

**Wildlife Toxicity Assessment for  
2,4-Dinitroanisole (DNAN)**

**WTA No. HEF-042019-004**

**Toxicology Directorate, Health Effects Research Division**

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**April 2020**



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The military energetic compound 2,4-dinitroanisole (DNAN, also known as 1-methoxy-2,4-dinitrobenzene) was historically used as an explosive in warheads containing Amatol 40 and is currently being investigated as a replacement for 2,4,6-trinitrotoluene (TNT) in melt-cast insensitive munition (IM) formulations. DNAN is also used industrially in the synthesis of dyes and insect repellants. This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of DNAN on wildlife. Evaluating the toxicity of DNAN will contribute to the derivation of toxicity reference values (TRVs) for use as screening-level benchmarks for wildlife near contaminated sites. The protocol for the performance of this WTA is available in detail in Technical Guide No. 254 (Standard Practice for Wildlife Toxicity Reference Values).

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**U.S. Army Public Health Center  
Department of the Army**

**Wildlife Toxicity Assessment for 2,4-Dinitroanisole (DNAN)**

CAS No. 119-27-7

April 2020

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## **1. INTRODUCTION**

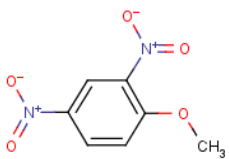
The military energetic compound 2,4-dinitroanisole (DNAN, also known as 1-methoxy-2,4-dinitrobenzene and referred to as DNA in early reports) was historically used as an explosive in warheads containing Amatol 40 and is currently being investigated as a replacement for 2,4,6-trinitrotoluene (TNT) in melt-cast insensitive munition (IM) formulations. DNAN is also used industrially in the synthesis of dyes and insect repellants. DNAN is a low sensitivity explosive organic compound. It has an anisole core with two nitro groups attached. (Defense Science and Technology Organisation, 2006) As compared with TNT, DNAN has 90% of the energetic or explosive power and is less dense with a higher melting point. Within the U.S. Army, DNAN is used in the following explosive formulations:

- IMX 101, 105 mm and 155 mm artillery high explosive (HE),
- IMX 104 (60/81/120 mm Mortar),
- OSX-12, PAX 21 (60 mm M720A1/M768 Mortars),
- PAX 41 (Spider Grenade), and
- PAX 48 (120 mm HE –t) (Defense Science and Technology Organisation 2006; USAPHC. 2014a).

DNAN is also used as a key component of MYL louse powder for the treatment of head lice, which is comprised of 0.2% permethrins, 2% IN-930, 0.3% phenol, 2% DNAN, and pyrophyllite inert diluent (Eddy 1948). Table 1 summarizes the chemical/physical properties of DNAN.

## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

**Table 1. Summary of the Physical-Chemical Properties of 2,4-DINITROANISOLE (DNAN)**

Structure	
	
CAS No.	119-27-7
Molecular weight <sup>3</sup>	198.1
Color <sup>2</sup>	Tan to yellow
Physical state	Crystalline solid
Melting point	94.5°C (202.1°F), 94-96°C
Boiling point (760 mmHg) <sup>2</sup>	351°C (664°F)
Density	1.34 at 25°C
Odor	No Data Available
Solubility in water <sup>1</sup>	0.216 mg/L
Solubility in other solvents <sup>2</sup>	Soluble in ethanol, ether, acetone, and benzene; very soluble in pyridine.
Partition coefficients:	
Log K <sub>ow</sub>	1.58
Log K <sub>oc</sub>	--
Vapor pressure at 25°C <sup>2</sup>	1.4 x 10 <sup>-4</sup> mm Hg
Henry's Law constant at 25°C <sup>4</sup>	7.2249 x 10 <sup>-6</sup> atm-m <sup>3</sup> /mole
Vapor density	6.8
Conversion factors	1 ppm = 8.10 mg/m <sup>3</sup> ; 1 mg/m <sup>3</sup> = 0.12 ppm

Source:

<sup>1</sup> Hawari et al., 2015

<sup>2</sup> OARS WEEL 2014

<sup>3</sup> Lent et al., 2016

<sup>4</sup> Boddu et al., 2008

## 2. TOXICITY PROFILE

### 2.1 Literature Review

Electronic searches of relevant biomedical, toxicological, and ecological databases were conducted on 16–17 August 2018, with the aim of identifying primary reported studies and reviews on the toxicology of DNAN. These databases include BIOSIS®, PubMed®, Defense Technical Information Center (DTIC®) online multisearch, Scopus, Web of Science, and TOXNET®, which is an aggregated tool for simultaneously searching the following databases: HSDB®, TOXLINE®, ChemIDplus®, IRIS; LactMed; DART®; TOXMAP®; TRI; CTD; Household

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Products Database; Haz-Map; ITER; ALTBIB; CCRIS; CPDB; and GENE-TOX.

Separate searches were conducted for general toxicology and specific searches for birds, reptiles, amphibians, and wildlife. Each database was searched using key words such as DNAN, 2,4-dinitroanisole, or 119-27-7, in addition to toxicity, ecotoxicology, wild, wildlife, avian, bird, frog, amphibian, reptile, or environment. Appendix A documents the details and results of the search strategies. The titles of articles identified in each search were reviewed for relevance. Potentially relevant articles focused on the toxicological effects of DNAN on terrestrial vertebrates or its environmental fate and transport. All potentially relevant articles were acquired as electronic files or by visiting the Johns Hopkins University School of Medicine's libraries. Review articles provided additional articles not identified during the initial database searches.

