carcinogenic to humans," Lifetime HAs are not recommended.

The Health Advisory evaluation of carcinogenic potential includes the U.S. EPA classification for the weight of evidence of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed as well as quantitative estimates of cancer potency (slope factor) where available. The cancer slope factor is the result of the application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The Health Advisory includes the drinking water concentration equivalent to cancer risks of one-in-ten-thousand (10^{-4}), one-in-one-hundred-thousand (10^{-5}), to one-in-one-million (10^{-6}).

Cancer assessments conducted before 1996 used the five-category, alphanumeric system for classifying carcinogens established by the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986). After 1999, assessments were conducted using *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996, 1999). Between 1996 and 2001, assessments were conducted using both the 1986 and 1996 guidelines. Currently, the Agency Administrator has issued a directive that all new cancer assessments should be in accordance to the 1999 *Draft Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2001). This has been superseded by the final guidelines, *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005).

2.0 GENERAL INFORMATION AND PROPERTIES

2.1 Chemical Identity

The 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) isomers, Chemical Abstracts Service (CAS) Nos. 121-14-2 and 606-20-2, respectively, are the major components of technical grade DNT (Tg-DNT). Other DNT isomers make up 5% of Tg-DNT (ATSDR, 1998). Analysis of Tg-DNT reveals the following composition: 76.49% 2,4-DNT, 18.83% 2,6-DNT, 0.65% 2,5-DNT, 2.43% 3,4-DNT, 1.54% 2,3-DNT, 0.040% 3,5-DNT, 0.050% trinitrotoluene (TNT), 0.005% cresols, 0.003% mononitrobenzene, and 0.003%, 0.0005%, and 0.006%, for ortho-, meta-, and para-, mononitrotoluenes, respectively (HSDB, 2004a,b,c).1994. The chemical structures of 2,4-DNT and 2,6-DNT are displayed in Figure 2-1.

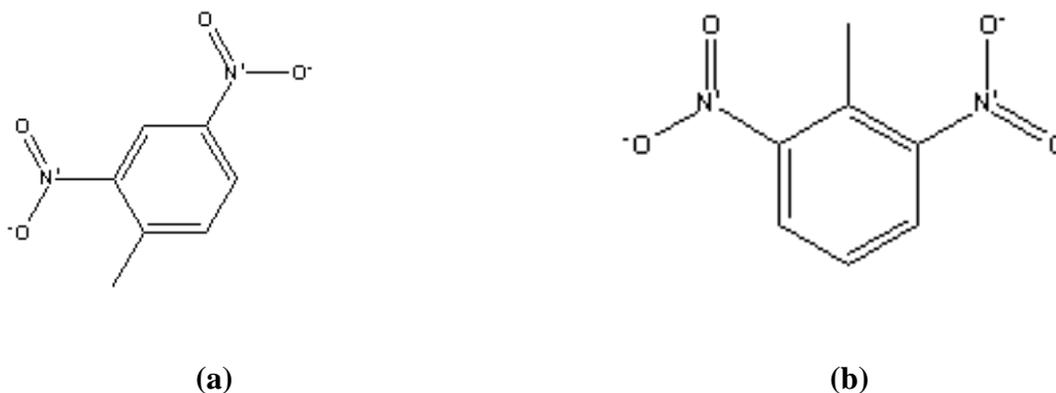


Figure 2-1. Chemical Structures of (a) 2,4-Dinitrotoluene and (b) 2,6-Dinitrotoluene (ChemFinder.com, 2004)

2.2 Physical and Chemical Properties

DNT is a white- to buff-colored solid at room temperature and exists as a mixture of two or more of its six isomers: 2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, 3,4-DNT, and 3,5-DNT. Upon heating, DNT forms an oily liquid that turns yellow when exposed to sunlight (Hartley et al., 1994). The chemical and physical properties of 2,4-DNT and 2,6-DNT are listed in Table 2-1.

The 2,4 and 2,6-DNT isomers are combustible, nitroaromatic compounds that are soluble in water (Hartley et al., 1994). At room temperature, 2,4-DNT appears as yellow or orange needles or monoclinic prisms (HSDB, 2004a). At room temperature, 2,6-DNT exists as yellow to red rhombic crystals (HSDB, 2004b).

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

Property	2,4-Dinitrotoluene	2,6-Dinitrotoluene
CAS No.	121-14-2	606-20-2
U.S. EPA Pesticide Chemical Code	NA	NA
Synonyms	1-methyl-2,4-dinitrobenzene, 2,4-dinitrotoluol 2,4-DNT	1-methyl-2,6-dinitrobenzene, 2,6-DNT
Registered Trade Name(s)	No data	NA
Chemical Formula	C ₇ H ₆ N ₂ O ₄	C ₇ H ₆ N ₂ O ₄
Molecular Weight	182.14	182.14
Physical State	Yellow solid	Yellow to red solid
Boiling Point	300 °C (slight decomposition)	285 °C
Melting Point	71 °C	66 °C
Density (at 71 °C)	1.3208	1.2833
Vapor Pressure:		
At 20 °C	0.0051 mm Hg	0.018 mm Hg
At 25 °C	1.4 × 10 ⁻⁴ mm Hg	5.67 × 10 ⁻⁴ mm Hg
Partition Coefficients:		
Log K _{ow} (octanol/water partition coefficient)	1.98	1.72 or 2.10
Log K _{oc} (organic carbon soil partition coefficient)	1.65	1.96
Solubility in:		
Water at 22 °C	300 mg/L	180 mg/L
Other Solvents	Acetone, alcohol, benzene, ethanol, diethyl ether, pyridine, CS ₂	Ethanol, chloroform
Conversion Factors (at 25 °C, 1 atm)	1 ppm = 7.40 mg/m ³ 1 mg/m ³ = 0.13 ppm	1 ppm = 7.40 mg/m ³ 1 mg/m ³ = 0.13 ppm

Sources: ATSDR, 1998; HSDB, 2004a,b; Hartley et al., 1994

3.0 Occurrence/Exposure

DNTs are not known to occur naturally in the environment but have been detected in the soil, surface water, and groundwater of hazardous waste sites that contain buried ammunition wastes and wastes from manufacturing facilities that release DNT (ATSDR, 1998). No recent quantitative estimates of DNT production or use are available. Combined 2,4- and 2,6-DNT production was $\sim 1.24 \times 10^6$ kg in 1975 (U.S. EPA, 1980). Tg-DNT produced in the United States was reported to be 3.27×10^9 g in 1982 (HSDB, 2004a,b,c).

Sources of exposure to 2,4-DNT and 2,6-DNT include facilities that manufacture or process DNT, as well as hazardous waste sites (ATSDR, 1998). 2,4-DNT and 2,6-DNT have been detected in soil, sediment, water, or air at 69 out of 1,467 and 53 out of 1,467, respectively, current or former National Priorities List (NPL) hazardous waste sites (HazDat, 1998). The general population may be exposed via inhalation, dermal contact, and incidental ingestion. Occupational exposure to DNTs, which is much more likely than general exposure, can occur by inhalation, skin absorption, or inadvertent ingestion during DNT production and DNT use as intermediates (ATSDR, 1998; International Agency for Research on Cancer [IARC], 1996; Tchounwou et al., 2003).

Both 2,4-DNT and 2,6-DNT are listed as Toxics Release Inventory (TRI) chemicals. TRI data for both isomers are reported for the years 1988-2002 (U.S. EPA 2004).

2,4-Dinitrotoluene

TRI releases for 2,4-DNT were reported from 21 States (AK, CA, FL, IA, IL, IN, KY, LA, MI, MO, MS, NE, NJ, NV, OH, SC, TN, TX, UT, VA, WV). Surface water discharges in 1988 and 1989 were slightly greater than 12,000 lbs/year. They declined significantly in the 1990s, ranging from 3,735 lbs/yr to as low as 90 lbs/yr. Surface water discharges were even lower during the years 2000, 2001, and 2002, at levels of 177, 10, and 6 lbs/yr, respectively. Combined releases of all kinds (i.e., onsite [air, surface water discharge, underground injection, releases to land] and offsite) declined in the early 1990s and then peaked again around 1999-2001 to a high of almost 700,000 lbs/yr (U.S. EPA, 2004).

2,6-Dinitrotoluene

TRI releases for 2,6-DNT were reported from 10 States (AR, CA, IN, KY, LA, MI, NV, OH, TX, WV), with no more than 9 States reporting in any one year. Surface water discharges in 1988 and 1989 were approximately 1,000 lbs/yr. They declined in the 1990s, ranging from 702 lbs/yr to as low as 24 lbs/yr. Surface water discharges remained low during the years 2000, 2001, and 2002, at levels of 32, 0, and 1 lbs/yr, respectively. Combined releases of all kinds (i.e., onsite [air, surface water discharge, underground injection, releases to land] and offsite) declined in the early 1990s and then peaked again around 1999-2001 to more than 1 million lbs/yr (U.S. EPA, 2004).

3.1 Production and Use

DNT is made by reacting toluene with a mixture of nitric and sulfuric acids (ATSDR, 1998). Typically, the process yields 75% 2,4-DNT, 19% 2,6-DNT, 2.5% 3,4-DNT, 1.0% 2,3-DNT, and 0.5% 2,5-DNT by weight (HSDB, 2004a). TNT and mononitrotoluenes account for the remaining percentage (ATSDR, 1998). This mixture typically is Tg-DNT. An alternative method for the production of DNT is the nitration of mononitrotoluene with mixed acid (HSDB, 2004c). Small concentrations of DNT isomers also occur as byproducts in the production of TNT (Hartley et al., 1994). The military requirement for DNT specifies a minimal melting point of 65.5 °C, which corresponds to a 2,4-DNT purity of 92%. Another specification for the production of military munitions requires the use of DNT mixture composed of at least 98.5% of the 2,4-isomer (Hartley et al., 1994).

Most DNT is produced for production of toluene diisocyanate, a precursor to polyurethane polymers. 2,4-DNT also is used in the production of TNT as a modifier for smokeless powders in the munitions industry, in the production of waterproofing for explosives, as a dye intermediate, as a plasticizer in propellants (HSDB, 2004a). The 2,4-DNT isomer is used in airbags of automobiles (ATSDR, 1998). Similar to 2,4-DNT, 2,6-DNT is used in the production of waterproofing for explosives, and as a plasticizer in propellants. It also is an intermediate in the production of TNT, urethane polymers, flexible and rigid foams, surface coatings, and dyes (HSDB, 2004b).

Currently, there are a small number of companies manufacturing DNT in the United States. They include U.S. Bayer Corporation, Pittsburgh, PA (production site: Baytown, TX) and Rubicon LLC, Ascension Parish, LA (production site: Geismar, LA) (HSDB, 2004c). Information from the late 1990s (SRI Consulting, 1999 and). Hartley et al., 1994) identified other companies that produced 2,4-DNT and/or 2,6-DNT [Air Products and Chemicals, Inc., Allentown, PA; Allied Chemical Corporation, Moundsville, WV, and E.I. Du Pont de Nemours and Company, Deepwater, NJ].

3.2 Air

Most measurements of 2,4-DNT and 2,6-DNT in air are from occupational environments where explosives or explosive devices are manufactured or processed (ATSDR, 1998). The TRI Program records air releases to the ambient environment. These releases for 2,4-DNT and 2,6-DNT were discussed in Section 3.0 of this document.

2,4-Dinitrotoluene

Ambient air concentrations of 2,4-DNT in the work environment have been reported to range from less than detectable levels up to 2,680 mg/m³ (Letzel et al., 2003; Woollen et al., 1985; Ahrenholz, 1980).

Personal (breathing zone) samples taken from workers at these same workplaces also ranged from less than detectable levels but only up to air concentrations of 440 mg/m³. In other airborne samples collected for the detection of nitroaromatics (from unspecified sources), Matsushita and Iida (1986) reported a concentration of 0.024 ng/m³ for 2,4-DNT.

2,6-Dinitrotoluene

Studies primarily measured DNT mixtures or 2,4-DNT in air samples. Levine et al. (1985a) detected personal breathing zone samples of 2,6-DNT at 50 mg/m³ to 590 mg/m³ in the workplace.

Dinitrotoluene Mixture

In biological monitoring studies among workers exposed to Tg-DNT in an explosives factory, routine personal air sampling revealed levels ranging from undetectable to 100 mg/m³. The detection limit was not reported by the ATSDR (1998); however, air monitoring analysis has a detection limit of 20 parts per trillion (ppt) for 2,4-DNT (Nacson et al., 1994). In the same study, static samples positioned near potentially dusty areas revealed atmospheric concentrations ranging from 20 mg/m³ to 2,680 mg/m³ (mean of 400 mg/m³) (Woollen et al., 1985). In another occupational environment, breathing zone concentrations of Tg-DNT ranged from undetectable to 23 mg/m³ (time-weighted average [TWA]) (Ahrenholz, 1980). Concentrations of Tg-DNT in area air samples ranged from undetectable to 420 mg/m³ (TWA). Ahrenholz and Meyer (1982) reported that area air samples in a manufacturing facility contained TWA concentrations of Tg-DNT that ranged from undetectable to 890 mg/m³.

3.3 Food

2,4-DNT or 2,6-DNT were not detected in fish samples from Lake Michigan tributaries, Grand Traverse Bay, and other Great Lakes harbors and tributaries in Ohio and Wisconsin (Camanzo et al., 1987; De Vault, 1985). Reports of other food sources that contained levels of 2,4-DNT were not found in the available literature.

3.4 Water

3.4.1 Drinking Water Occurrence

The first Unregulated Contaminant Monitoring Regulation (UCMR1) was established to satisfy the requirements of the 1996 Safe Drinking Water Act amendments. It was designed to collect information on the national occurrence of select emerging contaminants in drinking water. 2,4-DNT and 2,6-DNT were scheduled to be monitored by all large community water systems (CWSs) and non-transient non-community water systems (NTNCWSs) and a statistically representative sample of qualifying small CWSs and NTNCWSs. Although monitoring was conducted primarily between 2001 and 2003, some results were not collected and reported until as late as 2006. Because the health reference level (0.05 µg/L) for each

contaminant (2,4-DNT and 2,6-DNT) is less than the minimum reporting level (MRL) of 2 µg/L, the data are analyzed only as detections (\geq MRL).

Among small systems, there were no detections of 2,4-DNT. There was only one detection in large systems that reported results of 2,4-DNT; this surface water system represented 0.03% of reporting large systems and 0.02% of the population served by them. The analysis of samples from large and small systems did not detect any 2,6-DNT.

