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Wildlife Toxicity Assessment for 2,4,6-TRINITROTOLUENE (TNT)

OCTOBER 2000

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Health Effects Research Program
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Readiness Thru Health

Acknowledgements

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

Wildlife Toxicity Assessment for 2,4,6-Trinitrotoluene

CAS No. 118-96-7 **October 2000**

1. INTRODUCTION

This Wildlife Toxicity Assessment is the result of a thorough investigation of the scientific literature regarding the toxicological characteristics of 2,4,6-trinitrotoluene (TNT) that may be important for the health of wildlife (mammals, birds, reptiles and amphibians) exposed to the substance. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

This document is designed to support ecological risk assessment activities. The measures of toxicity derived in this document are intended to be used in screening-level assessments. By definition, the measures of toxicity presented herein evaluate the likelihood of effects in *individual* organisms that may be relevant to a *population* of organisms in the wild. This Wildlife Toxicity Assessment does not specifically address how the measures, or any resulting risk estimates, relate to demographic rates or outcomes for any particular population of interest. Assessing risk to populations involves using these methods and other lines of evidence before risk management actions to protect populations can be recommended based upon scientific information. Therefore, the toxicity measures in this document should not be used to demonstrate unacceptable population risks that require remedial action without further site-specific study.

2. TOXICITY PROFILE

2.1 Literature Review

Given the predominant military use of trinitrotoluene, many studies were found from U.S. Army sources. These military sponsored studies, and subsequent reports, were found through TOXLINE and DTIC searches. However, the most appropriate ones were found though traditional cross-referencing techniques and through individual queries to project investigators within the Army. Several databases were searched and Appendix A contains details of this search.

2.2 Environmental Fate and Transport

The distribution of TNT at many U.S. military sites is substantial. At least 17 Army installations have reported soil concentrations ranging from 0.08 to 64,000 micrograms per gram (µg/g) (Hovatter et al. 1997). Of those that had detectable concentrations, 5 installations had samples in which surface soils exceeded 10,000 µg TNT/g soil dry weight (Walsh and Jenkins 1992).

A summary of physical and chemical properties is provided in Table 1. An important route for the contamination of surface water, ground water, and surface soils with TNT has historically been due to large aqueous effluents of rinse water ("pink water," Walsh and Jenkins 1992, ATSDR 1995). Some sources have reported wastewater emissions ranging from 61 – 210 pounds/day (Rosenblatt et al. 1973). Due to its relatively low vapor pressure, and relatively high water solubility, TNT does not actively partition from surface waters to the atmosphere (ATSDR 1995). Photolysis studies, comparing river waters and distilled water, have shown that the rate of TNT photolysis is directly related to increases in pH and organic matter content (Spanggord et al. 1980). Generally, TNT is not expected to hydrolyze or bioconcentrate in aquatic systems under normal environmental conditions (HSDB 1997).

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

CAS No.	118-96-7
Molecular weight	227.13
Color	yellow-white
State	Monoclinic needles
Melting point	80.1°C
Boiling point	240°C
Odor	Odorless
Solubility	130 mg/L in water at 20°C; soluble in acetone, benzene, alcohol and ether
Partition coefficients	
Log K_{OW}	1.60; 2.2 (measured), 2.7 (estimated)
K_{OC}	300 (estimated), 1,100 (measured)
Vapor pressure (at 20°C)	1.99E-04 mm Hg
Henry's Law constant (at 20°C)	4.57E-07 atm m^3/mole
Conversion factors	1 ppm = 9.28 mg/m^3 1 mg/m^3 = 0.108 ppm

Source: ATSDR (1995)

Soil contamination of TNT can result from spills, disposal of solid waste, open incineration and detonation of explosives, or leaching from poorly engineered impoundments (Burrows et al. 1989). Retrieval and subsequent destruction of unexploded ordinance (UXO) can result in soil contamination as well (includes open burning/open detonation, OB/OD areas). Based primarily upon the physical and chemical properties of TNT (i.e., octanol-water partition coefficient (K_{ow}) and water solubility), TNT is not expected to bioaccumulate or biomagnify in terrestrial systems (HSDB 1997).

Based on the measured and estimated soil organic carbon adsorption coefficient (K_{oc}) of 300 – 1100, TNT is not expected to significantly partition to sediment (from surface waters) or sorb to soil particles (HSDB 1997, ATSDR 1995). However, the biotransformation of TNT in soil can be significant, and can be readily reduced under anaerobic conditions. These anaerobic reactions occur through microbial reduction, primarily through successive reduction of the nitro groups (Burrows et al. 1989). Several bacteria have been identified in these reactions. They include species of *Pseudomonas, Escherichia, Bacillus, Citrobacter, Enterobacter, Klebseilla, Veillonella,* and *Clostridium* (Burrows et al. 1989). Fungi are also capable of reducing TNT (Burrows et al. 1989, ATSDR 1995). Microbial transformation of TNT leads to a variety of reduction products, including 2-amino and 4-amino dinitrotoluene and azoxydimers (Burrows et al. 1989, HSDB 1997), though some oxidation products have been identified (Won et al. 1974). Biological transformation by bacterial and fungal species occurs slowly in the environment, with slightly higher rates in the presence of other carbon sources. However, biological degradation may not extend to cleavage of the TNT ring (the successive reductions of each of the nitro groups to amines followed by oxidative deamination to a phenol that releases an ammonia or nitrite has been described (HSDB 1997)). Accurate mass balance without the use of radio-labeled compound is difficult with TNT based on its crystal forming tendencies, low organic solubility, and relatively low water solubility (M. Major, USACHPPM, pers. comm.).