### 2.2 Environmental Fate and Transport

The fate and transport of DNAN in the environment is poorly documented or known and is very limited due in part to the sparsity of published work in the peer-reviewed literature or from the gray literature found elsewhere. Likewise, there is little information reported on the occupational exposure scenarios relevant to DNAN. DNAN discharged in effluent from production facilities is typically reduced or degraded to 2,4-dinitrophenol (2,4-DNP) following bacterial metabolic pathways. In addition, only a few studies have reported the biological metabolic transformation of DNAN under aerobic and anaerobic conditions (Perreault et al., 2012; Platten et al., 2010; Olivares et al., 2016).

Under aerobic conditions, a *Bacillus* strain was reported to transform DNAN to 2-amino-4-nitroanisole (2-ANAN) as the predominant end product of metabolism (Perreault et al., 2012). Others have demonstrated aerobic biodegradation of DNAN by *Nocardioides* sp. (Fida et al., 2014). The first reaction of microbial metabolism involves hydrolytic release of methanol to form 2, 4-dinitrophenol. The bacterial biotransformation of DNAN under conditions of anaerobic transformation with zero valent iron involves reduction of the nitroso group at the ortho position to yield 2-ANAN. Others have shown that regional selectivity is part of the liberation of 2-ANAN following abiotic transformation with zero valent iron or under conditions of bacterial metabolism, which under anaerobic conditions yields 2,4-diaminoanisole (DAAN) (Hawari et al., 2015). Hawari et al., (2015) also showed that reduction of the nitro groups in wet, anaerobic soils is expected to result in amines that are subsequently irreversibly bound to the humic fractions of the soil, similar to other nitroaromatics.

The aqueous solubility of DNAN (slightly soluble in water 0.213 grams per liter (g/L)) is much less than that of TNT (130 milligrams per liter (mg/L) in water). The lower hydrophobicity and tendency of DNAN to form amino derivatives that sorb irreversibly to soil, contributes to its purported much lower toxicity than the traditional explosive TNT (Hawari et al., 2015). Under conditions of sunlight exposure, DNAN is transformed to nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>) and 2,4-dinitrophenol via photo-oxidation. Photolysis of DNAN occurs rapidly in the presence of sunlight and the compound has a temporal half-life of 0.6 to 1.0 day (Rao et al., 2013). The photo degradation of DNAN was studied both in pure solid and as part of IMX formulations. The degradation products were found to be small <1% relative to DANA and products like methoxy nitrophenol and methoxy nitroaniline were consistently detected (Taylor et al., 2017). DNAN

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adsorption to Pahokee peat and montmorillonite was high, and DNAN could not be detected in aqueous solution after 7 days. DNAN also showed a stronger sorption affinity for the three soil types tested (Hawari, 2013; USAPHC, 2014a).

An ecotoxicological assessment of DNAN found decreased growth of algae ( $EC_{50}$  value of 4.0 mg/L) (Dodard et al., 2013). In soil, DNAN decreases the growth of perennial ryegrass ( $EC_{50} = 7$  milligrams per kilogram (mg/kg)) and it was found to be lethal to earthworms ( $LC_{50} = 47$  mg/kg). The microbial toxicity of DNAN in aerobic and anaerobic sludge was measured by the Microtox assay that uses bioluminescent bacterium *Aliivibrio fischeri* (Liang et al., 2013). DNAN undergoes facile microbial reduction to 2-methoxy-5-nitroaniline (MENA) and DAAN, which were found to produce inhibitory effects on growth of algae (Liang et al., 2013).

### 2.3 Bioaccumulation and Elimination

Detailed information on the metabolism or pharmacokinetics of DNAN is currently unavailable. Studies have demonstrated that DNAN is metabolized *in vivo* to 2,4-DNP via oxidative cleavage of the methoxy group. The extent and rate of metabolism are currently unknown (Agency for Toxic Substances and Disease Registry (ATSDR)), 1995; Harris and Cocoran, 1995). An acute inhalation and oral toxicity study was conducted in male and female rats (USAPHC, 2015). This study showed that DNAN is rapidly absorbed and metabolized to DNP by both exposure routes and is eliminated via the urine (USAPHC, 2015).

Others have dosed DNAN at 47 mg/kg by oral administration in corn oil to rhesus macaques (*Macaca mulatta*), and tracked levels of DNAN in the blood and urine over a 24-hour period (Hoyt et al., 2013). DNAN was rapidly metabolized to 2,4-DNP and was subsequently detected in both the blood and urine. The absorption of DNAN is lower during this 24-hr period, which demonstrated that the levels in the blood increased from approximately 1.7 to 2.7 micrograms per milliliter ( $\mu\text{g/mL}$ ) over a 5-hour period (Hoyt et al., 2013). In addition, DNAN was administered at a dose of 25 mg/kg, which showed peak levels in the blood within a 5-hour period. At the lowest dose tested of 5 mg/kg administered to monkeys, neither DNAN nor its metabolite DNP were detected in the serum or in urine during a 48-hour period.

### 2.4 Summary of Mammalian Toxicity

#### 2.4.1 Mammalian Oral Toxicity

##### 2.4.1.1 Acute

The acute oral lethal dose ( $LD_{50}$ ) in rats was found to be 199 mg/kg in both male and female rats. Clinical signs of toxicity included decreased activities, breathing abnormalities, salivation, and soft stools (Air Force Research Laboratory, 2002). No remarkable clinical findings or gross lesions were discovered at necropsy. Others have reported an approximate lethal dose (ALD) of DNAN, which was calculated as 300 mg/kg in rats (USAPHC, 2012a). In 14-day subacute toxicity studies conducted in rats at doses of 1.5, 3, 6, 12.5, 25, 50, and 100 mg/kg, no mortalities were observed. Clinical signs of toxicity observed in male rats included lethargy, and rapid respiration and labored breathing, a prostrate posture, and salivation. In addition, dark urine, orange feces, and barbering were observed in the 100 mg/kg dose group. Lethargy, dark



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urine, and congested breathing were noted in the 50 mg/kg dose group. The lowest observed adverse effect level (LOAEL) for DNAN was estimated as 50 mg/kg in subacute toxicity studies conducted in the rat (Lent et al., 2016). Table 2 summarizes the acute and subacute mammalian oral toxicity for DNAN.

