ERDC/CERL TR-05-10

Construction Engineering Research Laboratory

US Army Corps of Engineers®
Engineer Research and Development Center

Military Munitions-Related Compounds Fate and Effects

A Literature Review Relative to Threatened and Endangered Species

Katherine von Stackleberg, Craig Amos, and Thomas Smith

March 2005

ERDC/CERL TR-05-10
March 2005

Military Munitions-Related Compounds Fate and Effects

A Literature Review Relative to Threatened and Endangered Species

Katherine von Stackleberg and Craig Amos

Menzie-Cura and Associates, Inc.
8 Winchester Place, Suite 202
Winchester, MA 01890

Thomas Smith

Construction Engineering Research Laboratory
PO Box 9005
Champaign, IL 61826-9005

Final Report

Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000

Under Work Unit #T033

ABSTRACT: As with many anthropogenic substances, there is a general concern about the potential impacts on ecosystems and their component species resulting from military training and testing exercises using various forms of military munitions, which upon impact release or produce various chemical compounds. The biological species present on military training and testing lands may be exposed to these militarily prevalent chemical compounds during soldier training and testing exercises.

This report provides a review and summarization of the literature on the fate and effects of military munitions compounds (MMCs) in the environment. More than 340 reports and other scientific papers were identified that relate directly to primary military munitions constituents and species of high interest (species of high interest include those species considered to be threatened and endangered, as defined in accordance with the Endangered Species Act).

Contents

Conversion Factors ..... **vii**

Preface ..... **viii**

1 Introduction **1**

Background 1
Objective 1
Approach 2
Mode of Technology Transfer 3

2 Constituents of Concern and Data Sources **4**

3 Relevant Literature **6**

1,3,5-Trinitrobenzene 6
Physical-Chemical Properties *6*
Environmental Fate and Transport *6*
Bioaccumulation and Trophic Transfer *7*
Toxicity *7*
1,3-Dinitrobenzene 13
Physical-Chemical Properties *14*
Environmental Fate and Transport *14*
Bioaccumulation and Trophic Transfer *16*
Toxicity *16*
2,4-Dinitrophenol 20
Physical-Chemical Properties *21*
Environmental Fate and Transport *21*
Bioaccumulation and Trophic Transfer *21*
Toxicity *21*
Dinitrotoluene 25
Physical-Chemical Properties *26*
Environmental Fate and Transport *26*
Bioaccumulation and Trophic Transfer *29*
Toxicity *31*
Octahydro-1,3,57-tetranitro-1,3,5,7-tetrazocine (HMX) 35
Physical-Chemical Properties *35*
Environmental Fate and Transport *36*

Bioaccumulation and Trophic Transport *41*
Toxicity *41*
Nitrobenzene 45
Physical-Chemical Properties *47*
Environmental Fate and Transport *47*
Bioaccumulation and Trophic Transfer *53*
Toxicity *55*
Nitroglycerin 62
Physical-Chemical Properties *63*
Environmental Fate and Transport *63*
Bioaccumulation and Trophic Transfer *67*
Toxicity *67*
Nitrophenol 72
Physical-Chemical Properties *72*
Environmental Fate and Transport *72*
Bioaccumulation and Trophic Transfer *74*
Toxicity *76*
Pentaerythritol Tetranitrate (PETN) 81
Physical-Chemical Properties *81*
Environmental Fate and Transport *81*
Bioaccumulation and Trophic Transfer *84*
Toxicity *85*
Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) 86
Physical-Chemical Properties *87*
Environmental Fate and Transport *87*
Bioaccumulation and Trophic Transfer *98*
Toxicity *98*
Trinitrophenylmethylnitramine (Tetryl) 107
Physical-Chemical Properties *107*
Environmental Fate and Transport *107*
Bioaccumulation and Trophic Transfer *110*
Toxicity *110*
Trinitrotoluene (TNT) 114
Physical-Chemical Properties *114*
Environmental Fate and Transport *114*
Bioaccumulation and Trophic Transport *134*
Toxicity *135*

4 Database Adequacy 172

1,3,5-Trinitrobenzene 172
Environmental Fate and Transport *172*
Bioaccumulation and Trophic Transfer *172*

Toxicity *174*
1,3-Dinitrobenzene 175
Environmental Fate and Transport *175*
Bioaccumulation and Trophic Transfer *175*
Toxicity *176*
2,4-Dinitrophenol 176
Environmental Fate and Transport *176*
Bioaccumulation and Trophic Transfer *177*
Toxicity *178*
Dinitrotoluene 179
Environmental Fate and Transport *179*
Bioaccumulation and Trophic Transfer *180*
Toxicity *181*
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) 182
Environmental Fate and Transport *182*
Bioaccumulation and Trophic Transport *183*
Toxicity *183*
Nitrobenzene 185
Environmental Fate and Transport *185*
Bioaccumulation and Trophic Transfer *187*
Toxicity *187*
Nitroglycerin 188
Environmental Fate and Transport *188*
Bioaccumulation and Trophic Transfer *189*
Toxicity *189*
Nitrophenol 190
Environmental Fate and Transport *190*
Bioaccumulation and Trophic Transfer *191*
Toxicity *192*
Pentaerythritol Tetranitrate (PETN) 194
Environmental Fate and Transport *194*
Bioaccumulation and Trophic Transfer *194*
Toxicity *195*
Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) 195
Environmental Fate and Transport *195*
Bioaccumulation and Trophic Transfer *197*
Toxicity *197*
Trinitrophenylmethylnitramine (Tetryl) 198
Environmental Fate and Transport *198*
Bioaccumulation and Trophic Transfer *199*
Toxicity *200*

Trinitrotoluene (TNT)..... 200
Environmental Fate and Transport..... *200*
Bioaccumulation and Trophic Transport..... *203*
Toxicity..... *204*

References..... **207**

Report Documentation Page..... **224**

Conversion Factors

Non-SI* units of measurement used in this report can be converted to SI units as follows:

Multiply	By	To Obtain
acres	4,046.873	square meters
cubic feet	0.02831685	cubic meters
cubic inches	0.00001638706	cubic meters
degrees (angle)	0.01745329	radians
degrees Fahrenheit	(5/9) x (°F – 32)	degrees Celsius
degrees Fahrenheit	(5/9) x (°F – 32) + 273.15.	kelvins
feet	0.3048	meters
gallons (U.S. liquid)	0.003785412	cubic meters
horsepower (550 ft-lb force per second)	745.6999	watts
inches	0.0254	meters
kips per square foot	47.88026	kilopascals
kips per square inch	6.894757	megapascals
miles (U.S. statute)	1.609347	kilometers
pounds (force)	4.448222	newtons
pounds (force) per square inch	0.006894757	megapascals
pounds (mass)	0.4535924	kilograms
square feet	0.09290304	square meters
square miles	2,589,998	square meters
tons (force)	8,896.443	newtons
tons (2,000 pounds, mass)	907.1847	kilograms
yards	0.9144	meters

*_Système International d'Unités_ ("International System of Measurement"), commonly known as the "metric system."

Preface

This study was conducted for Office of the Commander, U.S. Army Corps of Engineers under project A896, "Base Facility Environmental Quality"; Work Unit T033, "threatened and Endangered Species." The technical monitor was Dr. Vic Diersing, DAIM-ED-N.

The work was performed by the Ecological Processes Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). The CERL Principal Investigator was Thomas Smith. Part of this work was done by Katherine von Stackleberg and Craig Amos of Menzie-Cura and Associated, Inc., under DACA88-89-D-0002 through the University of Illinois. The technical editor was Gloria J. Wienke, Information Technology Laboratory. Stephen E. Hodapp was Chief, CECER-CEN-N, and Dr. John T. Bandy is Chief, CEERD-CN. The associated Technical Director was Dr. William D. Severinghaus, CEERD-CV-T. The Director of CERL is Dr. Alan W. Moore.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Commander and Executive Director of ERDC is COL James R. Rowan, and the Director of ERDC is Dr. James R. Houston.

1 Introduction

Background

As with many anthropogenic substances, there is a general concern about the potential impacts on ecosystems and their component species resulting from military training and testing exercises using various forms of military munitions, which upon impact release or produce various chemical compounds (military munitions compounds [MMCs] in this report). The biological species present on military training and testing lands may be exposed to these militarily prevalent chemical compounds during soldier training and testing exercises. These compounds are contained in or a result of the use of various military weapons systems and ammunition. A variety of munitions are used on Army and other armed forces firing ranges including various configurations of 9mm, .38 cal, .45 cal, 5.56m, 7.62m, and 12 ga small arms ammunition; .50 cal heavy machine gun ammunition; 40mm rifle and other grenades (e.g., fragmentation); 84mm and other mortar rounds; 105mm and other artillery rounds; various tank munitions; various explosives (e.g., demolition, Claymore mines); illumination flares; and various colored signal smokes. (This report is not intended to provide a list or other identification of all munitions types that may be used at Army ranges and/or coincide with threatened and endangered species – rather it is intended to address relevant military munitions chemical constituents). Any potential effects of MMCs released would be dependent on any inherent toxicity of the material, the overlap or presence of the materials in the environment with the species of concern, and relevant exposure pathways.

Objective

The objective of this research is to provide a review and summarization of the literature and other reports on the fate and effects of MMCs (and other comparably used compounds) in the environment. More specifically, this research is focused on and related to species of high interest. For this document, species of high interest include those species considered to be threatened and endangered, as defined in accordance with the Endangered Species Act, and concurrently found on Army and other military installations where MMCs are released during military training and

testing activities. Concurrently, this review also focuses on ecological receptors. Consequently, literature, data, and information relating to laboratory animals, as well as human health effects, are not specifically addressed. The review and bibliography help to identify potential effects of MMCs on threatened and endangered species on or near military training areas, in accordance with the Endangered Species Act and implementing regulations (50 Code of Federal Regulations).

Approach

A literature search was conducted using bibliographic software EndNote® version 7 to search the National Library of Medicine's PUBMED database. Cambridge Scientific Abstracts was also searched, and the results imported into EndNote®.

This document provides a literature review for those topics where the literature is sufficient, and provides an annotated bibliography where the literature search revealed either a limited number of citations or a largely inaccessible primary literature. In some cases, if neither an abstract nor the full citation was available, we were unable to provide a summary of that citation.

In this review, over 340 reports and other scientific papers were identified. While effort has been made to identify all of the relevant literature, no doubt some have been missed and overlooked. This review focuses directly on primary military munitions constituents, and generally speaking, information and literature on related chemical compounds has not been included. As an example, there is a body of information on the fate and effects and other characteristics of petroleum products in natural and laboratory environments. This report does not address those types of broad-based topics, but rather attempts to look at only those military munitions related compounds and product fate and effects resulting from their generation and release.

This review attempts to provide a uniform level of review and discussion of each militarily prevalent chemical compound or constituent of concern and thus provide equal treatment of each. However, due in part to different amounts of information available for the different constituents, this has not always been possible. Also, in some instances the level of treatment differs because of subjective judgments made by the authors in determining what information to present, as well as judgments as to the current relative importance of the constituents to military training and other uses. A consideration in the level of discussion or detail presented has been not only the amount of data and information available, but also the relative or comparative

apparent toxicity of the constituents addressed. In all instances we have attempted to address all relative and salient information. In this review, relevant literature and reports are identified and summarized. However, as always, to ensure all relevant information in those reports is understood, the reader should consult directly with the report and not rely solely on the review presented here.

This review focuses on potential toxicity to ecological receptors. The goal is to identify previous studies that will be useful for assessing the potential risks to threatened and endangered species. The typical primary route of exposure is ingestion, and we give emphasis to those studies.

Chapter 2 describes the potential constituents of concern (COCs), and the sources of information consulted in developing the literature search. Chapter 3 provides the details of the literature search for each of the categories and constituents. Chapter 4 presents a discussion of the adequacy of the database (with a focus on ecological risk assessment purposes). Chapter 5 provides additional references.

Mode of Technology Transfer

This report will be made accessible through the World Wide Web (WWW) at URL: http://www.cecer.army.mil

2 Constituents of Concern and Data Sources

This review focuses on the following twelve constituents of concern. These COCs are the main topic headings and form the primary organizational basis of the review.

1,3,5-Trinitrobenzene (1,3,5-TNB)
1,3-Dinitrobenzene (1,3-DNB)
2,4 – Dinitrophenol (2,4-DNP)
Dinitrotoluene isomers (e.g., 2,4 – Dinitrotoluene; 2,6 – Dinitrotoluene)
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)
Nitrobenzene (NB)
Nitroglycerin (NG)
Nitrophenol isomers (e.g. 2 – Nitrophenol; 4 – Nitrophenol)
Pentaerythritol Tetranitrate (PETN)
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
Trinitrophenylmethylnitramine (Tetryl)
2,4,6-Trinitrotoluene (TNT)

These COCs were selected based on their known toxic characteristics and on their widespread and/or frequency of occurrence in terrestrial or aquatic environmental sites at Army and other military installations.

The literature summary and review was compiled by searching available databases for:

- toxicity/toxic properties/characteristics, including any threshold levels
- environmental fate and transport
- degradation/breakdown and processes
- bioaccumulation in terrestrial and aquatic environments
- trophic transfer in terrestrial and aquatic environments
- pathways for biological exposure.

The literature search used bibliographic software EndNote® version 7 to search the National Library of Medicine's PUBMED database. Cambridge Scientific Abstracts was also searched, and the results imported into EndNote®.

The review organizes the information into the following categories:

- Physical-chemical properties
- Environmental fate and transport
- Bioaccumulation and trophic transfer
- Toxicity

Table 1 (page 149; the key for the table is on page 157) provides a database summary of relevant articles. This database contains codes for each contaminant of concern that is addressed; the broad category that the citation applies to (e.g., physical and chemical properties, environmental fate and transport, bioaccumulation and trophic transfer, and toxicity); whether or not we were able to obtain the abstract (A), primary reference (C), or neither (N); a brief summary; and the full citation.

3 Relevant Literature

1,3,5-Trinitrobenzene

1,3,5-Trinitrobenzene (TNB) is formed by the nitration of benzene using nitric and sulfuric acids (ATSDR 1995). TNB is also formed as a by-product during TNT production, most likely due to oxidation of a methyl group (ATSDR 1995, Talmage et al. 1999).

TNB is used as an explosive by the military. TNB is less sensitive to shock and more powerful than TNT (Talmage et al. 1999). TNB is used as an explosive in the mining and drilling industries, and as a vulcanizing agent for processing rubber (ATSDR 1995).

ATSDR. 1995. Toxicological Profile for 1,3-Dinitrobenzene and 1,3,5-Trinitrobenzene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for 1,3,5-Trinitrobenzene.

Environmental Fate and Transport

TNB is expected to be highly mobile in soils (HSDB 2003). TNB will be degraded by photolysis on soil surfaces, and volatilization from moist soils is not expected to occur (HSDB 2003).

TNB is expected to adsorb to suspended solids or sediment in aquatic systems at moderate to low levels (HSDB 2003). Direct photolysis is the primary degradation mechanism for TNB in aquatic systems (HSDB 2003).

Based on an extrapolated vapor pressure of $3.2X10^{-6}$ mm Hg at 20º C, atmospheric TNB is expected to exist partly in the vapor phase and partly adsorbed to atmospheric particulate matter (HSDB 2003). Wet deposition is a possible fate of atmospheric TNB based on its water solubility of 340 mg/l at 20º C (HSDB 2003).

No other information for the environmental fate and transport of TNB was found. Due to the similar structure of TNB to other nitrobenzenes, the environmental fate and transport may be similar to other nitrobenzene compounds.

Bioaccumulation and Trophic Transfer

No information was found on the bioaccumulation and trophic transfer of TNB.

Toxicity

Deneer, J.W., van Leeuwen, C.J., Seinen, W., Maas-Diepeveen, J.L. and Hermens, J.L.M. 1989. QSAR Study of the Toxicity of Nitrobenzene Derivatives Towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum. *Aquatic Toxicology AQTODG.* 15(1): 83-98.

The toxicity of various mono and dinitrobenzene derivatives towards *Daphnia magna, Chlorella pyrenoidosa*, and *Photobacterium phosphoreum* is investigated, establishing quantitative structure-activity relationships (QSARs). For the mono-nitro compounds tested, it is observed that their acute and semi-chronic toxicity towards *D. magna* is only slightly, if at all, higher than the minimum toxicity expected on the basis of a narcosis type of action. Moreover, the logarithm of the octanol/water partition coefficient (log P) is found to be a sufficient descriptor of the toxicity of these compounds to *D. magna*. The statistical quality of the QSAR describing the toxicity of mono-nitro compounds to *C. pyrenoidosa* is found to be improved by incorporation of Hammett sigma constants and log P as parameters. For the dinitrobenzene derivatives, it is established that the description of their toxicity to *D. magna* and *C. pyrenoidosa* necessitates the use of sigma constants. Furthermore, the toxicity of several of these compounds is found to be more dependent upon the duration of the test than observed for the mono-nitro compounds. This is assumed to be due to the formation of reactive metabolites, which probably contribute substantially to the toxicity of most di-nitro compounds. For the toxicity of dinitrobenzenes to *P. phosporeum*, no significant QSAR could be established employing sigma constants and log P as parameters. The applicability of the Microtox test as a prescreening tool for the selection of compounds needing further evaluation is discussed. It is concluded that the usefulness of the test is only limited. There appears to be no obvious relationship between the susceptibilites of *P. phosphoreum* and other species to chemicals that cause toxic effects through modes of biological action different from general anaesthesia. (Author's abstract)

Fuller, M.E. and Manning, J.F., Jr. 1998. Evidence for differential effects of 2,4,6-trinitrotoluene and other munitions compounds on specific subpopulations of soil microbial communities. ***Environmental Toxicology and Chemistry.*** **17(11): 2185-2195.**

The effects of 2,4,6-trinitrotoluene (TNT) and other munitions compounds on indigenous microbial communities in several soils were examined. Culturable heterotrophs, concentrations of phospholipid fatty acid (PLFA), and basal respiration rates exhibited slight negative correlations with high TNT and 1,3,5-trinitrobenzene (TNB) levels. Heat-shock-resistant culturable heterotrophs, percentage of gram-positive soil isolates, mole percent of branched PLFA, and 10Me 18:0 (tuberculostearic acid) were observed to be significantly lower in highly contaminated soils. Total soil nitrogen levels were positively correlated with high TNT and TNB concentrations, whereas total soil carbon exhibited no significant correlation with either compound. Multivariate analysis of PLFA data resulted in distinct separation of soils with respect to their degree of contamination, with specific signature PLFAs for gram-positive bacteria, fungi, and protozoa being negatively associated with high contaminant levels. Apparent concentrations of TNT resulting in 50% reductions in indicators of gram-positive populations were much higher than values from pure culture experiments, possibly as a result of low bioavailability due to sorption onto clay and soil organic matter. Few effects of other munitions compounds were observed. Closer examination of a highly contaminated soil revealed that the number of culturable heterotrophs growing on 0.3% molasses plates decreased by 50% when 67 µg TNT/ml was added to the medium; a 99% decrease was observed for soil contaminated with less than 20 µg TNT/g. Highly contaminated soil harbored a greater number of organisms that were able to grow on plates amended with greater than 10 µg TNT/ml. Gram-positive isolates from both soils demonstrated marked growth inhibition when greater than 8-16 µg TNT/ml was present in the culture medium. These results indicate that chronic exposure to munitions compounds can dramatically alter soil microbial communities.

Gough, K.M., Belohorcova, K. and Kaiser, K.L.E. 1994. Quantitative structure-activity relationships (QSARs) of Photobacterium phosphoreum toxicity of nitrobenzene derivatives. ***Science of the Total Environment [SCI. TOTAL ENVIRON.].*** **142(3): 179-190.**

The Microtox super(TM) values (acute toxicities to Photobacterium phosphoreum) of 85 nitrobenzene derivatives are reported, along with the results of ab initio (minimal basis, STO-3G) and semi-empirical (AM1) molecular orbital calculations of electronic properties. QSARs are developed between the acute toxicities and the various calculated parameters. The more rapid semi-empirical calculations are compared to the ab initio results and the advantages and disadvantages of the two methods are

considered. As in earlier work, the change in the electronic charge distribution on the nitro group, (QO), in compounds with strongly electron withdrawing substituents, is found to be a significant indicator of high toxicities. The data set is comprised of di-, tri- and tetra substituted compounds. The effect of the increase in the number of substituents and their relative positions on the ring are considered as possible factors affecting acute toxicity. For compounds in which the nitro group is ortho to a substituent with which it may hydrogen-bond, the charge on the nitro group ceases to be a useful indicator of toxicity. The energy of the Highest Occupied Molecular Orbital (HOMO) is found to be highly correlated with QO and alternates with it as the significant parameter in some regressions.

Lachance, B., Robidoux, P.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. ***Mutation Research Genetic Toxicology and Environmental Mutagenesis.*** **444(1): 25-39.**

The mutagenicity and toxicity of energetic compounds such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and of amino/nitro derivatives of toluene were investigated in vitro. Mutagenicity was evaluated with the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay. Cytotoxicity was evaluated using V79 and TK6 human lymphoblastic cells. For the TK6 and V79 assays, TNB and 2,4,6-triaminotoluene were more toxic than TNT, whereas RDX and HMX were without effect at their maximal aqueous solubility limits. The primary TNT metabolites (2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene) were generally less cytotoxic than the parent compound. The FT results indicated that TNB, TNT and all the tested primary TNT metabolites were mutagenic. Except for the cases of 4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene in the TA98 strain, addition of rat liver S9 resulted in either no effect, or decreased activity. None of the tested compounds were mutagenic for the V79 mammalian cells with or without S9 metabolic activation. Thus, the FT assay was more sensitive to the genotoxic effects of energetic compounds than was the V79 test, suggesting that the FT might be a better screening tool for the presence of these explosives. The lack of mutagenicity of pure substances for V79 cells under the conditions used in this study does not preclude that genotoxicity could actually exist in other mammalian cells. In view of earlier reports and this study, mutagenicity testing of environmental samples should be considered as part of the hazard assessment of sites contaminated by TNT and related products.

Lotufo, G.R., Farrar, J.D., Inouye, L.S., Bridges, T.S. and Ringelberg, D.B. 2001. Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic invertebrates. ***Environmental toxicology and chemistry.*** **20(8): 1762-1771.**

The toxicity of nitroaromatic (2,4-diaminonitrotoluene [2,4-DANT] and 1,3,5-trinitrobenzene [TNB]) and ^{14}C-labeled cyclonitramine compounds (hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX] and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine [HMX]) to the marine polychaete *Neanthes arenaceodentata* and the estuarine amphipod *Leptocheirus plumulosus* following 10- or 28-day exposures to spiked sediments was investigated. Organismal-level effects on survival, growth, and reproduction and cellular-level effects on apoptosis (programmed cell death) were evaluated. Because cyclonitramines have low affinity for sediment, overlying water was not exchanged in the RDX and HMX exposures. Nitroaromatics sorbed strongly to sediment, resulting in near complete resistance to solvent extraction. Cyclonitramines sorbed weakly to sediment, as more ^{14}C-activity was found in the overlying water than in the sediment at exposure termination. No significant decrease in survival or growth was observed with cyclonitramines at initial sediment concentrations as high as 1,000 µg/g. Survival was significantly affected by nitroaromatics at nominal sediment concentrations as low as 200 µg/g, with *L. plumulosus* being more sensitive than *N. arenaceodentata*. Growth was significantly decreased at sublethal concentrations of 2,4-DANT for *N. arenaceodentata*. Reproduction, measured only with *L. plumulosus*, was significantly decreased only in the highest RDX treatment and also in the lower TNB treatment. However, no decrease was observed in higher concentrations of TNB. Body burden at exposure termination was below detection limit (1 µg/kg) for all compounds. Significant inhibition of apoptosis was not accompanied by significant decreases in growth or reproduction. Because of its critical function in many biological processes, alterations in this endpoint may result in adverse effects on the organism and could be used as an early indicator of toxicity.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L., Miller, K. and Saepoff, S. 2001. Development of Marine Toxicity Data for Ordnance Compounds. ***Archives of Environmental Contamination and Toxicology.*** **41(3): 308-318.**

A toxicity database for ordnance compounds was generated using eight compounds of concern and marine toxicity tests with five species from different phyla. Toxicity tests and endpoints included fertilization success and embryological development with the sea urchin *Arbacia punctulata*; zoospore germination, germling length, and cell number with the green macroalga *Ulva fasciata*; survival and reproductive success of the polychaete *Dinophilus gyrociliatus*; larvae hatching and survival with the redfish (*Sciaenops ocellatus*); and survival of juveniles of the opossum shrimp

(*Americamysis bahia*) (formerly *Mysidopsis bahia*). The studied ordnance compounds were 2,4- and 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2,4,6-trinitrophenylmethylnitramine (tetryl), 2,4,6-trinitrophenol (picric acid), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The most sensitive toxicity test endpoints overall were the macroalga zoospore germination and the polychaete reproduction tests. The most toxic ordnance compounds overall were tetryl and 1,3,5-trinitrobenzene. These were also the most degradable compounds, often being reduced to very low or below-detection levels at the end of the test exposure. Among the dinitro- and trinitrotoluenes and benzenes, toxicity tended to increase with the level of nitrogenation. Picric acid and RDX were the least toxic chemicals tested overall.

Reddy, G., Reddy, T.V., Choudhury, H., Daniel, F.B. and Leach, G.J. 1997. Assessment of environmental hazards of 1,3,5-trinitrobenzene. *Journal of Toxicology and Environmental Health.* 52(5): 447-460.

The remedial investigation/feasibility studies conducted at certain Army installations showed a need to clean up contaminated sites, where high levels of ammunition chemicals such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB), and their degradation products/metabolites were detected in surface soil and groundwater. TNB is a photodegradation product of TNT; it is not easily degraded, and persists in the environment. The toxicity data on TNB are scanty. Hence the U.S. Environmental Protection Agency in 1988 developed a reference dose (RfD) for TNB (0.00005 mg/kg/d for chronic toxicity) based on the toxicity of DNB, which is structurally similar to TNB. Since then we have completed acute, subacute, subchronic, chronic, reproductive, and developmental toxicity studies and toxicokinetics studies. We have reviewed the mammalian toxicity data for TNB and have determined the no observed adverse effect levels (NOAEL) and low observed adverse effect levels (LOAEL) for subchronic, chronic, reproductive, and developmental toxicity. Based on the newly determined NOAEL and LOAEL values, we have now developed a new RfD for TNB (0.03 mg/kg/d), based on the chronic toxic effects on hematology and histopathological changes in testes and kidney.

Schmitt, H., Altenburger, R., Jastorff, B. and Schuurmann, G. 2000. Quantitative structure-activity analysis of the algae toxicity of nitroaromatic compounds. *Chem Res Toxicol.* 13(6): 441-450.

Proliferation toxicity toward the algae *Scenedesmus vacuolatus* in a 24-hr one-generation reproduction assay was determined for nitrobenzene and 18 derivatives, including two phenols. The resultant EC_{50} values covering more than 4 orders of magnitude were subjected to a quantitative structure-activity analysis (QSAR) using hydrophobicity in terms of the octanol/water partition coefficient in logarithmic

form, log K_{ow}, and 16 quantum chemical descriptors of molecular reactivity that were calculated with the AM1 scheme. For 13 mononitro derivatives and the highly hydrophobic trifluralin, a narcotic-type mode of action can explain most of the toxicity variation. Correction of log K_{ow} for ionization for the phenols and quantification of the molecular susceptibility for one-electron reduction as apparently rate-determining biotransformation step by the energy of the lowest unoccupied molecular orbital, E(LUMO), yields a highly significant QSAR for all 19 compounds (r(adj)(2) = 0.90), which can be further improved when adding the maximum net atomic charge at the nitro nitrogen, q(nitro)(-)(N), as the third descriptor (r(adj)(2) = 0.93). Comparison of the energy of the singly occupied molecular orbital, E(SOMO), of the radical anions as initial metabolites with the E(SOMO) of known redox cyclers suggests that dinitrobenzenes and TFM as well as multiply chlorinated nitrobenzenes may also exert oxidative stress. This is based on an E(SOMO) window of -0.30 to 0.55 eV as a tentative criterion for molecular structures to have the potential for redox cycling, derived from a set of eight known redox cyclers. The discussion includes a detailed analysis of apparently relevant metabolic pathways and associated modes of toxic action of nitroaromatics.

Thomulka, K.W. and Lange, J.H. 1997. Mixture toxicity of nitrobenzene and trinitrobenzene using the marine bacterium Vibrio harveyi as the test organism. *Ecotoxicol Environ Saf.* 36(2): 189-195.

Vibrio harveyi, a bioluminescent marine bacterium, was used to evaluate combined or mixture toxicity of two organic compounds: nitrobenzene and trinitrobenzene. An estimated median effective concentration (EC_{50}) and confidence interval were determined for each chemical. These chemicals at their EC_{50} were evaluated in combination and an additive index method was used to determine a numerical toxicology value. Combinations at 20% intervals of the EC_{50} were performed using isopleths. The isopleths employed were the isobole plot and the isobologram. Bioluminescent change was also determined and graphed for evaluation of toxicity. Statistical evaluation of isopleths and the additive index method were employed by incorporating confidence intervals. Bioluminescent change and isopleths suggest that mixtures of nitrobenzene and trinitrobenzene are additive, while the additive index method is suggestive of synergism. Statistical evaluation between mixtures and single values, using the z test, was in some cases different at the 5% level. These data suggest that interaction of combinations should be evaluated and described by multiple methodologies. Evaluation of these data suggests, in part, that one mixture is statistically different for antagonism. This study supports the use of bioluminescent microbial toxicity tests with various evaluative methodologies for the determination of mixture interactions.

van der Schalie, W.H., Shedd, T.R. and Zeeman, M.G. 1988. Ventilatory and movement responses of Bluegills exposed to 1,3,5-trinitrobenzene. *Aquatic Toxicology and Hazard Assessment*. Philadelphia PA, American Society for Testing and Materials. 10: 307-315.

TNB (1,3,5-trinitrobenzene) is a by-product of the TNT (2,4,6-trinitrotoluene) manufacturing process and is also formed by the photolysis of TNT in natural waters. The effects of TNB on the ventilatory patterns and whole-body movement rates of bluegills (*Lepomis macrochirus*) were determined. Fish were exposed for six days to concentrations of TNB ranging from 6% (0.03 mg/l) to 108% (0.61 mg/l) of the 96 hr LC_{50}. The lowest TNB concentrations causing significant changes in the parameters monitored were 0.13 mg/l (ventilatory depth) and 0.61 mg/l (cough rate and body movement). These responses occurred within the first 4 hr of exposure. No effects on ventilatory rate were found. The large differences in sensitivity between the parameters monitored indicate that automated water or wastewater toxicity monitoring systems that utilize fish ventilatory or movement responses should use several end points to determine the presence or absence of toxicity. The most sensitive, short-term ventilatory changes measured in this test occurred at concentrations near the estimated chronic toxicity levels of TNB for fish. The range between the highest TNB concentration causing no responses and the lowest concentration causing short-term ventilatory responses was 0.06 to 0.13 mg/l. This is comparable to reported no effect/effect ranges for TNB in early life stage tests of 0.08 to 0.12 mg/l for fathead minnows (*Pimephales promelas*) and 0.08 to 0.17 mg/l for rainbow trout (*Onchorhynchus mykiss*).

1,3-Dinitrobenzene

1,3-Dinitrobenzene (DNB) is formed by a two-step nitration process of mixing benzene with fuming nitric and sulfuric acids (ATSDR 1995). Small quantities of DNB are also formed as a by-product during TNT production.

DNB is used as an explosive and camphor substitute in nitrocellulose, which is an explosive and propellant ingredient, by the military (ATSDR 1995). DNB has various commercial uses, but the majority of DNB is used as an intermediate organic for the production of aramid fibers and spandex (ATSDR 1995).

ATSDR. 1995. Toxicological Profile for 1,3-Dinitrobenzene and 1,3,5-Trinitrobenzene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for 1,3-Dinitrobenzene.

Environmental Fate and Transport

Hajjar, N.P., Brower, M.E., Turck, P.A., Kruger, C.L. and Hartley, W.R. 1992. 1,3-Dinitrobenzene (DNB). ***IN: Drinking Water Health Advisory: Munitions. Lewis Publishers, Boca Raton, FL.*** **1992: 49-86.**

1,3-Dinitrobenzene (1,3-DNB) is a colorless to yellowish crystalline solid at room temperature. Small volumes of dinitrobenzenes are formed as byproducts during the manufacture of nitrobenzene. In addition, 1,3-DNB is a byproduct of the manufacture of trinitrotoluene (TNT) explosives at the U.S. Army Ammunition plants (USAAP) located at Radford, VA, (Radford AAP) Chattanooga, TN (Volunteer AAP), Joliet, IL (Joliet AAP), and Newport, IN (Newport AAP). Du Pont, located in Deepwater, NJ, is listed as the only U.S. producer of 1,3-DNB for 1983 (more recent information is not available). The microbial degradation of 1,3-DNB was studied in sewage effluent maintained under aerobic or anaerobic conditions. The disappearance of 1,3-DNB under aerobic conditions was followed for 28 days: significant amounts of UV absorbency persisted. The decrease in initial absorbency was 58% by day 28. Microbial degradation of 1,3-DNB to CO_2 by *Candida pucherrima* occurred under aerobic conditions. The treatment of wastewater generated during dinitrobenzene production, involved slaking with lime, followed by removal of organics contained in the lime in a muffle furnace. The longer-term health advisory (HA) for a 10 kg child has been determined to be 0.04 mg/l. In the absence of adequate animal data to determine a 1 day or 10 day HA, the longer-term HA for a 10 kg child, 0.04 mg/l, is used as a conservative estimate of the 1 day or 10-day HA. The longer-term HA for a 70 kg adult has been determined to be 0.14 mg/l. A lifetime HA of 0.0010 mg/L for a 70 kg adult has been determined. Since no chronic toxicity or carcinogenicity studies with 1,3-DNB are currently available, 1,3-DNB is classified in group D: not classifiable as to human carcinogenicity.

Hallas, L.E. and Alexander, M. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. ***Applied and Environmental Microbiology.*** **45(4): 1234-1241.**

The transformation of mono-and dinitroaromatic compounds was measured in sewage effluent maintained under aerobic or anaerobic conditions. Most of the nitrobenzene, 3-and 4-nitrobenzoic acids, and 3- and 4-nitrotoluenes and much of the 1,2- and 1,3-dinitrobenzenes disappeared both in the presence and absence of oxygen. Under anaerobiosis, 2,6-dinitrotoluene and 3,5-dinitrobenzoic acid disappeared

slowly, but no loss was evident in 28 days in aerated sewage. Aromatic amines did not accumulate during the aerobic decomposition of the mononitro compounds. It is suggested that the transformations of widely used nitroaromatic compounds should be further studied because of the persistence and possible toxicity of products of their metabolism.

Mitchell, W.R. and Dennis, W. 1982. Biodegradation of 1,3-Dinitrobenzene. *Journal of Environmental Science and Health, Part A.* 17(6): 837-853.

1,3-Dinitrobenzene was degraded microbially in water samples taken downstream of the Volunteer Army Ammunition Plant, Chattanooga, TN, but not in water from streams near Frederick, MD. Degradation of the 5 µg/l solution proceeded after a 10 day lag and was complete by Day 15. Microorganisms from the Tennessee River were able to grow with 1,3-dinitrobenzene as the sole carbon source with a half-life of about 1 day. Enriching the cultures degraded the compound to carbon dioxide with a half-life of 9.7 days. The 1,3 dinitrobenzene-adapted organisms did not grow with the other munitions manufacturing wastes tested: 1,3 dinitrobenzene, 1,4-dinitrobenzene, 3,5-dinitroaniline, 1,3,5-trinitrobenzene, or nitrobenzene.

Trapido, M., Dello, A., Goi, A. and Munter, R. 2003. Degradation of nitroaromatics with the Fenton reagent. *Proceedings of the Estonian Academy of Sciences, Chemistry [Proc. Eston. Acad. Sci. Chem.].* 52(1): 38-47.

The feasibility of the Fenton reagent treatment for the degradation and detoxification of the nitroaromatic compounds (NAC) such as p-nitrotoluene, nitrobenzene, and m-dinitrobenzene was studied. The degradation rate of NAC with the Fenton treatment was strongly dependent on the molar ratio NAC/hydrogen peroxide/catalyst (Fe^{2+}). The 90% conversion times of NAC in the Fenton treatment followed the order p-nitrotoluene approximately nitrobenzene < m-dinitro-benzene. The degree of nitrogen conversion to nitrate with the Fenton treatment varied from 25% to 100%, depending on the treatment conditions. Total organic carbon removal of 45-47% was obtained when the Fenton reagent treatment with the concentration of hydrogen peroxide 10 mM and catalyst 1 mM was applied. According to the *Daphnia magna* toxicity test the Fenton reagent treatment enabled to reduce the toxicity of NAC.

Bioaccumulation and Trophic Transfer

Deneer, J.W., T.L. Sinnige, W. Seinen, and J.L.M. Hermens. 1987. Quantitative structure-activity relationships for the toxicity and bioaccumulation factors of nitrobenzene derivatives towards the guppy (*Poecilia reticulata*). *Aquatic Toxicology.* 10:115-129.

Nystrom, D.D. and Rickert, D.E. 1987. Metabolism and excretion of dinitrobenzenes by male Fischer-344 rats. *Drug Metab Dispos.* 15(6): 821-825.

All three dinitrobenzene (DNB) isomers cause methemoglobinemia, but only 1,3-DNB produces testicular toxicity in rats. In order to determine whether major differences exist in the routes of DNB metabolism, male Fischer-344 rats were given an oral dose (0.15 mmol/kg) of ^{14}C-labeled 1,2-, 1,3-, or 1,4-DNB, and excreta were collected over 48 hr. Elimination of radiolabel was rapid; 85%, 60%, and 75% of the 1,2-, 1,3-, and 1,4-DNB dose was recovered in 24 hr, respectively. Urine was the primary route of excretion, accounting for 82% of the total dose of 1,2-DNB and 75% of the dose of 1,4-DNB after 8 hr. Radiolabel from 1,3-DNB was excreted to a slightly lesser extent in the urine (63% of the dose). A greater portion of radiolabel was excreted in the feces than with the other isomers (18% of total dose, compared to 8% and 9% with 1,2-DNB and 1,4-DNB, respectively). The major urinary metabolites of 1,2-DNB were S-(2-nitrophenyl)-N-acetylcysteine (42% of the dose), 2-nitro-aniline-N-glucuronide (4%), 4-amino-3-nitrophenylsulfate (17%), 2-amino-3-nitrophenylsulfate (1.5%), and 2-(N-hydroxylamino)nitrobenzene (1-2%). The major urinary metabolites of 1,3-DNB were 3-aminoacetanilide (22%), 4-acetamido-phenylsulfate (6%), 1,3-diacetamidobenzene (7%), and 3-nitroaniline-N-glucuronide (4%). The major metabolites of 1,4-DNB were 2-amino-5-nitrophenylsulfate (35%), S-(4-nitrophenyl)-N-acetylcysteine (13%), and 1,4-diacetamidobenzene (7%). These results suggest that the DNB isomers are primarily metabolized by nitro group reduction and conjugation with glutathione. The testicular toxicant 1,3-DNB was apparently metabolized exclusively by reduction.

Toxicity

Allenby, G., Sharpe, R.M. and Foster, P.M. 1990. Changes in Sertoli cell function in vitro induced by nitrobenzene. *Fundam Appl Toxicol.* 14(2): 364-375.

Nitrobenzene (NB) has been identified as a testicular toxicant in vivo, but its site of action remains unknown. In the present study, the effect of NB on the Sertoli cell was assessed in vitro using Sertoli cell and Sertoli-germ cell cocultures. The parameters measured were the exfoliation of germ cells; the secretion of lactate, pyru-

vate, and inhibin; and gross cellular morphology. The effect of metadinitrobenzene (mDNB), a related compound which is a known Sertoli cell toxicant, was assessed for comparison. Gross morphological changes including vacuolation of Sertoli cells were observed following treatment of cultures with 10^{-3} M NB. Exposure of cocultures to NB also resulted in dose-dependent exfoliation of predominantly viable germ cells. NB (greater than $5x10^{-4}$ M) and mDNB at the single dose level used (10^{-4} M) stimulated the secretion of lactate and pyruvate significantly by Sertoli cells, an effect that was more marked in the absence of germ cells. Comparable changes were observed in follicle stimulating hormone (FSH)-stimulated cultures. Inhibin secretion by Sertoli cells was also altered by exposure to NB but in a biphasic manner, with low (10^{-8} to 10^{-6} M) and high (10^{-4} to 10^{-3} M) doses enhancing inhibin secretion while intermediate (10^{-5} M) doses had no effect. These effects were evident in both culture systems but inhibin secretion by Sertoli-germ cell cocultures was always greater than that by Sertoli cell cultures. However, these effects of NB on inhibin secretion were not evident in FSH-stimulated cultures. In contrast to the effects of NB, mDNB had no effect on basal secretion of inhibin but blocked the stimulatory effect of FSH. It is concluded that NB, like mDNB, is probably a Sertoli cell toxicant in view of its similar disruptive effects on various parameters of Sertoli cell function. However, NB is far less toxic than mDNB at equivalent concentrations in vitro. The present study is the first to evaluate the potential of inhibin secretion by Sertoli cells in culture as an additional marker of toxicant action, and concludes that it merits further study in this context.

Cave, D.A. and Foster, P.M. 1990. Modulation of m-dinitrobenzene and m-nitrosonitrobenzene toxicity in rat Sertoli–germ cell cocultures. *Fundam Appl Toxicol.* 14(1): 199-207.

Previous work has shown that m-dinitrobenzene is a testicular toxicant in rats in vivo, and in vitro produces comparable morphological changes in rat testicular Sertoli-germ cell cocultures. m-Dinitrobenzene is metabolized both in vivo and in the in vitro system to m-nitroaniline m-nitroaniline and m-nitroacetanilide. These metabolites do not provoke testicular toxicity in vivo or in vitro. We have therefore proposed a pathway for the metabolism of m-dinitrobenzene to m-nitroaniline and m-nitroacetanilide, which involved the intermediate m-nitrosonitrobenzene (1-nitroso-3-nitrobenzene, NNB). When tested, m-nitrosonitrobenzene, at equimolar doses to m-dinitrobenzene, produced similar morphological changes in the culture system to those exhibited by m-dinitrobenzene. However, m-nitrosonitrobenzene produced a greater toxicity than did m-dinitrobenzene (as measured by germ cell detachment). When the intracellular thiol levels were reduced in the cocultures pretreated with diethyl maleate, the toxicity of both m-dinitrobenzene and m-nitrosonitrobenzene was enhanced. In contrast, pretreatment of cocultures with agents known to increase cellular thiol (cysteamine) or scavenge reactive intermediates (cysteamine or

ascorbate) reduced the toxicity of m-dinitrobenzene and m-nitrosonitrobenzene. We propose that m-dinitrobenzene requires metabolic activation before it can exert its toxicity to Sertoli cells, and it appears that the toxic species is m-nitrosonitrobenzene or a further metabolite of m-nitrosonitrobenzene.

Deneer, J.W., vanLeeuwen, C.J., Seinen, W., Maas-Diepeveen, J.L. and Hermens, J.L.M. 1989. QSAR Study of the Toxicity of Nitrobenzene Derivatives Towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum. *Aquatic Toxicology AQTODG.* 15(1): 83-98.

The toxicity of various mono and dinitrobenzene derivatives towards *Daphnia magna*, *Chlorella pyrenoidosa*, and *Photobacterium phosphoreum* is investigated, establishing quantitative structure-activity relationships (QSARs). For the mono-nitro compounds tested, it is observed that their acute and semi-chronic toxicity towards *D. magna* is only slightly, if at all, higher than the minimum toxicity expected on the basis of a narcosis type of action. Moreover, the logarithm of the octanol/water partition coefficient (log P) is found to be a sufficient descriptor of the toxicity of these compounds to *D. magna*. The statistical quality of the QSAR describing the toxicity of mono-nitro compounds to *C. pyrenoidosa* is found to be improved by incorporation of Hammett sigma constants and log P as parameters. For the dinitrobenzene derivatives, it is established that the description of their toxicity to *D. magna* and *C. pyrenoidosa* necessitates the use of sigma constants. Furthermore, the toxicity of several of these compounds is found to be more dependent upon the duration of the test than observed for the mono-nitro compounds. This is assumed to be due to the formation of reactive metabolites, which probably contribute substantially to the toxicity of most di-nitro compounds. For the toxicity of dinitrobenzenes to *P. phosporeum*, no significant QSAR could be established employing sigma constants and log P as parameters. The applicability of the Microtox test as a prescreening tool for the selection of compounds needing further evaluation is discussed. It is concluded that the usefulness of the test is only limited. There appears to be no obvious relationship between the susceptibilites of *P. phosphoreum* and other species to chemicals that cause toxic effects through modes of biological action different from general anaesthesia.

Gough, K.M., Belohorcova, K. and Kaiser, K.L.E. 1994. Quantitative structure-activity relationships (QSARs) of Photobacterium phosphoreum toxicity of nitrobenzene derivatives. *Science of the Total Environment* 142(3): 179-190.

The Microtox™ values (acute toxicities to *Photobacterium phosphoreum*) of 85 nitrobenzene derivatives are reported, along with the results of ab initio (minimal basis, STO-3G) and semi-empirical (AM1) molecular orbital calculations of electronic

properties. QSARs are developed between the acute toxicities and the various calculated parameters. The more rapid semi-empirical calculations are compared to the ab initio results and the advantages and disadvantages of the two methods are considered. As in earlier work, the change in the electronic charge distribution on the nitro group, (QO), in compounds with strongly electron withdrawing substituents, is found to be a significant indicator of high toxicities. The data set is comprised of di-, tri- and tetra substituted compounds. The effect of the increase in the number of substituents and their relative positions on the ring are considered as possible factors affecting acute toxicity. For compounds in which the nitro group is ortho to a substituent with which it may hydrogen-bond, the charge on the nitro group ceases to be a useful indicator of toxicity. The energy of the Highest Occupied Molecular Orbital (HOMO) is found to be highly correlated with QO and alternates with it as the significant parameter in some regressions.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L., Miller, K. and Saepoff, S. 2001. Development of Marine Toxicity Data for Ordnance Compounds. *Archives of Environmental Contamination and Toxicology.* 41(3): 308-318.

A toxicity database for ordnance compounds was generated using eight compounds of concern and marine toxicity tests with five species from different phyla. Toxicity tests and endpoints included fertilization success and embryological development with the sea urchin *Arbacia punctulata*; zoospore germination, germling length, and cell number with the green macroalga *Ulva fasciata*; survival and reproductive success of the polychaete *Dinophilus gyrociliatus*; larvae hatching and survival with the redfish (*Sciaenops ocellatus*); and survival of juveniles of the opossum shrimp (*Americamysis bahia*, formerly *Mysidopsis bahia*). The studied ordnance compounds were 2,4- and 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2,4,6-trinitrophenylmethylnitramine (tetryl), 2,4,6-trinitro-phenol (picric acid), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The most sensitive toxicity test endpoints overall were the macroalga zoospore germination and the polychaete reproduction tests. The most toxic ordnance compounds overall were tetryl and 1,3,5-trinitrobenzene. These were also the most degradable compounds, often being reduced to very low or below-detection levels at the end of the test exposure. Among the dinitro- and trinitrotoluenes and benzenes, toxicity tended to increase with the level of nitrogenation. Picric acid and RDX were the least toxic chemicals tested overall.