The state of Illinois submitted data to EPA indicating that significant levels of DNT (2,4-DNT and 2,6-DNT; other isomers not specified) had been detected in groundwater at a number of sites within the state (Illinois EPA, 2007). DNT in potable groundwater at the Joliet Army Ammunition Plant and the Savannah Army Depot was detected at levels of 10,000 µg/L and 58.8 µg/L, respectively. Data were also obtained from the state of Wisconsin (2007) where the 2,3-, 2,5-, 3,4-, and 3,5-DNT isomers have been identified in both monitoring well and a few private water supply wells near the Badger Army Ammunition Plant site. These data demonstrate the risk for DNT contamination of groundwater at military bases where DNT munitions have been used or stored.

3.4.2 Bed Sediment

SVOCs such as 2,4-DNT and 2,6-DNT have a slight tendency to sorb to sediment and particles because of their low to moderate log octanol/water coefficients (K_{OWS}) (1.98 and 1.72, respectively). The NAWQA Pesticide National Synthesis Project includes an analysis of SVOC monitoring in bed sediment from representative watersheds across the country between 1992 and 2001. Sampling was conducted at 1,029 sites. The RL for all SVOCs was 50 µg/L (Nowell and Capel, 2003).

2,4-Dinitrotoluene

NAWQA data indicate that 2,4-DNT was not detected in bed sediment in agricultural, urban, or undeveloped settings. In mixed land use settings, 2,4-DNT was detected in 1.3% of samples, with a maximum concentration of 173 µg/kg dry weight (Nowell and Capel, 2003).

2,6-Dinitrotoluene

2,6-DNT was detected in bed sediment at frequencies ranging from 1.6% in urban settings to 4.4% in agricultural settings, 6.6% in mixed land use settings, and 6.9% in undeveloped settings. The 95th percentile concentrations were less than the RL in all settings. The highest concentration, 291 µg/kg dry weight, was found in an undeveloped setting (Nowell and Capel, 2003).

3.5 Soil

2,4-Dinitrotoluene

Concentrations of 2,4-DNT in soil ranged from <0.1 mg/kg to 117 mg/kg at the Joliet Army Ammunition Plant in Joliet, IL, an NPL site (Simini et al., 1995). The 2,4-DNT isomer was detected in the soil at 2.2% of hazardous waste sites, with a geometric mean concentration of 1.0 mg/kg (ATSDR, 1989). The concentration of 2,4-DNT in the soil in a waste lagoon abandoned for 20 years at the Iowa Army Ammunition Plant was 3.0 mg/kg (Ryon et al., 1984).

2,6-Dinitrotoluene

Concentrations of 2,6-DNT in soil ranged from <0.1 mg/kg to 8 mg/kg at the Joliet Army Ammunition Plant, an NPL site (Simini et al., 1995). The 2,6-DNT isomer was detected in the soil at 1.3% of hazardous waste sites, with a geometric mean concentration of 0.140 mg/kg (ATSDR, 1989).

4.0 ENVIRONMENTAL FATE

DNT has been found in the soil, surface water, and groundwater of hazardous waste sites that contain buried ammunition wastes and wastes from manufacturing facilities that release DNT (ATSDR, 1998). The water solubilities of 2,4-DNT and 2,6-DNT are moderate, and the log K_{ow} and log K_{oc} are low for both isomers (Table 2-1). Since the partitioning of organics to the sediment from the aqueous phase does not become a major loss until the log K_{oc} values exceed 3.5, the relatively low log K_{oc} values for 2,4-DNT and 2,6-DNT indicate that these compounds would have only a slight tendency to sorb to sediments, suspended solids, and biota. Therefore, there is potential for transport via surface water or groundwater. The low lipophilicity of this compound predicts it is not expected to bioaccumulate in animal tissues (ATSDR, 1998).

4.1 Environmental Media Transport

2,4-Dinitrotoluene

2,4-DNT adsorption to sediments, suspended solids, and biota is not a significant environmental fate due to its relatively low log K_{oc} (Spangord et al., 1980). Additionally, the low vapor pressure (1.4×10^{-4} mm Hg at 25 °C) and Henry's law constant (solubilities) (8.79×10^{-8} atm•m³•mol) of 2,4-DNT indicate that 2,4-DNT is not expected to volatilize from water or soil (ATSDR, 1998; HSDB, 2004a,b,c).

2,6-Dinitrotoluene

Measurements in two types of soil indicated that 2,6-DNT's low K_{oc} s were 1.86 and 1.28 (Kenaga, 1980). These values indicate that 2,6-DNT would have high mobility in soil (HSDB, 2004a,b,c). It is concluded that adsorption on sediments is not a significant environmental fate. Additionally, the low vapor pressure (5.67×10^{-4} mm Hg at 25 °C) and Henry's law constant (9.26×10^{-8} atm•m³•mol) of this DNT isomer indicate that it is not expected to volatilize from water or soil (ATSDR, 1998; HSDB, 2004a,b,c).

Dinitrotoluene Mixture

DNT may be released and transported in the air in the form of dusts or aerosols from manufacturing plants. It can enter surface water and groundwater by releases of wastewater from TNT manufacturing facilities and from buried munition wastes (ATSDR, 1998). The relatively low volatility and moderate solubility of DNT indicate that it will remain in water for long periods of time. DNT is degraded by light, oxygen, and biota. As a result, it can be transported to groundwater or surface water (ATSDR, 1998).

4.2 Environmental Degradation

2,4-Dinitrotoluene

Vapor-phase 2,4-DNT is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals and has an estimated half-life of 75 days (HSDB, 2004a,b,c; Meylan and Howard, 1993).

Ho (1986) found that intermediates formed during 2,4-DNT's degradation include 1,3-dinitrobenzene, hydroxynitrobenzene derivatives, and carboxylic acids. DNT's half-life following photolysis from sunlight ranged from 2.7 hours to 9.6 hours in natural waters (Spanggord et al., 1980). Dissolved humic substances in natural waters enhance the sunlight-induced photodegradation rates (by 10-17 times) compared with rates observed in distilled water (Simmons and Zepp, 1986). Chlorination and ozonation reduce 2,4-DNT 35% and 60%, respectively, regardless of length of contact time.

Microbial biodegradation of DNT in water has been observed under both aerobic and anaerobic conditions. Biotransformation occurs mainly through the reduction of the nitrogroup (Spanggord et al., 1981). Microorganisms isolated from DNT-contaminated sites are capable of growth on 2,4-DNT as their sole carbon and energy source (ATSDR, 1998; Lewis et al., 2004; Spanggord et al., 1980). 2,4-DNT degradation occurred in waters taken downstream from the Radford Army Ammunition Plant (Radford, VA) but not in those from a Maryland surface freshwater source (Bausum et al., 1992). Biotransformation of DNT by *Pseudomonas aeruginosa* isolated from a propellant wastewater treatment plant, was observed under both aerobic and anoxic conditions (Noguera and Freedman, 1996).

In soil, microorganisms can degrade DNT. Jenkins et al. (2001) determined that 2,4-DNT's half-life in soil was 25 days at 22 °C, from an initial concentration of 0.5 mg/kg, and concluded that it was concentration dependent. Microorganisms indigenous to surface soils from munitions-contaminated sites transformed 2,4-DNT to aminonitro intermediates within 70 days (Bradley et al., 1994). Lower temperatures slow 2,4-DNT's breakdown (Grant et al., 1995).

Since microorganisms readily metabolize 2,4-DNT to CO₂ as the final product, DNT is not expected to persist in the environment. However, studies show persistence is water-body dependent, and the length of time any particular nitroaromatic compound resides in the environment ultimately depends on the compound's unique interaction with the natural organics and biota in its surroundings (Spanggord et al., 1980; Liu et al., 1984). Multiple studies show that the breakdown/intermediate products of 2,4-DNT include 4-amino-2-nitrotoluene, 2-amino-4-nitrotoluene, and/or 2,4-diaminotoluene (Bradley et al., 1997; Cheng et al., 1996; Freedman et al., 1996; Liu et al., 1984; Noguera and Freedman, 1996, 1997).

2,6-Dinitrotoluene

Vapor-phase 2,6-DNT is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals (HSDB, 2004b). The half-life for this reaction in air is estimated to

be 75 days, as calculated from its rate constant of 2.2×10^{-13} cm³/molecule-sec at 25 °C, which was determined using a structure estimation method (Meylan and Howard, 1993).

In a study of chemicals associated with TNT in the soil, the half-life of 2,6-DNT was measured to be 20 days at 22 °C when the initial concentration of 2,6-DNT was 0.5 mg/kg. The study also showed that the half-life of 2,6-DNT was concentration dependent (Jenkins et al., 2001). In another study, microorganisms indigenous to surface soils collected at munitions-contaminated sites were reported to transform 2,4-DNT and 2,6-DNT to aminonitro intermediates within 70 days (Bradley et al., 1994). Degradation rates of 2,6-DNT measured in Mississippi and Texas soils were 0.5 mg/kg/day and 0.7 mg/kg/day, respectively, which correspond to half-lives of 92 and 73 days, respectively (Loehr, 1989).

In oxygenated waters, photolysis is probably the major route of degradation of DNT (ATSDR, 1998). Dillert et al. (1995) reported that degradation rates followed first-order kinetics, and were dependent on time, solution pH, and light intensity. Simmons and Zepp (1986) studied the influence of various humic substances on the photoreactions of 19 nitroaromatic substances, including 2,4-DNT and 2,6-DNT. The results observed indicate that dissolved humic substances in natural waters enhance the sunlight-induced photodegradation rates (by 10-17 times) compared with rates observed in distilled water. The half-life of 2,6-DNT in river water exposed to sunlight was measured to be 12 minutes, and the degradation was determined to be from an indirect photoreaction (Zepp et al., 1984).

Degradation of DNT by ozonation and chlorination was measured by Lee and Hunter (1985). 2,6-DNT was reduced by less than 17% with both chlorine and ozone, regardless of length of contact time.

Microbial biodegradation of DNT in water has been observed under both aerobic and anaerobic conditions. Biotransformation occurs mainly through the reduction of the nitrogroup (Spanggord et al., 1981). Several studies have isolated microorganisms from DNT-contaminated sites, which are capable of growth on 2,4-DNT and 2,6-DNT as their sole carbon and energy source (ATSDR, 1998; Lewis et al., 2004; Spanggord et al., 1980). Data from studies cited in Lewis et al. (2004) suggest that the initial biological breakdown of DNTs involves the activity of dioxygenase enzymes, leading to electrophilic degradation by a monooxygenase enzyme on an aromatic, nitro-substituted carbon atom.

Since microorganisms readily metabolize 2,6-DNT to CO₂ as the final product, it is not expected to persist in the environment. Persistence is water-body dependent, and the half-life ultimately depends on the compound's unique interaction with the natural organics and biota in its surroundings. Spanggord et al. (1980) observed biodegradation in an aerobic environment with a half-life of less than 1 hour. Complete degradation of 20 ppm of 2,6-DNT was observed in water samples taken downstream from the Radford Army Ammunition Plant. Up to 60% of the substrate carbon appeared as CO₂ (Bausum et al., 1992). In another study, 2,6-DNT was degraded to CO₂ more slowly than 2,4-DNT. The conversion rate was concentration dependent and increased with increasing concentration (Bausum et al., 1992). The bacterium *Burkholderia cepacia* was isolated from the Radford Army Ammunition plant in West

Virginia and can use 2,4-DNT as its sole source of carbon and nitrogen (Johnson et al., 2002). In contrast, degradation was not observed in samples taken from Maryland surface freshwater sources (Bausum et al., 1992).

Approximately 14% of 2,6-DNT was degraded by indigenous microorganisms in microcosms (30 mL vials) prepared from contaminated aquifer material within 30 days (Bradley et al., 1997). Following a 1-week acclimation period at a hydraulic residence time of 6 hours, 76% of the 2,6-DNT in the influent to a fluidized-bed biofilm reactor was degraded using a mixed bacterial culture (Lendenmann et al., 1998).

Under anaerobic conditions, the half-life of 2,6-DNT in non-acclimated sewage was found to be 28 days, with no loss of the compound under aerobic conditions during the same period (Hallas and Alexander, 1983). In anaerobic conditions, three of six methanogens studied were capable of degrading 55% to 95% of 2,6-DNT in an aqueous solution in 30 days; degradation was not observed for the other three organisms (Boopathy, 1994). Biotransformation of DNT by *Pseudomonas aeruginosa* isolated from a propellant wastewater treatment plant, was observed under both aerobic and anoxic conditions. The primary products of the biotransformation, which was mainly reductive, were 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene, with some 2,4-diaminotoluene. DNT metabolites from acetylation of the arylaminos were identified, including 2-acetamide-2-nitrotoluene, 2-acetamide-4-nitrotoluene, 4-acetamide-2-aminotoluene, and 2,4-diacetamidetoluene (Noguera and Freedman, 1996).

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. (1981). 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. The half-life was 8.3 days.

Dinitrotoluene Mixture

Studies indicate that DNT may be degraded through several mechanisms in the environment, including photolysis, microbial biodegradation, ozonation, chlorination, and oxidation by strong oxidants such as hydrogen peroxide, ozone, or oxone (potassium peroxomonosulfate) (ATSDR, 1998).

In the air, DNT is thought to break down by a variety of chemical reactions that take place upon exposure to sunlight (ATSDR, 1998). In oxygenated waters, photolysis is probably the major route of degradation of DNT (ATSDR, 1998). Based on its rapid photolysis in water, DNT presumably is subject to oxidation of its methyl group, decarboxylation, ring oxidation, and/or nitroreduction in air and sunlight (ATSDR, 1998). Degradation rates follow first-order kinetics and are dependent on time, solution pH, and light intensity (Dillert et al., 1995).

4.3 Bioaccumulation

2,4-Dinitrotoluene

2,4-DNT's relatively low log K_{OW} (1.98) indicates that it is not expected to bioaccumulate in animals (ATSDR, 1998; Callahan et al., 1979; Mabey et al., 1982). Calculated estimates of 2,4-DNT's BCF were 7 (Meylan et al., 1999; HSDB, 2004a) and 204 calculated for guppy (*Poecilia reticulata*) (Deneer et al., 1987); the first estimate suggests a low potential for bioconcentration in aquatic organisms while the second is an experimental value. Since DNT, however, is quite soluble in water, it is expected to accumulate readily in plants via root uptake from soils (ATSDR, 1998).

2,6-Dinitrotoluene

The relatively low log K_{OW} of 2,6-DNT (1.72) indicates that 2,6-DNT in the environment would not bioaccumulate in animals (ATSDR, 1998; Callahan et al., 1979; Mabey et al., 1982). However, since DNT is quite soluble in water, it can be transferred to plants via root uptake from soils and is expected to accumulate readily in plants (ATSDR, 1998). The structural analogy with 1,3-dinitrobenzene and 4-nitrotoluene suggests that 2,6-DNT would be readily taken up by plants (McFarlane et al., 1987; Nolt, 1988). , Davis et al. (1981) reported a bioconcentration factor (BCF) of 5225 L/kg.

