U.S. ARMY PUBLIC HEALTH CENTER
8252 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

Toxicology Report No. S.0012411a-18, June 2019
Toxicology Directorate

Toxicology Assessment of IMX-101 - Update, January 2019

Prepared by Emily May Lent, Toxicity Evaluation Division

Specialty: 500C, Toxicity Tests

ACKNOWLEDGEMENTS

We would like to acknowledge the support and encouragement provided to this effort by Ms. Kimberly Watts, Acting Director, U.S. Army Combat Capabilities Development Command (CCDC), Dr. John LaScala of the Environmental Quality Technology Program, Pollution Prevention Team (EQT P2), Brian Hubbard, ESOH Officer G-3 Programs Office, JPEO Armaments & Ammunition, and James Chang, Chief, Energetics Branch, Office of Program Management-Combat Ammunition Systems.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
17-07-2019	Technical Report	January 2019

4. TITLE AND SUBTITLE
Toxicology Assessment of IMX-101 - Update

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Emily May Lent

5d. PROJECT NUMBER
S.0012411a-18

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Public Health Center (APHC)
Toxicology Directorate (MCHB-PH-TEV)
8252 Blackhawk Road, Building 5158
Aberdeen Proving Ground, MD 21010-5403

8. PERFORMING ORGANIZATION REPORT NUMBER
S.0012411a-18

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
PM – Combat Ammunition Systems
SFAE-AMO-CAS
Picatinny Arsenal, NJ 07806-5000

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This report summarizes the available toxicological information for the insensitive munition IMX-101 and its components, dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine (NQ). The toxicity of the IMX-101 mixture is largely unknown, however, a single subacute test in rats suggests greater than additive mixture effects. In aquatic species, mixture effects of IMX-101 largely due to DNAN. NTO and NQ demonstrate limited oral toxicity while the oral toxicity of DNAN is moderate. Toxic effects of DNAN are consistent with other nitroaromatics, including the development of anemia. Testicular toxicity is the predominant effect of exposure to NTO. Exposure to NQ induces minimal, non-specific signs of toxicity. NTO demonstrates minimal ecotoxicity. DNAN demonstrates greater ecotoxicity, particularly in mammals and birds. Ecotoxicity of NQ is low; however, photodegradation products of NQ demonstrate increased aquatic toxicity.

15. SUBJECT TERMS
IMX-101, insensitive munition, toxicity, NTO, DNAN, nitroguanidine, NQ

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Emily May Lent
					19b. TELEPHONE NUMBER *(Include area code)*
UU	UU	UU	SAR	57	410-436-7749

TABLE OF CONTENTS

FIGURES

TABLES

TOXICOLOGY REPORT NO. S.0012411a-18
TOXICOLOGY ASSESSMENT OF IMX-101 - UPDATE
JANUARY 2019

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)-101, a new insensitive munition formulation, is planned for use in several weapons systems including the 105-millimeter (mm) M1, the 155mm M1122 trainer, and the 155mm M795 artillery projectiles. IMX-101 is a mixture of 3-nitro-1,2,4-triazol-5-one (NTO), 2,4-dinitroanisole (DNAN), and nitroguanidine (NQ). Two of these components, NTO and DNAN, have limited toxicity data. This report presents the latest toxicity data and interpretations for DNAN, NTO, and NQ, as well as the full IMX-101 combination (see paragraphs 7.3–7.6). This report also includes Workplace Environmental Exposure Limits (WEELs) and Occupational Exposure Limits (OELs) developed for 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. This toxicity assessment for IMX-101 is an update to the previous version published in 2012. The purpose of this effort is to evaluate and update the insensitive munition mixture, IMX-101.

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 2018b).

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 2018a).

NQ is not acutely toxic via the oral or dermal routes. No inhalation studies have been conducted. NQ does not cause skin or eye irritation or skin sensitization. In repeat-dose studies, non-specific toxicity was observed with greater severity in gavage studies relative to feeding studies. In developmental studies in rats and rabbits and multi-generation reproductive studies in rats, developmental effects occurred at high doses, often coincident with maternal toxicity. NQ was determined to be non-genotoxic based on a preponderance of negative findings in a battery of *in vitro* and *in vivo* tests. No chronic or carcinogenicity studies have been conducted. Because NQ is very soluble in water and has limited sorption to soils, it has the potential to contaminate groundwater and surface water. NQ demonstrates limited ecotoxicity; however, the compound is subject to photo degradation with end products having greater toxicity. The long-term health advisory of NQ is 10.5 milligrams per liter (mg/L); the Reference Dose (RfD) is 0.1 milligrams per kilogram per day (mg/kg-day); the Drinking Water Exposure Limit (DWEL) is 3.5 mg/L; and the Lifetime Health Advisory is 0.7 mg/L (USEPA 2018).

Evaluation of the toxicological effects of the complete IMX-101 formulation in rats revealed that the effects determined for the mixture did not bear a simple relationship to those of the components individually. The results indicate that the effects of the formulation are not simply an additive response to the individual components. When present as part of the mixture IMX-101, 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. In contrast to the findings in rats, in aquatic species evaluation of mixture interactions using the toxic units approach indicated that DNAN was responsible for the toxicity of IMX-101 with response-additive summation providing a conservative assessment of IMX-101 toxicity.

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-101 to understand the potential for toxicity from dermal contact.

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

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.

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-101 is planned for use in several weapons systems including the M795 155mm, M1122 155mm, and 105mm artillery projectiles.

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-101 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 used 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-101

Substance	CAS RN
2,4-dinitroanisole (DNAN)	119-27-7
3-nitro-1,2,4-triazol-5-one (NTO)	932-64-9
nitroguanidine (NQ)	556-88-7

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 U.S. Environmental Protection Agency (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 U.S. National Institute for Occupational Safety and Health (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. 10-1,000 mg/L log K_{OC} 2.0-1.0	Water sol. > 1,000 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 1500 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} is the concentration that results in 50 observation of a selected endpoint, (e.g., immobility); LOAEL is the lowest-observed adverse effect level; LC_{50} is the concentration expected to result in 50% lethality to a population of test animals; LD_{50} is the 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, then 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]	0.858[7] 0.802 ± 0.012[10] 1.58[4]	-0.38[6]	2.58E-13[6]	5.82E-07[6]
NQ	104.09[1]	225-250[12] (dec)	4400[11]	-0.89[5] 0.21[4] (exp)	1.4[4] 1.161[9] (exp)	4.49E-12[5] (est)	1.00E-09[5] (est)
IMX-101	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 (USEPA 2014);
[7]Experimental value, (USAPHC 2010);
[8](USARDEC 2009) via HPLC;
[9](USARDEC 2009) gravimetric;
[10](USARDEC 2011)
[6](USABRDL 1985);
[7](Williams 2013)

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 the 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[6]	Mixed[1, 7]	Possible[3]
NTO	>5000[8] (rat, mouse)	30[9] (LOAEL)	569.8[3]	0.18[10]	Mild irritant[8]	Moderate to severe irritant[8]	Negative[11]	Indeterminate[3]
NQ	>5000[12]	316[13] (NOAEL)	0.62[3]	0.56[3]	Not irritating or sensitizing[14]	Negative[11]	Mixed (largely Negative)[15, 16]	Negative[17]
IMX-101	924 (♀ rat)[18] 1237 (♂ rat)[18]	nd	nd	nd	nd	nd	nd	nd

Legend:
nd=no data;
Notes:
[1](USAFRL 2002);
[2](Mullins et al. 2016);
[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](LAIR 1988m);
[13](LAIR 1988b);
[14](LAIR 1988c);
[15](AMRL 1978);
[16](Ishidata et al. 1977);
[17](IRIS 2011);
[18](USAPHC 2012b)

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
NQ	Low	Low	Low	Low	Low	

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.
NQ	Low-Mod	Low	Moderate	Low	Unk	Photolytic breakdown product in water 100-1,000x more toxic than NQ

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-101, 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.

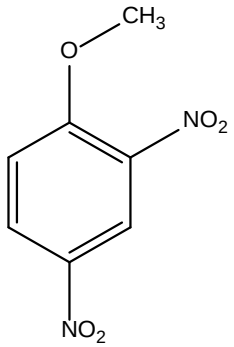

Figure 1. DNAN

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 2018b).

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 2012c); 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 (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 (ONTA) (BAE 2007).

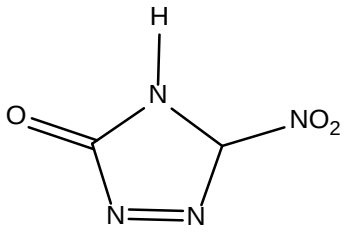

Figure 2. NTO

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 2018a).

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).

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 2012c). 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 2018a).

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 2014b).

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 Nitroguanidine

7.5.1 General Information

NQ is a legacy explosive that is the third component of IMX-101. The Army at Sunflower Army Ammunition Plant in DeSoto, Kansas manufactures NQ.

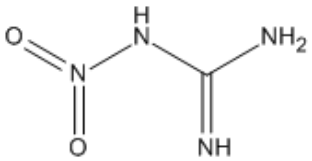

Figure 3. NQ

7.5.2 Toxicology Data

7.5.2.1 Oral

The acute LD_{50} is 3,850 mg/kg in mice and 3,120 mg/kg in guinea pig. Mortality is due to respiratory cyanosis. The acute LD_{50} in rats is 10,200 mg/kg (LAIR 1988m, 1988e; Lewis 2004). The predominant clinical signs associated with NQ administration were urinary excretion of a whitish precipitate (NQ) in the first 24 hours followed by reddish urine for up to a week. NQ also affected the gastrointestinal tract; it produced diarrhea with perianal staining and irritation of the mucosa of the stomach and small intestine. Excessive secretion from the Harderian gland was also observed as a red nasal discharge and staining around the nose and mouth. Based upon the very high LD50, NQ is classified as practically nontoxic (LAIR 1988m).