Schmitt, H., Altenburger, R., Jastorff, B. and Schuurmann, G. 2000. Quantitative structure-activity analysis of the algae toxicity of nitroaromatic compounds. *Chem Res Toxicol.* 13(6): 441-450.

Proliferation toxicity toward the algae *Scenedesmus vacuolatus* in a 24 hr one-generation reproduction assay was determined for nitrobenzene and 18 derivatives, including two phenols. The resultant EC_{50} values covering more than 4 orders of magnitude were subjected to a quantitative structure-activity analysis (QSAR) using hydrophobicity in terms of the octanol/water partition coefficient in logarithmic form, log K_{ow}, and 16 quantum chemical descriptors of molecular reactivity that were calculated with the AM1 scheme. For 13 mononitro derivatives and the highly hydrophobic trifluralin, a narcotic-type mode of action can explain most of the toxicity variation. Correction of log K_{ow} for ionization for the phenols and quantification of the molecular susceptibility for one-electron reduction as apparently rate-determining biotransformation step by the energy of the lowest unoccupied molecular orbital, E(LUMO), yields a highly significant QSAR for all 19 compounds (r(adj)(2) = 0.90), which can be further improved when adding the maximum net atomic charge at the nitro nitrogen, q(nitro)(-)(N), as the third descriptor (r(adj)(2) = 0.93). Comparison of the energy of the singly occupied molecular orbital, E(SOMO), of the radical anions as initial metabolites with the E(SOMO) of known redox cyclers suggests that dinitrobenzenes and TFM as well as multiply chlorinated nitrobenzenes may also exert oxidative stress. This is based on an E(SOMO) window of -0.30 to 0. 55 eV as a tentative criterion for molecular structures to have the potential for redox cycling, derived from a set of eight known redox cyclers. The discussion includes a detailed analysis of apparently relevant metabolic pathways and associated modes of toxic action of nitroaromatics.

2,4-Dinitrophenol

The commercial dinitrophenol mixture (a mixture of the 2,3- and 2,6- isomers (but mostly the 2,4- isomer) is produced by heating phenol with dilute sulfuric acid, cooling the product, and then nitrating while keeping the temperature below 50º C, or by nitrating with a mixed acid under careful temperature control. 2,6-DNP is prepared by sulfonation and nitration of *o*-nitrophenol. 2,6-DNP is also produced as a byproduct in the synthesis of 2,4-DNP by way of 2,4-dinitrochlorobenzene. Heating with 6% aqueous sodium hydroxide at 95-100º C for 4 hours hydrolyzes 2,4-dinitrochlorobenzene. 2,4-DNP in the hydrolyzed product is precipitated by adding acid; the precipitate is removed by filtration. The residue is washed to remove added acid and the more soluble 2,6-DNP.

The commercial dinitrophenol mixture is used in the synthesis of dyes, picric acid, picramic acid, wood preservatives, diaminophenol dihydrochloride (a photographic developer), explosives, and insecticides, and as a pH indicator. 2,4-DNP is also used as an insecticide, acaricide, and fungicide. In the 1930s, 2,4-DNP was used as a weight-reducing agent or treatment for obesity. It is estimated that 100,000 people took the drug during the first 15 months following its introduction.

ATSDR. 1995. Toxicological Profile for Dinitrophenols. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for 2,4-Dinitrophenol.

Environmental Fate and Transport

ATSDR. 1995. Toxicological Profile for Dinitrophenols. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Bioaccumulation and Trophic Transfer

ATSDR. 1995. Toxicological Profile for Dinitrophenols. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Toxicity

ATSDR. 1995. Toxicological Profile for Dinitrophenols. Prepared for Agency of Toxic Substances and Disease Registry.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Brecken-Folse, J.A., Mayer, F.L., Pedigo, L.E. and Marking, L.L. 1994. Acute toxicity of 4-nitrophenol, 2,4-dinitrophenol, terbufos and trichlorfon to grass shrimp (*Palaemonetes spp.*) and sheepshead minnows (*Cyprinodon variegatus*) as affected by salinity and temperature. *Environmental Toxicology and Chemistry.* 13(1): 67-77.

The toxicities of two industrial chemicals (4-nitrophenol and 2,4-dinitrophenol) and two organophosphate insecticides (terbufos and trichlorfon) to juvenile grass shrimp (*Palaemonetes spp*.) and sheepshead minnows (*Cyprinodon variegatus*) were determined by static, 96 hr toxicity tests in a factorial design with 12 combinations of salinity and temperature (15, 20, 25, 30 ppt x 17, 22, 27º C). Concentrations of the toxicants, including bioconcentration, were determined as appropriate by gas or liquid chromatography and the use of ^{14}C-labeled compounds. The 96 hr LC_{50}s for 4-nitrophenol ranged from 12 to 31 mg/l and for 2,4-dinitrophenol from 13 to 50 mg/l. Toxicity decreased as salinity increased for 4-nitrophenol and both test organisms. Toxicity decreased as salinity increased for 2,4-dinitrophenol and sheepshead minnows, but toxicity to grass shrimp increased as salinity increased. Toxicity decreased with increased temperature for grass shrimp exposed to 2,4-dinitrophenol and sheepshead minnows exposed to 4-nitrophenol, increased with temperature for sheepshead minnows exposed to 2,4-dinitrophenol, and no change was observed for grass shrimp exposed to 4-nitrophenol. Bioconcentration of phenols in both test organisms increased as concentration increased. The 96 hr LC_{50}s for terbufos ranged from 3.4 to 6.6 µg/l and for trichlorfon from 6.3 to 19,300 µg/l. Terbufos and trichlorfon toxicity to grass shrimp and sheepshead minnows increased with increased temperature. BCFs for terbufos were greater in sheepshead minnows than grass shrimp, but were reversed for trichlorfon.

Haghighi-Podeh, M.R., Bhattacharya, S.K. and Qu, M. 1995. Effects of nitrophenols on acetate utilizing methanogenic systems. *Water Research.* 29(2): 391-399.

Nitrophenols are widely used for manufacturing explosives, pharmaceuticals, pesticides, pigments, dyes, etc. A literature research shows that there is a lack of quantitative information on the effects of nitrophenols on anaerobic systems. The objective of this research was to study the toxic effects of nitrophenols on acetate enrichment, methanogenic systems. Anaerobic toxicity assays were performed with batch serum bottles. Results showed that among the selected nitrophenols, toxicity decreases in the following order: 2,4-dinitrophenol > 4-nitrophenol > 2-nitrophenol > 3-nitrophenol. Complete removals of mononitrophenols were seen in serum bottle systems which did not fail due to toxicity. Chemostats (15-day retention time) were used to study toxicity, acclimation, and removal of 4-nitrophenol in continuous systems. New steady-states with high effluent acetate (substrate) concentrations were at-

tained after spiking 5.5, 13.5, and 33.0 mg/l of 4-nitrophenol. A competitive inhibition coefficient model fits well with the experimental data from 4-nitrophenol studies. The competitive inhibition coefficient (K_I), for 4-nitrophenol varied between 0.077 and 0.089 mg/l. Fifty-five to 82% of the 4-nitrophenol was removed in the chemostats; HPLC analysis of effluents showed no intermediate products.

Howe, G.E., Marking, L.L., Bills, T.D., Boogaard, M.A. and Mayer, F.L., Jr. 1994a. Effects of water temperature on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to developing rainbow trout (Oncorhynchus mykiss). ***Environmental Toxicology and Chemistry.*** **13(1): 79-84.**

Early-life-stage (ELS) toxicity tests were conducted to determine the effect of selected water temperatures on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to rainbow trout (*Oncorhynchus mykiss*). NOECs were determined for growth and mortality at selected time intervals and water temperatures of 7, 12, and 17º C. As tests progressed, NOECs leveled to constant time-independent values that were similar for tests at each temperature. In 4-nitrophenol tests, the time-independent NOEC values at 7, 12, and 17º C, respectively, were 1.16, 1.20, and 1.16 mg/l for growth and 3.40, 3.38, and 2.20 mg/l for mortality. For 2,4-dinitrophenol, time-independent NOEC values at 7, 12, and 17º C, respectively, were 1.07, 0.50, and 0.80 mg/l for growth and 1.30, 1.89, and 1.60 mg/l for mortality. Temperature did, however, affect the rate at which time-independent NOECs were reached. More time was required to reach time-independent NOECs as temperature decreased. For example, the time-independent NOEC in 4-nitrophenol tests at 17º C was reached in 14 days, whereas it required 42 days at 7º C.

Howe, G.E., Marking, L.L., Bills, T.D., Rach, J.J. and Mayer, F.L., Jr. 1994b. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol and 2,4-dinitrophenol to the amphipod Gammarus pseudolimnaeus and rainbow trout (Oncorhynchus mykiss). ***Environmental Toxicology and Chemistry.*** **13(1): 51-66.**

Acute toxicity tests were conducted to determine (a) the individual and interactive effects of water temperature (7, 12, 17º C), pH (6.5, 7.5, 8.5, 9.5), and time on the toxicity of terbufos, trichlorfon, 4-nitrophenol, and 2,4-dinitrophenol to rainbow trout (*Oncorhynchus mykiss*) and the amphipod *Gammarus pseudolimnaeus*, and (b) the individual and interactive effects of water temperature and pH on chemical bioconcentration during acute tests with rainbow trout and Gammarus exposed to terbufos, 4-nitrophenol, and 2,4-dinitrophenol. The toxicity of all four chemicals was significantly affected by pH in all tests, except for Gammarus exposed to terbufos. The toxicity of terbufos to rainbow trout and Gammarus was less at pH 7.5 than at higher or lower pH. The toxicity of both nitrophenols decreased as pH increased,

whereas the toxicity of trichlorfon increased with pH. The effect of pH on trichlorfon toxicity decreased with temperature. Temperature significantly affected the toxicity of all four chemicals to both species. Toxicity increased with temperature in all tests, except for rainbow trout exposed to nitrophenols; toxicity decreased as temperature increased for rainbow trout. Chemical bioconcentration was also significantly affected by temperature and pH and was directly related to toxicity in most tests. Significant interactive effects between toxicity-modifying factors were also frequently observed. Temperature and pH effects on chemical toxicity need to be considered in chemical hazard assessment to ensure adequate protection of aquatic organisms.

Koizumi, M., Yamamoto, Y., Ito, Y., Takano, M., Enami, T., Kamata, E. and Hasegawa, R. 2001. Comparative study of toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats. *J Toxicol Sci.* 26(5): 299-311.

The toxicities of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats was examined and the susceptibility of newborn rats was analyzed in terms of presumed unequivocally toxic and no observed adverse effect levels (NOAELs). In the 18-day repeated dose newborn rat study, 4-nitrophenol was orally given from day 4 to day 21 after birth but did not induce any toxicity up to 160 mg/kg in the main study, although it induced death in one of six males at 160 mg/kg, and three of six males and one of six females at 230 mg/kg in a prior dose-finding study. In the 28 day repeated dose oral toxicity study starting at 6 weeks of age, 4-nitrophenol caused the death of most males and females at 1,000 mg/kg but was not toxic at 400 mg/kg except for male rat-specific renal toxicity. As unequivocally toxic levels were considered to be 230 mg/kg/day in newborn rats and 600 to 800 mg/kg/day in young rats, and NOAELs were 110 mg/kg/day in newborn rats and 400 mg/kg/day in young rats, the susceptibility of the newborn to 4-nitrophenol appears to be 2.5 to 4 times higher than that of young animals. In the newborn rat study of 2,4-dinitrophenol, animals died at 30 mg/kg in the dose-finding study and significant lowering of body and organ weights was observed at 20 mg/kg in the main study. In the 28-day young rat study, clear toxic signs followed by death occurred at 80 mg/kg but there was no definitive toxicity at 20 mg/kg. As unequivocally toxic levels and NOAELs were considered to be 30 and 10 mg/kg/day in newborn rats and 80 and 20 mg/kg/day in young rats, respectively, the toxicity of 2,4-dinitrophenol in newborns again seems to be 2 to 3 times stronger than in young rats. Abnormalities of external development and reflex ontogeny in the newborn were not observed with either chemical. Based on these results, it can be concluded that the toxic response in newborn rats is at most 4 times higher than that in young rats, at least in the cases of 4-nitrophenol and 2,4-dinitrophenol.

Phipps, G.L., Holcombe, G.W. and Fiandt, J.T. 1981. Acute Toxicity of Phenol and Substituted Phenols to the Fathead Minnow, *Bulletin of Environmental Contamination and Toxicology* 26(5):585-593.

The 96 hr and 192 hr LC_{50}s were determined for phenol and 11 substituted phenols. The freshwater flow-through tests with measured concentrations and nearly identical test conditions used the fathead minnow (*Pimephales promelas*) as the test organism. The fish were checked for uniformity in toxicity response using 48 h static reference tests with 2,4-dichlorophenol as the toxicant. The results of these 12 tests produced a mean 48 hr LC_{50} of 8.6 mg/l plus or minus 1.1 mg/l. The mean of duplicate 96 hr LC_{50}s (mg/l) in ascending order of toxicity were: pentachlorophenol, 0.22; 4,6-dinitro-o-cresol, 2.0; 2,4,6-tribromophenol, 6.6; 2,4-dichlorophenol, 8.2; 2-chlorophenol, 12; 2,4-dinitrophenol, 17; 2,4-dimethylphenol, 17; 2,6-dimethylphenol, greater than 27; phenol, 29; 4-nitrophenol, 61; and 3-methoxyphenol, 76.

Uberoi, V. and Bhattacharya, S.K. 1997. Toxicity and degradability of nitrophenols in anaerobic systems. *Water Environment Research.* 69(2): 146-156.

Nitrophenols are among the most important and versatile industrial organic compounds and are widely used in the chemical industry and are listed as priority pollutants by the U.S. EPA. The toxic effects and degradability of three selected nitrophenols (2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol) in anaerobic acetate and propionate enrichment systems were studied using batch serum bottles. The toxicity to both propionate- and acetate-fed systems decreased in the following order: 2,4-dinitrophenol > 4-nitrophenol > 2-nitrophenol. An up-flow fixed film, acetate-fed reactor was able to acclimate to 20 mg/l of 2- and 4-nitrophenol showing a 95% removal of each nitrophenol. The effect of biomass was studied using serum bottles for anaerobic toxicity assays with the design volatile suspended solids (VSS) concentrations of 500, 1,000, and 500 mg/l. With higher VSS the toxic effects of nitrophenols on methanogenesis were less severe. Nitrophenols were more inhibitory to acetate utilization than propionate utilization. Under anaerobic conditions 2-nitrophenol and 2,4-dinitrophenol were transformed both abiotically and biotically to 2-aminophenol and 2-amino,4-nitrophenol, respectively. The presence of propionate in propionate enrichment culture enhanced the removal rates of all three nitrophenols studied.

Dinitrotoluene

Dinitrotoluene (DNT) is formed by the nitration of toluene using concentrated nitric and sulfuric acids (ATSDR 1998).

Approximately 99% of all manufactured DNT is used as an intermediate for the production of polyurethane polymers (ATSDR 1998). DNT is also used for production of plasticizing agents, waterproofing agents, dyes, smokeless powder, and in automobile air bags (ATSDR 1998). DNT is used as a gelatinizing agent in military explosives (ATSDR 1998).

ATSDR. 1998. Toxicological Profile for 2,4- and 2,6-Dinitrotoluene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for dinitrotoluene.

Environmental Fate and Transport

Christopher, H.J., Boardman, G.D. and Freedman, D.L. 2000. Aerobic biological treatment of 2,4-dinitrotoluene in munitions plant wastewater. *Water Research [Water Res.].* 34(5): 1595-1603.

Wastewater from the manufacture of propellants typically contains 2,4-dinitrotoluene (DNT), a known animal carcinogen. Previous studies have indicated that DNT is aerobically biodegradable. However, inconsistent removal of DNT during aerobic treatment has been observed at a munitions wastewater treatment plant, necessitating the use of activated carbon pretreatment. The objective of this study was to evaluate the effect of nutrient and cosubstrate amendments on the rate and extent of DNT removal. Addition of ethanol (100-500 mg/l) and phosphate (0.8-3.3 mg/l) significantly accelerated the rate of aerobic DNT (0.3-5.6 mg/l) biodegradation. Addition of phosphate alone also increased the rate of DNT degradation, but to a lesser degree. The presence of ethyl ether, another substrate commonly found in munitions plant wastewater, had comparatively little effect on the rate of DNT removal. Interruptions in the DNT manufacturing process can result in DNT being absent from the munitions plant wastewater for extended periods. The effect of such interruptions was evaluated in semicontinuously operated reactors, fed daily with phosphate-amended wastewater (containing no detectable background level of DNT), at a hydraulic residence time of 3 days. DNT was added at varying intervals (from once every 3 days to once every 15 days). DNT removal resumed without a lag even after it was absent from the feed for periods up to 15 days. During aerobic biodegradation of DNT, reduction to 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene was consistently observed, with reduction at the para position predominating. The highest level of aminonitrotoluene formation was 23% of the total DNT degraded. Aminonitrotoluene isomers were consumed within 1 day after the DNT dis-

appeared in the semicontinuously operated reactors, confirming the potential for degradation of these metabolites. Although the aminonitrotoluene isomers are not currently regulated, their presence in treated munitions wastewater is a concern due to possible toxicity.

Davis, E.M., Murray, H.E., Liehr, J.G. and Powers, E.L. 1981. Basic Microbial Degradation Rates and Chemical Byproducts of Selected Organic Compounds. *Water Research.* 15(9): 1125-1127.

The authors investigated the biodegradation rates of eight organic compounds by *Acinetobacter*, *Pseudomonas alcaligenes*, *Flavobacterium*, and *Rhodotorula*. The compounds were benzene, toluene, phenol, nitrobenzene, methylene chloride, o-dichlorobenzene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene. The purpose for this investigation was to determine the toxicity levels, degradation rate or loss by volatilization, and identification of metabolic byproducts formed from the parent compounds.

Davis, E.M., Turley, J.E., Casserly, D.M. and Guthrie, R.K. 1983. Partitioning of selected organic pollutants in aquatic ecosystems. 5. International Biodeterioration Symposium, Aberdeen (UK), Sep 1981.

This research project was undertaken to quantify and identify the routes taken by twelve xenobiotic compounds in model waste stabilization ponds. It was not the intent to achieve maximum degradation of the compounds. The compounds which were tested were benzene; toluene; chlorobenzene; 1,2-dichlorobenzene; nitrobenzene; napthalene; 2,6-dinitrotoluene; phenanthrene; pyrene; di-n-butylphthalate; di-n-octylphthalate; and bis-2-ethylhexylphthalate. These were selected for their broad range of n-octanol partition coefficients, water solubilities, wide use in industry and because there were no data available on their fate in waste stabilization ponds. A mass balance was obtained which incorporated the amount of each chemical lost by volatilization, sedimentation, degradation, bioconcentration, the amount in the pond effluent, and the amount remaining in the water column.

Hallas, L.E. and Alexander, M. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. *Applied and Environmental Microbiology.* 45(4): 1234-1241.

The transformation of mono-and dinitroaromatic compounds was measured in sewage effluent maintained under aerobic or anaerobic conditions. Most of the nitrobenzene, 3-and 4-nitrobenzoic acids, and 3- and 4-nitrotoluenes and much of the 1,2- and 1,3-dinitrobenzenes disappeared both in the presence and absence of oxygen. Under anaerobiosis, 2,6-dinitrotoluene and 3,5-dinitrobenzoic acid disappeared

slowly, but no loss was evident in 28 days in aerated sewage. Aromatic amines did not accumulate during the aerobic decomposition of the mononitro compounds. It is suggested that the transformations of widely used nitroaromatic compounds should be further studied because of the persistence and possible toxicity of products of their metabolism.

Razo-Flores, E., Lettinga, G. and Field, J.A. 1999. Biotransformation and biodegradation of selected nitroaromatics under anaerobic conditions. *Biotechnol Prog.* 15(3): 358-365.

The fate of four nitroaromatic compounds (5-nitrosalicylate, 5NSA; 4-nitrobenzoate, 4NBc; 2,4-dinitrotoluene, 2,4DNT; and nitrobenzene, NB) was studied in 160 ml laboratory-scale upward-flow anaerobic sludge bed reactors supplied with a mixture of volatile fatty acids and/or glucose as electron donors. All the nitroaromatics were transformed stoichiometrically to their corresponding aromatic amines. After prolonged reactor operation, 5NSA and 4NBc were completely mineralized to CH_4 and CO_2, whereas 2,4DNT was partially transformed to a nonidentified and nondegradable metabolite. Batch nitro-reduction experiments indicated that the position of the nitro group in relation to the other substituents in the aromatic ring plays a key role in the rate of the nitro-group reduction. The results obtained indicate that certain nitroaromatic compounds can be completely mineralized and serve as a carbon and energy source for anaerobic bacteria.

Riefler, R.G. and Smets, B.F. 2000. Enzymatic Reduction of 2,4,6-Trinitrotoluene and Related Nitroarenes: Kinetics Linked to One-Electron Redox Potentials. *Environmental Science & Technology [Environ. Sci. Technol.].* 34(18): 3900-3906.

At military bases and munitions factories, 2,4,6-trinitrotoluene (TNT) is a common soil and groundwater contaminant. Although the reduction of nitro groups in TNT and related nitroarenes has been extensively investigated, few researchers have studied the link between reduction rates and the electrochemical properties of these compounds. In this work, the standard one-electron redox potentials at pH 7 (E_1°) for six important nitroarenes have been measured by pulse radiolysis. The internally consistent values were -0.253 V for TNT, -0.417 V for 2-amino-4,6-dinitrotoluene, -0.449 V for 4-amino-2,6-dinitrotoluene, -0.397 V for 2,4-dinitrotoluene, -0.402 V for 2,6-dinitrotoluene, and -0.502 V for 2,4-diamino-6-nitrotoluene. The reduction kinetics of these nitroarenes was investigated using a bacterial nitroreductase, NAD(P)H:FMN oxidoreductase that uses NADH times H^+ as a cosubstrate. A log-linear relationship was observed between the E_1° values and the enzymatic reduction rates for five nitroarenes, suggesting that transfer of the first electron is the rate-limiting step in nitroreduction.

Bioaccumulation and Trophic Transfer

The relatively low K_{ow} values of 1.98 for 2,4-DNT and 1.72 for 2,6-DNT indicate that DNT will not bioaccumulate (ATSDR 1998). A measeured BCF of 11 also indicates potential for bioaccumulation is low (HSDB 2003).

BCF values of 2,4-DNT of 9.15 and 4.15 were measured in carp (Lang et al. 1997). Metabolites in the carp liver were identified as 4-amino-2-nitrotoluene and 2,4-diamino-toluene, indicating that the low BCF values were a result of metabolism. Major urinary metabolites of rats dosed with DNT were 2,4-dinitrobenzyl alcohol and 2,6-dinitrobenzyl alcohol (Mori et al. 1996). Mori et al. (1997) found that 2,4-dinitrobenzaldehyde and 2,6-dinitrobenzaldehyde, which are potent mutagens, are formed by the hepatic metabolism of 2,4-dinitrobenzene and 2,6- dinitrobenzene which is a biliary hepatic metabolite of 2,4-DNT and 2,6-DNT.

Lang, P.Z., Wang, Y., Chen, D.B., Wang, N., Zhao, X.M. and Ding, Y.Z. 1997. Bioconcentration, elimination and metabolism of 2,4-dinitrotoluene in carps(Cyprinus Carpio L.). *Chemosphere.* 35(8): 1799-1815.

Bioconcentration curves of 2,4-dinitrotoluene(2,4-DNT) in carp (whole fish, liver, intestine and muscle) were investigated using semistatic system. For whole fish, its curve could be described as a gentle peak which began with a rise in concentration to summit or steady state, then declined and reached lower level followed by another steady state. For liver and intestine, their curves both contained two successive peaks, with the second peak followed by slight fluctuation. Bioconcentration factors of 2,4-DNT in whole fish during the first and second steady state were 9.15 and 4.15, (97.86 and 44.39, based on lipid content), respectively. By logarithmic plotting, two straight-lines with different slopes (3.6 and 0.1) were measured for elimination. According to peaky curves of 2,4-DNT in whole fish, liver and intestine, smaller BCFs than calculated BCFs based on the regression equations for inert chemicals, and large rate constants of elimination, biotransformation was inferred to have happened in tissues such as liver, intestine, and other tissues. Two metabolites were separated from liver and identified as 4-amino-2-nitrotoluene (4A2NT) and 2,4-diamino-toluene(2,4-DAT) on HPLC, their retention times were 23.1 and 8.8 min, respectively. In bioconcentration test of 2,4-DNT in liver, two metabolites and parent were determined at the same time at intervals, higher concentrations of 4A2NT and 2,4-DAT were found when level of 2,4-DNT declined. Such results demonstrated our inference that metabolism caused the declines in bioconcentration curves. A one-compartment model was set up to simulate the bioconcentration, in which biotransformation adhered to Delayed Enzyme-Catalytic Logarithmic Kinetics. Good fit of model curves with measured values could be observed.

Mori, M.A., Shoji, M., Dohrin, M., Kawagoshi, T., Honda, T. and Kozuka, H. 1996. Further studies on the urinary metabolites of 2,4-dinitrotoluene and 2,6-dinitrotoluene in the male Wistar rat. *Xenobiotica.* 26(1): 79-88.

Conjugates of 2,4-dinitrobenzyl alcohol (2,4-DNB) and 2,6-dinitrobenzyl alcohol (2,6-DNB), which were major urinary metabolites of the male Wistar rat dosed orally with 2,4-dinitrotoluene (2,4-DNT) or 2,6-dinitrotoluene (2,6-DNT), were examined by hplc using potassium 2,4-dinitrobenzyl glucuronide (2,4-DNB-G), potassium 2,6-dinitrobenzyl glucuronide (2,6-DNB-G), pyridinium 2,4-dinitrobenzyl sulphate (2,4-DNB-S), and pyridinium 2,6-dinitrobenzyl sulphate (2,6-DNB-S) as authentic compounds. Other metabolites were also examined by hplc. Conjugates detected from urine following administration of 2,4-DNT and 2,6-DNT were 2,4-DNB-G and 2,6-DNB-G, which accounted for about 10.7 and 17.4% of the administered dose respectively. No peaks corresponding to pyridinium 2,4-DNB-S and pyridinium 2,6-DNB-S were detected in urine samples. 2-Amino-4-nitrobenzoic acid (0.71%), 4-amino-2-nitrobenzoic acid (0.52%) and 4-acetylamino-2-nitrobenzoic acid (3.9%), in addition to known metabolites 4-amino-2-nitrotoluene (0.04%), 2,4-DNB (0.25%), 2,4-dinitrobenzoic acid (6.9%) and 4-acetylamino-2-aminobenzoic acid (3.4%), were detected in ether extracts of urine of rat given 2,4-DNT. 2,6-Dinitrobenzoic acid (0.17%) and two known metabolites, 2-amino-6-nitrotoluene (0.44%) and 2,6-DNB (0.53%), were detected in ether extracts of urine of rat given 2,6-DNT.

Mori, M.A., Sayama, M., Shoji, M., Inoue, M., Kawagoshi, T., Maeda, M. and Honda, T. 1997. Biliary excretion and microfloral transformation of major conjugated metabolites of 2,4-dinitrotoluene and 2,6-dinitrotoluene in the male Wistar rat. *Xenobiotica.* 27(12): 1225-1236.

Major biliary conjugates of the male Wistar rat dosed orally with 2,4-dinitrotoluene (2,4-DNT) or 2,6-dinitrotoluene (2,6-DNT) were examined by hplc using potassium 2,4-dinitrobenzyl glucuronide (potassium 2,4-DNB-G), potassium 2,6-dinitrobenzyl glucuronide (potassium 2,6-DNB-G), pyridinium 2,4-dinitrobenzyl sulphate (pyridinium 2,4-DNB-S) and pyridinium 2,6-dinitrobenzyl sulphate (pyridinium 2,6-DNB-S) as authentic compounds. Other metabolites were also examined by hplc. In addition, metabolites formed by incubation of potassium 2,4-DNB-G and potassium 2,6-DNB-G with rat intestinal microflora under nitrogen were examined by hplc. Conjugates detected directly from bile following administration of 2,4-DNT and 2,6-DNT were 2,4-DNB-G and 2,6-DNB-G, which accounted for 35.0 and 51.5% of the administered dose respectively. No peaks corresponding to pyridinium 2,4-DNB-S and pyridinium 2,6-DNB-S were detected in bile samples. 2-Amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-diaminotoluene and 4-acetylamino-2-nitrobenzoic acid (0.02-0.12% of the dose excreted in 24 h), in addition to the known metabolites 2,4-dinitrobenzyl alcohol (2,4-DNB), 2,4-dinitrobenzaldehyde and 2,4-dinitrobenzoic

acid (0.09-0.14%), were detected in ether extracts of bile of rat given 2,4-DNT. 2,6-Dinitrobenzyl alcohol (2,6-DNB), 2-amino-6-nitrotoluene and 2,6-dinitrobenzaldehyde (0.02-0.03%), which are known metabolites, were detected in ether extracts of bile from rat given 2,6-DNT. 4.) Potassium 2,4-DNB-G was transformed by the anaerobic incubation of rat intestinal microflora into 2,4-DNB, 4-amino-2-nitrobenzyl alcohol and 2-amino-4-nitrobenzyl alcohol. Potassium 2,6-DNB-G was transformed into 2,6-DNB and 2-amino-6-nitrobenzyl alcohol by the anaerobic incubation. Time-course studies showed that 2,4-DNB, 4-amino-2-nitrobenzyl alcohol, 2-amino-4-nitrobenzyl alcohol and 2,6-DNB, 2-amino-6-nitrobenzyl alcohol peaked at 30, 75, 120 and 10, 50 min respectively. 5.) These results, together with previous findings, show that 2,4-dinitrobenzaldehyde and 2,6-dinitrobenzaldehyde, which are potent mutagens, are formed either by the hepatic metabolism of 2,4-DNB and 2,6-DNB formed by the intestinal metabolism of 2,4-DNB-G and 2,6-DNB-G excreted in bile or by the direct hepatic metabolism of 2,4-DNT and 2,6-DNT.

Toxicity

Dave, G., Nilsson, E. and Wernersson, A.S. 2000. Sediment and water phase toxicity and UV-activation of six chemicals used in military explosives. *Aquatic Ecosystem Health & Management.* 3(3): 291-299.

Explosives used in ammunition have been dumped in both lakes and oceans before the potential environmental effects of these chemicals were understood. Growing environmental concern in society and in the Swedish military resulted in a project dealing with the aquatic toxicology of explosives. The aim of the present study was to assess the hazard of six explosives by determining the acute sediment and water phase toxicity for two crustaceans (*Daphnia magna* and *Nitocra spinipes*). Detoxification during storage was used as an indication of degradability (hydrolysis and biodegradation). The effect of ultraviolet light on the toxicity of these compounds was determined by post-exposure to ultraviolet light and determination of toxicity enhancement. The explosives were picric acid, trinitrotoluene, (2,4-dinitrotoluene), hexahydro-1,3,5-trinitro-1,3,5-trazine, nitroguanidine, and pentyl. The stabilizing agent diphenylamine was tested in the same way. For the major explosive, trinitrotoluene, the water-phase EC_{50}/LC_{50}s were between 5 and 20 mg/l^{-1} and the toxicity was not significantly affected by storage of test solutions for 30 days, indicating hydrolytic stability. The toxicity was not affected by addition of sediment, indicating that trinitrotoluene was not bound appreciably to sediment, but toxicity decreased after storage for 30 days in the presence of sediment, indicating biological degradation or slow adsorption of the chemical. The toxicity of the other explosives was similar or lower than for trinitrotoluene, but the stabilizing agent diphenylamine was slightly more toxic with EC_{50}/LC_{50}s between 0.5 and 5 mg l^{-1}. Photoinduced toxicity by ultraviolet light is known for many chemicals including polycyclic aromatic

hydrocarbons and trinitrotoluene. The latter was confirmed in this study and especially for degraded trinitrotoluene. 2,4-Dinitrotoluene, which is one degradation product of trinitrotoluene was also activated by ultraviolet light. The toxicity of diphenylamine decreased after storage in water (hydrolysis) and with sediment (biodegradation), but both diphenylamine and its degradation products exhibited photoinduced toxicity.

Davenport, R., Johnson, L.R., Schaeffer, D.J. and Balbach, H. 1994. Phototoxicology. 1. Light-enhanced toxicity of TNT and some related compounds to Daphnia magna and Lytechinus variagatus embryos. *Ecotoxicol Environ Saf.* 27(1): 14-22.

Many environmental pollutants interact with solar near-ultraviolet (nuv) light in a manner which greatly increases their toxic effects. The phenomenon of light-mediated toxicity (phototoxicity) is only now becoming generally recognized to any significant degree. Manufacture of, and loading munitions with, the explosive 2,4,6-trinitrotoluene (TNT) in past decades caused contamination of soils and sediments at levels exceeding 1000 ppm and of waters at levels near saturation (100 ppm). Manufacture of TNT produces numerous nitrated by-products, and most of these compounds, including TNT, can be metabolized by many species, including bacteria, fungi, plants, and mammals. This study investigated the phototoxicity of TNT, and 2,3-, 2,4-, 2,6-, and 3,4-dinitrotoluene (DNT) and -diaminotoluene (DAT), and the major metabolites 2-amino-4,6-dinitrotoluene (2A) and 4-amino-2,6-dinitrotoluene (4A), to *Daphnia magna* (acute toxicity) and *Lytechinus variagatus* (sea urchin) embryos (subacute, developmental toxicity). Most of the compounds were weakly toxic or nontoxic in the dark. All were phototoxic to sea urchins. In *D. magna*, 2,3- and 3,4-DNT/DAT and 4A were not toxic but were phototoxic, and 2A was toxic and phototoxic; the other isomers were not toxic or phototoxic to this species.

Dodard, S.G., Renoux, A.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Ecotoxicity characterization of dinitrotoluenes and some of their reduced metabolites. *Chemosphere.* 38(9): 2071-2079.

In the present study, the toxic effects of 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) and a selection of their respective metabolites were examined and compared to 2,4,6-trinitrotoluene (TNT) using the 15 min Microtox (*Vibrio fischeri*) and 96 hr freshwater green alga (*Selenastrum capricornutum*) growth inhibition tests. All of the compounds tested were less toxic than TNT. Using the Microtox assay, 2,6-DNT was more toxic than 2,4-DNT and the order of toxicity for 2,6-DNT and its metabolites was : 2,6-DNT greater than or equal to 2A-6NT >> 2,6-DAT; whereas that for 2,4-DNT was: 4A-2NT > 2A-4NT > 2,4-DNT > 2,4-DAT. For the algal test, 2,4-DNT was more toxic than 2,6-DNT and the order of toxicity for

2,4-DNT and its metabolites was: 2,4-DNT > 2,4-DAT approximately equal to 4A-2NT = 2A-4NT. The order of toxicity for 2,6-DNT and its reduced metabolites using the algal test was very similar to the Microtox bioassay. These results demonstrate that the reduced metabolites of 2,6-DNT tested in this study were less toxic than that of the parent compound, but certain partially reduced metabolites of 2,4-DNT can be more toxic than the parent molecule. These data put into question the general hypothesis that reductive metabolism of nitro-aromatics is associated with a sequential detoxification process.

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology.* 28(2): 229-235.

Aqueous samples containing various explosives, their reduced metabolites, as well as related compounds were subjected to the luminescent bacterium Vibrio fischeri NRRL-B-11177 to determine their ecotoxicological potential. As the most important parameter, the EC_{50} values of 24 test compounds were calculated. The EC_{50} value means the concentration of a chemical compound that is needed to reduce bacterial luminescence by 50%. According to the widely accepted classification scheme of Strupp et al. (1990) and in consideration of an incubation period of 30 min TNT, 26DNT, 2A6NT, 4A2NT, 34DNT, TNB, TNBA, TETRYL and HEXYL must be classified in the category "very toxic to aquatic organisms"; 2A46DNT, 4A26DNT, 24DA6NT, 24DNT, 2A4NT, RDX, HMX and PETN must be classified in the category "toxic to aquatic organisms"; and 26DA4NT, TAT, TNPh, 26DAT, 24DAT, HMT and NQ can be classified in the category "less toxic to aquatic organisms." EC_{50} values after 30, 60, and 90 min of incubation of the test compounds are presented and discussed. For many of the compounds tested in this study, there are no, or only a few, toxicological data in the literature available.

Holen, I., Mikalsen, S.O. and Sanner, T. 1990. Effects of dinitrotoluenes on morphological cell transformation and intercellular communication in Syrian hamster embryo cells. *J Toxicol Environ Health.* 29(1): 89-98.

The effects of four isomers of dinitrotoluene (DNT) and technical DNT (a mixture of DNT isomers and other compounds, with 2,4-DNT as the major constituent) were studied in two short-term in vitro assays. None of the isomers or technical DNT induced an increase in morphological transformation of Syrian hamster embryo (SHE) cells. Four DNT metabolites (2,4-diaminotoluene, 2-amino-4-nitrotoluene, 2-amino-6-nitrotoluene, and 2,4-dinitobenzoic acid), representing different stages in reduction or oxidation of DNT isomers, were also negative for induction of morphological transformation. The DNT isomers were tested in an intercellular communication

assay based on dye transfer. 2,4-DNT, 2,6-DNT, and technical DNT inhibited intercellular communication in the SHE cell line BPNi at toxic concentrations. This may be reminiscent of in vivo data showing promoting activity of these compounds. 2,3-DNT and 3,4-DNT did not inhibit communication.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L., Miller, K. and Saepoff, S. 2001. Development of Marine Toxicity Data for Ordnance Compounds. *Archives of Environmental Contamination and Toxicology.* 41(3): 308-318.

A toxicity database for ordnance compounds was generated using eight compounds of concern and marine toxicity tests with five species from different phyla. Toxicity tests and endpoints included fertilization success and embryological development with the sea urchin *Arbacia punctulata*; zoospore germination, germling length, and cell number with the green macroalga *Ulva fasciata*; survival and reproductive success of the polychaete *Dinophilus gyrociliatus*; larvae hatching and survival with the redfish (*Sciaenops ocellatus*); and survival of juveniles of the opossum shrimp (*Americamysis bahia,* formerly *Mysidopsis bahia*). The studied ordnance compounds were 2,4- and 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2,4,6-trinitrophenylmethylnitramine (tetryl), 2,4,6-trinitro-phenol (picric acid), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The most sensitive toxicity test endpoints overall were the macroalga zoospore germination and the polychaete reproduction tests. The most toxic ordnance compounds overall were tetryl and 1,3,5-trinitrobenzene. These were also the most degradable compounds, often being reduced to very low or below-detection levels at the end of the test exposure. Among the dinitro- and trinitrotoluenes and benzenes, toxicity tended to increase with the level of nitrogenation. Picric acid and RDX were the least toxic chemicals tested overall.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L. and Miller, K. 2002. Toxicological and chemical assessment of ordinance compounds in marine sediments and porewaters. *Mar Pollut Bull.* 44(8): 789-806.

Toxicological and chemical studies were performed with a silty and a sandy marine sediment spiked with 2,6-dinitrotoluene (2,6-DNT), 2,4,6-trinitrophenyl-methylnitramine (tetryl), or 2,4,6-trinitrophenol (picric acid). Whole sediment toxicity was analyzed by the 10 day survival test with the amphipod Ampelisca abdita, and porewater toxicity tests assessed macro-algae (*Ulva fasciata*) zoospore germination and germling growth, sea urchin (*Arbacia punctulata*) embryological development, and polychaete (*Dinophilus gyrociliatus*) survival and reproduction. Whole sediments spiked with 2,6-DNT were not toxic to amphipods. The fine-grained sediment spiked with tetryl was also not acutely toxic. The tetryl and picric acid LC_{50} values

in the sandy sediment were 3.24 and 144 mg/kg dry weight, respectively. The fine-grained sediment spiked with picric acid generated a U-shaped concentration-response curve in the amphipod test, with increased survival both in the lowest and highest concentration. Grain-size distribution and organic carbon content strongly influenced the behavior of ordnance compounds in spiked sediments. Very low concentrations were measured in some of the treatments and irreversible binding and biodegradation are suggested as the processes responsible for the low measurements. Porewater toxicity varied with its sedimentary origin and with ordnance compound. The sea urchin embryological development test tended to be the least sensitive. Tetryl was the most toxic chemical in all porewater tests, and picric acid the least toxic. Samples spiked with 2,6-DNT contained a degradation product identified as 2-methyl-3-nitroaniline (also known as 2-amino-6-nitrotoluene), and unidentified peaks, possibly degradation products, were also seen in some of the picric acid- and tetryl-spiked samples. Degradation products may have played a role in observed toxicity.

Octahydro-1,3,57-tetranitro-1,3,5,7-tetrazocine (HMX)

Octahydro-1,3,57-tetranitro-1,3,5,7-tetrazocine (HMX) is formed by the nitration of hexamine with ammonium nitrate and nitric acid in an acetic acid/acetic anhydride solvent at 44º C (ATSDR 1997).

HMX is used as a component in plastic-bonded explosives, a solid fuel rocket propellant, and a burster charge for military munitions (ATSDR 1997). HMX is used to implode fissionable material in nuclear devices to achieve critical mass (ATSDR 1997).

ATSDR. 1997. Toxicological Profile for HMX (Octahydro-1,3,57-tetranitro-1,3,5,7-tetrazocine). Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for HMX.

Environmental Fate and Transport

Bhadra, R., Wayment, D.G., Williams, R.K., Barman, S.N., Stone, M.B., Hughes, J.B. and Shanks, J.V. 2001. Studies on plant-mediated fate of the explosives RDX and HMX. *Chemosphere.* 44(5): 1259-1264.

The fate of the explosives RDX and HMX on exposure to plants was investigated in 'natural' aquatic systems of Myriophyllum aquaticum for 16 days, and in axenic hairy root cultures of Catharanthus roseus for > or = 9 wks. Exposure levels were: HMX, 5 mg/l; and RDX, approximately 8 mg/l. Exposure outcomes observed include: HMX, no transformation by aquatic plants, and minimal biological activity by axenic roots; and RDX, removal by both plant systems. In the case of RDX exposure to axenic roots, since ^{14}C-RDX was included, removal was confirmed by the accumulation of ^{14}C-label in the biomass. The intracellular ^{14}C-label in these RDX studies was detected in two forms: intact RDX and bound unknown(s).

Boopathy, R. 2001. Enhanced biodegradation of cyclotetramethylenetetranitramine (HMX) under mixed electron-acceptor condition. *Bioresour Technol.* 76(3): 241-244.

The biodegradation of cyclotetramethylenetetranitramine, commonly known as 'high melting explosive' (HMX), under various electron-acceptor conditions was investigated using enrichment cultures developed from the anaerobic digester sludge of Thibodaux sewage treatment plant. The results indicated that the HMX was biodegraded under sulfate reducing, nitrate reducing, fermenting, methanogenic, and mixed electron accepting conditions. However, the rates of degradation varied among the various conditions studied. The fastest removal of HMX (from 22 ppm on day 0 to < 0.05 ppm on day 11) was observed under mixed electron-acceptor conditions, followed in order by sulfate reducing, fermenting, methanogenic, and nitrate reducing conditions. Under aerobic conditions, HMX was not biodegraded, which indicated that HMX degradation takes place under anaerobic conditions via reduction. HMX was converted to methanol and chloroform under mixed electron-acceptor conditions. This study showed evidence for HMX degradation under anaerobic conditions in a mixed microbial population system similar to any contaminated field sites, where a heterogeneous population exists.

Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. *Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).* Weston Roy F., Inc., West Chester, PA (USA).

The primary objective of this task was to determine the feasibility of using GAC to treat ground water contaminated by explosives at the Milan Army Ammunition Plant (MAAP) in Milan, Tennessee. Laboratory GAC isotherm studies were conducted and two carbons, Atochem, Inc. GAC 830 and Calgon Filtrasorb 300, were selected for further testing in continuous flow GAC columns. Three pilot scale continuous flow GAC column tests were performed at MAAP using the two carbons selected from the laboratory GAC isotherm studies. The results from the laboratory and pilot studies are presented in this report. They show that concurrent removal of explosives such as TNT, RDX, HMX, Tetryl, and nitrobenzene from ground water using continuous flow granular activated carbon is feasible.

Groom, C.A., Halasz, A., Paquet, L., Morris, N., Olivier, L., Dubois, C. and Hawari, J. 2002. Accumulation of HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in Indigenous and agricultural plants grown in HMX-contaminated anti-tank firing-range soil. *Environmental Science & Technology.* 36(1): 112-118.

To investigate their potential for phytoremediation, selected agricultural and indigenous terrestrial plants were examined for their capacity to accumulate and degrade the explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). Plant tissue and soil extracts were analyzed for the presence of HMX and possible degradative metabolites using high-performance liquid chromatography with diode-array UV detection (HPLC-UV), micellar electrokinetic chromatography with diode-array UV detection (MEKC-UV), and HPLC with electrospray ionization mass spectrometry (LC-MS). The pattern of HMX accumulation for alfalfa (*Medicago sativa*), bush bean (*Phaseolus vulgaris*), canola (*Brassica rapa*), wheat (*Triticum aestivum*), and perennial ryegrass (*Lolium perenne*) grown in a controlled environment on contaminated soil from an anti-tank firing range was similar to that observed for plants (wild bergamot (*Monarda fistulosa*), western wheat grass (*Agropyron smithii*), brome grass (*Bromus sitchensis*), koeleria (*Koeleria gracilis*), goldenrod (*Solidago sp.*), blueberry (*Vaccinium sp.*), anemone (*Anemone sp.*), common thistle (*Circium vulgare*), wax-berry (*Symphoricarpos* albus), western sage (Artemisia gnaphalodes), and Drummond's milk vetch (Astragalus *drummondii*)) collected from the range. No direct evidence of plant-mediated HMX (bio)chemical transformation was provided by the available analytical methods. Traces of mononitroso-HMX were found in contaminated soil extracts and were also observed in leaf extracts. The dominant mechanism for HMX translocation and accumulation in foliar tissue was concluded

to be aqueous transpirational flux and evaporation. The accumulation of HMX in the leaves of most of the selected species to levels significantly above soil concentration is relevant to the assessment of both phytoremediation potential and environmental risks.

Harkins, V.R., Mollhagen, T., Heintz, C., and Rainwater, K. 1999. Aerobic biodegradation of high explosives, phase I - HMX. *Bioremediation Journal.* 4: 285-290.