5.0 TOXICOKINETICS

5.1 Absorption

2,4-Dinitrotoluene

No experimental studies were identified that characterize how 2,4-DNT is absorbed in humans and other animals. However, studies that demonstrate distribution, metabolism, and excretion suggest that in both humans (Woollen et al., 1985) and animals (Medinsky and Dent, 1983; Lee et al., 1975, 1978; Mori et al., 1977, 1978), 2,4-DNT is absorbed rapidly, within 24 hours to 72 hours post-exposure or post-dosing.

2,6-Dinitrotoluene

No experimental studies were identified in the literature that characterize how 2,6-DNT is absorbed in humans and animals. Studies suggest that humans absorb both DNT isomers following dermal and inhalation exposure (Woollen et al., 1985; Medinsky and Dent, 1983; Lee et al., 1975, 1978; Mori et al., 1977, 1978).

Dinitrotoluene Mixture

Absorption of Tg-DNT following oral exposure in humans has not been determined. Occupational studies indicate that after inhalation or dermal exposure, Tg-DNT is absorbed by humans and excreted in the urine. Absorption of DNT and excretion from the body occur rapidly and are usually complete within 24 hours to 72 hours post-dosing. Percentage absorption of the DNT isomers is difficult to estimate since biliary excretion is significant in most animals leading to fecal excretion of a portion of the absorbed material. The amount of DNT reabsorbed from bile also varies among species.

Two studies in which a total of 33 workers in an explosives factory were exposed to Tg-DNT (20% 2,6-DNT) were published by Woollen et al. (1985). The authors suggest that the skin was probably the primary route of exposure for these workers, and the lungs constituted the secondary route because of low atmospheric levels of DNT (0.02 – 2.68 mg/m³). Blood levels of DNT (2,4-DNT and 2,6-DNT combined) were low before the workday began (<10 ng/mL), gradually increased during the exposure period (20-90 ng/mL), and peaked at the end of the work shift (70-250 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.

5.2 Distribution

2,4-Dinitrotoluene

Oral administration of a single dose of 2,4-DNT in rats, mice, rabbits, and monkeys show that both the unchanged compound and its metabolites distribute to the liver, kidneys, lung, brain, skeletal muscle, blood, and adipose tissue (Lee et al., 1975; Rickert and Long, 1980; Mori et al., 1977, 1978). Most tissues acquired between 0.36% and 1.6% of DNT and its metabolites within 24 hours; the liver, kidneys, and lungs showed a preferential initial uptake. Very little 2,4-DNT was retained in the single-dose studies after 24 hours (Lee et al., 1975, 1978). Results were similar in dogs given multiple doses of 2,4-DNT for 5 days; however, tissue concentrations were two to four times greater than those of single-dose studies, indicating that 2,4-DNT and/or its metabolites can accumulate in the body (Lee et al., 1978). Schut et al. (1981, 1982) reported similar tissue distribution in male mice given single intraperitoneal (ip) doses of 2,4-DNT, except that blood and tissues reached peak levels quicker (30 minutes to 2 hours). There was no indication in the data that any organ had preferential uptake.

Radioactivity was detected in the placenta and amniotic fluid of rats (strain not given) administered a single oral dose of ^{14}C -2,4-DNT on gestation day 20 (Rickert et al., 1980). The investigators reported that approximately 10% to 50% of the ^{14}C dose was recovered from these two fractions; however, the time of sacrifice was not reported. The concentrations of ^{14}C in fetal tissues were reported to be similar to those in maternal tissues, but supporting data were not provided.

2,6-Dinitrotoluene

Schut et al. (1983) examined the disposition of a single oral or ip dose of [^3H]2,6-DNT in male A/J mice. Animals were given 1-, 10-, or 100-mg/kg doses of the radiolabeled compound (2.5 $\mu\text{Ci}/\text{mouse}$). Blood and liver levels of ^3H were similar in orally dosed mice and remained reasonably constant for the first 8 hours after dosing. In contrast, hepatic concentrations of radioactivity in ip-dosed mice peaked during the first hour post-dosing and decreased steadily thereafter. The amount of ^3H in the blood was two to four times lower than that in the liver through the first 2 hours after compound administration. Kidney ^3H residue levels reportedly were 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. The maximum concentration was higher in orally dosed mice (9.5-16.9% of the administered ^3H) than in ip-dosed animals (5.2-8.9%). 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 μ Ci of [ring-UL-¹⁴C]2,6-DNT at a dose equivalent to 1/10 of 50% of the lethal dose (LD₅₀) (~80 mg/kg).

5.3 Metabolism

Covalent binding of DNT to hepatic macromolecules may be particularly important due to data showing a higher incidence of hepatic carcinomas and hepatic neoplastic nodules in DNT-exposed male rats compared with treated female rats (Rickert et al., 1983; Swenberg et al., 1983). 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 (Rickert et al., 1983), and binding of DNT isomers appears to be lower in females than in males. Diet (i.e., as it affects microbial activity and number) also may influence the degree to which binding of DNT metabolites occurs.

Hartley et al., (1994) summarized DNT biotransformation from gastric absorption to urinary excretion. After DNT is absorbed from the gastrointestinal tract, it is oxidized in the liver and forms metabolites. Some metabolites are conjugated, which then allows them to be excreted in urine or into bile. Metabolites that move from the bile to the gut are hydrolyzed and reduced by intestinal microflora (e.g., *Escherichia coli*, from human intestines). Many of these compounds, then, 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 may occur again, resulting in additional reduction by intestinal bacteria prior to elimination from the body.

2,4-Dinitrotoluene

The effect on tissue uptake, following repeated administration of 2,4-DNT in the diet, was examined in rats. Animals given both the treated and untreated (controls) diets were given a single oral dose of 2,4-DNT after cessation of the feeding regimen. Ellis et al. (1979) found that after 24 hours, tissue levels of 2,4-DNT were comparable in those that received the chemical in feed for 20 months when compared with untreated controls. Mori et al. (1980) found slightly lower levels of 2,4-DNT in the tissues of rats fed the chemical in the diet compared with those on a standard diet. Both studies suggest that prior exposure to 2,4-DNT does not stimulate metabolism.

Humans and experimental laboratory animals (i.e., rats, mice, rabbits, dogs, monkeys) transform 2,4-DNT to metabolites that are excreted in urine or into bile. The types of metabolites formed vary among species and include reduction, oxidation, acetylation, and glucuronidation products (Turner, 1986; Shoji et al., 1985; Turner et al., 1985; Levine et al., 1985a; Woollen et al., 1985; Schut et al., 1985; Medinsky and Dent, 1983; Mori et al., 1981a; Rickert et al., 1981; Rickert and Long, 1981; Ellis et al., 1979; Lee et al., 1978). Some studies show that the microsomal metabolism of 2,4-DNT involves cytochrome monooxygenases (e.g., P-450 and P-

448) to produce compounds such as 2,4-diaminotoulene (2,4-DAT) (Bond and Rickert, 1981; Mori et al., 1981b) and dinitrobenzaldehyde (2,4-DNBal) (Sayama et al., 1989a). A more detailed summary of 2,4-DNT's microbial metabolism can be found in Hartley et al. (1994).

2,6-Dinitrotoluene

The metabolism of 2,6-DNT has not been studied extensively. The primary urinary metabolites of 2,6-DNT excreted by individuals (14 men, 3 women) exposed occupationally to Tg-DNT (0.05-0.59 mg/m³) were the corresponding 2-6 dinitrobenzyl alcohols (2,6-DNBalc) and their glucuronides (2,6-DNBalcG), the corresponding dinitrobenzoic acids (2,6-DNBacid), and metabolite (2-amino-6-nitrobenzoic acid (2A4NBacid) (Levine et al., 1985a; Turner, 1986; Turner et al., 1985). Males excreted more DNBacid (2,4- and 2,6- combined) than females (52.5% vs. 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% vs. 33.3%, respectively). Approximately 73.9% of the urinary DNBalcG metabolites in females and 35.6% in males were the 2,6- isomeric forms. Urinary excretion of 2,6-DNBacid was slightly higher in males (2.2-14.3% of all metabolites) than in females (2.5%), and levels of 2,6-DNBalc were comparable between the sexes (4.8-6.6%). The unchanged parent compound (i.e., 2,6-DNT) also was recovered from the urine, and the hydrolyzed urine of one individual contained trace amounts of 4-amino-2-nitrobenzoic acid (4A2NBacid) and 4-(N-acetyl)amino-2-nitrobenzoic acid. Reduction of both nitro groups was not evident. Wide variations in urinary metabolite profiles were attributed to differences in exposure and in the pathways by which an individual may metabolize DNT.

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 ³H-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 ³H recovered from the urine of these animals during the 8-hour post-dosing 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. 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 ¹⁴C excreted by male and female Fischer 344 rats given a single oral dose of [ring-UL-¹⁴C]-2,6-DNT: 2,6-DNBacid, 2,6-DNBalcG, and 2A6NBacid (Long and Rickert, 1982). In males, these metabolites accounted for about 21, 22, and 14% of the ¹⁴C dose, respectively; in females, the corresponding values were 20, 19, and 11%.

The primary metabolite of 2,6-DNT produced by hepatocytes under aerobic conditions from male A/J mice and male Fischer 344 rats was 2,6-DNBalc; most (61.3-70.9%) was conjugated (Dixit et al., 1986). 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).

The role of gut microflora in the metabolism of 2,6-DNT has been examined *in vivo* and *in vitro*. In a study by Mori et al. (1984), *Escherichia coli* isolated from human intestines converted 2,6-DNT to 2-amino-6-nitrotoluene (2A6NT) via the corresponding hydroxylaminonitrotoluenes. Nitrosotoluenes, however, were not recovered in any human samples (Mori et al., 1984). Microflora in the cecal contents of male A/J mice and male Fischer 344 rats anaerobically converted [³H]2,6-DNT to 2A6NT, 2-acetylamino-6-nitrotoluene (2Ac6NT), and 2,6-diaminotoluene (2,6-DAT) (Dixit et al., 1986). Most of the parent compound (82.1-88.7%) was recovered as unchanged 2,6-DNT at the end of the 30-minute incubation period.

Dinitrotoluene Mixture

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 urine or into bile. Metabolites that are transported from the bile to 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. 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.

Several investigators conclude that, in humans exposed occupationally to Tg-DNT, metabolism is similar to that in DNT-exposed Fischer rats (Levine et al., 1985a; Turner, 1986; Turner et al., 1985). The primary urinary metabolites formed include dinitrobenzyl alcohols and their glucuronides, the corresponding dinitrobenzoic acids, and 2-amino-4-nitrobenzoic acid (2A4NBacid). Sex-related differences in the metabolism of 2,4-DNT have been observed only in Fischer rats and humans (Levine et al., 1985a; Turner, 1986; Turner et al., 1985). Females produce up to three times more 2,4-DNBalc and/or 2,4-DNBalcG than males, and in humans, men excrete almost double the 2,4-DNBacid as women.

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 2A4NBacid, 4A2NBacid, 2A6NBacid, and 4Ac2NBacid. The urine of these workers contained no 2,4-DNBalc or 2,6-DNBalc.

Mori et al. (1989) supported the finding of Sayama et al. (1989b) that dinitrobenzaldehyde is a metabolite of DNT and that the metabolism of DNT isomers is strain dependent, which was evident in Wistar and Sprague-Dawley rats.

5.4 Excretion

Absorption of DNT and excretion from the body occur rapidly and are usually complete within 24 hours to 72 hours post-dosing. Elimination via feces or lungs has not been examined in humans. In explosives workers, urinary excretion of Tg-DNT's primary metabolite (2,4-DNBacid) was highest at the end of the work shift and generally was much greater at the end of each workweek than at the beginning. This suggests that DNT is cleared from the body readily and does not accumulate (Woollen et al., 1985). Elimination half-lives for all five DNT metabolites excreted in the urine of three workers exposed to 0.05-0.59 mg Tg-DNT/m³ were between 0.88 hour and 2.76 hours (Turner, 1986; Turner et al., 1985). Values for individual metabolites were between 0.80 and 4.26 hours. The urine is the primary route of elimination for both DNT isomers in most animals, including rodents, rabbits, dogs, and monkeys.

2,4-Dinitrotoluene

2,4-DNT is eliminated rapidly in humans through urine and in laboratory animals through feces and urine. Information concerning the elimination of 2,4-DNT from humans via feces or lungs was not found in the literature. Woollen et al. (1985) estimated that the half-life for the urinary elimination of 2,4-DNT was between 2 hours and 5 hours. Low, but detectable, levels of 2,4-DNBacid were found in the urine 3 days after exposure, suggesting that urinary elimination of DNT is biphasic (Turner, 1986; Turner et al., 1985; Woollen et al., 1985).

In animals, the primary elimination route varies by species or strain. In Fischer 344 and CD rats, rabbits, dogs, and monkeys, urine is the primary excretion route, and up to 90% is eliminated within 24 hours to 72 hours (Rickert et al., 1981, 1984; Ellis et al., 1979, 1985; Lee et al., 1975, 1978). In CD-1 and female B6C3F1 mice (Lee et al., 1978) and in Wistar rats (Mori et al., 1977), however, up to 84% of administered 2,4-DNT was eliminated in feces between 24 hours and 7 days. Similar results were observed in male AJ mice given ip doses of 2,4-DNT (Schut et al., 1982, 1985). Rickert et al. (1981) observed that axenic (lacking intestinal bacteria) male Fischer 344 rats eliminated significantly ($p < .05$) less 2,4-DNT in urine and feces, suggesting that metabolism by gut flora may have a role in excretion. The only report of elimination of 2,4-DNT by exhalation was noted in a study with A/J mice, where only 0.20% was detected in the breath.

2,6-Dinitrotoluene

Orally administered 2,6-DNT was eliminated primarily via urine. Male AJ mice excreted about 54, 54, and 49% of a single oral dose of 1, 10, or 100 mg [³H]2,6-DNT/kg, respectively, in urine within 8 hours post-dosing (Schut et al., 1983). Feces contained no more than 2.1% of the ³H dose, and levels in the small intestine accounted for about 3.0% to 3.6%. Elimination via the lungs was negligible (<0.35%).

Twenty-four hours following administration of a single oral dose of uniformly ¹⁴C labeled ring [ring-UL-¹⁴C]2,6-DNT (80 mg/kg) to female CD rats, about 60% of the ¹⁴C was recovered from urine, and 40% was recovered from 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.

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 into 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 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 μM [ring-UL- ^{14}C]2,6-DNT. At 70 μM , however, total recovery of this metabolite in the bile was significantly less ($p < .05$) in females than in males. These data indicate a possible saturation point in the metabolism and excretion of 2,6-DNT in females. Biliary flow rates were similar for both sexes, and disappearance of parent compound (20 μM or 70 μM) from the perfusate was biphasic: half-times of elimination were 7.5 minutes and 8.4 minutes for males and females, respectively, for the initial phase, and 53.9 minutes and 52.7 minutes, respectively, for the second phase.