The 14-day subacute oral toxicity of NQ was evaluated in male and female rats. NQ was administered in the diet at dose levels of 0, 100, 316, or 1,000 mg/kg-day for 14 days. The addition of NQ to the diet did not have an effect on food consumption, but water consumption increased with NQ dose. Clinical signs attributable to the test compound were not observed during the study. At necropsy, blood samples were taken for hematological and serum clinical analyses. Serum K+ and Ca2+ values were decreased in the treated dose groups. Microscopic examination of tissues from the control and 1,000 mg/kg-day dose group animals revealed no lesions attributable to the administration of NQ. These findings indicate that NQ is nontoxic in rats at doses as high as 1,000 mg/kg-day for 14 days. The combination of serum electrolyte decreasing with an increased water consumption, suggests that NQ, which is excreted unchanged in the rat's urine, may be acting as an osmotic diuretic (LAIR 1988g).

Subchronic oral toxicity of NQ was evaluated in male and female Sprague-Dawley rats (LAIR 1988j). Nitroguanidine was administered in the diet at dose levels of 0, 100, 316, and 1,000 mg/kg-day for 90 days. There were 15 animals/sex/dose. The addition of nitroguanidine to the diet consistently reduced food consumption, while increasing water consumption. Blood samples taken at necropsy for hematology and serum chemistry analysis exhibited no significant abnormalities that could be attributed to nitroguanidine dosing. Microscopic examination of tissues from the control and 1,000 mg/kg-day dose group animals revealed no lesions attributable to the administration of nitroguanidine. Thus, NQ is nontoxic to rats when administered at doses as high as 1,000 mg/kg-day for 14 days. The NOAEL was 316 mg/kg-day, based on a decrease in the rate of growth of female rats at 1,000 mg/kg-day on weeks 5, 6, 8, 9, and 12 (LAIR 1988j).

The 90-day subchronic oral toxicity of nitroguanidine was evaluated in male and female ICR mice (LAIR 1988b). There were 15 animals/sex/dose. Nitroguanidine was administered in the diet at dose levels of 0, 100, 316, and 1,000 mg/kg-day for 90 days. The addition of nitroguanidine to the diet had no effect on food consumption or weight gains, but there was a significant dose-response increase in water consumption. Several serum chemistry parameters did exhibit alterations from control values, but these changes were isolated occurrences with no consistent dose-related trends being noted. With the exception of the brain-to-body weight ratio in the high-dose males at interim sacrifice, organ weights and their respective ratios were not affected by dosing. Microscopic examination of tissues from the control and 1,000 mg/kg-day dose group animals revealed no lesions attributable to the administration of nitroguanidine. The

findings of increased water consumption suggest that nitroguanidine, which is excreted unchanged in the mouse's urine, may be acting as an osmotic diuretic. The finding of increased brain-to-body weight ratios in male mice at 1,000 mg/kg-day at interim sacrifice is supportive of a 316 mg/kg-day NOAEL (LAIR 1988b).

Korolev et al. (1980) conducted a chronic toxicity study in animals given 5, 50 or 500 µg/kg. The species of animals used, the method of administration and duration of study were not reported. However, a NOAEL was reported to be 5 µg/kg-day, the lowest dose tested. TOPKAT modeling of nitroguanidine predicts a chronic LOAEL of 620.9 µg/kg-day with high confidence.

7.5.2.2 Inhalation

No experimental data were found. TOPKAT modeling predicts an LC_{50} of 557.5 mg/m^3-hour with high confidence.

7.5.2.3 Dermal

Nitroguanidine was found to not be irritating in male and female New Zealand White rabbits using a modified Draize method (LAIR 1986). Neither erythema, edema, nor any other recognizable skin reaction was detected at 1, 24, 48, or 72 hours after dosing or during the remaining period before terminal sacrifice 14 days after dosing (LAIR 1986).

Acute dermal toxicity potential of NQ was evaluated in male and female New Zealand White rabbits. A limit dose of 2000 mg/kg of NQ was not lethal following dermal exposure for 24 hours and produced no compound-related clinical signs or dermal irritation during the 15-day observation period (LAIR 1988l).

NQ was tested for its potential to produce sensitization via contact with the skin in guinea pigs. Testing on male guinea pigs was performed using the Buehler Dermal Sensitization Method demonstrated no evidence of dermal sensitization to NQ (LAIR 1988c).

Dermal absorption of NQ was evaluated in an *in vitro* test system using frozen human epidermal membranes (without stratum corneum) in a static Franz cell system based on OECD 428 testing guidelines. NQ was applied to the skin at an infinite dose (100 mg) powder and samples were withdrawn from the receptor fluid at 1, 2, 4, 6, or 8 hours and analyzed for NQ by HPLC. The authors estimated a steady state flux of 31.25 micrograms per square centimeter per hour (µg/cm^2-hour) for neat NQ (USAPHC 2012c). 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 NQ.

7.5.2.4 Ocular

Nitroguanidine was found to not be irritating to rabbit eyes using the standard Draize technique (LAIR 1988d).

7.5.2.5 Developmental and Reproductive

Nitroguanidine has been tested and found to not be teratogenic in both the rat and rabbit (Korte et al. 1990; Schardein 2000). However, in rats nitroguanidine produced maternal and fetal toxicity at the 1,000 mg/kg-day dose level; the NOEL was 316 mg/kg-day (LAIR 1988f). Although there were no dose-related malformations in rabbits, fetuses in the 1,000 mg/kg-day group were lighter in weight and had an increased incidence of retarded ossification of the sternebrae, olecranon, patellae, and phalanges (LAIR 1988k).

The potential of NQ to produce reproductive toxicity was evaluated in Sprague-Dawley rats. NQ was mixed into the diet at 0, 1.3, 4.0, and 12.7 parts per thousand (ppt). In young adult rats, these dose levels in ppt approximated the 100, 316, and 1,000 mg/kg-day NQ dose levels in developmental toxicity studies in rats and rabbits. The diet was fed to the parental males and females starting at 56 to 58 days of age and continued throughout their lives and to the F1 and F2 generation animals. Parental males and females were paired for mating. All matings were within the same dose group. The parental males and females that did not breed were euthanized after the mating period. Litters were examined and weighed at 0, 4, 7, 14, and 21 days of age. On day 4, litters of more than eight pups were culled to eight remaining pups, four males and 4 females where possible. When the pups were weaned at 21 days of age, one male and one female from each litter were selected to continue as parents for the next generation. Unselected and dams were euthanized. When the F1 animals were 20 weeks of age the breeding procedure was repeated. The F2 pups and dams were euthanized at weaning. NQ caused a decrease in some of the weekly body weights in the high-dose animals, but the decrease was not consistent throughout the study. Terminal body weights were lower for the high-dose F1 males and females and low-dose F1 females. There were no dose-related effects on clinical signs, mating, fertility, gestation, litter size, pup weights, or survival. Histopathological examination of the reproductive organs on adult animals and gross examination of weanlings showed no lesions attributable to NQ in any of the generations (LAIR 1990b, 1990a)

The potential of NQ to produce developmental toxicity was evaluated in pregnant Sprague-Dawley rats. NQ, suspended in 1% carboxymethylcellulose, was administered at doses of 0, 100, 316, or 1,000 mg/kg-day by oral gavage on days 6 through 15 of gestation. Fetuses were delivered by cesarean section on day 20; they were weighed, examined externally, and processed in either Bouin's solution for visceral examination or alizarin red stain for skeletal examination. Following a generalized failure to thrive, two animals in the 1,000 mg/kg-day group died and one was terminated in a moribund condition. At necropsy, significant quantities of NQ were present in the stomachs of these three animals. NQ given at 1,000 mg/kg-day produced decreased food consumption, weight loss, dehydration, red urine, and red material on nose/whiskers in the dams during the treatment period. Dams also exhibited decreased weight gain from Day 0 to Day 20 of gestation. Fetuses from the 1,000 mg/kg-day group were significantly smaller than controls with an increased incidence of retarded ossification of the sternebrae, caudal vertebrae, and pubis. There was no evidence of developmental toxicity of NQ in rats under conditions of this study. NQ produced maternal and fetal toxicity at the 1,000 mg/kg-day dose level. The NOAEL was 316 mg/kg-day (LAIR 1988f).

The potential of NQ to produce developmental toxicity was evaluated in pregnant New Zealand White rabbits. NQ in 1% carboxymethylcellulose was administered at doses of 0, 100, 316, or

1,000 mg/kg-day by oral gavage on days 6 through 18 of gestation. Fetuses were delivered by cesarean section on day 29; they were weighed and examined externally. The soft tissues were examined while the body was being eviscerated for subsequent processing in alizarin red stain for skeletal examination. Ten dams in the 1,000 mg/kg-day group died or were terminated in a moribund condition following a generalized failure to thrive. The dams administered 1,000 mg/kg-day exhibited weight loss and decreased food consumption. Signs of developmental toxicity associated with NQ administration were an increased incidence of resorptions in all dose groups. Fetuses in the 1,000 mg/kg-day group were lighter in weight and had an increased incidence of retarded ossification of the sternebrae, olecranon, patellae, and phalanges. There were no dose-related malformations. The authors concluded that NQ was not a teratogen, but had the potential to cause developmental toxicity (LAIR 1988k). Based on the patterns of toxicity observed, NQ has the potential to cause developmental toxicity (increased resorptions) in rabbits only at doses that are maternally toxic. A LOAEL of 1,000 mg/kg/day and a NOAEL of 316 mg/kg/day were identified for maternal and fetal toxicity (WEEL 2017).

7.5.2.6 Mutagenicity

NQ was non-mutagenic in the Ames *Salmonella* test (TA98, TA100, TA102, TA1535, TA1537, and TA1538) with and without activation doses near the limit of solubility (2.8 mg/plate) (AMRL 1978; McGregor et al. 1980; LAIR 1988i).

NQ did not induce sister chromatid exchange (SCE) in CHO cells at doses near the limit of solubility of NQ, with or without S9 activation (LAIR 1988h).

NQ tested in the mouse lymphoma assay did not induce gene mutation in the TK+/- gene of mouse lymphoma cells (L5178Y) at doses that approached the solubility limit, in the presence and absence of S9 activation (AMRL 1978; LAIR 1987).

NQ (10 mg/plate) was not active in the DNA repair assay using the *Escherichia coli* W3110/polA+, p3478/polA- system (McGregor et al. 1980).

Nitroguanidine-associated recombinant activity was not observed in *Saccharomyces cerevisiae* at doses of 22.7 mg/mL (McGregor et al. 1980).