The Pantex facility near Amarillo, TX, has soil and groundwater contaminated with differing combinations of high explosives (HEs), including hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6-trinitrotoluene (TNT). This project was concerned with direct treatment of HMX in groundwater withdrawn at this plant. Several physical and chemical treatment schemes for the treatment of HMX have been successful. However, the successful biological treatment of HMX has been limited to anaerobic environments. The objective of this work was to identify microbial consortia and amendments capable of aerobically biodegrading HMX in water. Microbial consortia and amendments employed were provided as livestock manure and soil with its indigenous flora from nearby historically contaminated sites. Possible losses of HMX by nonbiological means such as adsorption and photolysis were accounted for by appropriate abiotic experiments. Loss of the parent compound was measured by high-performance liquid chromatography, using a modification of U.S. Environmental Protection Agency (EPA) Method 8330. Results varied from no degradation to a reduction of parent HMX from 6 to 1 mg/l in 5.2 days. Evidence for biodegradation was supported by the appearance of metabolites. Metabolite identification was performed at Oak Ridge National Laboratory, TN. Five metabolites (four intermediate and one final) were identified.

Hawari, J., Halasz, A., Beaudet, S., Paquet, L., Ampleman, G. and Thiboutot, S. 2001. Biotransformation routes of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by municipal anaerobic sludge. *Environ Sci Technol.* 35(1): 70-75.

Recently we demonstrated that hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), a trimer of methylene nitramine (CH_2=N-NO_2) undergoes spontaneous decomposition following an initial microbial attack using a mixed microbial culture at pH 7 in the presence of glucose as carbon source. The present study describes whether the second cyclic nitramine octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), a more strained tetramer of CH_2=N-NO_2, degrades similarly using sludge of the same source. Part of HMX biotransformed to give products that are tentatively identified as the nitroso derivatives octahydro-1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazocine

(mNs-HMX) and octahydro-1,3-dinitroso-5,7-dinitro-1,3,5,7-tetrazocine and its isomer octahydro-1,5-dinitroso-3,7-dinitro-1,3,5,7-tetrazocine (dNs-HMX). Another fraction of HMX biotransformed, apparently via ring cleavage, to produce products that are tentatively identified as methylenedinitramine (O_2NNHCH_2-$NHNO_2$) and bis(hydroxymethyl)nitramine ($(HOCH_2)2NNO_2$). None of the above intermediates accumulated indefinitely; they disappeared to predominantly form nitrous oxide (N_2O) and formaldehyde (HCHO). Formaldehyde biotransformed further to eventually produce carbon dioxide ($^{14}CO_2$). Nitrous oxide persisted in HMX microcosms containing glucose but denitrified rapidly to nitrogen in the absence of glucose. The presence of nitrous oxide was accompanied by the presence of appreciable amounts of hydrogen sulfide, a known inhibitor of denitrification.

Lynch, J.C., Brannon, J.M., Hatfield, K. and Delfino, J.J. 2003. An exploratory approach to modeling explosive compound persistence and flux using dissolution kinetics. *J Contam Hydrol.* 66(3-4): 147-159.

Recent advances in the description of aqueous dissolution rates for explosive compounds enhance the ability to describe these compounds as a contaminant source term and to model the behavior of these compounds in a field environment. The objective of this study is to make predictions concerning the persistence of 2,4,6-trinitrotoluene (TNT) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in solid form both as individual explosive compounds and components of octol, and the resultant concentrations of explosives in water as a result of dissolution using three exploratory modeling approaches. The selection of dissolution model and rate greatly affect not only the predicted persistence of explosive compound sources but also their resulting concentrations in solution. This study identifies the wide range in possible predictions using existing information and these modeling approaches to highlight the need for further research to ensure that risk assessment, remediation and predicted fate and transport are appropriately presented and interpreted.

Monteil-Rivera, F., Groom, C. and Hawari, J. 2003. Sorption and degradation of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in soil. *Environ Sci Technol.* 37(17): 3878-3884.

The sorption/desorption and long-term fate of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) was examined using sterilized and nonsterilized soils. Two soils were used that differ mainly by the amount of total organic carbon (TOC): an agricultural topsoil (VT, 8.4% TOC) and a sandy soil (SSL, 0.33% TOC). The adsorption isotherms performed at room temperature were well-described by a linear model, which led to sorption distribution coefficients of 2.5 and 0.7 l/kg for VT and SSL soils, respectively. The organic content of soil did not significantly affect HMX sorption. Over a period of 20 weeks, HMX degraded (60% disappearance) in static an-

aerobic nonsterile VT soil preparations. In separate experiments using UL-[^{14}C]-HMX, 19% mineralization (liberated $^{14}CO_2$) was obtained in 30 wks. In addition, four nitroso derivatives of HMX were detected. Knowing the sorption/desorption behavior and the long-term fate of HMX in soil will help assess the effectiveness of natural attenuation for HMX removal.

Williams, R.T., Ziegenfuss, P.S., Mohrman, G.B. and Sisk, W.E. 1989. Composting of Explosives and Propellant Contaminated Sediments. ***IN: Hazardous and Industrial Wastes: Proceedings of the 21st Mid Atlantic Industrial Waste Conference. Technomic Publishing Co., Inc., Lancaster, Pennsylvania.*** **1989: 599-611.**

Two field-scale demonstrations were conducted to investigate composting as a technology for remediating explosives and propellant contaminated sediments. Test sediments at the Louisiana Army Ammunition Plant contained approximately 76,000 parts per million of total explosives, including TNT (2,4,6 ,-trinitrotoluene) (66% of total explosive), 25% RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), 9% HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine), and 0.3% tetryl. The mixture that was composed consisted of straw/horse manure, alfalfa, horse feed, and sediment. Two 12 yd^3 piles were constructed, one was maintained at approximately 35º C and the second at approximately 55º C. After 22 weeks, total explosives were reduced by 99% (from 17,872 to 74 ppm) in the thermophilic pile (55º C). Transformation products peaked in concentration at approximately 20 days and subsequently fell to near detection limits. At the Badger Army Ammunition Plant, WI, test sediments contained approximately 18,000 parts per million of nitrocellulose. Nitrocellulose was reduced from 13,086 ppm to 16 ppm after 101 days in a thermophilic pile.

Yoon, J.M., Oh, B.T., Just, C.L. and Schnoor, J.L. 2002. Uptake and leaching of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by hybrid poplar trees. ***Environmental Science & Technology.*** **36(21): 4649-4655.**

The feasibility of remediating a high explosive, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), using hybrid poplar trees (*Populus deltoides x nigra*, DN34) was investigated. The fate, transport, and toxicity were determined. HMX was taken up by poplar cuttings from hydroponic solutions in long-term experiments (65 days) without evidence of toxicity. HMX was not toxic to actively growing hybrid poplar cuttings, even under saturated conditions. The measured log K_{ow} for HMX was 0.19, less than other explosives, TNT, and RDX. However, the calculated transpiration stream concentration factor (TSCF) and root concentration factor (RCF) for HMX from an uptake study using radiolabeled [U-^{14}C]HMX were 0.21 +/- 0.07 and 5.55 +/- 1.78 ml/g, respectively, both of which were intermediate between the values for TNT and ROX in previous reports. A 70% uptake of [U-^{14}C]HMX was translocated and

accumulated in leaves, and no metabolites were observed during a 65 day exposure using radiochromatography of plant tissue extracts. Most of the accumulated HMX (57%) in dried (fallen) poplar leaves was leached by deionized water after 5 days. Bioaccumulation in poplar trees and resolublization of HMX from leaves would be of significant ecological concern, and phytoremediation may not be warranted as a treatment option unless other processes occur under field conditions that degrade HMX to innocuous end products (e.g., photolysis, hydrolysis, or microbial degradation).

Bioaccumulation and Trophic Transport

Cameron BD. 1986. HMX: Toxicokinetics of (^{14})C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171600.

Henderson MS. 1985. HMX: Analysis in Plasma Obtained after 90 day Toxicity Studies with Rats and Mice. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171603.

Toxicity

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology*. 28(2): 229-235.

Aqueous samples containing various explosives, their reduced metabolites, as well as related compounds were subjected to the luminescent bacterium Vibrio fischeri NRRL-B-11177 to determine their ecotoxicological potential. As the most important parameter, the EC_{50} values of 24 test compounds were calculated. The EC_{50} value means the concentration of a chemical compound that is needed to reduce bacterial luminescence by 50%. According to the widely accepted classification scheme of Strupp et al. (1990) and in consideration of an incubation period of 30 min TNT, 26DNT, 2A6NT, 4A2NT, 34DNT, TNB, TNBA, TETRYL and HEXYL must be classified in the category "very toxic to aquatic organisms"; 2A46DNT, 4A26DNT, 24DA6NT, 24DNT, 2A4NT, RDX, HMX and PETN must be classified in the category "toxic to aquatic organisms"; and 26DA4NT, TAT, TNPh, 26DAT, 24DAT, HMT and NQ can be classified in the category "less toxic to aquatic organisms." EC_{50} values after 30, 60, and 90 min of incubation of the test compounds are presented and discussed. For many of the compounds tested in this study, there are no, or only a few, toxicological data in the literature available.

Everett, D.J. and Maddock, S.M. 1985. HMX: 13 Week Toxicity Study in Mice by Dietary Administration. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171602.

Greenough R.J. and McDonald, P. 1985. HMX: 14 Day Toxicity in Mice by Dietary Administration. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171596.

Lachance, B., Robidoux, P.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. *Mutation Research Genetic Toxicology and Environmental Mutagenesis.* 444(1): 25-39.

The mutagenicity and toxicity of energetic compounds such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and of amino/nitro derivatives of toluene were investigated in vitro. Mutagenicity was evaluated with the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay. Cytotoxicity was evaluated using V79 and TK6 human lymphoblastic cells. For the TK6 and V79 assays, TNB and 2,4,6-triaminotoluene were more toxic than TNT, whereas RDX and HMX were without effect at their maximal aqueous solubility limits. The primary TNT metabolites (2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene) were generally less cytotoxic than the parent compound. The FT results indicated that TNB, TNT and all the tested primary TNT metabolites were mutagenic. Except for the cases of 4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene in the TA98 strain, addition of rat liver S9 resulted in either no effect, or decreased activity. None of the tested compounds were mutagenic for the V79 mammalian cells with or without S9 metabolic activation. Thus, the FT assay was more sensitive to the genotoxic effects of energetic compounds than was the V79 test, suggesting that the FT might be a better screening tool for the presence of these explosives. The lack of mutagenicity of pure substances for V79 cells under the conditions used in this study does not preclude that genotoxicity could actually exist in other mammalian cells. In view of earlier reports and this study, mutagenicity testing of environmental samples should be considered as part of the hazard assessment of sites contaminated by TNT and related products.

Robidoux, P.Y., Hawari, J., Thiboutot, S., Ampleman, G. and Sunahara, G.I. 2001. Chronic toxicity of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in soil determined using the earthworm (Eisenia andrei) reproduction test. *Environ Pollut.* 111(2): 283-292.

The sublethal and chronic effects of the environmental contaminant and explosive octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in artificial soil were assessed using the earthworm (*Eisenia andrei*). Based on various reproduction parameters (total and hatched number of cocoons, number of juveniles and their biomass), fecundity was reduced at the different concentrations of HMX tested (from 280.0 +/- 12.3 to 2502.9 +/- 230.0 mg/kg dry soil) in spiked artificial soil (LOEC: 280.0 +/- 12.3 mg/kg dry soil). The growth of adult *E. andrei* was also reduced at the different concentrations tested, though no mortality occurred, even at the highest tested concentrations. The number of juveniles produced was correlated with the number of total and hatched cocoons, and the biomass of juveniles was correlated with the number of cocoons. Pooled results of these and earlier studies on explosives (TNT, RDX) using the *E. andre*i reproduction test confirm that effects of HMX on cocoon production are indicative of some reproductive consequences (number of juvenile and their biomass), whereas adult growth, in general, does not correlate strongly with change in reproduction capacity.

Robidoux, P.Y., Hawari, J., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 2002a. TNT, RDX, and HMX decrease earthworm (Eisenia andrei) life-cycle responses in a spiked natural forest soil. *Archives of Environmental Contamination and Toxicology.* 43(4): 379-388.

Sublethal and chronic toxicities of 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) on earthworm Eisenia andrei in a sandy forest soil were assessed. Various reproduction parameters of fecundity (total and hatched number of cocoons, number of juveniles, and their biomass) were significantly decreased by TNT (> or = 58.8 +/- 5.1 mg/kg dry soil), RDX (> or = 46.7 +/- 2.6 mg/kg), and HMX (> or = 15.6 +/- 4.6 mg/kg). These effects occurred at much lower concentrations than those reported earlier using artificial soil preparations. Growth of adults was significantly decreased in the TNT-spiked natural soils at 136.2 +/- 25.6 mg/kg dry soil, the highest concentration having no significant mortality. In contrast, survival and growth were not significantly reduced at relatively high measured concentrations of RDX (167.3 mg/kg) and HMX (711.0 mg/kg). Although TNT, RDX, and HMX share a common life-cycle response (i.e., decreased juvenile counts), a number of differences related to other reproduction parameters (e.g., productivity of cocoons) was observed. These results indicate that the tested explosives do not support a common mechanism of

toxicity, at least in the earthworm, probably due to differences in their physical-chemical properties as well as metabolites formed during exposure.

Robidoux, P.Y., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S., Hawari, J. and Sunahara, G.I. 2003. Phytotoxicity of 2,4,6-trinitrotoluene (TNT) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in spiked artificial and natural forest soils. ***Archives of environmental contamination and toxicology.*** **44(2): 198-209.**

Toxicity of 2,4,6-trinitrotoluene (TNT) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) using two terrestrial plant species, lettuce (*Lactuca sativa*) and barley (*Hordeum vugare*), was assessed in artificial soil (silica) and forest soil. Lettuce emergence was significantly decreased after 5 days of exposure to TNT nominal spiked concentrations >/= 1,040 mg/kg dry soil in silica. Barley emergence was significantly reduced after 14 days of exposure at initial (t = 0) TNT concentrations >/= 55.9 +/- 4.5 mg/kg dry soil in silica and at >/= 291.9 +/- 42.8 mg/kg dry forest soil. Biomasses of shoot and roots of barley seeds were significantly reduced after 14 days of exposure at TNT initial exposure concentrations >/= 55.9 +/- 4.5 (LOEC) mg/kg dry soil in silica. Results were similar with the forest soil (LOEC = 91.4 +/- 7.9 mg TNT/kg dry soil) using the root growth parameter, but the shoot biomass was reduced only at concentrations >/= 291.9 +/- 42.8 mg TNT/kg dry soil. Plants were not affected by an HMX exposure up to 3,320 +/- 1,019 mg/kg dry soil using silica or 1,866 +/- 438 mg/kg dry soil using a forest soil. During the 14-day experiments, TNT was partially transformed in the spiked soil samples, as indicated by the presence of its amino metabolites (2-ADNT and 4-ADNT). Higher quantities of metabolites were detected in forest soils having higher initial TNT concentrations (</= 1,849.4 +/- 228.2 mg/kg) compared to silica (</= 239.3 +/- 88.0 mg TNT/kg). After 14 days, TNT concentrations in spiked silica and forest soil were reduced up to 80.5% at 55.9 +/- 4.5 mg/kg initial concentration and 94.4% at 91.4 +/- 7.9 mg/kg initial concentration, respectively. Data indicate that TNT is the probable phytotoxicant because it decreased plant emergence and growth in the presence and absence of the ADNT metabolites.

Steevens, J.A., Duke, B.M., Lotufo, G.R. and Bridges, T.S. 2002. Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to Chironomus tentans and Hyalella azteca: low-dose hormesis and high-dose mortality. ***Environmental toxicology and chemistry.*** **21(7): 1475-1482.**

The toxicity of the explosives 2,4,6-trinitrotoluene (TNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive [RDX]); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive [HMX]), was evaluated in spiked sedi-

ment with two freshwater invertebrates. The midge *Chironomus tentans* and the amphipod *Hyalella azteca* demonstrated significant toxic effects after exposure to TNT and its degradation products, 1,3,5-trinitrobenzene (TNB) and 2,4-diamino-6-nitrotoluene (2,4-DANT). Significant reductions in survival of *C. tentans* exposed to TNT, TNB, and 2,4-DANT were observed at nominal sediment concentrations as low as 200 mg/kg. *Hyalella azteca* was more sensitive to TNT, TNB, and 2,4-DANT than the midge, where significant reductions in survival were observed at nominal concentrations of 50, 100, and 200 mg/kg, respectively. Survival of the midge and the amphipod was unaffected after exposure to RDX or HMX at the highest concentrations of 1,000 and 400 mg/kg, respectively. Growth of the midge, measured as total weight, was significantly reduced by 2,4-DANT. However, significantly increased growth was observed after exposure to sublethal concentrations of RDX and HMX. Although significant reductions in amphipod survival were observed at high concentrations of TNB, growth was significantly increased at sublethal concentrations. The results of the current investigation suggest that organisms exposed to explosives at contaminated sites may be affected at concentrations less than 25 mg/kg through hormetic growth enhancement and at higher concentrations through increased mortality.

Talmage, S.S., Opresko, D.M., Maxwell, C.J., Welsh, C.J., Cretella, F.M., Reno, P.H. and Daniel, F.B. 1999. Nitroaromatic munition compounds: environmental effects and screening values. *Reviews of Environmental Contamination and Toxicology.* 161: 1-156.

Nitroaromatic compounds are potentially toxic and found at a number of U.S. Army Ammunition Plants and other military facilities. This review presents a summary and analysis of available data on eight nitroaromatic compounds including environmental concentrations, environmental fate and transport processes, and ecotoxicity and bioaccumulation for aquatic and terrestrial biota. For those groups of organisms for which there are sufficient data, ecological criteria and screening benchmarks were developed. Staff at Oak Ridge National Laboratory (ORNL) under a project jointly sponsored by the U.S. Army and the U.S. EPA developed these criteria and screening benchmarks. The review discusses the methodologies for development of the screening criteria and benchmarks.

Nitrobenzene

Nitrobenzene is produced by reacting benzene with fuming nitric acid in the presence of a sulfuric acid catalyst between 50 – 65º C (ATSDR 1990). Isomers of nitrobenzene are produced by nitration of nitrobenzene using fuming nitric and sulfuric acids, or as by-products during production of TNT (Talmage et al. 1999).

Approximately 95% of nitrobenzene is used for the production of aniline, which is used for the manufacturing of polyurethanes. Nitrobenzene is also used for the production of acetaminophen, as a flavoring agent, as a perfume for soaps, and a solvent for shoe polish (ATSDR 1990).

1,3-Dinitrobenzene is the primary nitrobenzene isomer used for military applications. 1,3-Dinitrobenzne is used as an explosive and for the production of higher explosives (Hajjar et al. 1992, Talmage et al. 1999).

ATSDR. 1990. Toxicological Profile for Nitrobenzene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Hajjar, N.P., Brower, M.E., Turck, P.A., Kruger, C.L. and Hartley, W.R. 1992. 1,3-Dinitrobenzene (DNB). ***IN: Drinking Water Health Advisory: Munitions. Lewis Publishers, Boca Raton, FL.*** **1992: 49-86.**

1,3-Dinitrobenzene (1,3-DNB) is a colorless to yellowish crystalline solid at room temperature. Small volumes of dinitrobenzenes are formed as byproducts during the manufacture of nitrobenzene. In addition, 1,3-DNB is a byproduct of the manufacture of trinitrotoluene (TNT) explosives at the U.S. Army Ammunition plants (U.S. AAP) located at Radford, VA, (Radford AAP) Chattanooga, TN (Volunteer AAP), Joliet, IL (Joliet AAP), and Newport, IN (Newport AAP). Du Pont, located in Deepwater, NJ, is listed as the only US producer of 1,3-DNB for 1983 (more recent information is not available). The microbial degradation of 1,3-DNB was studied in sewage effluent maintained under aerobic or anaerobic conditions. The disappearance of 1,3-DNB under aerobic conditions was followed for 28 days: significant amounts of UV absorbency persisted. The decrease in initial absorbency was 58% by day 28. Microbial degradation of 1,3-DNB to CO_2 by *Candida pucherrima* occurred under aerobic conditions. The treatment of wastewater generated during dinitrobenzene production, involved slaking with lime, followed by removal of organics contained in the lime in a muffle furnace. The longer-term health advisory (HA) for a 10 kg child has been determined to be 0.04 mg/l. In the absence of adequate animal data to determine a 1 day or 10 day HA, the longer-term HA for a 10 kg child, 0.04 mg/l, is used as a conservative estimate of the 1 day or 10 day HA. The longer-term HA for a 70 kg adult has been determined to be 0.14 mg/l. A lifetime HA of 0.0010 mg/l for a 70 kg adult has been determined. Since no chronic toxicity or carcinogenicity studies with 1,3-DNB are currently available, 1,3-DNB is classified in group D: not classifiable as to human carcinogenicity.

Talmage, S.S., Opresko, D.M., Maxwell, C.J., Welsh, C.J., Cretella, F.M., Reno, P.H. and Daniel, F.B. 1999. Nitroaromatic munition compounds: environmental effects and screening values. *Reviews of Environmental Contamination and Toxicology.* 161: 1-156.

Nitroaromatic compounds are potentially toxic and found at a number of U.S. Army Ammunition Plants and other military facilities. This review presents a summary and analysis of available data on eight nitroaromatic compounds including environmental concentrations, environmental fate and transport processes, and ecotoxicity and bioaccumulation for aquatic and terrestrial biota. For those groups of organisms for which there are sufficient data, ecological criteria and screening benchmarks were developed. Staff at Oak Ridge National Laboratory (ORNL) under a project jointly sponsored by the U.S. Army and the U.S. EPA developed these criteria and screening benchmarks. The review discusses the methodologies for development of the screening criteria and benchmarks.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for nitrobenzene and nitrobenzene isomers.

Environmental Fate and Transport

Davis, E.M., Murray, H.E., Liehr, J.G. and Powers, E.L. 1981. Basic Microbial Degradation Rates and Chemical Byproducts of Selected Organic Compounds. *Water Research.* 15(9): 1125-1127.

The authors investigated the biodegradation rates of eight organic compounds by *Acinetobacter sp.*, *Pseudomonas alcaligenes*, *Flavobacterium sp.* and *Rhodotorula sp*. The compounds were benzene, toluene, phenol, nitrobenzene, methylene chloride, o-dichlorobenzene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene. The purpose for this investigation was to determine the toxicity levels, degradation rate or loss by volatilization, and identification of metabolic byproducts formed from the parent compounds.

Davis, E.M., Turley, J.E., Casserly, D.M. and Guthrie, R.K. 1983. Partitioning of selected organic pollutants in aquatic ecosystems. 5. International Biodeterioration Symposium, Aberdeen (UK), Sep 1981.

This research project was undertaken to quantify and identify the routes taken by twelve xenobiotic compounds in model waste stabilization ponds. It was not the intent to achieve maximum degradation of the compounds the compounds which were

tested were benzene; toluene; chlorobenzene; 1,2-dichlorobenzene; nitrobenzene; napthalene; 2,6-dinitrotoluene; phenanthrene; pyrene; di-n-butylphthalate; di-n-octylphthalate; and bis-2-ethylhexylphthalate. These were selected for their broad range of n-octanol partition coefficients, water solubilities, wide use in industry and because there were no data available on their fate in waste stabilization ponds. A mass balance was obtained which incorporated the amount of each chemical lost by volatilization, sedimentation, degradation, bioconcentration, the amount in the pond effluent, and the amount remaining in the water column.

Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. *Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).* Weston Roy F., Inc., West Chester, PA (USA).

The primary objective of this task was to determine the feasibility of using GAC to treat ground water contaminated by explosives at the Milan Army Ammunition Plant (MAAP) in Milan, TN. Laboratory GAC isotherm studies were conducted and two carbons, Atochem, Inc. GAC 830 and Calgon Filtrasorb 300, were selected for further testing in continuous flow GAC columns. Three pilot scale continuous flow GAC column tests were performed at MAAP using the two carbons selected from the laboratory GAC isotherm studies. The results from the laboratory and pilot studies are presented in this report. They show that concurrent removal of explosives such as TNT, RDX, HMX, Tetryl, and nitrobenzene from ground water using continuous flow granular activated carbon is feasible.

Haigler, B.E. and Spain, J.C. 1991. Biotransformation of nitrobenzene by bacteria containing toluene degradative pathways. *Appl Environ Microbiol.* 57(11): 3156-3162.

Nonpolar nitroaromatic compounds have been considered resistant to attack by oxygenases because of the electron withdrawing properties of the nitro group. We have investigated the ability of seven bacterial strains containing toluene degradative pathways to oxidize nitrobenzene. Cultures were induced with toluene vapor prior to incubation with nitrobenzene, and products were identified by high-performance liquid chromatography and gas chromatography-mass spectrometry. Pseudomonas cepacia G4 and a strain of *Pseudomonas* harboring the TOL plasmid (pTN2) did not transform nitrobenzene. Cells of *Pseudomonas putida* F1 and *Pseudomonas sp*. strain JS150 converted nitrobenzene to 3-nitrocatechol. Transformation of nitrobenzene in the presence of 18O2 indicated that the reaction in JS150 involved the incorporation of both atoms of oxygen in the 3-nitrocatechol, which suggests a dioxygenase mechanism. *P. putida* 39/D, a mutant strain of *P. putida* F1, converted nitrobenzene to a compound tentatively identified as cis-1,2-dihydroxy-3-

nitrocyclohexa-3,5-diene. This compound was rapidly converted to 3-nitrocatechol by cells of strain JS150. Cultures of *Pseudomonas mendocina* KR-1 converted nitrobenzene to a mixture of 3- and 4-nitrophenol (10 and 63%, respectively). *Pseudomonas pickettii* PKO1 converted nitrobenzene to 3- and 4-nitrocatechol via 3- and 4-nitrophenol. The nitrocatechols were slowly degraded to unidentified metabolites. Nitrobenzene did not serve as an inducer for the enzymes that catalyzed its oxidation. These results indicate that the nitrobenzene ring is subject to initial attack by both mono- and dioxygenase enzymes.

Hallas, L.E. and Alexander, M. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. *Applied and Environmental Microbiology.* 45(4): 1234-1241.

The transformation of mono-and dinitroaromatic compounds was measured in sewage effluent maintained under aerobic or anaerobic conditions. Most of the nitrobenzene, 3-and 4-nitrobenzoic acids, and 3- and 4-nitrotoluenes and much of the 1,2- and 1,3-dinitrobenzenes disappeared both in the presence and absence of oxygen. Under anaerobiosis, 2,6-dinitrotoluene and 3,5-dinitrobenzoic acid disappeared slowly, but no loss was evident in 28 days in aerated sewage. Aromatic amines did not accumulate during the aerobic decomposition of the mononitro compounds. It is suggested that the transformations of widely used nitroaromatic compounds should be further studies because of the persistence and possible toxicity of products of their metabolism.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Ince, N. and Inel, Y. 1989. Volatilization of Organic Chemicals from Water. *Water, Air and Soil Pollution WAPLAC.* 47: 1-2.

Liquid-phase transfer coefficients of 10 selected organic pollutants were measured simultaneously with the oxygen reaeration coefficient in a 2-liter reactor. The contents of the reactor were stirred in the laboratory by a 0.15 m diameter propeller attached to a constant speed motor. The ratio of the mass transfer coefficient of the chemical to that of oxygen is found to vary between 0.43 and 0.60 for 8 of the chemicals tested (toluene, o-xylene, naphthalene, biphenyl, tetrachloroethylene, chlorobenzene, ethylbenzene, trichloroethylene). This result is in good agreement with the literature concerning high volatility chemicals. The value of this ratio was 0.10 for nitrobenzene and 0.01 for phenol, confirming the predominance of gas phase resistances in the transport process for these two compounds.

Lee, D.S. and Park, S.D. 1996. Decomposition of nitrobenzene in supercritical water. *Journal of Hazardous Materials.* 51(1-3): 67-76.

This work was conducted to extend the understanding of the reaction kinetics and mechanisms of nitrogen containing compounds in SCW. The main objectives were to investigate the reaction kinetics and pathways for the decomposition of nitrobenzene in SCW, and the fate of nitrogen upon the decomposition. The experimental conditions included the temperature range from 440º to 550º C and the supercritical water density range from 0.09 to 0.23 g/ml^{-1}. A continuous flow stirred tank reactor system and a batch reactor system were used for the decomposition experiments without and with oxygen, respectively. In the absence of oxygen, nitrobenzene decomposed in SCW to form primarily benzene and nitrite. The decomposition kinetics had an activation energy of 68.0 plus or minus 9.0 kJ mol^{-1}. Nitrobenzene concentration showed a weak effect on the decomposition kinetics whereas the density of SCW had no effect. Additional decomposition products such as carbon monoxide and carbon dioxide suggested the occurrence of oxidation driven possibly by the nitro-group as well as SCW. The oxidative role of the nitro-group was supported by the observation of the reduction of nitrite to nitrogen gas. The decomposition rate was substantially enhanced in the presence of oxygen. The oxidation yielded a number of aromatic reaction products including aniline, phenol, 2-(2-pyridinyl)-benzonitrile, and dibenzofuran. The nitrogen in the decomposed nitrobenzene appeared to form mainly nitrogen gas even in the oxidative environments.

Lu, P.Y. and Metcalf, R.L. 1975. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. *Environ Health Perspect.* 10: 269-284.

A model aquatic ecosystem is devised for studying relatively volatile organic compounds and simulating direct discharge of chemical wastes into aquatic ecosystems. Six simple benzene derivatives (aniline, anisole, benzoic acid, chlorobenzene, nitrobenzene, and phthalic anhydride) and other important specialty chemicals: hexachlorobenzene, pentachlorophenol, 2,6-diethylaniline, and 3,5,6-trichloro-2-pyridinol were also chosen for study of environmental behavior and fate in the model aquatic ecosystem. Quantitative relationships of the intrinsic molecular properties of the environmental micropollutants with biological responses are established, e.g., water solubility, partition coefficient, pi constant, sigma constant, ecological magnification, biodegradability index, and comparative detoxication mechanisms, respectively. Water solubility, pi constant, and sigma constant are the most significant factors and control the biological responses of the food chain members. Water solubility and pi constant control the degree of bioaccumulation, and sigma constant limits the metabolism of the xenobiotics via microsomal detoxication enzymes. These highly sig-

nificant correlations should be useful for predicting environmental fate of organic chemicals.

Mc Farlane, C., Pfleeger, T. and Fletcher, J. 1990. Effect, uptake and disposition of nitrobenzene in several terrestrial plants. *Environmental Toxicology and Chemistry.* 9(4): 513-520.

Eight species of plants were exposed to nitrobenzene in a hydroponic solution. Four species experienced no depression of either transpiration or photosynthetic rates, while one was rapidly killed and the other three were temporarily affected but recovered from the treatment. Uptake of nitrobenzene was passive and was shown to be proportional to the rate of water flux in each species. The transpiration stream concentration factor (TSCF) was 0.72. The root concentration factor (RCF) was variable between the species and was higher than expected, presumably due to deposits of insoluble metabolic products. All of the species examined displayed a capacity to chemically alter nonpolar nitrobenzene into both polar and insoluble products.

Mitchell, W.R. and Dennis, W. 1982. Biodegradation of 1,3-Dinitrobenzene. *Journal of Environmental Science and Health, Part A.* 17(6): 837-853.

1,3-Dinitrobenzene was degraded microbially in water samples taken downstream of the Volunteer Army Ammunition Plant, Chattanooga, TN, but not in water from streams near Frederick, MD. Degradation of the 5 microgram per liter solution proceeded after a 10 day lag and was complete by day 15. Microorganisms from the Tennessee River were able to grow with 1,3-dinitrobenzene as the sole carbon source with a half-life of about 1 day. Enriching the cultures degraded the compound to carbon dioxide with a half-life of 9.7 days. The 1,3 dinitrobenzene-adapted organisms did not grow with the other munitions manufacturing wastes tested: 1,3 dinitrobenzene, 1 ,4-dinitrobenzene, 3,5-dinitroaniline, 1,3,5-trinitrobenzene, or nitrobenzene.

Nielsen, P.H., Bjarnadottir, H., Winter, P.L. and Christensen, T.H. 1995. In situ and laboratory studies on the fate of specific organic compounds in an anaerobic landfill leachate plume, 2. Fate of aromatic and chlorinated aliphatic compounds. *Journal of Contaminant Hydrology.* 20(1-2): 51-66.

The transformation of specific organic compounds was investigated by in situ and laboratory experiments in an anaerobic landfill leachate pollution plume at four different distances from the landfill. In a previous paper (Part 1, also published in this issue) we described the in situ microcosm and laboratory batch microcosm experiments performed focusing on redox conditions, microbiology and the fate of 7 phenolic compounds. In this paper we present the results on the fate of 8 aromatic com-

pounds and 4 chlorinated aliphatic compounds. Nitrobenzene was transformed at all distances from the landfill in methanogenic, and Fe(III)- and NO^-_3-reducing conditions. Toluene was transformed slowly in one out of three in situ experiments at the distance of 250 m from the landfill in the Fe(III)-reducing part of the plume after a lag phase of similar to 3 months. Benzene, o-xylene, p-dichlorobenzene, o-dichlorobenzene, naphthalene and biphenyl were not transformed at any of the investigated distances from the landfill, neither in in situ nor in laboratory experiments. In the methanogenic part of the aquifer 2 m from the landfill, 1,1,1-trichloroethane, tetrachloromethane and tetrachloroethene were transformed in in situ experiments while trichloroethene was not. Lag phase periods were up to 40 days for 1,1,1-trichloroethane and up to 100 days for tetrachloroethene. No or only short lag phases (< 10 days) were observed for tetrachloromethane. Tetrachloromethane was furthermore transformed at distances of up to 250 m from the landfill in Fe(III)-reducing conditions but not in NO^-_3-reducing conditions at 350 m from the landfill. Abiotic processes apparently contributed to the transformation of tetrachloromethane. A local variation in the transformation of the chlorinated aliphatic hydrocarbons was observed at 2 m from the landfill. In general, good accordance with respect to compound transformation was observed between in situ and laboratory experiments, but in a few cases more compounds were transformed in in situ experiments than in the corresponding laboratory experiments.

Shah, M.M. and Campbell, J.A. 1997. Transformation of nitrobenzene by ferredoxin NADP oxidoreductase from spinach leaves. *Biochem Biophys Res Commun.* 241(3): 794-796.

Nitrobenzene was reduced in a solution containing ferredoxin NADP oxidoreductase (FNR) from spinach leaves and NADPH generating system. The product of nitrobenzene was identified as phenylhydroxylamine (PHA) on 1:1 basis.

Trapido, M., Dello, A., Goi, A. and Munter, R. 2003. Degradation of nitroaromatics with the Fenton reagent. *Proceedings of the Estonian Academy of Sciences, Chemistry [Proc. Eston. Acad. Sci. Chem.].* 52(1): 38-47.

The feasibility of the Fenton reagent treatment for the degradation and detoxification of the nitroaromatic compounds (NAC) such as p-nitrotoluene, nitrobenzene, and m-dinitrobenzene was studied. The degradation rate of NAC with the Fenton treatment was strongly dependent on the molar ratio NAC/hydrogen peroxide/catalyst (Fe^{2+}). The 90% conversion times of NAC in the Fenton treatment followed the order p-nitrotoluene approximately nitrobenzene < m-dinitro-benzene. The degree of nitrogen conversion to nitrate with the Fenton treatment varied from 25% to 100%, depending on the treatment conditions. Total organic carbon removal of 45-47% was obtained when the Fenton reagent treatment with the concentration

of hydrogen peroxide and catalyst was applied. According to the *Daphnia magna* toxicity test the Fenton reagent treatment enabled to reduce the toxicity of NAC.

Wilson, J.T., Enfield, C.G., Dunlap, W.J., Cosby, R.L., Foster, D.A. and Baskin, L.B. 1981. Transport and Fate of Selected Organic Pollutants in a Sandy Soil. *Journal of Environmental Quality.* 10(4): 501-506.

Ground waters are increasingly vulnerable to pollution by organic chemicals that migrate through the soil mantle. To assess the minimal protection that soil can be expected to provide ground water, the transport and fates of 13 organic pollutants were studied in a sandy soil with low organic matter content (0.087% organic C). Chloroform (trichloromethane), 1,2-dibromo-3-chloropropane, dichlorobromomethane, 1,2-dichloroethane, tetrachloroethene, 1,1,2-trichloroethane, and trichloroethene were not degraded in this soil. Chlorobenzene, 1,4-dichlorobenzene, and 1,2,4-trichlorobenzene also percolated through the soil; retardation factors were 1.7,3.4, and 9.4, respectively. Between 26 and 49% of the material applied reached 140 cm. Toluene and nitrobenzene degraded in some of the columns but not in others. In the absence of degradation, 60 and 80% of nitrobenzene and 13% of toluene applied to the soil surface reached 140 cm. The retardation factors were 2.3 or less. Bis(2-chloroethyl)ether did not degrade; 86% of the material applied reached 140 cm, and the compound's retardation factor was < 1.5.

Bioaccumulation and Trophic Transfer

Harada, N. and Omura, T. 1980. Participation of cytochrome P-450 in the reduction of nitro compounds by rat liver microsomes. *J Biochem (Tokyo).* 87(5): 1539-1554.

1.) The subcellular distribution of nitrobenzene reduction activity in rat liver cells indicated the existence of two different enzyme systems, one localized in microsomes and the other localized in cytosol. The activity in the cytosol was mainly attributable to xanthine oxidase, judging from its substrate specificity and the inhibition by allopurinol. 2.) The participation of the microsomal electron transport system in nitrobenzene reduction was examined by using antibodies against four components of the system, NADPH-cytochrome c reductase (fpT), NADH-cytochrome b5 reductase (fpD), cytochrome b5, and cytochrome P-450. Both NADH- and NADPH-dependent nitrobenzene reduction activities were strongly inhibited by anti-fpT IG and also by anti-P450 IG, but not inhibited by anti-fpD IG or anti-b5 IG. The reduction of nitrosobenzene and phenylhydroxylamine, which are supposed to be the intermediates of nitrobenzene reduction, was also examined, and it was found that NADH- and NADPH-dependent reduction of both compounds were strongly inhibited by anti-fpT IG and anti-P450 IG, but not by anti-fpD IG or anti-b5 IG. 3.) Reconstruction ex-

periments using purified NADPH-cytochrome P-450 reductase and cytochrome P-450 were also carried out and it was confirmed that the reduction of nitrobenzene, nitrosobenzene, and phenylhydroxylamine to aniline could be effected by these two components. 4.) Nitrobenzene reduction by microsomes exhibited a short initial time lag and was activated by the addition of purified NADPH-cytochrome c reductase, whereas nitrosobenzene and phenylhydroxylamine reductions did not show any initial time lag and were not activated by the reductase. These observations suggest that the reduction of nitrobenzene to an intermediate, possibly nitrosobenzene or phenylhydroxylamine, limits the rate of aniline formation, and such an initial step of nitrobenzene reduction can be catalyzed by NADPH-cytochrome c reductase alone. Cytochrome P-450 is essential at least in the final step of nitrobenzene reduction to aniline. This conclusion was further confirmed by determination of these intermediates in nitrobenzene reduction.

Nystrom, D.D. and Rickert, D.E. 1987. Metabolism and excretion of dinitrobenzenes by male Fischer-344 rats. *Drug Metab Dispos.* 15(6): 821-825.

All three dinitrobenzene (DNB) isomers cause methemoglobinemia, but only 1,3-DNB produces testicular toxicity in rats. In order to determine whether major differences exist in the routes of DNB metabolism, male Fischer-344 rats were given an oral dose (0.15 mmol/kg) of ^{14}C-labeled 1,2-, 1,3-, or 1,4-DNB, and excreta were collected over 48 hr. Elimination of radiolabel was rapid; 85%, 60%, and 75% of the 1,2-, 1,3-, and 1,4-DNB dose was recovered in 24 hrs, respectively. Urine was the primary route of excretion, accounting for 82% of the total dose of 1,2-DNB and 75% of the dose of 1,4-DNB after 48 hrs. Radiolabel from 1,3-DNB was excreted to a slightly lesser extent in the urine (63% of the dose). A greater portion of radiolabel was excreted in the feces than with the other isomers (18% of total dose, compared to 8% and 9% with 1,2-DNB and 1,4-DNB, respectively). The major urinary metabolites of 1,2-DNB were S-(2-nitrophenyl)-N-acetylcysteine (42% of the dose), 2-nitroaniline-N-glucuronide (4%), 4-amino-3-nitrophenylsulfate (17%), 2-amino-3-nitrophenylsulfate (1.5%), and 2-(N-hydroxylamino)nitrobenzene (1-2%). The major urinary metabolites of 1,3-DNB were 3-aminoacetanilide (22%), 4-acetamidophenylsulfate (6%), 1,3-diacetamidobenzene (7%), and 3-nitroaniline-N-glucuronide (4%). The major metabolites of 1,4-DNB were 2-amino-5-nitrophenylsulfate (35%), S-(4-nitrophenyl)-N-acetylcysteine (13%), and 1,4-diacetamidobenzene (7%). These results suggest that the DNB isomers are primarily metabolized by nitro group reduction and conjugation with glutathione. The testicular toxicant 1,3-DNB was apparently metabolized exclusively by reduction.

Toxicity

Allenby, G., Sharpe, R.M. and Foster, P.M. 1990. Changes in Sertoli cell function in vitro induced by nitrobenzene. *Fundam Appl Toxicol.* 14(2): 364-375.

Nitrobenzene (NB) has been identified as a testicular toxicant in vivo, but its site of action remains unknown. In the present study, the effect of NB on the Sertoli cell was assessed in vitro using Sertoli cell and Sertoli-germ cell cocultures. The parameters measured were the exfoliation of germ cells; the secretion of lactate, pyruvate, and inhibin; and gross cellular morphology. The effect of metadinitrobenzene (mDNB), a related compound which is a known Sertoli cell toxicant, was assessed for comparison. Gross morphological changes including vacuolation of Sertoli cells were observed following treatment of cultures with 10^{-3} M NB. Exposure of cocultures to NB also resulted in dose-dependent exfoliation of predominantly viable germ cells. NB (greater than 5 X 10^{-4} M) and mDNB at the single dose level used (10^{-4} M) stimulated the secretion of lactate and pyruvate significantly by Sertoli cells, an effect that was more marked in the absence of germ cells. Comparable changes were observed in follicle stimulating hormone (FSH)-stimulated cultures. Inhibin secretion by Sertoli cells was also altered by exposure to NB but in a biphasic manner, with low (10^{-8} to 10^{-6} M) and high (10^{-4} to 10^{-3} M) doses enhancing inhibin secretion while intermediate (10^{-5} M) doses had no effect. These effects were evident in both culture systems but inhibin secretion by Sertoli-germ cell cocultures was always greater than that by Sertoli cell cultures. However, these effects of NB on inhibin secretion were not evident in FSH-stimulated cultures. In contrast to the effects of NB, mDNB had no effect on basal secretion of inhibin but blocked the stimulatory effect of FSH. It is concluded that NB, like mDNB, is probably a Sertoli cell toxicant in view of its similar disruptive effects on various parameters of Sertoli cell function. However, NB is far less toxic than mDNB at equivalent concentrations in vitro. The present study is the first to evaluate the potential of inhibin secretion by Sertoli cells in culture as an additional marker of toxicant action, and concludes that it merits further study in this context.

Black, J.A., Birge, W.J., McDonnell, W.E., Westerman, A.G. and Ramey, B.A. 1982. The Aquatic Toxicity of Organic Compounds to Embryo-Larval Stages of Fish and Amphibians. University of Kentucky Water Resource Research Institute, Lexington, KY. Report No. 133.

Aquatic toxicity tests were conducted on 11 organic compounds considered hazardous to water resources. The toxicity of each compound was evaluated using embryo-larval stages of up to eight fish and amphibian species. Exposure was initiated at fertilization and maintained through 4 days posthatching. The animal test species

exhibited varying degrees of sensitivity to the selected toxicants. Combined frequencies for mortality and teratogenesis at 4 days posthatching gave LC_{50} ranges of 3.66 to 8.25 mg/l for benzene, 1.16 to 22.42 mg/l for carbon tetrachloride, 0.11 to 1.20 mg/l for chlorogenzene, 2.03 to 68 mg/l for chlorogrorm, 3.01 to 5.56 mg/l for 1,2 dichlorobenzene, 2.54 to 34 mg/l for 1,2 dichloroethane, 13.16 to 48 mg/l for methylene chloride, 0.002 to 0.64 mg/l for nitrobenzene, 0.04 to 32 mg/l for phenol, 0.02 to 0.85 mg/l for toluene, and 3.53 to 3.77 mg/l for m-xylene. The species that exhibited the greatest susceptibility to organic compounds were the rainbow trout (*Onchorhynchus mykiss*), *Rana pipiens*, and *Rana temporaria*. The more sensitive amphibian species generally were those that normally are restricted to aquatic or moist terrestrial habitats, whereas the more tolerant amphibians included those semi-aquatic and terrestrial species which appear to be more broadly adapted ecologically. Of the 11 test compounds, nitrobenzene, toluene, chlorobenzene, and phenol were the most toxic. The least toxic organics included dicloroethane and methylene chloride. For three chlorinated alkanes, including methylene chloride, chloroform, and carbin tetrachloride toxicity was found to increase with the degree of chlorination.

Bond, J.A., Chism, J.P., Rickert, D.E. and Popp, J.A. 1981. Induction of hepatic and testicular lesions in Fischer-344 rats by single oral doses of nitrobenzene. *Fundam Appl Toxicol.* 1(5): 389-394.

Since the acute toxicity of nitrobenzene (NB) in rats has not been characterized, experiments were performed to ascertain the possible deleterious effects of NB in different tissues of the male Fischer-344 rat. Rats were given single oral doses of NB (50-450 mg/kg) and at the time of sacrifice, 25 tissues were removed and examined histologically by light microscopy. Histopathological changes induced by a single oral dose of NB consistently involved only the liver and testes. One rat receiving 450 mg NB/kg had a microscopic cerebellar lesion. Hepatic centrolobular necrosis appeared inconsistently in rats given various doses of NB, while hepatocellular nucleolar enlargement was consistently detected in rats given doses of NB as low as 110 mg/kg. These data suggest that nucleolar enlargement was independent of cell death and subsequent regeneration. Testicular lesions were confined to the seminiferous tubules and consisted of necrosis of the primary and secondary spermatocytes with the appearance of multinucleated giant cells between one and four days after administration of NB 300 mg/kg. Necrotic debris and decreased numbers of spermatozoa were seen in the epididymis as early as three days after NB administration. The NB-induced methemoglobinemia does not appear to be solely responsible for the formation of early lesions in the rat liver, testes, or brain, since sodium nitrite administration, at dosages which produced methemoglobinemia equivalent to that of NB, did not produce any histopathological changes. Thus, the observed liver and testicular damage are probably due to a direct effect of NB or its metabolites.

Burns, L.A., Bradley, S.G., White, K.L., Jr., McCay, J.A., Fuchs, B.A., Stern, M., Brown, R.D., Musgrove, D.L., Holsapple, M.P., Luster, M.I., et al. 1994. Immunotoxicity of nitrobenzene in female B6C3F1 mice. *Drug Chem Toxicol.* 17(3): 271-315.