**Table 2. Summary of Acute and Subacute Oral Toxicity for DNAN in Mammals**

Test Organism	LD <sub>50</sub> (mg/kg)	Test Results			Study
		NOAEL (mg/kg)	LOAEL (mg/kg)	Effects Observed at the LOAEL	
Rats	199	NA	NA	Acute oral both male and female rats combined. Clinical observations included decreased activities, breathing abnormalities and salivation	Air Force Research Laboratory 2002
Rats	ALD 300	NA	50	Lethargy, rapid respiration, salivation, dark urine	Lent et al., 2016

Legend:

NA = not available/not reported

ALD = Approximate Lethal Dose

### 2.4.1.2 Subchronic

Lent et al., (2016) conducted a subchronic toxicity of DNAN in male and female Sprague-Dawley rats in which rats were dosed with DNAN via oral gavage in corn oil at 0, 1.25, 5, 20, and 80 milligrams per kilogram per day (mg/kg-day) for 90 days. Mortality was observed at the highest dose; three males died on days 50, 63, and 77, and one female died on day 26 of the study. Rats from the 80 mg/kg-day dose group experienced lethargy, labored/rapid respiration, prostrate and/or recumbent posture, hunched posture, ear twitching, squinting, curled tail, and gait irregularities. A functional observation battery (FOB) and motor activity analysis at week 13 indicated that rats treated with 80 mg/kg-day DNAN were presented with altered neuromuscular function and decreased activity levels. Also, in the 80 mg/kg-day group, female rats displayed reduced sensorimotor responses, while male rats displayed increased excitability responses. Neurobehavioral evaluations did not indicate any treatment-related effects at a dose of less than or equal to 20 mg/kg-day (Lent et al., 2016).

Although food intake was similar among groups of male rats, animals from the 80 mg/kg-day dosed group exhibited reduced body mass and a reduced food efficiency ratio (Lent et al., 2016). Body mass did not differ among dosed female rats; however, female rats in the 80 mg/kg-day dose group displayed a reduced food efficiency ratio. Although these animals had elevated food consumption at several of the time points throughout the study.

Female rats in the 80 mg/kg-day dose group and male rats in the 20 mg/kg-day group produced higher volumes of urine and with a lower specific gravity (Lent et al., 2016). Despite the increase in volume, urine color in both male and female rats was darker in the 20 and 80 mg/kg-day dosed groups. Increased mean kidney, liver, and spleen mass were observed in male and

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female rats given 80 mg/kg-day DNAN (Lent et al., 2016). In male rats, increased mean kidney and liver mass were also noted in the 20 mg/kg-day dosed group. These changes were not associated with treatment-related microscopic abnormalities or altered clinical chemistry parameters. However, several clinical chemistry parameters in the controls exceeded reported normal levels for this strain (Sprague-Dawley) of rat (Lent et al., 2016).

Decreased mass of the testes and epididymides, and degeneration of the testicular seminiferous tubules with atrophy and aspermia were observed in rats from the 80 mg/kg-day group (Lent et al., 2016). In female rats, altered hematology, which was indicative of anemia, including decreased red blood cell count, hematocrit, and hemoglobin; increased red blood cell distribution width were observed in the 80 mg/kg-day dosed group. A dose-dependent increase in extramedullary hematopoiesis (HME) was noted in the spleens of female rats that were administered 20 and 80 mg/kg-day (Lent et al., 2016). Glial lesions within the cerebellum were noted in four of the rats (i.e., 1 female and 3 males) in the 80 mg/kg-day group.

The no observed adverse effect level (NOAEL) from this study was 5 mg/kg-day. Benchmark Dose Software (BMDS v.2.1.2) was used to fit mathematical models to the extramedullary hematopoiesis (EMH) incidence dose response data and calculate a lower-bound 95% confidence limit on a dose corresponding to a 10% response rate (BMDL10). The Log Logistic model was selected based on goodness-of-fit and statistical parameters and a mean BMDL10 value of 0.93 mg/kg-day. The benchmark dose (BMD) of 1.76 mg/kg-day value was determined (Lent et al., 2016; Occupational Alliance for Risk Science (OARS) Workplace Environmental Exposure Level (WEEL), 2014).

### **2.4.1.3 Subchronic: Reproductive and Developmental Toxicity**

Only a few studies were available for the reproductive or developmental toxicity of DNAN. The Air Force Research Laboratory (2002) conducted a prenatal developmental toxicity study with Composition B Replacement #12E (i.e., CBR-12 or PAX-21), which contained 34% DNAN. Pregnant rats were orally exposed via gavage at 0, 15, 30, or 60 mg/kg-day on gestational days 6–19. The 60 mg/kg-day exposure level (and an attempted lower dose of 45 mg/kg-day) caused maternal mortality and morbidity that precluded analysis of developmental effects. Adverse effects were observed in the 30 mg/kg-day dose group that included decreased maternal body weight and weight gain. The maternal and fetal NOAEL values were 15 mg/kg-day (Air Force Research Laboratory 2002).

In another developmental toxicity study (Gao et al., 2016), adult Sprague-Dawley rats were mated at ratios of 1:1, and the pregnant rats were randomly divided into five groups at the confirmed time of pregnancy. The negative control groups were oral gavaged with a 4% weight/volume solution of starch, and the three experimental groups were gavaged with a DNAN suspension at respective doses of 5, 15, and 45 mg/kg-day (Gao et al., 2016). The positive control for this study was pregnant rats that received aspirin at a dose of 280 mg/kg-day. All rats from each of the five groups were administered the respective test article or control from gestation day (GD) 5 to GD19, which were administered continuously. On day 20, embryo and fetal developmental toxicity were evaluated. The net weight growth for all three dosed groups was found to be lower than their counterparts in the negative controls. The body weight,

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body and tail lengths, and anal-genital distances for fetal rats in the high dose group were all less than their counterparts in the negative control groups.