When tested in the unscheduled DNA synthesis (UDS) assay, both in absence and presence of S9, NQ (0.1-5 mg/mL) did not produce evidence of primary DNA damage in human embryonic lung cells (WI-38 cells) (AMRL 1978). However, NQ did show evidence of clastogenicity in a screening test in Chinese hamster lung cells. Chromosomal aberrations observed included chromatid gaps, chromatid or chromosomal breaks, and translocation (Ishidata et al. 1977).

NQ was evaluated for mutagenic potential in the *Drosophila melanogaster* Sex-linked Recessive Lethal test. NQ was non-mutagenic following 72-hour feeding exposures to concentrations ranging from 2.08 µg/mL to 20.8 µg/mL (Gupta et al. 1993; LAIR 1988a).

NQ was evaluated in dominant lethal assays in mice and rats at doses of 200, 670, and 2000 mg/kg, respectively. The study authors reported that NQ did not cause a clastogenic response in either species (AMRL 1978).

7.5.2.7 Carcinogenicity

NQ is classified in group D as: not classifiable as to human carcinogenicity (USEPA 1992; IRIS 2011; USEPA 2009).

7.5.3 Ecotoxicology Data

7.5.3.1 Fate and transport

If released into water, NQ is not expected to adsorb to suspended solids or sediments in the water, but to migrate through subsurface soil and reach the water table causing groundwater contamination (DRDC 2011). Volatilization from water surfaces is not expected to be an important environmental fate based upon the low Henry's Law constant.

If released to air, an estimated vapor pressure of 0.03 mmHg at 25 ºC indicates NQ will exist solely in the vapor phase in the ambient atmosphere. Vapor phase NQ will be degraded by reaction with photochemically-produced hydroxyl radicals, with an estimated half-life of 18-20 hours. NQ has poor sorption in soil and should be mobile in soil with an estimated log K_{OC} of 1.40 (DRDC 2011).

An estimated bioconcentration factor of 3.2 suggests the potential for bioconcentration is low (HSDB 2003; USABRDL 1985).

7.5.3.2 Ecotoxicity

The acute toxicity of NQ was determined in 10 species of freshwater aquatic organisms (USABRDL 1985). Fish exposed to NQ for 96 hours included fathead minnows (*Pimephales promelas*), bluegill (*Lepomis macrochirus*), Channel catfish (*Ictalurus punctatus*), and rainbow trout (*Salmo gairdneri*). Invertebrates were exposed for 48 hours and included *Daphnia magna*, amphipods (*Hyallela azteca* and *Gammarus minus*), midge larvae (*Paratanytarsus dissimilis*), and aquatic worms (*Lumbriculus variegatus*). The acute toxicity of NQ was very low; fewer than 50% of the exposed organisms experienced mortality at concentrations up to the solubility limit of NQ in water (1,700 mg/mL at 12ºC for trout to about 3,000 mg/L at 22 ºC for most other species). The alga *Selenastrum capricornutum* was slightly more sensitive, with 120-hour EC_{50}'s of about 2,000 mg/L.

Because preliminary studies indicated that NQ undergoes photolysis in bright sunlight, aquatic toxicity tests were repeated with photolyzed NQ in *P. promelas*, *D. magna*, and *S. capricornutum* to determine if photo-NQ is more or less toxic than NQ (USABRDL 1985). Photo-NQ solutions were prepared by exposing NQ solutions (in distilled water) to UV light, using a 450 watt mercury-vapor immersion lamp, until NQ reached non-detectable levels. Photolysis increased the acidity of the solutions; based on regulatory control of pH of wastewater discharges, the pH was adjusted to 7.8. Complete photolyzation of NQ increased the toxicity of NQ approximately 100-fold. The 96-hour LC_{50} for fathead minnows decreased from greater than 2,714 mg/L to 34.5 mg/L. The EC_{50}s for *S. capricornutum* and *D. magna* decreased from 2,146 and >2,838 mg/L to 32.3 and 24.6 mg/L, respectively.

Acute lethality tests (96-hour LC_{50}) were similarly conducted in zebrafish larvae exposed to NQ alone and as part of IMX-101 both before and after photodegradation (Gust et al. 2017). Photo-NQ solutions were prepared by placing NQ stock solution (3,000 mg/L in dechlorinated tap water) in a photoreactor equipped with 16 black lamps (300nm, 14-W) for 4 hours. The exposure correlated to 96 hours of sunlight in Vicksburg, Mississippi at 450 lux. UV-treatment resulted in degradation of 26% of parent NQ and a reduction in pH from 6.6–6.8 in parent NQ exposure to 3.9–5.7 in photo-NQ exposures. UV-treatment of NQ increased the toxicity of NQ approximately 17-fold, reducing the LC_{50} from 1,323 to 77 mg/L. Photodegradation of IMX-101 resulted in a modest (2-fold) increase in toxicity. Assay pH was 7.16 to 8.21 for both parent and UV-treatments.

Similarly, *Ceriodaphnia* were exposed to NQ alone and as part of IMX-101 both before and after photodegradation (Kennedy et al. 2017). Photo-NQ solutions were prepared by placing two NQ solutions (high=1,619 mg/L and low=108 mg/L in synthetic freshwater bioassay medium [moderately hard reconstituted water]) in a photoreactor equipped with 16 black lamps (300 nm, 14-W) for 4 hours. The exposure correlated to 48 hours of sunlight in Vicksburg, Mississippi at 450 lux. Photolyzation resulted in 42 and 99% reduction in starting NQ concentrations in the high and low NQ solutions, respectively. The 48-hour LC_{50} value for NQ decreased from 1,174.2 mg/L for parent NQ to 16.1 and 0.76 mg/L for photo-NQ in the high and low concentration exposures, respectively. The difference in LC_{50} values for photo-NQ was attributed to the efficiency of photolysis at lower concentrations. Photodegradation of IMX-101 resulted in a 100-fold increase in toxicity, with photodegraded NQ products being the predominant source of toxicity.

Although the degradation products responsible for the observed increase in NQ toxicity have not been confirmed, with the exception of urea and nitrate ion, the identified photolysis end products (guanidine, urea, cyanoguanidine and nitrite ion, and ammonia) are more toxic to aquatic organisms than the parent compound. However, in photodegradation of studies with 50 mg/L NQ, only nitrite ion concentrations were high enough to contribute to enhanced toxicity of photo-NQ. The remaining degradation products were present at concentrations well below their respective LC_{50} values in aquatic organisms (USABRDL 1988). Given the photolytic half-life of 1–2 days for NQ in natural waters, and considering the dilution that would take place in that timeframe, it is highly unlikely that wastewaters discharged to a body of moving water could present a hazard to aquatic life. (The National Pollutant Discharge Elimination System (NPDES) established average daily discharge limit for NQ at Sunflower Army Ammunition plant is 25 mg/L).

The U.S. Department of Agriculture (USDA (1989) conducted a study to determine the uptake and distribution of NQ in selected plants and the effects on plant growth and physiology. Selected plants were grown in the presence of NQ or NQ-contaminated wastewater. Effects of NQ uptake and distribution on biomass production, reproductive capacity, and physiological parameters such as photosynthesis and respiration were determined using radioisotope methods. Adverse effects of NQ on plant growth and physiology were observed at threshold concentrations of 2 mM for soybeans and 8 mM for two grass species. (These concentrations substantially exceed maximum concentrations used in land application of wastewater at Sunflower AAP.) Uptake, metabolism, and distribution studies employing ^{14}C-labeled NQ in

soybeans and tall fescue were conducted. Uptake from both water and soil was found to be linear with concentration of applied NQ in each case. Results of the distribution and metabolism studies indicated NQ was taken up in the roots and translocated to the leaves where it was metabolized to carbon dioxide (Heitholt et al. 1990; USDA 1989).

In a 96-hour freshwater green algae (*P. subcapitata*) inhibition test, NQ had an EC_{20} of 760 mg/L (DRDC 2011).

7.5.3.3 Degradation/Treatment

NQ was co-metabolized in by soil microorganisms under reducing conditions to nitrosoguanidine (NSQ) after acclimation. No further microbial reduction occurred (no aminoguanidine, hydrazine, or urea were detected in culture abstracts). NSQ decomposed non-biologically, and formed cyanamide, cyanoguanidine, melamine, and guanidine. All products were identified by thin layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS). No ammeline, ammelide, or cyanuric acid was detected. NQ and NSQ were sensitive to UV light (NRDC 1981).

NQ is not susceptible to aerobic biodegradation in activated sludge, and is stable under sterile reducing conditions. After acclimation, NQ co-metabolizes to form NSQ. In an anaerobic continuous culture test, no NQ was present after 7 days (Kaplan et al. 1982).

Degradation of NQ in soil was studied with continuous flow soil columns. NQ was biodegraded when sufficient supplementary carbon was provided in the wastewater. The primary product formed during the biodegradation of NQ in soil was ammonia. Only trace amounts of NQ were detected and no significant levels of other organic nitrogen compounds, nitrates, or nitrites were present in column leachates (NRDC 1985).

NQ is readily degraded by ultraviolet photolysis, exhibiting zero-order kinetics through complete disappearance, regardless of pH. The principal products from unbuffered NQ solutions are guanidine, urea and nitrite ion, with lesser quantities of cyanoguanidine, nitrate ion, and ammonia. NSQ is a transient intermediate that is converted to guanidinium nitrate at a rate slightly faster than for NQ (USABRDL 1985). At pH 10, the product mix is different; elemental nitrogen is a significant product while less than 25% of NQ carbon is accounted for as urea, guanidine, and cyanoguanidine (USABRDL 1988). NQ will photolyze in clear surface water with a half-life of 1–2 days in summer and winter, respectively. The presence of humic substances may decrease NQ photolysis rates due to screening effects (USAMRDC 1987).

NQ is co-metabolically degraded in sewage sludge and soil. In sewage, NQ is reduced to NSQ that is subsequently abiotically transformed. In soil, only traces of NSQ were detected with 85% of NQ being degraded to ammonia. Supplemental carbon, added in the form of glucose (0.5–1.0%), was required for this transformation to occur (Walker et al. 1992).

The degradation of nitroguanidine manufacture wastewater components was examined in continuous flow (271 days) and soil perfusion columns (84 days) studies. Guanidine nitrate and sulfate were the most readily transformed wastewater components, while NQ was only partially removed. Mineralization was enhanced by addition of carbon (Williams et al. 1989).