Nitrobenzene (NBZ) is primarily employed as an oxidizing agent in the synthesis of analine and benzene compounds. It produces myelotoxic effects and effects on erythrocytes in both animal models and man. Reported hepatosplenomegaly and effects on the bone marrow are indicators that NBZ may be immunotoxic. In these studies, female B6C3F1 mice were exposed to 30, 100 and 300 mg/kg of NBZ in corn oil by gavage for 14 consecutive days. To assess the immunotoxic potential of NBZ, body and organ weights were determined and selected immunologic and host resistance responses were studied. In these studies, the liver and spleen appeared to be the primary target organs. Both liver and spleen weights were dose dependently increased. Gross histopathologic examinations revealed significant changes in the spleen, consisting of severe congestion of the red pulp areas with erythrocytes and reticulocytes. Serum chemistry profiles showed increases in alanine aminotransferase and aspartate aminotransferase activities, indicating liver toxicity. Hematologic studies showed a decrease in erythrocyte number and a concomitant increase in mean corpuscular hemoglobin and mean corpuscular volume. A dose-dependent increase in peripheral reticulocytes was also seen. DNA synthesis was enhanced, as was the number of formed elements and the number of monocyte/granulocyte stem cells in the bone marrow of treated mice. IgM responses were decreased and the phagocytic activity of macrophages in the liver was dose dependently increased with a concomitant decrease in the activities in the spleen and lung. Other immunological parameters examined were unchanged. Host resistance to microbial or viral infection was not markedly altered by NBZ; however, there were trends towards increased susceptibility where T-cell function contributes to host defense. These data indicate that NBZ-induced hemolysis and liver injury are linked to the observed alterations in bone marrow activity.

Cattley, R.C., Everitt, J.I., Gross, E.A., Moss, O.R., Hamm, T.E., Jr. and Popp, J.A. 1994. Carcinogenicity and toxicity of inhaled nitrobenzene in B6C3F1 mice and F344 and CD rats. *Fundam Appl Toxicol.* 22(3): 328-340.

The potential carcinogenicity and toxicity of inhaled nitrobenzene were evaluated following chronic (2 year) exposure in mice and rats. Male and female B6C3F1 mice were exposed to 0, 5, 25, or 50 ppm nitrobenzene, while male and female F344 rats and male CD rats were exposed to 0, 1, 5, or 25 ppm nitrobenzene. All exposures were for 6 hr/day, 5 days/week excluding holidays, for a total of 505 days over 2 yrs. Survival was not adversely affected by nitrobenzene exposure, and only mild exposure-related decreases in body weights (< 10% of control) were occasionally noted.

Nitrobenzene exposure resulted in increased incidence of neoplasia in male B6C3F1 mice (pulmonary alveolar/bronchiolar and thyroid follicular cell neoplasms), female B6C3F1 mice (mammary gland neoplasms), male F344 rats (hepatocellular and renal neoplasms), female F344 rats (endometrial stromal neoplasms), and male CD rats (hepatocellular neoplasms). In addition, there were marginal increases in the incidence of hepatocellular neoplasia in female B6C3F1 mice and thyroid follicular neoplasia in male F344 rats. Groups of nitrobenzene-exposed mice and rats with increased incidence of renal and thyroid neoplasia also had increased incidences of hyperplasia in these tissues. Toxicity resulting from chronic inhalation of nitrobenzene was manifested by methemoglobinemia, anemia, and adaptive or degenerative changes in the nose, liver, and testis. The results indicate that inhaled nitrobenzene is carcinogenic and toxic in mice and rats, and that the spectrum of these responses in animals is dependent on species, sex, and genetic background.

Cave, D.A. and Foster, P.M. 1990. Modulation of m-dinitrobenzene and m-nitrosonitrobenzene toxicity in rat Sertoli–germ cell cocultures. *Fundam Appl Toxicol.* 14(1): 199-207.

Previous work has shown that m-dinitrobenzene is a testicular toxicant in rats in vivo, and in vitro produces comparable morphological changes in rat testicular Sertoli-germ cell cocultures. m-Dinitrobenzene is metabolized both in vivo and in the in vitro system to m-nitroaniline m-nitroaniline and m-nitroacetanilide. These metabolites do not provoke testicular toxicity in vivo or in vitro. We have therefore proposed a pathway for the metabolism of m-dinitrobenzene to m-nitroaniline and m-nitroacetanilide, which involved the intermediate m-nitrosonitrobenzene (1-nitroso-3-nitrobenzene, NNB). When tested, m-nitrosonitrobenzene, at equimolar doses to m-dinitrobenzene, produced similar morphological changes in the culture system to those exhibited by m-dinitrobenzene. However, m-nitrosonitrobenzene produced a greater toxicity than did m-dinitrobenzene (as measured by germ cell detachment). When the intracellular thiol levels were reduced in the cocultures pretreated with diethyl maleate, the toxicity of both m-dinitrobenzene and m-nitrosonitrobenzene was enhanced. In contrast, pretreatment of cocultures with agents known to increase cellular thiol (cysteamine) or scavenge reactive intermediates (cysteamine or ascorbate) reduced the toxicity of m-dinitrobenzene and m-nitrosonitrobenzene. We propose that m-dinitrobenzene requires metabolic activation before it can exert its toxicity to Sertoli cells, and it appears that the toxic species is m-nitrosonitrobenzene or a further metabolite of m-nitrosonitrobenzene.

Dodd, D.E., Fowler, E.H., Snellings, W.M., Pritts, I.M., Tyl, R.W., Lyon, J.P., O'Neal, F.O. and Kimmerle, G. 1987. Reproduction and fertility evaluations in CD rats following nitrobenzene inhalation. *Fundam Appl Toxicol.* 8(4): 493-505.

A two-generation reproduction study was performed by exposure of Sprague-Dawley CD rats to concentrations of 40, 10, 1, or 0 (control) ppm of nitrobenzene (NB) vapor. No NB-related effects on reproduction were observed at 10 or 1 ppm. At 40 ppm, a decrease in the fertility index of the F0 and F1 generations occurred, which was associated with alterations in the male reproductive organs. Specifically, weights of testes and epididymides were reduced and seminiferous tubule atrophy, spermatocyte degeneration, and the presence of giant syncytial spermatocytes were observed. The only significant finding in the litters derived from rats exposed to 40 ppm was an approximate 12% decrease in the mean body weight of F1 pups on postnatal day 21. Survival indices were unaltered. To examine the reversibility of this selective effect on male gonads, the F1 males from the 40-ppm group were allowed a 9 week nonexposure period and mated to naive females. An almost fivefold increase in the fertility index was observed, indicating at least partial functional reversibility upon removal from NB exposure. Also, the numbers of giant syncytial spermatocytes and degenerated spermatocytes were greatly reduced. The results of this study support the selection of 10 ppm of NB as the no-observable-effect level for reproduction and fertility effects in rats.

Iida, S., Misaka, H. and Naya, M. 1997. A flow cytometric analysis of cytotoxic effects of nitrobenzene on rat spermatogenesis. *J Toxicol Sci.* 22(5): 397-407.

Cytotoxic effects of nitrobenzene (NB) on spermatogenesis of mature Sprague-Dawley (Crj:CD) rats were analyzed by measuring the DNA content distribution and testicular weight at 1, 2, and 3 weeks of daily oral dose of NB (60 mg/kg/day). Rats at the age of 9 weeks were used because the ratios of 1C, 2C, S, 4C to total testicular cells were stabilized after the age of 52 days. Within a week of administration, a large number of 1C cells were lost but 2C cells proliferated, resulting in little change of testicular weight. In another week that followed, the number of 1C cells and testicular weight were decreased, but the ratio of S-4C cells was increased, indicating that an appreciable number of 2C cells could progress to the 4C compartment. The data indicated that (1) 1C cells were destroyed, and (2) meiosis of secondary spermatocytes was suppressed, but (3) NB had little effect on the spermatocytes prior to the early pachytene stage. This interpretation was reinforced by the observation that (4) the ratio of 1C cells returned to a nearly normal level during a recovery period of 2 weeks. In conclusion, flow cytometry could offer an efficient method for the quantitative analysis of male reproductive toxicity.

Levin, A.A., Bosakowski, T., Earle, L.L. and Butterworth, B.E. 1988. The reversibility of nitrobenzene-induced testicular toxicity: continuous monitoring of sperm output from vasocystotomized rats. *Toxicology*. 53(2-3): 219-230.

Exposure of rats to nitrobenzene produces a degeneration of the seminiferous epithelium of the testes. Sperm production was continuously monitored in rats surgically prepared by anastomosing the vas deferentia with the urinary bladder to evaluate the reversibility of nitrobenzene toxicity. Rates of sperm production were monitored by collecting urine and counting sperm microscopically with a hemocytometer. Six weeks after surgery, rats were dosed p.o. with a single dose of 300 mg/kg of nitrobenzene in corn oil. Sperm were not detected in the urine of treated rats between 32 and 48 days after treatment. Despite the fact that degenerative changes in the seminiferous tubules were observed histologically as early as 3 days after dosing, there was a 32 day lag period between treatment and cessation of sperm output in treated rats. Histological examination showed that pachytene spermatocytes and step 1-2 spermatids were the most susceptible cell stages to nitrobenzene and were observed forming into giant cells as early as 3 days after treatment. However, repair was substantial by 3 wks after treatment and by days 76-100, the rate of sperm output reached 78% of the control group. By 100 days after treatment, there was greater than 90% regeneration of the seminiferous epithelium. Thus, a single oral dose of nitrobenzene induced testicular degeneration and approximately a 17 day period of aspermia resulted. Back-dating of the aspermic period to the timing of the spermatogenic cycle closely corresponded with the same germ cell stages that were observed degenerating in histologic examinations. Thus, changes in sperm output from vasocystotomized rats correlated well with histopathologic changes, demonstrating the value of this technique for toxicity studies.

Morgan, K.T., Gross, E.A., Lyght, O. and Bond, J.A. 1985. Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. *Neurotoxicology*. 6(1): 105-116.

Administration of single oral doses (550 mg/kg body wt) of nitrobenzene to male F-344 rats induced petechial hemorrhages in the brain stem and cerebellum, and bilaterally symmetric degeneration (malacia) in the cerebellum and cerebellar peduncles, within 48 hours of treatment. The malacia, which was lateral and dorsal to the fourth ventricle and involved the vestibular nuclei, was attributed to edematous swelling of a membrane bounded tissue compartment. Degenerating neurons present in, and adjacent to, areas of malacia exhibited severe watery swelling of mitochondria. Myelinated axons showed moderate to severe condensation of the axoplasm and accumulation of electron-lucent fluid between the inner myelin leaflets and less frequently in the periaxonal space. Blood vessels appeared morphologi-

cally normal, while leakage of intravascularly administered horseradish peroxidase from blood vessels in the brain accompanied the hemorrhages in a small number of animals. Extravasation of this tracer did not precede the onset of hemorrhages or malacia. Autoradiographic and analytical studies demonstrated that a very small percentage (0.02%) of the administered dose reached the brain; it was present as the parent compound and accumulated in higher concentration in grey matter than white matter. There was no preferential accumulation of nitrobenzene in the areas in which lesions occurred, which may reflect a regional susceptibility to nitrobenzene or an indirect mechanism of nitrobenzene neurotoxicity.

Thomulka, K.W. and Lange, J.H. 1997. Mixture toxicity of nitrobenzene and trinitrobenzene using the marine bacterium Vibrio harveyi as the test organism. *Ecotoxicol Environ Saf.* 36(2): 189-195.

Vibrio harveyi, a bioluminescent marine bacterium, was used to evaluate combined or mixture toxicity of two organic compounds, nitrobenzene and trinitrobenzene. An estimated median effective concentration (EC_{50}) and confidence interval were determined for each chemical. These chemicals at their EC_{50} were evaluated in combination and an additive index method was used to determine a numerical toxicology value. Combinations at 20% intervals of the EC_{50} were performed using isopleths. The isopleths employed were the isobole plot and the isobologram. Bioluminescent change was also determined and graphed for evaluation of toxicity. Statistical evaluation of isopleths and the additive index method were employed by incorporating confidence intervals. Bioluminescent change and isopleths suggest that mixtures of nitrobenzene and trinitrobenzene are additive, while the additive index method is suggestive of synergism. Statistical evaluation between mixtures and single values, using the z test, was in some cases different at the 5% level. These data suggest that interaction of combinations should be evaluated and described by multiple methodologies. Evaluation of these data suggests, in part, that one mixture is statistically different for antagonism. This study supports the use of bioluminescent microbial toxicity tests with various evaluative methodologies for the determination of mixture interactions.

Tyl, R.W., France, K.A., Fisher, L.C., Dodd, D.E., Pritts, I.M., Lyon, J.P., O'Neal, F.O. and Kimmerle, G. 1987. Development toxicity evaluation of inhaled nitrobenzene in CD rats. *Fundam Appl Toxicol.* 8(4): 482-492.

Pregnant CD (Sprague-Dawley) rats were exposed to nitrobenzene vapor (CAS Registry No. 98-95-3) at 0, 1, 10, and 40 ppm (mean analytical values of 0.0, 1.06, 9.8, and 39.4 ppm, respectively) on gestational days (gd) 6 through 15 for 6 hr/day. At sacrifice on gd 21, fetuses were evaluated for external, visceral, and skeletal malformations and variations. Maternal toxicity was observed: weight gain was reduced

during exposure (gd 6-9 and 6-15) to 40 ppm, with full recovery by gd 21, and absolute and relative spleen weights were increased at 10 and 40 ppm. There was no effect of treatment on maternal liver, kidney, or gravid uterine weights, on pre- or postimplantation loss including resorptions or dead fetuses, on sex ratio of live fetuses, or on fetal body weights (male, female, or total) per litter. There were also no treatment-related effects on the incidence of fetal malformations or variations. In summary, during organogenesis in CD rats, there was no developmental toxicity (including teratogenicity) associated with exposure to nitrobenzene concentrations that produced some maternal toxicity (10 and 40 ppm) or that produced no observable maternal toxicity (1 ppm).

Nitroglycerin

Nitroglycerin is formed by mixing glycerol with concentrated nitric and phosphoric acids (Encyclopedia Britannica 1997).

Nitroglycerin is used as a primary component for the AA2 double-base propellant (Cropek et al 2002). The AA2 double base-propellant is composed of nitroglycerin and nitrocellulose. Nitroglycerin is also used as a vasodilatory compound for the treatment of angina pectoris (HSDB 2003).

Cropek, D.M., Kemme, P.A., Day, J.M. and Cochran, J. 2002. Use of Pyrolysis GC/MS for Predicting Emission Byproducts from the Incineration of Double-Base Propellant. *Environmental Science & Technology* 36(20): 4346-4351.

Gas chromatography/mass spectrometry was used to analyze the pyrolytic byproducts from an Army-unique propellant compound (AA2) that is composed of predominantly nitrocellulose and nitroglycerin. Compounds produced by AA2 pyrolysis were compared to compounds detected in the gaseous effluent from AA2 incineration. The light permanent gases and most of the higher molecular weight byproducts produced by AA2 incineration are replicated by laboratory pyrolysis on AA2. The reverse case also holds whereby 18 out of 24 high molecular weight AA2 pyrolytic byproducts are found in the incinerator emissions. Poor matching, however, was obtained between the two processes for the volatile, water-soluble species. None of these low molecular weight compounds produced under pyrolytic conditions were detected in the AA2 incinerator samples, likely indicating inefficient capture of these compounds from the effluent stream. Separate pyrolytic degradation of the individual components of AA2 provides evidence that nearly all of the incomplete combustion products detected during incineration originate not from the prevalent energetic ingredients but rather from the minor and trace additives in AA2. In addi-

tion, pyrolysis successfully identified the AA2 components capable of surviving the incineration process intact. This work illustrates the potential of bench-scale pyrolysis for predicting incineration behavior.

Encyclopedia Brittannica. 1997. Guide to the Nobel Prizes: Nitroglycerine. Online at: http://www.britannica.com/nobel/micro/426_77.html

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for nitroglycerin.

Environmental Fate and Transport

Accashian, J.V., Vinopal, R.T., Kim, B.J. and Smets, B.F. 1998. Aerobic growth on nitroglycerin as the sole carbon, nitrogen, and energy source by a mixed bacterial culture. *Appl Environ Microbiol.* 64(9): 3300-3304.

Nitroglycerin (glycerol trinitrate [GTN]), an explosive and vasodilatory compound, was metabolized by mixed microbial cultures from aeration tank sludge previously exposed to GTN. Aerobic enrichment cultures removed GTN rapidly in the absence of a supplemental carbon source. Complete denitration of GTN, provided as the sole C and N source, was observed in aerobic batch cultures and proceeded stepwise via the dinitrate and mononitrate isomers, with successive steps occurring at lower rates. The denitration of all glycerol nitrate esters was found to be concomitant, and 1, 2-glycerol dinitrate (1,2-GDN) and 2-glycerol mononitrate (2-GMN) were the primary GDN and GMN isomers observed. Denitration of GTN resulted in release of primarily nitrite-N, indicating a reductive denitration mechanism. Biomass growth at the expense of GTN was verified by optical density and plate count measurements. The kinetics of GTN biotransformation were 10-fold faster than reported for complete GTN denitration under anaerobic conditions. A maximum specific growth rate of 0.048 +/- 0.005 h-1 (mean +/- standard deviation) was estimated for the mixed culture at 25º C. Evidence of GTN toxicity was observed at GTN concentrations above 0. 3 mM. To our knowledge, this is the first report of complete denitration of GTN used as a primary growth substrate by a bacterial culture under aerobic conditions.

Bhaumik, S., Christodoulatos, C., Brodman, B.W. and Pal, N. 1998. Biodegradation of glycerol trinitrate by activated sludge: Cosubstrate requirements, inhibition, and kinetics. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering [J. Environ. Sci. Health, Pt. A: Toxic/Hazard. Subst. Environ. Eng.].*(4): 574-571.

Nitroglycerin or glycerol trinitrate (GTN) is an energetic and toxic substance with a wide range of military and pharmaceutical applications. Studies conducted with activated sludge showed that GTN is amenable to aerobic degradation only in the presence of a primary carbon source, such as glucose. Kinetic experiments indicated that GTN is an inhibitory substrate whose presence during biodegradation, reduces substantially the microbial yield and the apparent maximum specific growth rate coefficient of primary substrates. However, in the range of concentrations tested (50 to 200 mg/l of GTN) its inhibitory effects are reversible. The biodegradation mechanism proceeds via a set of successive denitration reactions to form isomers of glycerol dinitrate (1,2-GDN and 1,3-GDN) and glycerol mononitrate (1-GMN and 2-GMN), which are subsequently degraded. Significant regioselectivity was observed during denitration of GTN and 1,2-GDN favoring production of 1,3-GDN and 1-GMN. The rates of degradation of the metabolic products of GTN were slower at each denitration step with 2-GMN exhibiting the lowest denitration rate. Aerobic GTN degradation ceased upon exhaustion of the primary substrate. Although, cosubstrate requirements during aerobic bioconversion of GTN were relatively high, in field applications the need for addition of external carbon sources will be minimal since GTN waste streams usually contain high amounts of BOD.

Christodoulatos, C., Bhaumik, S. and Brodman, B.W. 1997. Anaerobic biodegradation of nitroglycerin. *Water Research [WATER RES.].* 31(6): 1462-1470.

Nitroglycerin $(C_3H_5ONO_2)_3$ or glycerol trinitrate (GTN), a constituent of various propellant formulations and a vasodilator prescribed to treat angina pectoris and other heart diseases, was completely mineralized in serum vials under strict anaerobiosis by mixed cultures from an anaerobic digester. Anaerobic biodegradation occurred via successive denitration of the parent molecule and production of glycerol dinitrate (GDN), and glycerol mononitrate (GMN), which was converted to a utilizable carbon source, most likely glycerol. Both isomeric forms of GDN were detected, namely glycerol 1,3-dinitrate (1,3-GDN) and glycerol 1,2-dinitrate (1,2-GDN), which were converted to glycerol 1-mononitrate (1-GMN) and glycerol 2-mononitrate (2-GMN). Significant regioselectivity of the enzymatic degradation was observed with preferential attack at the secondary (C2) nitro group, which favored production of 1,3-GDN and 1-GMN. Addition of cosubstrates substantially enhanced the rates of

conversion of GTN; however, biodegradation also occurred in the absence of external carbon sources. The rates of biodegradation were significantly decreased at each successive denitration, with conversion of GMN to glycerol being the rate limiting step. The destruction of the GDN and GMN intermediates, which are more soluble than the parent compound, and possibly more toxic and mutagenic, is extremely important for the treatment of wastewater and soils contaminated with GTN. Nitrate and nitrite, the main nitrogen products of denitration, were also removed under anaerobic conditions.

Hempfling, C. 1997. Ultraviolet/oxidation treatment of explosive wastewaters using a commercial process. ***Environmental Progress [Environ. Prog.]. no.*** **3(164).**

The Indian Head Division, Naval Surface Warfare Center evaluated the performance of two pilot-scale ultraviolet/oxidation systems for treatment of nitroglycerin production wastewater, Solarchem Environmental Systems and Peroxidation Systems, Inc. A commercial-scale system was purchased from Solarchem and its performance compared to that of the pilot-scale system using standard reaction kinetics procedures. The first-order reaction rate expression for nitroglycerin decomposition was developed for the commercial-scale Solarchem system. Studies were conducted using the commercial-scale UV/oxidation system to determine the treatment by-products from nitroglycerin decomposition. Nitroglycerin and partially nitrated derivatives of nitroglycerin, inorganic nitrates, nitrites, and ammonia, and total organic carbon analyses were conducted to determine the extent of nitroglycerin decomposition and probable by-products. Studies were also conducted on nitroguanidine wastewater to determine the effectiveness of UV/oxidation wastewater treatment for the nitroguanidine production process.

Rosser, S.J., French, C.E. and Bruce, N.C. 2001. Engineering plants for the phytodetoxification of explosives. ***In Vitro Cellular & Developmental Biology Plant [In Vitro Cell. Dev. Biol. Plant].*** **37(3): 330-333.**

Widespread contamination of the environment by explosives resulting from the manufacture, disposal and testing of munitions is becoming a matter of increasing concern. Most explosives are considered to be a major hazard to biological systems due to their toxic and mutagenic effects. Interest on the bioremediation of land contaminated with explosives has recently been focused on phytoremediation. Unfortunately, whilst plants have many advantages for the remediation of contaminated land and water, they lack the catabolic versatility which enables microorganisms to mineralize such a wide diversity of xenobiotic compounds. This raised the interesting question as to whether the impressive biodegradative capabilities of soil bacteria could be combined with the high biomass and stability of plants to yield an op-

timal system for in situ bioremediation of explosive residues in soil. Our investigation into the degradation of explosive residues by soil bacteria resulted in the isolation of *Enterobacter cloacae* PB2, which is capable of utilizing nitrate ester explosives such as pentaerythritol tetranitrate (PETN) and nitroglycerin as the sole source of nitrogen for growth. We have successfully introduced PETN reductase, the enzyme initiating explosive degradation in this organism, into plants to create transgenic plants that degrade explosives. Since the bacterial degradative pathways for many classes of organic pollutant have been elucidated, this may be a generally applicable method of achieving bioremediation of contaminated soil in the environment.

USACHPPM. 2001. Wildlife Toxicity Assessment for Nitroglycerin (NG). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01F.

Williams, R.E., Rathbone, D.A., Moody, P.C., Scrutton, N.S. and Bruce, N.C. 2001. Degradation of explosives by nitrate ester reductases. *Biochemical Society symposium.* 68: 143-153.

Explosive-contaminated land poses a hazard both to the environment and to human health. Microbial enzymes, either in their native or heterologous hosts, are a powerful and low-cost tool for eliminating this environmental hazard. As many explosives have only been present in the environment for 10 yrs, and with similar molecules not known in Nature, the origin of enzymes specialized for the breakdown of explosives is of particular interest. Screening of environmental isolates resulted in the discovery of flavoproteins capable of denitrating the explosives pentaerythritol tetranitrate (PETN) and glycerol trinitrate. These nitrate ester reductases are related in sequence and structure to Old Yellow Enzyme from *Saccharomyces carlsbergenisis*. All the members of this family have alpha/beta barrel structures and FMN as a prosthetic group, and reduce various electrophilic substrates. The nitrate ester reductases are, however, unusual in that they display activity towards the highly recalcitrant, aromatic explosive 2,4,6-trinitrotoluene, via a reductive pathway resulting in nitrogen liberation. We have embarked on a detailed study of the structure and mechanism of PETN reductase from a strain of *Enterobacter cloacae*. Work is focused currently on relating structure and function within this growing family of enzymes, with a view to engineering novel enzymes exhibiting useful characteristics.

Bioaccumulation and Trophic Transfer

King, S.Y. and Fung, H.L. 1984. Rapid microbial degradation of organic nitrates in rat excreta. Re-examination of the urinary and fecal metabolite profiles of pentaerythritol tetranitrate in the rat. *Drug Metab Dispos.* 12(3): 353-357.

The in vitro stabilities of three organic nitrates, viz. pentaerythritol tetranitrate (PETN), nitroglycerin (NTG), and isosorbide dinitrate (ISDN) in rat urine, and of PETN in rat feces were examined. PETN , NTG, and ISDN degraded completely in rat urine following incubation for 24 hr at either 25º or 37º C. Degradation of PETN , NTG, and ISDN was absent in sterilized urine under the same conditions. These data suggested that decomposition of organic nitrates in untreated urine was usually rapid and extensive, and was primarily of microbial origin. Stability of these organic nitrates could also be maintained when urine samples were stored in packed ice. Rapid and extensive microbial degradation of PETN was also found in rat fecal homogenates, suggesting the possibility of organic nitrate metabolism by intestinal microflora. The metabolic profiles of PETN in rat urine and feces following intra-arterial and oral dosing of this organic nitrate were then re-examined. The data confirmed a previous finding that in vivo PETN excretion in urine was minimal. However, contrary to data, which showed about 8% fecal recovery after oral dosing, our results suggested a smaller (2%) fecal PETN recovery with oral dosing. It appeared likely then that unabsorbed PETN might be further metabolized by gut flora.

Toxicity

Anderson, J.A., McGuire, E.J., Watkins, J.R., Fitzgerald, J.E. and de la Iglesia, F.A. 1983. Toxicology studies with a stable intravenous formulation of nitroglycerin. *J Appl Toxicol.* 3(3): 161-165.

The preclinical toxicologic profile of Nitrostat, a stable parenteral formulation of nitroglycerin, was determined in mice, rats, rabbits and dogs. Single-dose i.v. studies in rodents yielded LD_{50} values of 17.3 and 18.2 mg/kg-1 in male and female mice, and 24.4 and 23.2 mg/kg-1 in male and female rats, respectively. Subacute i.v. studies in rats at doses of 2.5, 5.0 and 10.0 mg/kg/day, and in dogs at doses 1.0 and 3.0 mg/kg/day for two wks, elicited minimal reactions. In rats, suppression of bodyweight gain and food consumption occurred among treated and vehicle-control animals. Mild tissue irritation at injection sites was noted in treated and vehicle-control groups. There were no clearly drug-related clinical or pathological findings in dogs. In rabbits, repeated intravenous administration of Nitrostat did not induce significant local venous irritation. The results of these studies indicated that the

stabilized parenteral formulation of nitroglycerin did not elicit unusual toxic properties in intravenous infusion studies.

Burton DT, SD Turley, GT Peters. 1993. Toxicity of Nitroguanidine, Nitroglycerin, Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX), and 2,4,6-Trinitrotoluene (TNT) to Selected Freshwater Aquatic Organisms. Maryland University, College Park. Agriculture Experiment Station. AD-A267 467.

Fehrenbach, A., Wittwer, T., Meyer, D., von Vietinghoff, S., Viehover, M., Fehrenbach, H., Richter, J. and Wahlers, T. 2001. Nitroglycerin alters alveolar type II cell ultrastructure after ischemia and reperfusion. *J Heart Lung Transplant.* 20(8): 876-888.

BACKGROUND: Although administration of nitric oxide (NO) has been suggested to reduce pulmonary reimplantation response, concerns remain about cytotoxic side effects. METHODS: Using light and electron microscopy, we examined the effects of the NO donor nitroglycerin (NTG) (0.1 mg/ml) as a supplement to the preservation solution Celsior on the structural integrity of rat lungs after extracorporeal ischemia (4 hours at 10º C) and reperfusion (50 min) (IR). We performed evaluation in comparison with Celsior alone after IR using either standard antegrade perfusion through the pulmonary artery or retrograde perfusion through the left atrium as an alternative way to improve the preservation quality. Untreated, non-ischemic lungs served as controls (n = 5 per group). We recorded respiratory and hemodynamic parameters during reperfusion. Tissue collection using systematic uniform random sampling was representative for the whole organ and allowed stereologic quantification of structures. RESULTS: After IR, histochemistry revealed no breaks in the alveolo-capillary barrier and we detected no alveolar flooding. Edema formed in the peribronchovascular cuffs, of which the volume fraction was increased (p =.008). Vasoconstriction of the smaller arteries accompanied antegrade flush, which occurred neither after administration of NTG nor after retrograde flush, as shown by immunostaining for alpha-smooth muscle actin. Treatment with NTG was associated with focal disintegration of Type II cells, which displayed edematous swelling of distinct cell compartments and lysis of mitochondria and cells. Nitroglycerin prevented alveolar collapse, which was increased in the other IR groups (p = 0.013). We observed alterations in intra-alveolar surfactant components. CONCLUSION: These findings indicate pathologic effects of NTG treatment on alveolar epithelial integrity. Therefore, we suggest further critical evaluation of NTG/NO for therapeutic use in lung transplantation.

Imoto, S., Nakao, M., Kuramoto, M., Takeuchi, M., Shimpo, K. and Tanabe, T. 1986a. Teratological test of 10% nitroglycerin (NT-1 ointment) in rabbits. *J Toxicol Sci.* 11 Suppl 2: 59-70.

A teratological test of 10% nitroglycerin (NT-1 ointment) was carried out in New Zealand White rabbits. Pregnant rabbits were treated percutaneously with NT-1 ointment from day 6 to 18 of gestation at dose levels of 15, 60 and 240 mg/kg/day as nitroglycerin itself. All pregnant rabbits were killed on day 29 of gestation, and the influences of NT-1 ointment upon the performances of dams and fetuses were examined. During the treatment period, erythema was observed on the treated dorsal skin in all groups excluding the nontreatment control group. However, it disappeared soon after the cessation of NT-1 ointment administration. Influences of NT-1 ointment administration were not found at any dose levels on the food consumption and body weight changes of pregnant rabbits. In addition, influences of NT-1 ointment on the reproductive performance of dams and the development of fetuses were not observed at any dose levels. Therefore, the non-effect dose of NT-1 ointment on reproductive performance of dams and fetal development in rabbits was estimated to be 240 mg/kg/day and more as nitroglycerin itself.

Imoto, S., Kuramoto, M., Iwabuchi, K., Nagai, H. and Shimpo, K. 1986b. Percutaneous chronic toxicity study of 10% nitroglycerin (NT-1 ointment) in rabbits. *J Toxicol Sci.* 11 Suppl 2: 31-57.

A chronic toxicity test of 10% nitroglycerin (NT-1 ointment) was carried out in male NZW rabbits. NT-1 ointment was applied to the back skin for 26 weeks at daily doses of 15, 60 and 240 mg/kg as nitroglycerin itself, and 5-week withdrawal period was followed. Topical dermal responses to NT-1: Macroscopically, erythema, edema, scales, papules and dermal thickening were observed in response to NT-1 ointment. In the withdrawal period, however, all of them disappeared. Histopathologically, thickening of epidermis and cell infiltration were observed in response to NT-1 ointment. In addition, elongation of rete ridges and Touton giant cells were found only in 60 and 240 mg/kg groups, and hyperkeratosis only in 240 mg/kg group. At the end of withdrawal period, Touton giant cells were still found in 60 and 240 mg/kg groups, although the other dermal reactions disappeared. Systemic responses to NT-1 ointment: A slight increase in the excretion of loose or mucous feces was observed in 240 mg/kg group. The weights of right and left kidneys were increased by the administration of 240 mg/kg NT-1 ointment, and a similar trend was seen also in the weight of heart, although there found no such among-group differences at the end of withdrawal period. White blood cells, especially neutrophils, and gamma-globulin fraction were increased in response to 240 mg/kg NT-1 ointment. In conclusion, a no-toxic effect dose of NT-1 ointment as nitroglycerin itself was considered to be 15 mg/kg/day for the skin and 60 mg/kg/day for the general somatic system.

Maragos, C.M., Andrews, A.W., Keefer, L.K. and Elespuru, R.K. 1993. Mutagenicity of glyceryl trinitrate (nitroglycerin) in Salmonella typhimurium. *Mutat Res.* 298(3): 187-195.

The recent finding that the clinical nitrovasodilator, glyceryl trinitrate (GTN), is mutagenic in *Salmonella typhimurium* strain TA1535 has been examined in closer detail, with emphasis on its mechanism of action. GTN increased the number of His+ revertants to a maximum of 4 times over background at a GTN dose of 5 mumol/plate. Hamster liver S9 depressed the toxicity of high GTN doses and increased the maximum number of revertants to 5 times over background at 10 mumol/plate. GTN did not cause significant reversion in any of the six other *S. typhimurium* strains tested (TA1975, TA102, TA1538, TA100, TA100NR, YG1026), although signs of toxicity were observed. Therefore, the mutagenicity of GTN was manifest only in the repair-deficient (uvrB and lacking in pKM101) strain which is responsive to single base changes. Oligonucleotide probe hybridization of TA1535 revertants showed that virtually all of the GTN-induced mutants contained C-->T transitions in either the first or second base of the hisG46 (CCC) target codon, with a preference for the latter. A similar mutational spectrum was seen previously with a complex of spermine and nitric oxide (NO) which releases nitric oxide. This suggests that NO, which can be derived from GTN via metabolic reduction, may be responsible for GTN's mutagenic action. The known NO scavenger oxymyoglobin did not substantially alter the dose response of GTN, indicating that extracellular NO was not mediating reversion. The data are consistent with the hypothesis that intracellular nitric oxide is responsible for the observed mutations.

Tamano, S., Ward, J.M., Diwan, B.A., Keefer, L.K., Weghorst, C.M., Calvert, R.J., Henneman, J.R., Ramljak, D. and Rice, J.M. 1996. Histogenesis and the role of p53 and K-ras mutations in hepatocarcinogenesis by glyceryl trinitrate (nitroglycerin) in male F344 rats. *Carcinogenesis.* 17(11): 2477-2486.

Glyceryl trinitrate (GTN) was previously reported to induce hepatocellular carcinoma (HCC) in rats after prolonged feeding. The present experiments were undertaken to evaluate the histogenesis and molecular biology of these tumors and the possible role of nitric oxide (NO), a GTN metabolite, in their development. Male F344 rats received a single i.g. intubation of GTN (1.2 g/kg) at 6 wks of age and/or a diet containing 1% GTN from 8 weeks of age until necropsy, i.e. for up to 78 wks. Some animals were subjected to 2/3 partial hepatectomy (PH) at 9 wks of age. Five sequential sacrifices (14, 32, 52, 78 and 84 wks of age) were performed. No liver tumors developed in control rats or in rats that received GTN only by a single i.g. intubation, even when intubation was followed by PH. Preneoplastic foci, mainly of clear cell and mixed cell type (identified as positive for glutathione S-transferase placental form) were found from 14 wks of age in rats receiving GTN in the diet. Fo-

cal eosinophilic areas (atypical foci) composed of atypical hepatocytes that often extended into the veins were observed beginning at 52 wks of age. Some mixed hepatocholangiocellular adenomas and carcinomas arose in eosinophilic lesions. HCCs were seen beginning at 78 wks of age, but only in rats receiving dietary GTN. Incidence of HCC in the latter animals was 50-75%. Most HCCs were well differentiated. The carcinogenic effect of GTN given in the diet was not affected by prior intubation of a large single dose followed by PH. No p53 mutations were found in 18 tumors but K-ras point mutations, all within codon 12, were found in 8/18 tumors, mostly those with cholangiocellular elements. These were first or second position G-->T transversions or second position G-->A transitions. While these mutation types have also been commonly seen in bacteria after NO-related DNA damage, the fact that tumors arose only on prolonged feeding of this potently bioactive agent at massive doses seems consistent with a more complex mechanism involving multiple (i.e., genetic and/or epigenetic) factors in carcinogenesis by GTN.

Taniguchi, Y., Shimada, K., Kohyama, H., Hamai, Y., Hirao, R., Nishimori, T., Kikumori, M. and Yamamoto, H. 1986. Percutaneous subacute toxicity study of 10% nitroglycerin (NT-1 ointment) in rabbits. *J Toxicol Sci.* 11 Suppl 2: 11-29.

NT-1 ointment is a compound containing 10% nitroglycerin which is topically applied to the skin for angina pectoris. The subacute toxicity of this compound was examined by the continuous application for 5 wks to the shaven skin of rabbit back at dose levels of 240, 120 and 60 mg/kg, and the recovery was examined 3 wks after withdrawal of the drug. No special skin response to the NT-1 ointment was observed other than an erythema of the grading 2-3 Draize points, while the control ointment, which was the base ointment without nitroglycerin, showed a higher grade of erythema equivalent to 4 Draize point with crusta formation. No relationship between the dose level of NT-1 ointment and the skin response was observed, and skin response was thought to be caused mainly by the base ointment. The skin response gradually reduced even during the application period, and the skin response disappeared within a few days after withdrawal of the ointment. Histological examination of the treated skin at the respective ends of the application and recovery periods showed thickening of the corneum and epithelial layer, round cell infiltration and fibrosis in the corium, and development of hair folliculi in the subdermis. No systemic effect of the topically applied NT-1 ointment was observed in the majority of the animals, either in behavior, hematologic and electrocardiographic examination, food consumption, body weight change or urinalysis.

Nitrophenol

2-Nitrophenol is produced by the catalytic hydrolysis of 2-nitrochlorobenzene with NaOH. Alternatively, 2-nitrophenol can be produced by the action of dilute HNO_3 on phenol with subsequent steam distillation for separation from 4-nitrophenol. 4-Nitrophenol can also be produced in one of two ways. Like 2-nitrophneol, it can be produced by the catalytic hydrolysis of 4-ntrochlorobenzene or alternatively by the reaction of dilute HNO_3 on phenol and subsequent steam distillation to separate the 4- from the 2- isomer.

Nitrophenols may be released into the environment during their use as intermediates and indicators. 2-Nitrophenol is used as an intermediate for the production of dyestuffs, pigments, rubber chemicals, and fungicides. It is used in small amounts as an acid-base indicator and as a reagent for glucose. 4-Nitrophenol is used for the production of marathion, methyl parathion, and N-acetyl-p-aminophenol. 4-Nitrophenol is also used in the parathion-containing insecticide, Thiophos, and in fungicide for military footwear. Also, both 2- and 4- isomers may be released in vehicular exhaust from both gasoline and diesel engines.

ATSDR. 1992. Toxicological Profile for Nitrophenols: 2-Nitrophenol, 4-Nitrophenol. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for nitrophenol.

Environmental Fate and Transport

Megharaj, M., Rao, A.S. and Venkateswarlu, K. 1993. Influence of rice straw amendment on persistence and algal toxicity of p-nitrophenol in soil. *Soil Biology and Biochemistry.* 25(9): 1185-1188.

A laboratory investigation was made of the persistence of p-nitrophenol (PNP) with or without rice straw amendment to soil under non-flooded and flooded conditions. PNP disappeared more rapidly in flooded soil than in non-flooded soil. Addition of rice straw increased the persistence of PNP in soil under flooded conditions. The

toxicity of PNP towards indigenous populations of microalgae and cyanobacteria was related to its persistence in soil.

Heim, K., Schuphan, I. and Schmidt, B. 1994. Behavior of [^{14}C]-4-nitrophenol and [^{14}C]-3,4-dichloroaniline in lab sediment-water systems. 1. Metabolic fate and partitioning of radioactivity. *Environmental Toxicology and Chemistry.* 13(6): 879-888.

Using a standard screening procedure, the fate of [^{14}C]-4-nitrophenol and [^{14}C]-3,4-dichloroaniline was studied in lab sediment-water systems; incubation intervals ranged from 4 hrs to 90 days. The sediments were collected from a creek, a pond, and a drainage ditch of a fruit-growing plantation, and were characterized. Both compounds and their transformation products were sorbed to the sediments in large quantities; radioactivity left in the water phase was below 11% after termination of the experiments. Besides minor amounts of $^{14}CO_2$, 3,4-dichloroaniline was converted to predominantly nonextractable residues, presumably without preceding microbial transformation of the 3,4-dichloroaniline moiety. Two main end products were observed in the 4-nitrophenol experiments, namely nonextractable residues and $^{14}CO_2$. Data obtained from the sediment exhibiting highest microbial activity probably indicated a further mineralization of 4-nitrophenol-derived sediment-bound residues. On the whole, 4-nitrophenol presumably shows low persistence and accumulation in sediment-water systems, whereas due to its stability the toxicity of sediment-sorbed 3,4-dichloroaniline should be examined.

Haghighi-Podeh, M.R. and Bhattacharya, S.K. 1996. Fate and toxic effects of nitrophenols on anaerobic treatment systems. 18. Biennial Conference of the International Association on Water Quality, (Singapore), 23-28 Jun 1996.

Modern industrial societies discharge vast quantities of chemicals into the environment. Removal of priority pollutants is a vital task of increasing importance. A literature search shows that more quantitative information about the effects and fates of these pollutants during anaerobic treatment is needed. The objective of this research was to study the fate and toxic effects of nitrophenols (2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol) on methanogenic bacteria. Both batch and continuous flow experiments were performed. Batch anaerobic toxicity assay (ATA) were performed in methanogenic enrichment cultures. The effects of 4-nitrophenol were also studied using chemostats at 15 day solids retention time. Stock acetate enrichment cultures were developed and used in this research. An inhibition coefficient model was used to quantify the effects of 4-nitrophenol. The competitive inhibition coefficient model can adequately describe the fate of the systems exposed to 4-nitrophenol. The results of this research showed that about 81%

of 4-nitrophenol can be removed by biodegradation. Among the nitrophenols studied, 4-nitrophenol and 2,4-dinitrophenol were most toxic.

Uberoi, V. and Bhattacharya, S.K. 1997. Toxicity and degradability of nitrophenols in anaerobic systems. *Water Environment Research.* 69(2): 146-156.

Nitrophenols are among the most important and versatile industrial organic compounds and are widely used in the chemical industry and are listed as priority pollutants by the U.S. EPA. The toxic effects and degradability of three selected nitrophenols (2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol) in anaerobic acetate and propionate enrichment systems were studied using batch serum bottles. The toxicity to both propionate- and acetate-fed systems decreased in the following order: 2,4-dinitrophenol > 4-nitrophenol > 2-nitrophenol. An up-flow fixed film, acetate-fed reactor was able to acclimate to 20 mg/L of 2- and 4-nitrophenol showing a 95% removal of each nitrophenol. The effect of biomass was studied using serum bottles for anaerobic toxicity assays with the design volatile suspended solids (VSS) concentrations of 500, 1000, and 1500 mg/l. With higher VSS the toxic effects of nitrophenols on methanogenesis were less severe. Nitrophenols were more inhibitory to acetate utilization than propionate utilization. Under anaerobic conditions 2-nitrophenol and 2,4-dinitrophenol were transformed both abiotically and biotically to 2-aminophenol and 2-amino,4-nitrophenol, respectively. The presence of propionate in propionate enrichment culture enhanced the removal rates of all three nitrophenols studied.

Bioaccumulation and Trophic Transfer

Shafer, W.E. and Schonherr, J. 1985. Accumulation and transport of phenol, 2-nitrophenol, and 4-nitrophenol in plant cuticles. *Ecotoxicol Environ Saf.* 10(2): 239-252.

Partition (K) and permeance (P) coefficients have been determined for phenol, 2-nitrophenol, and 4-nitrophenol with isolated cuticles from mature tomato (*Lycopersicon*) and green pepper (*Capsicum*) fruits and from the adaxial surface of rubber (*Ficus*) leaves. Plant cuticular membranes (CM) are composed of a lipophilic, insoluble polymer matrix (MX) membrane and soluble cuticular lipids (SCL). Partition coefficients of the phenols (pH 3.0) for the system MX/buffer (MX/b) ranged from 43.6 to 164.9 and could be predicted from n-octanol/buffer (o/b) partition coefficients using the equation $\log K_{MX/b} = 0.363 + 0.952 \log K_{o/b}$ where ($r = 0.986$). In CM the K values were lower, especially for 4-nitrophenol, ranging from 32.4 to 110.8. The role of hydrogen bonding in partitioning of phenols into cuticles is discussed. Permeance coefficients for the cuticular membranes [P(CM)] ranged from 10^{-10} (*Ficus*) to 10^{-8}

m/sec (*Lycopersicon*, *Capsicum*), with 2-nitrophenol permeating more rapidly than the other two phenols. Extraction of the SCL increased the permeance coefficients [P(MX)] by factors of approximately 5 (*Lycopersicon*), 50 (*Capsicum*), and 1000 (*Ficus*), respectively. The transport-limiting layer in plant cuticles acts as a diffusion and solubility barrier.

Pena-Egido, M.J., Marino-Hernandez, E.L., Santos-Buelga, C. and Rivas-Gonzalo, J.C. 1988. Urinary excretion kinetics of p-nitrophenol following oral administration of parathion in the rabbit. *Arch Toxicol.* 62(5): 351-354.

The urinary excretion kinetics of p-nitrophenol were studied in rabbits following oral administration of parathion at a dose of 3 mg/kg. Elimination of p-nitrophenol began rapidly, and of the total amount excreted during the study period, 46% was excreted in the first 3 hrs; 85% was excreted at 6 hrs after administration of the pesticide. The mean maximum excretion rate of p-nitrophenol was 111.15 +/- 61.02 µg/hr reached in a time of 0.77 +/- 0.26 hr. The formation and disappearance rate constants of the metabolite were 2.85 +/- 2.80 h-1 and 0.80 +/- 0.28 h-1, respectively. A linear relationship was observed between the plasma concentrations of parathion and the urinary excretion rate of p-nitrophenol.

Fischer, E., Rafiel, A. and Bojcsev, S. 1995. Intestinal elimination of p-nitrophenol in the rat. *Acta Physiol Hung.* 83(4): 355-362.

The intestinal metabolism and metabolite transport of p-nitrophenol (PNP), as a model compound have been investigated in an in vivo isolated intestinal loop preparation in the rat. Different PNP concentrations (20 microM, 100 microM and 500 microM) were recirculated to determine the formation and transport of PNP-metabolites (PNP-glucuronide: PNP-G and PNP-sulphate: PNP-S) in the jejunal loop. It was found that the jejunum of the rat was able to metabolize PNP rapidly and to transport the metabolites efficiently back into the luminal solution. About 21, 16 and 6% of recirculated amount of PNP could be detected in 90 min as PNP-G in the lumen of jejunal loop when 20, 100 or 500 microM PNP was perfused. These results show that the luminal appearance of PNP-G tended to saturability. Sulphate conjugate of PNP was undetectable in the intestinal lumen at 20 and 100 microM PNP concentrations and PNP-S amounted to 0.07% of recirculated amount of PNP when it was used in concentrations of 500 microM. These results indicate that the intestinal metabolism and the excretion of metabolites may play a role in the elimination of xenobiotics containing phenolic hydroxyl groups and that the small intestine of the rat forms predominantly PNP-G after luminal administration of PNP.

