

U.S. Army Center for Health Promotion
and Preventive Medicine

**Wildlife Toxicity Assessment for
2,4 & 2,6-DINITROTOLUENE**

**FINAL REPORT
JANUARY 2006**

**Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program**

**USACHPPM Document No: 39-EJ-1138-01D
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Readiness Thru Health

Wildlife Toxicity Assessment for
2,4-Dinitrotoluene CAS No. 121-14-2
2,6-Dinitrotoluene CAS No. 606-20-2

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When referencing this document use the following citation

USACHPPM. 2006 Wildlife Toxicity Assessment for 2,4 & 2,6-Dinitrotoluene, Project Number 39-EJ-1138-01D, U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland.

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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for 2,4- and 2,6-Dinitrotoluene

CAS No. 121-14-2 and 606-20-2

January 2006

1. INTRODUCTION

The DNT's, 2,4-dinitrotoluene (CAS No. 121-14-2) and 2,6-dinitrotoluene (CAS No. 606-20-2), have been associated, either as precursors or by-products, with the synthesis of polyurethane foams, coatings, elastomers and explosives. DNTs are particularly important used a primary product in propellants and also found in by-products in the manufacture of the common military explosive, 2,4,6-trinitrotoluene (TNT). The importance of the DNTs as environmental contaminants is related to its distribution at and around military sites and its potential toxicity to wildlife and other ecological receptors.

Technical grade DNT is composed of approximately 75% 2,4-DNT, 20% 2,6-DNT, and 5% other isomers (USEPA 1993). It is often found in soils associated with firing points at artillery ranges, and with low order detonations (Pennington et al. 2004). This Wildlife Toxicity Assessment summarizes the current knowledge of the toxicological impacts of 2,4- and 2,6-dinitrotoluene on vertebrates. Evaluating the toxicity of 2,4- and 2,6-dinitrotoluene contributes to the derivation of toxicity reference values (TRVs) that could serve as screening-level benchmarks for wildlife in the vicinity of contaminated sites. The protocol for the development of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Given the common military use of 2,4,6-trinitrotoluene and its by-products, 2,4- and 2,6-dinitrotoluene, many studies were found in U.S. Army sources. These and other studies were found through TOXLINE and DTIC searches. In addition, many appropriate studies were found through traditional cross-referencing techniques and through individual queries to project investigators within the Army. Several databases were searched and the details of these searches are provided in Appendix A.

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

	2,4-DNT ¹	2,6-DNT ¹
CAS No.	121-14-2	606-20-2
Molecular weight	182.14	182.14
Color	Yellow	Yellow to red
State	Needles or monoclinic prisms	Solid or rhombic needles from alcohol
Melting point	71°C	66°C
Boiling point	300°C with slight decomposition	285°C
Odor	Slight	Slight
Solubility	270 mg/L in water @ 22°C; soluble in alcohol, ether, acetone, benzene, pyridine	180 mg/L in water @ 22°C; soluble in alcohol
Partition coefficients		
Log K _{ow}	1.98	1.72 (estimated)
Log K _{oc}	2.40 (estimated) ²	1.89 (estimated) ²
Vapor pressure, torr (at 25°C)	1.4 X 10 ⁻⁴	5.67 x 10 ⁻⁴
Henry's Law constant (at 20°C)	8.79E-08 atm m ³ /mole (calculated)	9.26E-08 atm m ³ /mole (estimated)
Conversion factors	1 ppm = 7.4 mg/m ³ 1 mg/m ³ = 0.13 ppm	1 ppm = 7.4 mg/m ³ 1 mg/m ³ = 0.13 ppm

¹ All values from HSDB 2000 and ATSDR 1998 unless otherwise noted

² Burrows et al. 1989

2.2 Environmental Fate and Transport

The DNTs (both 2,4-DNT and 2,6-DNT) have been used as a precursor or produced as a by-product in the synthesis or manufacture of polyurethane foams, coatings, elastomers and explosives. The manufacture of DNT generates a technical-grade (tg) mixture that consists of 75% 2,4-DNT, 20% 2,6-DNT and 5% other isomers (USEPA 1993). In addition, the by-products of the manufacture of 2,4,6-trinitrotoluene, 2,4- and 2,6-DNT, are common soil contaminants at munitions manufacturing sites (Nishino and Spain 1995). The discharge of waste streams generated during munitions manufacturing and processing to surface water is the major route of environmental entry for these compounds. Sampling of TNT effluents showed that 2,4-DNT, 2,6-DNT and 1,3-dinitrobenzene (1,3-DNB) were, in order, the major effluent constituents and accounted for approximately 75% by weight of total organics. Soil contamination by munitions compounds may also occur through open burning, incineration, operational spills and seepage from landfills and wastewater holding facilities (Burrows et al. 1989). The DNTs have frequently been detected in both soil (35 of 69 and 20 of 53 sites, respectively) and sediment (16 of 19

and 9 of 53 sites, respectively) at NPL hazardous waste sites (ASTDR 1998). A summary of physical and chemical properties is provided in Table 1.

The fate and distribution of munitions pollutants in the environment are principally driven by microbial and photochemical transformations (photolysis). Microbial transformations have occurred with common bacterial genera such as *Pseudomonas*, *Janthinobacterium*, *Actinobacter*, *Alcaligenes* and *Flavobacterium*, as well as some yeast and fungal species (Noguera and Freedman 1996, Burrows et al. 1989, Kaplan 1992, Brower et al. 1994). Nishino and Spain (1995) hypothesized that although bacteria able to degrade 2,4-DNT exist at most of the sites they studied, the presence of these two isomers in the original 4:1 production ratio suggests that the presence of 2,6-DNT may be inhibitory to the degradation of 2,4-DNT by 2,4-DNT-degrading bacteria.

The mobility of 2,4-DNT in soil is expected to be slight ($\log K_{oc} = 2.4$). Based on the results of aqueous tests, biodegradation of 2,4-DNT in both aerobic and anaerobic zones of the soil may occur. Hydrolysis is not expected to occur and photochemical transformation in soil should not be significant (HSDB 2000, ATSDR 1998). In water, 2,6-DNT should biodegrade (though more slowly than the 2,4 isomer) and photolysis should be rapid (half life = 12 mins) in surface layers. Absorption of 2,6-DNT to sediments or suspended solids should not be appreciable and volatilization from water should be insignificant (HSDB 2000, ATSDR 1998). The moderate water solubilities and relatively low octanol-water partition coefficients of the two isomers indicate a potential for transport by surface and groundwater (ATSDR 1998).

The most important removal process for 2,4-DNT from water will likely be photolysis. The photolytic half lives for 2,4-DNT in river, bay and pond waters were 2.7, 9.6 and 3.7 hours, respectively. In aqueous biodegradation tests, 2,4-DNT was shown to biodegrade both aerobically and anaerobically; however, microbial degradation data for both 2,4- and, particularly, 2,6-DNT were inconsistent. The 2,4-DNT will have a slight tendency to partition to suspended and sediment organic matter ($\log K_{ow} = 1.98$). Volatilization of 2,4-DNT from water should be minimal due to its low vapor pressure and Henry's Law constant and significant bioaccumulation ($BAF = 204$) is not expected to occur (HSDB 2000, ATSDR 1998).