7.5.3.4 Combustion products

The Lawrence Livermore National Laboratory's CHEETAH code predicts combustion products that 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 at such low levels that there is no health concern as a result of a transient exposure.

7.6 IMX-101

7.6.1 General Information

IMX-101 is a mixture of NTO, DNAN, and NQ. This section addresses the effects of the mixture formulation currently under production, as opposed to the individual components. An updated medical surveillance protocol for the IMX-101 formulation was published in 2014 by the Occupational Medicine Program USAPHC (2014a).

7.6.2 Toxicology Data

Only limited toxicology data are available for the IMX-101 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

IMX-101 administered in corn oil via gavage had an LD_{50} in male rats of 1,237 mg/kg and in female rats of 924 mg/kg, with a combined (male-female average) value of 1,100 mg/kg (USACHPPM 2008). A toxic units approach indicates less than additive effects for lethality.

In a 14-day repeated-dose study, Sprague-Dawley rats were orally gavaged with IMX-101 in a solution of polyethylene glycol (PEG 200) at nominal doses of 31, 63, 125, 250, 500, or 1,000 mg/kg-day. Clinical signs of toxicity included lethargy, prostrate posture, and rapid respiration/labored breathing, which appeared within 15 minutes of dosing in the 1,000 mg/kg-day group. Males appeared to be more affected than females, but after an initial recovery period of about 30 minutes, all animals in the 1,000 mg/kg-day group died. Animals dosed at 500 mg/kg-day began to show lethargy shortly after the second or third dose, but recovered by 30 minutes. The urine of animals in this group appeared progressively darker, becoming brownish by the end of the first week. Animals of both sexes in this group died over the course of the first week; all were dead by the tenth dose (USAPHC 2012b).

In the subacute study, the notable adverse events were: 1) lethality in the 500 mg/kg-day and 1,000 mg/kg-day groups; 2) splenomegaly (increased spleen weight) primarily in females; and 3) testicular atrophy, histopathologic moderate to severe tubular degeneration, and decreased sperm density and motility in males. The LOAEL from this study was 125 mg/kg-day based on testicular mass with a calculated $BMDL_{10}$ of 30.6 mg/kg-day (USAPHC 2012b).

The lethality and splenomegaly observed in the oral testing are likely due to the DNAN component of the IMX-101 mixture; the adverse events occur at concentrations similar to those of DNAN alone and were not observed with NTO or NQ. The testicular toxicity of IMX-101 is consistent with that described for both NTO and DNAN (Crouse et al. 2015; Mullins et al. 2016; Lent et al. 2018). Evaluation of mixture interactions using the toxic units approach indicated greater than additive effects on the testes. When present in the mixture, the levels at which DNAN and NTO reduced testes weight (EC_{10}) decreased by approximately 5- and 30-fold, respectively. Interactions between the components in the mixture appear to increase testicular toxicity, particularly for NTO (USAPHC 2012b).

Subchronic and chronic effects of IMX-101 mixture are not available; direct modeling of mixtures using current QSAR models is not possible.

7.6.2.2 Inhalation

No experimental data on inhalation effects of IMX-101 are available.

7.6.2.3 Dermal

Dermal absorption of IMX-101 as both the mixture and the individual components was evaluated in an *in vitro* test system using frozen human epidermal membranes (without stratum corneum) in a static Franz cell system based on OECD 428 testing guidelines. Test chemicals were applied to the skin at an infinite dose (100 mg) powder and samples were withdrawn from the receptor fluid at 1, 2, 4, 6, or 8 hours and quantified for component content by HPLC. The authors estimated steady state flux rates of 332, 1.10, and 31.25 µg/cm^2-hour for neat NTO, DNAN, and NQ, respectively (USAPHC 2012c). When 100 mg of the IMX-101 mixture was applied to the cell, the steady fluxes for NTO, DNAN, and NQ were 135.9, 1.80, and 236 µg/cm^2-hour, respectively. Thus, the flux rate of NTO was decreased when present as a mixture (0.4x), but DNAN increased (1.6x) and NQ was greatly increased (7.6x). In contrast, when tested as part of the PAX-21 mixture, the flux rate of DNAN decreased (0.5x) from 1.55 µg/cm^2-hour for neat DNAN to 0.74 µg/cm^2-hour for the mixture (USAFRL 2000). The differences in effects are likely due both to differences in mixture compositions and because the stratum corneum (the primary barrier to dermal absorption) was absent in the IMX-101 testing.

7.6.2.4 Ocular

No experimental data on ocular sensitivity of IMX-101 are available.

7.6.2.5 Developmental and Reproductive

No experimental data on developmental or reproductive toxicity of IMX-101 are available.

7.6.2.6 Mutagenicity

No experimental data on mutagenicity of IMX-101 are available.

7.6.2.7 Carcinogenicity

No experimental data on carcinogenicity of IMX-101 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, a formulation very similar to IMX-101 that substitutes RDX for NQ, only 0.4–1.2% of the NTO component remained as undetonated residue (CRREL 2013).

IMX-101 samples were included in a study of insensitive munitions by Cold Regions Research and Engineering Laboratory (CRREL) researchers that evaluated environmental dissolution. IMX-101 forms inhomogeneous particles with NTO and NQ embedded in a DNAN matrix. As the IMX-101 particles progressively dissolve when exposed to the environment, NTO and NQ 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. 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 IMX-101 was tested in soil columns (lysimeters) using two different soil types, breakthrough curves were demonstrated for the two soil types selected for the experiment (Camp Swift and Camp Guernsey soils). Behavior of the three IMX constituents was consistent with previously observed fate and transport parameters and the IMX dissolution observed in drip studies. NTO was the first compound to break through, followed soon by NQ. DNAN was the last IM constituent to break through. An additional compound, 2-methoxy-5-nitroaniline (MENA) was also observed in column effluents. MENA is believed to be a degradation product of DNAN (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 and NQ demonstrated limited sorption to soils and eluted first in a high concentration pulse. DNAN eluted last and demonstrated appreciable absorption.
The fate and transport of IMX-101 components was studied in static and running soil column experiments. In both studies, DNAN and NTO started to degrade within 24 hours in high organic content soils. Within 60 days, DNAN and NTO were completely degraded, while 80% of the NQ remained. These results are consistent with work on the individual components, indicating interactions did not occur in the soil to affect fate and transport (Temple et al. 2018).

7.6.3.2 Ecotoxicity

In *Hyalella azteca* exposed to IMX-101 components individually or in the mixture for 10 days or 35 days; a 10-day LC_{50} of 37.8 mg/L was determined for IMX-101. Lethality occurred at lower concentrations in the 35-day exposure, resulting in an LC_{50} approximately 3-fold lower than the 10-day LC_{50}. A reduction in growth was observed in the 10-day exposure (IC_{50}=15.8 mg/L), but not the 35-day exposure. Decreased reproduction, the most sensitive endpoint, was observed in the 35-day exposure with an IC_{50} of 7.0 mg/L. Evaluation of mixture interactions using the toxic units approach indicated that DNAN was responsible for the toxicity of IMX-101 and response-additive summation will provide a conservative assessment of IMX-101 mixture effects in *H. azteca* (Lotufo et al. 2018).

The effects of photodegradation on IMX-101 toxicity were studied in *Ceriodaphnia dubia* exposed to each IMX-101 constituent individually and to the IMX-101 mixture, both before and after the exposure solutions were irradiated in a UV photoreactor. Without photodegradation, the effects were similar to those reported for *H. Azteca*, with DNAN being predominantly responsible for the toxicity of IMX-101 based on toxic units. In contrast, in photodegraded IMX-101, NQ photoproducts were the predominant source of toxicity, demonstrating a 100–1,000-fold increase in toxicity. NTO and DNAN demonstrated only modest increases in toxicity (2–100-fold) following photodegradation. It is remains unclear whether intermediate or final breakdown products were responsible for the observed toxicity (Kennedy et al. 2017).

In contrast, in *Pimphales promelas* exposed to IMX-101 components individually or in the mixture in an acute (48-hour) test, DNAN was the most toxic constituent (LC_{50} of 36.1 mg/L, NTO and NQ did not elicit significant mortality) and was responsible for the toxicity of IMX-101 based on toxic units. Transcriptomic analyses indicated that transcriptomic responses of IMX-101 were driven by DNAN, indicating non-interactive effects among mixture components (Gust et al. 2018).

7.6.3.3 Degradation/Treatment

The field study conducted by CRREL (2014) determined that fragments of unexploded IMX-101 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.

When present in the IMX-101 mixture, photodegradation rates of DNAN, NQ, and NTO were lower than when present alone. Degradation products not observed during photonitration of the individual components were attributed to renitration of photoproducts during simultaneous photodenitration of other components. This indicates that the primary degradation products can be influenced by interactions with formulation ingredients and their degradation products (Halasz et al. 2018).

When present in the mixture IMX-101, degradation of NTO in static soil experiments and soil columns was similar to the individual component, with NTO degradation evident within 24 hours and complete within 60 days (Temple et al. 2018).

In phytoremediation studies of soil contaminated with up to 50 mg/kg IMX-101, DNAN and NQ were taken up by the roots and shoots of big bluestem grass, Nash Indian grass, and switchgrass. IMX-101 was degraded to below detection limits after 225 days (Richard et al. 2014b, 2014a).

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 (Indest et al. 2017).

8. DISCUSSION

8.1 Overall Formulation Effects

Oral dosing studies of the IMX-101 mixture indicate that NTO is a more potent testicular toxicant in the mixture than by itself, potentially supporting an additional level of safety required for those exposed to the mixture at high levels. The data from the *in vivo* rat 14-day subacute oral toxicity study (USAPHC 2012b) indicate that IMX-101 exhibits toxicity in male rats at concentrations approximately 2-fold lower than any of the individual components.

In contrast, in aquatic organisms interactive effects of the components of IMX-101 were not demonstrated. Toxicity was largely attributed to DNAN in exposures without photodegradation and to NQ photoproducts when photodegradation was present (Gust et al. 2017; Kennedy et al. 2017; Lotufo et al. 2018).

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 2018a).

