U.S. Army Public Health Command

Public Health Notice

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

Approved for public release; distribution unlimited

PHN No. 0512-1, May 2012

USAPHC

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

---

Acknowledgements

Key Technical Authors:	Christopher J. Salice, Ph.D.	USAPHC; Portfolio of Toxicology, Health Effects Research Program
	Mark S. Johnson, Ph.D.	USAPHC; Portfolio of Toxicology, Health Effects Research Program
	Heidi L. Ilg, B.S.	Oak Ridge Institute of Science and Education, Oak Ridge, TN
	Leonora P. Midgeley, Ph.D., M.P.H.	USAPHC; Portfolio of Toxicology, Health Effects Research Program
	Craig A. McFarland, DVM, Ph.D.	USAPHC; Portfolio of Toxicology, Health Effects Research Program
Contributors:	Matthew J. McAtee	USACHPPM; Directorate of Environmental Health Engineering, Environmental Health Risk Assessment Program
	Michael J. Quinn, Jr., Ph.D.	USAPHC; Portfolio of Toxicology, Health Effects Research Program
Reviewers:	Greg Linder, Ph.D. John Newsted, Ph.D. Steven Sheffield, Ph.D.	USGS/ BRD /CERC Entrix, Inc. Self

Point of Contact

For further information or assistance contact the following:

Michael J. Quinn, Jr., Ph.D.
U.S. Army Center for Health Promotion and Preventive Medicine
Toxicology Portfolio: Health Effects Research Program
ATTN: MCHB-IP-THE, Bldg, E2100
Aberdeen Proving Ground, MD 21010-5403
(410) 436-1064
michael.james.quinn@us.army.mil

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

---

When referencing this document use the following citation:

USAPHC. 2011. Wildlife Toxicity Assessment for 2,4 & 2,6 Dinitrotoluenes.

Table of Contents

List of Tables

1 Introduction

The DNTs, 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 a primary product in propellants and are also produced as 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 their distribution at and around military sites and their 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 military-sponsored and found using 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 U.S. Army. Several databases were searched and the details of these searches are provided in Appendix A.

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 descending order of contribution, 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 (2,4- and 2,6-DNT) 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, Walker & 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 Koc = 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). 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, 2,6-DNT should be fairly mobile despite its estimated log Koc of 1.89 and Kow of 1.72. Volatilization from soil and photo-oxidation of 2,6-DNT in soil should not be significant. The log Kow indicates that significant bioaccumulation is unlikely (HSDB 2000, ATSDR 2000).

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. 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. 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 Kow = 1.98). Adsorption of 2,6-DNT to sediments or suspended solids should not be appreciable (HSDB 2000, ATSDR 1998). Volatilization of 2,4- and 2,6-DNT from water should be minimal due to their low vapor pressure and Henry's Law constant . 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). Significant bioaccumulation is not expected to occur (HSDB 2000, ATSDR 1998).

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.

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
Physical state	Needles or monoclinic prisms	Solid or rhombic needles from alcohol
Melting point	71^{o}C	66^{o}C
Boiling point	300^{o}C with slight decomposition	285^{o}C

Density		
Odor	Slight	Slight
Solubility in water	270 mg/L @ 22^{o}C	180mk/L @ 22^{o}C
Partition coefficients:		
Log K_{ow}	1.98	1.72 (estimated)[2]
Log K_{oc}	2.40 (estimated)[2]	1.89 (estimated)[2]
Vapor pressure at 25 ^{o}C	1.4 X 10^{-4}	5.67 X 10^{-4}
Henry's Law constant at 25 ^{o}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	1ppm = 7.4 mg/m^3
	1 mg/m^3 = 0.13 ppm	1 mg/m^3 = 0.13 ppm

Notes:
[1] Sources: All values from HSDB 2000 and ATSDR 1998 unless otherwise noted
[2] Burrows et al., 1989

2.3 Mammalian Toxicology

2.3.1 Mammalian Oral Toxicity

Following oral administration, DNT isomers are rapidly absorbed, distributed and eliminated (Rickert and Long 1980, Rickert et al. 1983, 1984; 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 LD_{50} 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 oligospermatism 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 group lost up to 13% of their body weight by the eighth day of dosing. By the eighth day, all mice in treatment groups receiving ≥1250 mg/kg-d died. Adverse observations recorded for the 310 mg/kg-d group included lethargy (10) and hunched posture (1).

Lent et al. (In press) exposed Sprague Dawley rats to either 2,4-DNT (9, 18, 36, 71, 142, and 284 mg/kg) or 2,6-DNT (4, 7, 14, 35, 68, and 134 mg/kg) daily for 14 days. A LOAEL of 284 mg/kg bw-d was observed for rats exposed to 2,4-DNT due to mortality, body weight loss, and neurological effects (front limb paralysis); the NOAEL for each above described effect was 142 mg/kg bw-d. Hepatotoxicity was observed in rats that were dosed with 2,6-DNT: hepatocellular hyperplasia, oval cell hyperplasia, and hepatocellular hypertrophy were observed in rats belonging to the 35 mg/kg bw-d and higher dose groups. Testicular atrophy was observed at doses as low as 68 mg 2,6-DNT/kg bw-d.

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 for 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, hepato-cellular changes and altered liver weights/function, severe 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 at 8 and 17 weeks to study reversibility. Several dogs in the high-dose group were moribund and euthanized prior to 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 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 immunoglobin (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 male and female rats, respectively, was 34.4 and 38.3 mg/kg/day for the low-dose, 92.8 and 108.3 mg/kg/day for mid-dose, and 265.6 and 145.2 mg/kg/day for the high-dose.. 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 dog study (Lee et al. 1978, Hong et al. 1985). The average intake mice, respectively, was 47 and 52 mg/kg/day for the low-dose mice, 137 and 147 mg/kg/day for the mid-dose mice, and 413 and 468 mg/kg/day for the high-dose mice.