The presence of external fetal malformations were examined for all three dose groups with no significant differences observed when compared to the negative control group (Gao et al., 2016). However, the prevalence of skeletal malformations in the high dose group (i.e., 45 mg/kg-day) and evidence of internal organ malformations in the median (15 mg/kg-day) and high dose (45 mg/kg-day) treated groups appeared to be higher than found for the negative controls. There were no effects reported for any of the rats in the 5 mg/kg group. From this study, the NOAEL was 5 mg/kg-day for developmental toxicological effects (Gao et al., 2016). Table 3 summarizes the subchronic mammalian oral toxicity for DNAN.

**Table 3. Summary of Subchronic Oral Toxicity for DNAN in Mammals**

Test Organism	Test Duration	Test Results				Effects Observed at the LOAEL	Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD	BMDL <sub>10</sub>		
Sprague – Dawley rats	90 day oral	5	20			Increased kidney and liver mass	Lent et al., 2016
Sprague-Dawley rats	90 day oral			1.76	0.93	Extra medullary hematopoiesis	Lent et al., 2016
Sprague - Dawley rats	GD 5 to 19 day	NA	15			No significant differences in fetus external malformation, skeleton malformation in high dose and internal malformation in mid and high dose group	Gao et al., 2016

Legend:

NA=not available.

### 2.4.2 Mammalian Inhalation Toxicity

#### 2.4.2.1 Acute

An acute inhalation exposure was performed in Sprague-Dawley rats by heating DNAN to 175 degrees Celsius (°C) to generate DNAN vapors. The target concentration was 1 to 5 milligrams per cubic meter (mg/m<sup>3</sup>) (actual average concentration, 2.8 mg/m<sup>3</sup>). A second phase of the study involved dissolving DNAN in acetone and generating an aerosol to achieve a target concentration of 2,000 mg/m<sup>3</sup> (actual average concentration, 2,933 mg/m<sup>3</sup>). No mortalities were observed in either group. No clinical signs of toxicity were observed at the lower vapor-based exposure. Clinical signs of toxicity during the aerosol exposure consisted of decreased activity and labored breathing. Clinical signs observed post-exposure included increased salivation, lacrimation, and red or clear nasal discharge. These symptoms resolved in all animals within several days. There were no macroscopic post-mortem findings at the end of the 14-day post-exposure period that were considered treatment related. The inhalation LC<sub>50</sub> was thus judged to

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be greater than 2.9 grams per cubic meter (g/m<sup>3</sup>) (Hoffman, 2000a; OARS WEEL, 2014). Table 4 summarizes the acute and subacute mammalian inhalation toxicity for DNAN.

**Table 4. Summary of Acute and Subacute Inhalation Toxicity for DNAN in Mammals**

Test Organism	LC <sub>50</sub> (mg/m <sup>3</sup> )	Test Results		Effects Observed at the LOAEC	Study
		NOAEL (mg/m <sup>3</sup> )	LOAEL (mg/m <sup>3</sup> )		
Rats	2,933	NA	NA	Acute 4-hr study; no signs of toxicity	Hoffman 2000a; Air Force Research Laboratory 2002
Rats	NA	NA	150	2-week study (6-hr exposure per day at 150, 500, and 1,500 mg/m <sup>3</sup> ) to aerosolized DNAN for a total of 11-days. Decreased food consumption, irregular gait, lethargy, labored breathing, nasal discharge, decreased blood urea nitrogen, increased kidney weight; microscopic findings were non-specific; minimal metaplasia of the laryngeal epithelium observed	Hoffman 2000b; Air Force Research Laboratory 2002
Rats	>2.4 mg/L	NA	NA	Acute 4-hr study; no compound mortality observed; no adverse effects observed by gross necropsy and pathology, no remarkable effects by blood analysis	USAPHC 2015

Legend:

NA=not applicable.

In a study that evaluated the acute inhalational toxicity of DNAN and its blood absorption (USAPHC, 2015), male and female Sprague-Dawley rats were exposed by nose-only inhalation of aerosolized DNAN at a dose of 2.4 mg/L (USAPHC, 2015). DNAN did not induce any compound-related mortality, adverse toxicological signs from exposure, altered body weight, or remarkable findings from gross necropsy. The LC<sub>50</sub> of DNAN by acute exposure was determined to be greater than 2.4 mg/L. Blood sample analysis showed rapid absorption and metabolism of DNAN. The mean whole blood concentration of DNAN in male rats was 16.7 µg/mL following 4.2–4.3 hours of inhalational exposure (USAPHC, 2015). In female rats, the mean whole blood DNAN concentration was 12.5 µg/mL following 4.2 hours of exposure. DNAN was rapidly metabolized to DNP as detected in the blood of rats.

### 2.4.2.2 Subacute

Male and female Sprague-Dawley rats (5 animals/sex/group) were exposed to DNAN that had been dissolved in acetone at nominal concentrations of 150, 500, and 1,500 mg/m<sup>3</sup> of aerosol

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vapor for 6 hours per day; 5 days per week, for a total of 11 days. The actual average inhalational exposure concentrations were 165, 535, and 1,313 mg/m<sup>3</sup> (Hoffman, 2000b; OARS WEEL, 2014). Control animals were exposed to acetone aerosol or vapor alone (at a dose of 23,727 mg/m<sup>3</sup>). All animals in the 1,500 mg/m<sup>3</sup> exposure group and 8/10 animals in the 500 mg/m<sup>3</sup> exposure group were found dead or were euthanized during the exposure period. Clinical signs of toxicity observed prior to euthanasia included decreased food consumption, prostration, irregular gait, lethargy, head bobbing, poor physical condition, ambulatory movement that was directionally backwards, labored breathing, and red nasal discharge. Animals exposed to DNAN at a concentration of 500 mg/m<sup>3</sup> gained less weight and consumed less feed during the first week of exposure than did the acetone-exposed controls (Hoffman, 2000b; OARS WEEL, 2014).