If released to soil, data suggests that 2,6-DNT would degrade, though to a lesser degree than 2,4-DNT. Based on experiments in sandy loam and sandy silt loam, it should be fairly mobile despite its estimated $\log K_{oc}$ of 1.89 and K_{ow} of 1.72. Volatilization from soil and photo-oxidation of 2,6-DNT in soil should not be significant. The $\log K_{ow}$ indicates that significant bioaccumulation is unlikely (HSDB 2000, ATSDR 2000).

Given the relatively low water solubility and affinity to lipids, the DNTs are not expected to appreciably accumulate in plants or animals; therefore, exposure through food is expected to be minimal.

Primary exposures to wildlife are likely to be through inadvertent ingestions of soils (through grooming and feeding) and potentially through the skin.

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Toxicity: Oral

Following oral administration, DNT isomers are rapidly absorbed, distributed and eliminated (Rickert and Long 1980, ATSDR 1998). In rats, rabbits, dogs and monkeys, the bulk of radioactivity from radio-labeled oral DNT exposure was excreted in the urine. In mice, ³H-labeled 2,6-DNT was excreted primarily in the urine while ¹⁴C-labeled 2,4-DNT was excreted mostly in the feces (ATSDR 1998).

2.3.1.1 Mammalian Oral Toxicity – Acute

Oral LD₅₀ values for 2,4-DNT ranged from 240 to 650 mg/kg in rats and 1340 to 1954 mg/kg in mice. Toxicity observed in both species included ataxia and cyanosis, with death occurring within the first 24 hours. No treatment-related gross pathology was observed in dead animals and survivors recovered completely within 48 hours (Lee et al. 1975, Ellis et al. 1978, Vernot et al. 1977, Lane et al. 1985). These data are similar to those for TNT exposure in these species.

The oral LD₅₀ value for 2,6-DNT ranged from 180 to 795 mg/kg in rats and 621 to 1000 mg/kg in mice. Toxic symptoms, time to death, gross pathology and recovery observations were similar to 2,4-DNT. Females appeared slightly more tolerant of 2,6-DNT than males (Lee et al. 1975, Ellis et al. 1978, Vernot et al. 1977).

2.3.1.2 Mammalian Oral Toxicity - Subacute

McGowan et al. (1983) fed rats (5 per sex per group) 0.9, 1.2, 1.9 or 3 g 2,4-DNT/kg in diet for 14 days. Administration of 2,4-DNT resulted in dose-dependent decreases in food consumption and body weight gain, elevated blood cholesterol and glucose, and oligospermatisms with degenerative changes of the testes in males. The authors did not estimate average daily intake of 2,4-DNT.

As part of the determination of the Maximum Tolerated Dose for a developmental study, Smith (1983) exposed groups of 10 female CD-1 mice to oral doses of 0, 310, 525, 1250, 2500, and 3500 mg/kg-d of 2,4-DNT for eight days. Mice in the 525 mg/kg-d groups lost up to 13% of their body weight by the eighth day of dosing. By the eighth day, all mice in the higher dose groups died. Adverse observations recorded for the 310 mg/kg-d group included lethargy (10) and hunched posture (1).

2.3.1.3 Mammalian Oral Toxicity - Subchronic

Bloch et al. (1988) administered 0%, 0.1% and 0.2% 2,4-DNT in feed to Sprague-Dawley rats for 3 weeks. Average daily intakes were not estimated. Marked changes in Sertoli cell morphology and increases in circulating levels of follicle stimulating hormone and luteinizing hormone were observed in the 0.2% dose group. Vesicles of varying size associated with swollen mitochondria, reduced epididymis weights, and decreased epididymal sperm reserves were observed in 2,4-DNT treated animals. Rats in both treatment groups had decreased body weight gain and multinucleated spermatids, mild irregularity of basal lamina, vacuolation and lipid accumulation in Sertoli cells. The authors concluded that DNT is capable of testicular injury and altering spermatogenesis, however, the effect of this injury on reproductive function was not evaluated.

Lee et al. (1978) studied the effects of oral 2,4-DNT administration of up to 13 weeks in dogs, rats and mice. The toxic effects observed from 2,4-DNT administration included death, decreased body weight and food consumption, methemoglobinemia, reticulocytosis, hemosiderosis, anemia, hepatocellular changes and altered liver weights/function, serious neurological effects (dogs and rats), and testicular degeneration and decreased spermatogenesis in males. Dogs were the most sensitive species tested followed by rats and then mice. Reversibility of adverse effects, mutagenicity of the compound, immunologic response, and disposition and metabolism of radio-labeled compound were also determined.

A total of 32 beagle dogs were divided into four groups (4/sex/group) and administered 0, 1, 5, or 25 mg/kg/day of 2,4-DNT in capsules. One male and one female from each dose group were euthanized at 4 and 13 weeks, and then at 8 and 17 weeks to study reversibility. Several dogs in the high-dose group were moribund and euthanized ahead of schedule; two dogs in the high-dose 4-week group were not euthanized until 8 months later to investigate the potential for reversibility over a longer time period. Daily administration of 2,4-DNT at 1 and 5 mg/kg/day resulted in no adverse effects in dogs. Dosages of 25 mg/kg/day resulted in toxicity at 12 to 22 days and were lethal after 22 or more days. Toxic effects included anorexia and weight loss; neuromuscular incoordination and rigid paralysis (particularly in the hind legs); gliosis and demyelination in the brain; methemoglobinemia and anemia with reticulocytosis and Heinz bodies, hemosiderosis and extramedullary hematopoiesis; and atrophy of the testes with aspermatogenesis. Dogs recovered partially 4 weeks post-treatment and completely after eight months. Treatment with 2,4-DNT did not alter serum immunoglobulin (IgE) levels (Lee et al. 1978).

A total of 64 male and 64 female CD rats were divided into four equal groups (16/sex/group) and administered 0%, 0.07%, 0.2%, or 0.7% 2,4-DNT in their feed for 4 or 16 weeks in a protocol similar to that used for dogs (Lee et al. 1978). The average intake for the low-dose rats was 34.4 and 38.3 mg/kg/day, for mid-dose animals 92.8 and 108.3 mg/kg/day, for the high-dose rats 265.6 and 145.2

mg/kg/day, for males and females, respectively. The lower intake in high-dose females was the result of a marked decrease in feed consumption.

The only effect observed in the low-dose animals was a slight decrease in weight gain. The mid-dose animals had a greater decrease in weight gain, reticulocytosis, hemosiderosis in the spleen and decrease or cessation of spermatogenesis. Gliosis and demyelination was observed in the cerebellum of one mid-dose rat. High-dose rats had severe weight loss, anemia with reticulocytosis, greater and earlier onset of splenic hemosiderosis and aspermatogenesis, neuromuscular effects (unusual gait with wide stance and stiff hind legs), and mild to moderate gliosis and/or demyelination in the central nervous system. Eight of 16 high-dose males and one mid-dose male died ahead of schedule. Ten of 16 high-dose females died in the first 3 weeks of the study. Survivors across treatments partially recovered 4 weeks after cessation of exposure (weight gain, anemia, but not aspermatogenesis and splenic hemosiderin incidence. No effects on serum IgE were observed (Lee et al. 1978, Lee et al. 1985). These data are consistent with those effects observed in a 30-day study of technical grade (tg)-DNT in Fischer 344 rats (Hazelton Laboratories 1977).