6.0 HEALTH EFFECTS DATA

6.1 Human Studies

In humans, the toxic effects of 2,4-DNT or 2,6-DNT are on the central nervous system (CNS) and also may be on the heart and circulatory system.

6.1.1 Short-Term Exposure

Reports of short-term exposure of 2,4-DNT or 2,6-DNT on humans were not located in the available literature.

6.1.2 Long-Term Exposure

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; Levine et al., 1985b; 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).

Letzel et al. (2003) performed a cross-sectional study of 82 employees from a munitions dismantling mechanical plant in the Free State of Saxony, Germany. The workers were exposed to TNT and DNT regularly (51 persons) or occasionally (19) or were unexposed (12) for a median period of 59 months. Air analyses yielded maximum concentrations of 20 $\mu\text{g}/\text{m}^3$ for 2,4-DNT and 3,250 $\mu\text{g}/\text{m}^3$ for 2,4,6-TNT, respectively. In 63 workers where TNT, DNT, and/or their metabolites were detected in their urine, workers frequently reported symptoms such as bitter taste, burning eyes, and discoloration of skin and hair.

6.1.3 Reproductive and Developmental Effects

Limited evidence suggests that neither 2,4-DNT, 2,6-DNT, nor the DNT mixture causes adverse effects on human reproductive performance (Hamill et al., 1982; Ahrenholz and Meyer, 1982).

6.1.4 Carcinogenicity

An epidemiology study was performed by Stayner et al. (1993) to evaluate the relationship between DNT exposure and increased risk of cancers of the liver and biliary tract. The study included a total of 4,989 white male workers exposed to DNT and 7,436 white male unexposed workers who had worked for at least 5 months at the Radford Army Ammunition Plant between January 1, 1949, and January 21, 1980. Women and non-Caucasian employees were a small percentage of the worker population and therefore were excluded from the analysis. Workers were considered exposed if they had worked at least 1 day on a job with probable exposure to DNT; however, exposure data were not available. An excess of hepatobiliary cancer was

observed among workers exposed to DNT, but the increase was not statistically significant compared with the general population.

Brüning et al. (1999, 2001, 2002) investigated the carcinogenicity of DNT on the urinary tract of underground mining workers. Between 1984 and 1997, 6 cases of urothelial cancer and 14 cases of renal cell cancer were diagnosed in a group of 500 underground mining workers. They worked in the copper mining industry of the former German Democratic Republic (GDR) (East Germany) and had high exposures to explosives containing Tg-DNT. The incidences of both urothelial and renal cell tumors in this group were 4.5 and 14.3 times higher, respectively, than anticipated on the basis of the cancer registers of the GDR. A group of 161 miners highly exposed to DNT was investigated for signs of subclinical renal damage. Biomarker excretion (α_1 -microglobulin, glutathione S-transferases α and π) indicated that DNT-induced damage was directed toward the tubular system. The authors indicate that their observations appear consistent with the concept of cancer initiation by DNT isomers and the subsequent promotion of renal carcinogenesis by selective damage to the proximal tubule. The differential pathways of metabolic activation of DNT appear to apply to the proximal tubule of the kidney and to the urothelium of the renal pelvis and lower urinary tract as target tissues of carcinogenicity.

These studies are limited by a variety of determinants including inadequate exposure information (i.e. concentrations and duration). Confounding information on exposure to other chemicals and lifestyles factors such as smoking behavior was also not reported.

6.2 Animal Studies

There are several short- and long-term animal studies with 2,4-DNT and 2,6-DNT that demonstrate acute, subchronic, and chronic adverse effects. Both DNT isomers cause adverse neurological, hematological, reproductive, hepatic, and renal effects in rats, mice, and dogs. Dogs generally are the most sensitive of the three species.

6.2.1 Short-Term Exposure

LD₅₀ studies indicate that both 2,4-DNT and 2,6-DNT are moderately to highly toxic to rats and mice (Hartley et al., 1994). Times to death, recovery, and gross pathology were similar for both isomers (Lee et al., 1975; Vernot et al., 1977).

2,4-Dinitrotoluene

Acute oral toxicity studies indicate that rats are more susceptible to 2,4-DNT than mice. The LD₅₀ values ranged from 1,340 mg/kg 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.

Lane et al. (1985) orally administered 2,4-DNT (purity not specified) daily in corn oil to groups of 10 male Sprague-Dawley rats by gavage as part of a study of reproductive toxicity. The dose levels were 0, 60, 180, or 240 mg/kg/day for 5 consecutive days. High mortality was observed in the high-dose group; therefore, 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, while BW loss was seen at the high dose.

Groups of 10 female CD-1 mice were given oral doses of 2,4-DNT (purity not specified) by gavage in corn oil daily for 8 consecutive days (Smith, 1983). Dose levels were 0, 310, 525, 1,250, 2,500, or 3,500 mg/kg/day BW. There was 100% mortality in all groups receiving $\geq 1,250$ mg/kg/day and 60% mortality in the 525-mg/kg/day group. No treatment-related mortality was seen at the low dose. The survivors of the 525-mg/kg/day group had significantly ($p < .007$) lower mean BW and exhibited toxicity characterized by lethargy, dyspnea, rough hair coat, hunched posture, tilted head, tremors, ataxia, and prostration. The no observed adverse effect level (NOAEL) was 310 mg/kg/day.

As reported by Hartley et al. (1984), Sprague-Dawley rats (5/sex/dose) were fed diets containing 0, 900, 1,200, 1,900, or 3,000 mg of 2,4-DNT kg/day for 14 days (McGown et al., 1983). The chemical administered contained 97% 2,4-DNT, 2% 2,6-DNT, and 1% unspecified contaminants. The intake was 0, 97, 126, 180, or 257 mg/kg/day 2,4-DNT for males and 0, 96, 121, 186, or 254 mg/kg/day for females. 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 elevated significantly in dosed males and/or females. Hyaline droplets were found histologically in the epithelium of the proximal convoluted tubules of the kidneys of all dosed rats, but lacked a dose-response trend; the males appeared to be more susceptible than females. Oligospermia was found in a dose-related manner in males, with accompanying degenerative changes of the testes. Based on decreased BW gain, decreased food consumption, and changes in serum chemistry levels in females, the lowest observed adverse effect level (LOAEL) was 96 mg/kg/day.

Groups of 10 male Sprague-Dawley rats were fed a diet containing 0, 0.1, or 0.2% 2,4-DNT (equivalent to 50 or 100 mg/kg/day, based on Lehman [1959]) for 3 weeks as part of a study of reproductive toxicity (Bloch et al., 1988). The final BWs were significantly lower ($p < .01$) in treated animals at both dietary levels compared with controls. No systemic toxicity was seen.

In a mouse study (8/sex/dose), groups of animals were fed diets containing 0, 0.07, 0.20, or 0.70% 2,4-DNT (98% pure) daily for 4 weeks (Lee et al., 1978). This represents daily intakes of 0, 47, 137, or 413 mg/kg/day for males and 0, 52, 147, or 468 mg/kg/day for females (U.S. EPA, 1986). No mortality occurred in the mice. The low- and mid-dose levels were nontoxic, and mice at the high-dose level showed slight BW 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 BW 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.

In the companion study, Lee et al. (1978) fed the same DNT concentrations (and purity) in feed to groups of rats (8/sex/dose), where the corresponding daily intakes were 0, 34, 93, or 266

mg/kg/day for males and 0, 38, 108, or 145 mg/kg/day for females (U.S. EPA, 1986). 2,4-DNT was toxic to both sexes at all levels. At the high dose, two males and two females died; the surviving rats showed BW loss and decreased food consumption. Rats dosed at the lower levels exhibited a slight depression in BW gain and food consumption. No consistent changes were observed in hematology or clinical chemistry parameters. A significant ($p < .05$) 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 BW lost, but the hemosiderosis and testicular lesions were not reversible. Based on BW loss and decreased food consumption in both sexes, the LOAEL was 34 mg/kg/day in males and 38 mg/kg/day in females; a NOAEL was not established.

In a dog study, Lee et al. (1978) gave groups of two males and two females 2,4-DNT in capsules at doses of 0, 1, 5, or 25 mg/kg/day for 4 weeks. No treatment-related toxicity was observed in dogs given 1 or 5 mg/kg/day. At the high dose (25 mg/kg/day), dogs showed signs of toxicity, including decreased food consumption, BW loss, yellow stain on and near hind legs, pale gums, neuromuscular incoordination, and paralysis. Histopathology of the high-dose dogs revealed hemosiderosis in the liver, cloudy swelling and tubular degeneration of the kidneys, and lesions of the brain and spinal cord in both sexes. Males exhibited aspermatogenesis. After 4 weeks, the animals partially recovered, and two kept for 8 months recovered completely. Based on decreased BW gain, decreased food consumption, neurotoxic signs, and histopathology, the LOAEL was 25 mg/kg/day, and the NOAEL was 5 mg/kg/day.

2,6-Dinitrotoluene

The oral LD₅₀ values for 2,6-DNT ranged from 621 to 1,000 mg/kg in mice and from 180 mg/kg to 795 mg/kg in rats. The acute toxicity of 2,6-DNT appears to be less species specific than 2,4-DNT. Male rats were slightly less tolerant than females (Lee et al., 1975; Vernot et al., 1977).

Lee et al. (1976) fed 2,6-DNT (>99% pure) in the diet to mice (8/sex/dose) for 4 weeks at levels of 0.01, 0.05, or 0.25%, equivalent to a daily intake of 0, 11, 51, or 289 mg/kg/day for males and 0, 11, 55, or 299 mg/kg/day for females. No treatment-related effects were observed in the 11-mg/kg/day dose group. No treatment-related deaths occurred in any dose group. The mid- and high-dose levels caused decreased BW gain and decreased food consumption. The high-dose males and females exhibited extramedullary hematopoiesis in the spleen and the liver. High-dose males developed aspermatogenesis and testicular atrophy that reversed 4 weeks after treatment was discontinued. The NOAEL was 11 mg/kg/day for males and females, based on decreased BW gain and decreased food consumption.

In a similar study, Lee et al. (1976) fed 2,6-DNT (>99% pure) in the diet to rats (8/sex/dose) for 4 weeks at levels of 0.01, 0.05, or 0.25%, equivalent to a daily intake of 0, 7, 35, or 145 mg/kg/day for males and 0, 7, 37, or 155 mg/kg/day for females. No treatment-related deaths were observed in rats fed 2,6-DNT. The rate of BW gain was decreased in a dose-related manner

in treated males and females but was not significantly different from controls. Food consumption was also depressed in a dose-related manner. Histopathology revealed hematopoiesis in the spleen and liver of both sexes and degeneration of spermatogenesis in males. The LOAEL was the lowest dose tested, 7 mg/kg/day for both sexes, based on decreased BW gain and decreased food consumption.

Dogs were given doses of 0, 4, 20, or 100 mg/kg/day 2,6-DNT in capsules for 4 weeks by Lee et al. (1976). There were no signs of toxicity at 4 mg/kg/day, but the 20- and 100-mg/kg/day groups had BW loss and reduced food consumption. The affected animals showed listlessness, incoordination, lack of balance, pale gums, dark urine, and hind limb weakness. They also were anemic, with decreased hematocrit, decreased hemoglobin concentration, and compensatory reticulocytosis. Histopathology revealed extramedullary hematopoiesis in the liver and spleen and bile duct hyperplasia in both sexes. There also was decreased spermatogenesis in males. After 4 weeks of recovery, the dogs showed some improvement, with lesser amounts of extramedullary hematopoiesis and testicular lesions, and two high-dose dogs that were allowed to recover for 19 weeks showed complete recovery. The NOAEL was 4 mg/kg/day, based on decreased BW gain and decreased food consumption in both sexes.

Dinitrotoluene Mixture

Reports of LD₅₀ studies with Tg-DNT or any other mixture in animals were not located in the available literature.

The Chemical Industry Institute of Toxicology (CIIT, 1977) conducted a 30-day toxicity study in rats with Tg-DNT. Groups of Fischer 344 rats (10/sex/dose) were fed diets containing 0, 37.5, 75, or 150 mg/kg/day for 30 days. There were no deaths during the treatment period. 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 BWs of females in the high-dose group and of males in all groups were significantly ($p < .05$) lower than those of the controls and were most severe in rats fed the high-dose diet. A significant ($p < .05$), dose-related increase in mean values was observed for methemoglobin, reticulocyte counts, and Heinz body formation, with the exception of mean percentage 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 ($p < .05$) 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 BW gain and decreased food consumption, blood effects, and gross pathological changes in males, the LOAEL was 37.5 mg/kg/day, the lowest dose tested.

6.2.2 Dermal/Ocular Effects

Lee et al. (1975) reported that neither 2,4-DNT nor 2,6-DNT produced ocular irritation when instilled as a 50% paste in peanut oil into the eyes of groups of six New Zealand white rabbits.

As a result of primary skin irritation tests to New Zealand white rabbits (sex not reported), 2,4-DNT (98% pure) and 2,6-DNT (>99% pure), as a 50% paste with peanut oil, were classified as very mild skin irritants (Lee et al., 1975).

6.2.3 Long-Term Exposure

2,4-Dinitrotoluene

Groups of CD rats (16/sex/dose) were fed diets of 2,4-DNT (98% pure) for up to 13 weeks, at intake levels of 0, 34, 93, or 266 mg/kg/day for males and 0, 38, 108, or 145 mg/kg/day for females (Lee et al., 1978; Ellis et al., 1985). Four animals/sex/group were sacrificed at 4 and 13 weeks after being returned to normal diets for 1 month. All high-dose females died within 3 weeks. One male in the mid-dose group and 6 in the high-dose group died between weeks 4 and 13. All surviving animals exhibited dose-dependent decreases in BW gain. Orange to yellowish urine stains were observed on the fur of high-dose rats, and one male had widespread and stiff hind legs. Mid- and high-dose animals of both sexes were anemic, characterized by decreases in erythrocyte count, hematocrit, and hemoglobin and concurrent reticulocytosis. Absolute liver and kidney weights were slightly increased in mid-dose males, and relative weights of these organs were significantly increased. There was splenic hemosiderosis in mid- and high-dose males and females. Spermatogenesis was decreased in mid-dose males and 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 based on decreased BW gain and decreased food consumption in males. There was no NOAEL because effects occurred at all doses tested.

