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Toxicology Assessment of IMX-104, January 2019

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14. ABSTRACT
This report summarizes the available toxicological information for the insensitive munition IMX-104 and its components, dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The toxicity of the IMX-104 mixture is unknown in mammalian models. In aquatic species, acute mixture effects of IMX-104 were species dependent while sublethal effects were largely due to DNAN. DNAN and RDX demonstrate moderate oral toxicity while the oral toxicity of NTO is low. Toxic effects of DNAN are consistent with other nitroaromatics, including the development of anemia. Testicular toxicity is the predominant effect of exposure to NTO, while neurotoxicity and carcinogenesis are associated with exposure to RDX. NTO demonstrates minimal ecotoxicity. DNAN and RDX demonstrate greater ecotoxicity, particularly in mammals and birds.

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IMX-104, insensitive munition, toxicity, NTO, DNAN, RDX

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1. SUMMARY

1.1 Overview

The U.S. Department of Defense (DOD) has an initiative to improve the safety of munitions, and the U.S. Army has responded by developing insensitive munitions for use in future weapon systems (Duncan 2002). Insensitive munitions are "munitions which reliably fulfill their performance, readiness and operational requirements on demand, and which minimize the probability of inadvertent initiation and severity of subsequent collateral damage to weapon platforms, logistic systems, and personnel when subjected to unplanned stimuli" (NAVSEA 1994). New insensitive munitions are being developed to minimize the acute hazards associated with sympathetic and non-intentional detonation of warheads.

Insensitive Munition Explosives (IMX)-104, a new insensitive munition formulation, is planned for use in several weapons systems including 81-millimeter (mm) and 60mm mortar cartridges and is being evaluated in the 120mm mortars. IMX-104 is a mixture of 3-nitro-1,2,4-triazol-5-one (NTO), 2,4-dinitroanisole (DNAN), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Two of these components, NTO and DNAN, have limited toxicity data. This report presents the latest toxicity data and interpretations for DNAN, NTO, and RDX, as well as the full IMX-104 combination (see paragraphs 7.3–7.6). This report also includes Workplace Environmental Exposure Limits (WEELs) and Occupational Exposure Limits (OELs) developed for two of the individual components.

1.2 Purpose

Safeguarding the health of Soldiers, Civilians, and the environment requires a toxicity assessment prior to fielding of alternatives to existing weapons and energetics. Consequently, research, development, testing, training, and use of substances that are potentially less hazardous to human health and the environment is vital to the readiness of the U.S. Army. Continuous assessments of the potential alternatives, initiated early in the Research, Development, Testing, and Evaluation (RDT&E) process, can save significant time, cost, and effort during RDT&E, as well as over the life cycle of the items developed.

The Army Environmental Quality Technology (EQT) Ordnance Environmental Program (OEP) is dedicated to finding replacements for substances causing environmental and/or occupational risks to health. As part of this program, each project is evaluated for environmental and occupational health impacts. The purpose of this effort is to evaluate and update the insensitive munition mixture, IMX-104.

1.3 Conclusions

DNAN is moderately toxic via the oral route and slightly toxic via inhalation. DNAN is mildly irritating to the skin, causes cataracts, and is not a sensitizer. Although *in vitro* studies have shown that DNAN does penetrate intact skin, no studies have determined the toxicity of DNAN following dermal exposure. In the subchronic study in rats, effects from DNAN exposure included anemia and testicular atrophy. Reproductive and developmental toxicity studies have not been conducted with DNAN. The subchronic study indicates male reproductive toxicity, albeit at near lethal doses, and limited developmental studies suggest potential fetal effects. Additionally, the metabolite 2,4-DNP is fetotoxic in animals and has been associated with menstrual irregularities in humans (ATSDR 1995). Although DNAN is mutagenic in the *Salmonella* test system, results in mammalian systems (Chinese Hamster Ovary (CHO) and micronucleus) are negative, suggesting DNAN is not likely to be a human mutagen. No chronic or carcinogenicity studies have been conducted. DNAN is likely to have limited transport to groundwater and may demonstrate considerable natural attenuation due to sorption to soils and (bio)transformation. DNAN demonstrates limited ecotoxicity, with the most significant effects occurring in plants and birds. A WEEL assessment by the Occupational Alliance for Risk Science (OARS), determined the 8-hour time-weighted average for inhalation exposure should not exceed 0.1 milligrams per cubic meter (mg/m^3) (0.01 parts per million (ppm)) (WEEL 2018a).

Acute oral and inhalation toxicity of NTO is low. No acute dermal toxicity data are available for NTO and the data on dermal absorption are inadequate. NTO is mildly irritating to the skin and eyes, but is not a sensitizer and is negative in both *in vitro* and *in vivo* genotoxicity tests. In the subchronic oral study and subsequent reproductive and developmental studies effects on the male reproductive system were the primary adverse effect, and are the basis for occupational health and safety standards. Although the alteration of growth of male secondary sex organs in the pubertal development study in rats suggests altered endocrine function, additional *in vitro* and *in vivo* endocrine disruptor screening studies have demonstrated no effects. According to time-course studies of testicular toxicity, the Sertoli cell was the initial target of NTO toxicity in both rats and mice. NTO is readily soluble in water and represents a hazard for environmental transport and the potential to contaminate groundwater and surface water. However, microbial degradation (mineralization) and sorption to some soil types may limit transport. While acute chemical toxicity to aquatic species is generally low, the ability of NTO to alter the pH of aqueous environments presents a hazard, depending upon quantity released. The WEEL assessment by the OARS for NTO determined the 8-hour time-weighted average for inhalation exposure should not exceed 2 mg/m^3. No additional hazard notations were assigned (WEEL 2018b).

RDX is moderately toxic via the oral route but relatively non-toxic via the dermal route due to the limited dermal absorption of RDX. No reliable inhalation studies have been conducted. RDX does not cause skin irritation or skin sensitization, but may cause eye irritation. In repeat-dose studies, central nervous system (CNS) effects including gasping, labored breathing, and convulsions were the primary effects. Toxicity varied with route of administration, granularity of compound, and among species. Greater toxicity was observed in gavage studies relative to feeding studies. Finely ground RDX was more toxic than course preparations, and mice were less sensitive than other species. In developmental studies in rats and rabbits and multi-generation reproductive studies in rats, minimal developmental effects occurred at high doses,

coincident with maternal toxicity. RDX was not genotoxic in a battery of *in vitro* and *in vivo* tests. RDX is classified by the U.S. Environmental Protection Agency (USEPA) as having suggestive evidence of carcinogenic potential based upon production of benign and malignant tumors in the liver and lungs of female B6C3F1 mice in chronic studies (USAMRDC 1984a). An oral slope factor of 0.08 milligrams per kilogram per day (mg/kg-day)$^{-1}$ was derived based on the combined incidence of these tumors (USEPA 2018b). Mobility of RDX in soil is expected to be moderate to high and RDX is expected to leach into groundwater (USAMRDC 1980b). RDX is resistant to aerobic bacterial degradation in soil but is rapidly degraded by photolysis. Volatilization from wet surfaces is limited. RDX demonstrates low aquatic toxicity, but is highly toxic to birds, reptiles, and mammals. The USEPA has established a Long-Term Health Advisory (LTHA) for RDX of 0.1 milligrams per liter (mg/L), a Lifetime health advisory of 2 micrograms per liter (μg/L), and an Oral Slope Factor of 0.08 (mg/kg-day)$^{-1}$ (USEPA 2018b, 2018a). The USEPA has determined a residential soil screening level (SSL) of 6.1 milligrams per kilogram (mg/kg) and an industrial SSL of 28 mg/kg (USEPA 2017). ACGIH has established a Threshold Limit Value (TLV) (8-hour Time-Weighted Average (TWA)) of 0.5 mg/m^3 for RDX (ACGIH 2008). U.S. National Institute for Occupational Safety and Health (NIOSH) has established a Recommended Exposure Limit (REL) (10-hour TWA) of 1.5 mg/m^3 and a Short Term Exposure Limit (STEL) (15 minute) of 3.0 mg/m^3 with a skin notation (NIOSH 2005).

Evaluation of the toxicological effects of the complete IMX-104 formulation in aquatic species demonstrated interactive effects in acute toxicity tests; however, the nature of the interactive effect differed among the species tested (Gust et al. 2018; Lotufo et al. 2018). In sublethal tests, toxicity was largely attributed to DNAN. The IMX-104 mixture has not been tested in a mammalian model. When present as part of the mixture IMX-104, the individual components were found to dissolve according to their aqueous solubility and the components did not interact in the soil to affect fate and transport.

1.4 Recommendations

Reproductive toxicity is an important data gap for DNAN. Reproductive/developmental and chronic rodent studies of DNAN are suggested to reduce uncertainty associated with derivation of environmental criteria (e.g., reference dose) and to enable sustained use.

Mechanisms of toxicity, including the underlying testicular toxicity and the mechanism of reduction of DNAN to 2,4-DNP in mammals, should be more fully explored as they will inform future hazard and risk assessments.

In vitro data suggesting skin absorption of NTO and the IMX mixture require additional testing, preferably in an animal model. Dermal absorption and *in vivo* dermal toxicity studies are needed for NTO, DNAN, and IMX-104 to understand the potential for toxicity from dermal contact.

Additional ecotoxicological testing, to include terrestrial invertebrates, reptiles, and birds is recommended.

An investigation of chronic oral toxicity and potential subchronic avian effects are indicated for DNAN.

Studies to clarify the interactive effects in the mixture are recommended, particularly in a mammalian model.

2. REFERENCES

See Appendix A for list of references.

3. AUTHORITY

This Toxicology Assessment addresses, in part, the environment, safety and occupational health (ESOH) requirements outlined in Department of the Army Regulation (AR) 200-1, AR 40-5, and AR 70-1; Department of Defense Instruction (DoDI) 4715.4; and 2018 Army Environmental Research and Technology Assessment (AERTA) requirement PP-3-02-07.

4. BACKGROUND

As a result of the DOD-wide initiative to improve the safety of munitions, the U.S. Army is developing insensitive munitions for use in future weapon systems (Duncan 2002). Insensitive munitions are "munitions which reliably fulfill their performance, readiness and operational requirements on demand, and which minimize the probability of inadvertent initiation and severity of subsequent collateral damage to weapon platforms, logistic systems, and personnel when subjected to unplanned stimuli" (NAVSEA 1994). In addition to minimizing collateral damage from weapon or ordnance accidents, insensitive munitions offer logistical advantages on the battlefield—more munitions can be stored in a given area if quantity-distance requirements are reduced, resulting in more efficient use of available land and smaller targets for potential enemy action. As modern battlefields increasingly shift into populated urban centers, insensitive munition inventories represent a less desirable target for terrorists and minimize the threat to surrounding communities. IMX-104 is planned for use in several weapons systems including the 81, 60, and 120mm mortar.

Current regulations require assessment of human health and environmental effects arising from exposure to substances in soil, surface water, and groundwater. Applied after an item has been fielded, these assessments can reveal the existence of adverse environmental and human health effects that must be addressed, often at substantial cost. It is more efficient to begin the assessment of exposure, effects, and environmental transport of military-related compounds/substances early in the RDT&E process to avoid unnecessary costs, conserve physical resources, and sustain the health of our forces and others potentially exposed.

To support this preventive approach, the U.S. Army Public Health Center (APHC) has created a phased approach to toxicity testing designed to reduce adverse ESOH effects impacting readiness, training, and development costs. Evaluation of new materials is an on-going effort, and this report represents the status of information available for this project as of the date of publication. Summary interpretations of this information have been provided to the sponsor in support of Ordnance Environmental Program In-Progress Reviews (IPR) and to the proponent of the technology (PM-CAS).

5. STATEMENT OF PROBLEM

Throughout their history, the U.S. Armed Forces have experienced needless loss of life and equipment due to inadvertent detonation of munitions via sympathetic detonation, non-explosive impact, or the action of fire upon munitions. The objective of the IMX program is to develop an explosive formulation that will only detonate when desired, and will be insensitive to other environmental effectors. IMX-104 is intended to provide explosive performance comparable to current explosive formulations, but to be less sensitive to unplanned stimuli, whether by accident or enemy action. The new formulations must also demonstrate an improvement in human health and environmental outcomes.

6. METHODS

To determine the human health and ecological impact of compounds employed in these formulations, it is necessary to identify each compound correctly and to determine its physical, chemical, and toxicological properties. The primary means of identification employed for each compound in this program is its Chemical Abstracts Service Registry Number (CAS RN) (see Table 1). The CAS RN is an unambiguous way of accessing information for chemical substances, though all compounds do not necessarily have a single CAS RN. The CAS RN is readily used as a keyword for searching online databases and is often cross-referenced with both systematic and trivial (i.e., common) names for chemical substances. In some cases, synonyms and trade names are also used to identify structures.

This report addresses compounds investigated as part of this work unit through the end of Q1 FY2019. Basic physical and chemical properties are usually determined by consulting tertiary sources when such information is available. The properties necessary to assess fate and transport in the environment (FTE) include—

- Molecular weight (MW).
- Henry's law constant (KH).
- Octanol-water partition coefficient (log K_{OW}).
- Water solubility
- Boiling point (bp).
- Organic carbon partition coefficient (log K_{OC}).
- Vapor pressure (vp).