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 give 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 sensitive 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 per group at the time of dosing cessation, while the other pair was allowed to recover for 4 weeks prior to being 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 animals 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 remaining animals 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 more than half of 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 that of other dose groups, anemia with many Heinz bodies, and non-functioning gonads in both sexes. Generalized pigmentation was observed in many tissues of the high-dose animals. Effects were more severe in males than females (Ellis et al. 1979, 1980; 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 hepatocarcinogenicity, other parameters were measured. Twenty-eight rats 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, while the remaining rats (20) were 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 chronically exposed to 2,6-DNT were consistent with those observed for subchronic 2,6-DNT administration. These effects included decreased body weights, hepatocellular degeneration and vacuolation, acidophilic and basophilic foci of hepatocellular 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 to evaluate developmental effects were conducted by Smith (1983) and Hardin et al. (1987). In both studies female CD-1 mice were exposed to 2,4-DNT at 0 and 390 mg/kg-d on GD-6 or 7 through 13. Smith (1987) reported that 10 of 50 mice exposed to 390 mg/kg-d 2,4-DNT died, in which 4/10 were pregnant. An additional 5 died in the post-dosing observation period, two of which were pregnant. A total of sixteen of the 50 mated female mice were pregnant. Five additional litters were found to be reabsorbed. The reproductive index in the test group (0.68) was significantly lower than that of the control group (0.91). No other measures of reproductive toxicity were statistically significant.

Only one of 50 mice 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 of 14 to 150 mg/kg-d on gestational days 7-20. Overt toxicity included rough coat, lethargy, and hind limb weakness. Blood effects (decreases in red blood cell 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 concluded that DNT was not teratogenic but may cause embryo/fetal toxicity at levels where maternal mortality occurs.

Lent et al. (In press) administered 2,3-, 2,5-, 3,4-, and 3,5-DNT to male Sprague-Dawley rats in addition to the above mentioned 2,4-, and 2,6-DNT for 14 days. The 3,5-DNT isomer (LD-50 = 310 mg/kg) was found to be the most toxic with death, body weight loss, and neurological effects being observed at 39 mg/kg bw-d (LOAEL); the NOAEL was determined to be 19 mg/kg bw-d. Neurological effects included facial twitching, altered head carriage, and paralysis that was restricted to the front limbs. A LOAEL of 550 mg/kg bw-d was determined for 2,3-DNT (LD-50 = 1100 mg/kg) and was based on mortality, body weight loss, and testicular atrophy. Single exposures to 3,4-DNT (LD-50 = 454 mg/kg) caused convulsions at 1000 mg/kg-d, however neurological effects at 227 mg/kg bw-d for the 14d exposure were limited to hypoactivity/staring, and facial twitches (NOAEL); the LOAEL for these responses was determined to be 113 mg/kg-d. The mildest effects were observed in rats exposed to 2,5-DNT (LD-50 = 616 mg-kg) with the most sensitive response being trace to mild extramedullary hematopoiesis of the spleen observed in the 39 mg/kg bw-d and higher dose groups.

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 reports.

The USACHPPM 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 7 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 for 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	Test Results			
			LD50 (mg/kg)	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at LOAEL
Lee et al. 1975 Ellis et al. 1978	Rat/CD/gavage	Single, acute	568 (M) 650 (F)			
Vernot et al. 1977	Rat/Sprague Dawley/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 1996	White-footed mouse (Peromyscus leucopus)/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)	45 (M)	Severe atrophy/degeneration of seminiferous tubules
				5(F)	45 (F)	Difficult parturition; decreased pup viability.
Lee et al. 1978, 1985	Rats/CD/feed/ad lib	4 or 13 weeks			34 (M) 38 (F)	Moderate decrease body weight gain

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

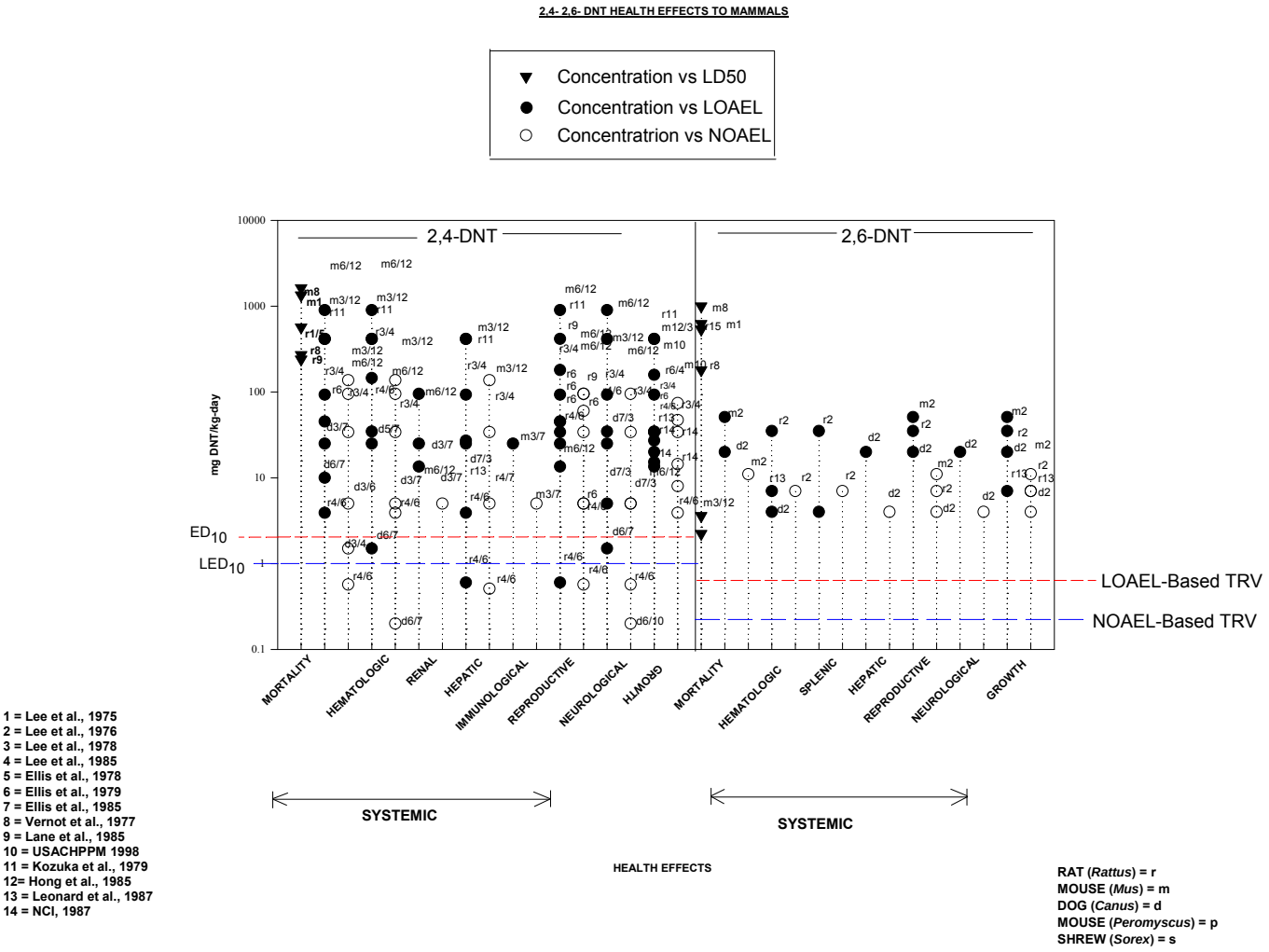
Study	Test Organism/route	Test Duration	Test Results			
			LD50 (mg/kg)	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at LOAEL
				34 (M) 38 (F)	93 (M) 108 (F)	Decreased food consumption
				34 (M) 108 (F)	266 (M) 145 (F)	Reticulocytosis and hemosiderosis. (anemia at 266 (M) and 145 (F))
				34 (M)	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 Lee et al. 1978	Mice/CD-1/feed/ad lib	4 or 13 weeks		137 (M) 147 (F)	413 (M) 468 (F)	Mild anemia and reticulocytosis
				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) 95 (F)	Atrophy of seminiferous tubules, aspermatogenesis