Sixty-four male and 64 female albino Swiss mice were divided into four equal groups (16/sex/group) and administered 0%, 0.07%, 0.2%, or 0.7% 2,4-DNT in feed for 4 or 16 weeks in a experimental protocol similar to the dogs (Lee et al. 1978, Hong et al. 1985). The average intake for the low-dose animals was 47 and 52 mg/kg/day, for the mid-dose mice 137 and 147 mg/kg/day, and for the high-dose mice 413 and 468 mg/kg/day for males and females, respectively.

No adverse effects from 2,4-DNT were observed in either the low or mid-dose animals, except for a statistically significant decreased weight gain in males at both doses. High-dose mice had weight loss and decreased food consumption, mild anemia, mild depression of spermatogenesis (decreasing fertility), and a few deaths. Surviving mice recovered completely by 4 weeks after cessation of treatment (Lee et al. 1978, Hong et al. 1985).

Lee et al. (1976) studied the effects of oral 2,6-DNT exposure for up to 13 weeks in dogs, rats, and mice in a protocol similar to that used for 2,4-DNT (Lee et al. 1978). The effects of the test article relative to drug metabolizing enzymes, mutagenicity, immunologic response and reversibility of adverse effects were also evaluated. Treatment-related effects resulting from 2,6-DNT exposure were similar to those observed for 2,4-DNT. Toxic effects included mortality, decreased body weight gain and food consumption, hemosiderosis in spleen and liver, neuromuscular effects in dogs, and degeneration of the testes and decreased spermatogenesis in males. Although dogs remained the most sensitive species, the relative species sensitivities of rats and mice were reversed when compared to 2,4-DNT.

Four groups of 8 dogs (4 per sex) were given 0, 4, 20 or 100 mg/kg/day of 2,6-DNT in capsules for 4 or 13 weeks. One male and one female from each dose group were euthanized at 4 and 13 weeks,

then at 8 and 17 weeks to study reversibility. Because of severity of symptoms, dogs in the high-dose reversibility group were continued for an additional 2 weeks (19 weeks total) before they were euthanized.

Toxicity occurred in a dose-dependent manner and included decreased body weight and food consumption; listlessness, incoordination leading to rigid paralysis with occasional tremors; methemoglobinemia with Heinz bodies and anemia with compensatory reticulocytosis and extramedullary hematopoiesis; lymphoid depression with peripheral lymphocytopenia; bile duct hyperplasia, inflammatory and degenerative changes in liver and kidney with elevated serum chemistries; and degeneration and atrophy of the spermatogenic cells of the testes. Administration of 4 mg/kg/day had no remarkable symptoms, while all dogs given 100 mg/kg/day died between the second and eighth weeks. The toxic effects of oral 2,6-DNT exposure were partially reversed at 4 weeks and completely reversed at 19 weeks post-treatment. Treatment-related effects on serum immunoglobulin E-titers were not observed (Lee et al. 1976).

Four groups of 32 CD rats each (16 males and 16 females) were given 0%, 0.01%, 0.05% or 0.25% 2,6-DNT in their feed within the same experimental design as for dogs, except that four animals per sex per group were euthanized at each time point (Lee et al. 1976). The average intakes of 2,6-DNT were 7.2 and 7.4, 35.1 and 37.1, and 144.7 and 155 mg/kg/day for males and females in low-, mid- and high-dose groups, respectively.

No treatment-related effects from 2,6-DNT were observed at the low dose. At the mid-dose, effects observed included decreased weight gain and food consumption, extramedullary hematopoiesis in spleen and/or liver, bile duct hyperplasia, and depression of spermatogenesis and atrophy in the testes. Animals in the high-dose group were more severely affected, and exhibited weight loss, decreased erythrocyte count with compensatory reticulocytosis, methemoglobinemia with Heinz bodies, severe testicular lesions, and more prevalent and severe bile duct hyperplasia and extramedullary hematopoiesis. Rats recovered only partially 4 weeks after treatment cessation (Lee et al. 1976).

Mice were fed 0%, 0.01%, 0.05% or 0.25% 2,6-DNT in a protocol similar to the one used for dogs and rats (16 animals per sex/group; Lee et al. 1976). The average intakes for mice fed the low-, mid- and high-dose 2,6-DNT were 11.1 and 11.0, 50.8 and 55.2, 288.8 and 298.8 mg/kg/day for male and female mice, respectively. Three males from the control group, two males from the low-dose group, eight males and one female from the mid-dose group, and eight males and six females in the high-dose group died on study. The increases in deaths in the mid- and high-dose groups were considered treatment-related. The mid- and high-dose animals also exhibited decreased feed consumption and weight gain, extramedullary hematopoiesis, depression of spermatogenesis and atrophy of the testes, and bile duct hyperplasia. Partial recovery was observed in mice 4 weeks after cessation of treatment. No toxic effects

were observed in the low-dose animals. Unlike 2,4-DNT, where mice appeared to be almost unaffected by doses lethal to rats, mice were more susceptible to 2,6-DNT (Lee et al. 1976).

2.3.1.4 Mammalian Oral Toxicity – Chronic

Ellis et al. (1979) administered 0, 0.2, 1.5 or 10.0 mg/kg/day of 2,4-DNT in capsules to dogs for up to 24 months. The starting group of animals was 12 per group, with equal numbers of males and females. After 12 months of dosing, treatment was stopped for two males and two females from each group. One pair was euthanized at the time of dosing cessation, the other pair was allowed to recover for 4 weeks then euthanized. The remaining animals were dosed for 12 more months (24 months total), then two pairs from each group euthanized at dosing cessation and the two remaining pairs from each group allowed to recover for 4 weeks before sacrifice.

No adverse effects from 2,4-DNT were observed at the low-dose (0.2 mg/kg/day) group. Toxicity was observed in some, but not all dogs in the mid-dose (1.5 mg/kg/day) group, while administration of the high-dose (10 mg/kg/day) was toxic to all dogs and lethal to some. Principal target organs were the erythrocytes, nervous system and biliary tract. Observed effects include methemoglobinemia, anemia, reticulocytosis and Heinz bodies; neuromuscular incoordination and paralysis (especially of the hind legs), degenerative lesions of the cerebellum; and hyperplasia of the biliary tract and gallbladder epithelium. Recovery appeared to occur after dosing cessation; however, animal numbers were very low.

CD rats were administered 0, 0.0015%, 0.01% or 0.07% 2,4-DNT in their feed for up to 24 months, with the average intake for low-, mid- and high-dose animals being 0.57, 3.9, or 34 mg/kg/day for males and 0.71, 5.1 or 45 mg/kg/day for females, respectively. The experimental schedule was similar to that with dogs, except there were initially 38 animals per sex per group. Four per sex per group were euthanized at the interim (12 and 13 mo) time points, and the remaining survivors euthanized at 24 and 25 months (Ellis et al. 1979).

No toxicity from 2,4-DNT was noted in rats in the low-dose group. Toxicity occurred in the mid-dose animals and included a statistically significant decreased weight gain, indicators of liver toxicity, anemia, and incidences of mammary tumors. Increased toxicity was observed in the high-dose group and included severely decreased weight gain, shortened life span, livers with hyperplastic foci to hepatocellular carcinoma, decreased spermatogenesis or aspermatogenesis, and increases in usual background tumors, such as of the connective (fibromas in males) and mammary (fibroadenomas in females) tissues. Occasional neurological symptoms, such as straddling gait, were observed in some high-dose rats but without accompanying histopathological lesions (Ellis et al. 1979, Lee et al. 1985).