Table 1. Components of IMX-104

Substance	CAS RN
2,4-dinitroanisole (DNAN)	119-27-7
3-nitro-1,2,4-triazol-5-one (NTO)	932-64-9
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4

Information on combustion, explosion, and thermal decomposition products is also collected, if available. Toxicological information needed to estimate potential human health risks includes the—

- Reported toxicity effects of oral, inhalation, dermal, and ocular exposures;
- Potential for developmental or reproductive toxicity, mutagenesis and carcinogenesis; and
- Mode(s) and mechanisms of toxicity. Toxicological information is derived directly from primary sources whenever possible.

Sources used in this search included The Merck Index (Williams 2013); the U.S. National Library of Medicine's (NLM) Toxicology Data Network (TOXNET®) providing access to information from the National Institutes of Health (NIH) and the USEPA; the U.S. Department of Health and Human Services' Agency for Toxic Substances and Disease Registry (ATSDR); the USEPA ECOTOXicology Database System (ECOTOX); and the Defense Technical Information Center (DTIC®). Additional sources may include publications from the NIOSH, the World Health Organization (WHO), and the International Agency for Research on Cancer (IARC).

Primary references are identified and retrieved by PubMed® and the EBSCOhost® Research Databases. TOXNET simultaneously searches a database suite including ChemIDPlus® (chemical structures, registration numbers, and links to other sites providing physical/chemical properties of a compound), the Hazardous Substances Data Bank (HSDB®), TOXLINE (primary literature for physiological and toxicological effects of drugs and other chemicals), Developmental and Reproductive Toxicology (DART) database, Comparative Toxicogenomics Database (CTD), the Integrated Risk Information System (IRIS), and Animal Testing Alternatives (ALTBIB) database, and others including archived databases for the Chemical Carcinogenesis Research Information System (CCRIS), the Carcinogenic Potency Database (CPDB), and GENE-TOX genetic toxicity database. Commercial suppliers may provide results of in-house research absent from the open literature.

Persistence, bioaccumulation, human health toxicity, and ecotoxicity were assigned to general categories of risk (e.g., low, moderate, or high) using criteria modified from Howe et al. (2006). Table 2 describes the criteria used in the categorization, though the relative proportions of each substance were also factored into the final assessment.

Table 2. Categorization Criteria Used in the Development of Environmental Safety and Occupational Health Severity (modified from Howe et al. 2006)

	Low	Moderate	High
PERSISTENCE	Readily biodegrades (<28 days)	Degradation ½ life: water <40 days , soil <120 days	Degradation ½ life: water >40 days soil > 120 days
TRANSPORT	Water sol. < 10 mg/L log K_{OC} > 2.0	Water sol. 10-1000 mg/L log K_{OC} 2.0-1.0	Water sol. > 1000 mg/L log Koc <1.0
BIOACCUMULATION	log K_{OW} <3.0	log K_{OW} 3.0-4.5	log K_{OW} >4.5
TOXICITY	No evidence of carcinogenicity/ mutagenicity; Subchronic LOAEL > 200 mg/kg-day	Mixed evidence for carcinogenicity/mutagenicity (B2, 2); Subchronic LOAEL 5-200 mg/kg-day	Positive corroborative evidence for carcinogenicity /mutagenicity; LOAEL < 5 mg/kg-day
ECOTOXICITY	Acute LC_{50}/LD_{50} >1 mg/L or 1,500 mg/kg; Subchronic EC_{50} >100 µg/L or LOAEL >100 mg/kg-day	Acute LC_{50}/LD_{50} 1-0.1 mg/L or 1500-150 mg/kg; Subchronic EC_{50} 100-10 µg/L or LOAEL – 10-100 mg/kg-day	Acute LC_{50}/LD_{50}<100 µg/L or <150 mg/kg; Subchronic LOAEL <10 mg/kg-day

Legend:
mg/L=milligrams per liter
mg/kg-day=milligram per kilogram per day;
µg/L=microgram per liter
Notes:
EC_{50} – concentration that results in 50 observation of a selected endpoint, e.g., immobility; LOAEL –lowest-observed adverse effect level; LC_{50} – concentration expected to result in 50% lethality to a population of test animals; LD_{50} – total dose expected to result in 50% lethality to a population of test animals;

If no experimental data were identified in the literature, toxicity values for the various parameters were predicted using Quantitative Structure Activity Relationship (QSAR) software where possible. Modeling packages include USEPA's EPI Suite™ 4.11 (USEPA 2014), ECOSAR™ (USEPA 2014) and TOPKAT® (BIOVIA, formerly Accelrys Inc.).

7. RESULTS

7.1 Physical and Chemical Properties

Table 3 summarizes physical and chemical properties. When data were not found, "nd" (no data) is inserted. In some cases, the property named is not applicable "n/a" to the substance being described. For example, if the compound is a nonvolatile solid or an inorganic salt, vapor pressure, K_{OW}, K_{OC}, and the Henry's Law constant (KH) are typically negligible.

Table 3. List of Physical and Chemical Properties

Compound	Molecular Weight	Boiling Point (°C)	Aqueous solubility (mg/L)	log K_{ow}	log K_{oc}	Henry's Law Constant (at-m^3/mol) @ 25 °C	Vapor Pressure (mmHg) @ 25 °C
DNAN	198.15[1]	390[2] (exp) (76mm Hg)	207[3] (20°C) 216[4] (22 °C)	1.710[5,6] 1.58[4]	2.362[6]	4.96E-09[5]	1.38E-04[5]
NTO	130.08[1]	nd	2.00E+03[7] 1.72E+04[4](25 °C) 1.56E+04[8] 1.50E+04[9]	0.858[7] 0.802 ± 0.012[10] 1.58[4]	-0.38[6]	2.58E-13[6]	5.82E-07[6]
RDX	222.15[1] (calc)	407[11]	59.7[5] (exp)	0.87[5] (exp)	2.145 (calc)	2.53E-06[12] (est)	4.10E-09[11]
IMX-104	n/a	nd	insoluble	n/a	n/a	n/a	n/a

Legend:
nd = no data;
n/a = not applicable;
est = estimated;
exp= experimental;
dec = decomposes;
Notes:
[1]Calculated from molecular formula and standard atomic weights;
[2]ChemSpider (2015);
[3]USARDEC (2009);
[4]NRC (2013);
[5]CIDPL 2009.;
[6]EPI Suites 4.11;
[7]Experimental value, DLS USACHPPM, 2009;
[8]USARDEC (2009) via HPLC;
[9]USARDEC (2009) gravimetric;
[10]USARDEC (2011);
[11]HSDB (2012);
[12]USEPA (2018a)

7.2 Summaries

Table 4 presents the summaries of toxicology data for each of the formula components; Tables 5 and 6 present the summary assessments of human health and environmental toxicity. Each characterization is generally based on the criteria provided in Table 2. The final risk characterization also incorporates assessment of the uncertainty associated with available data, the amount of each compound present in the formulation, and the nature of potential exposure associated with use of the end item.

Table 4. Toxicity Data

Compound	Acute Oral LD_{50} (mg/kg)	Sub-chronic NOAEL/ LOAEL	Chronic Oral LOAEL (mg/kg-day)	Inhalation LC_{50} (g/m^3-h)	Dermal	Ocular	Mutagenicity	Carcinogenicity
DNAN	199[1]	50[2] (LOAEL)	17.3[3]	>2.4[4] >2.9[5]	Mild irritant[1]	Mild irritant[1] Cataracts	Mixed[7]	Possible[3]
NTO	>5,000[8] (rat, mouse)	30[9] (LOAEL)	569.8[3]	0.18[10]	Mild irritant[8]	Moderate to severe irritant[8]	Negative[11]	Indeterminate[3]
RDX	59[12,13](mouse) 68-300[12-15](rat) 500[12](rabbit)	28[12] (diet) 8[14] (gavage) (LOAEL)	0.3[14] (NOAEL)	nd (lethal effects observed in swine)	Negative	Irritant	Negative	Suggestive
IMX-101	924 (♀ rat)[8] 1,237 (♂ rat)[8]	nd	nd	nd	nd	nd	nd	nd

Legend:
nd=no data;
NOAEL=no-observed adverse effect level
LOAEL=lowest observed adverse effect level
Notes:
[1]USAFRL (2002);
[2]Lent et al. (2016b);
[3]TOPKAT modeling;
[4]USAPHC (2013);
[5]Huntingdon (2000);
[6]Horner (1942);
[7]Dodd et al. (2002);
[8]LANL (1985);
[9]Crouse et al. (2015);
[10]USAPHC (2013);
[11]Reddy et al. (2011);
[12]USAMRDC (1980a);
[13]USAMRDC (1979);
[14]USACHPPM (2006);
[15]Schneider et al. (1977)

Table 5. Toxicity Assessment

Compound	Oral	Inhalation	Dermal	Ocular	Carcinogenicity	Comments
DNAN	Moderate	Low	Unknown	Moderate	Unknown	Effects consistent with other nitroaromatics
NTO	Low	Mod	Unknown	Moderate	Unlikely	Lacks dermal toxicity data
RDX	Moderate	Unknown	Low	Low	Possible	Evidence of carcinogenicity only in female mice, rated as a poor predictor of carcinogenicity in other species.

Note:

Evaluations are based on weight of evidence, physicochemical properties, and professional judgment using criteria presented in Table 2.

Table 6. Ecotoxicity Assessment

Compound	Aquatic	Invertebrate	Plants	Mammalian	Avian	Comments
DNAN	Low-Mod	Low	Moderate	Moderate	Moderate	Causes cataracts in Japanese quail
NTO	Low	Low	Low	Low	Unk	Appears readily biodegradable; readily taken up from soil by plants.
RDX	Low	Low	Low	High	High	Causes seizure activity in mammals and birds.

Note:
Evaluations are based on weight of evidence and professional judgment using criteria presented in Table 2.

7.3 2,4-Dinitroanisole [DNAN]

7.3.1 General Information

In addition to its role in IMX-104, DNAN (dinitroanisole or 1-methoxy-2,4-dinitrobenzene) is also a component of the explosive formulation known as PAX-21, which was fielded for several years before problems with incomplete detonation were detected and production ceased.

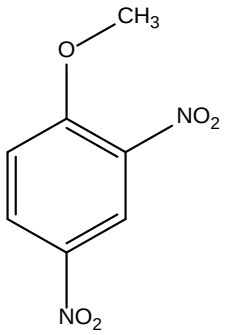

Figure 1. DNAN

7.3.2 Toxicology Data

7.3.2.1 Oral

As part of a study on new explosive formulation PAX-21, an acute oral LD_{50} in rats was determined on DNAN, and was 199 mg/kg in both sexes of rats. Clinical signs of toxicity included decreased activities, breathing abnormalities, salivation, and soft stools. No remarkable clinical findings or gross lesions were discovered at necropsy (USAFRL 2002).

Lent et al. (2016b) conducted an Approximate Lethal Dose (ALD) study, a 14-day repeated dose study, and a 90-day subchronic study of DNAN. The ALD was determined to be 300

mg/kg, with test animals exhibiting clinical signs including lethargy, rapid respiration/labored breathing, prostrate posture, and salivation in male rats at doses of 88.9 mg/kg and greater, and in female rats at doses of 133.3 mg/kg and greater. Female rats also exhibited chromodacryorrhea (red tears).

In the subacute study, male and female Sprague-Dawley rats were administered DNAN in corn oil via oral gavage at doses of 0, 1.56, 3.13, 6.25, 12.5, 25, 50, or 100 mg/kg-day for 14 days. Clinical effects were observed at doses of 50 mg/kg-day and higher. The NOAEL for this study was determined to be 25 mg/kg-day (Lent et al. 2016b).

In the subchronic study, male and female Sprague-Dawley rats were administered DNAN via oral gavage at 0, 1.25, 5, 20, or 80 mg/kg-day for 90 days. Mortality occurred in three male rats (days 50, 63, and 77) and one female rat (day 26), all in the 80 mg/kg-day dose group. Rats in the highest dose group experienced lethargy, labored/rapid respiration, prostrate or recumbent posture, hunched posture, ear twitching, squinting, curled tail, and gait irregularities. A functional observational battery and analysis of motor activity at week 13 indicated that rats given 80 mg/kg-day had altered neuromuscular function and decreased activity levels. In the 80 mg/kg-day group, female rats also had reduced sensorimotor responses, while male rats had increased excitability responses. The neurobehavioral evaluations indicated no treatment-related effects at 20 mg/kg-day or below. Decreased weight of the testes and epididymides as well as degeneration and atrophy of the testicular seminiferous tubules and aspermia were also observed. A dose-related increase of extramedullary hematopoiesis (EMH) was noted in spleens of female rats at 20 and 80 mg/kg-day. While the NOAEL for this study was 5 mg/kg-day, EMH was observed at the lowest dose with no dose-response relationship observed in the lower dose range. The incidence of EMH was modeled using Benchmark Dose software to obtain a $BMDL_{10}$ value of 0.93 mg/kg-day (Lent et al. 2016b; WEEL 2018a).

No chronic oral experimental data were found in the literature. TOPKAT modeling predicted a chronic LOAEL of 17.3 mg/kg-day at high confidence.

