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

USCHPPM

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

DECEMBER 2005

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

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Acknowledgements

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

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

CAS Nos. 35572-78-2 and 19406-51-0

1. INTRODUCTION

This Wildlife Toxicity Assessment is based on a thorough review of the scientific literature regarding the toxicological characteristics of nitroglycerin that may pertain to 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 TG254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000). This document is designed to support ecological risk assessment activities.

The compounds 2-amino-4,6-dinitrotoluene (2A-DNT) and 4-amino-2,6-nitrotoulene (4A-DNT) are the primary reduction products of 2,4,6-trinitrotoluene (TNT). Both occur from the reduction of one of the nitro groups on the benzene ring. Hovatter et al. (1997) included 2A-DNT in their survey of the ecological toxicity of nitroaromatic compounds released from U.S. Army Superfund sites, because the compound is known to be a microbial degradation product of the military explosive, trinitrotoluene (TNT). Although less well characterized, the compound's structural isomer, 4A-DNT also may be produced from TNT under similar circumstances with the release of TNT from Army ammunition plants (AAPs) in large quantities (as "pink water"), the 2-amino isomer has been demonstrated to occur at sites within and around army munitions factories and during load, assembly and pack (LAP) activities at AAPs and other military installations.

This Wildlife Toxicity Assessment summarizes the current state of knowledge of the likely harmful impacts of 2A-DNT and 4A-DNT on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of the compound. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for all wildlife ranging in the vicinity of affected sites. 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). Since both 2A- and 4A-DNTs are often found *in vivo* and are the

primary reduction products in the metabolism of TNT, more information can be found in the Wildlife Toxicity Assessment for TNT.

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched on May 5, 2000 in DIALOG to identify primary reports of studies and reviews on the toxicology of 2A-DNT and 4A-DNT. Separate searches were carried out linking each compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. Abstracts of all hits were downloaded directly because of the comparative small number of identified articles. For 2A-DNT, four of thirteen reports of studies were retrieved for evaluation, whereas two of thirteen reports were retrieved for 4A-DNT. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to DIALOG searching, the Defense Technical Information Center was searched for relevant U.S. Army reports on the compounds, though without result. No other secondary sources of information were located.

2.2 Environmental Fate and Transport

Talmage et al. (1999) discussed the widespread occurrence of 2A-DNT at military sites as TNT is produced and released to the environment. The compound was detected in the soil at Joliet Army Ammunition Plant, Illinois, in concentrations of up to 19 mg/kg and up to 37 mg/kg at Raritan Arsenal, New Jersey. 2A-DNT is photosensitive at wavelengths >290 nm, suggesting that it may be able to undergo photolytic degradation . The biotic degradation of 2A-DNT has been demonstrated in the presence of mixed microbial isolates, undifferentiated sludge samples, and in the presence of pure cultures of *Pseudomonas* spp. Since prolonged incubation with these preparations can result in a mixture of degradation products including triaminotoluene, it is possible that microbial degradation is a relatively non-stereospecific enzymatic reduction process. This would suggest that 4A-DNT also may be able to undergo a similar range of interactions.

Empirical evidence exists that the amino dinitrotoluenes are formed relatively rapidly when TNT is released to the soil, but that they then persist. Degradation pathways in soil have also been outlined in Walsh (1990). This led Bumpus and Tatarko (1994) to suggest that degradation of the amino-dinitrotoluene isomers may be the rate-limiting step in the biodegradation of TNT. Some metabolic information supports this contention in animal systems as well (Yinon 1990, Johnson et al. 2000a).

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

CAS No.	35572-78-2
Molecular weight	197.17
Color	ND
State	crystals
Melting point	173-176^{o}C
Boiling point	ND
Odor	ND
Solubility	2A-DNT - 38 mg/L at 20 ^{o}C* 4A-DNT - 43 mg/L at 20 ^{o}C*
Partition coefficients Log K_{OW} K_{OC}	 1.06, 1.94, 0.5 (all estimated) ND
Vapor pressure (at 20°C)	4 x 10^{-5} mm Hg (estimated)
Henry's Law constant (at 25°C)	3 x 10^{-3} L-torr/mole (estimated)
Conversion factors	1 ppm = 8.06 mg/m^3 1 mg/m^3 = 0.124 ppm

Sources: Talmage et al. (1999), *Empirically determined (Allen and Major 2001).

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

There are very few data on the toxicity of the amino-dinitrotoluenes in experimental studies, and the only studies that were found addressed the acute lethality of the compound. For example, Ellis et al. (1980) reported data on the acute toxicity testing of a number of substituted and unsubstituted dinitrotoluenes including 2A-DNT and 4A-DNT in CD rats and Swiss or B6C3F1 mice. For 2A-DNT, LD_{50} values ranged from 1394 (female) to 2240 (male) mg/kg in the rats and 1522 (female) to 1722 (male) mg/kg in the mice. For 4A-DNT, the values ranged from 939 (female) to 1360 (male) mg/kg in the rats and from 1342 (male) to 1495 (female) mg/kg in the mice. (Table 2).