Another process that can affect the fate and transport of TNT in the environment is photolysis. Photolysis has been reported to produce "pink water" from TNT-contaminated surface water (ATSDR 1995). Numerous transformation products have been identified in pink water, the predominant ones including 1,3,5-trinitrobenzene, 4,6-dinitroanthranil, 2,4,6-trinitrobezaldehyde, 2,4,6-trinitrobenzonitrile, in addition to several azo and azoxy derivatives formed by the coupling of nitroso and hydroxyamine products (Jerger et al. 1976, Spanggord et al. 1980).

2.3 Summary of Mammalian Toxicology

2.3.1 Mammalian Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

Oral lethal dose to 50% of the exposed population (LD_{50}) values of 660 milligrams per kilogram (mg/kg) in male and female mice and 1320 and 795 mg/kg in male and female rats, respectively, have been reported (Dilley et al. 1982a). These animals developed seizures (grand mal), followed by mild convulsions 1 – 2 hours after exposure. All deaths occurred within 24 hours after exposure; red urine and lethargy were other signs of exposure (Dilley et al. 1982a). Animals that survived the convulsions were still alive 14 days following the exposure (Dilley et al. 1982b). Variation in response for dogs was considered significant (Voegtlin et al. 1921). Cyanosis was evident 12 hours following administration of 100 mg/kg TNT. Severe incoordination and tremors followed. However, the authors note that some dogs receiving 100% of the 100 mg/kg dose did not exhibit the same symptoms as those receiving 50% or less (Voegtlin et al. 1921). Most species showed signs of ataxia after dosing (Voegtlin et al. 1921, Dilley et al. 1982b).

Cats injected intraperitoneally with 0.10 to 0.15 grams per kilogram (g/kg) TNT died within 5.5 hours (Bredow and Jung 1942). Injections of 0.04 g/kg caused convulsions, paralysis of the hindlimbs, decrease in body temperature, and enhanced saliva secretion. Methemoglobin was also present in the blood. Cats given daily subcutaneous injections of 50 mg/kg TNT died within 4 to 9 days (Lillie 1943). Each showed signs of splenic congestion. Livers had fat accumulation (steatosis) and Kupffer cell hemosiderosis.

White-footed mice (*Peromyscus leucopus*; 10/group/sex) were exposed to one of five treatments of 0, 0.042, 0.083, 0.165, and 0.330% TNT in feed for 14 days (Johnson et al. 2000a). These treatments were calculated by the authors to be equivalent to 66, 145, 275, and 602 mg TNT/kg body weight per day (bw/d) for males and 70, 142, 283, and 550 mg/kg/d for females for the 0.042, 0.083, 0.165, and 0.330% TNT, respectively. Indicators suggesting hemolysis were evident in the 0.330% treatment for both sexes, where only males had suppressed splenic phagocyte hydrogen peroxide production for the 0.165 and the 0.330% treatments, and a reported reduction in phagocytosis for males in all TNT exposures. However, the authors note that the significance of the latter endpoint (i.e., inhibited phagocytosis for males and not females) is questionable.

Oral LD_{50} estimates for cotton rats (*Sigmodon hispidus*) exposed to TNT in corn oil were 607 and 767 mg TNT/kg bw for males and females, respectively (Reddy et al. 2000). Animals exhibited an increased respiratory rate within 90 minutes after dosing. Orange-colored urine and urinary bladder distension was observed in all animals at necropsy. No other abnormal histological observations were reported.

A 7-day gavage exposure representing 1/8, 1/4, and 1/2 the LD_{50} for male (75.9, 151.8, and 303.5 mg TNT/kg bw/d) and female cotton rats (96, 192, and 384 mg TNT/kg bw/d) was conducted using corn oil (Reddy et al. 2000). Histopathology of major organs as well as hematology, hepatic metabolizing enzymes, and clinical chemistry of the sera were evaluated. Splenic weights were increased in the 192 (females only) and the 384 mg/kg/d treatments; and liver weights were increased in the 151.8 (males only) and 303.5 mg/kg/d treatments. These two high dose groups also showed hematological results consistent with erythrolytic anemia. Hemosiderin laden macrophages were noted in the spleen of rats receiving the lowest dose. Subtle testicular lesions were noted in the two high dose groups.

2.3.1.2 Mammalian Oral Toxicity - Subchronic

Subchronic exposures to rats, mice, and dogs have produced consistent hematologic effects (Von Oettingen et al. 1944, Dilley et al. 1982b, Levine et al. 1990a, b). Exposures of 13 weeks were sufficient to produce anemia (consisting of reduced number of red blood cells, reduced hemoglobin and hematocrit) in all of these species. Increases in immature red blood cells (reticulocytes), reduction in blood, hematocrit, and corpuscle volumes were evident after only 15 days in dogs administered TNT in gelatin capsules of dosages ranging 5 – 33 mg (Voegtlin et al. 1921). TNT exposure is reported to result in direct hemolysis within circulating blood, leading to an increase in spleen weight. Dilley et al. (1982a, b) reported similar findings including pathological assessment of the spleen that suggested hemolytic anemia in beagles. Other important effects included increased liver weight (including hepatocytomegaly), intestinal inflammation (and mucoid stools), enlarged kidneys, and splenic congestion in mice, rats, and dogs (Dilley et al. 1982b, Levine et al. 1990a, b). Most animals in the highest dose group of all species displayed some degree of hemosiderosis of the spleen (Dilley et al. 1982b). Rats and dogs had dose-related increased serum cholesterol and lower iron and serum glutamic-pyruvic transaminase (SGPT) levels following the 13-week exposure period; mice seemed to be more resistant to treatment (Dilley et al. 1982b). Increased serum cholesterol was consistent with doses in rats and dogs (Levine et al. 1984, Dilley et al. 1982b). Other endpoints consistent with anemia were decreased erythrocyte numbers, hemoglobin and hematocrit values, and occasionally bone marrow hyperplasia.