7.3.2.2 Inhalation

In an acute inhalation study, Sprague-Dawley rats were exposed to DNAN in two phases due to difficulties achieving target concentrations. In the first phase, DNAN was heated to 175 degrees Celsius (ºC) to generate vapors and a target concentration of 1 to 5 mg/m^3; however, actual concentrations were only 2.8 mg/m^3. In the second phase, DNAN was dissolved in acetone to achieve a higher exposure level for 4 hours (target concentration of 2,000 mg/m^3; actual average concentration 2,933 mg/m^3). No mortalities were observed in either group. No clinical signs of toxicity were observed at the lower vapor-based exposure. In the aerosol exposure, animals exhibited decreased activity and labored breathing during the exposure and increased salivation, lacrimation, and red or clear nasal discharge post-exposure. These clinical signs resolved in all animals within several days. No treatment-related macroscopic postmortem findings were noted at the end of the 14-day post-exposure period. The inhalation LC_{50} was judged to be >2.9 g/m^3 (USAFRL 2002; Huntingdon 2000).

Rats exposed for 2 weeks (6 hours/day; 5 days/week) to aerosol concentrations of DNAN ≥ 545 mg/m^3 in an acetone vehicle experienced mortality (80%), lethargy, labored breathing, irregular

gait, nasal discharge, decreased fecal volume, yellow staining of the vent, decreased food consumption, and decreased body weight gain. Control (acetone vehicle only) animals also exhibited signs of CNS depression (irregular gait). Mild signs of toxicity and non-specific minimal metaplasia of laryngeal epithelium were observed at 165 mg/m^3. An inhalation LOAEL of 165 mg/m^3 was determined for DNAN based upon mild signs of toxicity and non-specific minimal metaplasia of laryngeal epithelium; no NOAEL was determined (USAFRL 2002; Huntingdon 2000).

In a study to determine DNAN absorption following inhalation and oral exposure, rats were exposed nose-only to a 2.4 mg/L aerosol atmosphere of DNAN for a single 4-hour exposure. No test compound-related mortalities occurred and no adverse toxic signs, body weight changes, or gross necropsy findings were observed in exposed rats (USAPHC 2015). The multi-time point blood absorption data indicated that acute exposure to DNAN via oral gavage appears to induce higher DNAN whole blood concentrations in laboratory rats compared to those exposed via inhalation. Oral exposure to DNAN also resulted in an insignificant increase in whole blood concentrations of the metabolite 2,4-dinitrophenol (2,4-DNP) compared to those animals exposed via inhalation.

7.3.2.3 Dermal

Skin irritation studies were conducted as a part of the PAX-21 studies. Rabbits exposed to DNAN exhibited slight dermal irritation that was reversible within 24-48 hours. Studies in guinea pigs using a modified Buehler method indicated DNAN was not a sensitizer (USAFRL 2002).

Steady state flux of pure DNAN and DNAN as part of the mixture PAX-21 (34% DNAN) was determined through dermatomed rat skin in static diffusion cells over 6 hours at 32ºC. The rate of penetration was 1.55 micrograms per square centimeter per hour (µg/cm^2-hour) for neat DNAN and 0.74 µg/cm^2-hour for DNAN in the mixture. DNAN was applied to skin as a powder, the same form that would be encountered by workers (USAFRL 2000). *In vitro* penetration of powdered DNAN through human epidermal membranes (without stratum corneum) was also evaluated (USAPHC 2012b); the rate of skin penetration was determined to be 1.1 µg/cm^2-hour. When applied as part of the IMX-101 mixture, a penetration rate of 1.8 µg/cm^2-hour was obtained in human epidermal membranes. Because the stratum corneum (i.e., the primary barrier to dermal absorption) was removed prior to testing, no conclusions can be drawn regarding the dermal absorption of DNAN in this test.

7.3.2.4 Ocular

No experimental data were found. TOPKAT modeling predicts DNAN will not be an ocular irritant.

Exposure to DNAN is associated with development of cataracts, which may be due to the metabolism of DNAN to 2,4-DNP. Horner (Horner 1942) reported a relatively low rate of cataract development in humans who were consuming 2,4-DNP, a metabolite of DNAN, in order to lose weight. Takahashi et al. (1988) reported that 63% of Japanese quail administered DNAN at a dose of 120 mg/kg and 100% at 150 mg/kg developed reversible cataracts within 1 to 4 hours

after administration. Mortality among the 120 mg/kg group was approximately 20% and about 55% among the 150 mg/kg group.

7.3.2.5 Developmental and Reproductive

TOPKAT modeling predicts DNAN is not likely to be a developmental or reproductive toxicant.

DNAN has been observed to be a testicular toxicant, causing decreased mass of the testes and epididymides, epididymal aspermia, and degeneration and atrophy of the testicular seminiferous tubules at levels that cause mortality (Mullins et al. 2016). It is not known if the observed lesions result in impaired reproductive ability.

In a study of a PAX-21-equivalent formulation, pregnant rats were dosed by gastric intubation with 0, 5.1, 10.2, or 20.4 mg equivalents of DNAN on gestation days 6 to 9. Maternal mortality was observed at the highest dose and maternal toxicity including decreased body weight and food consumption were observed in the 10.2 mg/kg DNAN group. No treatment-related macroscopic post-mortem findings were observed in females treated at up to 10.2 mg/kg-day. A slight decrease in fetal body weight was observed in the 10.2 mg/kg-day group that was attributed to maternal toxicity. No malformations or variations were observed. The maternal and fetal NOAEL values corresponded to the 5.1 mg/kg-day dose (USAFRL 2002).

In a developmental toxicity study, timed-pregnant rats were orally dosed with DNAN at 5, 15, and 45 mg/kg-day on gestation days 5 through 19. Examination on gestation day 20 indicated reduced fetal survival, weight, and size in the 45 mg/kg group. Prevalence of skeletal malformations was increased in the high-dose group and internal malformations were increased in the 15 and 45 mg/kg groups. Maternal toxicity (reduced body weight gain) was observed in all dose groups (Gao et al. 2016). Low confidence is given to this data, as only the abstract was available.

7.3.2.6 Mutagenicity

DNAN tested positive in the Ames *Salmonella* histidine reversion test (strains TA 98, 100, 102, 1535, and 1537), both with and without S9 metabolic activation (CCRIS 2010; Chiu et al. 1978; GENETOX 2009; McMahon et al. 1979; USAFRL 2002).

DNAN tested negative in CHO cells (AS52/XPRT) at concentrations of 0.0625 to 1.0 milligrams per milliliter (mg/mL) (USAFRL 2002; Dodd et al. 2002).

In both males and females, DNAN was negative in the *in vivo* mouse bone marrow micronucleus assay at exposures of 10–90 mg/kg (USAFRL 2002; Dodd et al. 2002). The highest dose demonstrated erythrocyte toxicity indicating appropriate exposure levels.

7.3.2.7 Carcinogenicity

No experimental data were found. TOPKAT modeling is indeterminate, with six models each predicting positive and negative outcomes. There is some suggestion in the modeling outcome that rats may be more likely than mice to develop cancer.

7.3.2.8 Metabolism

Adult male rhesus monkeys received oral doses of DNAN at 50, 25, or 5 mg/kg followed by serial blood and urine sampling up to 48 hours post exposure (Hoyt et al. 2013). Results showed that DNAN had a complex temporal profile over 48 hours with consistently low blood, serum, and urine levels and without an evident peak. However, 2,4-DNP (the reductive metabolite of DNAN), appeared in blood at concentrations 10-fold higher than the parent compound at 50 and 25 mg/kg. Yet, neither DNAN nor DNP were detected in biosamples at doses of 5 mg/kg. Rodents dosed with DNAN showed a similar blood profile. Therefore, it appears that the primary metabolite in primates for DNAN exposure is DNP, which cannot be detected in urine when exposure levels approximate the OEL, at least in single doses.

2,4-DNP has an oral lethal dose of 14-43 mg/kg in humans, with the cause of death generally attributed to the pyretic effect of 2,4-DNP, produced by an increase in metabolic rate. This rate increase is due to uncoupling of oxidative phosphorylation in mitochondria, leading to a rapid consumption of energy without generation of adenosine triphosphate (ATP) (Ray et al. 2008; Hutanu et al. 2013; Hoyt et al. 2013).

7.3.3 Ecotoxicology Data

7.3.3.1 Fate and Transport

Because DNAN is relatively hydrophobic and has a moderate log K_{OC} value, it is not expected to adsorb strongly to soil and its aqueous solubility is projected to be moderate. DNAN is expected to only be a moderate groundwater transport risk.

Dissolution of DNAN is a quasi-linear function of water volume and occurs slowly under simulated rainfall conditions (Richard et al. 2014b; Taylor et al. 2015). Particles ranging in mass from 0.3 to 3.5 grams were estimated to be completely dissolved in 3–21 years, given 100 centimeters (cm) annual precipitation (Taylor et al. 2015).

DNAN sorbs reversibly to soils (Boddu et al. 2009; Hawari et al. 2015), largely as a function of organic carbon content, binding strongly to lignin (Saad et al. 2012); however, DNAN also binds to K+-montmorillonite, a secondary clay mineral in soils (Linker et al. 2015). DNAN (bio) transformation products containing amino groups sorb irreversibly to soils (Hawari et al. 2015).

In lysimeter studies that evaluated DNAN transport in a spectrum of soil types exposed to simulated rainfall, DNAN was not detected in leachate samples; thus indicating low solubility/soil mobility. DNAN was primarily located in the top 5 cm of the lysimeter devices, indicating it was migrating very slowly, and was taken-up by Rye grass sprouts planted on the surface of the soil in the lysimeter (USARDEC 2009, 2011).
Bioconcentration and bioaccumulation are expected to be low, based upon the log K_{OW}. In a study of the bioaccumulation kinetics of TNT, RDX, DNAN, and NTO in *Rana pipiens* tadpoles, these compounds demonstrated relatively slow uptake and fast elimination rates (when returned to uncontaminated water). Short elimination half-lives (1.2 hours or less) and a preliminary bioconcentration factor of 0.25 liters per kilogram (L/kg) were determined (Lotufo et al. 2015).

7.3.3.2 Ecotoxicity

ECOSAR modeling predicts a 96-hour LC_{50} in freshwater fish of 9.003 mg/L, a 48-hour LC_{50} of 72.778 mg/L for *Daphnids*, and a 96-hour EC_{50} for green algae of 0.818 mg/L. TOPKAT modeling projects an LC_{50} in fathead minnow of 24.9 mg/L at high confidence, and an EC_{50} in *Daphnia* of 7.0 mg/L, also at high confidence.

DNAN decreased green algae (*Pseudokirchneriella subcaptata*) growth (EC_{50} = 4.0 mg/L), bacterial (*Vibrio fischeri*) bioluminescence (Microtox, EC_{50} = 60.3 mg/L), ryegrass (*Lolium perenne*) growth (EC_{50} = 7 mg/kg), earthworm survival (LC_{50} = 47 mg/kg), and increased earthworm avoidance response (EC_{50} = 31 mg/kg) (Dodard et al. 2013).

Acute and chronic aquatic toxicity bioassays were conducted using standard fish (*Pimephales promelas*) and invertebrate (*Ceriodaphnia dubia* and *Daphnia pulex*) models. Chemical analysis of test water indicated that DNAN concentrations were relatively stable during the bioassays. The 48-hour median lethal concentrations (LC_{50}) ranged from 14.2 to 42.0 mg/L DNAN. Survival in the chronic toxicity tests indicated that fish (7-day LC_{50} = 10 mg/L) were more sensitive than the cladocerans (LC_{50} = 13.7 to >24 mg/L). However, reproduction endpoints in the cladocerans (IC_{50} 2.7-10.6 mg/L) were equally or more sensitive to DNAN than fish survival. The lowest observable adverse effect concentrations (LOAECs) in the chronic tests ranged from 10 to 12 mg/L DNAN and median effects on sub-lethal endpoints (growth, reproduction) ranged from 2.7 to 15 mg/L DNAN. Chronic no-effect concentrations ranged from approximately 6 to 8 mg/L DNAN, which is less than that reported for TNT (Kennedy et al. 2015).

In *Hyalella azteca* exposed to DNAN as a single chemical or in IM mixtures for 10 or 35 days, DNAN was the most toxic of the IM constituents, with a 10-day LC_{50} of 16.0 mg/L (NTO = 891 mg/L and NQ = 565 mg/L). Reduction in growth (IC_{50} = 12.1 mg/L) was observed in the 10-day exposure, while the 35-day exposure resulted in greater sensitivity for lethality (LC_{50} = 3.5 mg/L) and decreased reproduction (IC_{50} = 2.0 mg/L) (Lotufo et al. 2018).

Similarly, in *Pimphales promelas* exposed to DNAN as a single chemical or in IM mixtures, DNAN was the most toxic constituent (LC_{50} of 36.1 mg/L), responsible for the toxicity of IMX-101, and acted synergistically with RDX in the toxicity of IMX-104 (Gust et al. 2018).

Exposure to DNAN reduced survival in *Lithobates (=Rana) pipiens* tadpoles exposed for 96 hours (LC_{50} = 24.3 mg/L) or 28 days (LOEC = 2.4 mg/L). Tadpole growth and development were not affected by DNAN exposures up to 8.1 mg/L in the 28-day study (Stanley et al. 2015).