Ellis et al. (1980) also carried out a toxicokinetic study in which male CD rats were given a single oral dose of ^{14}C 4A-DNT at 1/10 the LD_{50}. Animals were kept in metabolic cages for the collection of expired air, feces and urine. At termination, major organs were excised to measure the radiological activity. The data suggested an absorption factor of approximately 50%, with most of the radioactivity being cleared to the urine. By contrast, there was very little tissue deposition of radioactivity and very little was expired on the breath.

Table 2. Acute data of oral exposures from 2A-DNT and 4A-DNT.

Compound	LD50 Mouse*	LD50 Rat*
2A-DNT	1722 ± 154 (m)	2240 ± 85 (m)
	1522 ± 71 (f)	1394 ± 191 (f)
4A-DNT	1342 ± 107 (m)	1360 ± 53 (m)
	1495 ± 90 (f)	959 ± 76 (f)

* mg/kg ± S.D., from Ellis et al. (1980).

2.3.1.2 Mammalian Oral Toxicity - Subchronic

No data are available.

2.3.1.3 Mammalian Oral Toxicity – Chronic

No data are available.

2.3.1.4 Mammalian Oral Toxicity – Other

No data are available.

2.3.1.5 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Few data are available for the monoamine DNTs. These data represent studies in rats and mice from acute exposures only (Ellis et al. 1980). Since these data are relatively similar, both 2A-and 4A-DNTs were treated together and used to develop values.

2.3.2 Mammalian Inhalation Toxicity

No data are available.

2.3.3 Mammalian Dermal Toxicity

No data are available.

2.4 Summary of Avian Toxicology

Toxicological data for the effects of amino-DNTs in avian species were not found. Ecotoxicological research on the effects of this compound in birds is recommended.

2.5 Summary of Amphibian Toxicology

Johnson et al. (2000b) exposed 9 tiger salamanders (*Ambystoma tigrinum*) to soil and earthworms containing TNT and 2A-and 4A-DNT. Initial soil concentrations (at time of initial exposure) were 280, 39, and 62 mg/kg soil of TNT, 2A- and 4A-DNT, respectively. Appropriate controls were used. At the termination of exposure (14 days later) soil concentrations were 59, 58, and 78 mg/kg soil of TNT, 2A- and 4A-DNT, respectively. Salamanders were fed earthworms in identical soil preparations. Earthworm concentrations ranged from 0.25- 0.79 µg/g TNT, 2.1 – 2.6 µg/g 2A-DNT, 2.1-2.5 µg/g 4A-DNT, and had trace amounts of 2,4-diamino-6-nitrotoluene. Salamanders were evaluated for immunological indicators of effects (phagocytosis, radical oxygen intermediate production), blood parameters (5 part differentials, total protein, hematocrit, etc.), and for histopathological indicators of the liver and kidney. No adverse effects were reported for any endpoint, and it was remarked that the animals appeared healthy and maintained an appetite. No adverse effects were reported from exposure to these conditions.

2.5.1 Studies Relevant for Amphibian TRV Development for All Exposures

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 2A- and 4A-DNT were monitored, these data are used to derive a NOAEL for terrestrial salamanders. A minimum soil concentration of 40 mg/kg was selected, which reflects all exposure pathways. Since adverse effects were not observed in the study, a LOAEL is not available.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of DNTs in reptilian species was not located. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Only acute data were available for 2A- and 4A-DNT [see Ellis et al (1980)]. The data are fairly consistent and the studies were reported and evaluated properly. Given these data, a TRV may be approximated through the application of uncertainty factors prescribed by TG254 (USACHPPM 2000).

Using the lowest LD50 value (for each sex and species) and applying an uncertainty factor of 100 and 20, a NOAEL-based and LOAEL-based TRV can be derived, respectively. These values are presented in Table 3.

Since these values are based only upon acute data, these TRVs are given a **LOW** degree of confidence.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	9.0 mg/kg/d	Low
LOAEL-based	48.0 mg/kg-d	Low

Both isomers have relatively low water solubility. This suggests that the bioavailability of these compounds is low. These facts coupled with the relative toxicity of TNT suggest that these values should be protective for mammals and that incidence of toxicity is considered low.

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

This assessment is not yet complete pending available data.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

This assessment is not yet complete pending available data.

3.2 Toxicity Reference Values for Birds

No data to derive values for birds were found.

3.3 Toxicity Reference Values for Reptiles

No data to derive values for reptiles were found.