Because earlier studies had shown that 2,4-DNT was less toxic in mice than rats, CD-1 mice were given 0%, 0.01%, 0.07%, or 0.5% in their feed for up to 24 months (Ellis et al. 1979). There were only

small differences in intake between sexes; the average intake for low-, mid- and high-dose animals were 13.5, 95 and 900 mg/kg/day, respectively. The experimental design was similar to the design used in the dog study, except the researchers used 58 animals per sex per group. Four animals per sex per group were euthanized at 12 and 13 months (recovery animals); the remainder were euthanized at 24 and 25 months (Ellis et al. 1979).

Low-dose administration (13.5 mg/kg/day) of 2,4-DNT to mice resulted in toxic nephropathy, excessive pigmentation, liver dysplasia and renal tumors (in males). The mid-dose (95 mg/kg/day) was very toxic and resulted in a higher incidence and more severe effects than those observed at the low dose, including cystic renal tumors in over half the males and atrophy of the testes. High-dose toxic effects were severe and included even greater decreases in weight gain and food consumption, a life span shortened to half of other dose groups, anemia with many Heinz bodies, and non-functioning gonads in both sexes. A general pigmentation was observed in many tissues of the high-dose animals. Effects were more severe in males than females (Ellis et al. 1979, Hong et al. 1985).

One study on the chronic toxicity of 2,6-DNT was located (Leonard et al. 1987). Although the study was designed to evaluate the hepatocarcinogenicity, other parameters were measured. Twenty-eight rats total were used in the study with a control and a low (7 mg/kg/day) and high (14 mg/kg/day) treatment of 2,6-DNT in feed. Four rats were euthanized at weeks 4 and 26 with remaining rats (20) exposed for 52 weeks total. Throughout the study, concentrations of 2,6-DNT were adjusted based on food consumption and average body weight to maintain target doses. The effects observed for rats exposed to 2,6-DNT were consistent with those observed for subchronic 2,6-DNT administration. These effects included decreased body weights and hepatocellular degeneration, vacuolation, acidophilic and basophilic foci of cellular alteration, cholangiocarcinoma and hepatocellular carcinoma (Leonard et al. 1987). The lowest observed adverse effect level (LOAEL) for reduced body weight was 7 mg/kg/day. Since this dose was the lowest used, a no-observed-adverse-effect-level (NOAEL) could not be derived.

2.3.1.5 Mammalian Oral Toxicity – Other

Studies were conducted to evaluate developmental effects by Smith (1983) and Hardin et al. (1987). In both cases, female CD-1 mice were exposed to 2,4-DNT at 0, 310, and 525; and 0, and 390 mg/kg-d on GD-6 through 13 on the Smith and Hardin et al. studies, respectively. Smith (1987) reported that 10 out of 50 exposed to 390 mg/kg-d 2,4-DNT were died, and four of which were pregnant. An additional 5 died in the post-dosing observation period, two of which were pregnant. Sixteen out of 50 mated female mice in total were pregnant. Five additional litters were found to be reabsorbed (reproductive index 0.68). No other measures of reproductive toxicity were statistically significant.

Only one mouse out of 50 was found dead following oral doses of 390 mg/kg-d in the Hardin et al. (1987) study. No other measures of reproductive toxicity were statistically significant.

Price et al. (1985) and Jones-Price et al. (1980) investigated developmental effects of technical grade DNT by exposing pregnant rats to oral doses from 14 to 150 mg/kg-d from gestational days 7-20. Overt toxicity included rough coat, lethargy, and hind limb weakness. Blood effects (decreases in red blood count, PCV; increases in methemoglobin, reticulocyte count) occurred in the 100 mg/kg-d group. No adverse reproductive effects were observed consistent with DNT exposure. The authors conclude that DNT is not teratogenic but may cause embryo/fetal toxicity at levels where maternal mortality occurs.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

2,4-Dinitrotoluene

The studies selected to derive a TRV for 2,4- DNT were chronic ingestion exposures in several species of mammals. Effects from chronic 2,4-DNT exposures were consistent with those of subchronic exposures, and included decreased survival, decreased body weights and food consumption, anemia, hepatocellular dysplasia, kidney tumors in mice, serious neuromuscular effects in dogs, ovarian atrophy in female mice, and decreased spermatogenesis and testicular atrophy in males. The most relevant chronic studies were those conducted by Ellis et al. (1979 and 1985), Lee et al. (1985) and Hong et al. (1985) in dogs, rats, and mice, respectively. These were well-conducted, of suitable duration, and provided an evaluation of a number of relevant endpoints. Additionally, results from these studies are corroborated by results from a number of other chronic (Leonard et al. 1987, NCI 1978) and subchronic (Lee et al. 1978, Kozuka et al. 1979, Bloch et al. 1988) studies. Acute studies provided additional information but were not considered relevant to the development of TRVs. Table 1 summarizes the data from these studies and Figure 1 presents the data in a scatter diagram. All major studies were conducted on contract to the U.S Army and provided detailed methods and results. These studies are considered high quality and sufficient for use in derivation of a toxicity reference value. Most of these studies have also been presented as peer reviewed report.

Technical Guide 254 states that Toxicity Reference Values should be based on ecologically-relevant endpoints. For 2,4-DNT, the neuromuscular effect in dogs (Ellis et al.. 1979, 1985) was chosen as the basis for TRV development. The severe paralysis and incoordination induced by 2,4-DNT occurred at relatively low concentrations (LOAEL was 1.5 mg/kg/day and NOAEL was 0.2 mg/kg/day) and in some cases, precluded dogs from eating and drinking normally. In less severe cases, hind leg, lip and tongue control were hampered. These neuromuscular effects would likely lead to difficulties in foraging, avoiding predators and mating in wild populations. Concomitant with the neuromuscular effects were increased methemoglobinemia and incidence of Heinz bodies. In addition, the onset of these effects in

dogs occurred at lower concentrations than the onset of effects such as hepatocellular dysplasia, kidney tumors, ovarian and testicular atrophy and decreased spermatogenesis seen in other mammalian species. Hence, a TRV based on neuromuscular effects in dogs would likely be protective of other endpoints.

2,6-Dinitrotoluene

There was one study that characterized the chronic toxicity of 2,6-DNT in rats. Leonard et al. (1987) showed that rats dosed with 14 mg 2,6-DNT/kg/day (LOAEL) for one year were significantly smaller than control rats. Some effects associated with subchronic 2,6-DNT exposures included decreased body weights and food consumption, bile duct hyperplasia, testicular degeneration, decreased spermatogenesis, hepatocellular degenerations, and splenic hemosiderosis. In addition, similar to 2,4-DNT, 2,6-DNT elicited a neuromuscular effect in dogs that manifested as incoordination and lack of balance. The consistency in response among test species and between 2,4- and 2,6-DNT provides a fairly strong case of the toxicity of these compounds. The studies on 2,6-DNT were well-conducted and provided an evaluation of a number of relevant endpoints; however, only one (Leonard et al., 1987) was of suitable duration to derive a TRV with minimal use of uncertainty factors.