The toxicity of DNAN in *Ceriodaphnia dubia* increased (2–100-fold) following photodegradation, which used simulated UV exposure of the exposure solution in a photoreactor (Kennedy et al. 2017).
Carp exposed to DNAN at a concentration of 117–270 mg/L experienced mortality (ECOTOX 2009).

Administration of DNAN to Japanese quail resulted in rapid production of cataracts. Quail developed cataracts within 4 hours of treatment (100%) when receiving oral doses of 120 or 150

mg/kg. Mortality rates in these groups were 1 of 5 at the lower dose and 5 of 9 at the higher dose (Takahashi et al. 1988).

A 1-hour treatment of wheat seeds with DNAN ($3x10^{-3}$ M) reduced germination, plantlet size, mass, and root mass (Amalia et al. 2009).

7.3.3.3 Degradation/Treatment

Biotransformation is an important component in the environmental fate of DNAN. Degradation of DNAN has been studied under aerobic and anaerobic conditions in enriched cultures and natural systems (Hawari et al. 2015; Olivares et al. 2013; Olivares et al. 2016a; Perreault et al. 2012; Platten et al. 2010). Under aerobic conditions, DNAN is initially removed due to sorption onto soils followed by slow transformation of a small fraction of DNAN. Although aerobic mineralization of DNAN has been observed in wastewater, reduction to MENA was observed in soils. Studies using sterilized soils indicate that under anaerobic conditions, both microbial and abiotic, using metallic or ferrous iron, reduction of DNAN occur (Ahn et al. 2011; Hawari et al. 2015; Niedzwiecka et al. 2017; USARDEC 2011). DNAN is reduced to the aromatic amines 2-methoxy-5-nitroaniline (MENA) and 2,4-diaminoanisole (DAAN) via nitroso and hydoxylamino intermediates (e.g., 2-amino-4-nitroanisole (2-ANAN), 4-amino-2-nitroanisole (4-ANAN)), which can react with amines to form azo-dimers and are potentially toxic and mutagenic (Hawari et al. 2015; Olivares et al. 2013; Olivares et al. 2016a; Platten et al. 2010; Padda et al. 2003). Microbial O-demethylation of DNAN, yielding 2,4-dinitrophenol (2,4-DNP) has also been reported (Fida et al. 2014; Richard et al. 2014a).

The rate of transformation is positively correlated with soil organic content (OC), which may act as an electron shuttle to stimulate reduction of DNAN and may also be a source of bacteria to catalyze nitroreduction (Glaus et al. 1992; Olivares et al. 2016b; Schwarzenbach et al. 1990). DNAN demonstrates zero-order transformation rates of 38.9-73.1 micromoles (µM) DNAN/day in fast soils and 4.51-11.6 µM DNAN/day in slow soils. Complete DNAN removal has been demonstrated in natural soils within 6 days in fast soils (OC-rich), while less than one-third of DNAN was removed in slow transforming soils after 9 days (Olivares et al. 2016b). Available evidence suggests DNAN is capable of undergoing natural biodegradation (Perreault et al. 2012; Saad et al. 2012).

Reduction of nitro groups to amines increases water solubility of the degradation products; however, the aminoderivatives sorb irreversibly to soil, reducing mobility in groundwater (Hawari et al. 2015). This reaction is shared by many nitroaromatics in wet soils. 2,4-DAAN is observed to be rapidly biodegraded under aerobic conditions.

Treatment of wastewater has been accomplished using zero-valent iron, iron-based bimetals, and magnesium-based bimetals (Hadnagy et al. 2018; Kitcher et al. 2017; Shen et al. 2013). Treatment of waste effluents via alkaline hydrolysis was also effective in destroying DNAN, probably producing 2,4-dinitrophenol in the process (USARDEC 2011).

Photochemical transformation of DNAN may represent an important degradation pathway in surface water. Studies in solar simulating photoreactors indicate that photodegradation of DNAN in water follows pseudo-first order decay kinetics with reported half-lives ($t_{1/2}$) of 330 minutes to

3.1 days (NRC 2013; Rao et al. 2013; CRREL 2013). Photo-oxidation is the dominant mechanism of degradation, producing 2,4-DNP as a minor species. The phototransformation of DNAN is dependent on the wavelength of the light source, but is not influenced by environmental factors including temperature, pH, and the presence of organic matter (Rao et al. 2013). As a pure solid, photodegradation of DNAN produced small quantities (<1% relative to DNAN) of degradation products that included methoxy nitrophenols and methoxy nitroanilines (Taylor et al. 2017).

7.4 3-Nitro-1,2,4-triazol-5-one [NTO]

7.4.1 General Information

NTO was developed by the Los Alamos National Laboratory in 1984 (LANL 1985); it is a candidate compound to replace RDX in explosive formulations and fulfill insensitive munitions (IM) requirements. This compound is also known as oxynitrotriazole (BAE 2007).

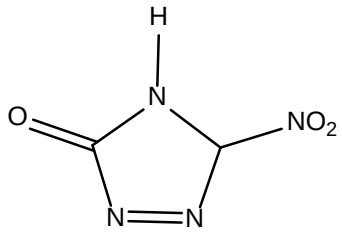

Figure 2. NTO

7.4.2 Toxicology Data

Testing of NTO for toxicity effects must take into consideration both the structure of the molecule and the acidity resulting from the presence of a dissociable hydrogen associated with nitrogen heterocyclic compounds. NTO produces a concentration-dependent decrease in pH, with a minimum of approximately pH = 3.

7.4.2.1 Oral

The oral LD_{50} for NTO is reported to be >5,000 mg/kg in both the rat and mouse (LANL 1985).

Sarlauskas et al. (2004) conducted an investigation into the mechanism of toxicity of NTO and the related ANTA (5-nitro-1,2,4-triazol-3-amine) by evaluating their reactions with one-electron and two-electron reductions by flavoproteins and oxyhemoglobin oxidation. Both NTO and ANTA were found to undergo cyclic oxidation-reduction reactions with production of superoxide (O_2), but to a lesser degree than TNT. Less production of free radicals is correlated with the lower toxicity of NTO and ANTA compared to TNT (Sarlauskas et al. 2004).

Results from a 14-day subacute oral toxicity study in rats showed significantly smaller testes weights in the high-dose groups (≥500 mg/kg) (Crouse et al. 2015).

A 90-day oral gavage study in rats was performed with doses of 0, 30, 100, 315, and 1,000 mg/kg. Significantly, smaller testes were observed in the 315 and 1,000 mg/kg dose groups (Crouse et al. 2015). Testicular effects are considered the critical effect for the development of exposure criteria. Benchmark dose analysis of the data from this study gave a $BMDL_{10}$ estimated to range from 22-47 mg/kg-day, depending upon the subset of acceptable models (WEEL 2018b).

In a chronic oral study, male and female Sprague Dawley rats were given *ad libitum* acess to NTO in drinking water at 0, 36, 110, 360, 1,100, and 3,600 mg/L for 1 year. Calculated average daily doses were 2, 6, 20, 50, and 170 mg/kg-day in males and 3, 9, 30, 80, and 260 mg/kg-day in females. Survival did not differ among groups and no treatment-related clinical signs were observed. NTO did not affect body weight, food consumption, clinical chemistry, or hematology parameters. The incidence of common neoplasms, including adenoma of the pituitary gland and benign mammary gland neoplasm and fibroadenoma, did not differ among treatment groups. In contrast to previous studies, testis mass and sperm count were not affected by NTO treatment. However, the high-dose group did exhibit a higher incidence of mild testicular tubular atrophy than the controls. In this study, the reduced severity of male testicular toxicity was likely due to a combination of reduced water intake resulting in NTO doses below those previously observed to induce testicular toxicity and differences in kinetics between oral gavage and drinking water studies (USAPHC 2019 (in prep)).

7.4.2.2 Inhalation

USAPHC (2013) performed an acute inhalation study with NTO to estimate a 4-hour LC_{50} value in rats. Since NTO is an explosive compound, a vapor or dry dust exposure could not be conducted, so exposure was effected via an aerosolized aqueous solution and a nose-only exposure. No compound-related animal deaths were noted at the highest air concentration achieved (0.184 mg/L).

7.4.2.3 Dermal

The manufacturers' Safety Data Sheet (SDS) suggests NTO may cause dermal irritation (BAE 2007). Administration of 500 mg NTO to the skin of a rabbit for 24 hours had a mild irritant effect (LANL 1985).

Dermal absorption of NTO was evaluated in an *in vitro* test using frozen human cadaver epidermal membranes (without stratum corneum) in a static Franz cell system based on OECD 428 testing guidelines. NTO was applied to the skin as a powder, and liquid samples were withdrawn from the receptor fluid at 1, 2, 4, 6, or 8 hours and analyzed for NTO. The authors estimated a steady state flux of 332 µg/cm^2-hour for NTO (USAPHC 2012b). Because the stratum corneum (the primary barrier to dermal absorption) was removed prior to testing, no conclusions can be drawn regarding the dermal absorption of NTO. Similarly, no definitive statement can be made regarding potential systemic toxicity following dermal exposure (WEEL 2018b).

NTO was not a dermal sensitizer in the guinea pig (LANL 1985).

7.4.2.4 Ocular

Eye irritation was tested in white rabbits using the Draize protocol. NTO (100 mg) was placed into the conjunctival envelope of six rabbits; the compound was not rinsed out in two animals, but was rinsed out of the eyes at 30 seconds for two animals and 5 minutes for the other two animals. Ocular erythema was graded at 24, 48, and 72 hours. All rabbits showed erythema at 1 and 4 hours, and by 72 hours the response had resolved in all but one rabbit (LANL 1985).

7.4.2.5 Developmental and Reproductive

To confirm the observations of the 90-day subchronic study, a test was conducted according to OECD 422 test guidelines. Groups of 10 male and 10 female rats were administered NTO at 0, 31, 125, or 500 mg/kg-day. An additional 20 males were included to serve as a satellite group to evaluate recovery or delayed effects. Male rats were dosed for 28 days including 2 weeks prior to and following mating. A complete necropsy was performed at the end of the dosing period. Female rats were dosed for 2 weeks prior to mating, during pregnancy, and through post-partum day 4. Treatment with NTO resulted in significant reductions in testes and epididymes mass and mass ratios in male rats given 500 mg/kg-day. Microscopic evaluation of these tissues revealed severe degeneration and atrophy of testicular seminiferous tubules along with moderate to severe hypospermia and cribriform change of the epididymes. Sperm counts were significantly reduced in the high-dose group (500 mg/kg-day). Despite the testicular atrophy and reduced sperm counts, there were no changes in reproductive success. Exposure and recovery periods were probably insufficient to observe full effects of dosing. The NOAEL from this study was 125 mg/kg-day (USAPHC 2014).

The reproductive and developmental toxicity of NTO was assessed in an Extended One Generation Reproductive Toxicity Test according to OECD 443 test guidelines. Groups of 25 male and 25 female rats were given *ad libitum* access to NTO in drinking water at four concentrations (0, 144, 720, or 3,600 mg/L NTO). Treatment of the parental (P) generation began 2 (females) to 4 (males) weeks pre-mating and continued until weaning of the litters. Direct dosing of offspring (F1) occurred from weaning through puberty. Additionally, two recovery groups (10 control and 10 high dose) were dosed concurrently with the main study animals and held for a period of 10 weeks following cessation of dosing to evaluate the reversibility of testicular toxicity. Mating index, pre-coital interval, gestation index, litter size, number of live and stillborn pups, and sex ratio did not differ among control and NTO-treated groups. The fertility index was slightly reduced in the 3,600 mg/L NTO group (88%) compared to the control (96%). Reproductive development of male, but not female, offspring was altered by exposure to NTO. Both the proportion of pups that had retained nipples and the number of nipples retained were increased in NTO-exposed males compared to controls. Attainment of puberty was delayed by 2.6 days in the 3,600 mg/L NTO exposed males. Pubertal males in the 3,600 mg/L NTO group exhibited reduced mass of the testis, epididymides, and accessory sex organs and associated histologic changes consistent with seminiferous tubule hypoplasia or degeneration/atrophy. P generation males in the high-dose group exhibited testicular seminiferous tubule degeneration and reduced sperm counts. Partial recovery was observed in the recovery group. This study indicates that testicular toxicity of NTO does not result in impaired fertility in rats. However, because humans have much lower sperm reserves, similar

testicular toxicity in humans may result in impaired fertility. Male reproductive development was likely altered because of testicular toxicity and resulting changes in testosterone levels (Lent et al. 2016a).

NTO was tested in a battery of *in vitro* and *in vivo* tests for endocrine disruption, including assays for estrogen receptor binding, androgen receptor binding, estrogen transactivation, aromatase, and steroidogenesis. All *in vitro* tests were negative (USAPHC 2012a).

In vivo endocrine disruption assays included a pubertal assay, Hershberger assay, and uterotrophic assay. The pubertal test evaluated the potential to affect pubertal development and thyroid function in male and female rats. NTO did not affect pubertal development in either sex and measured hormone levels were also not affected. Male rats exhibited reduced testis mass and tubular degeneration. These effects were associated with less-pronounced reductions in the mass of androgen-dependent accessory reproductive tissues (Lent et al. 2015). The uterotrophic and Hershberger assays assessed NTO's potential to act as an estrogen or androgen agonist/antagonist through changes in sex steroid sensitive organ weights in ovarectomized or castrated Sprague-Dawley rats. The results of these two screens do not provide evidence for endocrine disrupting activity at the dose levels tested (Quinn et al. 2014).