Frank, G. and Beyer, J. 1986. Metabolism of 3-nitrophenol by the frog Rana temporaria. *Xenobiotica* 16(4): 291-294.

Frogs (*Rana temporaria*) injected with 3-nitrophenol excreted 85-93% of the administered dose within 17 hrs; 70-90% dose was metabolized. Metabolites identified comprise 3-nitrophenyl glucuronide (57% dose), 3-nitrophenyl sulphate (24% dose), and 3-acetamidophenyl sulphate (2% dose). Traces of the following metabolites were found: 3-acetamidophenyl glucuronide, 3-acetamidophenol, 4-nitrocatechol, nitroquinol, 4-nitrocatechol sulphate and nitroquinol sulphate.

Gorge, G., Beyer, J. and Urich, K. 1987. Excretion and metabolism of phenol, 4-nitrophenol and 2-methylphenol by the frogs Rana temporaria and Xenopus laevis. *Xenobiotica* 17(11): 1293-1298.

1.) *Rana* and *Xenopus* excrete 90-95% dose, and metabolize 50-65% dose of phenol, 4-nitrophenol and 2-methylphenol within 24 hrs, to about the same extent. 2.) Kinetic data for the excretion of phenols from both species fit a two-compartment model. The elimination constants of *Rana* and *Xenopus* are not significantly different. 3.) Metabolism is mostly conjugation by glucuronidation and sulphation of the original phenols. Additionally, oxidations leading to dihydroxyphenols and benzoic acid from 2-methylphenol, and reduction of 4-nitrophenol occur, followed by conjugation. 4.) There is an important difference between the metabolite patterns of *Rana* and *Xenopus* in that the latter is unable to glucuronidate phenols. As the amount of metabolites produced is similar in both species. *Xenopus* compensates for its inability to glucuronidate by increasing other metabolites.

Toxicity

Brecken-Folse, J.A., Mayer, F.L., Pedigo, L.E. and Marking, L.L. 1994. Acute toxicity of 4-nitrophenol, 2,4-dinitrophenol, terbufos and trichlorfon to grass shrimp (Palaemonetes spp.) and sheepshead minnows (Cyprinodon variegatus) as affected by salinity and temperature. *Environmental Toxicology and Chemistry.* 13(1): 67-77.

The toxicities of two industrial chemicals (4-nitrophenol and 2,4-dinitrophenol) and two organophosphate insecticides (terbufos and trichlorfon) to juvenile grass shrimp (*Palaemonetes spp*.) and sheepshead minnows (*Cyprinodon variegatus*) were determined by static, 96 hr toxicity tests in a factorial design with 12 combinations of salinity and temperature (15, 20, 25, 30 ppt x 17, 22, 27º C). Concentrations of the toxicants, including bioconcentration, were determined as appropriate by gas or liquid chromatography and the use of ^{14}C-labeled compounds. The 96 hr LC_{50}s for 4-nitrophenol ranged from 12 to 31 mg/l and for 2,4-dinitrophenol from 13 to 50 mg/l.

Toxicity decreased as salinity increased for 4-nitrophenol and both test organisms. Toxicity decreased as salinity increased for 2,4-dinitrophenol and sheepshead minnows, but toxicity to grass shrimp increased as salinity increased. Toxicity decreased with increased temperature for grass shrimp exposed to 2,4-dinitrophenol and sheepshead minnows exposed to 4-nitrophenol, increased with temperature for sheepshead minnows exposed to 2,4-dinitrophenol, and no change was observed for grass shrimp exposed to 4-nitrophenol. Bioconcentration of phenols in both test organisms increased as concentration increased. The 96 hr LC_{50}s for terbufos ranged from 3.4 to 6.6 µg/l and for trichlorfon from 6.3 to 19,300 µg/l. Terbufos and trichlorfon toxicity to grass shrimp and sheepshead minnows increased with increased temperature. BCFs for terbufos were greater in sheepshead minnows than grass shrimp, but were reversed for trichlorfon.

Braunbeck, T., Storch, V. and Nagel, R. 1989. Sex-specific reaction of liver ultrastructure in zebra fish (Brachydanio rerio) after prolonged sublethal exposure to 4-nitrophenol. *Aquatic Toxicology.* 14(3): 185-202.

Morphological alterations of the liver of zebra fish (*Brachydanio rerio*) following prolonged exposure to 0.1, 1 and 5 mg/l 4-nitrophenol (4-NP) were investigated by means of light and electron microscopy. Based on a marked sexual dimorphism in control animals, liver reactions were both sex- and dose-dependent. Whereas at 0.1 mg/l only minor changes could be revealed, there were numerous structural modification at 1 mg/l: Whereas male fish primarily react with a proliferation of smooth endoplasmic reticulum, female fish display a high degree of fenestration within cisternae of the rough endoplasmic reticulum. Both sexes exhibit infiltration of macrophages and lymphocytes, an increase in the number of binucleate cells, of lysosomes, autophagosomes and myelinated bodies, but a depletion of hepatic glycogen. At 5 mg/l, deformations of the nuclear membrane and partial lysis of mitochondria could be observed. At 1 and 5 mg/l 4-NP in about 25% of the animals investigated showed symptoms of degenerative transformations of the liver tissue into huge multinucleate cell masses with completely different ultrastructure.

Hodson, P.V., Parisella, R., Blunt, B.R., Gray, B. and Kaiser, K.L.E. 1991. *Quantitative structure-activity relationships for chronic toxicity of phenol, p-chlorophenol, 2,4-dichlorophenol, pentachlorophenol, p-nitrophenol and 1,2,4-trichlorobenzene to early life stages of rainbow trout (Oncorhynchus mykiss).* Department of Fisheries and Oceans, Mont-Joli, Que. (Canada). Physical and Chemical Sciences Branch.

Rainbow trout (*Oncorhynchus mykiss*) were exposed to waterborne phenol, p-chlorophenol, 2,4-dichlorophenol, p-nitrophenol or 1,2,4-trichlorobenzene for 85 days. This period included full egg development from the day of fertilization, plus

hatching, yolk resorption and four weeks of feeding as freely-swimming fry. The primary effects of exposure were to reduce growth rate and to increase mortality rate. Growth inhibition was the most sensitive response since it occurred at exposure levels equal to or lower than those that increased mortality rates.

Linton, T.K., Mayer, F.L., Simon, T.L., Malone, J.A. and Marking, L.L. 1994. Salinity and temperature effects on chronic toxicity of 2,4-dinitrophenol and 4-nitrophenol to sheepshead minnows (Cyprinodon variegatus). ***Environmental Toxicology and Chemistry.*** **13(1): 85-92.**

Toxicity tests (28 day early-life-stage) were conducted to determine the effects of nine combinations of salinity (15, 20, 25 ppt) and temperature (22, 27, 32º C) on the toxicity of 2,4-dinitrophenol (2,4-DNP) and 4-nitrophenol (4-NP) to sheepshead minnows (Cyprinodon variegatus). The highest tested concentration having no observed effect (NOEC) on mortality and growth was derived weekly. The NOECs at test termination indicated that the survival and growth of fish exposed to 2,4-dinitrophenol were not significantly affected by salinity, temperature, or the salinity temperature interaction. However, 28 day NOECs of fish surviving from 4-nitrophenol exposures were significantly affected by temperature, but the highest value exceeded the lowest by only a factor of two. The overall data suggest that variations of salinity and temperature do not change the NOEC; only the exposure time required to attain the same NOEC is altered.

Howe, G.E., Marking, L.L., Bills, T.D., Rach, J.J. and Mayer, F.L., Jr. 1994b. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol and 2,4-dinitrophenol to the amphipod Gammarus pseudolimnaeus and rainbow trout (Oncorhynchus mykiss). ***Environmental Toxicology and Chemistry.*** **13(1): 51-66.**

Acute toxicity tests were conducted to determine (a) the individual and interactive effects of water temperature (7, 12, 17º C), pH (6.5, 7.5, 8.5, 9.5), and time on the toxicity of terbufos, trichlorfon, 4-nitrophenol, and 2,4-dinitrophenol to rainbow trout (*Oncorhynchus mykiss*) and the amphipod *Gammarus pseudolimnaeus*, and (b) the individual and interactive effects of water temperature and pH on chemical bioconcentration during acute tests with rainbow trout and *Gammarus* exposed to terbufos, 4-nitrophenol, and 2,4-dinitrophenol. The toxicity of all four chemicals was significantly affected by pH in all tests, except for *Gammarus* exposed to terbufos. The toxicity of terbufos to rainbow trout and *Gammarus* was less at pH 7.5 than at higher or lower pH. The toxicity of both nitrophenols decreased as pH increased, whereas the toxicity of trichlorfon increased with pH. The effect of pH on trichlorfon toxicity decreased with temperature. Temperature significantly affected the toxicity of all four chemicals to both species. Toxicity increased with temperature in all

tests, except for rainbow trout exposed to nitrophenols; toxicity decreased as temperature increased for rainbow trout. Chemical bioconcentration was also significantly affected by temperature and pH and was directly related to toxicity in most tests. Significant interactive effects between toxicity-modifying factors were also frequently observed. Temperature and pH effects on chemical toxicity need to be considered in chemical hazard assessment to ensure adequate protection of aquatic organisms.

Howe, G.E., Marking, L.L., Bills, T.D., Boogaard, M.A. and Mayer, F.L., Jr. 1994a. Effects of water temperature on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to developing rainbow trout (Oncorhynchus mykiss). ***Environmental Toxicology and Chemistry.*** **13(1): 79-84.**

Early-life-stage (ELS) toxicity tests were conducted to determine the effect of selected water temperatures on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to rainbow trout (*Oncorhynchus mykiss*). NOECs were determined for growth and mortality at selected time intervals and water temperatures of 7, 12, and 17º C. As tests progressed, NOECs leveled to constant time-independent values that were similar for tests at each temperature. In 4-nitrophenol tests, the time-independent NOEC values at 7, 12, and 17º C, respectively, were 1.16, 1.20, and 1.16 mg/l for growth and 3.40, 3.38, and 2.20 mg/l for mortality. For 2,4-dinitrophenol, time-independent NOEC values at 7, 12, and 17º C, respectively, were 1.07, 0.50, and 0.80 mg/l for growth and 1.30, 1.89, and 1.60 mg/l for mortality. Temperature did, however, affect the rate at which time-independent NOECs were reached. More time was required to reach time-independent NOECs as temperature decreased. For example, the time-independent NOEC in 4-nitrophenol tests at 17º C was reached in 14 days, whereas it required 42 days at 7º C.

Koizumi, M., Yamamoto, Y., Ito, Y., Takano, M., Enami, T., Kamata, E. and Hasegawa, R. 2001. Comparative study of toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats. ***J Toxicol Sci.*** **26(5): 299-311.**

The toxicities of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats was examined and the susceptibility of newborn rats was analyzed in terms of presumed unequivocally toxic and no observed adverse effect levels (NOAELs). In the 18-day repeated dose newborn rat study, 4-nitrophenol was orally given from day 4 to day 21 after birth but did not induce any toxicity up to 160 mg/kg in the main study, although it induced death in one of six males at 160 mg/kg, and three of six males and one of six females at 230 mg/kg in a prior dose-finding study. In the 28 day repeated dose oral toxicity study starting at 6 weeks of age, 4-nitrophenol caused the death of most males and females at 1,000 mg/kg but was not toxic at 400 mg/kg except for male rat-specific renal toxicity. As unequivocally toxic levels were considered to be

230 mg/kg/day in newborn rats and 600 to 800 mg/kg/day in young rats, and NOAELs were 110 mg/kg/day in newborn rats and 400 mg/kg/day in young rats, the susceptibility of the newborn to 4-nitrophenol appears to be 2.5 to 4 times higher than that of young animals. In the newborn rat study of 2,4-dinitrophenol, animals died at 30 mg/kg in the dose-finding study and significant lowering of body and organ weights was observed at 20 mg/kg in the main study. In the 28 day young rat study, clear toxic signs followed by death occurred at 80 mg/kg but there was no definitive toxicity at 20 mg/kg. As unequivocally toxic levels and NOAELs were considered to be 30 and 10 mg/kg/day in newborn rats and 80 and 20 mg/kg/day in young rats, respectively, the toxicity of 2,4-dinitrophenol in newborns again seems to be 2 to 3 times stronger than in young rats. Abnormalities of external development and reflex ontogeny in the newborn were not observed with either chemical. Based on these results, it can be concluded that the toxic response in newborn rats is at most 4 times higher than that in young rats, at least in the cases of 4-nitrophenol and 2,4-dinitrophenol.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L. and Miller, K. 2002. Toxicological and chemical assessment of ordinance compounds in marine sediments and porewaters. *Mar Pollut Bull.* 44(8): 789-806.

Toxicological and chemical studies were performed with a silty and a sandy marine sediment spiked with 2,6-dinitrotoluene (2,6-DNT), 2,4,6-trinitrophenylmethylnitramine (tetryl), or 2,4,6-trinitrophenol (picric acid). Whole sediment toxicity was analyzed by the 10 day survival test with the amphipod *Ampelisca abdita*, and porewater toxicity tests assessed macro-algae (*Ulva fasciata*) zoospore germination and germling growth, sea urchin (*Arbacia punctulata*) embryological development, and polychaete (*Dinophilus gyrociliatus*) survival and reproduction. Whole sediments spiked with 2,6-DNT were not toxic to amphipods. The fine-grained sediment spiked with tetryl was also not acutely toxic. The tetryl and picric acid LC_{50} values in the sandy sediment were 3.24 and 144 mg/kg dry weight, respectively. The fine-grained sediment spiked with picric acid generated a U-shaped concentration-response curve in the amphipod test, with increased survival both in the lowest and highest concentration. Grain-size distribution and organic carbon content strongly influenced the behavior of ordnance compounds in spiked sediments. Very low concentrations were measured in some of the treatments and irreversible binding and biodegradation are suggested as the processes responsible for the low measurements. Porewater toxicity varied with its sedimentary origin and with ordnance compound. The sea urchin embryological development test tended to be the least sensitive. Tetryl was the most toxic chemical in all porewater tests, and picric acid the least toxic. Samples spiked with 2,6-DNT contained a degradation product identified as 2-methyl-3-nitroaniline (also known as 2-amino-6-nitrotoluene), and unidentified peaks, possibly degradation products, were also seen in some of the picric

acid- and tetryl-spiked samples. Degradation products may have played a role in observed toxicity.

Pentaerythritol Tetranitrate (PETN)

Pentaerythritol Tetranitrate (PETN) is formed by nitration of pentaerythritol in nitric acid, followed by recrystalization in acetone (ICI 2004). PETN is primarily used by the military as a detonator charge for higher explosives such as TNT or as part of an explosives mixture (USACHPPM 2001). PETN has been widely replaced by RDX, which is less sensitive to shock and more chemically stable (Akhavan 2004). PETN has also been utilized as a vasodilator theraputic agent for treatment of angina pectoris (Bucher et al. 1990).

Akhavan J. 2004. The Chemistry of Explosives. The Royal Society of Chemistry. Online at: http://www.rsc.org/is/books/chem_expl.htm. Accessed January 21, 2004.

ICI Explosives, Inc. 2004. Fact Sheet No.55. Online at: http://www.slv2000.qc.ca/bibliotheque/centre_docum/protection/55_98_a.pdf. Accessed January 21, 2004.

USACHPPM. 2001. Wildlife Toxicity Assessment for Pentaerythritol Tetranitrate (PETN). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01G.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for pentaerythritol tetranitrate.

Environmental Fate and Transport

Binks, P.R., French, C.E., Nicklin, S. and Bruce, N.C. 1996. Degradation of pentaerythritol tetranitrate by Enterobacter cloacae PB2. *Applied and Environmental Microbiology.* 62(4): 1214-1219.

A mixed microbial culture capable of metabolizing the explosive pentaerythritol tetranitrate (PETN) was obtained from soil enrichments under aerobic and nitrogen-limiting conditions. A strain of *Enterobacter cloacae*, designated PB2, was isolated from this culture and was found to use PETN as a sole source of nitrogen for growth. Growth yields suggested that 2 to 3 mol of nitrogen was utilized per mol of

PETN. The metabolites pentaerythritol dinitrate, 3-hydroxy-2,2-bis-[(nitrooxy)-methyl]propanal, and 2,2-bis-[(nitrooxy)methyl]-propanedial were identified by mass spectrometry magnetic resonance. An NADPH-dependent PETN reductase was isolated from cell extracts and shown to liberate nitrite from PETN, producing pentaerythritol tri- and dinitrates which were identified by mass spectrometry. PETN reductase was purified to apparent homogeneity by ion-exchange and affinity chromatography. The purified enzyme was found to be a monomeric flavoprotein with a M_r of approximately 40,000, binding flavin mononucleotide noncovalently.

French, C.E., Nicklin, S. and Bruce, N.C. 1998. Aerobic degradation of 2,4,6-trinitrotoluene by Enterobacter cloacae PB2 and by pentaerythritol tetranitrate reductase. *Applied and Environmental Microbiology [Appl. Environ. Microbiol.].* 64(8): 2864-2868.

Enterobacter cloacae PB2 was originally isolated on the basis of its ability to utilize nitrate esters, such as pentaerythritol tetranitrate (PETN) and glycerol trinitrate, as the sole nitrogen source for growth. The enzyme responsible is an NADPH-dependent reductase designated PETN reductase. *E. cloacae* PB2 was found to be capable of slow aerobic growth with 2,4,6-trinitrotoluene (TNT) as the sole nitrogen source. Dinitrotoluenes were not produced and could not be used as nitrogen sources. Purified PETN reductase was found to reduce TNT to its hydride-Meisenheimer complex, which was further reduced to the dihydride-Meisenheimer complex. Purified PETN reductase and recombinant Escherichia coli expressing PETN reductase were able to liberate nitrogen as nitrite from TNT. The ability to remove nitrogen from TNT suggests that PB2 or recombinant organisms expressing PETN reductase may be useful for bioremediation of TNT-contaminated soil and water.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Nicklin, S., Binks, P.O., Bruce, N.H. and French, C.D. 1999. Detection and biodegradation of explosives, The Secretary of State for Defence in her Britannic Majesty's Government.

An enzyme capable of catalyzing the removal of nitrite from pentaerythritol tetranitrate (PETN) is provided. The enzyme (known as PETN reductase enzyme) is produced by culturing a novel strain of the *Enterobacter cloacae* bacterium isolated from nature. The strain designated PB2 has been deposited as NCIMB 40718. The amino acid sequence of the enzyme and the genetic sequence which encodes for this enzyme have also been determined. A PETN reductase enzyme encoded by the on

gene is provided. A method for producing PETN reductase enzyme in large quantities and methods of bioremediation using the enzyme so produced are also provided. Additionally there is provided a method of detecting the presence of PETN in a sample together with a biosensor for use in such a method.

Rosser, S.J., French, C.E. and Bruce, N.C. 2001. Engineering plants for the phytodetoxification of explosives. ***In Vitro Cellular & Developmental Biology Plant [In Vitro Cell. Dev. Biol. Plant].*** **37(3): 330-333.**

Widespread contamination of the environment by explosives resulting from the manufacture, disposal and testing of munitions is becoming a matter of increasing concern. Most explosives are considered to be a major hazard to biological systems due to their toxic and mutagenic effects. Interest on the bioremediation of land contaminated with explosives has recently been focused on phytoremediation. Unfortunately, whilst plants have many advantages for the remediation of contaminated land and water, they lack the catabolic versatility which enables microorganisms to mineralize such a wide diversity of xenobiotic compounds. This raised the interesting question as to whether the impressive biodegradative capabilities of soil bacteria could be combined with the high biomass and stability of plants to yield an optimal system for in situ bioremediation of explosive residues in soil. Our investigation into the degradation of explosive residues by soil bacteria resulted in the isolation of *Enterobacter cloacae* PB2, which is capable of utilizing nitrate ester explosives such as pentaerythritol tetranitrate (PETN) and nitroglycerin as the sole source of nitrogen for growth. We have successfully introduced PETN reductase, the enzyme initiating explosive degradation in this organism, into plants to create transgenic plants that degrade explosives. Since the bacterial degradative pathways for many classes of organic pollutant have been elucidated, this may be a generally applicable method of achieving bioremediation of contaminated soil in the environment.

Williams, R.E., Rathbone, D.A., Moody, P.C., Scrutton, N.S. and Bruce, N.C. 2001. Degradation of explosives by nitrate ester reductases. ***Biochemical Society symposium.*** **68: 143-153.**

Explosive-contaminated land poses a hazard both to the environment and to human health. Microbial enzymes, either in their native or heterologous hosts, are a powerful and low-cost tool for eliminating this environmental hazard. As many explosives have only been present in the environment for 10 yrs, and with similar molecules not known in nature, the origin of enzymes specialized for the breakdown of explosives is of particular interest. Screening of environmental isolates resulted in the discovery of flavoproteins capable of denitrating the explosives pentaerythritol tetranitrate (PETN) and glycerol trinitrate. These nitrate ester reductases are re-

lated in sequence and structure to Old Yellow Enzyme from *Saccharomyces carlsbergenisis*. All the members of this family have alpha/beta barrel structures and FMN as a prosthetic group, and reduce various electrophilic substrates. The nitrate ester reductases are, however, unusual in that they display activity towards the highly recalcitrant, aromatic explosive 2,4,6-trinitrotoluene, via a reductive pathway resulting in nitrogen liberation. We have embarked on a detailed study of the structure and mechanism of PETN reductase from a strain of *Enterobacter cloacae*. Work is focused currently on relating structure and function within this growing family of enzymes, with a view to engineering novel enzymes exhibiting useful characteristics.

Bioaccumulation and Trophic Transfer

King, S.Y. and Fung, H.L. 1984. Rapid microbial degradation of organic nitrates in rat excreta. Re-examination of the urinary and fecal metabolite profiles of pentaerythritol tetranitrate in the rat. *Drug Metab Dispos.* 12(3): 353-357.

The in vitro stabilities of three organic nitrates, viz. pentaerythritol tetranitrate (PETN), nitroglycerin (NTG), and isosorbide dinitrate (ISDN) in rat urine, and of PETN in rat feces were examined. PETN, NTG, and ISDN degraded completely in rat urine following incubation for 24 hrs at either 25 or 37º C. Degradation of PETN, NTG, and ISDN was absent in sterilized urine under the same conditions. These data suggested that decomposition of organic nitrates in untreated urine was usually rapid and extensive, and was primarily of microbial origin. Stability of these organic nitrates could also be maintained when urine samples were stored in packed ice. Rapid and extensive microbial degradation of PETN was also found in rat fecal homogenates, suggesting the possibility of organic nitrate metabolism by intestinal microflora. The metabolic profiles of PETN in rat urine and feces following intra-arterial and oral dosing of this organic nitrate were then re-examined. The data confirmed a previous finding that in vivo PETN excretion in urine was minimal. However, contrary to data which showed about 8% fecal recovery after oral dosing, our results suggested a smaller (2%) fecal PETN recovery with oral dosing. It appeared likely then that unabsorbed PETN might be further metabolized by gut flora.

Posadas del Rio FA, FJ Juarez, RC Garcia. 1988. Biotransformation of organic nitrate esters in vitro by human liver, kidney, intestine, and blood serum. *Drug Metab Disposition* 16(3):477-481.

The biotransformation of glycerol trinitrate (GTN), isosorbide dinitrate (ISD), pentaerythritol tetranitrate (PETN), erythritol tetranitrate (ETN), and mannitol hex-

anitrate (MHN) by extracts from human liver, small intestine mucosa, kidney, and blood serum was investigated. The glutathione-dependent organic nitrate ester reductase activity of the intestinal mucosa was 21, 4, 4, and 2 times higher than the liver activity for ISD, PETN, GTN, and ETN, respectively. The liver enzymatic activity for MHN was 35% higher than the intestinal activity and 56% higher than kidney enzyme activity. The order of increasing enzymatic rates was: ISD = PETN < GTN < ETN < MHN in the intestinal mucosa; ISD < PETN < GTN < ETN < MHN in the liver; and ISD < PETN = GTN < ETN < MHN in the kidney. Human serum also metabolized these organic nitrates at lower rates than the studied organs. Thus, the serum specific activities were 1/5 for MHN, 1/30 for ETN, 1/40 for GTN, 1/44 for ISD, and 1/2000 for PETN of the activity present in kidney.

Toxicity

Bucher, J.R., Huff, J., Haseman, J.K., Eustis, S.L., Lilja, H.S. and Murthy, A.S. 1990. No evidence of toxicity or carcinogenicity of pentaerythritol tetranitrate given in the diet to F344 rats and B6C3F1 mice for up to two years. *J Appl Toxicol.* 10(5): 353-357.

Toxicology and carcinogenesis studies of pentaerythritol tetranitrate (PETN), an organic nitrate used in explosives and as a therapeutic agent for angina pectoris, were conducted by administering diets containing PETN,NF (National Formulary Grade, a 1:4 mixture of PETN and lactose) to both sexes of F344 rats and B6C3F1 mice in 14 day, 13 wk and 2 yr studies. PETN was found to be essentially non-toxic in 14 day and 13 wk studies at dietary concentrations as high as 10,000 ppm; the weight gain of female rats was lower than that of controls at 5000 and 10,000 ppm in the 13 wk study. In the 13 wk studies, one in ten high-dose female rats had an adenoma of the Zymbal gland and one in ten high-dose female mice had a hepatocellular adenoma. Dietary concentrations chosen for the 2 yr studies were 5000 and 10,000 ppm for male rats and male and female mice, and 1240 and 2500 ppm for female rats. In the 2 yr studies, there were no adverse effects on survival or body weight gains in either sex of rats or mice. No neoplastic or non-neoplastic lesions were considered to be related clearly to PETN administration. Neoplasms of the Zymbal gland occurred at low incidences in PETN-exposed groups of both sexes of rats in the 2 yr study.

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology.* 28(2): 229-235.

Aqueous samples containing various explosives, their reduced metabolites, as well as related compounds were subjected to the luminescent bacterium *Vibrio fischeri*

NRRL-B-11177 to determine their ecotoxicological potential. As the most important parameter, the EC_{50} values of 24 test compounds were calculated. The EC_{50} value means the concentration of a chemical compound that is needed to reduce bacterial luminescence by 50%. According to the widely accepted classification scheme of Strupp et al. (1990) and in consideration of an incubation period of 30 min (Deutsche Einheitsverfahren zur Wasser-, Abwasser-, und Schlammuntersuchung-Testverfahren mit Wasserorganismen; Gruppe L; DIN 38412, L34; DEV 1991) TNT, 26DNT, 2A6NT, 4A2NT, 34DNT, TNB, TNBA, TETRYL and HEXYL must be classified in the category "very toxic to aquatic organisms"; 2A46DNT, 4A26DNT, 24DA6NT, 24DNT, 2A4NT, RDX, HMX and PETN must be classified in the category "toxic to aquatic organisms"; and 26DA4NT, TAT, TNPh, 26DAT, 24DAT, HMT and NQ can be classified in the category "less toxic to aquatic organisms". EC_{50} values after 30, 60, and 90 min of incubation of the test compounds are presented and discussed. For many of the compounds tested in this study, there are no, or only a few, toxicological data in the literature available.

Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX)

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is prepared by two methods: the continuous Bachmann process and direct nitration of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). The continuous Bachmann process involves reacting hexamine with nitric acid, ammonium nitrate, glacial acetic acid, and acetic anhydride followed by recrystallization (ATSDR 1995). The direct nitration of HMX to form RDX has a smaller yield.

Military application of RDX include as a base charge for detonation of higher explosives or as a primary component of an explosive mixture (ATSDR 1995). Civilian uses of RDX include fireworks, demolition blocks, heating fuel, and occasionally as a rodenticide (ATSRD 1995, Anon 1992).

Anon. 1992. RDX. *Dangerous Properties of Industrial Materials Report.* 12(2): 248-256.

RDX is used in the manufacture of explosives and rat poisons. An overview of its toxicological parameters is presented with attention given to oral toxicity thresholds.

ATSDR. 1995. Toxicological Profile for RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine). Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for RDX.

Environmental Fate and Transport

Adrian, N.R. and Chow, T. 2001. Identification of hydroxylamino-dinitroso-1,3,5-triazine as a transient intermediate formed during the anaerobic biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Environ Toxicol Chem.* 20(9): 1874-1877.

The metabolic fate of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in a mixed culture incubated under methanogenic conditions was studied. Analysis by high-performance liquid chromatography (HPLC) confirmed the loss of RDX and the formation of mono-, di-, and trinitroso-RDX as transient biodegradation intermediates. An additional peak observed in the HPLC chromatograms was identified by liquid chromatography-mass spectrometry as hydroxylamino-dinitroso-1,3,5-triazine. This is the first report identifying hydroxylamino-dinitroso-1,3,5-triazine as a transient intermediate produced during the anaerobic biodegradation of RDX.

Beller, H.R. 2002. Anaerobic biotransformation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by aquifer bacteria using hydrogen as the sole electron donor. *Water Res.* 36(10): 2533-2540.

RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) is a nitramine explosive that has contaminated soil and groundwater at military installations throughout the U.S. Although anaerobic RDX metabolism has been reported, the process is not well understood, as past studies have typically involved complex, undefined media with multiple potential electron donors and acceptors. In this study, bacteria enriched from RDX-contaminated aquifer sediments consumed RDX in a defined, bicarbonate-buffered, anaerobic medium containing hydrogen as the sole electron donor and RDX as a potential electron acceptor and sole nitrogen source. RDX was not consumed in live controls that did not contain hydrogen. Transient formation of mononitroso- and dinitroso-RDX metabolites (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine and hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine, respectively) was documented by liquid chromatography-mass spectrometry. However, studies with ^{14}C-labeled RDX suggested that mineralization to carbon dioxide was negligible (<2%), which is consistent with cometabolic transformation. Several lines of evidence suggest that the RDX-transforming bacteria under study were homoacetogens, including correlations between RDX consumption and acetate production. Methanogens were unlikely to be responsible for RDX metabolism, as the presence of 2-bromoethanesulfonate, an inhibitor of methanogenesis, did not appear to affect RDX

metabolism. The presence of nitrate reversibly halted RDX metabolism, whereas ammonium had no discernible effect, which implies that: (i) nitrate, which commonly occurs in RDX-contaminated groundwater, may inhibit in situ RDX metabolism, and (ii) although RDX may act as both a nitrogen source and cometabolic electron sink, the latter role predominates, as RDX reduction will proceed regardless of whether or not a more favorable nitrogen source is present.

Best, E.P., Sprecher, S.L., Larson, S.L., Fredrickson, H.L. and Bader, D.F. 1999. Environmental behavior of explosives in groundwater from the Milan Army Ammunition Plant in aquatic and wetland plant treatments. Uptake and fate of TNT and RDX in plants. *Chemosphere.* 39(12): 2057-2072.

Uptake and fate of TNT and RDX by three aquatic and four wetland plants were studied using hydroponic, batch, incubations in explosives-contaminated groundwater amended with [U-^{14}C]-TNT or [U-^{14}C]-RDX in the laboratory. Substrates in which the plants were rooted were also tested. Plants and substrates were collected from a small-scale wetland constructed for explosives removal, and groundwater originated from a local aquifer at the Milan Army Ammunition Plant, TN. This study demonstrated rapid uptake of [U-^{14}C]-TNT derived ^{14}C, concentration at the uptake sites and limited transport in all plants. Per unit of mass, uptake was higher in submersed than in emergent species. Biotransformation of TNT had occurred in all plant treatments after 7-day incubation in 1.6 to 3.4 mg TNT L-i, with labeled amino-dinitrotoluenes (ADNTs), three unidentified compounds unique for plants, and mostly polar products as results. Biotransformation occurred also in the substrates, yielding labeled ADNT, one unidentified compound unique for substrates, and polar products. TNT was not recovered by HPLC in plants and substrates after incubation. Uptake of [U-^{14}C]-RDX derived ^{14}C in plants was slower than that of TNT, transport was substantial, and concentration occurred at sites where new plant material was synthesized. As for TNT, uptake per unit of mass was higher in submersed than in emergent species. Biotransformation of RDX had occurred in all plant treatments after 13-day incubation in 1.5 mg RDX L-1, with one unidentified compound unique for plants, and mostly polar products as results. Biotransformation had occurred also in the substrates, but to a far lower extent than in plants. Substrates and plants had one unidentified ^{14}C-RDX metabolite in common. HPLC analysis confirmed the presence of RDX in most plants and in three out of four substrates at the end of the incubation period.

Best, E.P., Miller, J.L. and Larson, S.L. 2001. Tolerance towards explosives, and explosives removal from groundwater in treatment wetland mesocosms. *Water Sci Technol.* 44(11-12): 515-521.

A short-term study was performed to determine the feasibility of using constructed wetlands to remove explosives from groundwater, and to assess accumulation of parent explosives compounds and their known degradation compounds in wetland plants. Tolerance towards explosives in submersed and emergent plants was screened over a range of 0 to 40 mg/l^{-1}. Tolerance varied per compound, with TNT evoking the highest, 2NT the lowest, and 24DNT, 26DNT, and RDX an intermediate growth reducing effect. Submersed plants were more sensitive to TNT than emergent ones. A small-scale 4-month field study was carried out at the Volunteer Army Ammunition Plant, Chattanooga, TN. In this surface-flow, modular system, the influent contained high levels (>2.1 mg/l^{-1}) of TNT, 2,4DNT, 2,6DNT, 2NT, 3NT, and 4NT, and the HRT was 7 days. The performance criteria of U.S. EPA treatment goals for local discharge of 2,4DNT concentration <0.32 mg/l^{-1}, and 26DNT concentration <0.55 mg/l^{-1} were not met at the end of the experiment, although explosives levels were greatly reduced. Low levels of 2ADNT and 4ADNT were transiently observed in the plant biomass. Results of two other, older, constructed wetlands, however, indicated that in these systems treatment goals were met most of the time, residues of explosives parent compounds and known degradation compounds in plant tissues were low and/or transient, and in substrates were low.

Bhadra, R., Wayment, D.G., Williams, R.K., Barman, S.N., Stone, M.B., Hughes, J.B. and Shanks, J.V. 2001. Studies on plant-mediated fate of the explosives RDX and HMX. *Chemosphere.* 44(5): 1259-1264.

The fate of the explosives RDX and HMX on exposure to plants was investigated in 'natural' aquatic systems of Myriophyllum aquaticum for 16 days, and in axenic hairy root cultures of *Catharanthus roseus* for > or = 9 weeks. Exposure levels were: HMX, 5 mg/l; and RDX, approximately 8 mg/l. Exposure outcomes observed include: HMX, no transformation by aquatic plants, and minimal biological activity by axenic roots; and RDX, removal by both plant systems. In the case of RDX exposure to axenic roots, since ^{14}C-RDX was included, removal was confirmed by the accumulation of ^{14}C-label in the biomass. The intracellular ^{14}C-label in these RDX studies was detected in two forms: intact RDX and bound unknown(s).

Bhushan, B., Halasz, A., Spain, J., Thiboutot, S., Ampleman, G. and Hawari, J. 2002. Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-tiazine catalyzed by a NAD(P)H: nitrate oxidoreductase from Aspergillus niger. ***Environ Sci Technol.*** **36(14): 3104-3108.**

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) can be efficiently mineralized with anaerobic domestic sludge, but the initial enzymatic processes involved in its transformation are unknown. To test the hypothesis that the initial reaction involves reduction of nitro group(s), we designed experiments to test the ability of a nitrate reductase (EC 1.6.6.2) to catalyze the initial reaction leading to ring cleavage and subsequent decomposition. A nitrate reductase from Aspergillus niger catalyzed the biotransformation of RDX most effectively at pH 7.0 and 30 degrees C under anaerobic conditions using NADPH as electron donor. LC/MS (ES-) chromatograms showed the formation of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and methylenedinitramine as key initial products of RDX, but neither the dinitroso neither (DNX) nor trinitroso (TNX) derivatives were observed. None of the above detected products persisted, and their disappearance was accompanied by the accumulation of nitrous oxide (N2O), formaldehyde (HCHO), and ammonium ion (NH4+). Stoichiometric studies showed that three NADPH molecules were consumed, and one molecule of methylenedinitramine was produced per RDX molecule. The carbon and nitrogen mass balances were 96.14% and 82.10%, respectively. The stoichiometries and mass balance measurements supported a mechanism involving initial transformation of RDX to MNX via a two-electron reduction mechanism. Subsequent reduction of MNX followed by rapid ring cleavage gave methylenedinitramine which in turn decomposed in water to produce quantitatively N_2O and HCHO. The results clearly indicate that an initial reduction of a nitro group by nitrate reductase is sufficient for the decomposition of RDX.

Burton, D.T. and Turley, S.D. 1995. Reduction of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) toxicity to the cladoceran Ceriodaphnia dubia following photolysis in sunlight. ***Bull Environ Contam Toxicol.*** **55(1): 89-95.**

Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. ***Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).*** **Weston Roy F., Inc., West Chester, PA (USA).**

The primary objective of this task was to determine the feasibility of using GAC to treat ground water contaminated by explosives at the Milan Army Ammunition Plant (MAAP) in Milan, TN. Laboratory GAC isotherm studies were conducted and two carbons, Atochem, Inc. GAC 830 and Calgon Filtrasorb 300, were selected for further testing in continuous flow GAC columns. Three pilot scale continuous flow

GAC column tests were performed at MAAP using the two carbons selected from the laboratory GAC isotherm studies. The results from the laboratory and pilot studies are presented in this report. They show that concurrent removal of explosives such as TNT, RDX, HMX, Tetryl, and nitrobenzene from ground water using continuous flow granular activated carbon is feasible.

Fournier, D., Halasz, A., Spain, J., Fiurasek, P. and Hawari, J. 2002. Determination of key metabolites during biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine with Rhodococcus sp. strain DN22. *Appl Environ Microbiol.* 68(1): 166-172.

Rhodococcus sp. strain DN22 can convert hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to nitrite, but information on degradation products or the fate of carbon is not known. The present study describes aerobic biodegradation of RDX (175 microM) when used as an N source for strain DN22. RDX was converted to nitrite (NO_2^-) (30%), nitrous oxide (N_2O) (3.2%), ammonia (10%), and formaldehyde (HCHO) (27%), which later converted to carbon dioxide. In experiments with ring-labeled [$^{(15)}$N]-RDX, gas chromatographic/mass spectrophotometric (GC/MS) analysis revealed N_2O with two molecular mass ions: one at 44 Da, corresponding to $^{14}N^{14}NO$, and the second at 45 Da, corresponding to $^{15}N^{14}NO$. The nonlabeled N_2O could be formed only from $-NO_2$, whereas the ^{15}N-labeled one was presumed to originate from a nitramine group (^{15}N-$^{14}NO_2$) in RDX. Liquid chromatographic (LC)-MS electrospray analyses indicated the formation of a dead end product with a deprotonated molecular mass ion [M-H] at 118 Da. High-resolution MS indicated a molecular formula of $C_2H_5N_3O_3$. When the experiment was repeated with ring-labeled [^{15}N]-RDX, the [M-H] appeared at 120 Da, indicating that two of the three N atoms in the metabolite originated from the ring in RDX. When [U-^{14}C]-RDX was used in the experiment, 64% of the original radioactivity in RDX incorporated into the metabolite with a molecular weight (MW) of 119 (high-pressure LC/radioactivity) and 30% in $^{14}CO_2$ (mineralization) after 4 days of incubation, suggesting that one of the carbon atoms in RDX was converted to CO_2 and the other two were incorporated in the ring cleavage product with an MW of 119. Based on the above stoichiometry, we propose a degradation pathway for RDX based on initial denitration followed by ring cleavage to formaldehyde and the dead end product with an MW of 119.

Harvey, S.D., Fellows, R.J., Cataldo, D.A. and Bean, R.M. 1991. Fate of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants. *Environmental Toxicology and Chemistry.* 10(7): 845-855.

Soils amended with (^{14}C)hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) were sampled over 60 days and subjected to exhaustive Soxhlet extraction followed by HPLC

analysis. RDX was the only radiolabeled compound observed in soil extracts. Emission of volatile organics and $^{14}CO_2$ from soil accounted for only 0.31% of the amended radiolabel. Mass balance for RDX-amended soil was better than 84% throughout the two-month study. The analytical method developed for plants involved acid hydrolysis, solvent extraction, fractionation on Florisil) adsorbent and separation by HPLC. The described methodology allowed for RDX recovery of 86 plus or minus 3% from fortified bush bean leaf tissue. Further experiments were conducted with bush bean plants maintained on RDX-containing hydroponic solutions. Hydroponic plants did not emit detectable amounts of $^{14}CO_2$ or radiolabeled volatile organics.

Hawari, J., Beaudet, S., Halasz, A., Thiboutot, S. and Ampleman, G. 2000. Microbial degradation of explosives: biotransformation versus mineralization. *Appl Microbiol Biotechnol.* 54(5): 605-618.

The nitroaromatic explosive 2,4,6-trinitrotoluene (TNT) is a reactive molecule that biotransforms readily under both aerobic and anaerobic conditions to give aminodinitrotoluenes. The resulting amines biotransform to give several other products, including azo, azoxy, acetyl and phenolic derivatives, leaving the aromatic ring intact. Although some Meisenheimer complexes, initiated by hydride ion attack on the ring, can be formed during TNT biodegradation, little or no mineralization is encountered during bacterial treatment. Also, although the ligninolytic physiological phase and manganese peroxidase system of fungi can cause some TNT mineralization in liquid cultures, little to no mineralization is observed in soil. Therefore, despite more than two decades of intensive research to biodegrade TNT, no biomineralization-based technologies have been successful to date. The non-aromatic cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) lack the electronic stability enjoyed by TNT or its transformed products. Predictably, a successful enzymatic change on one of the N-NO_2 or C-H bonds of the cyclic nitramine would lead to a ring cleavage because the inner C-N bonds in RDX become very weak (<2 kcal/mol). Recently this hypothesis was tested and proved feasible, when RDX produced high amounts of carbon dioxide and nitrous oxide following its treatment with either municipal anaerobic sludge or the fungus *Phanaerocheate chrysosporium*. Research aimed at the discovery of new microorganisms and enzymes capable of mineralizing energetic chemicals and/or enhancing irreversible binding (immobilization) of their products to soil is presently receiving considerable attention from the scientific community.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Lynch, J.C., Brannon, J.M. and Delfino, J.J. 2002. Dissolution rates of three high explosive compounds: TNT, RDX, and HMX. *Chemosphere.* 47(7): 725-734.

Incidental exposure to high explosive compounds can cause subtle health effects to which a population could be more susceptible than injury by detonation. Proper source characterization is a key requirement in the conduct of risk assessments. For nonvolatile solid explosives, dissolution is one of the primary mechanisms that controls fate and transport, resulting in exposure to these compounds remote from their source. To date, information describing dissolution rates of high explosives has been sparse. The objective of this study was to determine the dissolution rates of three high explosive compounds, 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), in dilute aqueous solutions as a function of temperature, surface area, and energy input. To determine each variable's impact on dissolution rate, experiments were performed where one variable was changed while the other two were held constant. TNT demonstrated the fastest dissolution rate followed by HMX and then RDX. Dissolution rate correlation equations were developed for each explosive compound incorporating the three aforementioned variables, independently, and collectively in one correlation equation.

Price, R.A., Pennington, J.C., Larson, S.L., Neumann, D. and Hayes, C.A. 2002. Uptake of RDX and TNT by agronomic plants. *Journal of Soil Contamination.* 11(3): 307-326.

Process wastes from ordnance loading have created groundwater and soil contamination at numerous U. S. Army sites. Some of these sites are slated for return to public use after remediation. Potential hazards associated with use of these sites for vegetable gardening after remediation to low-level residual contamination are a special concern. The objective of this study was to quantify plant uptake of explosives from contaminated soil and irrigation water. Greenhouse studies were conducted with corn, tomato, lettuce, and radish to determine uptake of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT) from contaminated soil and uptake of RDX from contaminated irrigation water. A mass balance study of tomato, lettuce, and radish was conducted in chambers using carbon-14 labeled RDX. All crops accumulated RDX from soils contaminated at 5.8 mg/kg^{-1}, a remediation goal based on a site-specific risk assessment. All edible plant tissues accumulated RDX from soil except corn kernels. TNT was detected only in corn stover. At 100 $\mu g/l^{-1}$ RDX in irrigation water, accumulation of RDX by plants was undetectable. These results suggest that human health hazards from ingestion of vegetables growing in soils contaminated with low levels of RDX be carefully considered in plans for future use of remediated sites.

Ringelberg, D.B., Reynolds, C.M., Walsh, M.E. and Jenkins, T.F. 2003. RDX loss in a surface soil under saturated and well drained conditions. *J Environ Qual.* 32(4): 1244-1249.

On military training ranges, low-order, incomplete detonations deposit RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) into surface soils. In this study, we evaluated RDX biodegradation in surface soils obtained from a military training range in Alaska. Two factors were compared: (i) soil water potential during the incubations; and (ii) the use of acetonitrile (ACN) as an RDX carrier to spike samples. Organic solvents have been used in laboratory studies to dissolve slightly water-soluble contaminants before addition to soil. We added ACN to obtain final soil ACN concentrations of 0 mg/kg^{-1} (0%), 1000 mg/kg^{-1} (0.1%) and 10,000 mg/kg^{-1} (1%). We then compared RDX attenuation in the soil under saturated and unsaturated conditions. RDX fell below the limit of detection within 3 wks of study initiation under the saturated condition. A maximum degradation rate of 0.15 mg $RDX/l^{-1}/d^{-1}$ was measured. Under the unsaturated condition, 42% of the original RDX was still present at study termination (5 wk). The addition of acetonitrile at 0.1 or 1.0% had no affect on RDX loss in the saturated soil. In the unsaturated soil, however, ACN at 1.0% inhibited RDX loss by as much as 25%. These findings indicate that soil water potential and carrier solvent concentrations can impact the rate and extent to which RDX is attenuated in a surface soil.

Selim, H.M., Xue, S.K. and Iskandar, I.K. 1995. Transport of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science.* 160(5): 328-339.

We investigated the fate and transport of explosives in soils. Transport experiments were conducted to describe the mobility of 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in a SWy-1 reference clay (bentonite mixed with sand) and two selected soils (Norwood and Kolin). Miscible displacement experiments in packed soil columns under steady flow were used. For the bentonite/sand column, TNT was highly mobile and fully reversible when methanol was used as the background solution. In contrast, with 0.005 M $Ca(NO_3)_2$ as the background solution, the TNT pulse was strongly retarded with as much as 50% of that applied remaining within the bentonite/sand, Norwood, or Kolin columns. Products of the transformation of TNT to 4-Am-DNT and other compound were identified in the effluent solution. A 7 day flow interruption during the TNT pulse application resulted in decreased TNT levels in the effluent solution. This decrease corresponded to a sudden increase in the 4-Am-DNT concentration in the effluent, with peak concentrations of 0.60 mg/ml. For RDX, only limited retention was observed in all columns. These findings are consistent with results from adsorption-desorption batch experiments. The TNT and RDX transport results were successfully described

by a nonlinear multireaction and transport model (MRTM), which accounted for equilibrium and kinetic (reversible and irreversible) retention mechanisms. However, efforts to describe RDX transport were more successful than efforts to describe TNT when independently determined (batch) parameters were used. The mobility of TNT, RDX, and other compounds from a contaminated soil obtained from a Louisiana Army Ammunition Plant (AAP) site was also investigated. A gradual release and subsequent movement of various contaminants, including HMX, TNT, RDX, TNB, 2-Am-DNT, and 4-Am-DNT, was observed. The leaching patterns were consistent with results from uncontaminated Kolin soil columns and reflected the affinity of contaminants during leaching in the AAP soil.