Testicular atrophy was most pronounced in rats (Dilley et al. 1982b), and consisted of dose-related degeneration of the germinal epithelium lining the seminiferous tubules and hyperplasia of interstitial Leydig cells (in high dose group, 300 mg/kg/d; Levine et al. 1984). The No Observable Effect Levels (NOELs) for these three species were: dogs, 0.20; rats, 1.42; and mice, 7.76 mg/kg/d, suggesting that dogs were the most sensitive (Dilley et al. 1982b). Dilley et al. (1982b) also mention that the effects appear to be totally reversible (up to a 4-week exposure) following a 4-week recovery period.

A single study investigating the functional response of splenic phagocytes to TNT in NMRI mice was conducted (through chemiluminescent analysis) from exposure TNT metabolites (2,4-diamino-dinitrotoluene, 2,4,6 triaminotoluene, 2-amino-6-nitrotoluene, 4-amino-3,5-dinitrotoluene, and 2-amino-4,6-dinitrotoluene) *in vitro* (Thierfelder and Masihi 1995). This assay quantifies intracellular-activated oxygen species. Relatively high doses of metabolites were associated with reduced response relative to controls. Specifically, > 1 milligram per liter (mg/L) of 2,4-diaminotrinitrotoluene, >50 mg/L for 4-amino-3,5-dinitrotoluene, and > 100 mg/L for 2-amino-4,6-dinitrotoluene caused a plateau of 57 – 65% inhibition (Thierfelder and Masihi 1995).

Results of a 90-day feeding study using white-footed mice (*Peromyscus leucopus*) provided evidence that Nearctic mice may be more resistant than Palearctic (Old-World: *Mus)* species. McCain (1998) exposed 100 male and female *P. leucopus* to concentrations of 660, 1320, and 2640 parts per million (ppm) TNT in feed. The calculated dosage was about 165, 330, and 660 mg/kg/d, respectively. The highest concentration used in this study (2640 ppm; 660 mg/kg/d) was equivalent to the LD_{50} of 660 mg/kg reported by Dilley et al. (1982b) in *Mus*, yet none died during the study. Initial animal weight reduction consistent with reduced palatability was reported, yet all groups gained weight over time. McCain (1998) found only exposures to 1320 and 2640 ppm associated with adverse physiological changes (organ weight, incidence of chromaturia, hemosiderin, etc.), and established a No Observable Adverse Effect Level (NOAEL) of 660 ppm (165 mg/kg/d).

2.3.1.3 Mammalian Oral Toxicity - Chronic

Effects from chronic exposures were consistent with those of sub-chronic exposures. Two studies using Fisher 344 rats (Furedi et al. 1984) and beagle dogs (Levine et al. 1990a) reported dose-dependent indicators suggesting hemolytic anemia (e.g., reduced hemoglobin, hematocrit, and erythrocyte counts, increased quantities of reticulocytes). These effects were different from controls at doses ≥ 8.0 (i.e., and 32 mg/kg/d for dogs; Levine et al. 1990a) and for all TNT treatments for rats (i.e., 0.4, 2.0, 10.0, and 50.0 mg/kg/d; Furedi et al. 1984). Exposures for the rat study lasted 106 weeks and 26 weeks for dogs. Compensatory responses to anemia were minimal in rats (e.g., erythrocytic macrocytosis and reticulocytosis; Furedi et al. 1984). Methemoglobinemia was apparent in both studies in animals of the higher dose groups. Reduction in body weight was apparent in rats exposed to 10 mg/kg/d or greater, and at 8 mg/kg/d or greater for dogs (Furedi et al. 1984, Levine et al. 1990a). Dose-related hepatomegaly (and increased kidney weights) was evident in rats receiving > 2.0 mg/kg/d; this was only evident in the high dose group for dogs. Splenomegaly was evident in rats and dogs in the higher dose groups. Hemosiderosis in Kupfer's cells was seen in various dogs at most dose levels (Levine et al. 1990a). Renal injury was supported by gross and tissue morphological examinations (in high dose groups; Furedi et al.

1984). Increased pigment deposition occurred in the kidneys (as did evidence of bone marrow fibrosis) of rats exposed to 2.0 mg/kg/d or greater (Furedi et al. 1984). It was reported that the observed enteritis of the small intestine was related to TNT treatment in dogs (Levine et al. 1990a). Urinary bladder carcinomas were evident in some rats (2 males and 4 females of 1794 and 1754 rats, respectively) exposed for 106 weeks (Furedi et al. 1984). Given the rate of occurrence for these types of neoplasias, this finding was considered biologically significant. An NOEL was determined to be 0.4 mg/kg/d for rats (Furedi et al. 1984); none was found for dogs (Levine et al. 1990a). TNT was found to be mutagenic (without S9 activation) in *Salmonella typhimurium;* the reduced metabolites were less potent mutagens (Tan et al. 1992).