Time-course studies were conducted to identify the target cell(s) of NTO testicular toxicity of NTO. Groups of rats and BALB/c mice were exposed to NTO for 1, 3, 7, and 14 days to assess the timing and progression of injury to testicular cell populations. In both species, the earliest effects occurred at day 7. In rats, degeneration/apoptosis of pachytene spermatocytes and round and elongating spermatids, depletion of step 19 spermatids, luminal spermatogenic cell sloughing, multinucleate cells, and pronounced Sertoli cell vacuolation were observed. In mice, multinucleate cells, degeneration of spermatids and spermatocytes, and step 16 spermatid retention were noted. The Sertoli cell was indicated as the initial target of NTO toxicity in both species (Mullins et al. 2016).

7.4.2.6 Mutagenicity

NTO was evaluated for mutagenicity in *Salmonella typhimurium* and *Escherichia coli* plate incorporation assay both with and without S9 activation. Results were negative in *Salmonella* at up to 500 µg/plate without activation, and up to 5,000 µg/plate with activation. In *E. coli*, results were also negative at maximum concentrations up to 2,500 µg/plate without activation and 5,000 µg/plate with activation (Reddy et al. 2011).

NTO was also evaluated in the L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay. Cells were treated with NTO at concentrations up to 5,000 µg/mL, both with and without activation. Results of the assay were negative, either with or without activation (Reddy et al. 2011).

NTO was tested in CHO cells for clastogenicity. The test was conducted both with and without exogenous metabolic activation at concentrations up to 5,000 µg/mL; results were negative (Reddy et al. 2011).

A rat micronucleus assay was conducted in conjunction with a 14-day oral subacute study. Treatment of NTO did not produce a statistically significant increase in the frequency of

micronucleated reticulocytes in the peripheral blood of female or male rats. NTO is not genotoxic in rat peripheral blood at oral doses of up to 2,000 mg/kg in polyethylene glycol (Reddy et al. 2011).

7.4.2.7 Carcinogenicity

No experimental data were found. TOPKAT modeling of carcinogenicity produced an indeterminate result.

7.4.2.8 Metabolism

A study using ^{14}C-NTO found that metabolic degradation of this compound in rats appears to involve two separate enzymatic pathways. In the presence of oxygen, NTO is metabolized to two separate products: 5-amino-1,2,4-triazol-3-one (ATO) and 5-hydroxy-1,2,4-triazol-3-one (urazole). The presence of oxygen did not affect the overall conversion of NTO, but did alter the proportion of the metabolites. Under anaerobic conditions, the ATO is the primary product while urazole comprised only 5% of the product. Under aerobic conditions, urazole represented 40% of the product with a decrease in nitroreduction of 75%. Two separate pathways are represented here, since incubation of ATO with activated microsomes did not result in production of urazole, indicating that ATO does not represent an intermediate in this pathway and urazole is formed directly from NTO in mammalian systems (LeCampion et al. 1997).

Adult male rhesus monkeys received oral doses of either NTO at 50, 25, and 5 mg/kg followed by serial blood and urine sampling up to 48 hours post exposure. Results showed that NTO was absorbed quickly and eliminated by 8 hours, with urinary concentrations at least 100-fold higher than those of blood or serum. Screening of primate urine samples high in NTO for the metabolites ATO or urazole was negative. Rodents dosed with NTO showed similar blood profiles, but no NTO appeared in urine. This indicates potentially complete metabolism of NTO in rats, and functional differences between rodents and primates (Hoyt et al. 2013).

7.4.3 Ecotoxicology Data

7.4.3.1 Fate and Transport

NTO has a high solubility in water and a low log K_{OC}, indicating it will bind poorly to soil and have a high mobility in groundwater. This was confirmed experimentally in studies where NTO was allowed to percolate through different types of soil (Mark et al. 2016; Mark et al. 2017; USARDEC 2009, 2011). NTO was weakly adsorbed in a variety of clay mineral soil assemblages containing a range of organic matter, with adsorption decreasing with increasing soil pH (Mark et al. 2016). The amount of binding varied with soil type, with NTO being most strongly retained by the muck-peat, and least by the sandy-quartzose (USARDEC 2009, 2011). In contrast, NTO and ATO have been shown to adsorb to iron oxides occurring in soils (Khatiwada et al. 2018; Linker et al. 2015; Mark et al. 2017).

Uptake of NTO from soil by Rye grass grown on the top level of lysimeters was considerable. The amount varied from 398 mg/kg grass to 1,244 mg/kg grass, depending upon soil type.

Uptake by the grass was greater in soil where NTO was more strongly retained and lowest when NTO rapidly percolated through the soil (USARDEC 2009, 2011).

Based on the log K_{OW}, bioconcentration and bioaccumulation are expected to be low. In a study of the bioaccumulation kinetics of TNT, RDX, DNAN, and NTO in *Rana pipiens* tadpoles, all four compounds demonstrated relatively slow uptake and fast elimination rates (when returned to uncontaminated water). Short elimination half-lives (1.2 hours or less) and a preliminary bioconcentration factor of 0.25 L/kg were determined (Lotufo et al. 2015).

7.4.3.2 Ecotoxicity

TOPKAT modeling estimates an EC_{50} of 7.7 mg/L in *Daphnia* with low confidence. USEPA's ECOSAR program models NTO in the hydrazine class, with a 96-hour EC_{50} in green algae predicted to be 10.34 mg/L, the 48-hour LC_{50} in *Daphnia* to be 878.98 mg/L, and a 96-hour LC_{50} in fish of 61.52 mg/L.

Ceriodaphnia dubia was used in a 7-day survival and reproduction study and the unicellular green algae *Selenastrum capricornutum* in a 96-hour growth inhibition study. The addition of NTO to aqueous systems caused a concentration-related decrease in the pH of the system. This is likely from the ability of the ring-bonded hydrogen adjacent to the ring nitrogen to dissociate, producing a hydrogen ion. In 24- and 48-hour range finding studies, the pH of the NTO solution was found to affect the LC_{50}, with LC_{50} increasing approximately 7–13-fold when the pH was adjusted. Because of the impact of pH, all subsequent testing was done with NTO that had been adjusted for pH. In the definitive 7-day exposure study, the IC_{50}-value was 57 mg/L. The NOEC and LOEC values were 34 mg/L and 66 mg/L, respectively. While no mortality was observed at concentrations less than 523 mg/L, no eggs were produced at 262 mg/L, and eggs were produced but failed to develop at 133 mg/L (ECBC 2009).

In a 96-hour growth inhibition study using pH-adjusted NTO in *Selenastrum capricornutum*, the IC_{50} was estimated to be 3,465 mg/L, based upon a slight extrapolation of the IC_{20} value of 2,195 mg/L (ECBC 2009).

The acute toxicity of NTO and its breakdown product (ATO) was tested in methanogenic archaea, aerobic heterotrophs, and the bioluminescent marine bacterium, *Aliivibrio fischeri*. Anaerobic and aerobic sludge used for the methanogenic and aerobic heterotrophic inhibition assays, respectively, were dosed with NTO at concentrations up to 32 millimolar (mM) and samples from the headspace were analyzed for methane and oxygen, respectively. Inhibition of bioluminescence activity of *A. fischeri* was tested in the Microtox assay using pH adjusted NTO at concentrations of 0.12-30.4 mM (15.6-3954 mg/L). NTO was more inhibitory than ATO to methanogens (IC_{50}=1.2 mM, >62.8mM; 156 and >8,169 mg/L, respectively) and neither compound inhibited aerobic heterotrophs at the highest concentration tested. *A. fischeri* was inhibited only at high concentrations (IC_{50}=19.2mM; 2,498 mg/L) (Madeira et al. 2018).

In fathead minnow (*Pimphales promelas)* larvae exposed to NTO as a single chemical or in IM mixtures using 48-hour static, non-renewal tests with NTO buffered to pH 7.5, survival was not affected at concentrations up to 1,040 mg/L NTO (Gust et al. 2018).

In *Lithobates* (=*Rana) pipiens* tadpoles exposed to pH-adjusted NTO, a 96-hour range-finding study found no effects on survival at NTO exposure levels up to 500 mg/L (Stanley et al. 2015). The 48-hour and 7-day LC50s in *L. pipiens* exposed to unbuffered NTO were approximately 250 mg/L (Pillard et al. 2017). In long-term studies, a slight, non-monotonic reduction in survival was noted in tadpoles exposed for 28 days (LOEC 5.0 mg/L), while an LC_{50} of 3,670 mg/L was reported in the 70-day study (Pillard et al. 2017; Stanley et al. 2015). Tadpole growth and development was not affected by NTO exposures up to 100 mg/L in the 28-day study (Stanley et al. 2015). In the 70-day study, the number of organisms reaching complete metamorphosis was reduced by NTO (IC_{25} was 1,999 mg/L; NOEC for Time to Metamorphosis of 1,346 mg/L) (Pillard et al. 2017). A possible effect on the density of spermatogonia in NTO-exposed males was also suggested.

The toxicity of NTO and its breakdown product, ATO, was assessed in a zebrafish embryo assay. Mortality, morphology, and photomotor response were assessed in zebrafish embryos (6 hours post fertilization (hpf)) exposed to phosphate buffered NTO or ATO at concentrations of 0.075-750 µM (0.0097-97.56 mg/L) for 112 hours. No lethal or developmental effects were observed for either NTO or ATO. Abnormalities in swimming behavior were observed at the lowest concentration for both NTO and ATO; however, the degree that this response predicts adult behavior is unknown (Madeira et al. 2018).

A one-generation reproductive toxicity study was conducted in which parental generation Japanese quail (*Coturnix japonica*) were orally exposed to NTO at doses of 0, 20, 100, 500, and 1,000 mg/kg-day. Birds in the 1,000 and 500 mg/kg-day groups displayed neuromuscular anomalies including ataxia, convulsions, and opisthotonos; they were euthanized prior to reproductive testing. First generation off-spring (F1) were exposed to 20 or 100 mg/kg-day NTO only. Mild neuromuscular anomalies were observed in the 100 mg/kg-day group. Vacuolization of the cerebellum demonstrated a dose-dependent response and was identified as a critical endpoint for this study. A mean $BMDL_{10}$ of 35 mg/kg-day was derived based on the neurological effects in the parental generation (Jackovitz et al. 2018).

7.4.3.3 Decomposition/Treatment

Biodegradation was also explored by Haley and coworkers (ECBC 2009) using neutralized NTO in Ceriodaphnia growth medium. Under these conditions, approximately 10% of the NTO was lost over the course of a 7-day experiment. Assuming first order kinetics, this gives a rate constant of 0.015 d^{-1}, and a half-life of 46 days.

In column transport studies, smaller mass losses of NTO were observed in sterilized soils indicating that NTO is biodegraded by microorganisms (Mark et al. 2016; Mark et al. 2017). Biodegradation of NTO in soils was correlated positively with soil organic carbon content and exhibited half-lives of 72 hours to 2 days (Mark et al. 2016; Mark et al. 2017).

The ability of soil microbial communities to biodegrade NTO has been investigated in bioreactors inoculated with soil. NTO was readily biodegraded to ATO under anaerobic conditions, while ATO biodegradation occurred only under aerobic conditions (Krzmarzick et al. 2015; Madeira et al. 2017).

NTO is degraded by light, a process that can be replicated by means of a solar simulator (Hawari 2013). NTO photodegradation products are approximately 100 times more toxic to *Ceriodaphnia dubia* than parent NTO (Kennedy et al. 2017).

NTO is also reported to readily decompose by photodegradation in the presence of titanium dioxide. When exposed to light of wavelength >290 nanometers (nm), NTO solutions of concentration 150 mg/L in the presence of 0.4 grams per liter TiO_2 were completely degraded within 3 hours (Le Campion et al. 1999a). NTO is also readily degraded by a strain of the bacterium *Bacillus licheniformis* (Le Campion et al. 1999b). This degradation is reported to proceed through an oxygen-insensitive nitroreduction leading to production of the primary amine, ATO (5-amino-1,2,4-triazol-3-one), which is followed by cleavage of the triazone ring (Le Campion et al. 1998).

Treatment of an NTO waste stream was found to be unaffected by alkaline hydrolysis; however, treatment with bimetallic Fe/Cu particles rapidly degraded the compound (USARDEC 2011).

7.4.3.4 Combustion/Decomposition Products

Singh et al. (2001) recently prepared a short review on the chemistry and decomposition products of NTO. As is expected for a compound containing carbon and nitrogen, CO_2, NO_2, and N_2O are prominent combustion products, as well as water, carbon monoxide, diatomic nitrogen, hydrogen gas, and a product believed to be a polymeric form of 1,2,4-triazine-5-one (TO) with empirical formula $C_2H_3N_3O$. NO_2 free radicals are also believed to be produced by the cleavage of the nitrate group during the combustion process, but these would be too short-lived to have a biological effect.

7.5 RDX

7.5.1 General Information

RDX is a high explosive used extensively by the U.S. military since the late 1930's. It has been reported to cause convulsions in military field personnel who ingest it and in munitions workers inhaling its dust during manufacture. Because of its widespread use at military bases across the U.S., ranges have become contaminated with RDX due to its use in training, testing, and disposal. Due to this contamination, human exposure is possible both during remediation processes and through groundwater contamination. There is an active effort to find a safer replacement for RDX in modern military munitions.