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

Study	Test Organism/route	Test Duration	Test Results			
			LD50 (mg/kg)	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed 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
				95 (F)	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; 25% body weight decrease
NCI 1978	Rat/Fischer-344/feed/ad lib	78 weeks		8 (M) 8.8 (F)	20 (M) 22 (F)	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 LOAEL
Lee et al. 1975 Ellis et al. 1978	Rat/CD/gavage	Single dose	535 (M) 795 (F)			N/A
Vernot et . 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 (Peromyscus leucopus)/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
Lee et al. 1976	Dog/oral/feed	4 or 13 weeks			20 (F)	Death (2/8)
					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)	20% decrease in body weight



2.3.2 Mammalian Toxicity: Inhalation

Five rats/sex/group were exposed via the nasal passageways 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) LC_{50} 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

2,4-Dinitrotoluene

Northern Bobwhite 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.

2,6-Dinitrotoluene

The oral LD_{50} was estimated for Northern Bobwhite using the stagewise probit procedure. Males were orally dosed with 99% pure 2,6-DNT in a corn oil vehicle (Johnson et al. 2007). The oral LD_{50} was estimated to be 320 mg/kg (195-470 mg/kg 95% CI). Initial observations included loss of body mass, diarrhea, scant white feces, lethargy, and ataxia.

2.4.1.2 Avian Toxicity Oral – Subacute

2,4-Dinitrotoluene

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. Six treatment groups comprising seven birds of mixed sex were exposed to 0, 0.5, 5, 15, 35, or 55 mg/kg-day. Controls received doses of vehicle equivalent to the amount given to the high dose group, while doses for other groups were adjusted volumetrically to achieve the desired oral dose of 2,4-DNT. All birds in the 35 and 55 mg/kg-d groups died within 72 hours. Mortality was accompanied by weight loss (emaciation) and diarrhea. Non-significant trends in plasma triglyceride and electrolyte levels were suggestive of adverse kidney effects.

2,6-Dinitrotoluene

Northern Bobwhite were orally dosed with 2,6-DNT in a corn oil vehicle daily for 14 days (Johnson et al. 2007). Nine treatment groups comprising at least seven birds of mixed sex were exposed to 0, 50, 100, 190, or 350 mg/kg-day. Oral doses were determined volumetrically. Controls received doses of vehicle equivalent to the amount given to the high dose group. All birds in the 190 and 350 mg/kg-d groups died or were moribund within 14 days of exposure. Birds in the 350 mg 2,6-DNT/kg body mass-day treatment were lethargic, and had diarrhea, scant white feces and emaciation of the pectoralis muscle. Mean liver/body mass and kidney/body mass ratios were increased, relative to the control, in some treatment groups. Spleen/body mass ratio was decreased in the 100, 190, and 350 mg/kg-day

groups relative to both the control and low-dose treatment groups. Red blood cell counts, hemoglobin content, and packed cell volume (PCV) decreased with increasing dose, although PCV was not significant and hemoglobin did not differ among 2,6-DNT doses. Non-significant trends in reduction of serum glucose, globulin, and total protein were observed. Plasma uric acid levels increased with dose, suggesting kidney-related effects. Six of ten birds from the high-dose group (350 mg/kg-day) exhibited hepatocellular vacuolation consistent with glycogen accumulation which corresponded to higher liver weights. A LOAEL of 50 mg/kg-day was established based on anemia in exposed quail. No NOAEL could be established in this study.

2.4.1.3 Avian Toxicity Oral—Subchronic

2,4-Dinitrotoluene

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 (Section 2.4.1.2). 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. Increases in brain and liver/bw ratios occurred at levels where mortality occurred. Changes in kidney/bw levels were most sensitive, occurring at 5 mg/kg-d in females. Statistically significant increases in triglyceride levels and non-significant trends in plasma uric acid levels also occurred at 5mg/kg-day. 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 because they were within normal ranges for this species and occurred predominantly in females. Based on sensitive kidney effects (weight ratios, electrolytes, and trends in plasma uric acid concentrations), the authors report a NOAEL of 1 mg/kg-d and a LOAEL of 5 mg/kg-d.