Male rats that were exposed to 150 mg/m<sup>3</sup> had significantly decreased blood urea nitrogen (BUN) and had increased kidney weights. Females in the 150 mg/m<sup>3</sup> had statistically significant decreases in mean hemoglobin concentrations, mean corpuscular volume, and mean corpuscular hemoglobin with increased mean absolute monocyte counts and liver weight as compared to the acetone control group (Hoffman, 2000b; OARS WEEL, 2014). The urine of both male and female rats exposed to 150 mg/m<sup>3</sup> was darker than the acetone-treated controls. Irregular gait was periodically noted in the 150 mg/m<sup>3</sup> dose group; however, this was also observed in controls and was attributed to the CNS depressing effects from acetone exposure. The only reported DNAN-related microscopic finding was the observation of non-specific minimal metaplasia of the laryngeal epithelium in rats that were exposed to nominal concentrations of 500 and 150 mg/m<sup>3</sup> DNAN. The inhalational exposure concentration of 150 mg/m<sup>3</sup> (actual average concentration: 165 mg/m<sup>3</sup>) was therefore the DNAN LOEL of DNAN for this study (Hoffman, 2000b; OARS WEEL, 2014).

### 2.4.2.3 Subchronic and Chronic Toxicity

No studies on subchronic or chronic exposure to DNAN could be located.

### 2.4.3 Mammalian Toxicity—Other

Assays to assess potential eye irritation in rabbits indicated that DNAN was mildly irritating to the eye with a maximum average score of 12.0 at 1 hour and clearing observed by 48-hour post-treatment (Air Force Research Laboratory, 2002).

The skin irritation tests in rabbits indicated that DNAN produced slight dermal irritation (primary irritation index range from 0.08 to 0.25) which was transient, clearing after 24–48 hours (Air Force Research Laboratory, 2002). In the context of skin sensitization, this was not observed when DNAN was administered to guinea pigs in the standard sensitization assay (Air Force Research Laboratory, 2002).

The rate of absorption of pure DNAN was determined through a dermatome rat skin model in static diffusion cells over a 6-hour period at 32°C. The rate of penetration was 1.55 micrograms per square centimeters per hour (µg/cm<sup>2</sup>/hr). DNAN was applied to the skin as a powder, which was the same form encountered in the real-world situation by workers. When DNAN was applied to the skin as part of an explosive mixture (i.e., CBR-12, also known as PAX-21, which

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has a DNAN composition of approximately 34%), the dermal penetration rate of DNAN was estimated at 0.74 µg/cm<sup>2</sup>/hr. The rate by comparison, steady state flux of TNT from Composition B, and modeled in the same system, was 1.14 µg/cm<sup>2</sup>/hr (Air Force Research Laboratory (AFRL), 2002; AFRL, 2000).

Dermal penetration of DNAN has also been studied in static Franz diffusion cells (USAPHC, 2012b). Human epidermal membranes were prepared from frozen cadaver skin and were mounted on static diffusion receptor cells so that the visceral side was in contact with the receptor fluid. Test chemicals in powder form were administered at 100 mg by carefully placing the test compound on the skin in a donor chamber.

At different times following the application of the test compound (i.e., at 1, 2, 4, 6, and 8 hours), 0.1 mL of receptor fluid was collected and quantified for component content by HPLC analysis (USAPHC, 2012b). The dermal penetration rate showed that the steady state flux of neat powdered DNAN was 1.10 µg/cm<sup>2</sup>/hr (USAPHC, 2012b).

*In silico* modeling by Quantitative Structure-Activity Relationship (QSAR) predicted a chronic LOAEL in rats of 17.3 mg/kg-day (U.S. Army Public Health Command (USAPHC) 2014b).

### 2.5 Summary of Avian Toxicology

The available toxicological data on avian species is limited; therefore, it does not avail itself to an accurate determination of acute oral toxicity or LD<sub>50</sub> values. One group reported on administering DNAN by the oral route of exposure at doses of 120 or 150 mg/kg in 2-week old male Japanese quail (*Coturnix japonica*) (Takahashi et al., 1988). Mortality was noted at both dose levels, and found to be 20% (or 1/5 quail) in the 120 mg/kg exposure group and 50% (or 5/9) in the 150 mg/kg exposure group. All treated birds developed cataracts within 4 hours following exposure, which continued for 13 hours. The oral dose of 150 mg/kg DNAN was assigned as the LD<sub>50</sub> over a 24-hr period based on this reported mortality data. Table 5 summarizes the acute toxicity for DNAN in avian species.

**Table 5. Summary of Acute DNAN Toxicity in Avian Species**

Test Organism	Test Results				Study
	LD <sub>50</sub> (mg/kg)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Japanese Quail ( <i>Coturnix japonica</i> )	150	NA	NA	Mortality observed and cataract formation in an acute toxicity study	Takahashi <i>et al.</i> , 1988

Legend:  
NA=not applicable.