As outlined in Technical Guide 254 (USACHPPM, 2000), TRVs should be derived from ecologically-relevant endpoints. From an ecological perspective, reduced growth and /or associated reductions in food consumption as seen in rats exposed to 2,6-DNT for one year (Leonard et al., 1987) can affect the ecological performance of individuals by causing alterations in energy allocation patterns that could ultimately result in altered reproductive performance (Calow 1991, Congdon et al. 2001). On this premise, the Leonard et al. (1987) study on rats is most suitable for derivation of the TRV.

Table 2. Summary of Relevant Mammalian Data for 2,4-DNT

Study	Test Organism/route	Test Duration	LD50 mg/kg	NOAEL mg/kg/d	LOAEL mg/kg/d	Test Results
						Effects at LOAEL
Lee et al. 1975 Ellis et al. 1978	Rat/CD/gavage	single, acute	568 (M) 650 (F)			
Vernot et al. 1977	Rat/SpragueDawley/ gavage	Single, acute	270 (M)			
Lane et al. 1985	Rat/CharlesRiver/ gavage	single, acute	240 (M)			
Lee et al. 1975	Mouse/Swiss albino/gavage	single, acute	1954 (M) 1340 (F)			
Vernot et al. 1977	Mouse/CF-1/gavage	single, acute	1630 (M)			
Ellis et al. 1985 Lee et al. 1978	Dogs/oral/capsule	12 days		5	25	Incoordination, stiffness and abnormal gait
Lane et al. 1985	Rat/Sprague Dawley	5 days		60 (M)	180 (M)	Decreased (though reversible) fertility. Oral dog, 0, 0.01, 0.1 or 1 mg/kg/day for 4 weeks, then 0, 0.05, 0.5 and 5 mg/kg/day for 9 more weeks- NOAEL
Hardin et al. 1987	Mouse/CD-1	GD 6-13		390 (F)		No change in maternal bw, or developmental indices.
USACHPPM 1998	White-footed mouse (<i>Peromyscus leucopus</i>) feed	14 days		158 (M) 74(F)	286 (M) 158 (F)	Decreased body weight gain, Increased liver to body weight and brain/bw ratios
Ellis et al. 1979	Rat/CD/feed/ad lib	3 or 6 months			45.3 (F)	Increased incidence of death during parturition.
Kozuka et al. 1979	Rat/Wistar/feed/ad lib	6 months			415 (M)	71% died, humpback incoordination, testicular atrophy, 41% decrease in body weight. Hematologic (methemoglobinemia) and hepatic (liver weight and chemistry) changes, increase relative spleen weight NOAEL for renal effects.
Ellis et al. 1979, 1985	Dog/oral/capsule	6 months		1.5	10 (M)	4/6 animals died.
Ellis et al. 1985 Lee et al. 1978	Dog/oral/ capsule	4 or 13 weeks		5	25	5/8 died.
Ellis et al. 1979	Rats/CD/feed/ad lib	3 or 6 month			34 (M) 45 (F)	10-25% Decrease in body weight, decreased fertility (M), difficult parturition (F).
					34 (M)	Severe atrophy/degeneration of seminiferous tubules
					5(F)	Difficult parturition; decreased pup viability.

WILDLIFE TOXICITY ASSESSMENT FOR 2,4- AND 2,6-DINITROTOLUENE

Study	Test Organism/route	Test Duration	LD50 mg/kg	NOAEL mg/kg/d	LOAEL mg/kg/d	Test Results	
						Effects at LOAEL	
Lee et al. 1978, 1985	Rats/CD/feed/ad lib	4 or 13 weeks			34 (M) 38 (F)	Moderate decrease body weight gain	
					34 (M) 38 (F)	93 (M) 108 (F)	Both with decreased food consumption
					34 (M) 108 (F)	266 (M) 145 (F)	Reticulocytosis and hemosiderosis. (anemia at 266 (m) and 145 (f))
					34 (M)	93 (M) 145 (F)	Hepatic and renal effects, demyelination of cerebellum and brain stem, widespread and stiff-legged gait. Death in one male and 100% females.
					93 (M)	Severe decrease in spermatogenesis. Decrease in fertility.	
Hong et al. 1985	Mice/CD-1/feed/ad lib	4 or 13 weeks			137 (M) 147 (F)	413 (M) 468 (F)	Mild anemia and reticulocytosis.
Lee et al. 1978					137 (M) 147 (F)	413 (M) 468 (F)	Mild hepatocellular dysplasia 2/16 males and females died.
					47 (M) 52 (F)	413 (M) 468 (F)	Body weight loss with decreased food consumption. Also NOAEL for neurological effects.
Ellis et al. 1985, Lee et al. 1978	Dogs/oral/capsule	4 or 13 weeks			5	25	Anemia, Heinz bodies, hepatic, renal and immunological effects
					5	25	Incoordination, abnormal gait, paralysis
					5(M)	25 (M)	Testicular degeneration/decreased spermatogenesis.
Lee et al. 1985 Ellis et al. 1979	Rats/CD/feed/ad lib	1-2 years			.57 (M) .71 (F)	3.9 (M) 5.1 (F)	Decreased survival
					3.9 (M) 5.1 (F)	34.5 (M) 45.3 (F)	Decreased RBC count Marked anemia (males at 34.5)
						0.6 (M) 0.7 (F)	Preneoplastic foci of hepatocytes
					.51 (M) .71 (F)	3.9 (M) 5.1 (F)	Hepatocellular carcinoma, mammary and skin tumors
					3.9 (M) 5.1 (F)	34.5 (M) 45.3 (F)	30% decrease in body weight 27% decrease body weight-both with decreased food consumption
					.57 (M) .71 (F)	34.5 (M) 45. (F)	Wide-spread and stiff-legged gait
	.57 (M)	0.6 (M)	Atrophy of seminiferous tubules, aspermatogenesis				