2.3.1.4 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Primary target organs for TNT include the nervous system (primarily from acute effects) and blood (Table 2, Figure 1). Since TNT causes erythrolysis, the primary blood conditioning organs may also be affected (e.g., liver and kidney). These conditions were found in *Peromyscus* (McCain 1998), beagle dogs (Dilley et al. 1982b, Levine 1990a), rats (Furedi et al. 1984), and laboratory mice (*Mus*; Dilley et al. 1982b). Several studies were found that were current, well designed, and appropriate for the development of Toxicity Reference Values (TRVs) for mammals. The work of Dilley et al. (1982b), Levine et al. (1984, 1990a) and Furedi et al. (1984) are particularly valuable since they include chronic, subchronic and acute exposures, and use several species identified above. Two Orders and three families of *Mammalia* are represented that include: Carnivora: Canidae; Rodentia: Cricetidae, Muridae. Two wildlife species were also evaluated. Effects from exposure are consistent, yet slightly variable in magnitude of effect. Each study identifies several NOAELs and Low Observable Adverse Effect Levels (LOAELs) for various endpoints of effect, and the investigations are inclusive of other potential organ systems. It is for these reasons that this review is sufficient to derive class-specific TRVs for TNT.

With few exceptions, data from acute studies where gavage methods were employed were deemed irrelevant and not used for comparison (TRV derivation) purposes. Exceptions included acute or gavage studies that included other species not previously evaluated (e.g., Reddy et al. 2000). All other reports that evaluated TNT in feed were of sufficient quality and importance to include in this evaluation. These studies were consistent in quality and reporting of the methods.

2.3.2 Mammalian Oral Toxicity – Other

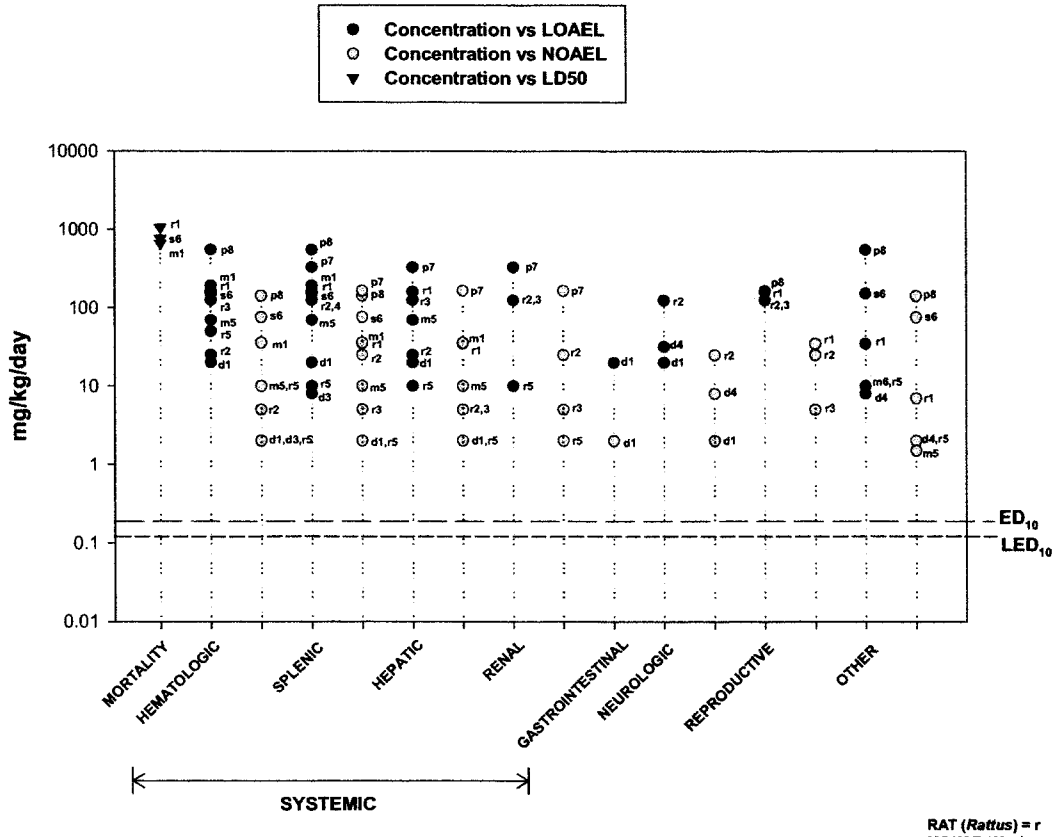
No other data relevant to oral exposures for mammals were found.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
McCain 1998	Mouse (*Peromyscus leucopus*)	90 d	165	330	Increased kidney, liver, spleen weights; presence of hemosiderin in spleen; chromaturia; increased extramedullary hematopoiesis in spleen
Johnson et al. 2000	Mouse (*Peromyscus leucopus*)	14 d	142	550 (♀)	Indicators of erythrolytic anemia (increased spleen weight, histopathology); decreased intracellular hydrogen peroxide of splenic phagocytes); phagocytosis results of uncertain biological significance
Reddy et al. 2000	Cotton rat (*Sigmodon hispidus*)	7 d	76 (♂)	152 (♂)	Erythrolytic anemia; changes in spleen and liver pathology, hematology; changes in hepatic glutathione S-transerfase for females, not males of uncertain biological significance; male dose protective of female dose for all other endpoints.
Dilley et al. 1982b	Rat (Sprague-Dawley)	13 wk	1.4	160	Anemia and leukocytosis
			34.7	160	Increased cholesterol, decreased body weight (10-20%), increased spleen weight, hemosiderosis, lymphocytosis; testicular atrophy
			7	34.7	Decreased food consumption
	Mouse (Wistar)	13 wk	35.7	193	Decreased hematocrit/RBC, liver necrosis
	Dog (Beagle)	13 wk	2	20	Mucoid stools (red), diarrhea, anemia, increased liver weight, bilirubin, and cholesterol; lethargy
Levine et al. 1984	Rat (Fisher 344)	13 wk	5	25 (♂)	Anemia, increased serum cholesterol
			25	125	Lipofuscin-like pigment in renal cortex, splenic enlargement with congestion, slight lethargy and ataxia; reduced food intake and body weight; atrophic seminiferous tubules, degenerated germinal epithelium
Levine et al. 1990a	Dog (Beagle)	6 mos	2 (♂) 8 (♀)	8 (♂) 32 (♀)	Anemia, methemoglobinemia, increased platelets, slight ataxia; chromaturia
			2 (♂)	8 (♂)	Decrease in body weight (16.4%; females at 32)
Levine et al. 1990b	Rat (Fisher 344)	13 wk	5	125	Increased spleen weight with diffuse congestion
Furedi et al. 1984	Rat (Fisher 344)	24 mos	10 (♀)	50 (♀)	Bone marrow fibrosis
			2 (♀)	10 (♀)	Increased cholesterol, enlarged liver; 14% decrease in body weight gain; splenic congestion, extramedullary hematopoiesis
Furedi et al. 1984	Mouse (B6C3F1)	24 mos	10 (♀)	70 (♀)	Mild anemia, increased liver weight, reduced serum globulin levels; 10-15% decrease in body weight gain; enlarged spleen and lymph nodes