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### 2.6 Summary of Amphibian Toxicology

Stanley et al., (2015) reported on the acute and chronic (28-day) toxicity of DNAN on exposing the Northern Leopard Frog (*Lithobates pipiens*). The 96-hour LC<sub>50</sub> value from DNAN exposure was 24.3 mg/L (95% CI - 21.3–27.6 mg/L). The lowest observed effect concentration (LOEC) for mortality from the 28-day exposure to DNAN was 2.4 mg/L. Changes in growth, swimming distance, and other non-lethal parameters did not differ when comparing the DNAN-exposed frogs and controls. Stanley et al., (2015) studied bioaccumulation kinetics of the munition chemicals TNT, RDX, and DNAN in *Rana pipiens* tadpoles, which showed that absorption of these chemicals was rapid, uptake and that clearance had slowed—characteristics that were similar among the different compounds (i.e., 1.32–2.19 liters per kilogram per hour). Upon transfer to uncontaminated water, the elimination rate was rapid and resulted in the prediction of a rapid period of time to approach a steady-state (i.e., 5 hours or less) and a short elimination half-life (i.e., 1.2 hours or less). Since DNAN is rapidly eliminated, there were no detectable metabolites of this compound demonstrated since it did not bioaccumulate in amphibians (Stanley et al., 2015). Table 6 summarizes the acute and subacute toxicity for DNAN in amphibian species.

**Table 6. Summary of Acute and Subacute of DNAN Toxicity in Amphibian Species**

Test Organism	Test Results				Study
	EC <sub>50</sub> (mg/L)	NOAEL (mg/L)	LOAEL (mg/L)	Effects Observed at the LOAEL	
Leopard frog ( <i>Lithobates pipiens</i> )	24.3 For 96 hrs	NA	NA	Mortality observed at the EC <sub>50</sub>	Stanley <i>et al.</i> , 2015
Leopard frog ( <i>Lithobates pipiens</i> )	NA	NA	2.4 For 28 days	No developmental stage effects nor significant altered growth effects seen at the LOAEL	Stanley <i>et al.</i> , 2015

Legend:  
NA=not applicable.

### 2.7 Summary of Reptilian Toxicology

No toxicological data were located for the effects of DNAN on reptiles in the primary or grey literature.

## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

### 3. RECOMMENDED TOXICITY REFERENCE VALUES (TRVs)

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#### 3.1 TRVs for Mammals

##### 3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The acute oral LD<sub>50</sub> was determined to be 199 mg/kg in both male and female rats (Air Force Research Laboratory, 2002). Others have reported an oral ALD for DNAN to be 300 mg/kg, and from a 14-day subacute toxicity study, a LOAEL of 50 mg/kg was reported for clinical toxicity end-points as determined in a rat model (Lent et al., 2016). There was only one subchronic oral toxicity study and one oral developmental toxicity study available on determining a TRV for mammals. No other data were located in other feral mammalian species.

In oral subchronic toxicity studies (Lent et al., 2016) reported, at the highest dose tested (i.e. 80 mg/kg), evidence of mortality and clinical signs of lethargy, labored respiration, and a posture that was characterized by prostrate, recumbent, hunched, and gait irregularities was observed (Lent et al., 2016). No treatment related FOBs were observed in the 20 mg/kg treated rats at week 13 of the study. Dose related increases in extramedullary hematopoiesis (EMH) were noted in the spleens of female rats at doses of 20 and 80 mg/kg.

Studies of EMH in a subchronic study of DNAN demonstrated that the sensitive effects of EMH were dose-dependent. The NOAEL of 5 mg/kg was also reported in rats for developmental toxicity (Gao et al., 2016). Lent et al., (2016) used a NOAEL to fit a mathematical model to the EMH dose response data and calculate a lower bound confidence limit on the dose response that corresponded to a 10% response rate (BMDL<sub>10</sub>). The BMDL<sub>10</sub> value of 0.93 mg/kg-day was derived. A BMD of 1.78 mg/kg-day was also derived and was based on the logistic model.

In addition, another developmental toxicity data showed a similarly derived NOAEL value of 5 mg/kg (Gao et al., 2016). The subchronic study (Lent et al., 2016) was conducted according to good laboratory practice (GLP) standards and standard test guidelines. The TRV derived from this data was determined according to Technical Guidance (TG) 254 (U.S. Army Center for Health Promotion and Preventive Medicine (USCHPPM), 2000). These results show a moderate degree of confidence in the reported subchronic data. Both studies were conducted in the rat, and both studies similarly derived bounded NOAELs of 5 mg/kg-day for subchronic toxicological end-points that are highly likely to adversely affect future fitness of the individual animals and the population. Lent et al., (2016) noted anemia, splenic enlargement, hemosiderosis, and extramedullary hematopoiesis in treated animals, which indicated the blood system as a target organ of DNAN, with females more sensitive than males. These observations indicate ecologically relevant adverse consequences to a population of exposed mammals. Furthermore, there was very good concordance in observations and dose-dependent effects between the studies of Lent et al., (2016) and Gao et al., (2016). For the above reasons and conclusions, we have assigned a moderate level of confidence in the derived BMD TRVs, where a BMD or effective dose (ED) of 1.78 mg/kg and a lower bound BMDL<sub>10</sub> or LED<sub>10</sub> of 0.93 mg/kg were derived points of departure doses for DNAN (Table 7).



## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

**Table 7. Selected Ingestion TRVs for Class Mammalia**

TRV	Dose	Confidence
TRV <sub>Low</sub>	0.93 mg/kg-day	Moderate
TRV <sub>High</sub>	1.78 mg/kg-day	Moderate

Notes:

Lent et al., 2016; OARS WEEL 2014: based on the determined BMD values.

### 3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Acute inhalation toxicity data from rat model studies informs that the derived LC<sub>50</sub> values were greater than 2.9 g/m<sup>3</sup> (Hoffman, 2000b). A complementary study found that treating rats with DNAN by the nose-only route of exposure at a dose of 2.4 mg/L did not provoke any toxicity or lead to any mortality (USAPHC, 2015).