2,6-Dinitrotoluene

Quinn et al. (2007) exposed 12 Northern Bobwhite per sex/dose to either 0, 5, 10, 40 or 60 mg/kg-d 2,6-DNT in corn oil for 60 days via oral gavage. One female and two males in the 40 mg/kg-day group and one female and three males from the 60 mg/kg-day group died or were euthanized due to excessive loss of body weight. Gross abnormalities of the intestinal tract, including enlarged gall bladder, green gizzard contents, and dark, shriveled descending colon and cecae were observed in many of the animals in the 40 and 60 mg/kg-day treatments. There was a non-significant decrease in body weight with increasing exposure level, whereas there were no treatment related differences in organ weights. Red blood cell count and hemoglobin content were significantly decreased in a dose-dependent manner; however, these values were within normal ranges for this species. Total protein, albumin, globulin, aspartate aminotransferase, and potassium, sodium, and chloride ions were significantly decreased in exposed animals. Levels of circulating uric acid were significantly increased in the high-dose groups. Histopathological abnormalities were observed in the liver (lesions) and kidneys (degeneration and multifocal tubulointerstitial nephritis) of birds exposed to 60 mg/kg-d. Although the ability to lay eggs, date of first egg laying, average number of eggs, and percentage of abnormal eggs were not significantly affected by treatment, the fewest eggs and the highest percentage of abnormal eggs (42%) were produced by birds in the 60 mg/kg-d group. Based on hematological parameters, the authors report a NOAEL of 10 mg/kg-d and a LOAEL of 40 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 pertaining to the kidney and are corroborated by 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 changes in organ mass respective to body weight.

The data from the Johnson et al. (2007) and Quinn et al. (2007) subchronic studies are relevant for TRV derivation for oral 2,6-DNT exposure in birds. Both studies indicate gastrointestinal disturbances such as diarrhea as well as adverse kidney and/or liver effects. Hematology and plasma chemistry data were the most sensitive endpoints. Plasma uric acid levels were increased in higher-dose groups in both studies which was further suggestive of kidney-related effects. The studies for both 2,4- and 2,6-DNT were well-conducted, high quality studies that assessed a variety of endpoints and thus are appropriate for use in TRV derivation.

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

Table 5. Summary of Relevant Avian Data from Oral Exposures to 2,6-DNT

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Johnson et al. 2007	Northern Bobwhite (quail)	LD50	320 mg/kg		Weight loss, diarrhea, scant white feces, lethargy, ataxia
		14-d		50	Increased uric acid, trends in reduction of serum glucose, globulin, and protein, decreased RBC and hemoglobin
Quinn et al. 2007	Northern Bobwhite (quail)	60-d	10	40	Mortality, enlarged gall bladder, edematous gastrointestinal tract, diarrhea, increased plasma protein, albumin, globulin, aspartate aminotransferase, sodium, potassium, chloride, and uric acid

2.4.2 Avian Toxicity: Inhalation

No data available.

2.4.3 Avian Toxicity: Dermal

No data available.

2.5 Summary of Amphibian Toxicology

2.5.1 Amphibian Toxicology: Oral

2.5.1.1 Amphibian Toxicity Oral—Acute

2,4-Dinitrotoluene / 2,6-Dinitrotoluene

In an acute experiment examining frogs, Paden et al. (2008) exposed male adult bullfrogs (Lithobates catesbeiana) using the Up-and-Down (UPD) method with 2,4-dinitrotoluene (2,4-DNT) and 2.6-dinitrotoluene (2,6-DNT) in a polyethylene glycol (PEG) vehicle administered with oral stainless steel gavage needles. Eight adult male bullfrogs for each compound were dosed with 175 to 2000 mg/kg body weight (BW). Three of three animals died in the highest dose group. The LD50 value for 2,4-DNT and 2,6-DNT was determined at 1,098 mg/kg with an approximate 95% confidence interval of 550 to 2000 mg/kg BW. Clinical signs observed in the high dose group included changes in respiratory rhythm (dyspnea, cyanosis, and tachypnea), decrease in spontaneous motor activities (somnolence, loss of righting reflex, prostration, tremors, tonic and clonic convulsion), salivation, muscle tone changes (hypertonia, hypotonia), gastrointestinal changes (vomiting), yellow color changes in the skin and urine, skin pigmentation, and ocular signs (relaxation of the nictitating membrane). Toxic signs for animals exposed to 2000 mg/kg BW started within the first 2 h after dosing with death at 80 h for 2,4-DNT and 72 h for 2,6-DNT. Tremors and convulsions appeared after 30 h and 28 h for 2,4-DNT and 2,6-DNT, respectively. A loss of appetite was observed in animals dosed at the highest level, however, a moderate increase in body weight, especially in 2,6-DNT exposed animals, was due to fluid retention giving frogs a bloated appearance. A strong yellow urine color was seen in frogs exposed to both isomers. Liver and kidney enlargement and necrosis (determined using gross morphological changes), particularly in 2000 mg/kg dosed animals, were seen at necropsy. An increase in spleen weight of 1.7- and 5.4-fold was seen in 2,4-DNT and 2,6-DNT animals, respectively. The authors state that the LD50 results, relative low concentrations typically observed in aquatic environments, and the short half-life of the compounds indicate that adult bullfrogs in the wild are at low risk for either exposure or toxicity.

2.5.1.2 Amphibian Toxicity Oral—Subchronic

No data were found for amphibians from subchronic exposures to either DNT isomer.

2.5.1.3 Amphibian Toxicity Oral—Chronic

No data were found for amphibians from subchronic exposures to either DNT isomer.

2.5.1.4 Studies Relevant for Amphibian TRV Development for Ingestion Exposures

Few data are available for DNT isomers. The acute LD50 study in bullfrogs by Paden et al. (2008) represents the only available study. Because the data are relatively similar for both isomers, 2,4-DNT and 2,6-DNT were treated together and used to develop values.

2.6 Summary of Reptilian Toxicology

2.6.1 Reptilian Toxicity: Oral

2.6.1.1 Reptilian Oral Toxicity – Acute

Western fence lizards (Sceloporus occidentalis) were orally dosed with 98% pure 2,4-DNT in a 1% methyl cellulose/0.02%Tween 80/deionized water vehicle (Suski et al. 2008). The oral LD50 was determined, using a stage-wise probit design, to be 380 mg/kg (149-515 mg/kg 95% CI) and 577 mg/kg (406-785 mg/kg 95% CI) and for male and female lizards, respectively. Initial signs associated with mortality included lethargy and ataxia.