In a longer, but limited (one-dose) study, Kozuka et al. (1979) reported similar effects observed in a group of 20 male Wistar rats given 0.5% 2,4-DNT (purity not reported) in feed for a period of 6 months. The estimated dose rate was 190 mg/kg/day for the first month and 214 mg/kg/day for the last 3 months. A total of 12 animals died before the end of treatment. Adverse neurological effects included piloerection, whitened skin color, humpback, jerky movements, decreased spontaneous movement, and general weakness. Toxic effects included decreased BW (58%), decreased BW gain, and significantly ($p < .01$) increased relative weights of the liver, spleen, and kidney.

Relative testicular weights were significantly lower. Methemoglobinemia was increased significantly ($p < .01$) in treated animals compared with controls, and there were significant ($p < .01$) differences in serum components (triglycerides, glucose, albumin, and albumin/globulin ratios) and serum enzymes (aspartate aminotransaminase, alanine aminotransferase, alkaline phosphatase, and acid-phosphatase). Gross pathology showed hepatic hypertrophy and testicular atrophy. No histopathology was conducted.

In a separate study (Lee et al., 1978; Ellis et al., 1985), CD-1 mice (16/sex/dose) were fed diets of 2,4-DNT (98% pure), with intakes equivalent to 0, 47, 137, or 413 mg/kg/day for males and 0, 52, 147, or 468 mg/kg/day for females for 13 weeks. Five mice died during the study—one low-dose male, two high-dose males, and two high-dose females. The males exhibited a dose-dependent decrease in BW. The high-dose groups of both sexes were anemic (decreased erythrocyte count, decreased hematocrit, and decreased hemoglobin) with concurrent reticulocytosis, mild hepatocellular dysplasia, and Kupffer cell dysplasia. High- and mid-dose males had mild degeneration of the seminiferous tubules or testicular degeneration. After 4 weeks off treatment, the mice recovered completely. The LOAEL was 47 mg/kg/day, based on BW loss in males. There was no NOAEL because effects occurred at all doses tested.

Beagle dogs (2/sex/dose) were exposed to 2,4-DNT in capsules at doses of 0, 1, 5, or 25 mg/kg/day for 13 weeks (Lee et al., 1978; Ellis et al., 1985). No treatment-related findings were observed in the mid- and low-dose groups. Mortality was observed after 22 days in the high-dose group. There was great variation in individual susceptibility in the high-dose group. All affected dogs exhibited decreased food consumption, BW loss, urine stains on the fur, pale gums, neuromuscular incoordination, and paralysis. Hematological indices showed methemoglobinemia, anemia, and Heinz bodies. The dogs were in fair to poor nutritional condition, with little or no body fat. Histologically, there was hemosiderosis in the liver and spleen, cloudy swelling of the kidneys in males and females, and aspermatogenesis in males. Dogs sacrificed during weeks 6 and 7 had brain lesions characterized by gliosis, edema, and demyelination of the cerebellum, spinal cord, and brain stem. Dogs were retained for 4 weeks without exposure to 2,4-DNT, and partial recovery from the various effects was observed. The LOAEL was 25 mg/kg/day, based on BW loss, hematological abnormalities, neurological signs, and histopathology. The NOAEL was 5 mg/kg/day.

Leonard et al. (1987) gave 24 male CDF Fischer 344/Cr1BR rats 27 mg/kg/day of 2,4-DNT (>99.4% pure) in the diet for 12 months, with a 6-month interim sacrifice of four rats. BW gain increased significantly ($p < .05$), and liver weight increased approximately 150% compared with control animals. Most of the animals exhibited liver histopathology characterized by hepatocyte degeneration and vacuolation and acidophilic and basophilic foci. Bile duct hyperplasia and a highly variable incidence of cholangiofibrosis occurred in the majority of the treated animals.

The National Cancer Institute (NCI) (1978) conducted a study with Fischer 344 rats (50/sex/dose) that were fed diets containing 0, 0.008% (80 ppm), or 0.02% (200 ppm) 2,4-DNT (>95% pure) for 78 weeks. Applying estimates by Lehman (1959), which assume that daily food consumption by rats is approximately 5% of their BW, the 2,4-DNT equivalent doses were 0, 4, or 10 mg/kg/day, respectively. Upon termination of dosing, the animals were observed for another 26 weeks. The only significant clinical observation was that high-dose males and females had mean average BWs that were 25% and 18%, respectively, lower than those of controls. The incidence and variety of nonneoplastic lesions in the major organs were similar in control and treated rats.

CD (Sprague-Dawley) rats (38/sex/dose) were fed 2,4-DNT (98% pure) in the diet for up to 2 years (Ellis et al., 1979; Lee et al., 1985). The intake of 2,4-DNT was 0, 0.57, 3.9, or 34 mg/kg/day for males and 0, 0.71, 5.1, or 45 mg/kg/day for females. There was an interim sacrifice (8/sex/group) after 12 months. In both sexes of high-dose rats, lifespan was shortened where there was 50% mortality by month 20; the same rate did not occur in controls until month 23. BW gains were reduced significantly (30-40%) in high-dose animals compared with controls. After 12 months of exposure, severe atrophy of the seminiferous tubules occurred in a dose-related manner in 16%, 26%, 33%, and 81% of the controls and low-, mid-, and high-dose groups, respectively. In some high-dose males, the atrophy caused almost complete aspermatogenesis. The spleen developed excessive pigmentation, and anemia and reticulocytosis occurred in mid- and high-dose males and in high-dose females after 12 months. The LOAEL was 34 mg/kg/day, based on seminiferous tubules effects in the males. The NOAEL was 3.9 mg/kg/day.

In a study of CD-1 mice (38/sex/dose), animals were fed (98% pure) 2,4-DNT up to 24 months (Ellis et al., 1979; Hong et al., 1985). The dose levels were 0, 14, 95, or 898 mg/kg/day. 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 BW 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 compared with 0/20 concurrent controls).

B6C3F1 mice (50/sex/dose) were given 2,4-DNT in feed at 0, 0.008, or 0.04% (0, 80, or 400 ppm, respectively) for 78 weeks, followed by 13 weeks without treatment (NCI, 1978). Following consumption estimates similar to the rat study, where daily food consumption by mice was approximately 15% of their BW (Lehman, 1959), the doses were 0, 11, or 57 mg/kg/day, respectively. BW gain depression decreased significantly in all treatment groups. At the end of the study, BW gain was depressed by 9% and 11% for low- and high-dose males, respectively; for females BW gain depression was 18% and 24%, respectively.

Beagle dogs (6/sex/dose) were fed 2,4-DNT (98% pure) in gelatin capsules at 0, 0.2, 1.5, or 10 mg/kg/day up to 24 months (Ellis et al., 1979, 1985). In the 10-mg/kg/day group, four of the six males were sacrificed due to moribund conditions by study week 19 after exhibiting progressive paralysis. The high-dose animals displayed incoordination and paralysis within 6 months of study initiation and during month 16 in one dog receiving 1.5 mg/kg/day. Histopathology of the CNS confirmed treatment-related lesions, which included vacuolization, endothelial proliferation, and gliosis of the cerebellum. In dogs fed 1.5 and 10 mg/kg/day, there was methemoglobinemia, with associated reticulocytosis and Heinz body formation. There also was biliary tract hyperplasia and pigmentation of the gallbladder, kidneys, and spleen. The hematologic effects were minimal during year 2, presumably due to an adaptive response. No males had testicular effects. The LOAEL in this study was 1.5 mg/kg/day based on neurotoxicity and the presence of Heinz bodies and biliary tract hyperplasia. The NOAEL was 0.2 mg/kg/day.

2,6-Dinitrotoluene

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.

CD rats were fed diets containing 0, 0.01, 0.05, or 0.25% 2,6-DNT for 13 weeks (Lee et al., 1976). The dose of 2,6-DNT was equivalent to 0, 7, 35, or 145 mg/kg/day for males and 0, 7, 37, or 155 mg/kg/day for females. No adverse effects were observed in the low-dose group. Both mid-dose males and females had decreases in BW gain and food consumption. They also exhibited extramedullary hematopoietic activity in the liver and spleen and bile duct hyperplasia. The males also developed depressed spermatogenesis and testicular atrophy. The high-dose animals developed a variety of adverse conditions, including decreases in BW, BW gain, and food consumption. They exhibited various hematological conditions such as methemoglobinemia, Heinz body formation, anemia, compensatory reticulocytosis, and severe extramedullary hematopoiesis in the spleen and liver. There was bile duct hyperplasia and renal degeneration in both sexes. By the end of 13 weeks, testicular lesions in males had progressed to encompass virtually all connective tissues. After 4 weeks, only partial recovery was observed; rats regained the BW lost, but lesions continued to occur in the spleen, liver, and testes of treated rats. The LOAEL was 35 mg/kg/day in males and 37 mg/kg/day in females, based on BW loss, hematological effects, and histopathology. The NOAEL was 7 mg/kg/day for both sexes.

Mice also were fed diets containing 0, 0.01, 0.05, or 0.25% 2,6-DNT for 13 weeks (Lee et al., 1976). Daily intake was 0, 11, 51, or 289 mg/kg/day for males and 0, 11, 55, or 299 mg/kg/day for females. The low-dose group did not show any treatment-related effects, and only a few animals in the mid-dose group had BW loss. The higher doses of 2,6-DNT caused decreases in BW gain and food consumption, hematopoiesis in the liver and/or spleen, and bile duct hyperplasia in both sexes. Males developed testicular atrophy and depression of spermatogenesis. There was partial recovery of some of the adverse effects after a 4-week recovery after removal of 2,6-DNT from the diet; however, hematopoiesis continued in the liver or spleen. The LOAEL was 51 mg/kg/day in males and 55 mg/kg/day in females, based on the effects to BW and food consumption and histopathologic changes in the spleen, liver, bile duct, and testes. The NOAEL was 11 mg/kg/day for both sexes.

Lee et al. (1976) also evaluated the effect of 2,6-DNT on dogs (4/sex/dose) that were given 2,6-DNT in capsules at doses of 0, 4, 20, or 100 mg/kg/day for 13 weeks. There were no adverse effects observed in the low-dose animals. 2,6-DNT did, however, produce toxicity at higher dose levels. All high-dose animals of both sexes and half of the females in the mid-dose group died before the end of the study. The animals had BW loss due to decreased food consumption. Adverse neurological effects observed were listlessness, incoordination leading to rigid paralysis, and occasional tremors. Clinical chemistry effects included elevations in serum alkaline phosphatase, alanine aminotransferase, and/or blood urea nitrogen. Adverse hematological effects included methemoglobinemia leading to Heinz body formation, anemia with compensatory reticulocytosis and extramedullary hematopoiesis, and lymphoid depression leading to peripheral lymphocytopenia. Bile duct hyperplasia and degenerative and inflammatory

changes in the liver and kidneys of both sexes were noted from histopathological examination. Testicular changes observed were degeneration and atrophy of the spermatogenic cells. 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 surviving animals completely recovered from the 2,6-DNT effects 19 weeks post-treatment. The NOAEL was 4 mg/kg/day, based on mortality, BW loss, hematology, neurological effects, and histopathology. The LOAEL was 20 mg/kg/day.

Leonard et al. (1987) studied the effects of purified 2,6-DNT (>99.4% pure) administered in the diet to 24 male CDF Fischer 344/CrIBR rats for 12 months. The dose was either 7 mg/kg/day or 14 mg/kg/day, with a 6-month interim sacrifice of four rats/group. After 1 year, serum alanine aminotransferase was elevated at both dose levels. Treatment-related liver effects were prominent at both treatment levels. Serum gamma-glutamyl transferase was increased in the high-dose group after both the 6-month and 1-year exposure periods. Histopathological changes in most animals included hepatocytic degeneration and vacuolization and acidophilic and basophilic foci. Most rats also exhibited bile duct hyperplasia.

Dinitrotoluene Mixture

Leonard et al. (1987) administered Tg-DNT in the diet to 24 male CDF Fischer 344/CrIBR rats for 12 months at concentrations that resulted in a dose of 35 mg/kg/day. There was a 6-month interim sacrifice of four animals. Both the 6-month and 1-year groups had significantly reduced BW gain. Liver weights were significantly increased at 1 year. There were no effects observed on serum alanine aminotransferase or serum gamma-glutamyl transferase activities. Hepatocyte histopathology (degeneration and vacuolization) was apparent in the majority of treated animals but was not dose dependent. Acidophilic and basophilic foci and bile duct hyperplasia were observed in most of the treated animals. There also was a highly variable incidence of cholangiofibrosis.

The CIIT (1982) studied the effects of Tg-DNT (composition not reported) in Charles River CDF Fischer 344 rats (130/sex/group) that were given the chemical in feed at doses of 0, 3.5, 14.0, or 35.0 mg/kg/day. Animals were sacrificed and necropsied at weeks 26 and 52 (10 rats/sex/group) and at week 78 (20 rats/sex/group). All surviving high-dose rats were sacrificed and necropsied at week 55, as were all the other surviving animals after 104 weeks. Treatment-related effects varied by exposure duration, but overall they included decreased BW gain, decreased mean BW, and anemia, characterized by increased hemosiderin and splenic extramedullary hematopoiesis. There were increases in relative liver, brain, kidney, and ovary weights and in absolute testicular weights in low-dose males. Adverse histopathological effects were noted for the liver, kidney, pancreas, testes, spleen, adrenal and parathyroid glands, and bone marrow. The LOAEL for Tg-DNT in this study was 3.5 mg/kg/day, the lowest dose tested, based on histopathological changes in the liver, kidney, and parathyroid gland.

6.2.4 Reproductive and Developmental Effects

2,4-Dinitrotoluene

Experimental studies with rats demonstrate that 2,4-DNT causes severe reproductive effects. A 5-day oral reproduction study in male Sprague-Dawley rats resulted in the deaths of 8 of 15 rats dosed at 240 mg/kg/day, the highest dose tested (Lane et al., 1985). Survivors exhibited BW loss and cyanosis; sharp decreases in the mating index and in the number of resulting sperm-positive and pregnant females were observed at 240 mg/kg/day. Cyanosis also was exhibited at 180 mg/kg/day.

A three-generation study was conducted by Ellis et al. (1979), where Sprague-Dawley rats (10-24/sex/dose) were fed approximately 0, 0.75, 5, or 35 mg/kg/day 2,4-DNT (98% pure) for up to 6 months prior to mating. The highest dose was associated with reduced parental BW, reduced pup survival, reduced fertility in F₁ animals, and slightly lower mean litter size and pup BW. At mid- and low-dose levels, there were slight reductions in BW for first- and third-generation pups; however, parental fertility and offspring viability were not affected. The LOAEL was 35 mg/kg/day, based on severe reductions in fertility. The NOAEL was 5 mg/kg/day.

Oral exposure to 2,4-DNT resulted 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 was reduced in both sexes. Also, 2,4-DNT caused reduced viability as well as decreases in the BW of offspring at birth and at weaning. Limited available data suggest that 2,4-DNT is not teratogenic in mice following ingestion (Hardin et al., 1987).