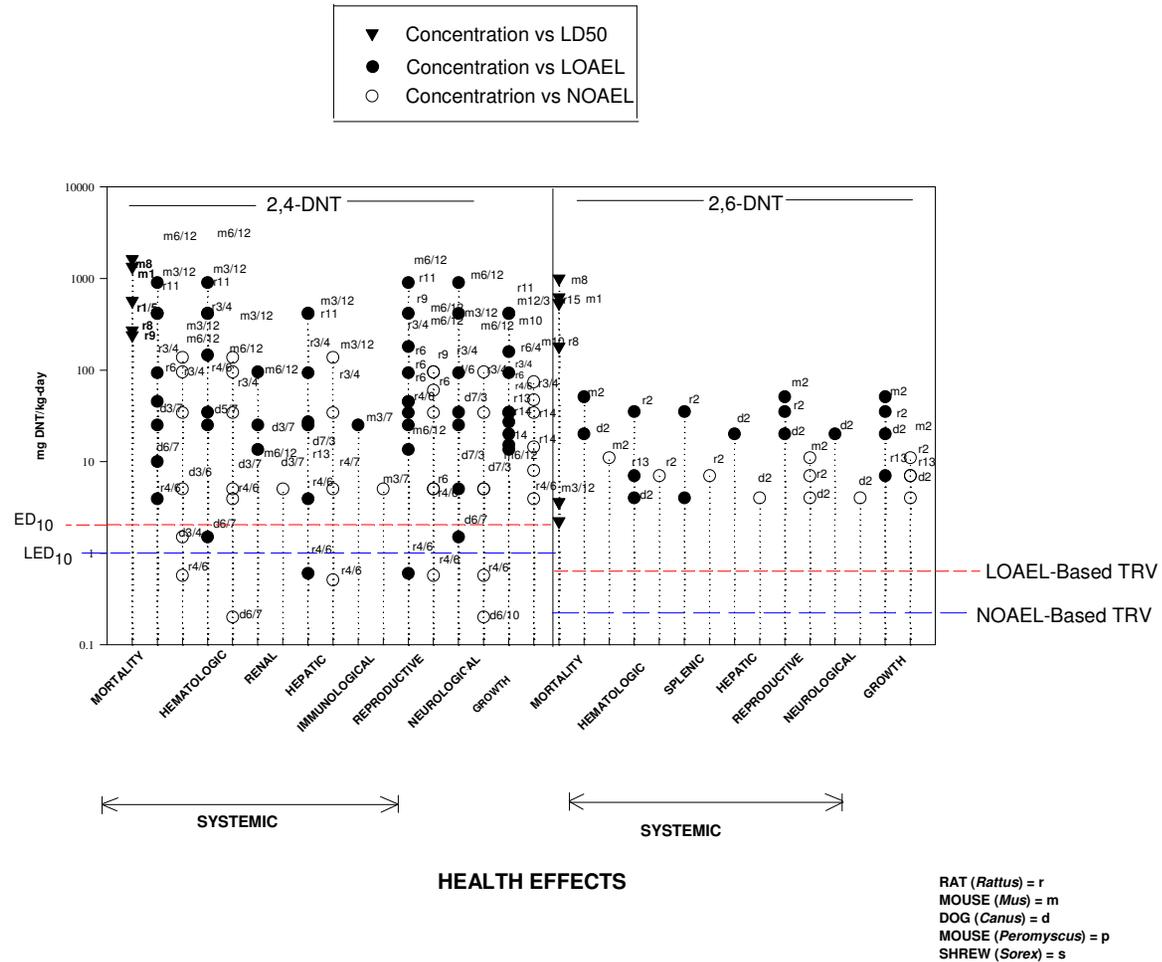
WILDLIFE TOXICITY ASSESSMENT FOR 2,4- AND 2,6-DINITROTOLUENE

Study	Test Organism/route	Test Duration	LD50 mg/kg	NOAEL mg/kg/d	LOAEL mg/kg/d	Test Results	
						Effects at LOAEL	
Ellis et al., 1979 Hong et al. 1985	Mice/CD-1/feed/ad lib	2 years		95	900	Decreased survival; Anemia; reticulocytosis; Heinz bodies; Stiff-legged gait; hyperactivity	
						13.5 (M) 13.5 (F)	Cystic dysplasia; toxic nephropathy
						13.5 (M) 900 (F)	16% decrease in body weight 20% decrease in body weight
						13.5 (M)	Decreased spermatogenesis and degenerative change; testicular atrophy
						95 (F) 900 (F)	Ovarian atrophy; nonfunctioning follicles
				95(M)	Renal solid carcinoma, cystic papillary carcinoma and adenoma, cystic adenoma		
Leonard et al. 1987	Rat/Fischer-344/feed/ad lib	1 year			27(M)	Non-neoplastic lesions of hepatocellular degeneration and vacuolation; basophilic and acidophilic foci of cellular alteration. Neoplastic nodule in only 1/20	
NCI 1978	Rat/Fischer-344/feed/ad lib	78 weeks		8 (M) 8.8 (F)	20 (M) 22 (F)	25% body weight decrease 25% decrease in body weight	
NCI 1978	Mouse/C57BL/6N/feed/ad lib	78 weeks		14.4 (M)	72 (M) 15.2 (F)	18% decrease in body weight 11% decrease in body weight-24% at 76	
Ellis et al. 1979, 1985	Dog/capsule/1xdaily	2 year		0.2	1.5	Methemoglobinemia, Heinz bodies, loss of hindquarter control, convulsions	

Table 3. Summary of Relevant Mammalian Data for 2,6-DNT

Study	Test Organism/route	Test Duration	Test Results			
			LD 50 mg/kg	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Lee et al. 1975	Rat/CD/gavage	Single dose	535 (M)			N/A
Ellis et al. 1978			795 (F)			
Vernot et al. 1977	Rat/SpragueDawley/gavage	Single dose	180 (M)			N/A
Lee et al. 1975	Mouse/CD	Single dose	621 (M) 807 (F)			N/A
Vernot et al. 1977	Mouse/CF-1/gavage	Single dose	1000 (M)			N/A
USACHPPM 1998	White-footed mouse (<i>Peromyscus leucopus</i>)/feed	14 day		103 (M) 44 (F)	238 (M) 103 (F)	Increased liver weight and liver to body and brain weight ratios
Lee et al. 1976	Mouse/Swiss-albino/feed	4 or 13 week		11 (M) 11 (F)	51 (M) 55 (F)	Death (8/16) Death (6/16)
				11	51 (M) 55 (F)	Weight loss
				11 (M)	51 (M)	Decreased spermatogenesis
					20 (F)	Death (2/8)
Lee et al. 1976	Dog/oral/feed	4 or 13 weeks			4	Mild extramedullary erythropoiesis and lymphoid depletion, mild splenic hematopoiesis
				4	20	Mortality. Bile duct hyperplasia; degenerative and inflammatory liver changes, dilated tubules, degenerative foci, weight loss with decreased food consumption, incoordination, lack of balance, testicular degeneration
				20	100	Thymic involution
Lee et al. 1976	Rat/CD/feed	4 or 13 weeks		7	35 (M) 37 (F)	Decreased weight gain, decreased spermatogenesis; degeneration of the testes, bile duct hyperplasia; hemosiderosis, splenic hemosiderosis extramedullary hematopoiesis
Leonard et al. 1987	Rat/Fischer-344/ feed	52 weeks			7 (M)	Hepatocellular degeneration, vacuolation; acidophilic and basophilic foci of cellular alteration, altered serum enzyme activities, 18% decrease in body weight
						7 (M)

2,4 – 2,6-DNT HEALTH EFFECTS TO MAMMALS



2.3.2 Mammalian Toxicity: Inhalation

Five rats/sex/group were exposed, nose only, to measured chamber concentrations of 0.026, 0.196, 0.473 and 0.696 mg 2,6-DNT/l for 6 hours. The acute (6-hour) LC₅₀ for 2,6-DNT aerosols were 0.24 mg/l for male and 0.66 mg/l for female Fischer 344 rats (Chemical Manufacturers Association 1991). Survivors had decreased body weight gain for 3-6 days, decreased food consumption, and small increases in methemoglobin levels, but no macroscopic abnormalities. Animals that died had higher lung to body weight ratios, congested lungs and a dark appearance to the liver. No longer-term inhalation studies were identified.

2.3.3 Mammalian Toxicity: Dermal

Neither isomer produced ocular irritation in rabbits and both isomers were very mild skin irritants. In guinea pigs, the 2,4-DNT isomer was not a skin sensitizer while the 2,6-DNT isomer was a mild sensitizer (Lee et al. 1975, Ellis et al. 1978).