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 NQ

The long-term health advisory of NQ is 10.5 mg/L; the RfD is 0.1 mg/kg-day; the Drinking Water Exposure Limit (DWEL) is 3.5 mg/L; and the Lifetime Health Advisory is 0.7 mg/L (USEPA 2018). A NOAEL and reference dose are listed in the Integrated Risk Information System (IRIS) database (IRIS 2011). The NOAEL value given, 316 mg/kg-day, suggests only moderate toxicity, and the cancer designation for NQ provided in IRIS is “D,” (inadequate information to assess carcinogenic potential). The reference dose derived for NQ in IRIS is 1 x 10-1 mg/kg-day. NQ is classified in group D as not classifiable as to human carcinogenicity (IRIS 2011; USEPA 1992, 2009). A drinking water guideline of 700 µg/L is established in Maine, and at the Federal level (HSDB 2003).

Although NQ is a legacy explosive and there is considerable experience with its behavior in high explosive mixtures, IMX-101 and PAX-21 are the first explosive formulations where low-order detonations could result in environmental distribution of NQ. Concerns regarding the photolytic product (NSQ) of aquatic NQ discharges suggest further studies are needed to quantify environmental half-lives and toxicity to aquatic organisms.

8.2.4 IMX-101

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-101 to understand the potential for toxicity from dermal contact.

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

10. POINT OF CONTACT

Questions pertaining to this report should be referred to Emily May Lent at DSN 584-3980, commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-aphc.mbx.tox-info@mail.mil.

Prepared By:

LENT.EMILY.MAY.1296114371 Digitally signed by LENT.EMILY.MAY.1296114371 Date: 2019.07.17 07:12:18 -04'00'

EMILY MAY LENT — Date
Toxicologist
Toxicity Evaluation Division

Approved By:

QUINN.MICHAEL.J.JR.1282372092 Digitally signed by QUINN.MICHAEL.J.JR.1282372092 Date: 2019.07.30 10:37:11 -04'00'

MICHAEL J. QUINN — Date
Division Chief, Health Effects

JOHNSON.MARK.STEVEN.1229380717 Digitally signed by JOHNSON.MARK.STEVEN.1229380717 Date: 2019.07.30 11:13:28 -04'00'

MARK S. JOHNSON — Date
Director, Toxicology

APPENDIX A

REFERENCES

Ahn, S.C., D.K. Cha, B.J. Kim, and S.Y. Oh. 2011. Detoxification of PAX-21 ammunitions wastewater by zero-valent iron for microbial reduction of perchlorate. *J Hazard Mater* 192 (2):909-14.

Amalia, C., A. Pui, S. Jurcoane, E. Rusu, and G. Drochioiu. 2009. Biological effect and the toxicity mechanisms of some dinitrophenyl ethers. *Romanian Biotechnological Letters* 14 (6):4893-4899.

6570th Aerospace Medical Research Laboratory. 1978. Mutagen and Oncogen Study on Nitroguanadine. Prepared by: Inc. Litton Bionetics, POC: M. Brusick and D.W. MathesonWright-Paterson AFB, Ohio. Technical Report AD-A064949.

Arthur, J.D., N.W. Mark, S. Taylor, J. Simunek, M.L. Brusseau, and K.M. Dontsova. 2018. Dissolution and transport of insensitive munitions formulations IMX-101 and IMX-104 in saturated soil columns. *Sci Total Environ* 624:758-768.

Agency for Toxic Substances and Disease Registry. 1995. Toxicological Profile for Dinitrophenols. Prepared by: U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia.

BAE Ordnance Systems, Inc. 2007. Material Safety Data Sheet (MSDS)-NTO. Prepared by: Holston Army Ammunition Plant, Kingsport, Tennessee. MSDS No. 6000.

Boddu, V.M., K. Abburi, A.J. Fredricksen, S.W. Maloney, and R. Damavarapu. 2009. Equilibrium and column adsorption studies of 2,4-dinitroanisole (DNAN) on surface modified granular activated carbons. *Environ Technol* 30 (2):173-81.

CCRIS. 2010. *Chemical Carcinogenesis Research Information System*. TOXNET, U.S. National Library of Medicine [cited multiple dates 2008-2019]. Available from http://toxnet.nlm.nih.gov/.

ChemSpider. 2015. 2,4-Dinitroanisole. http://www.chemspider.com/Chemical-Structure.8080.html: Royal Society of Chemistry. Date accessed: multiple: 2012-2019. http://www.chemspider.com/Chemical-Structure.8080.html

Chiu, C.W., L.H. Lee, C.Y. Wang, and G.T. Bryan. 1978. Mutagenicity of some commercially available nitro compounds for Salmonella typhimurium. *Mutation Research* 58 (1):11-22.

CIDPL. 2009. ChemIDPlusLite. http://toxnet.nlm.nih.gov/: National Library of Medicine, National Institutes of Health. Date accessed: multiple 2012-2019.

Crouse, L.C., E.M. Lent, and G.J. Leach. 2015. Oral toxicity of 3-nitro-1,2,4-triazol-5-one in rats. *Int J Toxicol* 34 (1):55-66.

Cold Regions Research and Engineering Laboratory. 2013. Field Detonation testing of 60- and 81-mm Mortar Bodies Containing IMX-104. Prepared by: M. Walsh, M. A. Walsh, J. Zufelt, C. Ramsey, S. Thiboutot, G. Ampleman, E. Diaz and L. Zunino, JBER-Richardson, Alaska.

Cold Regions Research and Engineering Laboratory. 2014. Dissolution of NTO, DNAN, and Insensitive Munitions Formulations and Their Fates in Soils. Prepared by: K. Dontsova, S. Taylor, R. Pesce-Rodriguez, M. Brusseau, J. Arther, N. Mark, M. Walsh, J. Lever and J. Šimůnek, Hanover, New Hampshire. Report No. ERDC/CRREL TR-14-23, DTIC Accession No. AD-A609594.

Dodard, S.G., M. Sarrazin, J. Hawari, L. Paquet, G. Ampleman, S. Thiboutot, and G.I. Sunahara. 2013. Ecotoxicological assessment of a high energetic and insensitive munitions compound: 2,4-dinitroanisole (DNAN). *J Hazard Mater* 262:143-50.

Dodd, D.E., S. Sharma, and G.M. Hoffman. 2002. Genotoxicity and 90-day developmental toxicity studies on an explosive formulation. *Toxicologist* 66 (1-S):267.

Defense Research and Development Canada. 2011. Environmental Fate and Ecological Impact of Emerging Energetic Chemicals (DNAN and its Amino-Derivatives, NTO, NQ, FOX-7, and FOX-12), Annual Report 2010-2011. Prepared by: J. Hawari, Montréal, Québec. NRC Report 53363.

Duncan, K. 2002. Insensitive Munitions and the Army: Improving Safety and Survivability *Army Logistician PB700-02-1* 34 (1):16-17.

Edgewood Chemical Biological Center. 2009. Aquatic toxicity of 3-nitro-1,2,4-triazol-5-one (NTO). Prepared by: M.V. Haley, R.G. Kuperman and R.T. Checkai, Aberdeen Proving Ground, Maryland.

ECOTOX. 2009. U.S. Environmental Protection Agency ECOTOX database. Washington, D.C.: U.S. Environmental Protection Agency. Date accessed: multiple: 2009-2019. http://cfpub.epa.gov/ecotox/

Fida, T.T., S. Palamuru, G. Pandey, and J.C. Spain. 2014. Aerobic biodegradation of 2,4-Dinitroanisole by Nocardioides sp. strain JS1661. *Appl Environ Microbiol* 80 (24):7725-31.

Gao, J., P. Zhang, Z. Liu, H. Wang, H. Yue, Q. Lu, and J. Dang. 2016. Study of embryo toxicity and the teratogenicity of 2, 4-dinitroanisole in rats. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 34 (1):50-2.

GENETOX. 2009. *Genetic Toxicology Database*. TOXNET, U.S. National Library of Medicine, Washington, D.C. [cited multiple 2009-2019]. Available from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX.

Glaus, M.A., C.G. Heijman, R.P. Schwarzenbach, and J. Zeyer. 1992. Reduction of nitroaromatic compounds mediated by Streptomyces sp. exudates. *Appl Environ Microbiol* 58 (6):1945-51.

Gupta, R.K., D.W. Korte, Jr. , and G. Reddy. 1993. Mutagenic Potential of Nitroguanidine in the Drosophila Melanogaster Sex-Linked Recessive Lethal Assay. *Journal of Applied Toxicology* 13:231-234.

Gust, K.A., J.K. Stanley, M.S. Wilbanks, M.L. Mayo, P. Chappell, S.M. Jordan, L.C. Moores, A.J. Kennedy, and N.D. Barker. 2017. The increased toxicity of UV-degraded nitroguanidine and IMX-101 to zebrafish larvae: Evidence implicating oxidative stress. *Aquat Toxicol* 190:228-245.

Gust, K.A., G.R. Lotufo, J.K. Stanley, M.S. Wilbanks, P. Chappell, and N.D. Barker. 2018. Transcriptomics provides mechanistic indicators of mixture toxicology for IMX-101 and IMX-104 formulations in fathead minnows (Pimephales promelas). *Aquat Toxicol* 199:138-151.

Hadnagy, E., A. Mai, B. Smolinski, W. Braida, and A. Koutsospyros. 2018. Characterization of Mg-based bimetal treatment of insensitive munition 2,4-dinitroanisole. *Environ Sci Pollut Res Int*.

Halasz, A., J. Hawari, and N.N. Perreault. 2018. New Insights into the Photochemical Degradation of the Insensitive Munition Formulation IMX-101 in Water. *Environ Sci Technol* 52 (2):589-596.

Hawari, J., F. Monteil-Rivera, N.N. Perreault, A. Halasz, L. Paquet, Z. Radovic-Hrapovic, S. Deschamps, S. Thiboutot, and G. Ampleman. 2015. Environmental fate of 2,4-dinitroanisole (DNAN) and its reduced products. *Chemosphere* 119:16-23.

Hayes, W.J. 1982. Nitro Compounds and Related Phenolic Pesticides. In *Pesticides Studied in Man*. Baltimore, Maryland: Williams and Wilkins.

Heitholt, J.J., R.H. Hodgson, and T.J. Tworkoski. 1990. Toxicity and uptake of nitroguanidine in plants. *Bull Environ Contam Toxicol* 44 (5):751-8.

Horner, W.D. 1942. Dinitrophenol and its relation to formation of cataracts. . *Arch Ophthalmol* 27:1097-1121.