Bloch et al. (1988) fed groups of 9-10 Sprague-Dawley rats 2,4-DNT (97% pure) 0, 100, or 200 mg/kg/day. Significant ($p < .05$) BW reduction was observed in the high-dose group. The high-dose group also showed significant ($p < .05$) increases in serum follicle stimulating hormone and luteinizing hormone, significantly ($p < .01$) reduced sperm count, disruption of spermatogenesis, and histological alterations or degeneration in Sertoli cells, spermatocytes, and spermatids. No significant effects were observed in the low-dose rats.

Studies of systemic toxicity reported in Sections 6.2.1 and 6.2.3 identified effects of 2,4-DNT on the testes and spermatogenesis following short and long term exposures (Ellis et al., 1979; Hong et al., 1985; Kazuka et al., 1979; Lee et al., 1978, 1985 and McGown et al., 1983).

2,6-Dinitrotoluene

No data on the reproductive effects or developmental effects of 2,6-DNT were found in the current literature. However, studies of systemic toxicity reported in Sections 6.2.1 and 6.2.3 identified effects of 2,4-DNT on the testes and spermatogenesis following short and long term exposures (Lee et al., 1976).

Dinitrotoluene Mixture

Tg-DNT (76% 2,4-DNT, 19% 2,6-DNT, 5% other isomers) was not teratogenic to time-mated female Fischer 344 rats administered gavage doses in corn oil (0, 14, 35, 37.5, 75, 100, or 150 mg/kg/day) (Price et al., 1985). Embryotoxicity, however, was observed at maternally toxic levels. In the 150-mg/kg/day group, there was 46% mortality, and clinical signs of toxicity began on gestation day 11. Corrected BW gain (minus gravid uterine weight) was significantly reduced in dams receiving ≥ 14 mg/kg/day. Relative liver weight increased significantly in the 75- and 100-mg/kg/day groups. Relative spleen weight was significantly increased at ≥ 35 mg/kg/day. There was a statistically insignificant increase in percentage resorption in the 150-mg/kg/day group, which was considered to be indicative of a compound-related effect. Developmental effects noted in the fetuses were reduced liver weight at 14 mg/kg/day and increased spleen weight at 35 and 75 mg/kg/day.

6.2.5 Mutagenicity

2,4-Dinitrotoluene

2,4-DNT is a weak mutagen in *Salmonella* test systems. A detailed summary of 2,4-DNT's mutagenic potential can be found in Hartley et al. (1994).

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). 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).

2,6-Dinitrotoluene

2,6-DNT is a weak mutagen in *Salmonella* test systems. A detailed summary of 2,6-DNT's mutagenic potential can be found in Hartley et al. (1994).

2,6-DNT appears to have both initiation and promoting activity (Popp and Leonard, 1982; Mirsalis et al., 1982; Doolittle et al., 1983).

Dinitrotoluene Mixture

DNTs are negative genotoxins in mammalian cells *in vitro*, in the dominant lethal test in mice and rats, and in *Drosophila melanogaster* 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). Tg-DNT gave negative responses for unscheduled DNA synthesis except when an *in vivo/in vitro* testing system was used (Bermudez et al., 1979; Mirsalis and Butterworth, 1982).

6.2.6 Carcinogenicity

2,4-Dinitrotoluene

In a 2-year NCI study (1978), 2,4-DNT (>95% pure) was administered in the diet of Fischer 344 rats (50/sex/dose) at doses of 80 and 200 ppm. Controls consisted of 75 rats/sex. The animals were tested for 78 weeks, followed by an additional observation period of 13-26 weeks. Survival was adequate in all groups, and a reduced BW gain in high-dose groups showed that a maximum tolerated dose (MTD) had been approached, indicating that study conditions were valid. Only benign tumors were noted. 2,4-DNT induced a statistically significant increase in fibromas of the skin and subcutaneous tissue in males (0/71, 7/49, 13/49) and fibroadenomas of the mammary gland in high-dose females (13/71, 12/49, 23/50).

CD (Sprague-Dawley) rats (38/sex/dose) were fed 2,4-DNT (98% pure, with 2% 2,6-DNT) in the diet, at concentrations of 0, 15, 100, or 700 ppm, for up to 2 years (Ellis et al., 1979; Lee et al., 1985). The intake of 2,4-DNT was 0, 0.57, 3.9, or 34 mg/kg/day for males and 0, 0.71, 5.1, or 45 mg/kg/day for females. Mortality was high in all treatment groups; the control group survival rate at 2 years was only 40-45%. The test chemical induced increased incidences of hepatocellular carcinomas in high-dose males (1/25, 2/28, 2/19, and 6/30, respectively) and a statistically significant increase in the same tumor type in high-dose females (0/23, 0/35, 1/27, and 19/35, respectively). The incidence of hepatocellular neoplastic nodules was not considered statistically significantly elevated in any of the treatment groups. A statistically significant increase in the incidence of benign mammary gland tumors was observed in high-dose females (8/23, 9/35, 16/27, and 33/35, respectively).

Leonard et al. (1987) treated 20 male Fischer 344 rats with 2,4-DNT (99.9% pure) in the diet for 1 year and compared results with an untreated control group of 20 rats. No tumors were found in controls or rats exposed to 2,4-DNT at 27 mg/kg/day.

In a 2-year NCI study (1978), 2,4-DNT (>95% pure) was administered in the diet of B6C3F1 mice (50/sex/dose) at concentrations of 80 ppm and 400 ppm. Controls consisted of 50 mice/sex. The animals were on test for 78 weeks, followed by an additional observation period of 13-26 weeks. Survival was adequate in all groups, and a reduced BW gain in high-dose groups showed that an MTD had been approached, indicating that the study conditions were valid. No statistically significant increase in incidence of tumors was noted in males or females.

In a study of CD-1 mice (38/sex/dose), the animals were fed 2,4-DNT (98% pure with 2% 2,6-DNT), at concentrations of 0, 100, 700, or 5,000 ppm, for up to 24 months (Ellis et al., 1979; Hong et al., 1985). The dose levels were 0, 14, 95, or 898 mg/kg/day. All the animals fed 898 mg/kg/day died by month 18 (males) or month 21 (females), and mortality was high in all treatment groups. The survival rate for the control group at 2 years was only 20% to 30%. All animals that died before 12 months were not included in the tumor incidence. In males, the incidence of kidney tumors (both benign and malignant) was 0/33, 8/33, and 19/28 for the control and low- and mid-dose groups, respectively. This was a significant ($p = .059$) elevation in the mid-dose group. No evidence of treatment-related increases in tumor frequency was noted

in females.

Ellis et al. (1979, 1985) gave groups of six male and six female beagle dogs oral doses of 2,4-DNT at 0, 0.2, 1.5, or 10.0 mg/kg/day in gelatin capsules for 2 years. The high dose was toxic to all dogs and lethal to five. The medium dose was toxic to some dogs, but the low dose had no apparent adverse effects. Each animal received a thorough clinical and histopathological examination following sacrifice. No evidence of carcinogenicity was seen in any of the dogs fed 2,4-DNT.

2,6-Dinitrotoluene

Leonard et al. (1987) treated 20 male Fischer 344 rats with 2,6-DNT (99.9% pure) in the diet for 1 year and compared results with an untreated control group of 20 rats. 2,6-DNT induced hepatocellular carcinomas in 100% (19/19) of the high-dose rats (14 mg/kg/day) and 85% (17/20) of the low-dose rats (7 mg/kg/day). No tumors were found in controls or rats exposed to 2,4-DNT at 27 mg/kg/day. Two low-dose males receiving 2,6-DNT and two males receiving Tg-DNT developed cholangiocarcinoma.

Dinitrotoluene Mixture

Leonard et al. (1987) treated 20 male Fischer 344 rats with 35 mg/kg/day Tg-DNT (76% 2,4-DNT, 19% 2,6-DNT) in the diet for 1 year and compared results with an untreated control group of 20 rats. Tg-DNT induced hepatocellular carcinomas in 47% (9/19) of the treated males. No tumors were found in controls. Two males receiving Tg-DNT developed cholangiocarcinoma. Although the duration of these studies was limited to 1 year and the number of animals tested was small, these results and those from the 2,4-DNT and 2,6-DNT studies suggest that 2,6-DNT accounts for much of the carcinogenic activity observed in mixed-isomer DNT bioassays.

Fischer 344 rats (130/sex/dose) were fed Tg-DNT (76% 2,4-DNT, 19% 2,6-DNT) at concentrations of 0, 3.5, 10.0, or 35.0 mg/kg/day (CIIT, 1982). All males and females in the high-dose group were sacrificed at 55 weeks because of significantly reduced survival. Histopathological studies were performed on sacrificed animals (20 rats/sex) with 100% incidence of hepatocellular carcinoma in males (20/20) and 55% incidence in females (11/20). Mid- and low-dose animals were kept on test for 104 weeks. The incidences of liver carcinoma in males at 104 weeks were 1/61 for the control group, 9/70 for the low-dose group, 22/23 for the mid-dose group, and 20/20 (at 55 weeks) for the high-dose group; the incidences in females at 104 weeks were 0/57 for the control group, 0/61 for the low-dose group, 40/68 for the mid-dose group, and 11/20 (at 55 weeks) for the high-dose group. The incidence of neoplastic nodules in males was 9/61, 11/70, 16/23, and 5/20, and the incidence in females was 5/57, 12/61, 53/68, and 12/20, at 104 weeks for the control and low- and mid-dose groups and (at 55 weeks) for the high-dose groups, respectively. Cholangiocarcinomas, presumably derived from the bile duct epithelium, also were observed in three high-dose males at 55 weeks and two mid-dose males at 104 weeks.

6.3 Sensitive Populations

Reports identifying populations or groups of people sensitive to 2,4-DNT or 2,6-DNT were not located in the available literature.

However, 2,4-DNT and 2,6-DNT are metabolic products of 2,4,6-trinitrotoluene (2,4,6-TNT). Therefore, it should be noted that TNT has been associated with the development of hemolytic crisis in individuals deficient in the G6PD enzyme (ATSDR, 1995). African Americans and people from Africa, the Middle East, and Southeast Asia exhibit higher incidences of G6PD deficiencies. G6PD deficiency is a genetic disorder and therefore can be passed on to offspring, who may display symptoms when stressed. Other populations that may show increased sensitivity to 2,4,6-TNT include very young children, who have immature hepatic detoxification systems; individuals with impaired liver function, including alcoholics, or impaired kidney function; and those who are prone to anemia or who are anemic. Also at increased risk may be individuals with sickle cell trait, genetically induced unstable hemoglobin forms, or congenital hypercholesterolemia (ATSDR, 1995).

Letzel et al. (2003) did not find the DNT-induced G6PD deficiency as the result of 2,4,6-TNT exposure that has been reported elsewhere.

6.4 Proposed Mode of Action

DNT and/or its metabolites oxidize the ferrous ion in hemoglobin and form methemoglobin (Ellis et al., 1979). Methemoglobin can form aggregates of hemoglobin degradation products called Heinz bodies, which is a sensitive indicator of hemoglobin destruction. High levels of methemoglobin lead to the development of anemia, which is compensated by reticulocytosis. When reticulocytosis cannot compensate adequately, then frank anemia develops.

The relationship between DNT metabolism and the formation of liver tumors is associated with the formation of reactive intermediates (ATSDR, 1998; Kedderis et al., 1984; Sayama et al., 1989b). When DNT is oxidized by cytochrome P450 and conjugated with glucuronic acid, it forms DNBAcG, which is excreted in urine and into bile (Long and Rickert, 1982; Medinsky and Dent, 1983). That in bile is metabolized further by intestinal microflora, is hydrolyzed, and then is reduced to form an aminonitrobenzyl alcohol (Chadwick et al., 1993; Guest et al., 1982; Mori et al., 1985). Nitroso and hydroxylamino derivatives probably are intermediates in the formation of the alcohol (ATSDR, 1998). Enterohepatic circulation allows the metabolites in the bile, which now are no longer conjugated, to transport back to the liver (Medinsky and Dent, 1983) where the amine group is N-hydroxylated by cytochrome P450 to form an unstable sulfate conjugate (Kedderis et al., 1984). The sulfate conjugate can decompose and form carbonium or nitrenium ions, which then can bind to hepatic macromolecules, leading to mutations and subsequently to liver tumors. This mechanism is thought to be applicable to the carcinogenicity of 2,6-DNT (Long and Rickert, 1982; Mirsalis and Butterworth, 1982).

Based on hepatic tumor initiation-promotion experiments, Leonard et al. (1983, 1986) and Mirsalis and Butterworth (1982) concluded that Tg-DNT has tumor-promoting and -initiating

activity. They further concluded that 2,6-DNT is a complete hepatocarcinogen and has the primary role in Tg-DNT's carcinogenic activity.

7.0 QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health advisories (HAs) generally are determined for 1-day, 10-day, longer term (up to 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:

$$HA = \frac{(\text{NOAEL or LOAEL}) \times (\text{BW})}{(\text{UF}) (\text{DWI})} = \text{mg/L } (\mu\text{g/L})$$

where:

HA = Health advisory

NOAEL or LOAEL = No or lowest observed adverse effect level (in mg/kg BW/day)

BW = Assumed body weight of a 10-kg child or a 70-kg adult

UF = Uncertainty factor (10, 100, 1,000, or 10,000) in accordance with the National Academy of Sciences (1983, 1994)

DWI = Drinking water ingestion; assumed daily water consumption of a 10-kg child (1 L/day) or a 70-kg adult (2 L/day)

7.1 1-Day Health Advisory

2,4-Dinitrotoluene

No suitable information was found in the available literature for determining a 1-day HA for 2,4-DNT. Due to the acute toxicity of 2,4-DNT, no appropriate NOAEL or LOAEL values were identified in the reviewed literature. The 5-day oral study in male Sprague-Dawley rats by Lane et al. (1985) was considered. Since only one sex was tested, this study was considered to be inappropriate for deriving a 1-day HA.

Since these data were not judged suitable for determining a 1-day HA value for 2,4-DNT, it is recommended that the 10-day HA for a 10-kg child (0.5 mg/L) be used as a conservative estimate of the 1-day HA value.

2,6-Dinitrotoluene

No suitable information was found in the available literature for determining the 1-day HA for 2,6-DNT. Due to the acute toxicity of 2,6-DNT, no appropriate NOAEL or LOAEL values were identified in the reviewed literature. 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 1-day HA value.

7.2 10-Day Health Advisory

2,4-Dinitrotoluene

The 14-day study with 2,4-DNT in Sprague-Dawley rats (McGown et al., 1983) is acceptable for derivation of the 10-day HA. Based on decreased BW gain, decreased food consumption, changes in serum chemistry levels in males and females, and testicular lesions in males, the LOAEL was 96 mg/kg/day, the lowest dose tested.