2.6.1.2 Reptilian Oral Toxicity – Subacute

Suski et al. (2008) assessed the toxicity of 2,4-DNT to western fence lizards (Sceloporus occidentalis) in a 14 day oral gavage experiment. Forty-two male lizards were dosed daily with 0, 6.25, 12.5, 25, 50, 100, or 200 mg/kg-day 2,4-DNT in a 1% methyl cellulose/0.02%Tween 80/deionized water vehicle. Doses were adjusted volumetrically to achieve the appropriate oral dose based on individual mass. All lizards in the 200mg/kg-d and 4 of 6 lizards in the 100 mg/kg-day groups were found dead or were moribund and euthanized. No differences in sub-lethal endpoints (body weight, organ weight, food consumption, hematology, or clinical chemistries) were observed in groups dosed with less than 50 mg/kg-day.

2.6.1.3 Reptilian Oral Toxicity – Subchronic

Suski et al. (2008) assessed the toxicity of 2,4-DNT to western fence lizards (Sceloporus occidentalis) in a 60 day oral gavage experiment. Sixty male lizards were dosed daily with 0, 9, 15, 25, 42, or 70 mg/kg-day 2,4-DNT in a 1% methyl cellulose/0.02%Tween 80/deionized water vehicle. Doses were adjusted volumetrically to achieve the appropriate oral dose based on individual mass. Survival was significantly reduced in the three highest dose groups (25, 42, and 70 mg/kg-day), whereas survival in the low-dose groups and control was 100%. Lizards from the 25, 42, and 70 mg/kg-day dose groups survived an average of 55, 51, and 40 days, respectively.

Body weight loss occurred early in the study in lizards dosed with 70 and 42 mg/kg-day (day 0-7 and 7-14, respectively), and occurred late in the study in the 25 and 15 mg/kg-day groups (day 14-21 and 56-60, respectively). The 15 mg/kg-day group also exhibited reduced feeding relative to the controls. The only significant effect on organ weight was an increase in kidney/body weight in the 15 mg/kg-day group relative to the control. Relative to the controls, this dose group also exhibited increased plasma uric acid and phosphorus levels. No other clinical chemistries or hematology measures were significantly affected by 2,4-DNT treatment. A trend suggesting a dose-related decrease in testosterone was observed; however, low sample sizes resulted in low statistical power.

Kidney (tubular degeneration, necrosis, and gout) and liver (cellular necrosis and lipogranulomas) effects were observed in the 9 mg/kg-day group. Mild tubular degeneration was observed in the testes in males exposed to 15 mg/kg-day, and this effect increased in severity in the higher dose groups. Lizards in the three highest dose groups also exhibited altered behavior, including arched posture, hanging, and increased skin pigmentation following dosing. Using the U.S. EPA's Benchmark Dose software, the authors derived a BMD of 15.8 mg/kg-day and a BMDL of 12.09 mg/kg-day based on survivability. A LOAEL of 15 mg/kg-day and a NOAEL of 9 mg/kg-day were indicated based on kidney effects (increased plasma uric acid and phosphorus levels and kidney/bw), weight loss, and food consumption.

2.6.1.4 Reptilian Toxicity Oral – Chronic

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

2.6.1.5 Studies Relevant for Reptilian TRV Development for Ingestion Exposures

The data from the Suski et al. (2008) subacute and subchronic studies are relevant for TRV derivation for oral 2,4-DNT exposures in reptiles. The most sensitive indicators of sublethal effect include weight loss, decreased feed consumption, and effects on the kidney, including increased relative kidney weight, plasma uric acid and phosphorus levels, and abnormal kidney histopathology.

Table 6. Summary of Relevant Reptilian 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
Suski et al. 2008	Western fence lizard	LD50	380 mg/kg (M) 577 mg/kg (F)		Lethargy, ataxia
		14-d	50 (M)	100 (M)	Mortality, lethargy, ataxia
		60-d	9 (M)	15 (M)	Increases in relative kidney weight, plasma uric acid, and phosphorus. Renal lesions, degeneration, necrosis and gout. Weight loss and decreased feed consumption. Mortality ≥ 25 mg/kg-d

2.6.2 Reptilian Toxicity: Inhalation

No data available.

2.6.3 Reptilian Toxicity: Dermal

No data available.

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 effects (loss of hindquarter control, convulsion) was the primary endpoint used to determine the TRV as this effect is likely to be most 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 (BMD) 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 7. 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 exhibited significantly smaller body size compared to control rats. This effect of 2,6-DNT was used to derive the TRV because 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 determined at 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 8. These TRVs are given a medium confidence rating since only one chronic study was located.

Table 8. 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 and the heterogeneous nature of these isomers likely to be experienced by wildlife, inhalation exposures are unlikely.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

2,4-Dinitrotoluene

Mortality occurred in Northern Bobwhite orally dosed at levels ≥ 15 mg/kg-d (Johnson et al. 2005). Effects on other endpoints, including egg production, triglyceride levels, and effects observed histologically (e.g. gout tophi of the kidney, hemosiderosis of the spleen), occurred at levels where mortality occurred. The most sensitive indicator of adverse effects was changes in kidney/bw ratio, which was 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 Approximation Approach was used to derive the TRV (USACHPPM 2000). An uncertainty factor of 10 to extrapolate from a subchronic NOAEL and an additional UF of 10 to account for species differences were used to arrive at the NOAEL-based TRV. The LOAEL-based TRV used a UF of 4 to extrapolate from a subchronic LOAEL and a UF of 10 was used to account for species differences. 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 9. Selected Ingestion TRVs for the Class Aves for 2,4-DNT