Figure 1.

TNT HEALTH EFFECTS TO MAMMALS



1= Dilley et.al. 1982b
2 = Levine et.al. 1984
3 = Levine et.al. 1990a
4 = Levine et.al. 1990b
5 = Furedi et al. 1984
6 = Reddy et al. 2000
7 = McCain 1998
8 = Johnson et al. 2000a

HEALTH EFFECTS

RAT (*Rattus*) = r
MOUSE (*Mus*) = m
DOG (*Canis*) = d
New World Mouse (*Peromyscus*) = p
New World Rat (*Sigmodon*) = s

2.3.3 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found.

2.3.4 Mammalian Dermal Toxicity

No dermal studies conducted using animals were found; however, information suggesting the importance of dermal exposures for humans has been reported (Hathaway 1977, Woollen et al. 1986). In addition, studies investigating the potential for TNT to transverse mammalian skin *in vitro* from a soil matrix have demonstrated that dermal exposures to TNT in soil may add to total systemic dose (Reifenrath 1994).

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity - Oral

2.4.1.1 Avian Oral Toxicity - Acute

Three experimental trials for the acute lethal dose (ALD) were recently performed on Northern Bobwhite (*Colinus virginianus*) (Gogal et al. *in draft*). Both male and female birds were gavaged with single oral doses of 4508, 3005, and 2003 mg TNT/kg bw and observed for 14 days. All birds except one female exposed to 3005 mg/kg died within 5 days. The female dosed at 2003 mg/kg exhibited extreme ataxia, yet survived until necropsy. Reddish-brown stool was observed 24-48 hrs following dosing, characteristic of hematuria seen in mammals. A single oral dose of 2003 mg/kg was determined to be the lowest concentration resulting in death to Northern Bobwhite.

2.4.1.2 Avian Oral Toxicity - Subchronic

Adult male and female Northern Bobwhite (*Colinus virginianus*; N = 50) were provided TNT in feed at concentrations of 3300, 1560, 863, and 160 mg TNT/kg feed for a 90-day exposure (Gogal et al. *in draft*.). Initially, 4/10 birds died from exposure to the 3300 mg/kg treatment, yet none thereafter. Histopathology and sensitive indicators of immune function were evaluated. The effects included a dose-dependant non-significant decreasing trend in: total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, blood lymphocytes, an increase in late apoptotic/necrotic blood leukocytic cells, and slight hemosiderosis in the liver. It was noted by the authors that significant erythrolytic anemia does not seem to be the major target of toxicity in quail, most likely due to the refractory nature of the avian hematological and vascular system. No adverse histopathology was associated with any animal exposed to the 160 mg/kg treatment.

2.4.1.3 Avian Oral Toxicity - Chronic

No data are available for chronic exposures.

2.4.1.4 Avian Oral Toxicity - Other

No other avian studies are available for TNT.

2.4.1.5 Studies Relevant for Avian TRV Development for Ingestion Exposures

The only study found that evaluated the effects of TNT to birds was Gogal et al. (*in draft*). The 90-day results suggest that birds are much less sensitive to the hemolytic mechanisms found in mammals, yet there is evidence of some mild erythrolytic effect. Given the refractory nature of the avian hematopoietic system and the magnitude of these observations, these findings are of uncertain biological significance. Consistent with the mammalian data are the initial central nervous system (CNS)-related effects of exposure where individuals exhibited ataxia and neuromuscular effects. These effects were observed prior to death of the quail in the high dose group (3300 mg/kg). Therefore, an NOAEL of 7 mg/kg/d was suggested by the authors based upon the lack of adverse pathological and immunotoxicological observations for any individual in the low dose group (160 mg/kg). These data are summarized in Table 3 and Figure 2. An LOAEL was identified as 178 mg/kg/d based on the four deaths that occurred in the high dose group, and that possible adverse histopathology was associated with some individuals in the 3300 mg/kg group.