Howe, P.D., T.T. Griffiths, S. Dobson, and H.M. Malcolm. 2006. *Environmental assessment of pyrotechnics compounds*. Washington, D.C.: Strategic Environmental Research and Development Program/Environmental Security Technology Certification Program

Symposium. Poster.https://symposiumarchive.serdp-estcp.org/symposium2007/upload/w65-griffiths.pdf

Hoyt, N., M. Brunell, K. Kroeck, M. Hable, L. Crouse, A. O'Neill, and D.I. Bannon. 2013. Biomarkers of oral exposure to 3-nitro-1,2,4-triazol-5-one (NTO) and 2,4-dinitroanisole (DNAN) in blood and urine of rhesus macaques (Macaca mulatta). *Biomarkers* 7:587-94.

HSDB. 2003. *Nitroguanidine*. TOXNET, U.S. National Library of Medicine [cited multiple 2009-2019]. Available from http://toxnet.nlm.nih.gov/.

Huntingdon Life Sciences Laboratory. 2000. 2,4-Dinitroanisole: An Acute (4-Hour) Inhalation Toxicity Study in the Rat via Nose-Only Exposure. Prepared by: ManTech Environmental Technology, POC: G.M. HoffmanDayton, Ohio. Project No. 00-5435.

Hutanu, C.A., M. Zaharia, and O. Pintilie. 2013. Quenching of tryptophan fluorescence in the presence of 2,4-DNP, 2,6-DNP, 2,4-DNA and DNOC and their mechanism of toxicity. *Molecules* 18 (2):2266-80.

Indest, K.J., D.E. Hancock, F.H. Crocker, J.O. Eberly, C.M. Jung, G.A. Blakeney, J. Brame, and M.A. Chappell. 2017. Biodegradation of insensitive munition formulations IMX101 and IMX104 in surface soils. *J Ind Microbiol Biotechnol* 44 (7):987-995.

IRIS. 2011. *Integrated Risk Information System (IRIS)- Nitroguanidine (CASRN 556-88-7)*. TOXNET, U.S. National Library of Medicine, Bethesda, MD. [cited multiple 2011-2019]. Available from http://www.epa.gov/iris/subst/0425.htm.

Ishidata, M., Jr., and S. Odashima. 1977. Chromosome tests with 134 compound on Chinese Hamster cells in vitro—a screening for chemical carcinogens. *Mutat Res* 48:337-353.

Jackovitz, A.M., K.A. Koistinen, E.M. Lent, D.I. Bannon, M.J. Quinn, Jr., and M.S. Johnson. 2018. Neuromuscular anomalies following oral exposure to 3-nitro-1,2,4-triazol-5-one (NTO) in a one-generation study with Japanese quail (Coturnix japonica). *J Toxicol Environ Health A* 81 (15):718-733.

Kaplan, D.L., J.H. Cornell, and A.M. Kaplan. 1982. Decomposition of Nitroguanidine. *Environ Sci Technol* 16 (8):488-492.

Kennedy, A.J., J.G. Laird, C. Lounds, P. Gong, N.D. Barker, S.M. Brasfield, A.L. Russell, and M.S. Johnson. 2015. Inter- and intraspecies chemical sensitivity: a case study using 2,4-dinitroanisole. *Environ Toxicol Chem* 34 (2):402-11.

Kennedy, A.J., A.R. Poda, N.L. Melby, L.C. Moores, S.M. Jordan, K.A. Gust, and A.J. Bednar. 2017. Aquatic toxicity of photo-degraded insensitive munition 101 (IMX-101) constituents. *Environ Toxicol Chem* 36 (8):2050-2057.

Khatiwada, R., L. Abrell, G. Li, R. Root, R. Sierra-Alvarez, J. Field, and J. Chorover. 2018. Adsorption and oxidation of 3-nitro-1,2,4-triazole-5-one (NTO) and its transformation

product (3-amino-1,2,4-triazole-5-one, ATO) at ferrihydrite and birnessite surfaces. *Environmental Pollution* 240:200-208.

Kitcher, E., W. Braida, A. Koutsospyros, J. Pavlov, and T.L. Su. 2017. Characteristics and products of the reductive degradation of 3-nitro-1,2,4-triazol-5-one (NTO) and 2,4-dinitroanisole (DNAN) in a Fe-Cu bimetal system. *Environ Sci Pollut Res Int* 24 (3):2744-2753.

Korolev, A.A., T.G. Shlepnina, N. Mikhailovskii, A. Zakharova, and V.P. Laskina. 1980. Hygienic basis for the maximum allowable concentration of diphenylnitrosamine and nitroguanidine in reservoir water. *Gig Sanit* 1:18-20.

Korte, D.W.J., V.C. Coppes, G.A. Orner, and C. Gomez. 1990. Developmental toxicity potential of nitroguanidine in rats and rabbits. *Toxicologist* 10:124.

Krzmarzick, M.J., R. Khatiwada, C.I. Olivares, L. Abrell, R. Sierra-Alvarez, J. Chorover, and J.A. Field. 2015. Biotransformation and Degradation of the Insensitive Munitions Compound, 3-Nitro-1,2,4-triazol-5-one, by Soil Bacterial Communities. *Environ Sci Technol* 49 (9):5681-8.

Letterman Army Institute of Research. 1986. Primary dermal irritation potential of nitroguanidine in rabbits. Prepared by: E.W. Morgan, S.K. Sano and D.W. Jr. Korte, Presidio of San Farncisco, California. LAIR Report No. 220.

Letterman Army Institute of Research. 1987. Mutagenic Potential of Nitroguanidine in the Mouse Lymphoma Forward Mutation Assay. Prepared by: J.W. Harbell and D.W. Korte, Jr., Presidio of San Farncisco, California. Toxicology Series 159, Technical Report No. 252.

Letterman Army Institute of Research. 1988a. Mutagenic Potential of Nitroguanidine in the Drosophila Melanogaster Sex-Linked Recessive Lethal Test. Prepared by: R.K. Gupta, L.D. Witcher, V.O. Villa and D.W. Korte, Jr., Presidio of San Francisco, California. Toxicology Series 222, Technical Report No. 275.

Letterman Army Institute of Research. 1988b. Ninety-day Subcrhonic Oral Toxicity Study of Nitroguanidine in Mice. Prepared by: D.F. Frost, E.W. Morgan and Y. Letellier, Presidio of San Farncisco, California. Report No. 319.

Letterman Army Institute of Research. 1988c. Dermal Sensitization Potential of Nitroguanidine in Guinea Pigs. Prepared by: E.W. Morgan, G.F.S. Hiatt and D.W. Jr. Korte, Presidio of San Farncisco, California. LAIR Report No. 263, DTIC Accession No. AD-A192693.

Letterman Army Institute of Research. 1988d. Primary eye irritation potential of nitroguanidine in rabbits. Prepared by: G.F.S. Hiatt, C.R. Wheeler and D.W. Jr. Korte, Presidio of San Farncisco, California. DTIC Accession No. AD-A164675.

Letterman Army Institute of Research. 1988e. Oral toxicity of nitroguanidine in mice. Prepared by: G.F.S. Hiatt, S.K. Sano, C.R. Wheeler and D.W. Jr. Korte, Presidio of San Farncisco, California. LAIR Report No. 265.

Letterman Army Institute of Research. 1988f. Developmental Toxicity Potential of Nitroguanidine in Rats. Report No. 257, Toxicology Series 174. Prepared by: V.G. Coppes, G.A. Orner and D.W. Korte, Jr., Presidio of San Farncisco, California.

Letterman Army Institute of Research. 1988g. Fourteen-day subchronic oral toxicity study of nitroguanidine in rats. Prepared by: E.W. Morgan, L.D. Brown, C.M. Lewis, R.R. Dahlgren and D.W. Jr. Korte, Presidio of San Francisco, California. Institute Report No. 272, DTIC Accession No. AD-A196666.

Letterman Army Institute of Research. 1988h. Sister Chromatid Exchange Assay of Nitroguanidine in Chinese Hamster Ovary Cells. Prepared by: J.W. Harbell, L.D. Witcher and D.W. Korte, Jr., Presidio of San Farncisco, California. Toxicology Series 191, Technical Report No. 273.

Letterman Army Institute of Research. 1988i. Mutagenic Potential of Nitrosoguanidine in the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Prepared by: S.E. Sebastian and D.W. Korte, Presidio of San Farncisco, California. Report No. 260.

Letterman Army Institute of Research. 1988j. Nintey-day Subschronic Oral Toxicity Study of Nitroguanidine in Rats. Prepared by: E.W. Morgan, M.J. Pierce, G.M. Zaucha, G.T. Makovec and D.W. Jr. Korte, Presidio of San Francisco, California. Institute Report No. 306, Toxicology Series 170, DTIC Accession No. AD-A203681.

Letterman Army Institute of Research. 1988k. Developmental Toxicity Potential of Nitroguanidine In Rabbits. Prepared by: V.G. Coppes, C.L. Gomez, D.K. Magnuson and D.W. Korte, Jr., Presidio of San Farncisco, California. Toxicology Series 184, Report No. 298.

Letterman Army Institute of Research. 1988l. Acute dermal toxicity of nitroguanidine in rabbits. Prepared by: C.M. Lewis, C.V. Okerberg and D.W. Jr. Korte, Presidio of San Francisco, California. Institute Report No. 254, DTIC Accession No. AD-A192693.

Letterman Army Institute of Research. 1988m. Acute Oral Toxicity of Nitroguanidine in Male and Female Rats. Prepared by: L.D. Brown, C.R. Wheeler and D.W. Jr. Korte, Presidio of San Farncisco, California. LAIR Report No. 264, DTIC Accession No. AD-A192694.

Letterman Army Institute of Research. 1990a. Toxic Potential of Nitroguanidine on Reproduction and Fertility in Rats. Volume 2 of 2. Prepared by: V.G. Coppes, C.B. Clifford, S. Ferraris and D.W. Korte, Jr., Presidio of San Farncisco, California. Report No. 434.

Letterman Army Institute of Research. 1990b. Toxic Potential of Nitroguanidine on Reproduction and Fertility in Rats. Volume 1 of 2. Prepared by: V.G. Coppes, C.B. Clifford, S. Ferraris and D.W. Korte, Jr., Presidio of San Farncisco, California. Report No. 434.