TRV	Dose	Confidence
NOAEL-based	0.01 mg/kg/d	Low
LOAEL-based	0.13 mg/kg/d	Low

2,6-Dinitrotoluene

Data on the toxicity of 2,6-DNT in birds was limited, as only two studies, conducted on Northern Bobwhite, were located (Johnson et al. 2007 and Quinn et al. 2007). Both studies indicate gastrointestinal disturbances such as diarrhea as well as adverse kidney and/or liver effects. Hematology and plasma chemistry data were the most sensitive endpoints. Uric acid levels were increased in higher-dose groups in both studies which was further suggestive of kidney-related effects. The LOAEL based on clinical chemistries was 40 mg/kg-day and the NOAEL was 10 mg/kg-day. These endpoints were used to derive the TRV as they are considered to be early indicators of adverse hepatic and renal effects which contributed to the observed gastrointestinal distress and ultimately to mortality. Because the two studies identified as relevant to TRV derivation were of subchronic duration and used only a single species, the data fail to meet the minimum requirements. As such, the Approximation Approach was used to obtain the NOAEL- and LOAEL-based TRV (USACHPPM 2000). An uncertainty factor of 10 to extrapolate from a subchronic NOAEL and an additional UF of 10 to account for species differences were used to arrive at the NOAEL-based TRV. The LOAEL-based TRV used a UF of 4 to extrapolate from a subchronic LOAEL and a UF of 10 was used to account for species differences. Because these subchronic studies represent the only data available in birds, and were conducted with a single species, the TRV was given a low degree of confidence. TRVs are presented below in Table 10.

Table 10. Selected Ingestion TRVs for the Class Aves for 2,6-DNT

TRV	Dose	Confidence
NOAEL-based	0.1 mg/kg/d	Low
LOAEL-based	1.0 mg/kg/d	Low

3.3 Toxicity Reference Values for Amphibians

3.3.1 TRVs for Ingestion Exposures for the Class Amphibia

The acute gavage study with bullfrogs (Paden et al. 2008) was considered sufficiently unique and able to provide relevant information on the toxicity of 2,4-DNT and 2,6-DNT in amphibians to warrant inclusion and TRV derivation. The reported LD50 value of 1,098 mg/kg BW for both isomers was divided by a UF of 1000 to estimate a chronic NOAEL (a UF of 100 for an LD50 value to a chronic NOEAL and a UF of 10 for interspecies). This resulted in an approximate NOAEL-based TRV of 1.10 mg/kg BW. To estimated a chronic LOAEL the LD50 of 1,098 mg/kg was divided by 200 (a UF of 20 for an LD50 value to a chronic LOAEL and a UF of 10 for interspecies). This resulted in an approximate LOAEL-based TRV of 5.49 mg/kg BW. The TRVs determined using acute LD50 tests have the least ecological relevance due to their short term exposures, but are provided because the study included important toxicological endpoints and a variety of intraspecific responses. A low level of confidence has been given because data from a single study was utilized, only one species was evaluated, and the short duration of the tests. Table 11 presents the TRVs for 2,4-DNT and 2,6-DNT for the bullfrog L. catesbeiana.

Table 11. Selected TRVs for the Class Amphibia for 2,4-DNT / 2,6-DNT

TRV	Dose	Confidence
NOAEL-based	1.10 mg/kg/d	Low
LOAEL-based	5.49 mg/kg/d	Low

3.4 Toxicity Reference Values for Reptiles

3.4.1 TRVs for Ingestion Exposures for the Class Reptilia

2,4-Dinitrotoluene

The subacute and subchronic studies conducted by Suski et al. (2008) were the only studies available on the oral toxicity of 2,4-DNT in reptiles. The most sensitive indicators of sublethal effects in this study were weight loss, decreased feed consumption, and effects on the kidney, including increased relative kidney weight, plasma uric acid and phosphorus levels, and abnormal kidney histopathology. A LOAEL of 15 mg/kg-day and a NOAEL of 9 mg/kg-day are indicated based on these effects. Because this study does not meet the minimum requirements (i.e. single study, single species, subchronic), the Approximation Approach was used to obtain the NOAEL- and LOAEL-based TRV (USACHPPM 2000). An uncertainty factor of 10 to extrapolate from a subchronic NOAEL and an additional UF of 10 to account for species differences were used to arrive at the NOAEL-based TRV. The LOAEL-based TRV used a UF of 4 to extrapolate from a subchronic LOAEL and a UF of 10 was used to account for species differences. Because the subchronic study only had data from a single reptile species, the TRV was given a low degree of confidence. TRVs are presented below in Table 12.

Table 12. Selected Ingestion TRVs for the Class Reptilia for 2,4-DNT

TRV	Dose	Confidence
NOAEL-based	0.09 mg/kg/d	Low
LOAEL-based	0.38 mg/kg/d	Low

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 toxicity data for 2,4- and 2,6-DNT in non-mammalian wildlife, such as birds, amphibians and reptiles. The avian, amphibian, and reptilian TRVs would benefit from data in additional species. Data on the toxicity of these compounds, particularly for 2,6-DNT, are needed for other classes of wildlife such as amphibians and reptiles.

5 References

Agency of Toxic Substances and Disease Registry (ATSDR). 1998. Toxicological profile for 2,4-dinitrotoluene and 2,6-dinitrotoluene. U.S. Department of Health & Human Services, Public Health Service.

Bloch, E., B. Gondos, M. Gatz, S.K. Varma and B. Thysen. 1988. Reproductive toxicity of 2,4-dinitrotoluene in the rat. *Toxicol. Appl. Pharmacol*., 94:466-472.

Brower, M.E., W.C. Roberts, W.R. Hartley, and C. Abernathy. 1994. 2,4- and 2,6-Dinitrotoluene (DNT). Pp. 39-151 in Drinking Water Health Advisory: Munitions II, (Hartley, W.R, Roberts, W.C and Commons, B.J., Eds.). USEPA Office of Drinking Water Health Advisories. Pub. by Professional Administrative Services.

Burrows, E. P, D. H. Rosenblatt, W. R. Mitchell, and D.L. Parmer. 1989. Organic explosives and related compounds: environmental and health considerations. Technical Report No. AD –8901. U. S. Army Biomedical Research and Development Laboratory, Ft. Detrick, Frederick, MD.

Calow, P. 1991. Physiological costs of combating toxicants: ecological implications. Comp. Biochem. Physiol. 100C: 3-6.

Congdon, J.D., A.E. Dunham, W.A. Hopkins, C.L. Rowe, and T.G. Hinton. 2001. Resource allocation-based life histories: a conceptual basis for studies of ecological toxicology. Environ. Toxicol. Chem., 20: 1698-1703.