Table 3. Summary of Relevant Avian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL mg/kg/d	LOAEL mg/kg/d	Effects at LOAEL
		Lowest lethal dose detected (LD_{LOW}) 2003 mg/kg	na	na	Male dies during the determination of the approximate lethal dose at 2003 mg/kg; female did not.
Gogal et al. (*in draft*)	Northern Bobwhite (*Colinus virginianus*)	90 d	7	175	4/10 initial deaths in high dose group (3300 mg/kg); dose-dependant non-significant decreasing trend in: total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, blood lymphocytes, an increase in late apoptotic/necrotic blood leukocytic cells, and slight hemosiderosis in the liver.

na – not applicable

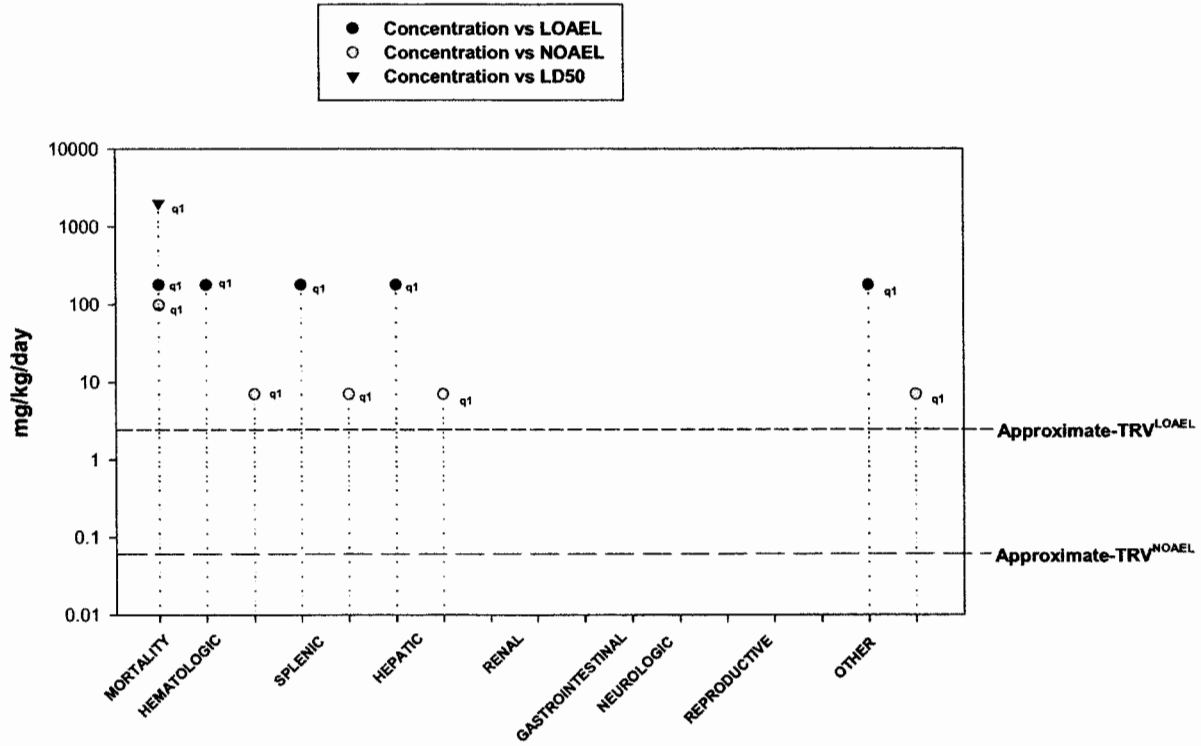
2.4.2 Avian Inhalation Toxicity

No data available.

2.4.3 Avian Dermal Toxicity

No data available.

TNT HEALTH EFFECTS TO BIRDS



1= Gogal et al. (draft)

Northern Bobwhite (*Colinus*) = q

2.5 Amphibian Toxicology

Only one study investigating 14-day exposures to TNT in soil in a terrestrial salamander was located.

2.5.1 Amphibian Microcosm Study

Tiger salamanders (*Ambystoma tigrinum*) were exposed to TNT in a soil matrix and were fed earthworms exposed to TNT in soil using a microcosm design for 14-days (Johnson et al. 2000b). Previous dermal exposures to TNT have been shown to be considerable compared to oral exposures in *Ambystomid* salamanders (Johnson et al. 1999). The TNT concentrations in soil reduced with time, ranging from 280 μg/g at the beginning to 59 μg/g at the conclusion. At which time the primary reduction products of TNT increased (39 and 62 μg/g at the beginning to 58 and 78 μg/g of 2-amino-4,6-dinitrotoluene and of 4-amino-2,6-dintitrotoluene at the conclusion, respectively). Concentrations of TNT in earthworms ranged from 0.25 – 0.62 μg/g, and from 2.1 – 2.6 μg/g of the primary reduction products mentioned previously. Immune function, histopathology, weight changes, and blood parameters were investigated. No adverse health effects were observed and the animals gained weight during exposure.

2.5.2 Relevance for Amphibian TRV Development

This study used a microcosm design that considered all pathways of exposure and potential variation in feeding regimes (Johnson et al. 2000b). Since soil concentrations of TNT were monitored, these data are used to derive a NOAEL for terrestrial salamanders, a soil concentration of 59 mg/kg that reflects all exposure pathways. Since adverse effects were not observed in the study, a LOAEL is not available.