In addition, at 4-hr post-exposure, the mean blood concentration of DNAN was 16 µg/mL in male rats and 12.5 µg/mL in female rats. In subacute exposure studies at exposure periods of 6-hr/day, 5 days/week for 11 days, minimal toxicological effects were found on treating animals at a dose of 150 mg/m<sup>3</sup> (i.e., 165 mg/m<sup>3</sup>, which was the actual DNAN dose metric). However, decreased blood urea levels and increased kidney weights with evidence of partial hematological effects were also observed (USAPHC 2015). A LOAEL of 150 mg/m<sup>3</sup> was derived from the USAPHC (2015) study.

The two higher doses, 500 and 1,500 mg/m<sup>3</sup> of DNAN, showed mortality of 80- to 100% (Hoffman, 2000b; OARS WEEL, 2014). For this study, there are no dose response data available to derive a BMD point of departure. Thus, according to TG 254 (USACHPPM, 2000), a LOAEL of 165 mg/m<sup>3</sup> was derived to account for subacute toxicity from exposure to DNAN. For these data, the uncertainty factors (UF) that were considered included a UF of 20 for the NOAEL and a UF of 4 for the LOAEL. Thus, the inhalation toxicity for class Mammalia TRV of LOAEL was 165 mg/m<sup>3</sup> and NOAEL was 4.1 mg/m<sup>3</sup> (Table 8). A low level of confidence was assigned given the general lack of data from inhalation exposures.

**Table 8. Selected Inhalation TRVs for Class Mammalia**

TRV	Dose	Confidence
TRV <sub>Low</sub>	4.1 mg/m <sup>3</sup>	Low
TRV <sub>High</sub>	165 mg/m <sup>3</sup>	Low

Legend:

TRV<sub>Low</sub> derived from the NOAEL (no observed adverse effect level)

TRV<sub>High</sub> derived from the LOAEL (lowest observed adverse effect level)

Notes:

Hoffman 2000b; OARS WEEL 2014; subchronic LOAEL was derived as 165 mg/m<sup>3</sup>, with a UF for the NOAEL= 20, and the UF for the LOAEL = 4.

## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

### 3.2 Toxicity Reference Value for Avian Oral Toxicity

Only a single oral dose toxicity study is available, which was conducted in Japanese quail; the study showed a mortality rate of 20% at a dose of 120 mg/kg and 50% at a dose of 150 mg/kg. The LD<sub>50</sub> value for this study was considered to be at 150 mg/kg. Quail developed cataracts within 4 hours of DNAN exposure (Takahashi et al., 1988). Based on an oral LD<sub>50</sub> value, the TRV derived from this data were determined according to the Approximation Approach outlined in TG 254 (USACHPPM, 2000). Because the available endpoint was an LD<sub>50</sub>, a UF of 100 was applied to calculate a NOAEL of 1.5 mg/kg and a UF of 20 was applied to calculate a LOAEL of 7.5 mg/kg. The confidence levels were considered low for these TRVs (Table 9).

**Table 9. Selected Ingestion TRVs for Class Avian**

TRV	Dose	Confidence
TRV <sub>Low</sub>	1.5 mg/kg	Low
TRV <sub>High</sub>	7.5 mg/kg	Low

Legend:

TRV<sub>Low</sub> derived from the NOAEL (no observed adverse effect level)

TRV<sub>High</sub> derived from the LOAEL (lowest observed adverse effect level)

Notes:

Takahashi et al., 1988. The selected UFs were based on an acute oral LD<sub>50</sub> value of 150 mg/kg. Thus, a UF of 100 was applied for the NOAEL TRV, and a UF of 20 was applied for the LOAEL TRV according to TG 245 (USACHPPM 2000).

### 3.3 Toxicity Reference Values for Amphibian Toxicity

The amphibian data for DNAN toxicology is limited to a single study of Northern Leopard frogs (*Lithobates pipiens*) by Stanley et al., (2015). This study reported a 96-hr LC<sub>50</sub> value of 24.3 mg/L. The LOEC for mortality from a 28-day chronic exposure was 2.4 mg/L. This single chronic study was used to derive LOAEL and NOAEL TRVs for amphibian species according to the Approximation Approach described in TG 254 (USACHPPM 2000). A UF 10 was applied to the chronic LOEC to derive a NOAEL of 0.24 mg/L, and a UF of 1 was applied to derive a LOAEL of 2.4 mg/L. Since these TRV derivations were from a single study, a low level of confidence is assigned. Table 10 presents the derived TRVs.

**Table 10. Selected Ingestion TRVs for Class Amphibian**

TRV	Dose	Confidence
TRV <sub>Low</sub>	0.24 mg/L	Low
TRV <sub>High</sub>	2.40 mg/L	Low

Source: Stanley et al., 2015.

### 3.4 Toxicity Reference Values for Reptilian Toxicity

No data are available to develop a toxicity reference value for reptiles.

## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

### **4. IMPORTANT RESEARCH NEEDS**

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The lack of data on the toxicity of DNAN to wildlife species weakens the development of TRVs. Hence, more toxicological studies of the compound and its derivatives are recommended. A large literature base exists for inhalation in mammals but for no other taxonomic group. Additional laboratory mammal testing is necessary for all toxicological routes of exposure. Data are also lacking for non-mammalian species. Adequate dermal, inhalational, and reproductive/developmental toxicity data are lacking for all groups. The toxicity literature is scant for all species described in this assessment, and completely lacking for reptiles. Thus, studies that focus on both acute and chronic toxicity studies on wild mammals as well as non-mammalian wildlife such as birds, reptiles, and amphibians are warranted.

## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

### APPENDIX A

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### APPENDIX B

#### LITERATURE REVIEW

A search on August 17, 2018 using DTIC's Multisearch function used the single search term, DNAN and then 2,4-dinitroanisole. This search identified 203 documents for DNAN and 89 documents for 2,4-Dinitroanisole for a total of 292 documents.