Chemical Manufacturers Association. 1991. 2,6-Dinitrotoluene acute (6-hour) inhalation toxicity study in rats. CMA, Washington, D.C. Submitted to USEPA under Toxic Substances Control Act Section 8(e).

Ellis, III, H.V., J.R. Hodgson, S.W. Hwang, L.M. Halpap, D.O. Helton, B.S. Anderson, D.L. VanGoethem and C.-C. Lee. 1978. Mammalian toxicity of munitions compounds phase I: acute oral toxicity, primary skin and eye irritation, dermal sensitization, disposition and metabolism, and Ames tests of additional compounds. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Ellis III, H.V., J.H. Hagensen, J.R. Hodgson, J.L. Minor, C.-B. Hong, E.R. Ellis, J.D. Girvin, D.O. Helton, B.L. Herndon and C.-C. Lee. 1979. Mammalian toxicity of munitions compounds phase III: effects of lifetime exposure part I: 2,4-dinitrotoluene. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Ellis, H.V., C.B. Hong, and C.C. Lee. 1980. Mammalian toxicity of munitions compounds-summary of toxicity of nitrotoluenes. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Ellis, H.V., C.B. Hong, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-Dinitrotoluene. Part I. Beagle Dogs. International Journal of Toxicology, 4: 233-242.

Hardin, B.D, R.L. Schuler, J.R. Burg, G.M. Booth, K.P. Hazelden, K.M. MacKenzie, V.J. Piccirillo, and K.N. Smith. 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratogenesis, Carcinogenesis, and Mutagenesis* 7: 29-48.

Hazardous Substance Data Base (HSDB). 2000. Online. Tomes/Micromedex Inc. National Library of Medicine, Bethesda, MD, April 1997.

Hazelton Laboratories. 1977. A thirty-day toxicology study in Fischer-344 rats given dinitrotoluene, technical grade. Performed for the Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Hong, C.B., H.V. Ellis III, C.-C. Lee, H. Sprinz, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part III. CD-1 Mice. *J. Am. Coll. Toxicol*. 4: 257-269.

Johnson, M.S., M.W. Michie, R.M. Gogal, Jr., and M.A. Bazar. 2005. Influence of oral 2,4-dinitrotoluene exposure to the northern bobwhite (Colinus virginianus). *International Journal of Toxicology*, 24: 265-274.

Johnson, M.S., M.J. Quinn, M.A. Bazar, C.A., K.A. Gust, B.L. Escalon, and E.J. Perkins. 2007. Subacute toxicity of oral 2,6-dinitrotoluene (DNT) and 1,3,5-trinitro-1,3,5-triazine (RDX) exposure to the northern bobwhite (Colinus virginianus). *Environ. Toxicol. and Chem*. 26 (7): 1481-1487.

Jones-Price, C., T.A. Marks, T.A. Ledoux, J.R. Reel, L. Lenghoff-Paschke, and R. Wolkowski-Tyl. 1980. Teratological and postnatal evaluation of dinitrotoluene in Fischer 344 rats. Chemistry and Life Sciences Group, Research Triangle Institute, RTP, NC and Chemical Institute of Toxicology, RTP, NC.

Kozuka, H., M. Mori and Y. Naruse. 1979. Studies of the metabolism and toxicity of dinitrotoluenes. Toxicological study of 2,4-dinitrotoluene (2,4-DNT) in rats in long term feeding study. *J. Toxicol. Sci*. 4: 221-228.

Lane, R.W., G.S. Simon, R.W. Dougherty, J.L. Egle Jr. and J.F. Borzelleca. 1985. Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotoluene in the male rat. Drug Chem. *Toxicol*. 8: 265-280.

Lee, C.C., J.V. Dilley, J.R. Hodgson, D.O. Helton and W.J. Wiegand. 1975. Mammalian toxicity of munitions compounds phase I: acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism. Midwest Research Institute, Kansas City, MO. Prepared for Environmental Protection Research Division, U.S. Army Medical Research and Development Command, Washington, DC.

Lee, C.C., H.V. Ellis III, J.J. Kowalski, J.R. Hodgson, R.D. Short, J.C. Bhandari, T.W. Reddig and J.L. Minor. 1976. Mammalian toxicity of munitions compounds Phase II: effects of multiple doses part III: 2,6-dinitrotoluene. ADA062015. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Lee, C.C., H.V. Ellis III, J.J. Kowalski, J.R. Hodgson, S.W. Hwang, R.D. Short, J.C. Bhandari, J.L. Sanyer, T.W. Reddig and J.L. Minor. 1978. Mammalian toxicity of munitions compounds Phase II: effects of multiple doses part II 2,4-dinitrotoluene. ADA061715. Midwest Research Institute, Kansas City, MO. Supported by U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Lee, C.C., C.B. Hong, H.V. Ellis III, J.C. Dacre and J.P. Glennon. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part II. CD rats. *J. Am. Coll. Toxicol*. 4: 243-256.

Lent, E.M., L.C.B. Crouse, M.J. Quinn, Jr., S.M. Wallace. In press. Comparison of the repeated dose toxicity of isomers of dinitrotoluene. *Int. J. Tocicol.*

Leonard, T.B., M.E. Graichen and J.A. Popp. 1987. Dinitrotoluene isomer-specific hepatocarcinogenesis in F344 rats. J. Natl. Canc. Inst. 79: 1313-1319.

McGown, E.L., J.J. Knudsen and G.E. Marrs, Jr. 1983. Fourteen-day feeding study of 2,4-dinitrotoluene in male and female rats. DTIC ADA126069. Letterman Army Institute of Research, The Presidio, San Francisco, CA.

National Cancer Institute. 1977. Bioassay of 2,4-dintrotoluene for possible carcinogenicity. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institute of Health, Bethesda, MD.

Nishino, S.F. and J.C. Spain. 1995. The effect of 2,6-dinitrotoluene on 2,4-dinitrotoluene degradation. USAF, Armstrong Laboratory, Tyndell AFB, FL.

Noguera, D.R. and D.L. Freedman. 1996. Reduction and acetylation of 2,4-dinitrotoluene by a Pseudomonas aeruginosa strain. *Appl. Environ. Microbiol*. 62: 2257-2263.