2.4 Summary of Avian Toxicology

2.4.1. Avian Toxicity Oral

2.4.1.1. Avian Toxicity Oral – Acute

Northern Bobwhite (*Colinus virginianus*) were orally dosed with 96% pure 2,4-DNT in a corn oil vehicle using the up/down procedure (Johnson et al. 2005). The oral LD₅₀ was determined to be 55 mg/kg (20-79 mg/kg 95% CI). Initial observations included diarrhea and lethargy. Most died within 72 hours post dosing.

2.4.1.2. Avian Toxicity Oral - Subacute

Northern Bobwhite were orally dosed with 2,4-DNT in a corn oil vehicle daily for 14 days (Johnson et al. 2005). Exposures were confirmed through analytical chemistry including stability and homogeneity in the vehicle. Treatments included six of seven birds of mixed sex to exposures of 0, 0.5, 5, 15, 35, or 55 mg/kg-day. Controls received an equivalent amount of vehicle as high dose and others were administered volumetrically to achieve oral dose. All birds in the 35 and 55 mg/kg-d groups died, accompanied by weight loss (emaciation) and diarrhea. Non-significant trends in plasma triglyceride and electrolyte levels were suggestive of adverse kidney effects.

2.4.1.3. Avian Toxicity Oral – Subchronic

Johnson et al. (2005) exposed a total of 12 Northern Bobwhite per sex/dose to either 0, 1, 5, 15 or 25 mg/kg-d 2,4-DNT in corn oil for 60 days in a similar manner as described previously. All females and 9/12 males died in the high dose group, most within the first week of exposure. Three males and four females died or were moribund in the 15 mg/kg-d groups. Again, lethargy, diarrhea, and weight loss were common accompanying observations. Brain, liver, and kidney/body weight ratios were affected by treatment. Brain and liver/bw ratios occurred at levels where mortality occurred. Changes in kidney/bw levels were most sensitive at 5 mg/kg-d. Statistically significant changes in triglyceride levels and non-significant trends in plasma uric acid levels also occurred at these levels. Incidences of uric acid accumulation were found in kidneys of some birds at levels that caused mortality. Changes in hematological parameters (packed cell volume, hemoglobin, and red blood cell counts) respective to treatment were significant, but were considered not to be biologically significant since they were within normal ranges for this species and predominantly for females only. Based on sensitive kidney effects (weight ratios, electrolytes, and trends in uric acid concentrations), the authors report a NOAEL of 1 mg/kg-d and a LOAEL of 5 mg/kg-d.

2.4.1.4. Avian Toxicity Oral – Chronic

No data were found for birds from chronic exposures to either DNT isomer.

2.4.1.5. Studies Relevant for Avian TRV Development for Ingestion Exposures

The data from the Johnson et al. (2005) subchronic studies are relevant for TRV derivation for oral 2,4-DNT exposures in birds. The sensitive indicators of effect are those that affected the kidney and are corroborated with plasma chemistry, histology, and indicators of dehydration (i.e. weight loss and diarrhea) at higher doses. The most sensitive indicator of adverse kidney and liver effects are organ mass changes respective to body weight.

Table 4. Summary of Relevant Avian Data from Oral Exposures to 2,4-DNT

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Johnson et al. 2005	Northern Bobwhite (quail)	LD50		55 mg/kg	Emaciation, diarrhea
		14-d	15	35	Mortality; changes in kidney/bw, diarrhea
		60-d	1	5	Increases in relative kidney weight, plasma uric acid trends. Mortality, diarrhea, weight loss ≥ 15 mg/kg-d

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of DNT isomers in amphibian species were not found. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of DNT isomers in reptilian species were not found. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

2,4-Dinitrotoluene

Data from at least 2 Orders and 3 species have been reported for each isomer of DNT. As described in Section 2.4, the dog appears to be the most sensitive to oral exposures of 2,4-DNT. Adverse neurological (loss of hindquarter control, convulsion) was the primary endpoint used to determine the TRV as this effect is likely to be relevant to the health of mammalian wildlife species. The data from the Ellis et al. 1979 dog study was used to derive a TRV using the benchmark dose approach (Appendix B). A dichotomous, multistage model was used to calculate the TRV. This model was chosen based on several goodness-of-fit tests. This TRV is given a high confidence level because the study was of suitable duration, study quality was high and results were consistent with those of other studies.

Table 3. Selected Ingestion TRVs for the Class Mammalia for 2,4-DNT

TRV	Dose	Confidence
LED ₁₀	0.67 mg/kg/d	High
ED ₁₀	1.4 mg/kg/d	High

2,6-Dinitrotoluene

Data on the chronic toxicity of 2,6-DNT was limited; only one study, conducted on rats, was located (Leonard et al. 1987). Rats exposed to 2,6-DNT orally for one year showed significantly smaller body size compared to control rats. This effect of 2,6-DNT was used to derive the TRV since smaller body size indicates a detrimental effect on the energy budgets of the organism and can lead to reductions in fitness as a result of altered reproductive schedules, increased risk of predation and overall poor condition. Derivation of the TRV using the Benchmark Dose Approach was unacceptable for this data set since it did not meet the necessary criteria. In the Leonard et al. (1987) study on which the TRV is based, all doses of 2,4-DNT showed a significant difference from control, hence a no-observed-adverse-effect-level (NOAEL) was not available while the lowest-observed-adverse-effect-level (LOAEL) was 7

mg/kg/day. The approximation approach was used to obtain the NOAEL-based TRV from the LOAEL using an uncertainty factor of 10 (based on TG254). The LOAEL-based TRV is the LOAEL reported from the Leonard et al. (1987) study. TRVs are presented below in Table 4. These TRVs are given a medium confidence rating since only one chronic study was located.

Table 4. Selected Ingestion TRVs for the Class Mammalia for 2,6-DNT

TRV	Dose	Confidence
NOAEL-based	0.7 mg/kg/d	Moderate
LOAEL-based	7.0 mg/kg/d	Moderate

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time, however, given the vapor pressure of this compound and the heterogeneous nature of this compound likely to be experienced by wildlife, inhalation exposures to these compound is unlikely.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time

3.2 Toxicity Reference Values for Birds

Mortality occurred in Northern Bobwhite orally dosed at levels ≥ 15 mg/kg-d (Johnson et al. 2005). Other effects observed histologically (e.g. gout topi of the kidney, hemosiderosis of the spleen), egg production, triglyceride levels occurred at levels where mortality occurred. The most sensitive indicator of adverse effects is changes in kidney/bw ratio, which is consistent with early onset of disease (uric acid trends, urate accumulation in the kidney, and diarrhea). The NOAEL for this study is 1 mg/kg-d. The study was conducted consistent with Good Laboratory Practices and quality was determined to be sufficient to superior. Therefore, the Approximate Method was used to derive the TRV (USACHPPM 2000). Uncertainty factors of 10 to extrapolate from a subchronic NOAEL and an additional UF of 10 was used to account for species differences to arrive at the NOAEL-based TRV. The LOAEL-based TRV

used a UF of 4 to extrapolate from a subchronic LOAEL. Since this study was conducted with a single species, and represents the only data available in birds, the TRV was given a low degree of confidence.

Table 5. Selected Ingestion TRVs for the Class Aves for 2,4-DNT

TRV	Dose	Confidence
NOAEL-based	0.01 mg/kg/d	Low
LOAEL-based	1.3 mg/kg/d	Low

3.3 Toxicity Reference Values for Amphibians

Not available at this time.