Sheremata, T.W., Halasz, A., Paquet, L., Thiboutot, S., Ampleman, G. and Hawari, J. 2001. The fate of the cyclic nitramine explosive RDX in natural soil. *Environmental science & technology.* 35(6): 1037-1040.

The sorption-desorption behavior and long-term fate of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was examined in sterilized and nonsterilized topsoil. Results of this study indicate that although RDX is not extensively sorbed by the topsoil (Ks(d) of 0.83 l/kg), sorption is nearly irreversible. Furthermore, there was no difference in the sorption behavior for sterile and nonsterile topsoil. However, over the longterm, RDX completely disappeared within 5 wks in nonsterile topsoil, and hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) metabolites formed in the aqueous phase. Over the same period, recovery of RDX from sterile topsoil was high (55-99%), and the nitroso metabolites were not detected. Only traces of RDX were mineralized to CO_2 and N_2O by the indigenous microorganisms in nonsterile topsoil. Of the RDX that was mineralized to N_2O, one N originated from the ring and the other from the nitro group substituent, as determined using N15 ring-labeled RDX. However, N_2O from RDX represented only 3% of the total N_2O that formed from the process of nitrification /denitrification.

Singh, J., Comfort, S.D., Hundal, L.S. and Shea, P.J. 1998. Long-term RDX sorption and fate in soil. *Journal of Environmental Quality.* 27(3): 572-577.

Soils at former munitions production facilities are often contaminated with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Contamination can be excessive and soils often contain precipitated or solid-phase RDX, resulting in soil solution concentrations at or near saturation. Sorption and long-term fate must be understood to predict RDX availability and develop remediation strategies. We characterized RDX sorption and availability in Sharpsburg (a fine, montmorillontic, mesic Typic Argiudoll) surface soil by equilibrating the soil with 32 mg RDX/l^{-1} (spiked with ^{14}C-labeled RDX) for 168 days; similar experiments were performed with contaminated

and uncontaminated subsurface soils. Surface soils exhibited rapid RDX sorption with 34% of the ^{14}C sorbed within 30 min. This sorbed fraction increased to only 37% at 168 days. During the 168 day equilibration, readily available RDX (sorbed RDX extractable with 3 mM $CaCl_2$ decreased from 75 to 52%, while potentially available RDX (acetonitrile-extractable) increased from 24 to 32%. Carbon-14 in the 0.5 M NaOH-extractable organic matter fraction increased from 0.8 (T = 30 min) to 3.8% (T = 168 days). Little ^{14}C was removed after eight extractions with 10% KOH in ethanol. Eight percent of the ^{14}C-label was unextractable (bound) residue at 168 days; no ^{14}C-bound residue formed in surface soil when solid-phase RDX was present in the equilibrating solution. Our experiments indicated limited RDX sorption and transformation in the Sharpsburg surface and subsurface soils. Most of the sorbed ^{14}C was potentially available for transport, indicating the importance of remediating RDX-contaminated soil to protect groundwater quality.

Thompson, P.L., Ramer, L.A. and Schnoor, J.L. 1999. Hexahydro-1,3,5-trinitro-1,3,5-triazine translocation in poplar trees. *Environmental Toxicology and Chemistry.* 18(2): 279-284.

This article evaluates the translocation of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in hybrid poplar trees (*Populus deltoides x nigra*, DN34) grown in hydroponic solutions. Mass balances with [U-^{14}C]RDX were used to assess RDX translocation. Up to 60% of the RDX uptaken by the tree accumulated in leaf tissues. Analysis of plant extracts by high-performance liquid chromatography equipped with radiochemical detection indicated that RDX was not significantly transformed during exposure periods of up to 7 days. The bioaccumulation of RDX may be an important concern for phytoremediation efforts.

Tucker, W.A., Murphy, G.J. and Arenberg, E.D. 2002. Adsorption of RDX to soil with low organic carbon: Laboratory results, field observations, remedial implications. *Soil and Sediment Contamination.* 11(6).

The purpose of this article is to review both laboratory and field observations of RDX adsorption to soils and to use those results to estimate the effects of a planned remedial action. Adsorption isotherms for RDX are generally observed to be linear and reversible. Statistical tests were performed to determine the relationship between Kd and various soil characteristics. A linear relationship between Kd and soil organic carbon was observed, as expected, but regression of Kd to organic carbon content indicated a non-zero intercept, suggesting that other sorbents may also be significant at low OC (e.g., < 0.5%). No other soil properties were significantly related to Kd so the mechanism of adsorption at low organic carbon was not determined. These results were used to interpret observations of RDX in the vadose zone at Milan Army Ammunition Plant (MAAP), TN. MAAP exhibits widespread soil con-

tamination by RDX. Depth to groundwater ranges from 40 to 80 ft. Unsaturated soils are fine grained near the surface, and sandy near the water table. RDX is concentrated in the upper 2 ft, where concentrations in some places exceed 1%. Subsurface concentrations are generally less than 50 mg/kg. The distribution of RDX in soil, soil moisture and groundwater, and soil physical testing data were interpreted using simple models. The distribution of RDX is consistent with the following conceptual model: Water containing RDX was discharged to the land surface (prior to 1983); Crystalline RDX remains in surface soil (remedial activities are ongoing); Infiltrating rainwater leaches RDX from surface soils; This leachate carries RDX through the deeper vadose zone, resulting in significant soil contamination throughout the full thickness of the vadose zone; these soils can generate leachate and adversely affect ground-water quality for many years to come.

Zhang, C. and Hughes, J.B. 2003. Biodegradation pathways of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Clostridium acetobutylicum cell-free extract. *Chemosphere.* 50(5): 665-671.

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), a military high explosive, is becoming an increasingly important pollutant in the U.S. The cleanup of RDX-contaminated soil and groundwater has been a serious challenge due to its recalcitrance in the environment. This study was conducted to determine the biodegradation kinetics of RDX by crude cell extract of *Clostridium acetobutylicum* (ATCC 824), and to examine whether this bacterium will carry out reductive transformation pathways similar to the transformation of 2,4,6-trinitrotoluene (TNT), 2,4- and 2,6-dinitrotoluenes (DNTs) we have reported previously. Batch studies on the anaerobic transformation of RDX were conducted in serum bottles with U-ring-^{14}C-RDX. RDX and its transformation products were quantified by HPLC and qualified by LC/MS interfaced to two soft ionization techniques -- an atmospheric pressure ionization and an electron spray ionization (API-ES). Results demonstrated that *C. acetobutylicum* is capable of transforming RDX with H_2 as the electron donor. The transformation followed a zero-order kinetics and the rates increased with increasing H_2. RDX was transformed into several polar intermediates that could not be separated by reverse-phase HPLC and its molecular ions were unstable under the condition of commonly used electron impact detector. Using a polar and water immiscible solvent (ethyl acetate) and the softer MS ionization techniques, mass spectroscopy detected the presence of several RDX derivatives including mononitroso-, monohydroxylamino-, mononitrosomonohydroxylamino-, monoamino-, diamino-, and triamino-compounds. The presence of hydroxylamino compounds is analogous to the transformation of TNT and DNTs we elucidated previously.

Bioaccumulation and Trophic Transfer

Little information was found regarding the bioaccumulation or trophic transfer of RDX. Several studies in the Environmental Fate and Transport section found that RDX would accumulate in plant tissues. Measured BCF values of 4.0 (catfish) and 5.9 (fathead minnow), indicate that bioconcentration in aquatic organisms is low (HSDB 2003). Talmage et al. (1999) found measured BCF values ranging from 1.5 (unspecified fish) to 11 (fathead minnow), also indicating potential for bioaccumulation in aquatic organisms is low.

Toxicity

Burton, D.T., Turley, S.D. and Peters, G.T. 1994b. The acute and chronic toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the fathead minnow (Pimephales promelas). *Chemosphere.* 29(3): 567-579.

The acute 96 hr LC_{50} for 15 to 17 day old fathead minnow (*Pimephales promelas*) exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in aqueous solution was 12.7 mg/l at 25º C. A 28 day early life stage (ELS) test with the fathead minnow produced a lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) based on growth (both wet and dry weight) of 2.4 and 1.4 mg/l, respectively. A review of the literature shows that the chronic toxicity of RDX to fathead minnow is similar when evaluated in ELS, partial life cycle, and complete life cycle tests. RDX is a high explosive used extensively by the military for various applications.

Burton, D.T., Turley, S.D. and Peters, G.T. 1994a. The toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the freshwater green alga Selenastrum capricornutum. *Water, Air, & Soil Pollution.* 76(3-4): 449-457.

RDX was not acutely toxic to the green alga *Selenastrum capricornutum* when tested at the solubility limit of the compound in algal assay media. A maximum reduction of 38% in cell density occurred after a 96 hr exposure to 36.7 mg/l; thus, an EC_{50} could not be determined. The lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) for the green alga were 4.8 and 0.5 mg/l (reduction in cell density), respectively, when chronic end points were used to analyze the data. A comparison of the data in this study with RDX toxicity data from the literature for the diatom Navicula pelliculosa, and the blue-green algae (*Cyanobacteria*), *Microcystis aeruginosa*, and *Anabeana flos-aquae* shows that *S. capricornutum* is the most sensitive to RDX. A U.S. Environmental protection Agency (EPA) numerical water quality criterion Final Plant Value based on the green alga data should protect the above algal groups.

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology.* 28(2): 229-235.

Aqueous samples containing various explosives, their reduced metabolites, as well as related compounds were subjected to the luminescent bacterium Vibrio fischeri NRRL-B-11177 to determine their ecotoxicological potential. As the most important parameter, the EC_{50} values of 24 test compounds were calculated. The EC_{50} value means the concentration of a chemical compound that is needed to reduce bacterial luminescence by 50%. According to the widely accepted classification scheme of Strupp et al. (1990) and in consideration of an incubation period of 30 min (Deutsche Einheitsverfahren zur Wasser-, Abwasser-, und Schlammuntersuchung-Testverfahren mit Wasserorganismen; Gruppe L; DIN 38412, L34; DEV 1991) TNT, 26DNT, 2A6NT, 4A2NT, 34DNT, TNB, TNBA, TETRYL and HEXYL must be classified in the category "very toxic to aquatic organisms"; 2A46DNT, 4A26DNT, 24DA6NT, 24DNT, 2A4NT, RDX, HMX and PETN must be classified in the category "toxic to aquatic organisms"; and 26DA4NT, TAT, TNPh, 26DAT, 24DAT, HMT and NQ can be classified in the category "less toxic to aquatic organisms". EC_{50} values after 30, 60, and 90 min of incubation of the test compounds are presented and discussed. For many of the compounds tested in this study, there are no, or only a few, toxicological data in the literature available.

Gogal, O.M., Jr., Johnson, M.S., Larsen, C.T., Prater, M.R., Duncan, R.B., Ward, D.L., Lee, R.B., Salice, C.J., Jortner, B. and Holladay, S.D. 2003. Dietary oral exposure to l,3,5-trinitro-1,3,5-triazine in the northern bobwhite (Colinus virginianus). *Environ Toxicol Chem.* 22(2): 381-387.

The potential risk to wildlife from exposure to explosives, including 1,3,5-trinitro-1,3,5-triazine (RDX), has been an issue at numerous U.S. military installations where these substances are found in soil and water. Presently, no data describing the effects of RDX exposure in avian species exist. Therefore, an acute lethal dose (ALD) and 14- and 90-d subchronic dietary exposures to RDX were evaluated in a species potentially present at many contaminated sites, i.e., the northern bobwhite (*Colinus virginianus*). The ALDs for females and males were 187 and 280 mg/kg, respectively. Data from the 14 day dietary trial suggested that RDX exposure inhibited food consumption, weight gain, and egg production. Dietary RDX exposure for 90 day produced a dose-dependant decreasing trend in total feed consumption, total egg production, and hen-housed production parameters. These collective data suggest that quail may respond differently to oral RDX exposure compared with mammals.

Gong, P., Hawari, J., Thiboutot, S., Ampleman, G. and Sunahara, G.I. 2001. Ecotoxicological effects of hexahydro-1,3,5-trinitro-1,3,5-triazine on soil microbial activities. *Environmental Toxicology and Chemistry.* 20(5): 947-951.

Although hexahydro-1,3,5-trinitro-1,3,5-triazine (also called RDX or hexogen) is a potentially toxic explosive compound that persists in soil, its ecotoxicological effects on soil organisms have rarely been assessed. In this study, two uncontaminated garden soils were spiked with 10 to 12,500 mg RDX/kg dry soil. Soil microbial activities, i.e., potential nitrification, nitrogen fixation, dehydrogenase, basal respiration, and substrate-induced respiration were chosen as bioindicators and were determined after 1-, 4-, and 12-weeks of exposure. Experimental results indicate that RDX showed significant inhibition (up to 36% of control) on indigenous soil microbial communities over the period of this study. All five bioindicators responded similarly to the RDX challenge. The length of exposure also affected the microbial toxicity of RDX, with 12-week exposure exerting more significant effects than the shorter exposure periods, suggesting that soil microorganisms might become more vulnerable to RDX when exposure is extended. The estimated lowest observable adverse effect concentration of RDX was 1,235 mg/kg. No biodegradation products of RDX were detected at all three sampling times. Compared with 2,4,6-trinitrotoluene (TNT), RDX is less toxic to microbes, probably because of its resistance to biodegradation under aerobic conditions, which precludes metabolic activation of nitro groups.

Jarvis, A.S., McFarland, V.A. and Honeycutt, M.E. 1998. Assessment of the effectiveness of composting for the reduction of toxicity and mutagenicity of explosive-contaminated soil. *Ecotoxicol Environ Saf.* 39(2): 131-135.

Composting is being developed as an economical method for remediating explosive-contaminated soils and has been found to reduce the concentrations of target contaminants such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). However, whether environmental safety is improved by composting can be determined only by assessing the effects of the treated material on living organisms. In this study, two bioassays, the Mutatox assay and the earthworm acute toxicity test, were used to evaluate the effectiveness of a pilotscale composting demonstration in reducing environmental hazard. Explosive-contaminated soil was collected from a military installation and amended for composting in two adiabatic reactors. The unamended soil was lethal to all exposed earthworms, as were both amended replicates, prior to composting. Serial dilutions of the finished composts with artificial soil had earthworm 14 day LC_{50} values of 35.7 and 100% finished compost: artificial soil. Extracts of the initial materials were also toxic to bacteria in the Mutatox assay. Dilutions of those extracts to sublethal concentrations revealed a

low level of mutagenicity. Extracts of the finished composts indicated reduced bacterial toxicity, but the mutagenicity was markedly increased by composting. The reduction in lethality reflected the attenuation of explosives caused by composting, as indicated by chemical analysis. However, the increased mutagenicity was a result that would not have been indicated by chemical analysis alone and is inferred to be the result of the formation of mutagenic metabolites of explosives during composting and their incomplete degradation.

Juck, D., Driscoll, B.T., Charles, T.C. and Greer, C.W. 2003. Effect of experimental contamination with the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine on soil bacterial communities. *FEMS Microbiology Ecology.* 43(2): 255-262.

The effect of contamination with the explosive hexahydro-1,3,5-trinitro- 1,3,5-triazine (RDX) on an indigenous soil bacterial community was examined in two uncontaminated loam soil columns possessing native grasses. One column was spiked twice with RDX crystals for a total RDX load of 1000 mg/kg soil. The reduced metabolite of RDX degradation, hexahydro-1-nitroso-3,5-dinitro- 1,3,5-triazine, was observed in the column leachate, suggesting anaerobic degradation of RDX. Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA from both contaminated and uncontaminated columns produced identical banding patterns which were stable over the course of the experimental period. The bacterial diversity remained high in the contaminated column, as determined by restriction fragment length polymorphism and rarefaction analyses of random 16S rDNA clones. These combined results suggested that long-term exposure to 1000 mg RDX/kg soil did not produce an observable effect on bacterial diversity or the numerically dominant members of the indigenous soil bacterial community.

Lachance, B., Robidoux, P.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. *Mutation Research Genetic Toxicology and Environmental Mutagenesis.* 444(1): 25-39.

The mutagenicity and toxicity of energetic compounds such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and of amino/nitro derivatives of toluene were investigated in vitro. Mutagenicity was evaluated with the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay. Cytotoxicity was evaluated using V79 and TK6 human lymphoblastic cells. For the TK6 and V79 assays, TNB and 2,4,6-triaminotoluene were more toxic than TNT, whereas RDX and HMX were without effect at their maximal aqueous solubility limits. The primary TNT metabolites (2-amino-4,6-dinitrotoluene, 4-

amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene) were generally less cytotoxic than the parent compound. The FT results indicated that TNB, TNT and all the tested primary TNT metabolites were mutagenic. Except for the cases of 4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene in the TA98 strain, addition of rat liver S9 resulted in either no effect, or decreased activity. None of the tested compounds were mutagenic for the V79 mammalian cells with or without S9 metabolic activation. Thus, the FT assay was more sensitive to the genotoxic effects of energetic compounds than was the V79 test, suggesting that the FT might be a better screening tool for the presence of these explosives. The lack of mutagenicity of pure substances for V79 cells under the conditions used in this study does not preclude that genotoxicity could actually exist in other mammalian cells. In view of earlier reports and this study, mutagenicity testing of environmental samples should be considered as part of the hazard assessment of sites contaminated by TNT and related products.

Levine, B.S., Furedi, E.M., Gordon, D.E., Burns, J.M. and Lish, P.M. 1981. Thirteen week toxicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fischer 344 Rats. *Toxicology Letters.* 8(4-5): 241-245.

Groups of Fischer 344 rats received hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the diet for up to 13 weeks at doses of 0, 1, 10, 30, 100, 300 or 600 mg/kg/day. Toxicological responses included slight reductions of food intake and body weight gain, hyperreactivity to approach, decreased serum triglyceride levels, marginal leukocytosis and mortality.

Levine, B.S., Furedi, E.M., Gordon, D.E., Barkley, J.J. and Lish, P.M. 1990. Toxic interactions of the munitions compounds TNT and RDX in F344 rats. *Fundamental and Applied Toxicology.* 15(2): 373-380.

The potential toxic interactions in F344 rats of the munitions compounds trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) were examined following their coadministration in the diet. Groups of 10 rats per sex received TNT at doses of 5 or 125 mg/kg/day, RDX at doses of 30, 100, or 300 mg/kg/day, and combinations thereof for 13 wks. Thirty rats per sex served as controls. Toxicologic endpoints included clinical observations, body weight, food consumption, hematology, clinical chemistry, organ weights, and tissue morphology. The major toxic effects following the dietary administration of TNT to rats included anemia, hypercholesterolemia, and hepatomegaly, splenomegaly, and testicular atrophy with their accompanying histologic lesions. RDX intoxication in rats included hypotriglyceridemia, behavioral changes, and mortality. Most of the toxic effects of these chemicals were partially antagonized following their coadministration.

Lotufo, G.R., Farrar, J.D., Inouye, L.S., Bridges, T.S. and Ringelberg, D.B. 2001. Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic invertebrates. ***Environmental Toxicology and Chemistry*** **20(8): 1762-1771.**

The toxicity of nitroaromatic (2,4-diaminonitrotoluene [2,4-DANT] and 1,3,5-trinitrobenzene [TNB]) and ^{14}C-labeled cyclonitramine compounds (hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX] and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazo-cine [HMX]) to the marine polychaete *Neanthes arenaceodentata* and the estuarine amphipod *Leptocheirus plumulosus* following 10 or 28 day exposures to spiked sediments was investigated. Organismal-level effects on survival, growth, and reproduction and cellular-level effects on apoptosis (programmed cell death) were evaluated. Because cyclonitramines have low affinity for sediment, overlying water was not exchanged in the RDX and HMX exposures. Nitroaromatics sorbed strongly to sediment, resulting in near complete resistance to solvent extraction. Cyclonitramines sorbed weakly to sediment, as more ^{14}C-activity was found in the overlying water than in the sediment at exposure termination. No significant decrease in survival or growth was observed with cyclonitramines at initial sediment concentrations as high as 1,000 µg/g. Survival was significantly affected by nitroaromatics at nominal sediment concentrations as low as 200 µg/g, with *L. plumulosus* being more sensitive than N. arenaceodentata. Growth was significantly decreased at sublethal concentrations of 2,4-DANT for *N. arenaceodentata*. Reproduction, measured only with *L. plumulosus*, was significantly decreased only in the highest RDX treatment and also in the lower TNB treatment. However, no decrease was observed in higher concentrations of TNB. Body burden at exposure termination was below detection limit (1 µg/kg) for all compounds. Significant inhibition of apoptosis was not accompanied by significant decreases in growth or reproduction. Because of its critical function in many biological processes, alterations in this endpoint may result in adverse effects on the organism and could be used as an early indicator of toxicity.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L., Miller, K. and Saepoff, S. 2001. Development of Marine Toxicity Data for Ordnance Compounds. ***Archives of Environmental Contamination and Toxicology.*** **41(3): 308-318.**

A toxicity database for ordnance compounds was generated using eight compounds of concern and marine toxicity tests with five species from different phyla. Toxicity tests and endpoints included fertilization success and embryological development with the sea urchin *Arbacia punctulata*; zoospore germination, germling length, and cell number with the green macroalga *Ulva fasciata*; survival and reproductive success of the polychaete *Dinophilus gyrociliatus*; larvae hatching and survival with the redfish (*Sciaenops ocellatus*); and survival of juveniles of the opossum shrimp

(*Americamysis bahia,* formerly *Mysidopsis bahia*). The studied ordnance compounds were 2,4- and 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2,4,6-trinitrophenylmethylnitramine (tetryl), 2,4,6-trinitro-phenol (picric acid), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The most sensitive toxicity test endpoints overall were the macroalga zoospore germination and the polychaete reproduction tests. The most toxic ordnance compounds overall were tetryl and 1,3,5-trinitrobenzene. These were also the most degradable compounds, often being reduced to very low or below-detection levels at the end of the test exposure. Among the dinitro- and trinitrotoluenes and benzenes, toxicity tended to increase with the level of nitrogenation. Picric acid and RDX were the least toxic chemicals tested overall.

Robidoux, P.Y., Svendsen, C., Caumartin, J., Hawari, J., Ampleman, G., Thiboutot, S., Weeks, J.M. and Sunahara, G.I. 2000. Chronic toxicity of energetic compounds in soil determined using the earthworm (Eisenia andrei) reproduction test. *Environmental Toxicology and Chemistry.* 7: 1764-1773.

Earthworm survival tests are commonly used in terrestrial ecotoxicology to assess the toxicity of compounds in soil. Earthworm (*Eisenia andrei*) reproduction tests were used to assess the sublethal and chronic effects of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5,7-triazacyclohexane (RDX). Effects on reproduction parameters (total number of cocoons, number of hatched cocoons, number of juveniles, juvenile biomass, and hatchability) were measured in TNT- and RDX-spiked artificial soil. For TNT, the lowest-observed-effect concentration (LOEC) was 110 mg/kg dry soil, and the no-observed-effect concentration (NOEC) was 55 mg/kg. For the RDX-spiked soil, the LOEC was 95 mg/kg dry soil, and the NOEC was <95 mg/kg. The growth of adult worms was also reduced when exposed to TNT-spiked soil at the highest tested concentration (881 mg/kg dry soil). Taken together, data analysis showed that the number of juveniles was strongly correlated with the number of cocoons but poorly correlated with the growth of adults. This information could permit one to optimize the application of the *Eisenia sp*. reproduction assay when used as a sublethal effect assessment tool for TNT- or RDX-contaminated soils.

Robidoux, P.Y., Hawari, J., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 2002a. TNT, RDX, and HMX decrease earthworm (Eisenia andrei) life-cycle responses in a spiked natural forest soil. *Archives of Environmental Contamination and Toxicology* 43(4): 379-388.

Sublethal and chronic toxicities of 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) on earthworm *Eisenia andrei* in a sandy forest soil were assessed. Various

reproduction parameters of fecundity (total and hatched number of cocoons, number of juveniles, and their biomass) were significantly decreased by TNT (> or = 58.8 +/- 5.1 mg/kg dry soil), RDX (> or = 46.7 +/- 2.6 mg/kg), and HMX (> or = 15.6 +/- 4.6 mg/kg). These effects occurred at much lower concentrations than those reported earlier using artificial soil preparations. Growth of adults was significantly decreased in the TNT-spiked natural soils at 136.2 +/- 25.6 mg/kg dry soil, the highest concentration having no significant mortality. In contrast, survival and growth were not significantly reduced at relatively high measured concentrations of RDX (167.3 mg/kg) and HMX (711.0 mg/kg). Although TNT, RDX, and HMX share a common life-cycle response (i.e., decreased juvenile counts), a number of differences related to other reproduction parameters (e.g., productivity of cocoons) was observed. These results indicate that the tested explosives do not support a common mechanism of toxicity, at least in the earthworm, probably due to differences in their physical-chemical properties as well as metabolites formed during exposure.

Simini, M., Wentsel, R.S., Checkai, R.T., Phillips, C.T., Chester, N.A., Major, M.A. and Amos, J.C. 1995. Evaluation of soil toxicity at Joliet Army Ammunition Plant. *Environmental Toxicology and Chemistry* 14(4): 623-630.

Environmental toxicity testing and chemical analyses of soil were performed as part of an ecological risk assessment at the Joliet Army Ammunition Plant (JAAP), Joliet, IL. Soils were collected from an area where munitions were loaded, assembled, and packed (area L7, group 1), and from an area where waste explosives were burned on unprotected soil (area L2). Control samples were collected from an adjacent field. Soil toxicity was determined using early seedling growth and vigor tests, earthworm survival and growth tests, and Microtox registered assays. Relative toxicity of soils was determined within each area based on statistical significance (p = 0.05) of plant and earthworm growth and survival, and the effective concentration at which luminescence of the bacterium *Photobacterium phosphoreum* was reduced by 50% (EC_{50}) in the Microtox assay. Samples were designated as having high, moderate, or no significant toxicity. Soil that had significant toxicity according to at least one test, and representative samples showing no toxicity, were analyzed for munitions via HPLC. Chemical residues found in soils were 2,4,6-trinitrotoluene (TNT); 1,3,5-trinitrobenzene (TNB); 2,4-dinitrotoluene (2,4-DNT); 2,6-dinitrotoluene; 2-amino-4,6-DNT; 4-amino-2,6-DNT; 1,3,5-trinitro-1,3,5-triazine (RDX); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). All soils with no significant toxicity were void of these chemicals. However, some soils void of munitions still showed toxicity that may have been caused by elevated levels of heavy metals. Linear regressions of toxicity test results vs. chemical concentrations showed that TNT and TNB accounted for most of the soil toxicity.

Steevens, J.A., Duke, B.M., Lotufo, G.R. and Bridges, T.S. 2002. Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to Chironomus tentans and Hyalella azteca: low-dose hormesis and high-dose mortality. *Environmental Toxicology and Chemistry* 21(7): 1475-1482.

The toxicity of the explosives 2,4,6-trinitrotoluene (TNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive [RDX]); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive [HMX]), was evaluated in spiked sediment with two freshwater invertebrates. The midge *Chironomus tentans* and the amphipod *Hyalella azteca* demonstrated significant toxic effects after exposure to TNT and its degradation products, 1,3,5-trinitrobenzene (TNB) and 2,4-diamino-6-nitrotoluene (2,4-DANT). Significant reductions in survival of *C. tentans* exposed to TNT, TNB, and 2,4-DANT were observed at nominal sediment concentrations as low as 200 mg/kg. Hyalella azteca was more sensitive to TNT, TNB, and 2,4-DANT than the midge, where significant reductions in survival were observed at nominal concentrations of 50, 100, and 200 mg/kg, respectively. Survival of the midge and the amphipod was unaffected after exposure to RDX or HMX at the highest concentrations of 1,000 and 400 mg/kg, respectively. Growth of the midge, measured as total weight, was significantly reduced by 2,4-DANT. However, significantly increased growth was observed after exposure to sublethal concentrations of RDX and HMX. Although significant reductions in amphipod survival were observed at high concentrations of TNB, growth was significantly increased at sublethal concentrations. The results of the current investigation suggest that organisms exposed to explosives at contaminated sites may be affected at concentrations less than 25 mg/kg through hormetic growth enhancement and at higher concentrations through increased mortality.

Talmage, S.S., Opresko, D.M., Maxwell, C.J., Welsh, C.J., Cretella, F.M., Reno, P.H. and Daniel, F.B. 1999. Nitroaromatic munition compounds: environmental effects and screening values. *Reviews of Environmental Contamination and Toxicology.* 161: 1-156.

Nitroaromatic compounds are potentially toxic and found at a number of U.S. Army Ammunition Plants and other military facilities. This review presents a summary and analysis of available data on eight nitroaromatic compounds including environmental concentrations, environmental fate and transport processes, and ecotoxicity and bioaccumulation for aquatic and terrestrial biota. For those groups of organisms for which there are sufficient data, ecological criteria and screening benchmarks were developed. Staff at Oak Ridge National Laboratory (ORNL) under a project jointly sponsored by the U.S. Army and the U.S. EPA developed these

criteria and screening benchmarks. The review discusses the methodologies for development of the screening criteria and benchmarks.

Trinitrophenylmethylnitramine (Tetryl)

Trinitrophenylmethylnitramine (Tetryl) is formed by dissolving dimethylaniline in an excess of concentrated sulfuric acid at temperature between 20-30º C. The resulting dimethylaniline sulfate is nitrated with a nitric and sulfuric acid mixture to produce 2,4-dinitrodimethylaniline and eventually crude tetryl. The crude tertyl is filtered, washed, and dissolved in acetone. The acetone is evaporated, yielding purified tetryl (ATSDR 1995).

Tetryl has been widely replaced by HMX for military uses (HSDB 2003). Military application of tetryl was primarily as a primer, detonator, or booster charge for less-sensitive explosives (ATSDR 1995).

ATSDR. 1995. Toxicological Profile for Tetryl (2,4,6-Trinitrophenyl-N-methylnitramine. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for tetryl.

Environmental Fate and Transport

Boopathy, R. 2000. Formation of aniline as a transient metabolite during the metabolism of tetryl by a sulfate-reducing bacterial consortium. *Curr Microbiol.* 40(3): 190-193.

A laboratory study was conducted to determine whether tetryl (2,4,6-trinitrophenylmethylnitramine) can be degraded by an anaerobic process. The results indicated that the metabolic conversion of tetryl to aniline is possible by a sulfate-reducing bacterial (SRB) consortium. This SRB consortium metabolized tetryl by co-metabolism with pyruvate as a growth substrate. For every mole of tetryl metabolized, 1 mole of aniline was produced, and the aniline was further metabolized. This metabolic conversion of tetryl is likely to be of value in the anaerobic treatment

of tetryl-contaminated soil and ground water, such as found at many military ammunition sites.

Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. *Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).* Weston Roy F., Inc., West Chester, PA (USA). [Contract DAAA15-88-D-0010.]

The primary objective of this task was to determine the feasibility of using GAC to treat ground water contaminated by explosives at the Milan Army Ammunition Plant (MAAP) in Milan, TN. Laboratory GAC isotherm studies were conducted and two carbons, Atochem, Inc. GAC 830 and Calgon Filtrasorb 300, were selected for further testing in continuous flow GAC columns. Three pilot scale continuous flow GAC column tests were performed at MAAP using the two carbons selected from the laboratory GAC isotherm studies. The results from the laboratory and pilot studies are presented in this report. They show that concurrent removal of explosives such as TNT, RDX, HMX, Tetryl, and nitrobenzene from ground water using continuous flow granular activated carbon is feasible.

Fuller, M.E., Kruczek, J., Schuster, R.L., Sheehan, P.L. and Arienti, P.M. 2003. Bioslurry treatment for soils contaminated with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl). *J Hazard Mater.* 100(1-3): 245-257.

Past and current DoD activities have resulted in the contamination of soil, sediment and groundwater with various explosive compounds. This research was undertaken to determine the effectiveness of a soil bioslurry process for remediation of soil with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl). A 99.9% reduction in tetryl concentrations (from 100,000 to below 100 mg/kg) was achieved in 180 to 200 days. A variety of process modifications (i.e., addition of fertilizer, microbial biomass, purging with nitrogen, etc.) that were performed during the course of the experiment did not increase the tetryl biodegradation rate beyond the rates of degradation without modifications. Subsequent batches of soil added as a 25% (v/v) replacement of the slurry were also degraded. These results indicate the potential for this process to remediate highly contaminated soils at many former and current ammunition manufacturing sites.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Shah, M.M. and Spain, J.C. 1996. Elimination of nitrite from the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) catalyzed by ferredoxin NADP oxidoreductase from spinach. *Biochem Biophys Res Commun.* 220(3): 563-568.

Nitroreductase enzymes generally catalyze the reduction of nitroaromatic compounds to the corresponding amines. In contrast, ferredoxin NADP oxidoreductase (FNR), glutathione reductase, xanthine oxidase, and cytochrome c reductase catalyze the NADPH dependent elimination of the nitramine nitro group from 2,4,6-trinitrophenylmethylnitramine to form N-methylpicramide (NMP). Nitrite elimination was inhibited under aerobic conditions. Our results suggest that under aerobic conditions, tetryl is enzymatically reduced to the nitroanion radical which is then involved in the reduction of molecular oxygen. Under anaerobic conditions, the radical is reduced to NMP and nitrite is eliminated.

Williams, R.T., Ziegenfuss, P.S., Mohrman, G.B. and Sisk, W.E. 1989. Composting of Explosives and Propellant Contaminated Sediments. *IN: Hazardous and Industrial Wastes: Proceedings of the 21st Mid Atlantic Industrial Waste Conference. Technomic Publishing Co., Inc., Lancaster, Pennsylvania.* 1989: 599-611.

Two field-scale demonstrations were conducted to investigate composting as a technology for remediating explosives and propellant contaminated sediments. Test sediments at the Louisiana Army Ammunition Plant contained approximately 76,000 parts per million of total explosives, including TNT (2,4,6 ,-trinitrotoluene) (66% of total explosive), 25% RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), 9% HMX (octahydro-1,3 ,5,7-tetranitro-1,3,5,7-tetraazocine), and 0.3% tetryl. The mixture that was composed consisted of straw/horse manure, alfalfa, horse feed, and sediment. Two 12 yd^3 piles were constructed, one was maintained at approximately 35º C and the second at approximately 55º C. After 22 wks, total explosives were reduced by 99% (from 17,872 to 74 ppm) in the thermophilic pile (55º C). Transformation products peaked in concentration at approximately 20 days and subsequently fell to near detection limits. At the Badger Army Ammunition Plant, test sediments contained approximately 18,000 ppm of nitrocellulose. Nitrocellulose was reduced from 13,086 ppm to 16 parts ppm after 101 days in a thermophilic pile.

Bioaccumulation and Trophic Transfer

Anusevicius, Z., Sarlauskas, J., Nivinskas, H., Segura-Aguilar, J. and Cenas, N. 1998. DT-diaphorase catalyzes N-denitration and redox cycling of tetryl. ***FEBS Lett.*** **436(2): 144-148.**

Rat liver DT-diaphorase (EC 1.6.99.2) catalyzed reductive N-denitration of tetryl (2,4,6-tri-nitrophenyl-N-methylnitramine) and 2,4-dinitrophenyl-N-methylnitramine, oxidizing the excess of NADPH. The reactions were accompanied by oxygen consumption and superoxide dismutase-sensitive reduction of added cytochrome c and reductive release of Fe2+ from ferritin. Quantitatively, the reactions of DT-diaphorase proceeded like single-electron reductive N-denitration of tetryl by ferredoxin:NADP+ reductase (EC 1.18.1.2) (Shah and Spain 1996), which was additionally checked up in this work. Thus, although reductive N-denitration of nitrophenyl-N-nitramines is a net two-electron (hydride) transfer process, DT-diaphorase catalyzed the reaction in a single-electron way. These data point out the possibility of single-electron transfer steps during obligatory two-electron (hydride) reduction of quinones and nitroaromatics by DT-diaphorase.

Toxicity

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. ***Archives of Environmental Contamination and Toxicology*** **28(2): 229-235.**

Aqueous samples containing various explosives, their reduced metabolites, as well as related compounds were subjected to the luminescent bacterium *Vibrio fischeri* NRRL-B-11177 to determine their ecotoxicological potential. As the most important parameter, the EC_{50} values of 24 test compounds were calculated. The EC_{50} value means the concentration of a chemical compound that is needed to reduce bacterial luminescence by 50%. According to the widely accepted classification scheme of Strupp et al. (1990) and in consideration of an incubation period of 30 min TNT, 26DNT, 2A6NT, 4A2NT, 34DNT, TNB, TNBA, TETRYL and HEXYL must be classified in the category "very toxic to aquatic organisms"; 2A46DNT, 4A26DNT, 24DA6NT, 24DNT, 2A4NT, RDX, HMX and PETN must be classified in the category "toxic to aquatic organisms"; and 26DA4NT, TAT, TNPh, 26DAT, 24DAT, HMT and NQ can be classified in the category "less toxic to aquatic organisms". EC_{50} values after 30, 60, and 90 min of incubation of the test compounds are presented and discussed. For many of the compounds tested in this study, there are no, or only a few, toxicological data in the literature available.

Hovatter, P.S., Talmage, S.S., Opresko, D.M. and Ross, R.H. 1997. Ecotoxicity of nitroaromatics to aquatic and terrestrial species at Army Superfund sites. The 1996 6th Symposium on Environmental Toxicology and Risk Assessment, Orlando, FL, USA, ASTM.

Nitroaromatic compounds, including 2, 4, 6-trinitrotoluene, RDX, HMX, tetryl, and associated degradation products, are released into the environment during the manufacturing, loading, packing, and assembling of munitions at U.S. Army Ammunition Plants. With the exception of 2A46DNT, nitroaromatic compounds are more toxic to freshwater fishes (LC_{50}s ranging from 0.4-32 mg/l) than to freshwater invertebrates (EC_{50}s ranging from 3-100 mg/l). Rainbow trout (*Onchorynchus mykiss*) were the most sensitive test species with LC_{50} values ranging from 0.43 to 6.4 mg/l for TNT, RDX, and HMX. These compounds produce negative effects on reproductive endpoints in terrestrial mammals at doses >1 mg/kg/day. Limited data indicate that nitroaromatics are not toxic to terrestrial plants (LOECs ranging from 25-100 µg/g in soil) and soil invertebrates (LOEC of 200 µg/g). Additional studies need to be undertaken to completely characterize the toxicity of these compounds; however, the criteria and screening benchmarks presented in this paper can be used to assess the risks to indigenous flora and fauna at Army Superfund sites.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L., Miller, K. and Saepoff, S. 2001. Development of Marine Toxicity Data for Ordnance Compounds. *Archives of Environmental Contamination and Toxicology* 41(3): 308-318.

A toxicity database for ordnance compounds was generated using eight compounds of concern and marine toxicity tests with five species from different phyla. Toxicity tests and endpoints included fertilization success and embryological development with the sea urchin *Arbacia punctulata*; zoospore germination, germling length, and cell number with the green macroalga *Ulva fasciata*; survival and reproductive success of the polychaete *Dinophilus gyrociliatus*; larvae hatching and survival with the redfish (*Sciaenops ocellatus*); and survival of juveniles of the opossum shrimp (*Americamysis bahia*, formerly *Mysidopsis bahia*). The studied ordnance compounds were 2,4- and 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2,4,6-trinitrophenylmethylnitramine (tetryl), 2,4,6-trinitro-phenol (picric acid), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The most sensitive toxicity test endpoints overall were the macroalga zoospore germination and the polychaete reproduction tests. The most toxic ordnance compounds overall were tetryl and 1,3,5-trinitrobenzene. These were also the most degradable compounds, often being reduced to very low or below-detection levels at the end of the test exposure. Among the dinitro- and trinitrotoluenes and benzenes, toxicity tended to in-

crease with the level of nitrogenation. Picric acid and RDX were the least toxic chemicals tested overall.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L. and Miller, K. 2002. Toxicological and chemical assessment of ordinance compounds in marine sediments and porewaters. *Mar Pollut Bull.* 44(8): 789-806.

Toxicological and chemical studies were performed with a silty and a sandy marine sediment spiked with 2,6-dinitrotoluene (2,6-DNT), 2,4,6-trinitrophenylmethylnitramine (tetryl), or 2,4,6-trinitrophenol (picric acid). Whole sediment toxicity was analyzed by the 10 day survival test with the amphipod Ampelisca abdita, and porewater toxicity tests assessed macro-algae (*Ulva fasciata*) zoospore germination and germling growth, sea urchin (*Arbacia punctulata*) embryological development, and polychaete (*Dinophilus gyrociliatus*) survival and reproduction. Whole sediments spiked with 2,6-DNT were not toxic to amphipods. The fine-grained sediment spiked with tetryl was also not acutely toxic. The tetryl and picric acid LC_{50} values in the sandy sediment were 3.24 and 144 mg/kg dry weight, respectively. The fine-grained sediment spiked with picric acid generated a U-shaped concentration-response curve in the amphipod test, with increased survival both in the lowest and highest concentration. Grain-size distribution and organic carbon content strongly influenced the behavior of ordnance compounds in spiked sediments. Very low concentrations were measured in some of the treatments and irreversible binding and biodegradation are suggested as the processes responsible for the low measurements. Porewater toxicity varied with its sedimentary origin and with ordnance compound. The sea urchin embryological development test tended to be the least sensitive. Tetryl was the most toxic chemical in all porewater tests, and picric acid the least toxic. Samples spiked with 2,6-DNT contained a degradation product identified as 2-methyl-3-nitroaniline (also known as 2-amino-6-nitrotoluene), and unidentified peaks, possibly degradation products, were also seen in some of the picric acid- and tetryl-spiked samples. Degradation products may have played a role in observed toxicity.

Reddy, T.V., Olson, G.R., Wiechman, B., Reddy, G., Torsella, J., Daniel, F.B. and Leach, G.J. 1999. Toxicity of tetryl (N-methyl-N,2,4,6-tetranitroaniline) in F344 rats. *International Journal of Toxicology* 18(2): 97-107.

The toxicity of tetryl (N-methyl-N,2,4,6-tetranitroaniline) in male and female F344 rats was evaluated after administration in the diet for 14 or 90 days. The 14 day study diet concentrations used were 0, 500, 1250, 2000, 2500, and 5000 ppm; the 90 day study diet concentrations were 0, 200, 1000, and 3000 ppm tetryl in the diet. The calculated average daily tetryl intake was 32.1, 82.5, 130.3, 178.9, and 374.4 mg/kg body weight (BW) for females and 31.8, 80.0, 121.0, 170.5, and 349.7 mg/kg

BW for males in the 14 day study. For the 90 day studies, the daily tetryl intake was 14.2, 68.8, and 199.0 mg/kg BW for females and 13.0, 62.4, and 179.6 mg/kg BW for males. In the 14 day study, there was a significant decrease in body weights (males), whereas relative (organ/body weight) liver and spleen (females), and kidney (males) weights were significantly increased in the 5000 ppm dose group. Hematological effects observed were decreased hemoglobin and hematocrit and an increased number of reticulocyts in females (2000 to 5000 ppm). Methemoglobin levels in males (2000 to 5000 ppm) and females (5000 ppm) and total blood protein and albumin levels in all groups of males and females (except 500 ppm) were significantly increased. Histopathological changes were observed in kidneys (deposition of cytoplasmic droplets) of all dose groups of male rats. In the subchronic (90 day) study, feed intake was reduced in all dose groups, but a significant decrease in terminal body weights was observed in females (1000 and 3000 ppm) and males (3000 ppm). An increase in the relative liver, kidney (1000-3000 ppm), and spleen (3000 ppm) weights were noted in both sexes. The hemoglobin content and red blood cell count were decreased whereas the reticulocyte count was elevated (3000 ppm) in both sexes at 45 and 90 days. Methemoglobin levels were increased in both sexes (1000 and 3000 ppm). Histopathological changes were noted in the spleen (pigment deposition and erythroid cell hyperplasia) of both sexes (3000 ppm) and kidneys (tubular degeneration and cytoplasmic droplets containing alpha-2-micro globulin) of male rats (1000 to 3000 ppm). A no observed adverse effect level (NOAEL) for both sexes was 13 mg/kg BW/day was determined.

Talmage, S.S., Opresko, D.M., Maxwell, C.J., Welsh, C.J., Cretella, F.M., Reno, P.H. and Daniel, F.B. 1999. Nitroaromatic munition compounds: environmental effects and screening values. ***Reviews of Environmental Contamination and Toxicology*** **161: 1-156.**

Nitroaromatic compounds are potentially toxic and found at a number of U.S. Army Ammunition Plants and other military facilities. This review presents a summary and analysis of available data on eight nitroaromatic compounds including environmental concentrations, environmental fate and transport processes, and ecotoxicity and bioaccumulation for aquatic and terrestrial biota. For those groups of organisms for which there are sufficient data, ecological criteria and screening benchmarks were developed. Staff at Oak Ridge National Laboratory (ORNL) under a project jointly sponsored by the U.S. Army and the USEPA developed these criteria and screening benchmarks. The review discusses the methodologies for development of the screening criteria and benchmarks.

Trinitrotoluene (TNT)

Trinitrotoluene (TNT) is formed by the nitration of toluene using of mixture of nitric and sulfuric acids (ATSDR 1995). The nitration is a three step process using increasing temperatures and mixed-acid concentrations, either completed in three individual steps or by continuous flow (ATSDR 1995).

TNT is used by the military as a high explosive, either alone or as part of a mixture. TNT is resistant to shock, making its use in firing shells and airborne demolition bombs (ATSDR 1995).

Commercial uses of TNT include as an explosive for mining or demolition, and as a chemical intermediate in the production of dyes and photographic chemicals (ATSDR 1995).

ATSDR. 1995. Toxicological Profile for 2,4,6-Trinitrotoluene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Physical-Chemical Properties

Table 2 (page 170) provides the physical-chemical properties for TNT.

Environmental Fate and Transport

Best, E.P., Sprecher, S.L., Larson, S.L., Fredrickson, H.L. and Bader, D.F. 1999. Environmental behavior of explosives in groundwater from the Milan Army Ammunition Plant in aquatic and wetland plant treatments. Uptake and fate of TNT and RDX in plants. *Chemosphere* 39(12): 2057-2072.