3.4 Toxicity Reference Values for Amphibians

Johnson et al. (2000b) identified values of 2A- and 4A-DNT in soil where no adverse effects were observed in tiger salamanders. 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 (39 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 a NOAEL-based TRV of 0.13 mg 2A- DNT or 4A-DNT/kg soil dry weight intended to be protective of terrestrial amphibians. However, since an

LOAEL was not identified, an approximation of a LOAEL-based TRV could not be derived. Table 4 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 4. Selected Soil TRVs for Terrestrial Amphibians

TRV	Dose	Confidence
NOAEL-based	0.13 mg/kg soil (dry weight)	Low
LOAEL-based	Not available	—

4. IMPORTANT RESEARCH NEEDS

Only acute data are available for mammals and for salamanders. No data are available for birds, reptiles, or other species of amphibians. Long-term (i.e., subchronic to chronic) oral toxicity testing is needed for all vertebrates, and acute data are needed for birds and other species of mammals (non-rodent). In addition, metabolic data that provides evidence of bioavailability of both compounds is also needed.

5. REFERENCES

Allen, N.A., and M.A. Major. 2001. The Re-evaluation of Water Solubility and Octanol/Water Partition Values for Military Explosives Commonly Found in Soil Contaminants. USACHPPM Report (*in prep*).

Bumpus, J.A., and M. Tatarko. 1994. Biodegradation of 2,4,6-trinitrotoluene by *Phanerochaete chrysosporium*: identification of initial degradation products and the discovery of a TNT metabolite that inhibits lignin peroxidases. Curr. Microbiol. 28: 185-190. (as cited in Talmage et al., 1999).

Ellis III, H.V., C-B Hong, and C-C. Lee. 1980. Mammalian toxicity of munitions compounds. Summary of toxicity of nitrotoluenes. AD A080146. Prepared by the Midwest Research Institute, Kansas City, MO for the U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

Hovatter, P.S., S.S. Talmage, D.M. Opresko, and R.H. Ross. 1997. Ecotoxicity of nitroaromatics to aquatic and terrestrial species at army Superfund sites. Pp. 117-129 *in* Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment. Sixth Vol. (F.J. Dwyer, T.R. Doane and M.L. Hinman, Eds.) American Society for Testing and Materials.

Johnson, M.S., J.K. Vodela, G. Reddy, and S.D. Holladay. 2000a. Fate and the biochemical effects of 2,4,6-Trinitrotoluene (TNT) exposure to tiger salamanders: (*Ambystoma tigrinum*). Ecotoxicology and Environ. Safety 46:186-191.

Johnson, M.S., S.D. Holladay, K.S. Lippenholz, J.L. Jenkins, and W.C. McCain. 2000b. Effects of 2,4,6-trinitrotoluene in a holistic environmental exposure regime to a terrestrial salamander: *Ambystoma tigrinum.* Toxicological Pathol. 28(2):334-341.

Talmage, S.S., D.M. Opresko, C.J. Maxwell et al. 1999. Nitroaromatic munition compounds: Environmental effects and screening values. Revs. Environ. Contam. Toxicol. 161:1-156.

U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, *Standard Practice for Wildlife Toxicity Reference Values*, USACHPPM 2000.

Walsh, M. 1990. Environmental Transformation Products of Nitroaromatics and Nitramines. Special Report No. 90. U.S. Army Corps of Engineers, Cold Regions Research & Engineering Laboratory, Hanover, New Hampshire.

Yinon, J. 1990. Toxicity and Metabolism of Explosives. CRC Press, Boca Raton, FL.

APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 10 AGRICOLA, File 203 AGRIS, File 399 Chemical Abstracts, File 337 CHEMTOX, File 77 Conference Papers Index, File 35 Dissertation Abstracts, File 40 ENVIRONLINE, File 68 Environmental Bibliography, File 76 Life Sciences Collection, File 41 Pollution Abstracts, File 336 RTECS, File 370 Science, File 143 Wilson Biological & Agricultural Index, File 185 Zoological Record, File 6 NTIS, File 50 CAB, File 144 PASCAL, File 34 SCISEARCH.

The search strategy for **Amphibians & Reptiles**:

- Chemical name, synonyms, CAS numbers
- AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- RD (reduce duplicates)

The search strategy for **Birds:**

- Chemical name, synonyms, CAS numbers
- And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- RD

The search strategy for **Laboratory Mammals:**

- Chemical name, synonyms, CAS numbers
- AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- NOT ((meeting()poster) or (meeting()abstract))
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- RD

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers

- And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?

- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))

- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. Since only a limited number of studies were identified by the search, all abstracts were downloaded at the time of the search.

As noted in Section 2.1, 13 hits on 2A-DNT and 13 hits on 4A-DNT were obtained in the searches, of which four (2A-DNT) and two (4A-DNT) were selected for retrieval.