2.6 Reptilian Toxicology

No data for reptiles are available.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Based on the information from five species, as described in Section 2.3.1.4, the dog appears to be the most sensitive mammal from oral exposures to TNT. The lowest LOAEL is 8 mg/kg/d, where Levine et al. (1990a) reported evidence of blood effects and decreased weight gain in dogs receiving 8 mg/kg/d but not at 2 mg/kg/d. The highest NOAEL within the same endpoints and species was the dose of 2 mg/kg/d reported by the same authors. Because decreased weight gain (an indicator of reduced growth and/or energy efficiency) and anemia have the potential to adversely effect future fitness, these endpoints are

considered to be ecologically relevant. In addition, this and the other studies satisfy the minimum data set requirement of the Standard Practice, Section 2.2 (USACHPPM 2000); thus, no uncertainty factors are needed to derive the TRVs. The data were appropriate for a benchmark dose derivation and are presented in Appendix B. A benchmark dose (BMD or ED_{10}) of 0.3 mg/kg/d was calculated from the model fit of the mean response at the 10% response level. A lower-bound on the benchmark dose (BMDL or LED_{10}) was calculated to be 0.2 mg/kg/d from the lower 95% confidence interval (CI) of the modeled curve. These values are selected as the class-specific TRVs (Table 4). Since these studies were well calibrated and the results are consistent with those of others, this TRV is given a high degree of confidence.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
LED_{10}	0.2 mg/kg/d	High
ED_{10}	0.3 mg/kg/d	High

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, since the dog is the most sensitive mammal tested, the class-specific TRVs shown in Table 4 are considered to be protective of non-carnivorous mammals. More specific TRVs may be developed considering the data provided in Table 2.

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.1.4 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

The only study that has evaluated the effects of TNT to birds is Gogal et al. (*in draft.*). These investigations evaluated hematological effects as well as systemic organ and sensitive immune parameters. Given the variation in response, only trends were evident. However, there were no incidences of adverse pathology associated with the low concentration treatment of 160 mg/kg. There were four mortalities in the high concentration treatment of 3300 mg/kg, and a non-significant dose-

related trend was evident in hematological and immune parameters. Though the biological significance concerning the magnitude of the hematological and immune parameters are questionable, the fact that mortality occurred initially in 4/10 animals is significant. The authors calculate an NOAEL at 7 mg TNT/kg bw/d at 160 mg TNT/kg feed dry weight treatment, and an LOAEL (serious) of 178 mg TNT/kg bw/d for the 3300 mg TNT/kg feed treatment. Since this is the only bird study, TRVs based on an approximation of the NOAEL and LOAEL were developed to represent the Class Aves. Given that the 90-d exposure regime represents <10% of the average lifespan of Northern Bobwhite it is considered a subchronic study. Therefore, an uncertainty factor (UF) of 100 was applied to account for interspecific variability (UF of 10) and to extrapolate from a single subchronic study (UF of 10). Table 5 presents the selected TRVs. A low level of confidence has been given to these TRVs because only one study is available, the single study only evaluates one species, and the study has relatively low power in its statistical comparisons.

Table 5. Selected Ingestion TRVs for the Class Aves

TRV	Dose	Confidence
NOAEL-based	0.07 mg/kg/d	Low
LOAEL-based	1.8 mg/kg-d	Low

3.2.2 TRVs for inhalation exposures for the Class Aves

Not available at this time.

3.2.3 TRVs for dermal exposures for the Class Aves

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Since the exposures were relatively brief, considering the average life span of *Ambystomid* salamanders (> 10 years), these were classified as acute exposures and an NOAEL was identified (Johnson et al. 2000b). In addition, since dermal exposures to TNT were reported to be considerable, a pathway-specific (i.e., oral) TRV would not be appropriate. However, since this study used a holistic exposure regime, a media-based value for soil could be derived. The acute (14-d) NOAEL of TNT in soil (59 µg/g) was divided by a UF of 300 to approximate a chronic NOAEL for terrestrial amphibians (a UF of 30 for an acute NOAEL to a chronic NOAEL and a UF of 10 to extrapolate across multiple species).

This resulted in an approximation of an NOAEL-based TRV of 0.2 mg TNT/kg soil dry weight intended to be protective of terrestrial amphibians. However, since an LOAEL was not identified, an approximation of an LOAEL-based TRV could not be derived. Table 6 presents the selected TRVs. A low confidence level has been assigned to the available TRV because a study observing adverse effects was not available, the only study is of limited length of exposure, and no other terrestrial amphibian data is available.

Table 6. Selected Soil TRVs for Terrestrial Amphibians

TRV	Dose	Confidence
NOAEL-based	0.2 mg/kg soil (dry weight)	Low
LOAEL-based	Not available	—

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

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APPENDIX A
LITERATURE REVIEW

The following databases were searched using the following keywords July 13, 1999:

TOXLINE & MEDLINE

Conditions: Two-word search; 1965 to present.

Trinitrotoluene and mammals - Trinitrotoluene = 911
Mammals = 471106
Combination = 158
Of these, 5 were appropriate and included.

Trinitrotoluene and birds - Trinitrotoluene = 911
Birds = 17894
Combination = 1
After review of the title, the single query result was not appropriate for this document.

Trinitrotoluene and wildlife - Trinitrotoluene = 911
Wildlife = 11830
Combination = 7
Of these, none were deemed appropriate for this document.

Trinitrotoluene and salamanders – Trinitrotoluene = 911
Salamanders = 398
Combination = 0

Trinitrotoluene and toads – Trinitrotoluene = 911
Toad = 411
Combination = 0

Trinitrotoluene and reptiles – Trinitrotoluene = 911
Reptiles = 4886
Combination = 0

Trinitrotoluene and snake – Trinitrotoluene = 911
Snake = 5825
Combination = 0

WinSPIRS 2.0

Conditions: Two-word conditional search; 1979-1997.