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

The limited availability of data on the toxicity of 2,6-DNT to wildlife species prevents the development of a high-confidence TRV for mammals. Hence, more research, particularly chronic studies, on the toxicity of 2,6-DNT to mammalian species, particularly mice are needed. At present, there are few data concerning the toxicity of both 2,4- and 2,6-DNT on non-mammalian wildlife such as birds, amphibian and reptiles. The avian TRV would benefit from data for additional species, particularly for 2,6-DNT. Data on the toxicity of these compounds are needed for other classes of wildlife such as amphibians and reptiles.

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APPENDIX A

LITERATURE REVIEW

A complete search for 2,4 and 2,6-dinitrotoluene was performed on Dialogue. Thirty-one references were considered appropriate for inclusion.

The following databases were searched using the following keywords:

TOXLINE & MEDLINE

Conditions: Two-word search; 1965 to present.

2,4-Dinitrotoluene and mammals - 2,4-Dinitrotoluene = 565
Mammals = 29565
Combination = 108

Of these, 4 were appropriate for inclusion.

2,6-Dinitrotoluene and mammals - 2,6-Dinitrotoluene = 340
Mammals = 29565
Combination = 99

Of these, 2 were appropriate for inclusion.

Dinitrotoluene and mammals - Dinitrotoluene = 951
Mammals = 29565
Combination = 203

Of these, 16 were appropriate for inclusion.

2,4-Dinitrotoluene and birds - 2,4-Dinitrotoluene = 565
Birds = 12168
Combination = 2

After review of the title, neither result was considered appropriate for inclusion.

2,4-Dinitrotoluene and birds - 2,6-Dinitrotoluene = 340
Birds = 12168
Combination = 0

Dinitrotoluene and birds - Dinitrotoluene = 951
Birds = 12168
Combination = 0

2,4-Dinitrotoluene and wildlife - 2,4-Dinitrotoluene = 565
Wildlife = 12095
Combination = 4

Of these, none were deemed appropriate for inclusion.

2,6-Dinitrotoluene and wildlife - 2,6-Dinitrotoluene = 340
Wildlife = 12095
Combination = 3

Of these, none were deemed appropriate for this document.

Dinitrotoluene and wildlife - Dinitrotoluene = 951
Wildlife = 12095
Combination = 6

Of these, none were deemed appropriate for this document.

Word search of Toxline & Medline for 2,4-Dinitrotoluene, 2,6-Dinitrotoluene and Dinitrotoluene in combination with

Salamanders = 425

Toads = 151

Reptiles = 4980

Snakes = 4403

Amphibians = 5687

yielded no hits.

* All but 2-3 Toxline/Medline hits were duplicates of the Dialogue searches.

BIOSIS

Conditions: One-word searches; 1984-1999.

DNTs -

Dinitrotoluene = 2

2,4-Dinitrotoluene = 0

2,6-Dinitrotoluene = 0

Of these, none were applicable for inclusion.

WORLD WILDLIFE

Conditions: One-word search

Dinitrotoluene = 0

STINET – DTIC

Conditions: One-word search

Dinitrotoluene = 25

Of these, two were relevant but were duplicates of the Dialogue search.

HSDB, RTEC and IRIS DATA BASES

Conditions: One-word search

A total of 26 articles were relevant and non-duplicate articles were evaluated.

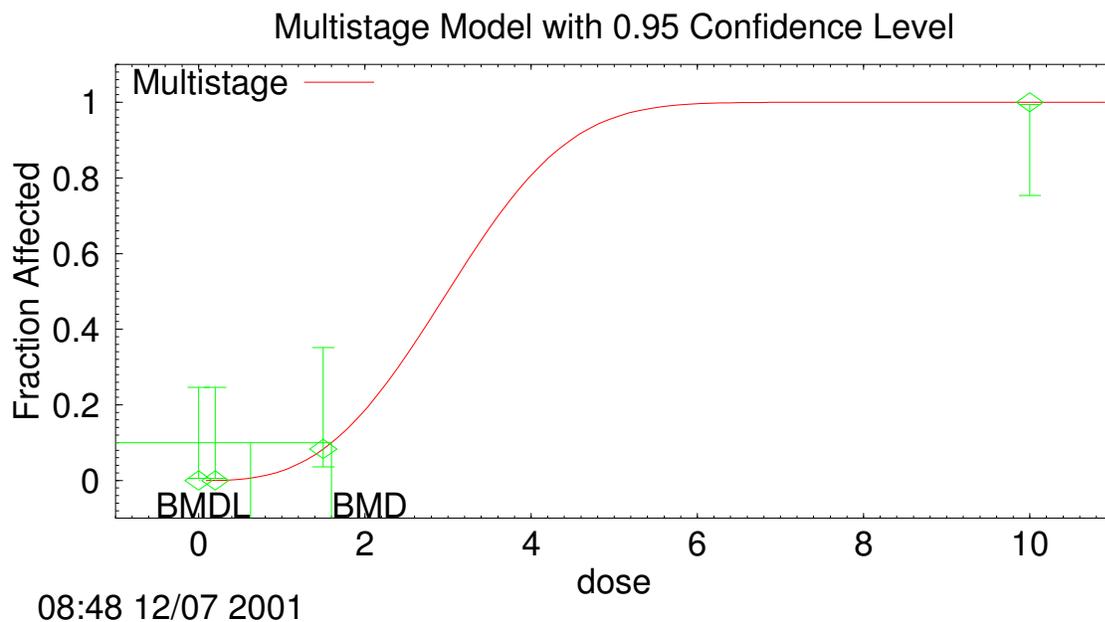
References from the USEPA (1994) Drinking Water Health Advisory for 2, 4- and 2,6-Dinitrotoluene, the ATSDR (1998) Toxicological Profile for 2,4- and 2,6-Dinitrotoluene and the Toxicity Summary for 2,4- and 2,6-dinitrotoluene (ORNL, 1995) were also reviewed and all relevant non-duplicate sources evaluated.

APPENDIX B

Benchmark Dose Calculation for Mammals

Benchmark dose for 2,4-DNT

The data presented in the graph below are from Ellis et al. 1979 and 1985, where he reported adverse neurological (loss of hindquarter control, convulsions) and pathologic (biliary hyperplasia) effects of 2,4-DNT in dogs. The model fit was sufficient, and a benchmark dose (BMD) and benchmark dose confidence limit (BMDL) were derived from this analysis.



The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2 - \beta_3 * \text{dose}^3)]$$

The parameter betas are restricted to be positive

Dependent variable = COLUMN3

Independent variable = COLUMN1

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

Beta(1) = 0

Beta(2) = 0

Beta(3) = 1.00112e+017

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) -Beta(2)

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

Beta(3)

Beta(3) 1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA

Beta(1)	0	NA
Beta(2)	0	NA
Beta(3)	0.0257177	0.0892218

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	-3.44203			
Fitted model	-3.4445	0.00494386	3	0.9999
Reduced model	-28.0361	49.1882	3	<.0001

AIC: 8.88901

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
0.0000	0.0000	0.000	0	12	0.000
0.2000	0.0002	0.002	0	12	-1.000
1.5000	0.0831	0.998	1	12	0.003
10.0000	1.0000	12.000	12	12	1.000
Chi-square =	0.00	DF = 3	P-value = 1.0000		

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.60011

BMDL = 0.626078