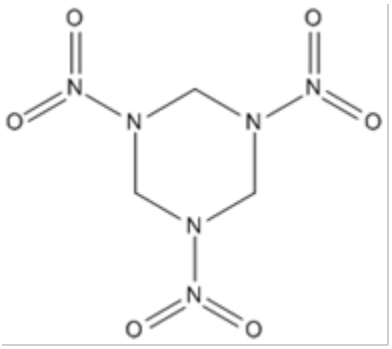

Figure 3. RDX

7.5.2 Toxicology Data

7.5.2.1 Oral

The LD_{50} values for RDX administered by oral gavage range from 59 to 97 mg/kg in mice (USAMRDC 1980a, 1979) and 68 to 300 mg/kg in rats (Schneider et al. 1977; USAMRDC 1980a; USACHPPM 2006; USAMRDC 1979). Mortality may be due to respiratory failure associated with repeated seizures. In the study by Crouse et al. (2006), tremors and convulsions appeared within 17–32 min at higher doses and the animals exhibited increased salivation. At 45 mg/kg, convulsions were observed 3 hours after dosing. Convulsions are consistently observed in all species examined including human, rat, pig, Northern Bobwhite, and Western fence lizard (Burdette et al. 1988; Barsotti et al. 1949; Kucukardalr et al. 2003).

The subchronic oral toxicity of RDX has been studied in rats, mice, beagle dogs, and rhesus monkeys. Five studies have been conducted in rats, three of these studies administered RDX in the diet (USAMRDC 1980a; IIT 1981; von Oettingen et al. 1949) while two studies used oral gavage (USACHPPM 2006; Schneider et al. 1978). In Fisher, 344 rats (60/sex/group) given RDX in the diet at concentrations providing 0, 10, 14, 20, 28, or 40 mg/kg-day RDX for 90 days, no mortality was observed. Reduced body weight in the 40 mg/kg-day group was noted in association with reduced food consumption. Reduced relative heart weight was noted in conjunction with increased myocardial degeneration in the 40 mg/kg-day group in both sexes. Anemia (reduced hematocrit and hemoglobin and increased reticulocytes) was observed in the 28 and 40 mg/kg-day groups (USAMRDC 1980a). In Fisher, 344 rats (10/sex/group) in the diet at concentrations providing 0, 10, 30, 100, 300, or 600 mg/kg-day RDX for 90 days, complete mortality was seen in the 300 and 600 mg/kg-day groups. A dose-related decrease in body weight gain was noted in males but not females. No biologically meaningful treatment-related effects were noted in the hematology or clinical chemistry parameters. Absolute and relative liver weight was increased in females in the 100 mg/kg-day group (IIT 1981). In a pair of studies with an unspecified strain of rats, RDX was given at concentrations providing 15, 50, or 100 mg/kg-day for 10 weeks and 15, 25, or 50 mg/kg-day for 12 weeks. In the first study, mortality occurred in the 50 and 100 mg/kg-day groups (60% and 87%) and was associated with congestion of the lungs and digestive tract. Hyperirritability and clonic/tonic seizures were noted in the 50 and 100 mg/kg-day groups. In the follow-up study, mortality (40%), body weight loss,

hyperirritability, and clonic/tonic seizures were noted in the 25 and 50 mg/kg-day groups (von Oettingen et al. 1949).

Sprague-Dawley rats given 20 mg/kg-day RDX via oral gavage for 90-days became lethargic and lost weight, but did not exhibit neurological signs of toxicity. Mortality (27%) was attributed to exacerbation of chronic respiratory disease (Schneider et al. 1978). In contrast, in F344 rats (10/sex/group) administered RDX via oral gavage at 0, 4, 8, 10, 12, or 15 mg/kg-day for 90 days, mortality was observed at dosages of 8 mg/kg-day and higher in both males and females (USACHPPM 2006). Visible signs of toxicity included changes in arousal, blepharitis (inflammation of the eyelid margins), increased salivation, bloodstains around eyes and mouth, rough hair coat, tremors, and convulsions. There were alterations in weights of brain, testes, kidney, and liver with associated alterations in hematology and clinical chemistry. There were no treatment-related alterations in histopathology, immunology, or behavior and motor activity. All visible and measured signs of toxicity were confined to dose groups that produced lethality. The NOAEL is 4 mg/kg-day based on lethality; LOAEL is 8 mg/kg-day (USACHPPM 2006). Similarly, in a study of the neurotoxicity of RDX, Sprague-Dawley rats given 0, 1, 3, or 10 mg/kg-day RDX via oral gavage for 30 days demonstrated no changes in motor activity, flavor aversion, schedule-controlled response, or acoustic startle (USEPA 1985). However, behavioral testing conducted 2 hours following acute doses of RDX (12.5-50 mg/kg) produced decreases in startle-response amplitude, figure-eight maze motor activity, landing foot spread, and rates of schedule-controlled response and increases in startle-response latency and conditioned flavor aversions (USEPA 1985).

Mice appear to be less sensitive to RDX than rats. B6C3F1 mice (10/sex/group) given RDX in the diet at concentrations providing 0, 10, 14, 20, 28, or 40 mg/kg-day for 90-day demonstrated no signs of toxicity (Cholakis 1980). In a follow-up study, B6C3F1 mice (10-12/sex/group) were given RDX in the diet at concentrations providing 0, 40, 60, or 80 mg/kg-day for 2 weeks followed by 0, 80, 160, or 320 mg/kg-day for 11 weeks. Mortality was observed in the 320 mg/kg-day group at week 11 in both males (40%) and females (17%). Only male (50%) mice in the 320 mg/kg-day group exhibited hyperactivity and/or nervousness. Males in the 160 mg/kg-day but not the 320 mg/kg-day exhibited anemia (decreased erythrocytes and hemoglobin and increased mean corpuscular volume). Absolute and relative liver weight was increased in both males and females in the 320 mg/kg-day group. Myocardial degeneration, tubular nephrosis, and periportal hepatocellular vacuolization were noted in the high-dose group (USAMRDC 1980a).

In beagle dogs given 50 mg/kg-day RDX in the diet for 6 weeks, hyperirritability, convulsions, and weight loss were observed (von Oettingen et al. 1949). When administered to dogs (3/sex/group) at lower doses (0, 0.1, 1, or 10 mg/kg-day) for 90 days, RDX caused no treatment-related effects (Litton 1974b).

One subchronic oral study was conducted in monkeys. Cynomolgus monkeys (3/sex/group) were given 0, 0.1, 1, or 10 mg/kg-day RDX in methylcellulose via oral gavage for 90 days. Dose-related vomiting was observed in both sexes. Central nervous system disturbances including tonic-type convulsions were noted in five animals in the 10 mg/kg-day group. No other biologically meaningful treatment-related findings were noted in organ weights, eye exams, clinical chemistry, hematology, and histopathology (Litton 1974a).

The chronic toxicity of RDX has been investigated in three studies in rats and mice (IIT 1983; USAMRDC 1984a; Litton 1976). A 2-year study in which Sprague-Dawley rats were given 0, 1.0, 3.1, or 10 mg/kg-day RDX in the diet demonstrated no effects on survival or histopathology. The only treatment-related effect observed was a reduction in body weight in females in the 3.1 and 10 mg/kg-day groups and in males in the 10 mg/kg-day group (Litton 1976). In a 2-year study in F344 rats (85/sex/group), RDX was administered at doses of 0.3, 1.5, 8.0, or 40 mg/kg-day in the diet. Mortality was higher in high-dose males (96%) and females (65%) than controls (52 and 48%, respectively). Tremors and convulsions were observed in the 40 mg/kg-day group; however, no lesions were noted in the CNS. Increased incidence of cataracts was noted in high-dose males but not females. Body weight gain was reduced in males given 8 mg/kg-day RDX and higher. Slight anemia (decreased hemoglobin, hematocrit, and erythrocytes) was noted in males and females in the 40 mg/kg-day group and was associated with splenic enlargement, extramedullary hematopoiesis, and sinusoidal congestion. Hepatotoxicity (increased liver weight and lactate dehydrogenase, and decreased cholesterol, triglycerides, albumin, and total protein) was noted in both sexes; renal toxicity (increased kidney weight, blood urea nitrogen (BUN), renal medullary papillary necrosis) was noted primarily in males in the 40 mg/kg-day group. Inflammation of the prostate and hemosiderin-like pigment deposits in the spleen (a secondary response to hemolytic anemia) were noted in males at doses of 1.5 mg/kg-day and higher (IIT 1983). In B6C3F1 mice (85/sex/group) given RDX in the diet at concentrations providing 0, 1.2, 7.0, 35.0, or 175 mg/kg-day for 2 years, high mortality was observed at 175 mg/kg-day and was reduced to 100 mg/kg-day at week 11. After the dose reduction, mortality was similar in control and RDX groups for the remainder of the study. Convulsions were noted in one male (35 mg/kg-day) and one female (100 mg/kg-day) during month 24. Absolute and relative liver weight was increased in males and females in the high-dose group. Relative kidney weights and the incidence of renal tubular cytoplasmic vacuolization were increased in the high-dose group. BUN levels were increased in high-dose females only. Brain weights were decreased and heart weights increased in the high-dose group. Incidence of testicular degeneration was increased in the 35 and 100 mg/kg-day group males (USAMRDC 1984a).

7.5.2.2 Inhalation

No studies in humans were found; epidemiological studies do not provide data suitable for dose-response analysis of inhalation exposure to RDX. One inhalation study reporting death attributed to impairment of the respiratory system in rabbits and guinea pigs exposed to an unspecified concentration of RDX is available; however, the study is of limited use as it does not include controls or complete reports of exposure levels (ATSDR 2012).

7.5.2.3 Dermal

Dermatitis that has been attributed to RDX manufacturing is possibly due to intermediates since significant dermatitis was not observed in individuals handling the final purified material (HSDB 2012). Patch testing did not produce irritation in an individual exposed for 2 days to an unknown dose (von Oettingen et al. 1949).

In a study of the acute dermal toxicity of RDX, rabbits, guinea pigs, and beagle dogs received single topical applications (1 ml) of RDX in three solvents (DMSO, cyclohexanone, and acetone) at concentrations of 33, 75, and 5.4% (weight per volume (w/v)). Animals were examined for

skin irritation and systemic toxicity for 30 days. No increase, relative to solvent control, in skin irritation, death, or systemic toxicity was noted. Dermatitis was observed in rabbits regardless of the solvent, but was most pronounced in rabbits exposed to RDX in DMSO (165 mg/kg) (EA 1974). Similarly, when the same RDX solutions were topically applied (0.1 ml and 1 ml) to rabbits (6/mixture/volume) 5 days/week for 4 weeks, no skin irritation was observed. Dermatitis was observed in the 1 ml RDX in DMSO (165 mg/kg) group, but not in the lower DMSO dose group or the other vehicles. In guinea pigs (4/group) given topical applications of 3% RDX in DMSO (w/v) at doses of 316, 510, 1,000, and 2,000 mg/kg, slight erythema was observed at 1,000 and 2,000 mg/kg. When the 1,000 mg/kg dose was repeated for 3 days, no further increase in erythema was observed (McNamara 1974). In beagle dogs (2/mixture), single topical applications of RDX in DMSO (33%, 289 mg/kg), cyclohexanone (7.5%, 65.7 mg/kg), or acetone (5.4%, 47.3 mg/kg) produced no treatment-related effects (EA 1974). When the same solutions were applied to the dorsal area of beagle dogs 5 days/week for 4 weeks, slight erythema and desquamation was noted in the RDX in DMSO and DMSO control treatments. No effects were noted for the other solvents. RDX in a 1% solution of carboxymethylcellulose (2,000 mg/kg) applied to the shaved skin of rabbits (5/sex) produced no signs of toxicity (USAMRDC 1984b).

Dermal absorption of RDX has been studied *in vitro* using human and pig skin. Limited dermal absorption of RDX from contaminated soils was observed in human (1.4–2.6%) and pig skin (<1%) (Reddy et al. 2008; Reifenrath et al. 2008). RDX in acetone also demonstrated limited dermal absorption (2.5%) (Reddy et al. 2008).

RDX produced no evidence of sensitization in guinea pigs given topical (0.5 mL) or intradermal (0.05 mL) dosed of RDX in DMSO, cyclohexanone, or acetone 3 days/week for 3 weeks. A challenge dose of RDX (1:1 solvent:saline mixture) was given via both routes after a 2-week rest period (EA 1974).

7.5.2.4 Ocular

RDX is reported to be an ocular irritant (HSDB 2012).

7.5.2.5 Developmental and Reproductive

A two-generation reproduction study with F344 rats was conducted using dietary delivery of RDX at rates of 5, 16, or 50 mg/kg-day over a 13-week treatment schedule for the F0 (22/sex/group) and F1 (26/sex/group) generations. After 13 weeks of treatment, animals were mated and dams were allowed to litter. After weaning, selected F1 animals were fed the same diet as their parents, F2 animals were necropsied. Elevated maternal mortality, neurotoxicity, and a higher percentage of stillborn pups were observed at 50 mg/kg. Pup viability (number of litters and litter size) was reduced in the 50 mg/kg-day group. Pup body weight was reduced in the 50 and 16 mg/kg groups in the F1 generation. Due to high mortality in the 50 mg/kg-day group in the F0 and F1 generations, too few animals were available in the high-dose F2 generation to permit analyses. No developmental effects were observed at 5 mg/kg, and no fertility effects were noted at either 5 or 16 mg/kg (USAMRDC 1980a).