Paden, N.E., E.E. Smith, and R.J. Kendall. 2008. Acute toxicity of 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene in the adult bullfrog (Lithobates catesbeiana). Bull. *Environ. Contam. Toxicol*. 80:487-491.

Pennington, J.C., T.F. Jenkins, G. Ampleman, S. Thiboutot, J. Brannon, J. Clausen, A.D. Hewitt, et al. 2004. Distribution and fate of energetics on DoD test and firing ranges. ERDC TR-04-4. Engineer Research and Development Center, Washington. D.C.

Price, C.J., R.W. Tyl, T.A. Marks, L.L. Paschke, T.A. Ledoux, and J.R. Reel. 1985. Teratologic evaluation of dinitrotoluene in the Fischer 344 rat. *Fund. Appl. Toxicol*. 948-961.

Quinn, M.J., M.A. Bazar, C.A. McFarland, E.J. Perkins, K.A. Gust, R.M. Gogal, and M.S. Johnson. 2007. Effects of subchronic exposure to 2,6-dinitrotoluene (DNT) in the northern bobwhite (Colinus virginianus). *Environ. Toxicol. and Chem*. 26 (10): 2202-2207.

Rickert, D.E. and R.M. Long. 1980. Tissue distribution of 2,4-dinitrotoluene and its metabolites in male and female Fischer-344 rats. *Toxicol. Appl. Pharmacol*. 56: 286-293.

Rickert, D.E., S.R. Schnell, and R M. Long. 1983. Hepatic macromolecular covalent binding and intestinal distribution of [14C] dinitrotoluenes. *J. Toxicol. Environ. Health* 11:555-567.

Rickert, D.E., B.E. Butterworth, and J.A. Popp. 1984. Dinitrotoluene: acute toxicity, oncogenicity, genotoxicity, and metabolism. CRC Crit. Rev. Toxicol. 13:217-234.

Smith, K.N. 1983. Determination of the reproductive effects in mice of nine selected chemicals. PB84 183-540. National Institute of Occupational Safety and Health (NIOSH), Cincinnati, OH.

Suski, J.G., C.S. Salice, J.T. Houpt, M.A. Bazar, L.G. Talent. 2008. Dose-related effects following oral exposure of 2,4-dinitrotoluene on the western fence lizard (Sceloporus occidentalis). *Environ. Toxicol. Chem*. 27: 352-359.

USACHPPM. 1996. Toxicological study No. 4152-31-96-06-14, 14-day feeding study of 2,4-dinitrotoluene in the white-footed mouse, Peromyscus leucopus, 1 July-19 July 1996. Sponsored by USEPA Ecological Exposure Research Laboratory, Cincinnati, OH.

USACHPPM, 1996. Toxicological study No. 85-4152-96, 14-day feeding study of 2,6-dinitrotoluene in the white-footed mouse, Peromyscus leucopus, 24 June-12 July 1996. Sponsored by USEPA Ecological Exposure Research Laboratory, Cincinnati, OH.

USACHPPM) 2000. Technical Guide 254, Standard Practice for Wildlife Toxicity Reference Values.

U.S. Environmental Protection Agency (USEPA), 1993. Overview of the Health Effects of Selected Munition Chemicals. EPA 822-R-93-022. Office of Water, Washington, D.C.

Vernot, E.H., J.D. MacEwen, C.C. Haun, and E.R. Kinkead. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. *Toxicol. Appl. Pharmacol*. 42: 417-423.

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.

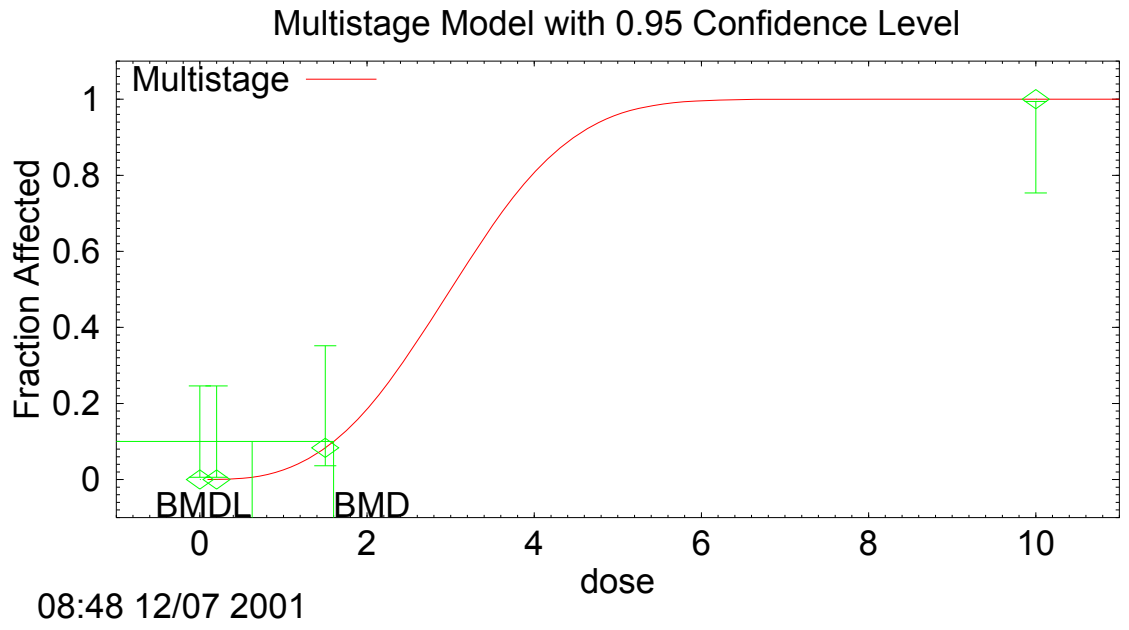
References from the USEPA (1994) Drinking Water Health Advisory for 2, 4- and 2,6-Dinitrotoluene, the ATSDR (1998) Toxicological Profile for 2,4- and 2,6-Dinitrotuluene and the Toxicity Summary for 2,4- and 2,6-dinitrotoluene (ORNL, 1995) were also reviewed and all relevant non-duplicate sources evaluated.

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[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2-beta3*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