Additional focused searches on August 17, 2018 using DTIC's Multisearch function used the terms:

- DNAN + quail\*. This search identified 6 documents.
- DNAN + mallard\*. This search identified 3 documents.
- DNAN + bird\*. This search identified 15 documents.
- DNAN + avian. This search identified 8 documents.
- DNAN + mouse. This search identified 20 documents.
- DNAN + mice. This search identified 22 documents.
- DNAN + rat. This search identified 48 documents.
- DNAN + mammal\*. This search identified 29 documents.
- DNAN + ecotox\*. This search identified 30 documents.
- DNAN + toxic\*. This search identified 76 documents.
- DNAN + amphib\*. This search identified 21 documents.
- DNAN + frog. This search identified 12 documents.
- DNAN + *Xenopus*. This search identified 7 documents.
- DNAN + reptil\*. This search identified 4 documents.

Additional focused searches on August 17, 2018 by DTIC's Multisearch function used the terms:

- 2,4-DINITROANISOLE + quail\*. This search identified no new documents.
- 2,4-DINITROANISOLE + mallard\*. This search identified no new documents.
- 2,4-DINITROANISOLE + bird\*. This search identified no new documents.
- 2,4-DINITROANISOLE + avian. This search identified no new documents.
- 2,4-DINITROANISOLE + mouse. This search identified one new document.
- 2,4-DINITROANISOLE + mice. This search identified one new document.
- 2,4-DINITROANISOLE + rat. This search identified two new documents.
- 2,4-DINITROANISOLE + mammal\*. This search identified no new documents.
- 2,4-DINITROANISOLE + ecotox\*. This search identified no new documents.
- 2,4-DINITROANISOLE + toxic\*. This search identified no new documents.
- 2,4-DINITROANISOLE + amphib\*. This search identified no new documents.
- 2,4-DINITROANISOLE + frog. This search identified no new documents.
- 2,4-DINITROANISOLE + *Xenopus*. This search identified no new documents.
- 2,4-DINITROANISOLE + reptil\*. This search identified no new documents.

## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

On August 17, 2018, a search of the U.S. Environmental Protection Agency's (EPA) online Ecotox database used the CAS No. 119-27-7 to identify additional articles. No references were returned for amphibians, reptiles, birds or mammals. Thus, no new references were identified. A search of the database of the National Library of Medicine's TOXNET system (<http://toxnet.nlm.nih.gov>), on August 17, 2018 used the CAS No. 119-27-7 as the search term. A total of 7920 articles were identified. This search was refined with—

- 119-27-7 and ecotox\* resulted in 2 hits
- 119-27-7 and reptil\* resulted in 0 hit
- 119-27-7 and amphib\* resulted in 1 hits
- 119-27-7 and *Xenopus* resulted in 0 hits
- 119-27-7 and frog resulted in 1 hits
- 119-27-7 and avian resulted in 0 hits
- 119-27-7 and mallard resulted in 0 hits
- 119-27-7 and quail resulted in 1 hits
- 119-27-7 and bird\* resulted in 1 hits
- 119-27-7 and wild\* resulted in 1 hits
- 119-27-7 and mammal\* resulted in 1 hits

The searches defined above identified many of the same articles. Additional references were identified during the review of individual articles. A total of 36 articles were reviewed, which included reports pulled from the grey literature.

In addition, on August 17, 2018, an additional literature search was conducted using the Johns Hopkins Welch Medical Library Multisearch Database. Using 2,4-DINITROANISOLE as a single search term in the title of the document, this search strategy identified 151 documents with 2,4-DINITROANISOLE in the title of the article in Web of Science; 87 documents in PubMed; 0 documents in the Cumulative Index to Nursing and Allied Health Literature (CINAHL) Plus; 0 documents in WorldCat® Advanced Search (FirstSearch), 85 articles in MEDLINE®; and 90 articles in Academic Search Complete. For additional targeted searches, a standard search of PubMed (National Library of Medicine, NIH) of 2,4-DINITROANISOLE [TI] as the anchored word in the title with the following search strings were selected using the wild-card (\*) function where appropriate for optimal returns on search terms and contexts. Species-specific search strings yielded the following hits from PubMed:

- 2,4-DINITROANISOLE [TI] AND mammal returned 4 hits
- 2,4-DINITROANISOLE [TI] AND animal returned 9 hits
- 2,4-DINITROANISOLE [TI] AND quail returned 0 hits
- 2,4-DINITROANISOLE [TI] AND mallard returned 0 hits
- 2,4-DINITROANISOLE [TI] AND bird returned 0 hits
- 2,4-DINITROANISOLE [TI] AND avian returned 0 hits
- 2,4-DINITROANISOLE [TI] AND mouse returned 0 hits
- 2,4-DINITROANISOLE [TI] AND mice returned 0 hits
- 2,4-DINITROANISOLE [TI] AND rat returned 4 hits
- 2,4-DINITROANISOLE [TI] AND wildlife returned 0 hits
- 2,4-DINITROANISOLE [TI] AND ecotox\* returned 3 hits



## WILDLIFE TOXICITY ASSESSMENT FOR 2,4-DINITROANISOLE (DNAN)

- 2,4-DINITROANISOLE [TI] AND amphib\* returned 0 hits
- 2,4-DINITROANISOLE [TI] AND amphibian returned 0 hits
- 2,4-DINITROANISOLE [TI] AND frog returned 0 hits
- 2,4-DINITROANISOLE [TI] AND *Xenopus* returned 0 hit
- 2,4-DINITROANISOLE [TI] AND reptile returned 0 hit
- 2,4-DINITROANISOLE [TI] AND reptil\* returned 0 hits