Developmental toxicity was investigated in four studies in rats and rabbits (USAEHA 1986; USAMRDC 1980a; USACHPPM 2007). In timed-pregnant F344 rats (24-25/group) given 0, 0.2, 2.0, or 20 mg/kg-day RDX via oral gavage on gestational days (GD) 6 through 19, maternal toxicity (mortality, convulsions, reduced body weight gain, and decreased food consumption) were observed in the 20 mg/kg-day group. Increased post-implantation loss was noted in the 20 mg/kg-day group. No teratogenicity was demonstrated (USAMRDC 1980a). In contrast, in timed-pregnant rabbits given 0, 0.2, 2.0, or 20 mg/kg-day RDX via oral gavage on GD 7 through 29, dams in the 20 mg/kg-day group exhibited slight maternal toxicity (reduced body weight gain). Implantation rates were not affected by RDX treatment; however, the incidence of fetal abnormalities was increased, though not statistically significantly, in the 20 mg/kg-day group. In a pair of developmental studies in rats, timed-pregnant Sprague-Dawley rats were given 0, 10, 20, 40, 80, or 120 mg/kg-day RDX via oral gavage on GD 6 through 15 in a range-finding study and 0, 2, 6, or 20 mg/kg-day on GD 6 through 15 in the main study. In the range-finding study, doses of 40 mg/kg-day and higher were lethal in all females. In the main study, increased mortality (31%) was observed in the 20 mg/kg-day group. Convulsions, prostration, and urogenital and nasal discharge were noted in the 20 mg/kg-day group. Fetal weight and length were reduced in the 20 mg/kg-day group. No teratogenic effects were observed (USAEHA 1986).

In a pilot developmental neurotoxicity test to determine if RDX is transferred to offspring during gestation and lactation, pregnant Sprague-Dawley rats were dosed with 6 mg/kg-day RDX on GD 6 through postnatal day 10. Brains from pups contained RDX at birth, but concentrations decreased by postnatal day 10 and were not detectable at weaning. RDX was, however, present in the dam's milk (USACHPPM 2007).

7.5.2.6 Mutagenesis

RDX is not genotoxic in the standard *in vitro* and *in vivo* genotoxicity assays (USAMRDC 1980a; Reddy et al. 2005; Whong et al. 1980; USAMRDC 1979, 1977). RDX was not mutagenic, both with and without S9 metabolic activation, in strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations of 0.24–14 µg/plate (USAMRDC 1977), 1–1,000 µg/plate (USAMRDC 1980a), and 0.625 or 1.25 mg/plate (Whong et al. 1980). Similarly, in *Saccharomyces cerevisiae* strain D3, RDX was not mutagenic when tested at concentrations ranging from 0.00004 to 0.0023% (USAMRDC 1977). RDX is, however, mutagenic after S9 incubation in the non-standard TA97a strain of *Salmonella typhimurium* (Pan et al. 2007).

RDX did not induce unscheduled DNA synthesis in human fibroblasts (WI-38) exposed to concentrations up to 4,000 mg/L RDX both with and without metabolic activation (USAMRDC 1979). RDX did not induce forward mutations at the thymidine kinase (TK) locus in mouse lymphoma cells (L5178Y) exposed to concentrations ranging from 3.93 to 500 µg/mL (Reddy et al. 2005).

RDX did not induce micronuclei in the bone marrow of male mice orally dosed with RDX at the maximum tolerated dose, 250 mg/kg (Reddy et al. 2005).

RDX was negative in a dominant lethal assay in male F344 rats given RDX at 0, 5, 16, or 50 mg/kg-day in the diet for 13 weeks. There was no evidence of pre- or post-implantation loss in untreated, virgin females mated to the treated males (USAMRDC 1980a).

7.5.2.7 Carcinogenesis

Evidence for carcinogenicity is drawn from the chronic studies discussed previously. Dose-related increases in the incidence of hepatocellular adenomas and carcinomas and alveolar/bronchiolar adenomas and carcinomas were noted in male and female B6C3F1 mice in the USAMRDC (1984a) study. A team of pathologists reassessed the histology of neoplasms found in female mice in the USAMRDC (1984a) study to determine the incidence of cancer using current criteria (Parker et al. 2006). Incidence of neoplasm for all groups was within the range of spontaneous neoplasms of female mice (incidence in Lish controls was inordinately low) and of significance only at 35 mg/kg-day (i.e., only equivocal evidence of carcinogenicity). In the Levine et al (1983) study, the incidence of hepatocellular carcinomas was increased in male but not female F344 rats. The Hart (1976) study did not demonstrate treatment-related increases in the incidence of adenomas or carcinomas.

RDX is classified by the USEPA as having suggestive evidence of carcinogenic potential, based upon production of benign and malignant tumors in the liver and lungs of female B6C3F1 mice (USAMRDC 1984a). An oral slope factor of 0.08 $(mg/kg\text{-}day)^{-1}$ was derived based on the combined incidence of these tumors using a maximum likelihood estimate of the combined risk at a 95% confidence level (USEPA 2018b). The American Conference of Governmental Industrial Hygienists lists RDX under category A4—not classifiable as a human carcinogen (HSDB, 2012).

7.5.3 Ecotoxicology Data

7.5.3.1 Fate and transport

The low vapor pressure of RDX indicates that in the atmosphere it will exist exclusively in the particulate form. Particulate RDX will be removed from the atmosphere by both wet and dry deposition. Solubility in water is low. With a K_{OC} range of 42–167 and aqueous solubility of 59.7 mg/L, mobility in soil is expected to be moderate to high and RDX is expected to leach into groundwater (USAMRDC 1980b). Soil column and lysimeter studies have demonstrated that RDX is not readily sorbed to soil (USAMRDC 1993; NRDC 1985; Selim et al. 1995); however, sorption is increased in soils with high organic matter or clay content (USAMRDC 1980b). The Henry's law constant for RDX suggests volatilization from wet surfaces will be slow.

Bioconcentration and bioaccumulation are expected to be low, based upon the log K_{OW}. In a study of the bioaccumulation kinetics of TNT, RDX, DNAN, and NTO in *Rana pipiens* tadpoles, all four compounds demonstrated relatively slow uptake and fast elimination rates (when returned to uncontaminated water). Elimination half-lives were 1.2 hours or less (Lotufo et al. 2015). Bioconcentration factors of 0.87 in Mediterranean mussels (*Mytilus galloprovincialis*), 0.6–0.9 in sheepshead minnows (*Cyprinodon variegatus*), 2.1 in aquatic oligochaetes, and 2.0 in channel catfish (*Ictalurus punctatus*) indicate the potential for bioconcentration in aquatic organisms is low (Rosen et al. 2007; Lotufo et al. 2010; Belden et al. 2005). A bioaccumulation factor of 3.6 was determined for earthworms exposed to RDX-contaminated soil (Sarrazin et al. 2009). Fish that were fed oligochaetes exposed to high concentrations of RDX demonstrated minimal bioaccumulation (Belden et al. 2005).

RDX is readily taken up by plants (Best et al. 1999b, 1999a; Harvey et al. 1991; Pennington et al. 2002; Simini et al. 1996). Uptake by plants is dependent on concentration, soil type, and plant species (USABRDL 1990; Simini et al. 1996). When grown in soil containing 58 mg/kg RDX, plants accumulated varying amounts of RDX—lettuce, 1,200 mg/kg; nutsedge, 62 mg/kg; tomato fruit, 7 mg/kg; corn kernels, 6 mg/kg; and corn stover (leaves and stalks of corn or other plants left behind in a field after harvest), 56 mg/kg (Pennington et al. 2002). Submerged aquatic plants Elodea, pondweed, and water star-grass grown in groundwater containing 1,529 µg/L RDX contained 976, 42, and 1,496 µg/L RDX, respectively, after 13 days (Best et al. 1999b). RDX is largely concentrated in leaves in most plants, with priority for storage in roots, stems, and seeds varying among plants (USABRDL 1990). RDX stored in leaves and seeds remains largely unchanged, but is metabolized to unidentified polar metabolites in other tissues (USABRDL 1990).

7.5.3.2 Ecotoxicity

RDX is not acutely toxic to species of green algae when tested at the solubility limit. At 32 mg/L RDX reduced cell density of several algae species by 17%, thus a 96-hr EC_{50} could not be determined (USABRDL 1977; Burton et al. 1994b).

RDX is not acutely toxic to several strains of invertebrates, including *Ceriodaphnia dubia, Hydra littoralis*, and a midge *(Paratanytarsus parthenogeneticus)* (Peters et al. 1991). The 48-hour EC_{50} for *Daphnia magna* is greater than 100 mg/L under static and greater than 15 mg/L under flow-through conditions; RDX did not affect survival of *Daphnia* exposed for 21 days to 5.8 mg/L (USABRDL 1977). RDX did not induce significant mortality or growth reduction in *Hyalella azteca* exposed RDX near its solubility limit (29.5 mg/L) for 10 days (Lotufo et al. 2018). Decreased reproduction was observed in *H. azteca* exposed for 35 days, with an IC_{50} of 18.5 mg/L (Lotufo et al. 2018). Soil RDX concentrations of 44–660 mg RDX/kg were not toxic to soil invertebrates including earthworms, potworms, and microarthropods (Dodard et al. 2005; ECBC 2003). Reproduction was reduced in soil invertebrates exposed to RDX contaminated soils, with EC_{20} values ranging from 5.0 to 8,797 mg/kg (Dodard et al. 2005; ECBC 2003). Adult survival and byssal thread formation, and larval development were not affected in *M. galloprovincialis* exposed to concentrations of RDX up to 28.4 mg/L for 96 hours (Rosen et al. 2007).

Several different life stages of the fathead minnow (*Pimephales promelas*) were tested for 96 hours in static tests. The egg and 1-day post-hatch fry were the least sensitive stages and the 7-day post-hatch was the most sensitive stage with an LC_{50} of 3.8 mg/L. In mature adults, the LC_{50} was 5.8 mg/L under static and 6.6 mg/kg under flow-through conditions (USABRDL 1977). The acute 96-hour LC_{50} for 15–17 day-old fathead minnows was 12.7 mg/L (Burton et al. 1994a). Exposure to 5.8 mg/L RDX for 21 days did not affect the survival of *Pimephales* (USABRDL 1977). In juvenile sheepshead minnows, the 10-day LC_{50} was 9.9 mg/L (Lotufo et al. 2010). In zebrafish (*Danio rerio*), the 96-hour LC_{50} was estimated at 23–26 mg/L. Vertebral deformities and behavioral abnormalities (whirling movement and lethargy) were noted with an EC_{50} of 20.8 mg/L (Mukhi et al. 2005).

RDX reduced swimming distance in *Lithobates (=Rana) pipiens* tadpoles exposed for 10 days (LOEC = 5.9 mg/L), but did not affect survival at the highest concentration tested (25.3 mg/L).

No effects on survival, growth, or development were observed in tadpoles exposed for 28 days at the highest concentration, 28.0 mg/L (Stanley et al. 2015).

The toxicity of RDX to the Northern Bobwhite (*Colinus virginianus*) was investigated in acute, subacute, and subchronic studies (Gogal et al. 2003). In the acute study, the approximate lethal dose (ALD) in male birds was 280 mg/kg, and for females, 187 mg/kg. In the subacute study, birds (6/sex/group) given RDX in the diet at concentrations providing 0, 8.7, 10.6, 13.4, 22.3, or 26.3 mg/kg RDX for 14-days demonstrated dose-dependent decreases in food consumption, body weight, and egg production. In the subchronic study, birds (10/sex/group) were given RDX in the diet at concentrations providing 0, 10.8, 13.4, 22.3, or 26.3 mg/kg RDX for 90 days. No treatment-related effects were observed in the subchronic study despite use of the same doses as the subacute study.

Two studies investigated the subacute toxicity of RDX in Northern Bobwhite when administered via oral gavage. In Northern Bobwhite given 0, 20, 80, 125, or 180 mg/kg-day RDX for 14-days, clonic/tonic convulsions followed by mortality (100%) occurred in all dose groups (Johnson et al. 2007). A subsequent study was conducted at lower doses (0, 0.5, 3, 8, 12, or 17 mg/kg RDX) to evaluate sublethal effects of RDX in birds; however, mortality was still observed (100, 67, and 25%) in the 17, 12, and 8 mg/kg-day dose groups, respectively (Quinn et al. 2009). Death was preceded by clonic/tonic convulsions and weight loss caused by gastrointestinal effects. Serum globulin and total leukocyte count were increased in the two highest dose groups. Degeneration of testicular and splenic tissue was also observed (Quinn et al. 2009).

In a study comparing the toxicity of RDX and 4A-DNT in Japanese quail (*Coturnix japonica*), Northern bobwhite, and Zebra finch (*Taeniopygia guttata*), birds were orally dosed with RDX at 0, 0.5, 1.5, 3, 6, or 12 mg/kg-day for 7 days. Bobwhite were more sensitive to RDX than Japanese quail, while finches were the most sensitive, demonstrating increased mortality and seizure activity (Quinn et al. 2013).