Los Alamos National Laboratory. 1985. A toxicological study of NTO. Prepared by: J.O. London and D.M. Smith, Los Alamos, New Mexico. Report No. LA-10533-MS.

Le Campion, L., M. Delaforge, J.P. Noel, and J. Ouazzani. 1998. Metabolism of 14C-labelled 5-nitro-1,2,4-triazol-3-one (NTO): comparison between rat liver microsomes and bacterial metabolic pathways. *Journal of Molecular Catalysis B: Enzymatic* 5 (1):395-402.

Le Campion, L., C. Giannotti, and J. Ouazzani. 1999a. Photocatalytic degradation of 5-nitro-1,2,4-triazol-3-one NTO in aqueous suspension of TiO2. Comparison with Fenton oxidation. *Chemosphere* 38 (7):1561-70.

Le Campion, L., A. Vandais, and J. Ouazzani. 1999b. Microbial remediation of NTO in aqueous industrial wastes. *FEMS Microbiol Lett* 176 (1):197-203.

LeCampion, L., M. DeLaForge, J.P. Noel, and J. Ouazzani. 1997. Metabolism of 14C-labelled 5-nitro-1,2,4-triazol-3-one by rat liver microsomes: Evidence for participation of cytochrome P-450. *Eur J Biochem* 248:401-406.

Lent, E.M., L.C. Crouse, S.M. Wallace, and E.E. Carroll. 2015. Peri-pubertal administration of 3-nitro-1,2,4-triazol-5-one (NTO) affects reproductive organ development in male but not female Sprague Dawley rats. *Reprod Toxicol* 57:1-9.

Lent, E.M., L.C. Crouse, A.M. Jackovitz, E.E. Carroll, and M.S. Johnson. 2016a. An extended one-generation reproductive toxicity test of 1,2,4-Triazol-5-one (NTO) in rats. *J Toxicol Environ Health A* 79 (24):1159-1178.

Lent, E.M., L.C. Crouse, and S.M. Wallace. 2016b. Oral Toxicity of 2,4-Dinitroanisole in Rats. *Int J Toxicol* 35 (6):692-711.

Lent, E.M., A.B. Mullins, A.D. May, C.L. Honnold, and K.E. Despain. 2018. Characterization of the Testicular Toxicity of 3-Nitro-1,2,4-Triazol-5-One and 2,4-Dinitroanisole in Rats (Rattus norvegicus). *Int J Toxicol* 37 (5):364-372.

Lewis, R.J. 2004. Nitroguanidine. In *SAX's Dangerous Properties of Industrial Materials*. New York: John Wiley & Sons, Inc.

Linker, B.R., R. Khatiwada, N. Perdrial, L.M. Abrell, M.R. Sierra Alvarez, J.A. Field, and J. Chorover. 2015. Adsorption of novel insensitive munitions compounds at clay mineral and metal oxide surfaces. *Environmental Chemistry* 12 (1):74-84.

Lotufo, G.R., J.M. Biedenbach, J.G. Sims, P. Chappell, J.K. Stanley, and K.A. Gust. 2015. Bioaccumulation kinetics of the conventional energetics TNT and RDX relative to insensitive munitions constituents DNAN and NTO in Rana pipiens tadpoles. *Environ Toxicol Chem* 34 (4):880-6.

Lotufo, G.R., J.K. Stanley, P. Chappell, N.L. Melby, M.S. Wilbanks, and K.A. Gust. 2018. Subchronic, chronic, lethal and sublethal toxicity of insensitive munitions mixture

formulations relative to individual constituents in Hyalella azteca. *Chemosphere* 210:795-804.

Madeira, C.L., S.A. Speet, C.A. Nieto, L. Abrell, J. Chorover, R. Sierra-Alvarez, and J.A. Field. 2017. Sequential anaerobic-aerobic biodegradation of emerging insensitive munitions compound 3-nitro-1,2,4-triazol-5-one (NTO). *Chemosphere* 167:478-484.

Madeira, C.L., J.A. Field, M.T. Simonich, R.L. Tanguay, J. Chorover, and R. Sierra-Alvarez. 2018. Ecotoxicity of the insensitive munitions compound 3-nitro-1,2,4-triazol-5-one (NTO) and its reduced metabolite 3-amino-1,2,4-triazol-5-one (ATO). *J Hazard Mater* 343:340-346.

Mark, N., J. Arthur, K. Dontsova, M. Brusseau, and S. Taylor. 2016. Adsorption and attenuation behavior of 3-nitro-1,2,4-triazol-5-one (NTO) in eleven soils. *Chemosphere* 144:1249-55.

Mark, N., J. Arthur, K. Dontsova, M. Brusseau, S. Taylor, and J. Simunek. 2017. Column transport studies of 3-nitro-1,2,4-triazol-5-one (NTO) in soils. *Chemosphere* 171:427-434.

McGregor, D.B., C.G. Riach, R.M. Hastwell, and J.C. Dacre. 1980. Genotoxic activity in microorganisms of tetry, 1,3-dinitrobenzene and 1,3,5-dinitrobenzene. *Environ Mutagen* 2:531-541.

McMahon, R.E., J.C. Cline, and C.Z. Thompson. 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. *Cancer Research* 39 (3):682-93.

Monosson, E. 2005. Chemical Mixtures: Considering the Evolution of Toxicology and Chemical Assessment. *Environ Health Persp* 113:383-390.

Mullins, A.B., K.E. Despain, S.M. Wallace, C.L. Honnold, and E. May Lent. 2016. Testicular effects of 3-nitro-1,2,4-triazol-5-one (NTO) in mice when exposed orally. *Toxicol Mech Methods* 26 (2):97-103.

NAVSEA Insensitive Munisitons Office, Insensitive Munitions Organization. 1994. The History of Insensitive Munitions. Prepared by: R.L. Beauregard, http://www.insensitivemunitions.org/history/preface.

Niedzwiecka, J.B., S.R. Drew, M.A. Schlautman, K.A. Millerick, E. Grubbs, N. Tharayil, and K.T. Finneran. 2017. Iron and Electron Shuttle Mediated (Bio)degradation of 2,4-Dinitroanisole (DNAN). *Environ Sci Technol* 51 (18):10729-10735.

National Research Council of Canada. 2013. Environmental Fate and Ecological Impact of Emerging Energetic Chemicals (ADN, DNAN and its Amino-derivatives, PETN, NTO, NQ, FOX-7, and FOX-12), Annual Report 2013-2014. Prepared by: J. Hawari, Montreal, Quebec. DRDC-RDDC-2014-C178.

U.S. Army Natick Research and Development Center. 1981. Microbiological and Chemical Transformations of Nitroguanidine. Prepared by: D.L. Kaplan, J.H. Cornell and A.M. Kaplan, Natick, Massachusetts. Report No. TR-81-019, DTIC Accession No. AD-A101631.

U.S. Army Natick Research and Development Center. 1985. Nitroguanidine Soil Degradation Mechanisms. Prepared Natick, Massachusetts. DTIC Accession No. EA055419.

Olivares, C., J. Liang, L. Abrell, R. Sierra-Alvarez, and J.A. Field. 2013. Pathways of reductive 2,4-dinitroanisole (DNAN) biotransformation in sludge. *Biotechnol Bioeng* 110 (6):1595-604.

Olivares, C.I., L. Abrell, R. Khatiwada, J. Chorover, R. Sierra-Alvarez, and J.A. Field. 2016a. (Bio)transformation of 2,4-dinitroanisole (DNAN) in soils. *J Hazard Mater* 304:214-21.

Olivares, C.I., R. Sierra-Alvarez, C. Alvarez-Nieto, L. Abrell, J. Chorover, and J.A. Field. 2016b. Microbial toxicity and characterization of DNAN (bio)transformation product mixtures. *Chemosphere* 154:499-506.

Padda, R.S., C. Wang, J.B. Hughes, R. Kutty, and G.N. Bennett. 2003. Mutagenicity of nitroaromatic degradation compounds. *Environ Toxicol Chem* 22 (10):2293-7.

Perreault, N.N., D. Manno, A. Halasz, S. Thiboutot, G. Ampleman, and J. Hawari. 2012. Aerobic biotransformation of 2,4-dinitroanisole in soil and soil Bacillus sp. *Biodegradation* 23 (2):287-95.

Pillard, D.A., W.S. Eck, M.S. Johnson, and S. Packard. 2017. Effects of 3-Nitro-1,2,4-triazol-5-one on Survival, Growth and Metamorphosis in the Northern Leopard Frog, Lithobates pipiens. *Ecotoxicology* 26 (9):1170-1180.

Platten, W.E., 3rd, D. Bailey, M.T. Suidan, and S.W. Maloney. 2010. Biological transformation pathways of 2,4-dinitro anisole and N-methyl paranitro aniline in anaerobic fluidized-bed bioreactors. *Chemosphere* 81 (9):1131-6.

Quinn, M.J., D.I. Bannon, A.M. Jackovitz, T.L. Hanna, A.A. Shiflett, and M.S. Johnson. 2014. Assessment of 3-nitro-1,2,4-triazol-5-one as a potential endocrine disrupting chemical in rats using the Hershberger and uterotrophic bioassays. *Int J Toxicol* 33 (5):367-372.

Rao, B., W. Wang, Q. Cai, T. Anderson, and B. Gu. 2013. Photochemical transformation of the insensitive munitions compound 2,4-dinitroanisole. *Sci Total Environ* 443:692-9.

Ray, S., and C.A. Peters. 2008. Changes in microbiological metabolism under chemical stress. *Chemosphere* 71 (3):474-83.

Reddy, G., J. Song, P. Kirby, E.M. Lent, L.C.B. Crouse, and M.S. Johnson. 2011. Genotoxicity assessment of an energetic propellant compound, 3-nitro-1,2,4-triazol-5-one (NTO). *Mutat Res* 719:35-40.

Richard, T., and J. Weidhaas. 2014a. Biodegradation of IMX-101 explosive formulation constituents: 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine. *J Hazard Mater* 280:372-9.

Richard, T., and J. Weidhaas. 2014b. Dissolution, sorption, and phytoremediation of IMX-101 explosive formulation constituents: 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazol-5-one (NTO), and nitroguanidine. *J Hazard Mater* 280:561-9.

Saad, R., Z. Radovic-Hrapovic, B. Ahvazi, S. Thiboutot, G. Ampleman, and J. Hawari. 2012. Sorption of 2,4-dinitroanisole (DNAN) on lignin. *J Environ Sci (China)* 24 (5):808-13.