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

$$\text{10-day HA} = \frac{(96 \text{ mg/kg/day}) (10 \text{ kg})}{(1,000) (1 \text{ L/day})} = 0.96 \text{ mg/L (rounded to 1 mg/L or 1,000 } \mu\text{g/L)}$$

where:

96 mg/kg/day = LOAEL, based on decreased BW gain, decreased food consumption and changes in serum chemistry levels in females following 14-day dietary dosing

10 kg = Assumed BW of a child

1000 = UF, which includes a tenfold UF for intraspecies variability, another tenfold UF to account for interspecies extrapolation, and another tenfold UF for use of a LOAEL in the absence of a NOAEL

1 L/day = Assumed DWI of a 10-kg child

2,6-Dinitrotoluene

No suitable information was found in the available literature for determining the 10-day HA for 2,6-DNT. Again, owing to the acute toxicity of 2,6-DNT, no appropriate NOAEL or LOAEL values were identified in the reviewed literature. 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 10-day HA value.

7.3 Longer Term Health Advisory

2,4-Dinitrotoluene

The 13-week feeding study in CD rats by Lee et al. (1978) (also reported by Ellis et al., 1985) is used to derive the longer term HA. Based on dose-related decreases in BW gain and food consumption, the LOAEL was 34 mg/kg/day for males and 38 mg/kg/day for females, the lowest doses tested. The LOAEL for males, 34 mg/kg/day, is used as the most conservative LOAEL for derivation of the longer term HA.

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

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

where:

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

10 kg = Assumed BW of a child

1000 = UF, which includes a tenfold UF for intraspecies variability, another tenfold UF to account for interspecies extrapolation, and another tenfold UF for use of a LOAEL in the absence of a NOAEL

1 L/day = Assumed DWI of a 10-kg child

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

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

where:

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

70 kg = Assumed BW of an adult

1000 = UF, which includes a tenfold UF for intraspecies variability, another tenfold UF to account for interspecies extrapolation, and another tenfold UF for use of a LOAEL in the absence of a NOAEL

2 L/day = Assumed DWI of a 70-kg adult

2,6-Dinitrotoluene

The 13-week dog study by Lee et al. (1976) was used to derive the longer term HA for 2,6-DNT. The animals (4/sex/dose) were administered 2,6-DNT in capsules at doses of 0, 4, 20, or 100 mg/kg/day for 13 weeks. All dogs in the high-dose group and two mid-dose females died before study termination. Toxic effects observed in the study included decreased food consumption leading to BW loss, adverse liver and kidney effects, bile duct hyperplasia, and atrophy of spermatogenic cells in males. There also were neurological, clinical chemistry, and hematological deficits. The LOAEL was 20 mg/kg/day based on BW loss, blood and neurological effects, and histopathology. The NOAEL was 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 BW of a child

100 = UF, which includes a tenfold UF for intraspecies variability and another tenfold UF to account for interspecies extrapolation

1 L/day = Assumed DWI of a 10-kg child

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

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

where:

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

70 kg = Assumed BW of an adult

100 = UF, which includes a tenfold UF for intraspecies variability and another tenfold UF to account for interspecies extrapolation

2 L/day = Assumed DWI of a 70-kg adult

7.4 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. The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious health effects during a lifetime. The RfD is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, and divided by the UF(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 BW of an adult and divided by the assumed daily drinking water ingestion (DWI) of a 70-kg adult (2 L/day). The Lifetime HA in drinking water alone is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water is based on actual exposure data or, if data are not available, a value of 20% is assumed.

For those substances that are “known or likely to be carcinogenic to humans” (U.S. EPA, 2005) or “carcinogenic to humans” or “probably carcinogenic to humans” (Group 1 and Group 2A, respectively, according to the IARC classification categories), the development of a Lifetime HA is not recommended. The risk manager must balance this assessment of carcinogenic potential and the quality of the data against the likelihood of occurrence and significance of health effects related to noncarcinogenic toxicity. To assist the risk manager in this process, drinking water concentrations associated with estimated excess lifetime cancer risks over the range of 1 in 10,000 to 1 in 1,000,000 for the 70-kg adult drinking 2 L water/day are provided in the Evaluation of Carcinogenic Potential, Section 7.5 below.

2,4-Dinitrotoluene

The 2-year chronic study with beagle dogs (Ellis et al., 1979, 1985) is used for derivation of lifetime values. The dogs (6/sex/dose) were fed 2,4-DNT (98% pure) in gelatin capsules at 0, 0.2, 1.5, or 10 mg/kg/day. In the 10-mg/kg/day group, four of the six males were sacrificed due to moribund conditions after exhibiting progressive paralysis early in the study. Typical effects observed in the remaining high-dose and in the mid-dose animals were methemoglobinemia, with associated reticulocytosis and Heinz body formation. There also was biliary tract hyperplasia and pigmentation of the gallbladder, kidneys, and spleen. The LOAEL in this study was 1.5 mg/kg/day based on neurotoxicity and the presence of Heinz bodies and biliary tract hyperplasia. The NOAEL was 0.2 mg/kg/day.

Using this study, the DWEL is derived as follows:

Step 1. Determination of the RfD

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

where:

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

100 = UF, which includes a tenfold UF for intraspecies variability and another tenfold UF to account for interspecies extrapolation

Step 2. Determination of the DWEL

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

where:

0.002 mg/kg/day	=	RfD
70 kg	=	Assumed BW of an adult
2L	=	Assumed DWI of a 70-kg adult

Step 3. Determination of Lifetime HA

The 2,4-DNT/2,6-DNT mixture is classified as “likely to be carcinogenic to humans” (U.S. EPA, 2005); thus, the development of a Lifetime HA for 2,4-DNT is not recommended.

2,6-Dinitrotoluene

The 13-week study by Lee et al. (1976) of 2,6-DNT effects on beagle dogs is used for derivation of lifetime values. The dogs (4/sex/dose) were given 2,6-DNT in capsules at doses of 0, 4, 20, or 100 mg/kg/day for 13 weeks. There were no adverse effects observed in the low-dose animals. 2,6-DNT did, however, produce toxicity at higher dose levels. All high-dose animals of both sexes and half of the females in the mid-dose group died before the end of the study. The animals had BW loss due to decreased food consumption. Adverse effects in this study were neurological and hematological, and there were altered clinical chemistry parameters. There also were bile duct hyperplasia and histopathological effects to the liver and kidneys of both sexes and to the testes in males. The LOAEL was 20 mg/kg/day, based on mortality, BW loss, hematology, neurological effects, and histopathology. The NOAEL was 4 mg/kg/day.

Using this study, the DWEL is derived as follows:

Step 1. Determination of the RfD

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

where:

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

3000 = UF, which includes a tenfold UF for intraspecies variability, another tenfold UF to account for interspecies extrapolation, and another tenfold UF 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 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 BW of an adult

2L = Assumed DWI of a 70-kg adult

Step 3. Determination of Lifetime HA

A 2,4-DNT/2,6-DNT mixture is classified as “likely to be carcinogenic to humans” (U.S. EPA, 2005); thus, the development of a Lifetime HA for 2,6-DNT is not recommended.

7.5 Evaluation of Carcinogenic Potential

The U.S. EPA reports the cancer classification for DNT as the 2,4-DNT/2,6-DNT mixture. Although usually accompanied by 2,6-DNT, 2,4-DNT is the more significant component of the mixture by volume in commercial formulations. For example, Tg-DNT is composed of approximately 76.5% 2,4-DNT and 18.8% 2,6-DNT. The carcinogenic assessment for lifetime exposure of DNT was determined using a chronic toxicity/oncogenicity study conducted with a mixture consisting of 98% 2,4-DNT and 2% 2,6-DNT (Ellis et al., 1979; Lee et al. 1985).

Therefore, in this HA, the cancer risk potential and estimates for each of the isomers (i.e., 2,4-DNT and 2,6-DNT) are the same as that of the mixture. The U.S. EPA classifies the 2,4-DNT/2,6-DNT mixture as “likely to be carcinogenic to humans.”

The cancer risk estimate for the 2,4-DNT/2,6-DNT mixture is derived from a study by Ellis et al. (1979) (also reported by Lee et al., 1985) where female rats were the sensitive species and mammary gland tumors were the critical endpoint. Selected information from the study is as follows:

- Tumor type: Liver: hepatocellular carcinomas, neoplastic nodules; mammary gland: adenomas, fibroadenomas, fibromas, adenocarcinomas/carcinomas
- Test animals: Female Sprague-Dawley rats
- Route: Oral (diet)
- References: Ellis et al., 1979; Lee et al., 1985

To estimate the potential cancer risk to exposed human populations from the combined incidence of mammary gland tumors developed by female rats in the Ellis et al. (1979) study, the doses administered to the animals are adjusted to a human equivalent exposure by using a surface area correction factor. The concentration of DNT administered in food (in ppm) was converted to dose (in mg/kg/day) using estimates from Lehman (1959), where 1 ppm = 0.05 mg/kg/day for the aging rat. The animal dose was divided by the ratio of the human BW to the aging rat BW raised to the 1/3 power, which is the human equivalent dose, as shown below.

Dose			Tumor Incidence
Administered (ppm)	Administered (mg/kg/day)	Human Equivalent* (mg/kg/day)	
0	0	0	11/23
15	0.71	0.129	12/35
100	5.10	0.927	17/27
700	45.00	7.557	34/35

*Human equivalent dose = (administered dose)/(70 kg/0.425 kg)^{0.33}

The dose-response data sets presented above were modeled using the Benchmark Dose Software system (Version 1.3.2) developed by the U.S. EPA National Center for Environmental Assessment (NCEA). The benchmark dose (BMD) was estimated using the numbers of female rats with mammary gland tumors, as indicated previously. For a benchmark risk (BMR) level of 0.10, the estimated BMD value is 0.25 mg/kg/day with a lower bound (95%) (BMDL) of 0.15 mg/kg/day using the multistage model. The multistage model had a chi square p value of 0.39 and an Akaike Information Criterion value of 127. The BMDL was used as the point of departure selected for the quantification of cancer risk from DNT. Additional information concerning BMD modeling and model output is in Appendices B and C, respectively. These values result in the following drinking water risk estimates:

- Oral slope factor (mg/kg/day)⁻¹—6.67 E-1
- Drinking water unit (µg/L) risk—1.90 E-5

The concentrations of DNT in drinking water at the 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk levels were calculated using the following equation:

$$\frac{35,000}{q1^*} \times R = C$$

where:

35,000	=	Conversion factor for mg to µg and exposure assumption that a 70-kg adult ingests 2 L water/day
q1*	=	(mg/kg/day) ⁻¹ , human oral slope factor
R	=	Risk at 10 ⁻⁴ , 10 ⁻⁵ , 10 ⁻⁶ , etc.
C	=	Concentration of chemical in µg/L

The resultant risk levels are 5 µg/L for the E-4 (1 in 10,000) risk level, 0.5 µg/L for the E-5 (1 in 100,000) risk level, and 0.05 µg/L for the E-6 (1 in 1,000,000) risk level.

8.0 OTHER CRITERIA, GUIDANCE, AND STANDARDS

- Ambient Water Quality Criteria to Protect Human Health for 2,4-DNT at a 10E-6 risk level (U.S. EPA, 1980):
 - Ingestion of water and organisms: 0.11 µg/L
 - Ingestion of organisms only: 9.10 µg/L

9.0 ANALYTICAL METHODS

Published analytical methods for DNT isomers 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.

Gas Chromatography

GC has been studied by a number of scientists utilizing different detection methods for various situations. Hartley et al. (1981) and Belkin et al. (1985) describe methods to detect and quantitate DNT in water using GC with electron capture detection (ECD). Richard and Junk (1986) describe a procedure for determining munitions in water utilizing a macroporous resin for extraction, elution with ethyl acetate, concentration of the eluate, separation by GC, and detection by ECD. Lichtenberg et al. (1987) described a study that utilized GC in conjunction with either ECD or flame ionization detection to identify and quantitate toxic organic substances in complex matrices. 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. Capillary GC or capillary GC/MS was used in conjunction with robotics to analyze wastewater samples for a variety of DNT isomers (Hornbrook and Ode, 1987). A method for determining DNT isomers in biosludge using GC and a thermal energy analyzer was described by Phillips et al. (1983). Air samples were collected on a quartz filter and extracted with a benzene/ethanol mixture by Matsushita and Iida (1986), who subsequently detected DNTs by analysis with GC using flame thermoionic detection.

High-Performance Liquid Chromatography

Krull et al. (1981) reported the use of HPLC with ECD and HPLC with GC/ECD for the analysis of 2,4-DNT. Lloyd (1983) described a technique for screening trace amounts of explosives that detected 2,4-DNT using a pendant mercury drop electrode in conjunction with HPLC. Reverse phase HPLC has been used in the analysis of munitions wastewater samples (Bauer et al., 1986; Jenkins et al., 1986). Preslan et al. (1991) modified a method for detecting TNT by adding an intermediate derivatization that allows the separation of 2-amino-4,6-DNT, 4-amino-2,6-DNT, and DNT. Bongiovanni et al. (1984) used a combination of HPLC and ultraviolet (UV) light to detect and analyze explosives-bearing soils for trace amounts of DNT isomers.

Other Methods

Yinon (1989) analyzed and identified a number of 2,4-DNT metabolites using electron impact and chemical ionization MS. Hable et al. (1991) detected DNT isomers in drinking water at levels below those measured by GC by using ECD together with a DB-1301 widebore-fused silica capillary column. Burns et al. (1987) reported on the possibility of identification and determination of 2,6-DNT by electron spin resonance spectrometry. McLuckey et al. (1985) studied the use of tandem MS for the analysis of explosives, where the first stage serves as a

separator; negative chemical ionization was the most sensitive detector for nitroaromatic compounds such as 2,4-DNT. Spanggord and Suta (1982) describe the use of cluster analysis to characterize the distribution of waste components resulting from the production and purification of TNT.

10.0 TREATMENT TECHNOLOGIES

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

The use of activated carbon for the adsorptive displacement of 2,4-DNT 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-DNT 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 nitrogroups, 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 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-DNT and 2,6-DNT were studied by Lee and Hunter (1985). Concentrations of 21.3 mg/L (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 effects of hydrogen peroxide and UV radiation on the decomposition of 2,4-DNT in water and found that at molar ratios of H₂O₂/DNT between 26 and 52, DNT disappeared very rapidly. The degradation rate of DNT in aqueous solution also was 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-DNT 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 photolytic 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).

Greater than 99.9% of 2,4-DNT (present at 2.1 mmoles) was removed in 162-202 days in an upflow anaerobic sludge bed reactor, using a granular sludge and glucose or a volatile fatty acid mixture as cosubstrate (Razo-Flores et al., 1997).