Red-backed salamanders (*Plethodon cinereus*; 20/group) were exposed to 5,000, 1,000, 100, 10, and 0 mg RDX/kg soil (dry weight) for 28 days using a microcosm design. Weight loss and neuromuscular effects (lethargy, gaping, hypersensitivity, and tremors) were observed in the high-dose group. No treatment-related effects were noted in the histopathologic evaluation (Johnson et al. 2004). The acute, subacute, and subchronic toxicity of RDX in reptiles was assessed in Western Fence Lizards (*Sceloporus occidentalis*) (McFarland et al. 2009). Median lethal doses were 72 and 88 mg/kg in males and females, respectively. In the subacute study, lizards were orally dosed with 0, 10, 20, 25, 30, 45, or 60 mg/kg-day RDX. Survival was reduced in the 20 mg/kg-day group. In lizards orally dosed with 0, 1, 2.5, 5, 8, or 11 mg/kg-day RDX for 60 days, mortality, lethargy, and anorexia were observed in the 8 and 11 mg/kg-day groups. Reduced body weight gain and food consumption were observed in the 5 mg/kg-day group.

Soil RDX had no effect on the growth of rice or perennial ryegrass (*Lolium perenne L.*) exposed for 40- or 21-days, respectively (Rocheleau et al. 2008; Vila et al. 2007). Rice plants, however, demonstrated necrosis at RDX concentrations over 500 mg/kg. Seed germination was reduced and seedling morphology altered in a woody shrub (*Morella cerifera*), grown in soils with RDX up to 1,500 ppm (Via et al. 2015).

7.5.3.3 Degradation/Treatment

RDX is expected to biodegrade in soil under anaerobic conditions, with complete degradation reported to have occurred within 24 days (Funk et al. 1993; Pennington et al. 2002). Its biodegradation products include hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX); hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX); hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX); hydrazine; 1,1-dimethyl-hydrazine; 1,2-dimethyl-hydrazine; formaldehyde; and methanol (McCormick et al. 1981). RDX is expected to be resistant to degradation under aerobic conditions. In water, degradation by hydrolysis is not expected to occur at pH levels of natural waters; however, alkaline hydrolysis has been demonstrated and represents a possible remediation method (Heilmann et al. 1996). The primary means of degradation of RDX is photolysis by sunlight; experimental half-lives of 9–13 hours are reported in translucent waters (HSDB 2012). Half-lives of several years have been reported in dark, tea-colored waters due to attenuation of sunlight penetration (USAMRDC 1983). Photoproducts include formaldehyde and nitrosamines.

7.5.3.4 Combustion products

The Lawrence Livermore National Laboratory's CHEETAH code predicts combustion products are consistent with the expected products of compounds containing carbon, nitrogen, and hydrogen (e.g., the oxides of these elements). Potential combustion products of concern such as ozone, superoxide, and nitrous oxide are predicted to be present at such low levels that there is no health concern because of a transient exposure.

7.6 IMX-104

7.6.1 General Information

IMX-104 is a mixture of NTO, DNAN, and RDX. This section addresses the effects of the mixture formulation currently under production, as opposed to the individual components.

7.6.2 Toxicology Data

Only limited toxicology data are available for the IMX-104 mixture. Traditional toxicology practice assumes that in the absence of a common mechanism, the effects of a mixture are merely a summation of the effects on the individual components (Monosson 2005). These studies were conducted to determine the validity of this assumption.

7.6.2.1 Oral

No experimental data on oral effects of IMX-104 are available.

7.6.2.2 Inhalation

No experimental data on inhalation effects of IMX-104 are available.

7.6.2.3 Dermal

No experimental data on dermal effects of IMX-104 are available.

7.6.2.4 Ocular

No experimental data on ocular sensitivity of IMX-104 are available.

7.6.2.5 Developmental and Reproductive

No experimental data on developmental or reproductive toxicity of IMX-104 are available.

7.6.2.6 Mutagenesis

No experimental data on mutagenicity of IMX-104 are available.

7.6.2.7 Carcinogenesis

No experimental data on carcinogenicity of IMX-104 are available.

7.6.3 Ecotoxicology Data

7.6.3.1 Fate and Transport

The insensitivity of IMX formulations was demonstrated after a blow-in-place (BIP) detonation, 45–50% of the NTO and 11–19% of the DNAN components remained as undetonated residue. However, after a high order detonation of IMX-104, only 0.4–1.2% of the NTO component remained as undetonated residue (CRREL 2013).

IMX-101 and IMX-104 samples were included in a study of insensitive munitions by CRREL researchers that evaluated environmental dissolution. IMX-104 forms inhomogeneous particles with NTO and RDX embedded in a DNAN matrix. As the IMX-104 particles progressively dissolve when exposed to the environment, NTO and RDX dissolve first, leaving a porous DNAN matrix that has an increased surface area and hence rate of dissolution. Outdoor testing also revealed particles of the IM mixtures were more friable than the legacy high explosive formulation particles (Walsh et al. 2014; Taylor et al. 2015). Outdoor testing also indicated phototransformation of the IM formulation, with pieces changing in color from cream or white to orange or brick red. Accompanying this color change is the appearance of an unidentified peak in the HPLC chromatogram where polar substances elute. The identity of the unknown compound was not determined. Phototransformation was determined to be a first-order kinetic process (CRREL 2014).

When present as part of the mixtures IMX-101 and IMX-104, the individual components were found to dissolve according to their aqueous solubility in soil column studies (Arthur et al. 2018; Richard et al. 2014b). NTO demonstrated limited sorption to soils and eluted first in a high concentration pulse. RDX and DNAN demonstrated appreciable absorption.

7.6.3.2 Ecotoxicity

In *Hyalella azteca* exposed to IMX-104 components individually or in the mixture for 10 days or 35 days; a 10-day LC_{50} of 98.1 mg/L was determined for IMX-104. Lethality occurred at lower concentrations in the 35-day exposure, resulting in an LC_{50} approximately 6-fold lower than the 10-day LC_{50}. A reduction in growth was observed in the 10-day exposure (IC_{20}=12.2 mg/L). Growth and reproduction were not affected at sublethal concentrations in the 35-day exposure. Evaluation of mixture interactions using the toxic units approach indicated interactive, potentially antagonistic effects, causing significantly decreased acute lethality of the components of IMX-104 in *H. azteca.* Sublethal toxicity, however, could be attributed solely to the DNAN component (Lotufo et al. 2018).

In contrast, in *Pimphales promelas* exposed to IMX-104 components individually or in the mixture in an acute (48-hour) test, LC_{50} values of 36.1, 28.9, and 66.1 were reported for DNAN, RDX, and IMX-104. NTO did not elicit significant mortality. Based on toxic units, the results indicate greater than concentration additive toxicity for the mixture (Gust et al. 2018).

7.6.3.3 Degradation/Treatment

The field study conducted by CRREL (2014) determined that fragments of unexploded IMX-101 and IMX-104 material could be found after incomplete detonation of rounds due to insensitivity of the munition. Fragments were found to be extremely friable and subject to differential leaching of the munition components. Components released individually to the environment in this manner would be expected to behave as described above in the individual compound discussions.

The biodegradation potential of IMX-101 and IMX-104 was studied in two un-amended training range soils under aerobic and anaerobic conditions. After 30 days, complete biotransformation was demonstrated in one soil type for DNAN and NTO under anaerobic conditions. Reduced transformation rates were observed under aerobic conditions and with the second soil type. RDX did not undergo significant transformation (Indest et al. 2017).

8. DISCUSSION

8.1 Overall Formulation Effects

The toxicity of the IMX-104 mixture has not been investigated in a mammalian model. In aquatic organisms, interactive effects were demonstrated in acute toxicity tests in *H. azteca* and *P. promelas*; however, the effects were antagonistic in *H. azteca* but greater than concentration additive in *P. promelas* (Gust et al. 2018; Lotufo et al. 2018). In sublethal tests, toxicity was largely attributed to DNAN.

8.2 Regulations and Standards

8.2.1 DNAN

A WEEL assessment by the OARS, determined the 8-hour time-weighted average for inhalation exposure should not exceed 0.1 mg/m^3 (0.01 ppm) (WEEL 2017)

Few experimental data are available for this compound. An investigation of chronic oral toxicity and potential subchronic avian effects are indicated. Although *in vitro* studies have shown that DNAN does penetrate intact skin, no studies have determined the toxicity of DNAN following dermal exposure. DNAN is metabolized in mammalian systems to 2,4-dinitrophenol, a significantly toxic substance with an oral lethal dose of 14–43 mg/kg in humans. However, both the rate of absorption and rate of demethylation may limit the concentration of the active toxicant (Hayes 1982). *In vivo* dermal toxicity studies are needed to determine the toxicity of DNAN associated with dermal exposure. Reproductive toxicity is an important data gap for DNAN. The subchronic study indicates male reproductive toxicity, albeit at near lethal doses, and limited developmental studies suggest potential fetal effects. Additionally, the metabolite 2,4-DNP is fetotoxic in animals and has been associated with menstrual irregularities in humans (ATSDR 1995). An investigation of the reproductive toxicity of DNAN is indicated. Although DNAN is mutagenic in the *Salmonella* test system, results in mammalian systems (CHO and micronucleus) are negative. This suggests DNAN is not likely to be a human mutagen. DNAN is likely to have limited transport to groundwater and may demonstrate considerable natural attenuation due to sorption to soils and (bio)transformation. DNAN demonstrates limited ecotoxicity, with the most significant effects occurring in plants and birds.

8.2.2 NTO

The WEEL assessment by the OARS for NTO determined the 8-hour time-weighted average for inhalation exposure should not exceed 2 mg/m^3. No additional hazard notations were assigned (WEEL 2018b).

Acute oral and inhalation toxicity of NTO is low, and a battery of tests show no indication that there is a hazard from genotoxicity. Effects to the male reproductive system are the most significant effect, and are the basis for occupational health and safety standards. While effects to testes and sperm development (at least in rodents) are pronounced, it is not found that these changes result in developmental abnormalities among offspring. The effects on humans are yet unquantified, and the mode of action for reproductive effects is unknown; test results for endocrine disrupting effects were all negative to date.

NTO is readily soluble in water and represents a hazard for environmental transport and the potential to contaminate groundwater and surface water. However, microbial degradation and sorption to some soil types may limit transport. While acute chemical toxicity to aquatic species is generally low, the ability of NTO to alter the pH of aqueous environments presents a hazard. The compound is also subject to photodegradation with end products of greater toxicity. The readiness with which nitrite appears to be split out of NTO may represent some hazard. The uptake of NTO by plant species could subject grazing animals to NTO exposures. Additional ecotoxicological testing, to include terrestrial invertebrates, reptiles, and birds is recommended.

8.2.3 RDX

The USEPA has established a Long-Term Health Advisory (LTHA) for RDX of 0.1 mg/L, a Lifetime health advisory of 2 µg/L, and an Oral Slope Factor of 0.08 $(mg/kg\text{-}day)^{-1}$ (USEPA 2018a, 2018b). The ATSDR has established a Minimal Risk Level of 0.2 mg/kg-day for acute duration oral exposures and 0.1 mg/kg-day for intermediate and chronic duration oral exposures (ATSDR 2012). The Federal Drinking Water Guideline is 2 µg/L. Several states have established groundwater or drinking water standards or guidelines for RDX, including Mississippi, Nebraska, and West Virginia (0.61 µg/L), Massachusetts (1 µg/L), Pennsylvania (2 µg/L), Maine (3 µg/L), and New Mexico and Indiana (7 µg/L) (USEPA 2017).

The USEPA has determined a residential SSL of 6.1 mg/kg and an industrial SSL of 28 mg/kg (USEPA 2017). Some states have established soil guidelines and standards for RDX ranging from 1 mg/kg in Massachusetts to 160 mg/kg in Pennsylvania. The USEPA Region II Biological Technical Assistance Group has established a freshwater sediment screening benchmark of 0.013 mg/kg.

The USEPA has not established ambient air level standards for RDX. ACGIH has established a TLV (8-hour TWA) of 0.5 mg/m^3 for RDX (ACGIH 2008). NIOSH has established a REL (10-hour TWA) of 1.5 mg/m^3 and a STEL (15 minute) of 3.0 mg/m^3 with a skin notation (NIOSH 2005).

8.2.4 IMX-104

No regulations for the mixture were found.

9. RECOMMENDATIONS

Reproductive/developmental and chronic rodent studies of DNAN are suggested to reduce uncertainty associated with reference dose derivation and to enable sustained use.

Mechanisms of toxicity, including the underlying testicular toxicity and the mechanism of reduction of DNAN to 2,4-DNP in mammals should be more fully explored, as they will inform future hazard and risk assessments.

In vitro data suggesting skin absorption of NTO and the IMX mixture require additional testing, preferably in an animal model. Dermal absorption and *in vivo* dermal toxicity studies are needed for NTO, DNAN, and IMX-104 to understand the potential for toxicity from dermal contact.

Studies are needed to identify the photoproducts associated with increased photodegraded-NTO aquatic toxicity and to quantify environmental half-lives of the product(s).

10. POINT OF CONTACT

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Appendix A

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