Trinitrotoluene and amphibian - Trinitrotoluene = 281
Amphibian = 2031
Combination = 0

Trinitrotoluene and salamander - Trinitrotoluene = 281
Salamander = 711
Combination = 0

Trinitrotoluene and frog - Trinitrotoluene = 281
Frog = 4412
Combination = 0

BIOSIS

Conditions: Two-word conditional search; 1984-1997.
Trinitrotoluene and wildlife - Trinitrotoluene = 1182
Wildlife = 17829
Combination = 73
Of these, most concerned the effects of effluent; duplicates with TOXLINE/MEDLINE search.

Trinitrotoluene and mammal - Trinitrotoluene = 1182
Mammal = 44329
Combination = 178
Of these, most concerned the effects of effluent; duplicates with TOXLINE/MEDLINE search.

Trinitrotoluene and bird - Trinitrotoluene = 1182
Bird = 24112
Combination = 3
These were not appropriate (non-laboratory evaluations).

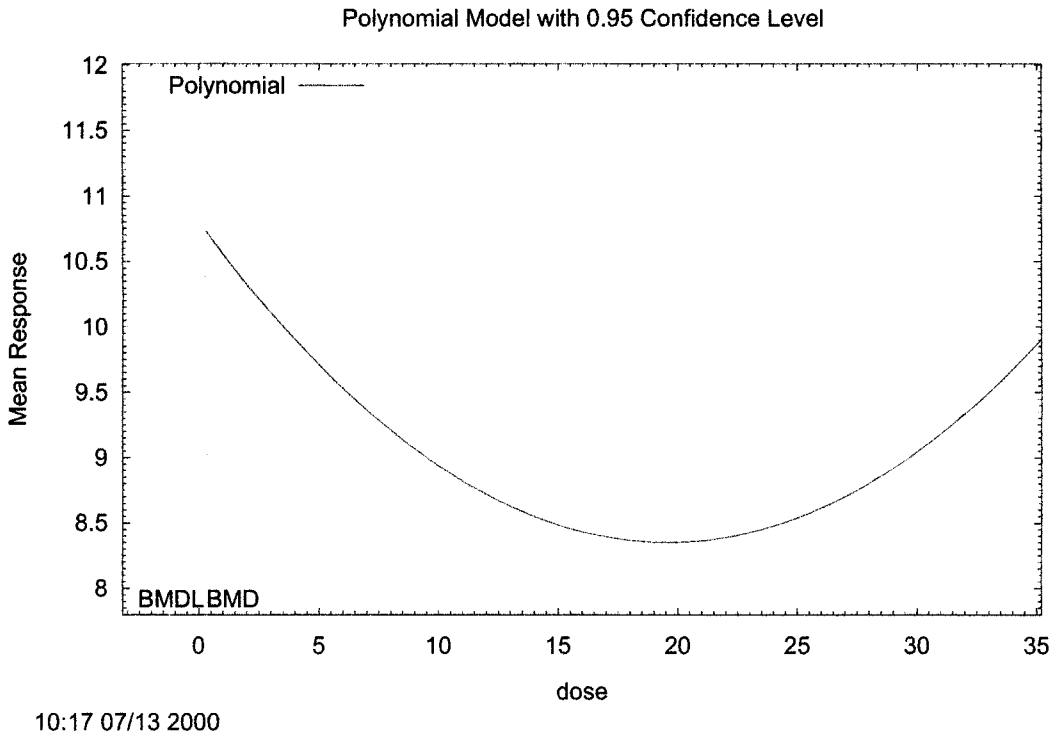
STINET – DTIC

Conditions: Two-word boolean search
Trinitrotoluene and mammal - Combination = 8
Original reports referenced (from which some peer reviewed submissions were based).

Trinitrotoluene and wildlife - Combination = 0
Trinitrotoluene and bird - Combination = 0
Trinitrotoluene and reptile - Combination = 0
Trinitrotoluene and amphibian - Combination = 0

APPENDIX B
Benchmark Dose Calculation for Mammals

The data presented below are from Levine et al. (1990a) where mean body weight (in kg = Mean Response) was measured in dogs from a 6-month feeding study. The data from the most sensitive sex was used in the calculation. Data from changes in hemoglobin and hematocrit followed the same trend and resulted in benchmark dose estimates that were statistically equivalent (One-way ANOVA on Ranks, P > 0.40).



Results of the model are presented below:

BMD = 0.324674
BMDL = 0.21622

==========

Polynomial Model. $Revision: 1.1.1.8 $ $Date: 2000/03/22 17:51:39 $
Input Data File: A:\TNT.(d)
Gnuplot Plotting File: A:\TNT.plt

Fri Jul 14 12:23:19 2000

==========

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
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

alpha =	0.75
beta_0 =	10.7721
beta_1 =	-0.249975
beta_2 =	0.00637527

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.647838	5.97832
beta_0	10.7721	6.80499
beta_1	-0.249975	100.578
beta_2	0.00637527	3122.43

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-1e-007	-1e-007	-1.2e-007
beta_0	-1e-007	1	0.58	0.48
beta_1	-1e-007	0.58	1	0.98
beta_2	-1.2e-007	0.48	0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	6	11	0.5	10.8	0.805	0.283
0.5	6	10.4	1.2	10.6	0.805	-0.309
2	6	10.3	0.9	10.3	0.805	0.0029
8	6	9.2	1	9.18	0.805	0.0244
32	6	9.3	0.5	9.3	0.805	-0.00146

Model Descriptions for Likelihoods Calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-7.94995	6	27.8999
A2	-4.40918	10	28.8184
fitted	-8.48827	4	24.9765
R	-16.93	2	37.86

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.0416	8	<.0001
Test 2	7.08154	4	0.1316
Test 3	1.07665	2	0.5837

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.950000

BMD = 0.324674
BMDL = 0.21622