Sarlauskas, J., A. Nemeikaite-Ceniene, Z. Anusevicius, L. Miseviciene, A. Maroziene, A. Markevicius, and N. Cenas. 2004. Enzymatic redox properties of novel nitrotriazole explosives implications for their toxicity. *Z Naturforsch C* 59:399-404.

(Schardein, J.L.). 2000. *Chemically Induced Birth Defects, Third Edition*. New York: Mercel Dekker, Inc.

Schwarzenbach, R.P., R. Stierli, K. Lanz, and J. Zeyer. 1990. Quinone and iron porphyrin mediated reduction of nitroaromatic compounds in homogeneous aqueous solution. *Environ Sci Technol* 24:1566-1574.

Shen, J., C. Ou, Z. Zhou, J. Chen, K. Fang, X. Sun, J. Li, L. Zhou, and L. Wang. 2013. Pretreatment of 2,4-dinitroanisole (DNAN) producing wastewater using a combined zero-valent iron (ZVI) reduction and Fenton oxidation process. *J Hazard Mater* 260:993-1000.

Singh, G., I.P.S. Kapoor, S.K. Tiwari, and P.S. Felix. 2001. Studies on energetic compounds Part 16: Chemistry and decomposition mechanisms of 5-nitro-2,4-dihydro-3H-1,2,4-triazole-3-one (NTO). *J Haz Matl* B81:67-82.

Stanley, J.K., G.R. Lotufo, J.M. Biedenbach, P. Chappell, and K.A. Gust. 2015. Toxicity of the conventional energetics TNT and RDX relative to new insensitive munitions constituents DNAN and NTO in Rana pipiens tadpoles. *Environ Toxicol Chem* 34 (4):873-9.

Takahashi, K.W., T.R. Saito, H. Amao, T. Kosaka, M. Obata, M. Umeda, and Y. Shirasu. 1988. Acute reversible cataract due to nitrocompounds in Japanese quail (Coturnix coturnix japonica) [English abstract]. *Jikken Dobutsu* 37:239-243.

Taylor, S., K. Dontsova, M.E. Walsh, and M.R. Walsh. 2015. Outdoor dissolution of detonation residues of three insensitive munitions (IM) formulations. *Chemosphere* 134:250-6.

Taylor, S., M.E. Walsh, J.B. Becher, D.B. Ringelberg, P.Z. Mannes, and G.W. Gribble. 2017. Photo-degradation of 2,4-dinitroanisole (DNAN): An emerging munitions compound. *Chemosphere* 167:193-203.

Temple, T., M. Ladyman, N. Mai, E. Galante, M. Ricamora, R. Shirazi, and F. Coulon. 2018. Investigation into the environmental fate of the combined Insensitive High Explosive

constituents 2,4-dinitroanisole (DNAN), 1-nitroguanidine (NQ) and nitrotriazolone (NTO) in soil. *Sci Total Environ* 625:1264-1271.

U.S. Army Medical Bioengineering Research and Development Laboratory. 1985. The toxicity of nitroguanidine and photolyzed nitroguanidine to freshwater aquatic organisms. Prepared by: W.H. van der Schalie, Fort Detrick, Maryland. Technical Report No. 8404, DTIC Accession No. AD-A153045.

U.S. Army Biomedical Research and Development Lab. 1988. Photochemistry of aqueous nitroguanidine. Prepared by: W.D. Burrows, M.O. Schmidt, R.H. Chyrek and C.I. Noss, Ft. Detrick, Maryland. Techinical Report 8808, DTIC Accession No. AD-A203200.

U.S. Army Center for Health Promotion and Preventive Medicine. 2008. Acute Oral Toxicity of OSX-CAN Type II Using the Sequential Stage-Wise Probit Method. Prepared by: L.C.B. Crouse, J. T. Houpt, A. O'Neill and E.M. LaFiandra, Aberdeen Proving Ground, Maryland. Toxicology Study No. 85-XC-OA6Va-08.

U.S. Air Force Research Laboratory. 2000. Dermal Absorption of Comp B and CRB-12 in Isolated Rat Skin. Prepared by: Mantech-Geo-Centers Joint Venture, POC: J.N. McDougal, D. Pollard, D.E. Dodd and R. DavisWright-Patterson AFB, Ohio. Report No. AFRL-HE-WP-TR-2001-0058.

U.S. Air Force Research Laboratory. 2002. Recommendation of an occupational exposure level for PAX-21. Prepared by: D.E. Dodd and J.N. McDougal, Wright-Patterson Air Force Base, Ohio. Report No. AFRL-HE-WP-TR-2001-0103.

U.S. Army Medical Research and Development Command. 1987. Environmental Fate of Nitroguanidine, Diethyleneglycol Dinitrate, and Hexachloroethane Smoke, Final Report, Phase II. Prepared by: R. Spanggord, T.W. Chou, T. Mill, W. Haag and W. Lau, Fort Detrick, Frederick, Maryland. SRI Project LSU-7706, DTIC AD-A200324.

U.S. Army Public Health Command (Provisional). 2010. Determination of the Water Solubility, Octanol-water Partition Coefficient and Biodegradation Potential of 3-Nitro-1,2,4-Triazol-5-One (NTO). Prepared by: J.T. Houpt and M.A. Hable, Aberdeen Proving Ground, Maryland. Toxicology Report No. 85-XC-OA6Wb-08.

U.S. Army Public Health Command. 2012a. In vitro endocrine disruption screening of 3-nitro-1,2,4-triazol-5-one (NTO). Prepared by: V.H. Adams, Aberdeen Proving Ground, Maryland. Toxicology Report No. S.0002745-12.

U.S. Army Public Health Command. 2012b. Effects of Sub-acute Oral IMX-101 Exposure to male and female Rats (Rattus norvegicus). Prepared by: L.R. Williams, V.H. Adams and S. Wallace., Aberdeen Proving Ground, Maryland. Toxicology Study No. S.0001797-12.

U.S. Army Public Health Command. 2012c. In vitro dermal absorption of insensitive munitions explosive 101 (IMX-101) and components. Prepared by: W.C. McCain, L. Williams and

G. Reddy, Aberdeen Proving Ground, Maryland. Report No. S.0002733-12, DTIC Accession No. ADA 584068.

U.S. Army Public Health Command. 2013. Acute inhalation toxicity and blood absorption of 3-nitro-1,2,4-triazol-5-one (NTO) in rats. Prepared by: L.C.B. Crouse and A.J. O'Neill, Aberdeen Proving Ground, Maryland. Report No. 885-XC-0ENTb-11, DTIC Accession No. ADA590390.

U.S. Army Public Health Command. 2014a. White Paper: Medical Surveillance Examination for Workers Exposed to IMX-101 and Its Components. Prepared by: W.S. Monks and R.A. Mirza, Aberdeen Proving Ground, Maryland.

U.S. Army Public Health Command. 2014b. Repeated dose and reproductive/developmental toxicity of NTO (3-nitro-1,2,4-triazol-5-one) in the rat. Prepared by: L.C.B. Crouse and E. M. Lent, Aberdeen Proving Ground, Maryland. Toxicology Study 85-XC-0FP4-12, DTIC Accession No. ADA597490.

U.S. Army Public Health Command. 2015. Acute Inhalation Toxicity and Blood Absorption of 2,4-Dinitroanisole (DNAN) in Rats. Prepared by: A.J. O'Neill and L.C.B. Crouse, Aberdeen Proving Ground, Maryland. Toxicology Study No. 85-XC-0ENTa-11.

U.S. Army Public Health Center. 2019. Chronic Toxicity Test in Rats Exposed to 3-nitro-1,2,4-triazol-5-one (NTO) in Drinking Water. Prepared by: Emily May Lent and Allison M. Narizzano (in Prep), Aberdeen Proving Ground, Maryland. Report no. S.0049418-17.

U.S. Army Armament Research, Development and Engineering Center. 2009. Environmental Effects of Insensitive Munitions. Prepared by: Stevens Institute of Technology Center for Environmental Systems, Hoboken, New Jersey. Contract No. W51QKN-05-D-0011, Task Order No. 41.

U.S. Army Armament Research, Development and Engineering Center. 2011. Evaluation of Treatment Options for Insensitive Munitions Industrial Laden Effluents. Prepared by: Stevens Institute of Technology Center for Environmental Systems, Hoboken, New Jersey. Contract No. W51QKN-05-D-0011, Task Order No. 64, Subtask 3.2.1.

U.S. Department of Agriculture. 1989. Effect of Nitroguanidine-Contaminated Wastewater on Physiology of Plants. Prepared by: R. H. Hodgson, Ft. Detrick, Maryland. DTIC Accession No. EA061579.

U.S. Environmental Protection Agency (USEPA). 1992. *Drinking Water Health Advisory: Munitions*. Edited by W.C. Roberts and W.R. Hartley: Office of Drinking Water Health Advisories; Lewis Publishers.

U.S. Environmental Protection Agency. 2009. Provisional Peer Reviewed Toxicity Values for Nitroguanidine (CASRN 556-88-7). Prepared by: National Center for Environmental Assessment Superfund Health Risk Technical Support Center, Office of Research and Development, Cincinnati, Ohio.

Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 2018. Drinking Water Standards and Health Advisory Tables, 2018 Edition. Prepared by: Office of Water, Washington, D.C. EPA 822-F-18-001.

Walker, J.E., and D.L. Kaplan. 1992. Biological degradation of explosives and chemical agents. *Biodegradation* 3 (2-3):369-385.

WEEL. 2017. Nitroguanidine (NQ) (2016). *Toxicology and Industrial Health* 34 (1):15-22.

WEEL. 2018a. 3-Nitro-1,2,4-triazol-5-one (2014). *Toxicol Ind Health* 34 (1):8-14.

WEEL. 2018b. 2,4-Dinitroanisole (DNAN) (2014). *Toxicol Ind Health* 34 (1):2-7.

Williams, M., ed. 2013. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 15th Edition*. Cambridge, United Kingdom: Royal Society of Chemistry.

Williams, R.T., W.E. Sisk, and A.R. MacGillivray. 1989. Degradation of nitroguanidine wastewater components in soil. *Environmental Toxicology and Chemistry* 8 (6):469-475.