Uptake and fate of TNT and RDX by three aquatic and four wetland plants were studied using hydroponic, batch, incubations in explosives-contaminated groundwater amended with [U-^{14}C]-TNT or [U-^{14}C]-RDX in the laboratory. Substrates in which the plants were rooted were also tested. Plants and substrates were collected from a small-scale wetland constructed for explosives removal, and groundwater originated from a local aquifer at the Milan Army Ammunition Plant. This study demonstrated rapid uptake of [U-^{14}C]-TNT derived ^{14}C, concentration at the uptake sites and limited transport in all plants. Per unit of mass, uptake was higher in submersed than in emergent species. Biotransformation of TNT had occurred in all plant treatments after 7-day incubation in 1.6 to 3.4 mg TNT/l-i, with labeled amino-dinitrotoluenes (ADNTs), three unidentified compounds unique for plants, and mostly polar products as results. Biotransformation occurred also in the substrates, yielding labeled ADNT, one unidentified compound unique for substrates, and polar products. TNT

was not recovered by HPLC in plants and substrates after incubation. Uptake of [U-^{14}C]-RDX derived ^{14}C in plants was slower than that of TNT, transport was substantial, and concentration occurred at sites where new plant material was synthesized. As for TNT, uptake per unit of mass was higher in submersed than in emergent species. Biotransformation of RDX had occurred in all plant treatments after 13-day incubation in 1.5 mg RDX/l-1, with one unidentified compound unique for plants, and mostly polar products as results. Biotransformation had occurred also in the substrates, but to a far lower extent than in plants. Substrates and plants had one unidentified ^{14}C-RDX metabolite in common. HPLC analysis confirmed the presence of RDX in most plants and in three out of four substrates at the end of the incubation period.

Boopathy, R. and Kulpa, C.F. 1994. Biotransformation of 2,4,6-trinitrotoluene (TNT) by a Methanococcus sp. (strain B) isolated from a lake sediment. *Can J Microbiol.* 40(4): 273-278.

A mesophilic, irregular coccoid methanogen, which shows close resemblance to *Methanococcus sp*., was isolated from a sediment sample of St. Joseph Lake located in the University of Notre Dame campus. Formate or hydrogen plus carbon dioxide served as substrate for methanogenesis in a mineral salt medium. This organism was studied for its ability to metabolize 2,4,6-trinitrotoluene (TNT). The result showed that this isolate could transform 100 ppm of TNT within 40-60 days of incubation at 30º C. The main intermediate produced was 2,4-diamino-6-nitrotoluene. The TNT transformation rates were higher in cells grown in hydrogen plus carbon dioxide than in cells grown in formate. The isolate did not use acetate and methanol as sole source of carbon and energy. The organism had an optimal pH range of 6.8-7.2. The optimal growth conditions for this isolate are described.

Boopathy, R., Manning, J. and Kulpa, C.F. 1997. Optimization of environmental factors for the biological treatment of trinitrotoluene-contaminated soil. *Arch Environ Contam Toxicol.* 32(1): 94-98.

In earlier studies, soil bacteria present in a TNT-contaminated site removed 2,4,6-trinitrotoluene (TNT). In this study the optimum conditions for the most efficient removal of TNT is discussed. The results suggest that the soil bacterial consortium has an optimal pH range of 6-7. Maximum growth was observed at pH 7. However, the TNT removal rate was higher at pH 6. Studies of the effects of temperature showed that the bacterial consortium had maximum metabolic activity at 20 to 22º C (ambient temperature). At a higher temperature (37º C) the TNT removal rate dropped significantly. The consortium could not use TNT as a nitrogen source but required the addition of ammonium. Optimal growth occurred with 0.25 g/l of ammonium chloride. Growing cells removed TNT significantly faster rates than resting

cells or cell-free extract. The operation of soil slurry reactors with the optimal conditions suggested that TNT can be removed effectively from the contaminated sites. These environmental conditions established as optimal can be used to improve the efficiency of large-scale soil slurry reactors for the treatment of soil contaminated with TNT.

Breitung, J., Bruns-Nagel, D., Steinbach, K., Kaminski, L., Gemsa, D. and von Low, E. 1996. Bioremediation of 2,4,6-trinitrotoluene-contaminated soils by two different aerated compost systems. ***Appl Microbiol Biotechnol.*** **44(6): 795-800.**

Two composting systems were compared on a laboratory scale as a bioremediation technology for degradation or immobilization of 2,4,6-trinitrotoluene (TNT) in contaminated soils. The first compost was aerated from the beginning whereas the second compost was only aerated after an anaerobic prephase of 65 days. In the first compost system the TNT concentration declined rapidly by 92% but, at the end, TNT could be partially recovered. During the anaerobic prephase of the second compost system, TNT was almost completely converted to aminodinitrotoluenes, which during the subsequent aeration almost entirely disappeared. In addition, the second compost generated less toxic material than the first one as confirmed by inhibition of bioluminescence of Vibrio fischeri. These data show that microbiological TNT-degradation systems can be successfully designed which are prerequisite for an efficient bioremediation of contaminated soils.

Bruns-Nagel, D., Brettung, J., von Loew, E., Steinbach, K., Gorontzy, T., Kahl, M., Blotevogel, K.H. and Gemsa, D. 1996. Microbial transformation of 2,4,6-trinitrotoluene in aerobic soil columns. ***Applied and Environmental Microbiology.*** **62(7): 2651-2656.**

2,4,6-Trinitrotoluene (TNT)-contaminated soil material of a former TNT production plant was percolated aerobically in soil columns. Nineteen days of percolation with a potassium phosphate buffer supplemented with glucose or glucose plus ammonium sulfate caused an over 90% decline in the amount of extractable nitroaromatics in soils containing 70 to 2,100 mg TNT/kg (dry weight). In the percolation solution, a complete elimination of TNT was achieved. Mutagenicity and soil toxicity were significantly reduced by the percolation process. 4-N-Acetylamino-2-amino-6-nitrotoluene was generated in soil and percolation fluid as a labile TNT metabolite.

Carpenter, D.F., McCormick, N.G., Cornell, J.H. and Kaplan, A.M. 1978. Microbial transformation of ^{14}C-labeled 2,4,6-trinitrotoluene in an activated-sludge system. *Appl Environ Microbiol.* 35(5): 949-954.

The fate of ^{14}C-labeled 2,4,6-trinitrotoluene (TNT) in an activated-sludge system was investigated. No [^{14}C]TNT could be detected in the contents of an aerated reactor after 3 to 5 days of incubation. No significant $^{14}CO_2$ was formed, and the radioactivity was about equally divided between the floc and the supernatant. The radioactive carbon present in the microflora was mainly associated with the lipid and protein components, but the characteristic constituents of these compounds (e.g., fatty acids and amino acids) were not radioactive. The major part of the ^{14}C present in the lipid and protein fractions was found in precipitates that formed in both fractions. The solubility properties and infrared spectra of these precipitates suggested that they are macromolecular structures of the polyamide type formed by the reaction of TNT biotransformation products with lipids, fatty acids, and protein constituents of the microbial flora. This hypothesis is further supported by the correspondence of the infrared spectrum of the lipid precipitate with that of a model compound synthesized from TNT transformation products and lipid precursors. The resistance of these macromolecules to further biodegradation was paralleled by the reported resistance to microbial attack of polyamides containing similar linkages.

Cui, H., Hwang, H.M., Cook, S. and Zeng, K. 2001. Effect of photosensitizer riboflavin on the fate of 2,4,6-trinitrotoluene in a freshwater environment. *Chemosphere* 44(4): 621-625.

The effect of riboflavin (1 microM) on the fate of TNT (20 mg/l) in a natural water environment was studied. The relative contribution of photolysis, microbial assemblages and freshwater matrix to TNT degradation was examined. The rates, extent and products of TNT and riboflavin transformation were compared under different experimental conditions. It was found that riboflavin significantly enhanced the degradation of TNT in natural water environment. Thus it is a potentially useful photosensitizing agent for the treatment of TNT-contaminated surface water. Furthermore, in the presence of riboflavin, two new intermediates with max. absorption wavelength of 230 nm were found, demonstrating that transformation of TNT in the presence of riboflavin undergoes different pathways.

Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. *Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).* Weston Roy F., Inc., West Chester, PA (USA).

The primary objective of this task was to determine the feasibility of using GAC to treat ground water contaminated by explosives at the Milan Army Ammunition Plant (MAAP) in Milan, TN. Laboratory GAC isotherm studies were conducted and two carbons, Atochem, Inc. GAC 830 and Calgon Filtrasorb 300, were selected for further testing in continuous flow GAC columns. Three pilot scale continuous flow GAC column tests were performed at MAAP using the two carbons selected from the laboratory GAC isotherm studies. The results from the laboratory and pilot studies are presented in this report. They show that concurrent removal of explosives such as TNT, RDX, HMX, Tetryl, and nitrobenzene from ground water using continuous flow granular activated carbon is feasible.

Drzyzga, O., Bruns-Nagel, D., Gorontzy, T., Blotevogel, K.H. and von Low, E. 1999. Anaerobic incorporation of the radiolabeled explosive TNT and metabolites into the organic soil matrix of contaminated soil after different treatment procedures. *Chemosphere* 38(9): 2081-2095.

Four bioreactor designs were performed to evaluate the level of incorporation of ^{14}C-labeled 2,4,6-trinitrotoluene (TNT) and metabolites into the organic soil matrix of different anaerobically treated contaminated soils. The contaminated soils were amended with molasses slivers (80:20% per weight) as auxiliary substrate to enhance microbial activity. After 5 wks (bioreactors 1 and 2), 8 wks (bioreactor 3) and 12 wks (bioreactor 4) of anaerobic incubation, we determined 41%, 58%, 72%, and 54%, respectively, of the initially applied radioactivity immobilized in various soil fractions. After alkaline hydrolyses of the solvent-extracted soils, low quantities of radiolabel were found in the humic and fulvic acid fractions, whereas the bulk of ^{14}C activity was found to be strongly bound to the humin fraction (solid soil residues). The amounts of solvent extractable radioactivity were 53%, 40%, 16%, and 29% for bioreactors 1, 2, 3, and 4, respectively. The level of TNT transformation at the end of the experiments was within 90-94%. Regarding the results presented in this study, we can assume that there is the possibility of high incorporation levels of TNT metabolites into the soil organic matrix mediated by microbial cometabolism under strictly anoxic conditions.

Eriksson, J. and Skyllberg, U. 2001. Binding of 2,4,6-trinitrotoluene and its degradation products in a soil organic matter two-phase system. *J Environ Qual.* 30(6): 2053-2061.

The widely used explosive 2,4,6-trinitrotoluene (TNT) and its degradation products are of large environmental concern because of their toxic properties and high concentrations encountered in contaminated soils. Batch experiments were used to study TNT* (the sum of TNT and its degradation products) bonding to dissolved (DOM) and particulate (POM) soil organic matter. Reversed-phase high performance liquid chromatography (RP-HPLC) was used as a separation technique in combination with ^{14}C-labeled TNT to determine free TNT and TNT* bound to DOM. By use of dialysis we showed that DOM did not interfere with the HPLC analysis of free TNT. Depending on pH and total TNT concentration, the relative distribution of TNT* among water, POM, and DOM varied between 60 to 90, 10 to 30, and 0.5 to 6%, respectively, after 22 hrs of equilibration. The association of TNT* to DOM was strongly pH dependent and followed a nonlinear Langmuir isotherm. The association of TNT* to POM was less pH dependent and data were equally well fitted by linear and nonlinear isotherms. Particulate organic matter had 6.4 (pH 6.2) to 22 (pH 5.2) times greater capacity to bind TNT* than DOM, but the binding strength (the slope of the isotherm) was greater for DOM. The TNT degradation was enhanced with increasing concentration of soil organic matter, resulting in a stronger bonding of TNT* to DOM and POM. Based on our results, combined with other recent findings, we suggest that it is mainly the degradation products of TNT that associate with DOM and POM, and that the association with DOM is mainly of ionic character involving specific DOM sites. The greater binding capacity and a weaker, linear type of isotherm suggests a nonspecific type of partitioning in POM, possibly of hydrophobic character.

Esteve-Nunez, A., Lucchesi, G., Philipp, B., Schink, B. and Ramos, J.L. 2000. Respiration of 2,4,6-trinitrotoluene by Pseudomonas sp. strain JLR11. *J Bacteriol.* 182(5): 1352-1355.

Under anoxic conditions *Pseudomonas sp*. strain JLR11 can use 2,4,6-trinitrotoluene (TNT) as the sole N source, releasing nitrite from the aromatic ring and subsequently reducing it to ammonium and incorporating it into C skeletons. This study shows that TNT can also be used as a terminal electron acceptor in respiratory chains under anoxic conditions by *Pseudomonas sp*. strain JLR11. TNT-dependent proton translocation coupled to the reduction of TNT to aminonitrotoluenes has been observed in TNT-grown cells. This extrusion did not occur in nitrate-grown cells or in anaerobic TNT-grown cells treated with cyanide, a respiratory chain inhibitor. We have shown that in a membrane fraction prepared from *Pseudomonas sp*. strain JLR11 grown on TNT under anaerobic conditions, the synthesis

of ATP was coupled to the oxidation of molecular hydrogen and to the reduction of TNT. This phosphorylation was uncoupled by gramicidin. Respiration by *Pseudomonas sp*. strain JLR11 is potentially useful for the biotreatment of TNT in polluted waters and soils, particularly in phytorhizoremediation, in which bacterial cells are transported to the deepest root zones, which are poor in oxygen.

French, C.E., Nicklin, S. and Bruce, N.C. 1998. Aerobic degradation of 2,4,6-trinitrotoluene by Enterobacter cloacae PB2 and by pentaerythritol tetranitrate reductase. *Applied and Environmental Microbiology* 64(8): 2864-2868.

Enterobacter cloacae PB2 was originally isolated on the basis of its ability to utilize nitrate esters, such as pentaerythritol tetranitrate (PETN) and glycerol trinitrate, as the sole nitrogen source for growth. The enzyme responsible is an NADPH-dependent reductase designated PETN reductase. *E. cloacae* PB2 was found to be capable of slow aerobic growth with 2,4,6-trinitrotoluene (TNT) as the sole nitrogen source. Dinitrotoluenes were not produced and could not be used as nitrogen sources. Purified PETN reductase was found to reduce TNT to its hydride-Meisenheimer complex, which was further reduced to the dihydride-Meisenheimer complex. Purified PETN reductase and recombinant *Escherichia coli* expressing PETN reductase were able to liberate nitrogen as nitrite from TNT. The ability to remove nitrogen from TNT suggests that PB2 or recombinant organisms expressing PETN reductase may be useful for bioremediation of TNT-contaminated soil and water.

Griest, W.H., Stewart, A.J., Tyndall, R.L., Caton, J.E., Ho, C.H., Ironside, K.S., Caldwell, W.M. and Tan, E. 1993. Chemical and toxicological testing of composted explosives-contaminated soil. *Environmental Toxicology and Chemistry* 12(6): 1105-1116.

Static-pile and mechanically stirred composts of explosives-contaminated soil at the Umatilla Army Depot Activity (UMDA, Umatilla, OR) in a field composting optimization study were characterized chemically and toxicologically. The concentrations of extractable explosives (e.g., 2,4,6-trinitrotoluene) in the composts and their aqueous leachates, the mutagenicity of organic solvent extracts from the composts, and the toxicity of compost aqueous leachates to *Ceriodaphnia dubia* all decreased considerably with 20 days of composting. After 44 days (mechanical composters) or 90 days (static piles) of composting, the toxicity, mutagenicity, and concentrations of extractable explosives decreased more than 90% in some cases. The composting efficiency was generally inversely proportional to the percentage (v/v) of contaminated soil. Composting in static piles was efficient up to about 20% (v/v) of contaminated soil; composting in the mechanically stirred compsters was efficient up to about 25%

soil. Mechanical composting was more efficient than composting in static piles. The main conclusion of the study is that composting can effectively remediate explosives contaminated soil and sediment. However, low levels of explosives and metabolites, bacterial mutagenicity, and leachable toxicity to *Ceriodaphnia* may remain after composting. The sources of residual toxicity and mutagenicity and the ultimate fate of the explosives are unknown.

Hannink, N., Rosser, S.J., French, C.E., Basran, A., Murray, J.A., Nicklin, S. and Bruce, N.C. 2001. Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. *Nat Biotechnol.* 19(12): 1168-1172.

There is major international concern over the wide-scale contamination of soil and associated ground water by persistent explosives residues. 2,4,6-Trinitrotoluene (TNT) is one of the most recalcitrant and toxic of all the military explosives. The lack of affordable and effective cleanup technologies for explosives contamination requires the development of better processes. Significant effort has recently been directed toward the use of plants to extract and detoxify TNT. To explore the possibility of overcoming the high phytotoxic effects of TNT, we expressed bacterial nitroreductase in tobacco plants. Nitroreductase catalyzes the reduction of TNT to hydroxyaminodinitrotoluene (HADNT), which is subsequently reduced to aminodinitrotoluene derivatives (ADNTs). Transgenic plants expressing nitroreductase show a striking increase in ability to tolerate, take up, and detoxify TNT. Our work suggests that expression of nitroreductase (NR) in plants suitable for phytoremediation could facilitate the effective cleanup of sites contaminated with high levels of explosives.

Hawari, J., Halasz, A., Paquet, L., Zhou, E., Spencer, B., Ampleman, G. and Thiboutot, S. 1998. Characterization of metabolites in the biotransformation of 2,4,6-trinitrotoluene with anaerobic sludge: role of triaminotoluene. *Appl Environ Microbiol.* 64(6): 2200-2206.

The present study describes the biotransformation of 2,4,6-trinitrotoluene (TNT) (220 microM) by using anaerobic sludge (10%, vol/vol) supplemented with molasses (3.3 g/liter). Despite the disappearance of TNT in less than 15 hrs, roughly 0.1% of TNT was attributed to mineralization ($^{14}CO_2$). A combination of solid-phase microextraction-gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry identified two distinctive cycles in the degradation of TNT. One cycle was responsible for the stepwise reduction of TNT to eventually produce triaminotoluene (TAT) in relatively high yield (160 microM). The other cycle involved TAT and was responsible for the production of azo derivatives, e.g., 2,2',4,4'-tetraamino-6,6'-azotoluene (2,2',4, 4'-TA-6,6'-azoT) and 2,2',6,6'-tetraamino-4,4'-azotoluene (2,2',6, 6'-TA-4,4'-azoT) at pH 7.2. These azo compounds were also detected when

TAT was treated with the anaerobic sludge but not with an autoclaved sludge, suggesting the biotic nature of their formation. When the anaerobic conditions in the TAT-containing culture medium were removed by aeration and/or acidification (pH 3), the corresponding phenolic compounds, e.g. hydroxy-diamino-toluenes and dihydroxy-aminotoluenes, were observed at room temperature. Trihydroxytoluene was detected only after heating TAT in water at 100º C. When $13CH_3$-labeled TNT was used as the N source in the above microcosms, we were unable to detect 13C-labeled p-cresol or [$13CH_3$]toluene, indicating the absence of denitration or deamination in the biodegradation process. The formation and disappearance of TAT were not accompanied by mineralization, suggesting that TAT acted as a dead-end metabolite.

Hitchcock, D.R., McCutcheon, S.C. and Smith, M.C. 2003. Using rotifer population demographic parameters to assess impacts of the degradation products from trinitrotoluene phytoremediation. *Ecotoxicol Environ Saf.* 55(2): 143-151.

The objective of this study was to evaluate the chronic lethal and sublethal aquatic toxicity effects associated with the phytoremediation of water contaminated with 2,4,6-trinitrotoluene (TNT) by the wetland plant species *Myriophyllum aquaticum* (parrot feather). Rotifers (*Brachionus calyciflorus*) feeding on an algal species (*Nannochloropsis spp.*) were used as the aquatic test organisms. Continuous flow laboratory microcosms were used to quantify effects on rotifer populations from TNT and the primary degradation product aminodinitrotoluene (ADNT) during and after phytoremediation. Rotifer demographic parameters from life tables, including survivorship, fecundity, reproductive values, net reproductive rate, generation time, intrinsic growth rate, and life expectancy, were used as measures of treatment effects. High-performance liquid chromatography analyses were performed to determine nitroaromatic concentrations. Results from this study have revealed significant differences in rotifer demographic parameters between microcosms with elevated initial TNT concentrations. Significant differences in demographic parameters also existed between the microcosms that did and did not receive phytoremediation treatment and the control microcosms. Study results have indicated that TNT phytoremediation via artificial wetlands not only may clean up hazardous waste at munitions sites but also may encourage the growth of aquatic populations such as rotifers.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov. Accessed December 17, 2003.

Hwang, P., Chow, T. and Adrian, N.R. 2000. Transformation of trinitrotoluene to triaminotoluene by mixed cultures incubated under methanogenic conditions. *Environmental Toxicology and Chemistry* 4: 836-841.

2,4,6-Trinitrotoluene (TNT) is an explosive widely used by the military. Although it is no longer manufactured in the U.S., large amounts of wastewater are generated annually from load, assembly, packing, and demilitarization operations. Granular-activated carbon adsorption is the standard technology for treating wastewater containing TNT and maintaining discharges within the limits established under the National Pollutant Discharge Elimination System. Studies evaluating biological treatment of pink water with an anaerobic fluidized-bed, granular-activated carbon bioreactor have been promising, but the fate of TNT is unknown. We investigated the anaerobic transformation of TNT by biofilm microorganisms obtained from a wastewater treatment plant receiving explosive manufacturing wastewater. The TNT was transformed to a mixture of 2-amino-4,6-dinitrotolune; 4-amino-2,6-dinitrotoluene; 2,4-diamino-6-nitrotoluene; and 2,6-diamino-4-nitrotoluene before culminating in the formation of triaminotoluene (TAT). Triaminotoluene was susceptible to further degradation under anaerobic conditions, but its fate was not determined. Methane formation was inhibited but resumed after the depletion of the diaminonitrotoluene isomers. These studies demonstrate near stoichiometric formation of TAT from TNT and the transformation of 2-amino-4,6-dinitrotoluene to 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene by a mixed culture incubated under methanogenic conditions. This evidence indicates TAT is also a likely end-product of TNT biodegradation in the anaerobic fluidized bed bioreactor.

Kalafut, T., Wales, M.E., Rastogi, V.K., Naumova, R.P., Zaripova, S.K. and Wild, J.R. 1998. Biotransformation patterns of 2,4,6-trinitrotoluene by aerobic bacteria. *Curr Microbiol.* 36(1): 45-54.

2,4,6-Trinitrotoluene (TNT), a toxic nitroaromatic explosive, accumulates in the environment, making necessary the remediation of contaminated areas and unused materials. Although bioremediation has been utilized to detoxify TNT, the metabolic processes involved in the metabolism of TNT have proven to be complex. The three aerobic bacterial strains reported here (*Pseudomonas aeruginosa*, *Bacillus sp*., and *Staphylococcus sp*.) differ in their ability to biotransform TNT and in their growth characteristics in the presence of TNT. In addition, enzymatic activities have been identified that differ in the reduction of nitro groups, cofactor preferences, and the ability to eliminate-NO_2 from the ring. The *Bacillus sp*. has the most diverse bioremediation potential owing to its growth in the presence of TNT, high level of reductive ability, and capability of removing-NO_2 from the nitroaromatic ring.

Krumholz, L.R., Li, J., Clarkson, W.W., Wilber, G.G. and Suflita, J.M. 1997. Transformations of TNT and related aminotoluenes in groundwater aquifer slurries under different electron-accepting conditions. *J Ind Microbiol Biotechnol.* 18(2-3): 161-169.

The transport and fate of pollutants is often governed by both their tendency to sorb as well as their susceptibility to biodegradation. We have evaluated these parameters for 2,4,6-trinitrotoluene (TNT) and several biodegradation products. Slurries of aquifer sediment and groundwater depleted TNT at rates of 27, 7.7 and 5.9 microM day-1 under methanogenic, sulfate-reducing and nitrate-reducing conditions, respectively. Abiotic losses of TNT were determined in autoclaved controls. Abiotic TNT loss and subsequent transformation of the products was also observed. These transformations were especially important during the first step in the reduction of TNT. Subsequent abiotic reactions could account for all of the transformations observed in bottles which were initially nitrate-reducing. Other controls removed TNT reduction products at much slower rates than slurries containing live organisms. 2-Amino-4,6-dinitrotoluene was produced in all slurries but disappeared in methanogenic and in sulfate-reducing slurries within several weeks. This compound was converted to 2,4-diamino-6-nitrotoluene in all slurries with subsequent removal of the latter from methanogenic and sulfate-reducing slurries, while it persisted in autoclaved controls and in the nitrate-reducing slurries. Aquifer slurries incubated with either 2,4- or 2,6-diaminotoluene showed losses of these compounds relative to autoclaved controls under nitrate-reducing conditions but not under sulfate-reducing or methanogenic conditions. These latter compounds are important as reduced intermediates in the biodegradation of dinitrotoluenes and as industrial chemicals. In experiments to examine sorption, exposure to landfill sediment resulted in losses of approximately 15% of diaminotoluene isomers and 25% of aminodinitrotoluene isomers from initial solution concentrations within 24 hrs. Isotherms confirmed that the diaminotoluenes were least strongly sorbed and the aminodinitrotoluenes most strongly sorbed to this sediment, while TNT sorption capacity was intermediate. In our studies, 2,4,6-triaminotoluene sorption capacity was indeterminate due to its chemical instability. Coupled with biodegradation information, isotherms help describe the likelihood of contaminant removal, persistence, and movement at impacted sites.

Lucero, M.E., Mueller, W., Hubstenberger, J., Phillips, G.C. and O'Connell, M.A. 1999. Tolerance to nitrogenous explosives and metabolism of TNT by cell suspensions of Datura innoxia. *In Vitro Cellular & Developmental Biology Plant.* 6: 480-486.

Cell suspension cultures of *Datura innoxia* were incubated in the presence of the nitro-substituted explosives 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazine

(RDX), and 1,3,5,7-tetranitro-1,3,5,7-tetraazocyclooctane (HMX). Cellular tolerance levels and TNT biotransformation kinetics were examined. Tolerance to TNT varied as cell suspensions aged. Concentrations of RDX or HMX in excess of reported solubility limits produced no observable changes in cell viability. GC/MS analysis of TNT-treated cell media and cell lysates revealed rapid removal of TNT. Within 12 hrs, less than 1% of the initial TNT remained in the growth medium. Aminodinitrotoluenes (ADNTs), known metabolites of TNT, accumulated transiently in cell lysates, and to a lesser extent in cell media. ADNT concentrations started to decrease after 3 hrs. After 12 hrs, less than 5% of the initial TNT could be detected as ADNT. Total ADNTs never exceeded 26% of initial TNT, suggesting that additional biotransformation steps also occurred. No other nitroaromatics were detected. A pseudo-first order rate constant for TNT clearance was calculated, k = 0.40 hr. *D. innoxia* cell suspension cultures demonstrated virtually complete clearance of TNT and of subsequent ADNT metabolites in less than 12 hrs. This rapid metabolism of nitroaromatics by the *Datura* cell suspension system indicates the utility of this system for further molecular and biochemical studies.

Lynch, J.C., Brannon, J.M. and Delfino, J.J. 2002. Dissolution rates of three high explosive compounds: TNT, RDX, and HMX. *Chemosphere* 47(7): 725-734.

Incidental exposure to high explosive compounds can cause subtle health effects to which a population could be more susceptible than injury by detonation. Proper source characterization is a key requirement in the conduct of risk assessments. For nonvolatile solid explosives, dissolution is one of the primary mechanisms that controls fate and transport, resulting in exposure to these compounds remote from their source. To date, information describing dissolution rates of high explosives has been sparse. The objective of this study was to determine the dissolution rates of three high explosive compounds, 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), in dilute aqueous solutions as a function of temperature, surface area, and energy input. To determine each variable's impact on dissolution rate, experiments were performed where one variable was changed while the other two were held constant. TNT demonstrated the fastest dissolution rate followed by HMX and then RDX. Dissolution rate correlation equations were developed for each explosive compound incorporating the three aforementioned variables, independently, and collectively in one correlation equation.

Pasti-Grigsby, M.B., Lewis, T.A., Crawford, D.L. and Crawford, R.L. 1996. Transformation of 2,4,6-trinitrotoluene (TNT) by actinomycetes isolated from TNT-contaminated and uncontaminated environments. *Appl Environ Microbiol.* 62(3): 1120-1123.

Actinomycete strains isolated from 2,4,6-trinitrotoluene (TNT)-contaminated and uncontaminated environments were compared for TNT tolerance and abilities to transform TNT. Regardless of previous TNT exposure history, no significant differences in TNT tolerance were seen among strains. Selected strains did not significantly mineralize [^{14}C]TNT. The actinomycetes did, however, transform TNT into reduced intermediates. The data indicate that, in actinomycete-rich aerobic environments like composts, actinomycetes will transform TNT into intermediates which are known to form recalcitrant polymers.

Price, R.A., Pennington, J.C., Larson, S.L., Neumann, D. and Hayes, C.A. 2002. Uptake of RDX and TNT by agronomic plants. *Journal of Soil Contamination* 11(3): 307-326.

Process wastes from ordnance loading have created groundwater and soil contamination at numerous U. S. Army sites. Some of these sites are slated for return to public use after remediation. Potential hazards associated with use of these sites for vegetable gardening after remediation to low-level residual contamination are a special concern. The objective of this study was to quantify plant uptake of explosives from contaminated soil and irrigation water. Greenhouse studies were conducted with corn, tomato, lettuce, and radish to determine uptake of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT) from contaminated soil and uptake of RDX from contaminated irrigation water. A mass balance study of tomato, lettuce, and radish was conducted in chambers using carbon-14 labeled RDX. All crops accumulated RDX from soils contaminated at 5.8 mg/kg, a remediation goal based on a site-specific risk assessment. All edible plant tissues accumulated RDX from soil except corn kernels. TNT was detected only in corn stover. At 100 µg/l RDX in irrigation water, accumulation of RDX by plants was undetectable. These results suggest that human health hazards from ingestion of vegetables growing in soils contaminated with low levels of RDX be carefully considered in plans for future use of remediated sites.

Qaisi, K.M., Ro, K.S., Constant, W.D. and Smith, M.L. 1996. Soil - water partitioning and mass transfer kinetics of 2,4,6 trinitrotoluene in highly contaminated soil. *Environmental Sciences and Pollution Mgmt.* A31(9): 2079-2085.

The soil distribution coefficient for highly concentrated TNT soil is determined. Different amounts of TNT soil are shaken with 250 ml of distilled water, and the concentration of TNT in water is monitored throughout the experiment. At the conclusion of the experiment, the remainder of TNT in the soil is determined. The soil distribution coefficient is calculated by dividing the concentration of TNT in the soil by the concentration of TNT in the water phase. The average soil distribution coefficient is found to be 4.88 l/kg. The mass transfer coefficient was determined under static conditions. This experiment involves a comprehensive study of distribution profiles for TNT in the soil and solvent phases. The experiment was conducted under a controlled temperature environment, for extensive periods of time. Samples were collected on a regular basis and were analyzed promptly. The average value of the mass transfer coefficient was determined to be 3.4×10^{-3}/hr.

Renoux, A.Y., Sarrazin, M., Hawari, J. and Sunahara, G.I. 2000. Transformation of 2,4,6-trinitrotoluene in soil in the presence of the earthworm Eisenia andrei. *Environmental Toxicology and Chemistry* 6: 1473-1480.

The ability of the earthworm *Eisenia andrei* to metabolize 2,4,6-trinitrotoluene (TNT) was studied in experiments with TNT-spiked soils, dermal contact tests, and with an in vitro assay. Lethality of TNT in a forest sandy soil was first determined (14 day LC_{50} = 143 mg/kg). Then TNT at lethal and sublethal concentrations was applied to the same soil and was monitored along with its metabolites in extracts of soil and earthworm tissue for up to 14 day postapplication. High performance liquid chromatography-ultra violet analyses indicated that TNT was transformed in the presence of *E. andrei* by a reductive pathway to 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,4-diamino-6-nitrotoluene (2,4-DANT), and traces of 2,6-diamino-4-nitrotoluene (2,6-DANT) in earthworm tissues. This transformation could be explained by either a metabolic mechanism within the earthworm or by the enhancement of an earthworm-associated microbial activity or both. The TNT concentrations decreased from the spiked soils. However, the monoamino-dinitrotoluene (2-ADNT and 4-ADNT) concentrations increased with exposure duration and were dependent on the initial TNT soil concentrations. This was also observed to a lesser extent in the TNT-spiked soils with no earthworms present. The biotransformation of TNT into 2-ADNT, 4-ADNT, and 2,4-DANT and the presence of these metabolites in *E. andrei* after dermal contact on TNT-spiked filter paper showed that dermal uptake can be a significant exposure route for TNT. In vitro experiments showed that earthworm homogenate could metabolize TNT and form 2-

ADNT and 4-ADNT at room temperature and at 37º C. This effect was inhibited by heat inactivation prior to incubation or by incubation at 4º C, suggesting that the biotransformation of TNT in the presence of *E. andrei* may be enzymatic in nature.

Rho, D., Hodgson, J., Thiboutot, S., Ampleman, G. and Hawari, J. 2001. Transformation of 2,4,6-trinitrotoluene (TNT) by immobilized Phanerochaete chrysosporium under fed-batch and continuous TNT feeding conditions. *Biotechnol Bioeng.* 73(4): 271-281.

The cometabolic transformation of 2,4,6-trinitrotoluene (TNT) by an immobilized *Phanerochaete chrysosporium* culture was investigated under different TNT and/or glycerol feeding conditions in a 5-L reactor. In the fed-batch feeding mode, as a result of four spiking events at an average feeding rate of 20 mg TNT/l/d and 250 mg glycerol/l/d, the initial TNT transformation rate and the glycerol uptake rate of the 7-day-old immobilized cell culture were 2.41 mg/l/h and 16.6 mg/l/h, respectively. Thereafter, the TNT fed into the reactor depicted a negative effect on the cell physiology of *P. chrysosporium*, i.e., both rates decreased constantly. At 32 mg TNT/l/d feeding rate, also in the presence of glycerol (200 mg/l/d), this effect on the fungal cell metabolism was even more significant. When TNT was fed alone at 3.7 mg/l/d, it showed an initial 0.75 mg/l/h rate of TNT transformation, i.e., one-third the initial level observed in the presence of glycerol. In contrast, in the continuous feeding mode (dilution rate, D = 0.11 d), at 5.5 mg TNT/l/d and 220 mg glycerol/l/d, the immobilized cell culture exhibited a constant TNT transformation rate for cultivation periods of 50 and 61 days, under uncontrolled and controlled pH conditions, respectively. Thereafter, during the latter experiment, 100% TNT biotransformation was achieved at 1,100 mg/l/d glycerol feeding rate. Immobilized cells (115-day-old), sampled from a continuous TNT feeding experiment, mineralized [^{14}C]-TNT to a level of 15.3% following a 41 day incubation period in a microcosm.

Selim, H.M., Xue, S.K. and Iskandar, I.K. 1995. Transport of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science* 160(5): 328-339.

We investigated the fate and transport of explosives in soils. Transport experiments were conducted to describe the mobility of 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in a SWy-1 reference clay (bentonite mixed with sand) and two selected soils (Norwood and Kolin). Miscible displacement experiments in packed soil columns under steady flow were used. For the bentonite/sand column, TNT was highly mobile and fully reversible when methanol was used as the background solution. In contrast, with 0.005 M $Ca(NO_3)_2$ as the background solution, the TNT pulse was strongly retarded with as much as 50% of that applied remaining within the bentonite/sand, Norwood, or Kolin columns. Products

of the transformation of TNT to 4-Am-DNT and other compound were identified in the effluent solution. A 7-day flow interruption during the TNT pulse application resulted in decreased TNT levels in the effluent solution. This decrease corresponded to a sudden increase in the 4-Am-DNT concentration in the effluent, with peak concentrations of 0.60 mg/ml. For RDX, only limited retention was observed in all columns. These findings are consistent with results from adsorption-desorption batch experiments. The TNT and RDX transport results were successfully described by a nonlinear multireaction and transport model (MRTM), which accounted for equilibrium and kinetic (reversible and irreversible) retention mechanisms. However, efforts to describe RDX transport were more successful than efforts to describe TNT when independently determined (batch) parameters were used. The mobility of TNT, RDX, and other compounds from a contaminated soil obtained from a Louisiana Army Ammunition Plant (AAP) site was also investigated. A gradual release and subsequent movement of various contaminants, including HMX, TNT, RDX, TNB, 2-Am-DNT, and 4-Am-DNT, was observed. The leaching patterns were consistent with results from uncontaminated Kolin soil columns and reflected the affinity of contaminants during leaching in the AAP soil.

Siciliano, S.D. and Greer, C.W. 2000. Plant-Bacterial Combinations to Phytoremediate Soil Contaminated with High Concentrations of 2,4,6-Trinitrotoluene. *Journal of Environmental Quality* 1: 311-316.

The explosive 2,4,6-trinitrotoluene (TNT) is a contaminant of concern at abandoned manufacturing and military sites because of its mobility and toxicity. Phytoremediation may play a role in natural attenuation scenarios by reducing TNT levels at point sources. The purpose of this study was to develop a phytoremediation system suitable for use in soils contaminated with high TNT levels. Sixteen grasses were screened for their tolerance to 41 g TNT/kg soil. Meadow bromegrass (*Bromus erectus* Huds.), perennial ryegrass (*Lolium perenne* L.) and sweet vernalgrass (*Anthoxanthum odoratum* L.) grew in this soil. Inoculating these grasses with *Pseudomonas sp*. Strain 14, capable of transforming TNT into mono- and di-amino metabolites, increased the growth of meadow bromegrass but was lethal to perennial ryegrass and sweet vernalgrass. Meadow bromegrass inoculated with Strain 14 reduced TNT levels by 30% compared with the control soil and had 50% more plant biomass than noninoculated plants. Meadow bromegrass, combined with Strain 14, increased the percentage of the culturable soil heterotrophic population containing the genes involved in 2-nitrotoluene (ntdAa) metabolism 3-fold, as well as the population containing the genes involved in 4-nitrotoluene (ntnM) metabolism 14-fold. Strain 14 inoculations of meadow bromegrass altered the portion of the rhizosphere community involved in nitroaromatic metabolism and led to a reduction in soil TNT levels.

Snellinx, Z., Nepovim, A., Taghavi, S., Vangronsveld, J., Vanek, T. and van der Lelie, D. 2002. Biological remediation of explosives and related nitroaromatic compounds. *Environ Sci Pollut Res Int.* 9(1): 48-61.

Nitroaromatics form an important group of recalcitrant xenobiotics. Only few aromatic compounds, bearing one nitro group as a substituent of the aromatic ring, are produced as secondary metabolites by microorganisms. The majority of nitroaromatic compounds in the biosphere are industrial chemicals such as explosives, dyes, polyurethane foams, herbicides, insecticides and solvents. These compounds are generally recalcitrant to biological treatment and remain in the biosphere, where they constitute a source of pollution due to both toxic and mutagenic effects on humans, fish, algae and microorganisms. However, relatively few microorganisms have been described as being able to use nitroaromatic compounds as nitrogen and/or carbon and energy source. The best-known nitroaromatic compound is the explosive TNT (2,4,6-trinitrotoluene). This article reviews the bioremediation strategies for TNT-contaminated soil and water. It comes to the following conclusion: The optimal remediation strategy for nitroaromatic compounds depends on many site-specific factors. Composting and the use of reactor systems lend themselves to treating soils contaminated with high levels of explosives (e.g., at former ammunition production facilities, where areas with a high contamination level are common). Compared to composting systems, bioreactors have the major advantage of a short treatment time, but the disadvantage of being more labor intensive and more expensive. Studies indicate that biological treatment systems, which are based on the activity of the fungus *Phanerochaete chrysosporium* or on *Pseudomonas sp*. ST53, might be used as effective methods for the remediation of highly contaminated soil and water. Phytoremediation, although not widely used now, has the potential to become an important strategy for the remediation of soil and water contaminated with explosives. It is best suited where contaminant levels are low (e.g., at military sites where pollution is rather diffuse) and where larger contaminated surfaces or volumes have to be treated. In addition, phytoremediation can be used as a polishing method after other remediation treatments, such as composting or bioslurry, have taken place. This in-situ treatment method has the advantage of lower treatment costs, but has the disadvantage of a considerable longer treatment time. In order to improve the cost-efficiency, phytoremediation of nitroaromatics (and other organic xenobiotics) could be combined with bio-energy production. This requires, however, detailed knowledge on the fate of the contaminants in the plants as well as the development of efficient treatment methods for the contaminated biomass that minimize the spreading of the contaminants into the environment during post harvest treatment.

Stahl, J.D. and Aust, S.D. 1993. Metabolism and detoxification of TNT by Phanerochaete chrysosporium. *Biochem Biophys Res Commun.* 192(2): 477-482.

Several lines of evidence suggest that TNT detoxification by *Phanerochaete chrysosporium* is through reduction. Rates of TNT reduction were directly correlated with mycelial mass and TNT concentration. Toxicity was inversely related to the amount of fungus. TNT toxicity was identical in both ligninolytic and nonligninolytic cultures. Rapid disappearance of the reduced metabolites coincided with production of the manganese-dependent peroxidases and mineralization of TNT was not observed until the lignin peroxidases were detected.

Thompson, P.L., Ramer, L.A. and Schnoor, J.L. 1998. Uptake and transformation of TNT by hybrid poplar trees. *Environmental Science & Technology* 32(7): 975-980.

This paper examines the potential for using hybrid poplar trees to remediate sites contaminated with the high explosive 2,4,6-trinitrotoluene (TNT). Laboratory experiments assessed the uptake of [U-^{14}C]TNT from both hydroponic and soil systems. TNT is strongly bound and transformed by root tissues, and it only translocates slightly to the leaves of poplar cuttings. TNT was more bioavailable in the hydroponic system, but this did not affect the distribution of radiolabel among root, stem, or leaf tissues. The translocation of TNT was found to be similar to that reported for other plant species with up to 75% of the explosive uptaken remaining in root tissues and up to 10% eventually being translocated to the leaves. The majority of TNT was not extractable from plant tissues, and less than 10% of the applied label was identifiable by HPLC/radiochromatograph. TNT was transformed by the tree to 4-amino-2,6-dinitrotoluene (4-ADNT), 2-amino-4,6-dinitrotoluene (2-ADNT), and to a number of unidentified compounds which are more polar than TNT. Phytoremediation efforts must consider the fate and toxicity of these metabolites.

Van Aken, B., Hofrichter, M., Scheibner, K., Hatakka, A.I., Naveau, H. and Agathos, S.N. 1999. Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete Phlebia radiata. *Biodegradation* 10(2): 83-91.

The degradation of the nitroaromatic pollutant 2,4,6-trinitrotoluene (TNT) by the manganese-dependent peroxidase (MnP) of the white-rot fungus *Phlebia radiata* and the main reduction products formed were investigated. In the presence of small amounts of reduced glutathione (10 mM), a concentrated cell-free preparation of MnP from *P. radiata* exhibiting an activity of 36 nkat/ml (36 nmol Mn(II) oxidized per sec and per ml) transformed 10 mg/l of TNT within three days. The same preparation was capable of completely transforming the reduced derivatives of TNT. When present at 10 mg/l, the aminodinitrotoluenes were transformed in less than two days and the diaminonitrotoluenes in less than three hours. Experiments with ^{14}C-U-ring labeled TNT and 2-amino-4,6-dinitrotoluene showed that these com-

pounds were mineralized by 22% and 76%, respectively, within 5 days. Higher concentrations of reduced glutathione (50 mM) led to a severe inhibition of the degradation process. It is concluded that *Phlebia radiata* is a good candidate for the biodegradation of TNT as well as its reduction metabolites.

Vanderberg, L.A., Perry, J.J. and Unkefer, P.J. 1995. Catabolism of 2,4,6-trinitrotoluene by Mycobacterium vaccae. *Appl Microbiol Biotechnol.* 43(5): 937-945.

Mycobacterium vaccae strain JOB-5 cometabolized 2,4,6-trinitrotoluene (TNT) in the presence of propane as a carbon and energy source. Two novel oxidized metabolites, as well as several known reduced products, were generated during catabolism of TNT by *M. vaccae*. During the cometabolic process, there was transient production of a brown chromophore. This compound was identified as 4-amino-2,6-dinitrobenzoic acid. When *M. vaccae* was incubated with [^{14}C]TNT and propane, 50% of the added radiolabel was incorporated into the cellular lipid fraction. These results suggest that ring cleavage occurred prior to the incorporation of radiolabelled carbon into phosphatidyl-L-serine, phosphatidylethanolamine, cardiolipin, and other polar lipids.

Vasilyeva, G.K., Kreslavski, V.D., Oh, B.T. and Shea, P.J. 2001. Potential of activated carbon to decrease 2,4,6-trinitrotoluene toxicity and accelerate soil decontamination. *Environ Toxicol Chem.* 20(5): 965-971.

Activated carbon can be used to decrease 2,4,6-trinitrotoluene (TNT) toxicity and promote bioremediation of highly contaminated soil. Adding activated carbon at 0.25, 0.75, and 1.0% (w/w) to Sharpsburg soil contaminated with 500, 1,000, and 2,000 mg TNT/kg decreased concentrations of TNT and its transformation products in soil solution to 5 mg/L or less, resulting in low toxicity to corn plants (*Zea mays* L.) and soil microorganisms. As much as 50% of the added TNT was rapidly bound to the soil-activated carbon matrix. Simultaneous accumulation of 2,4,6-trinitrobenzaldehyde (TNBAld) indicated that the activated carbon promoted oxidation of TNT. Some of the TNBAld was further oxidized to 1,3,5-trinitrobenzene, followed by reduction to 3,5-dinitroaniline. Reversibly bound TNT was gradually transformed to 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene, and both were bound to the soil-activated carbon matrix. The transformation and binding of TNT to soil were further promoted by incorporating shredded corn plants after growing for 52 days in the activated carbon-amended soil. After 120 days, these amendments reduced extractable TNT and transformation products by 91% in soil containing 2,000 mg TNT/kg, compared to 55% in unamended soil. These results demonstrate the potential use of activated carbon in combination with plants to promote in situ bioremediation of soils highly contaminated with explosives.

Williams, R.T., Ziegenfuss, P.S., Mohrman, G.B. and Sisk, W.E. 1989. Composting of Explosives and Propellant Contaminated Sediments. *IN: Hazardous and Industrial Wastes: Proceedings of the 21st Mid Atlantic Industrial Waste Conference. Technomic Publishing Co., Inc., Lancaster, Pennsylvania.* 1989: 599-611.

Two field-scale demonstrations were conducted to investigate composting as a technology for remediating explosives and propellant contaminated sediments. Test sediments at the Louisiana Army Ammunition Plant contained approximately 76,000 ppm of total explosives, including TNT (2,4,6 ,-trinitrotoluene) (66% of total explosive), 25% RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), 9% HMX (octahydro-1,3 ,5,7-tetranitro-1,3,5,7-tetraazocine), and 0.3% tetryl. The mixture that was composed consisted of straw/horse manure, alfalfa, horse feed, and sediment. Two 12 yd^3 piles were constructed; one was maintained at approximately 35º C and the second at approximately 55º C. After 22 wks, total explosives were reduced by 99% (from 17,872 to 74 parts per million) in the thermophilic pile (55º C). Transformation products peaked in concentration at approximately 20 days and subsequently fell to near detection limits. At the Badger Army Ammunition Plant, WI, test sediments contained approximately 18,000 parts per million of nitrocellulose. Nitrocellulose was reduced from 13,086 parts per million to 16 ppm after 101 days in a thermophilic pile.