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APPENDIX A. CALCULATION OF DINITROTOLUENE INGESTION BY RATS IN MCGOWN ET AL. (1983)

The previous version of the Drinking Water Health Advisory (HA) for 2,4-dinitrotoluene (2,4,-DNT) and 2,6-dinitrotoluene (2,6-DNT) (Hartley et al., 1994; ATSDR, 1998) describes the 14-day rat feeding study by McGown et al. (1983). The HA estimates DNT ingestion rates of 0, 45, 60, 94, or 143 mg/kg/day for both sexes. The ATSDR estimates the rates as 0, 78, 104, 165, or 261 mg/kg/day for males and 0, 82, 109, 173, or 273 mg/kg/day for females. Neither reference describes how the ingestion values were calculated.

The ingestion rates were recalculated from data derived from the report by McGown et al. (1983). The authors did not report the animals' body weights (BWs) or DNT consumption rates; however, these data were depicted in graphs. The values for the BW gain and food consumption were estimated from the graphs and are shown in Table A-1.

Table A-1. Estimated Body Weight Gain and Food Consumption

Dose Group (g/kg) ^a	Body Weight Gain (g)								Food Consumption (g/day) ^b						
	Day of Study								Day of Study						
	2	4	6	8	10	12	14	16	2	4	6	8	10	12	14
Males															
0.0	165	177	198	215	225	240	257	242	21	21.8	24.5	24.5	22.5	23.5	25
0.9	164	172	190	204	212	226	238	224	20.5	20	22	21.8	22	21.5	22
1.2	164	172	190	204	214	222	235	215	20	20	22	20	21	20	22
1.9	166	168	187	194	193	206	217	196	21	15.8	20	17	15.2	17.5	18.5
3.0	164	164	175	182	190	195	201	183	20	13	15	15	15	15	15
Females															
0.0	137	136	144	152	158	164	170	155	17.5	16	16.5	17	16	16	17
0.9	138	142	154	158	164	170	175	164	18	15	17	16	16.5	17.8	17
1.2	134	135	145	148	156	158	164	148	16	13	16	15	14.5	15	15
1.9	135	134	144	146	152	154	158	144	17.5	11.5	15	14	14.5	13	14
3.0	138	132	135	135	140	140	144	130	17.5	8.5	9.5	11	12	11.8	11.5

^agrams of DNT per kilogram of feed

^bgrams of feed per day

Table A-1 data were used to calculate the daily and mean DNT ingestion rates (Table A-2) with the formula:

$$\text{Ingestion rate} = (\text{dose} \times \text{food consumption}) \div \text{body weight}$$

Table A-2. Ingestion Rates of Dinitrotoluene (mg/kg/day)

Dose Group (g/kg) ^a	Day of Study							
	2	4	6	8	10	12	14	Mean ^b
Males								
.0	165	177	198	215	225	240	257	242
0.9	164	172	190	204	212	226	238	224
1.2	164	172	190	204	214	222	235	215
1.9	166	168	187	194	193	206	217	196
3.0	164	164	175	182	190	195	201	183
Females								
0.0	137	136	144	152	158	164	170	155
0.9	138	142	154	158	164	170	175	164
1.2	134	135	145	148	156	158	164	148
1.9	135	134	144	146	152	154	158	144
3.0	138	132	135	135	140	140	144	130

^agrams of DNT per kilogram of feed

^bMean values are reported in the HA.

APPENDIX B. BENCHMARK DOSE MODELING RESULTS FOR 2,4-DINITROTOLUENE (2,4-DNT)

Benchmark dose (BMD) modeling was performed to identify potential critical effect levels for derivation of the reference dose (RfD) for 2,4-DNT. The modeling was conducted according to draft U.S. Environmental Protection Agency guidelines (U.S. EPA, 2000c), using Benchmark Dose Software (BMDS) system Version 1.3.2, which is available from the U.S. EPA (U.S. EPA, 2002). The BMD modeling results are summarized in Table B-1 below, and selected output is attached as Appendix C. A brief discussion of the modeling results is presented below. Because the endpoint is a quantal tumor incidence, the multistage models available with BMDS were used. For all of the modeling conducted, the benchmark risk was defined as an excess risk of 10% (U.S. EPA, 2000c).

The incidence of mammary gland tumors (including benign and malignant tumors from epithelial or mesenchymal cells) in female CD (Sprague-Dawley) rats given 2,4-DNT in feed for 24 months (Ellis et al., 1979; Lee et al., 1985) was chosen as the endpoint to model. As summarized in Table B-1, BMD and benchmark dose level (BMDL) estimates were identical between the two-stage and three-stage multistage models. The goodness-of-fit p values calculated for these two models were identical, as was the Akaike Information Criterion (AIC), a measure of goodness of fit that takes into account the number of degrees of freedom. The two-stage multistage model was chosen as the basis for the BMDL for this endpoint, based on its simpler form.

Table B-1. Benchmark Dose Estimates of 2,4-DNT From Female Rat Mammary Gland Tumors

Model	BMD	BMDL	Chi square p value	AIC
Multistage (2)	0.25	0.15	0.39	127
Multistage (3)	0.25	0.15	0.39	127

APPENDIX C. BENCHMARK DOSE (BMD) MODELING OUTPUT

Multistage Model. \$Revision: 2.1 \$ \$Date: 2000/08/21 03:38:21 \$
Input Data File: E:\BMDS\DATA\DNT-CANCER.(d)
Gnuplot Plotting File: E:\BMDS\DATA\DNT-CANCER.plt
Thu Jun 09 13:35:51 2005

BMDS MODEL RUN 1

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2)]$$

The parameter betas are restricted to be positive

Dependent variable = Incidence

Independent variable = HED

Total number of observations = 4

Total number of records with missing values = 0

Total number of parameters in model = 3

Total number of specified parameters = 0

Degree of polynomial = 2

Maximum number of iterations = 250

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

Background = 0.420273

Beta(1) = 0.399193

Beta(2) = 0

Asymptotic Correlation Matrix of Parameter Estimates

(***The model parameter(s) -Beta(2) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix.)

	Background	Beta(1)
Background	1	-0.37
Beta(1)	-0.37	1

Parameter Estimates		
Variable	Estimate	Standard Error
Background	0.392887	0.0966033
Beta(1)	0.420126	0.138015
Beta(2)	0	NA*

*Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

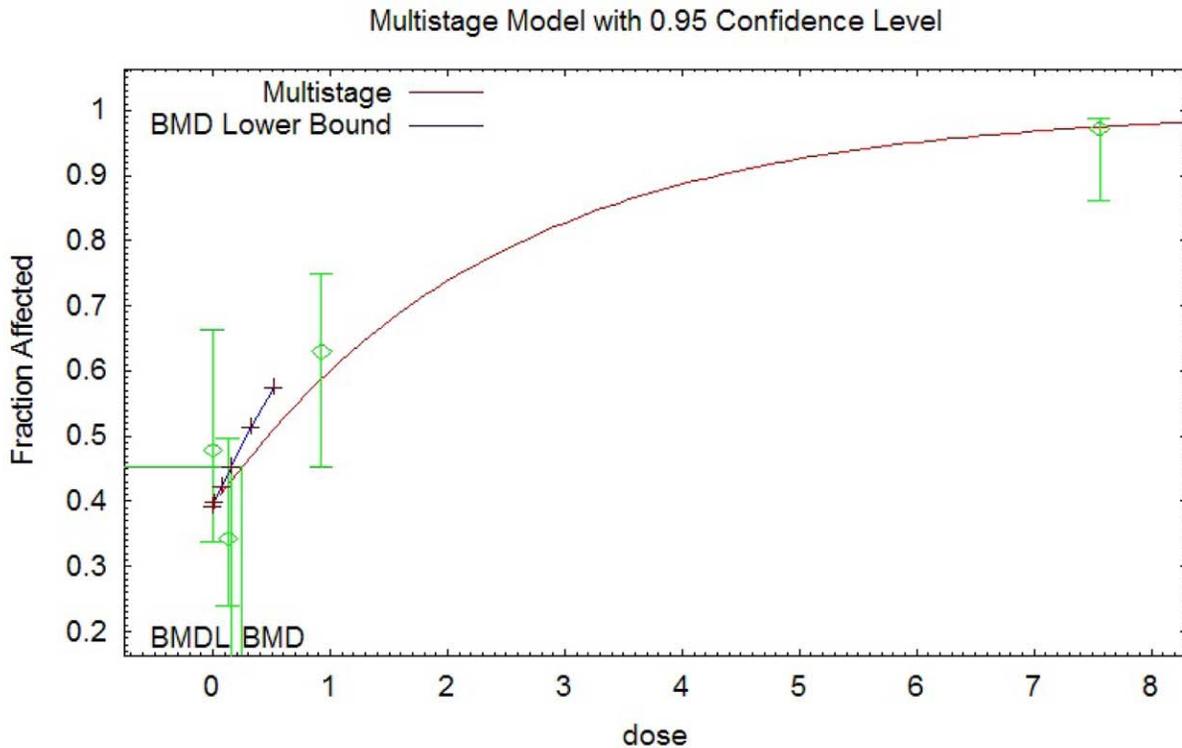
Analysis of Deviance Table				
Model	Log(likelihood)	Deviance	Test DF	p Value
Full model	-60.7606			
Fitted model	-61.6992	1.87722	2	0.3912
Reduced model	-79.8807	38.2401	3	<.0001

AIC: 127.398

Goodness of Fit					
Dose	Est. Prob.	Expected	Observed	Size	Chi Sq. Res.
i: 1	0.0000	9.036	11	23	0.358
i: 2	0.1290	14.872	12	35	-0.336
i: 3	0.9270	15.896	17	27	0.169
i: 4	7.5570	34.112	34	35	-0.129

Chi square = 1.87 DF = 2 p value = 0.3929

BMD Computation	
Specified effect	= 0.1
Risk type	= Extra risk
Confidence level	= 0.95
BMD	= 0.250783
BMDL	= 0.154257



Multistage Model. \$Revision: 2.1 \$ \$Date: 2000/08/21 03:38:21 \$
 Input Data File: E:\BMDS\DATA\DNT-CANCER.(d)
 Gnuplot Plotting File: E:\BMDS\DATA\DNT-CANCER.plt
 Thu Jun 09 13:37:22 2005

BMDS MODEL RUN 2

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 - \text{beta}3 * \text{dose}^3)]$$

The parameter betas are restricted to be positive

Dependent variable = Incidence

Independent variable = HED

Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3

Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

Background = 0.420273
 Beta(1) = 0.399193
 Beta(2) = 0
 Beta(3) = 0

Asymptotic Correlation Matrix of Parameter Estimates

(***The model parameter(s) -Beta(2) -Beta(3) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix.)

	Background	Beta(1)
Background	1	-0.37
Beta(1)	-0.37	1

Parameter Estimates		
Variable	Estimate	Std. Err.
Background	0.392887	0.0966033
Beta(1)	0.420126	0.138015
Beta(2)	0	NA*
Beta(3)	0	NA*

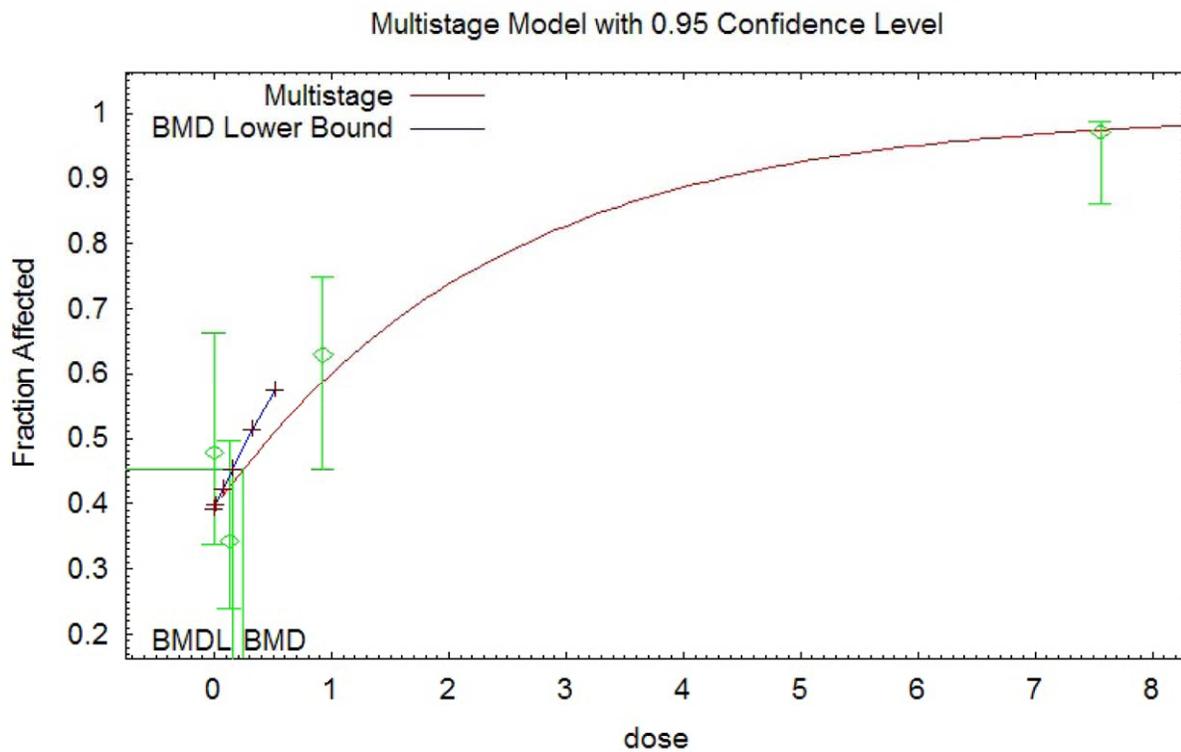
*Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table				
Model	Log(likelihood)	Deviance	Test DF	p Value
Full model	-60.7606			
Fitted model	-61.6992	1.87722	2	0.3912
Reduced model	-79.8807	38.2401	3	<.0001
AIC:	127.398			

Goodness of Fit					
Dose	Est. Prob.	Expected	Observed	Size	Chi Sq. Res.
i: 1	0.0000	9.036	11	23	0.358
i: 2	0.1290	14.872	12	35	-0.336
i: 3	0.9270	15.896	17	27	0.169
i: 4	7.5570	34.112	34	35	-0.129
Chi square =	1.87	DF = 2	p value = 0.3929		

Benchmark Dose Computation

Specified effect = 0.1
 Risk Type = Extra risk
 Confidence level = 0.95
 BMD = 0.250783
 BMDL = 0.154257



13:37 06/09 2005