Zoh, K.D. and Horne, A.J. 1999. Removal of TNT using plants in constructed wetlands. Wetlands and Remediation: An International Conference, Salt Lake City, UT, Battelle Press, 505 King Ave Columbus OH 43201.

The TNT degradation study was conducted using three typical wetlands plant litter types (straw, cattail, and bulrush) in hydroponic solution. TNT (220 mu M) was rapidly and completely removed with all plants (10 g/l) within three to five days at 20º C. The first order rate constant of TNT disappearance using straw litter was 0.59 day at 20º C, and similar rates were observed with bulrush and cattail ranging from 0.56 to 1.33 days. TNT removal first resulted in an initial increase of 4-aminodinitrotoluene (4-ADNT). The concentration of 4-ADNT also decreased and was below detectable limits after further incubation. Disappearance of 4-ADNT could have been due to further reduction to 2,4-diamino-6-nitrotoluene (2,4-DANT). The same patterns of metabolites formation and degradation were observed with other bulrush, and cattail carbon sources. A significant difference in TNT degradation rates (0.12 day at 10º C) was observed with 10º C difference. The maximum combined level of metabolites concentration was only 10 to 18% of the initial TNT. From extraction study, we found the trace amounts of 4-ANDT and 2,4-DANT, but no TNT was detected. The ^{14}C study and sorption experiment also showed that TNT mineralization did not take place, and the TNT disappearance have been the result

of a combination of two processes; initial sorption followed by bacterial degradation. A mesocosm scale constructed wetlands for treating TNT is now underway at the Richmond Field Station (Richmond, CA).

Bioaccumulation and Trophic Transport

Johnson, M.S., Franke, L.S., Lee, R.B. and Holladay, S.D. 1999. Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: Is the dermal route significant? *Environmental Toxicology and Chemistry* 5: 873-878.

Tiger salamanders (*Ambystoma tigrinum*) were exposed via soil and/or food (earthworms) to 2,4,6-trinitrotoluene (TNT) and a PCB mixture (Aroclor 1260) at environmentally relevant concentrations. Four exposures were considered: (1) uncontaminated food + uncontaminated soil (control group); (2) contaminated soil + uncontaminated food (dermal group); (3) contaminated food + uncontaminated soil (oral group); and, (4) contaminated soil + contaminated food (dual-exposure group). The chemical exposure was estimated for each group by analysis of both soil and earthworms. Body burdens of TNT and its primary metabolites were highest in the dermal groups while PCB burdens were highest in the oral groups. Concentrations of the primary TNT metabolites evaluated, 2-amino-dinitrotoluene (DNT) and 4-amino-DNT, exceeded that of unmetabolized TNT and accumulated to 116 and 670 ng/g, respectively. These results provide evidence that dermal exposures to nitroaromatics in terrestrial salamanders may make an important contribution to total body burden and thus may be important when considering the health consequences of such exposures. Further, the demonstration of the accumulation of TNT and TNT metabolites in a primitive vertebrate may have food web modeling implications.

Leung, K.H., Yao, M., Stearns, R. and Chiu, S.H. 1995. Mechanism of bioactivation and covalent binding of 2,4,6-trinitrotoluene. *Chem Biol Interact.* 97(1): 37-51.

Studies were undertaken to investigate the mechanism of bioactivation and covalent binding of TNT. Incubation of [^{14}C]TNT with rat liver microsomes in the presence of an NADPH generating system resulted in metabolism and covalent binding to microsomal proteins. Time-dependence studies showed that TNT was rapidly reduced to yield 4-hydroxylamino-2,6-dinitrotoluene (4HA), 4-amino-2,6-dinitrotoluene (4A) and 2-amino-4,6-dinitrotoluene (2A) as intermediates which were further metabolized to form 2,4-diamino-6-nitrotoluene (2,4DA) and 2,6-diamino-4-nitrotoluene (2,6DA). In contrast to the rapid disappearance of TNT, formation of covalent protein adducts increased with time, suggesting that the reactive intermediate was likely to be formed not directly from TNT but from proximal intermedi-

ates such as 4HA. The hypothesis that 4HA was more readily converted to the reactive intermediate than TNT was further supported by the increased levels of covalent adduct formation when [^{14}C]4HA was incubated directly with liver microsomes. Covalent binding of TNT and 4HA was dependent on oxygen concentration. Higher levels of covalent adducts were formed when TNT was incubated aerobically (up to 50% oxygen concentration) than under anaerobic conditions. Covalent binding of [^{14}C]4HA also increased with increasing oxygen concentrations. These results suggest that the reactive intermediate is likely to be an oxidized metabolite of 4HA, e.g. 4-nitroso-2,6-dinitrotoluene. Compounds containing a free sulfhydryl group (cysteine, N-acetylcysteine, GSH or 3,4-dichlorobenzenethiol) decreased the amount of covalent binding to various degrees, suggesting the involvement of the sulfhydryl group in adduct formation with TNT following bioactivation. Metabolic activation of TNT by liver microsomes required NADPH but not NADH as the cofactor. Incubation of [^{14}C]TNT with purified rat liver NADPH cytochrome P450 reductase under either aerobic or anaerobic conditions yielded exclusively 4HA. In contrast, 2A and 4A were formed following incubation of TNT with the reconstituted system containing cytochrome P450, NADPH cytochrome P450, reductase and dilauroyl phosphatidylcholine. These observations suggest that the initial reduction of the nitro group can be catalyzed by NADPH cytochrome P450 reductase alone but cytochrome P450 is needed in the reduction of the hydroxylamine to the amine.

Talmage, S.S., Opresko, D.M., Maxwell, C.J., Welsh, C.J., Cretella, F.M., Reno, P.H. and Daniel, F.B. 1999. Nitroaromatic munition compounds: environmental effects and screening values. ***Reviews of Environmental Contamination and Toxicology*** **161: 1-156.**

Nitroaromatic compounds are potentially toxic and found at a number of U.S. Army Ammunition Plants and other military facilities. This review presents a summary and analysis of available data on eight nitroaromatic compounds including environmental concentrations, environmental fate and transport processes, and ecotoxicity and bioaccumulation for aquatic and terrestrial biota. For those groups of organisms for which there are sufficient data, ecological criteria and screening benchmarks were developed. Staff at Oak Ridge National Laboratory (ORNL) under a project jointly sponsored by the U.S. Army and the U.S. EPA developed these criteria and screening benchmarks. The review discusses the methodologies for development of the screening criteria and benchmarks.

Toxicity

Arfsten, D.P., Davenport, R. and Schaeffer, D.J. 1994. UV-A coexposure enhances the toxicity of aromatic hydrocarbons, munitions, and metals to Photobacterium phosphoreum. ***Biomed Environ Sci.*** **7(2): 101-108.**

Previous studies showed that coexposure to UV-A between 300-400 nm enhanced the toxicity of nitrotoluenes to Photobacterium phosphoreum, a marine bioluminescent bacteria used in the Microtox test (Microbics Inc.). This paper reports that UV-A photoenhanced the toxicity of polynuclear aromatic hydrocarbons, other types of organic compounds, and some transition metals to P. phosphoreum. Coexposure to 400 muw/cm^2 for 15 min increased the toxicity of psoralen, alpha-terthienyl, anthracene, acridine, fluoranthene, TNT, Cu_2, As_3-, Ni_2, and Cd2+. Phenanthrene was photoenhanced after 30 min coexposure at 400 muw/cm^2-, and Mn2+ at 800 muw/cm^2 after 15 min. Naphthalene was not enhanced at 800 muw/cm^2 for 30 min.

Davenport, R., Johnson, L.R., Schaeffer, D.J. and Balbach, H. 1994. Phototoxicology. 1. Light-enhanced toxicity of TNT and some related compounds to Daphnia magna and Lytechinus variagatus embryos. *Ecotoxicol Environ Saf.* 27(1): 14-22.

Many environmental pollutants interact with solar near-ultraviolet (nuv) light in a manner which greatly increases their toxic effects. The phenomenon of light-mediated toxicity (phototoxicity) is only now becoming generally recognized to any significant degree. Manufacture of, and loading munitions with, the explosive 2,4,6-trinitrotoluene (TNT) in past decades caused contamination of soils and sediments at levels exceeding 1000 ppm and of waters at levels near saturation (100 ppm). Manufacture of TNT produces numerous nitrated by-products, and most of these compounds, including TNT, can be metabolized by many species, including bacteria, fungi, plants, and mammals. This study investigated the phototoxicity of TNT, and 2,3-, 2,4-, 2,6-, and 3,4-dinitrotoluene (DNT) and -diaminotoluene (DAT), and the major metabolites 2-amino-4,6-dinitrotoluene (2A) and 4-amino-2,6-dinitrotoluene (4A), to *Daphnia magna* (acute toxicity) and *Lytechinus variagatus* (sea urchin) embryos (subacute, developmental toxicity). Most of the compounds were weakly toxic or nontoxic in the dark. All were phototoxic to sea urchins. In *D. magna*, 2,3- and 3,4-DNT/DAT and 4A were not toxic but were phototoxic, and 2A was toxic and phototoxic; the other isomers were not toxic or phototoxic to this species.

Dilley, J.V., Tyson, C.A., Spanggord, R.J., Sasmore, D.P., Newell, G.W. and Dacre, J.C. 1982. Short-term oral toxicity of 2,4,6-trinitrotoluene in mice, rats, and dogs. *Journal of Toxicology and Environmental Health* 9(4): 565-585.

The short-term oral toxicity of 2,4,6-trinitrotoluene (alpha-TNT) was determined in dogs, rats, and mice. All species receiving the highest doses exhibited anemia, with reduced erythrocytes, hemoglobin, and hematocrit. Alterations were observed in organ weights, including enlarged spleens (accompanied by hemosiderosis) and livers, and depressed body weight and/or body weight gain (temporary in dogs and mice).

Alterations in clinical chemistry values included elevated cholesterol and depressed serum glutamicpyruvic transaminase activity in dogs and rats; no effect on serum glutamic-oxaloacetic transaminase activity was observed. Some effects, such as SGPT depression in rats, appeared after 13 wks, suggesting a cumulative toxicity. Reduced testes size was observed in rats at the highest dose regardless of length of exposure. Most of the toxic effects were reversible, but testicular atrophy was not in rats allowed a 4 wks recovery period after treatment. Signs of anemia were present at intermediate dose levels. "No observable effects" levels for alpha-TNT were: dogs, 0.20; rats, 1.42; and mice, 7.76 mg/kg/d.

Dodard, S.G., Renoux, A.Y., Powlowski, J. and Sunahara, G.I. 2003. Lethal and subchronic effects of 2,4,6-trinitrotoluene (TNT) on Enchytraeus albidus in spiked artificial soil. *Ecotoxicology and Environmental Safety* 54(2): 131-138.

The effects of 2,4,6-trinitrotoluene (TNT) exposure in spiked artificial soil on the survival and reproduction rate of the white potworm *Enchytraeus albidus* were studied. Based on the initial concentrations, TNT in freshly spiked soil decreased enchytraeid survival (21 day LC_{50} = 422 (plus or minus 63 (SD)) mg/kg, N = 3) and fecundity (42 day EC_{50} = 111 (plus or minus 34), N = 4). Data also indicated that TNT was 5-10 times more lethal to juveniles than adults, and lethality was less pronounced in TNT-spiked soils aged for 21 days. A time-dependent decrease in the TNT concentrations, as well as a concomitant increase in the levels of 2- and 4-aminodinitrotoluene, was observed during the 42 day toxicity test. Taken together, TNT (or one of its metabolites) is more lethal to juvenile than adult enchytraeids. This effect may explain, at least in part, the ability of TNT to decrease fecundity as determined using the enchytraeid mortality-reproduction test.

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology* 28(2): 229-235.

Aqueous samples containing various explosives, their reduced metabolites, as well as related compounds were subjected to the luminescent bacterium *Vibrio fischeri* NRRL-B-11177 to determine their ecotoxicological potential. As the most important parameter, the EC_{50} values of 24 test compounds were calculated. The EC_{50} value means the concentration of a chemical compound that is needed to reduce bacterial luminescence by 50%. According to the widely accepted classification scheme of Strupp et al. (1990) and in consideration of an incubation period of 30 min TNT, 26DNT, 2A6NT, 4A2NT, 34DNT, TNB, TNBA, TETRYL and HEXYL must be classified in the category "very toxic to aquatic organisms"; 2A46DNT, 4A26DNT,

24DA6NT, 24DNT, 2A4NT, RDX, HMX and PETN must be classified in the category "toxic to aquatic organisms"; and 26DA4NT, TAT, TNPh, 26DAT, 24DAT, HMT and NQ can be classified in the category "less toxic to aquatic organisms". EC_{50} values after 30, 60, and 90 min of incubation of the test compounds are presented and discussed. For many of the compounds tested in this study, there are no, or only a few, toxicological data in the literature available.

Fuller, M.E. and Manning, J.F., Jr. 1998. Evidence for differential effects of 2,4,6-trinitrotoluene and other munitions compounds on specific subpopulations of soil microbial communities. *Environmental Toxicology and Chemistry* 17(11): 2185-2195.

The effects of 2,4,6-trinitrotoluene (TNT) and other munitions compounds on indigenous microbial communities in several soils were examined. Culturable heterotrophs, concentrations of phospholipid fatty acid (PLFA), and basal respiration rates exhibited slight negative correlations with high TNT and 1,3,5-trinitrobenzene (TNB) levels. Heat-shock-resistant culturable heterotrophs, percentage of gram-positive soil isolates, mole percent of branched PLFA, and 10Me 18:0 (tuberculostearic acid) were observed to be significantly lower in highly contaminated soils. Total soil nitrogen levels were positively correlated with high TNT and TNB concentrations, whereas total soil carbon exhibited no significant correlation with either compound. Multivariate analysis of PLFA data resulted in distinct separation of soils with respect to their degree of contamination, with specific signature PLFAs for gram-positive bacteria, fungi, and protozoa being negatively associated with high contaminant levels. Apparent concentrations of TNT resulting in 50% reductions in indicators of gram-positive populations were much higher than values from pure culture experiments, possibly as a result of low bioavailability due to sorption onto clay and soil organic matter. Few effects of other munitions compounds were observed. Closer examination of a highly contaminated soil revealed that the number of culturable heterotrophs growing on 0.3% molasses plates decreased by 50% when 67 µg TNT/ml was added to the medium; a 99% decrease was observed for soil contaminated with less than 20 µg TNT/g. Highly contaminated soil harbored a greater number of organisms that were able to grow on plates amended with greater than 10 µg TNT/ml. Gram-positive isolates from both soils demonstrated marked growth inhibition when greater than 8-16 µg TNT/ml was present in the culture medium. These results indicate that chronic exposure to munitions compounds can dramatically alter soil microbial communities.

Gogal, R.M., Jr., Johnson, M.S., Larsen, C.T., Prater, M.R., Duncan, R.B., Ward, D.L. and Holladay, S.D. 2002. Influence of dietary 2,4,6-trinitrotoluene exposure in the northern bobwhite (*Colinus virginianus*). *Environmental Toxicology and Chemistry* 21(1): 81-86.

The risk to wildlife from exposure to the explosive, 2,4,6-trinitrotoluene (TNT) has been a concern at numerous military installations where it has been found in the soil. To date, no published data are available describing effects of TNT exposure in an avian species. Subchronic dietary exposure to TNT was therefore evaluated in a species of management concern at military installations, the northern bobwhite (*Colinus virginianus*). Adult male and female quail (n = 5/sex/dose) were given commercial feed containing 3,000, 1,500, 750, and 100 mg/kg TNT for 90 days following the determination of an acute lethal dose and a 14 day range finding study. Dietary TNT intake caused a dose-dependent decrease in total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, and blood lymphocytes. An increased trend in late apoptotic/necrotic blood leukocytic cells was also observed in TNT-exposed birds, as was hemosiderosis in the liver. With the exception of hemosiderosis, these trends were statistically significant yet of questionable biological significance. Since treatment-related responses in this preliminary study were variable, a conservative interpretation is suggested. However, since these treatments had concentrations that were a log-fold or more than doses in similar studies using mammals, these data suggest that northern bobwhite are less sensitive to oral exposures of TNT than mammals.

Gong, P., Siciliano, S.D., Greer, C.W., Paquet, L., Hawari, J. and Sunahara, G.I. 1999. Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field-contaminated soils to indigenous microorganisms. *Environmental Toxicology and Chemistry* 12: 2681-2688.

The response of potential nitrification activity (PNA), nitrogen-fixation activity (NFA), and dehydrogenase activity (DHA) in soil to 2,4,6-trinitrotoluene (TNT) was assessed. Two garden soils of contrasting texture (sandy loam vs. clay loam) were spiked with TNT (25-1,000 mg TNT/kg). Soil microbial activities and TNT residues were analyzed 1 week later. The estimated IC50 (concentration causing 50% inhibition) ranged from 39 to 533 mg/kg of the acetonitrile-extractable (AE) TNT (1 week after spiking), depending on indicators and soils. The lowest LOEC (lowest-observed-effect concentration) was 1 mg AE TNT/kg. Field soil (0-15 cm) was collected from three known contaminated sites in an abandoned TNT manufacturing facility. Microbial toxicity significantly correlated to TNT levels in these soils. The LOEC and NOEC (no-observed-effect concentration) values were site and indicator specific, with the lowest LOEC being 1 mg AE TNT/kg and the lowest NOEC being 0.4 mg AE TNT/kg. The LC_{50} of the pooled field samples was 51 mg AE TNT/kg for PNA or 157 mg AE TNT/kg for DHA. These results indicate that microbial responses were consistent and comparable between the laboratory and the field and that TNT could significantly inhibit soil microbial activities at very low levels. Both AE TNT and deionized water-extractable (DW) TNT concentrations correlated well

with microbial toxicity, but AE TNT provided a better evaluation of TNT bioavailability than did DW TNT.

Green, A., Moore, D. and Farrar, D. 1999. Chronic toxicity of 2,4,6-trinitrotoluene to a marine polychaete and an estuarine amphipod. *Environmental Toxicology and Chemistry* 8: 1783-1790.

The chronic toxicity of sediment-associated 2,4,6-trinitrotoluene (TNT) to the marine polychaete *Neanthes arenaceodentata* and the estuarine amphipod *Leptocheirus plumulosus* was evaluated. Test organisms were exposed to sediments spiked with radiolabeled TNT for 28 days, after which time the endpoints of mortality, growth, and reproduction (*L. plumulosus* only) were assayed and compared against the TNT tissue concentrations as well as the TNT sediment concentrations. Survival was significantly reduced at a tissue concentration of 61 µg TNT/g wet wt tissue in *N. arenaceodentata* and at 6.3 µg TNT/g wet wt tissue in *L. plumulosus*. The growth end point demonstrated an apparent hormesis effect in both *N. arenaceodentata* and *L. plumulosus* with enhancement (significant for *N. arenaceodentata*) occurring at the lower TNT concentrations. Growth was significantly reduced at the highest TNT exposure of 10.0 µg TNT/g wet wt tissue in *L. plumulosus*. Reproduction was significantly reduced at a tissue concentration of 6.3 µg TNT/g wet wt tissue in *L. plumulosus*. The results of this study demonstrate that both *N. arenaceodentata* and *L. plumulosus* are sensitive to the presence of sediment-associated TNT and that more information is needed about the toxicity of TNT to benthic fauna to facilitate risk assessment and management of TNT-contaminated sites.

Hankenson, K. and Schaeffer, D. 1991. Microtox Assay of Trinitrotoluene, Diaminonitrotoluene, Dinitromethylaniline Mixtures. *Bulletin of Environmental Contamination and Toxicology* 46(4): 550-553.

The toxicity of aqueous solutions of 2,4,6-trinitrotoluene (TNT), 2,6-diamino-4-nitrotoluene (DANT), and 2 ,6-dinitro-4-methylaniline (DNMA) alone and as binary mixtures was evaluated using the Microtox test. TNT was found to be 20 to 50 times as toxic as DANT or DNMA. The results indicated that the acute toxicity of mixtures of TNT with related compounds was often not described by simple similar addition. Because TNT was more toxic than the other compounds studied, and because it is the predominant compound found in TNT-contaminated sites, preliminary risk assessments based on chemical analyses can probably proceed on the assumption of additivity. However, subsequent specific toxicity testing using several compounds found in the mixture is recommended.

Johnson, L.R., Davenport, R., Balbach, H. and Schaeffer, D.J. 1994. Comparative toxicity of trinitrotoluene and aminodinitrotoluenes to Daphnia

magna, Dugesia dorotocephala, and sheep erythrocytes. ***Ecotoxicology and Environmental Safety*** **27(1): 34-49.**

2,4,6-Trinitrotoluene (TNT) and compounds associated with its production are toxic and phototoxic to a wide range of biota. The planarian *Dugesia dorotocephala*, but not *Daphnia magna*, metabolized TNT (1 mg/l) to 4-amino-2,6-dinitrotoluene (4A; 0.4 mg/l) and 2-amino-4,6-dinitrotoluene (2A; 0.2 mg/l). Coexposure to near-ultraviolet (nuv) light enhanced the toxicity of 2A more than that of TNT and 4A. The toxicities of TNT, 4A, and 2A to *D. dorotocephala* were all decreased by glutathione (GSH) conjugation. This suggests that all had mechanisms of toxic action involving formation of quinone-GSH conjugates. Dark and light mechanisms for TNT and 2A depended on GSH conjugation, but the specific mechanisms may be different for each compound. The dark and light mechanisms of toxic action for 4A appeared to be fundamentally different in that the dark toxic mechanism of action was less dependent on GSH conjugation. Hemolysis studies using sheep erythrocytes showed that the light-enhanced toxic mechanism of action for TNT, 2A, and/or 4A did not involve cellular membrane damage in response to nuv-induced anions.

Johnson, M.S., Ferguson, J.W. and Holladay, S.D. 2000. Immune effects of oral 2,4,6-trinitrotoluene (TNT) exposure to the white-footed mouse, Peromyscus leucopus. ***International Journal of Toxicology*** **1: 5-11.**

Immune toxicity associated with exposure to 2,4,6-trinitrotoluene (TNT) in a 14-day feeding study was examined in male and female white-footed mice (*Peromyscus leucopus*). Mice (10/group/sex) were exposed to 0, 0.042, 0.083, 0.165, and 0.330% TNT in feed for 14 days. Based upon average feed consumption and body weight, these diets resulted in approximate daily doses of 66, 145, 275, and 601 mg TNT/kg body weight (bw) for males and 70, 142, 283, and 550 mg/kg/day for females. At the end of the exposure period the mice were euthanized and several indicators of nonspecific immunity were examined. These indicators included primary and secondary lymphoid organ/body weight ratio (spleen and thymus), and characterization of nonspecific immune responses (phagocytosis and radical oxygen intermediate [ROI] production). No deaths occurred, even though the high-dose group approached the reported LD_{50} in Swiss-Webster mice (Dilley et al. 1982). Spleen weight was significantly increased in the high-dose group (0.330% TNT) for both sexes, whereas a dose-related trend in thymus cellularity was suggestive for males, but not females. In addition, males, but not females, displayed inhibited splenic macrophage phagocytosis and ROI production. Splenic congestion and extramedullary hematopoiesis were observed in both sexes in the two highest dose groups. These results suggest species-specific differences in relative subacute toxicity between laboratory (genus *Mus*) and Nearctic (genus *Peromyscus*) mice. In addition, these immunological indi-

cators appear more sensitive than other toxicological endpoints that have been reported as most descriptive of TNT-related effects in mammals.

Lachance, B., Robidoux, P.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. ***Mutation Research Genetic Toxicology and Environmental Mutagenesis*** **444(1): 25-39.**

The mutagenicity and toxicity of energetic compounds such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and of amino/nitro derivatives of toluene were investigated in vitro. Mutagenicity was evaluated with the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay. Cytotoxicity was evaluated using V79 and TK6 human lymphoblastic cells. For the TK6 and V79 assays, TNB and 2,4,6-triaminotoluene were more toxic than TNT, whereas RDX and HMX were without effect at their maximal aqueous solubility limits. The primary TNT metabolites (2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene) were generally less cytotoxic than the parent compound. The FT results indicated that TNB, TNT, and all the tested primary TNT metabolites were mutagenic. Except for the cases of 4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene in the TA98 strain, addition of rat liver S9 resulted in either no effect, or decreased activity. None of the tested compounds were mutagenic for the V79 mammalian cells with or without S9 metabolic activation. Thus, the FT assay was more sensitive to the genotoxic effects of energetic compounds than was the V79 test, suggesting that the FT might be a better screening tool for the presence of these explosives. The lack of mutagenicity of pure substances for V79 cells under the conditions used in this study does not preclude that genotoxicity could actually exist in other mammalian cells. In view of earlier reports and this study, mutagenicity testing of environmental samples should be considered as part of the hazard assessment of sites contaminated by TNT and related products.

Levine, B.S., Furedi, E.M., Gordon, D.E., Lish, P.M. and Barkley, J.J. 1984. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. ***Toxicology*** **32(3): 253-265.**

This study was conducted to evaluate the toxicity of trinitrotoluene (TNT) in Fischer 344 rats when administered in the diet for 13 wks. Toxic effects following 125 mg/kg/day or greater included decreased food intake and body weight gain, elevated serum cholesterol levels, and anemia (reduced hemoglobin, hematocrit and RBC counts). Splenomegaly, hepatomegaly/hepatocytomegaly and testicular atrophy with degeneration of the seminiferous tubular epithelium were also seen at 125 and 300

mg/kg/day. Hemosiderin-laden macrophages, congestion of the splenic red pulp, methomoglobin production indicative of the oxidizing activity of TNT and/or its metabolites, and the lack of bone marrow toxicity suggested hemolysis as the mechanism of anemia.

Levine, B.S., Furedi, E.M., Gordon, D.E., Barkley, J.J. and Lish, P.M. 1990. Toxic interactions of the munitions compounds TNT and RDX in F344 rats. ***Fundamental and Applied Toxicology*** **15(2): 373-380.**

The potential toxic interactions in F344 rats of the munitions compounds trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) were examined following their coadministration in the diet. Groups of 10 rats per sex received TNT at doses of 5 or 125 mg/kg/day, RDX at doses of 30, 100, or 300 mg/kg/day, and combinations thereof for 13 weeks. Thirty rats per sex served as controls. Toxicologic endpoints included clinical observations, body weight, food consumption, hematology, clinical chemistry, organ weights, and tissue morphology. The major toxic effects following the dietary administration of TNT to rats included anemia, hypercholesterolemia, and hepatomegaly, splenomegaly, and testicular atrophy with their accompanying histologic lesions. RDX intoxication in rats included hypotriglyceridemia, behavioral changes, and mortality. Most of the toxic effects of these chemicals were partially antagonized following their coadministration.

Levine, B.S., Rust, J.H., Barkley, J.J., Furedi, E.M. and Lish, P.M. 1990. Six month oral toxicity study of trinitrotoluene in beagle dogs. ***Toxicology*** **63(2): 233-244.**

This study was conducted to evaluate the toxicity of the munitions compound 2,4,6-trinitrotoluene (TNT; CAS Reg. No. 118-96-7) in beagle dogs when administered daily for 26 wks by capsule. Groups of six dogs per sex received TNT at doses of 0 (vehicle controls), 0.5, 2, 8, or 32 mg/kg/day. Toxicologic endpoints included clinical signs, body weights, food consumption, clinical biochemistry, hematology, urinalyses, organ weights, and gross and tissue morphology. The major toxic effects following the oral administration of TNT to dogs included hemolytic anemia, methemoglobinemia, liver injury, splenomegaly with accompanying histologic lesions, and death. Only the highest dose given proved to be lethal. Hepatocytic cloudy swelling and hepatocytomegaly were apparent at all doses tested. Thus, a no observable effect level was not established in this investigation.

Pederson, G.L. 1970. ***Evaluation of toxicity of selected TNT wastes on fish, phase i-acute toxicity of alpha-TNT to Bluegills.*** **Army Environmental Hygiene Agency, 1 January 1970-31 October 1970.**

The acute toxicity of alpha-TNT (2,4,6 trinitrotluene) to bluegills (*Lepomas microchirus*) was determined relative to variations in water temperature or in water hardness. Ninety-six hr LC_{50} values ranged from 2.3 to 2.8 mg/l of alpha-TNT. Water temperature significantly affected the toxicity of alpha-TNT, i.e., lower concentrations were required to elicit toxicity at 10º C than at 25ºC. Water hardness had no apparent effect.

Reddy, G., Chandra, S.A.M., Lish, J.W. and Qualls, C.W., Jr. 2000. Toxicity of 2,4,6-trinitrotoluene (TNT) in hispid cotton rats (Sigmodon hispidus): Hematological, biochemical, and pathological effects. *International Journal of Toxicology* 19(3): 169-177.

The contamination of soil and water with munitions chemicals and their degradation products has been reported at certain munitions production waste disposal sites and at certain U.S. Army installations. The effects of 2,4,6-trinitrotoluene (TNT) on wild cotton rats (*Sigmodon hispidus*) were evaluated to identify target organ toxicity that could be used to develop biomarkers for exposure assessment for ecological and health risks. The oral LD_{50} values for TNT in corn oil were 607 and 767 mg/kg body weights for male and female cotton rats respectively. Hematological, pathological, and biochemical effects of TNT were determined after daily oral gavage of TNT in corn oil at doses of 0, 75.9, 151.8, and 303.5 (males) or 0, 96, 192, and 384 mg/kg (females) for 7 days. Cotton rats treated with TNT showed an increase in spleen weights in males (303.5 mg/kg) and in females (192 and 384 mg/kg). Cotton rats of both sexes treated for 7 days with TNT had marked hemolytic anemia with reduced erythrocytes, hemoglobin, and hematocrit in high-dose groups; methemoglobin levels were elevated significantly in males at mid and high dose. Hepatic drug metabolizing enzyme analysis revealed that microsomal O-dealkylation of methoxy, ethoxy, and pentoxy resorufin were elevated in male (high dose) cotton rats. The activity of hepatic glutathione S-transferases (GST) was significantly elevated in male (mid and high dose) and female (all doses) cotton rats exposed to TNT. Histopathological analysis of spleen revealed mild to marked splenic congestion with mild extramedullary hematopoiesis, hemosiderosis, and lymphoid hyperplasia in male and female cotton rats treated with TNT (all doses). Liver weights were increased in males (mid and high dose) and in females (high-dose group). In the high-dose groups, histological changes in liver (mild to moderate hepatocellular hypertrophy, increased hemosiderin pigment in Kupffer cells) in both sexes, and in testis (premature exfoliation of spermatozoa from dilated seminiferous tubules) were observed (mid and high dose). These results suggest that hepatic GST and hemolytic anemia may be biomarkers in cotton rats of terrestrial contamination with TNT or other nitroaromatic explosive compounds.

Robidoux, P.Y., Hawari, J., Thiboutot, S., Ampleman, G. and Sunahara, G.I. 1999. Acute toxicity of 2,4,6-trinitrotoluene in earthworm (Eisenia andrei). ***Ecotoxicology and Environmental Safety*** **44(3): 311-321.**

2,4,6-Trinitrotoluene (TNT) is a worldwide recalcitrant environmental contaminant and is toxic to a number of organisms including humans. This study examines the acute effects (lethal and biomass changes) of TNT on the oligochaetes species *Eisenia andrei*, using the 3-day filter paper, and the 7- and 14-day direct contact spiked soil (OECD artificial and forest soil) toxicity tests. Studies using the filter paper test indicated that the lethality of TNT could be detected in the range 1.5 to 14.2 µg /cm^2, with significant biomass (body weight) changes occurring at the lowest concentration. Acute effects (lethality) could not be measured when earthworms were placed on filter paper containing a saturated aqueous solution of TNT. This may indicate that with these exposure conditions, TNT may have been adsorbed to the filter paper, and that this matrix should be saturated with TNT before becoming available to the earthworms. Spiked soil toxicity tests indicated that the *E. andrei* lethality by TNT was >1.5 times higher when earthworms were exposed to TNT-spiked forest soil (LOEC: 260 mg/kg; LC_{50}: 14 days 222.4 mg/kg) than to spiked OECD artificial soil (LOEC: 420 mg/kg; LC_{50}: 14 days 364.9 mg/kg). The sublethal effect on biomass change at the selected TNT concentrations in soil was not significant compared to controls. Results indicate that the bioanalytical methods described in this article could be used as TNT toxicity assessment tools. This soil quality test method gives valuable information for the screening of soil toxicity.

Robidoux, P.Y., Svendsen, C., Caumartin, J., Hawari, J., Ampleman, G., Thiboutot, S., Weeks, J.M. and Sunahara, G.I. 2000. Chronic toxicity of energetic compounds in soil determined using the earthworm (Eisenia andrei) reproduction test. ***Environmental Toxicology and Chemistry.*** **7: 1764-1773.**

Earthworm survival tests are commonly used in terrestrial ecotoxicology to assess the toxicity of compounds in soil. Earthworm (*Eisenia andrei*) reproduction tests were used to assess the sublethal and chronic effects of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5,7-triazacyclohexane (RDX). Effects on reproduction parameters (total number of cocoons, number of hatched cocoons, number of juveniles, juvenile biomass, and hatchability) were measured in TNT- and RDX-spiked artificial soil. For TNT, the lowest-observed-effect concentration (LOEC) was 110 mg/kg dry soil, and the no-observed-effect concentration (NOEC) was 55 mg/kg. For the RDX-spiked soil, the LOEC was 95 mg/kg dry soil, and the NOEC was <95 mg/kg. The growth of adult worms was also reduced when exposed to TNT-spiked soil at the highest tested concentration (881 mg/kg dry soil). Taken together, data analysis showed that the number of juveniles was strongly correlated with the number of co-

coons but poorly correlated with the growth of adults. This information could permit one to optimize the application of the *Eisenia sp*. reproduction assay when used as a sublethal effect assessment tool for TNT- or RDX-contaminated soils.

Robidoux, P.Y., Hawari, J., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 2002a. TNT, RDX, and HMX decrease earthworm (Eisenia andrei) life-cycle responses in a spiked natural forest soil. *Archives of Environmental Contamination and Toxicology* 43(4): 379-388.

Sublethal and chronic toxicities of 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) on earthworm *Eisenia andrei* in a sandy forest soil were assessed. Various reproduction parameters of fecundity (total and hatched number of cocoons, number of juveniles, and their biomass) were significantly decreased by TNT (> or = 58.8 +/- 5.1 mg/kg dry soil), RDX (> or = 46.7 +/- 2.6 mg/kg), and HMX (> or = 15.6 +/- 4.6 mg/kg). These effects occurred at much lower concentrations than those reported earlier using artificial soil preparations. Growth of adults was significantly decreased in the TNT-spiked natural soils at 136.2 +/- 25.6 mg/kg dry soil, the highest concentration having no significant mortality. In contrast, survival and growth were not significantly reduced at relatively high measured concentrations of RDX (167.3 mg/kg) and HMX (711.0 mg/kg). Although TNT, RDX, and HMX share a common life-cycle response (i.e., decreased juvenile counts), a number of differences related to other reproduction parameters (e.g., productivity of cocoons) was observed. These results indicate that the tested explosives do not support a common mechanism of toxicity, at least in the earthworm, probably due to differences in their physical-chemical properties as well as metabolites formed during exposure.

Smock, L.A., Stoneburner, D.L. and Clark, J.R. 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. *Water Res.* 10(6): 539-543.

The effects of alpha-trinitrotoluene (alpha-TNT) and its primary degradation product (TNT), commonly referred to as 'pink water', were determined on members of 2 trophic levels. The growth responses f the algae *Selenastrum capricornutum* and *Microcystis aeruginosa* were examined through static bioassays. Death and behavioral responses of the fathead minnow (*Pimephales promelas*) were determined using a proportional diluter. Alpha TNT and TNTcc were both more tonic to the fathead minnow than to either species of alga. Five and 15 mg/l alpha TNT inhibited *S. capriconrutum* and *M. aeuruginosa* growth, respectively. TNTcc inhibited *S. capricornutum* growth at concs > 9 mg/1; it was lethal to *M. aeruginosa* at 50 mg/1, but stimulated growth at lower cons. The 96 hr LC_{50} values based on the death response of the fathead minnow to alpha TNT and TNTcc were 2.58 and 1.60 mg/1,

respectively. The 96 hr LC_{50} values based on the behavioral responses were 0.46 and 0.64 mg/1, respectively. There was no response to concs of 0.05 mg v alpha TNT and 0.07 mg/1 TNTcc.

Steevens, J.A., Duke, B.M., Lotufo, G.R. and Bridges, T.S. 2002. Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to Chironomus tentans and Hyalella azteca: low-dose hormesis and high-dose mortality. *Environmental Toxicology and Chemistry.* 21(7): 1475-1482.

The toxicity of the explosives 2,4,6-trinitrotoluene (TNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive [RDX]); and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive [HMX]), was evaluated in spiked sediment with two freshwater invertebrates. The midge *Chironomus tentans* and the amphipod *Hyalella azteca* demonstrated significant toxic effects after exposure to TNT and its degradation products, 1,3,5-trinitrobenzene (TNB) and 2,4-diamino-6-nitrotoluene (2,4-DANT). Significant reductions in survival of *C. tentans* exposed to TNT, TNB, and 2,4-DANT were observed at nominal sediment concentrations as low as 200 mg/kg. *Hyalella azteca* was more sensitive to TNT, TNB, and 2,4-DANT than the midge, where significant reductions in survival were observed at nominal concentrations of 50, 100, and 200 mg/kg, respectively. Survival of the midge and the amphipod was unaffected after exposure to RDX or HMX at the highest concentrations of 1,000 and 400 mg/kg, respectively. Growth of the midge, measured as total weight, was significantly reduced by 2,4-DANT. However, significantly increased growth was observed after exposure to sublethal concentrations of RDX and HMX. Although significant reductions in amphipod survival were observed at high concentrations of TNB, growth was significantly increased at sublethal concentrations. The results of the current investigation suggest that organisms exposed to explosives at contaminated sites may be affected at concentrations less than 25 mg/kg through hormetic growth enhancement and at higher concentrations through increased mortality.

Key for Table 1.

Categories	
P	Physical / Chemical Properties
E	Environmental Fate and Transport
T	Toxicity
B	Bioaccumulation
Agents	
DNB	1,3-Dinitrobenzene
DNP	2,4-Dinitrophenol
DNT	Dinitrotoluene and isomers
HMX	High Melting Point Explosive
NB	Nitrobenzene and isomers
NG	Nitroglycerin
NP	Nitrophenol and isomers
PETN	Pentaerythritol Tetranitrate
RDX	Cyclotrimethylenetrinitramine
TA	Terephthalic Acid
TET	Trinitrophenylmethylnitramine (Tetryl)
TNB	1,3,5-Trinitrobenzene
TNT	Trinitrotoluene
UG	Unspecified or General
Status	
A	Abstract Available
C	Complete Citation Available
N	No Abstract or Citation Available

Table 1. Database of citations for military-related compounds.

Agent	Category	Status	Notes	Citation
DNB	T	C	Toxicological profile for DNB	ATSDR. 1995. Toxicological Profile for 1,3-Dinitrobenzene and 1,3,5-Trinitrobenzene. Prepared for Agency of Toxic Substances and Disease Registry.
DNB	E	C	Biodegradation of NB	Mitchell, W.R. and Dennis, W. 1982. Biodegradation of 1,3-Dinitrobenzene. *Journal of Environmental Science and Health, Part A.* 17(6): 837-853.
DNP	T	C	Toxicological profile of NP	ATSDR. 1995. Toxicological Profile for Dinitrophenols. Prepared for Agency of Toxic Substances and Disease Registry.
DNT	T	C	Toxicological profile for DNT	ATSDR. 1998. Toxicological Profile for 2,4- and 2,6-Dinitrotoluene. Prepared for Agency of Toxic Substances and Disease Registry.
DNT	T	N	Reproductive toxicity tests of DNT to rats	Bloch E, B Gondas, M Gatz, SK Varma, B Thysen. 1988. Reproductive toxicity of 2,4-dinitrotoluene in the rat. *Toxicol Appl Pharmacol.* 94(3):466-472.
DNT	E	A	Aerobic treatment of DNT	Christopher, H.J., Boardman, G.D. and Freedman, D.L. 2000. Aerobic biological treatment of 2,4-dinitrotoluene in munitions plant wastewater. *Water Research [Water Res.].* 34(5): 1595-1603.
DNT	T	N	Toxicity test of DNT to mice	Dacre JC, CB Hong, HV Ellis III, CC Lee, H Spirnz, JP Glennon. 1985. Subchronic and chronic studies of 2,4-dinitrotoluene. Part III. Mice *J Am Coll Toxicol.* 4(4):257-269.
DNT	T	N	Toxicity test of DNT to beagle dogs	Dacre JC, HV Ellis III, CB Hong, CC Lee, JP Glennon. 1985. Subchronic and chronic studies of 2,4-dinitrotoluene. Part I. Beagle dogs. *J Am Coll Toxicol.* 4(4):233-242.
DNT	T	C	Toxicity of DNT and metabolites	Dodard SG, AY Renoux, J Hawari, G Ampleman, S Thiboutot, GI Sunahara. 1999. Ecotoxicity characterization of dinitrotoluenes and some of their reduced metabolites. *Chemosphere.* 38(9):2071-2079.
DNT	T	N	Chronic toxicity of DNT in rats	Ellis HV, CB Hong, JC Dacre, CC Lee. 1978. Chronic toxicity of 2,4-dinitrotoluene in the rat. *Toxicol Appl Pharmacol.* 45:245-246.
DNT	T	N	Mammalian toxicity of DNT	Ellis HV, JH Hagensen, JR Hodgson, JL Minor, C Hong. Mammalian toxicity of munitions compounds. Phase III. Effects of lifetime exposure. Part I. 2,4-dinitrotoluene. Midwest Research Institute, Kansas City, MO. Report No. AD-A077692.
DNT	E	A	Water quality criteria for DNT	Etnier EL. 1987. Water Quality Criteria for 1,4-dinitrotoluene and 2,6-dinitrotoluene: Final Report. Department of Energy, Washington, DC. Aug:149.
DNT	T	A	Toxicity of DNT to aquatic organisms	Hartley WR. 1981. Evaluation of Selected Subacute Effects of the Nitrotoluene Group of Munitions Compounds on Fish and Potential Use in Aquatic Toxicity Evaluation. Tulane University, New Orleans, LA. School of Public Health and Tropical Medicine. 1981:223.
DNT	T	C	Toxicity of several DNT isomers	Holen, I., Mikalsen, S.O. and Sanner, T. 1990. Effects of dinitrotoluenes on morphological cell transformation and intercellular communication in Syrian hamster embryo cells. *J Toxicol Environ Health.* 29(1): 89-98.
DNT	T	N	Metabolism and toxicity of DNT to rats	Kozuka H, M-A Mori, Y Naruse. 1979. Metabolism and toxicity of dinitrotoluene: Toxicological study of 2,4-dinitrotoluene in rats in long-term feeding. *J Toxicol Sci.* 4(3):221-228.
DNT	T	N	Toxicity of DNT in rats	Lane RW, GS Simon, RW Dougherty, JL Egle Jr., JF Borzelleca. 1985. Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotoluene in the male rat. *Drug Chem Toxicol.* 8:265-280.
DNT	B	A	Toxicokinetics of DNT	Lang, P.Z., Wang, Y., Chen, D.B., Wang, N., Zhao, X.M. and Ding, Y.Z. 1997. Bioconcentration, elimination and metabolism of 2,4-dinitrotoluene in carps(Cyprinus Carpio L.). *Chemosphere.* 35(8): 1799-1815.
DNT	T	N	Toxicity test of DNT to rats	Lee CC, CB Hong, HV Ellis III, JC Dacre, JP Glennon. 1985. Subchronic and chronic studies of 2,4-dinitrotoluene. Part II. Rats *J Am Coll Toxicol.* 4(4):243-256.

Agent	Category	Status	Notes	Citation
DNT	B	A	Toxicokinetics of DNT	Mori, M.A., Sayama, M., Shoji, M., Inoue, M., Kawagoshi, T., Maeda, M. and Honda, T. 1997. Biliary excretion and microfloral transformation of major conjugated metabolites of 2,4-dinitrotoluene and 2,6-dinitrotoluene in the male Wistar rat. *Xenobiotica.* 27(12): 1225-1236.
DNT	B	A	Toxicity and metabolism of DNT in rats	Mori, M.A., Shoji, M., Dohrin, M., Kawagoshi, T., Honda, T. and Kozuka, H. 1996. Further studies on the urinary metabolites of 2,4-dinitrotoluene and 2,6-dinitrotoluene in the male Wistar rat. Xenobiotica. 26(1): 79-88..
DNT	T	C	Toxicity and metabolism of DNT in rats	Rickert DE, BE Butterworth, JA Popp. 1984. Dinitrotoluene: Acute toxicity, oncogenicity, genotoxicity, and metabolism. *Crit Rev Toxicol.* 13(3)217-234.
DNT	T	N	Toxicity and elimination of DNT in male and female rats	Rickert DE, RM Long. 1980. Tissue distribution of 2,4-dinitrotoluene and its metabolites in male and female Fischer-344 rats. *Toxicol Appl Pharmacol.* 56(2):286-293.
DNT	E	A	Biodegradation of DNT	Riefler, R.G. and Smets, B.F. 2000. Enzymatic Reduction of 2,4,6-Trinitrotoluene and Related Nitroarenes: Kinetics Linked to One-Electron Redox Potentials. *Environmental Science & Technology [Environ. Sci. Technol.].* 34(18): 3900-3906.
DNT	T	N	Toxicity of DNT to carp	Xu J and J Tisong. 1998. The acute and subacute toxicity of 2,4-DNT to carp. *Huanjing Kexue.* 19(2):89-91.
DNT DNB	E	A	Biodegradation of DNT and DNB	Hallas, L.E. and Alexander, M. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. *Applied and Environmental Microbiology.* 45(4): 1234-1241.
DNT NB	E	N	Study of degradation pathways of DNT and NB	Johnson GR and JC Spain. 2003. Evolution of catabolic pathways for synthetic compounds: Bacterial pathways for degradation of 2,4-dinitrotoluene and nitrobenzene. *App Microbiol Biotechnol.* 62(2-3):110-123.
DNT NB	E	A	Biodegradation of compounds under anaerobic conditions	Razo-Flores E, G Lettings, JA Field. 1999. Biotransformation and biodegradation of selected nitroaromatics under anaerobic conditions. *Biotechnol Prog.* 15(3):258-265.
DNT NB	T	C	Toxicity of mixtures to Vibrio fischeri	Yuan X, G Lu, J Zhao. 2002. QSAR study on the joint toxicity of 2,4-dinitrotoluene with aromatic compounds to Vibrio fischeri. *J Environ Sci Health.* A37(4):573-578.
DNT NB TNB	T	C	Toxicity of munitions compounds to aquatic organisms	Wellington DR and WR Mitchell. 1991. In vitro cytotoxicity of certain munitions nitroaromatic compounds. *Chemosphere.* 23(3):363-374
DNT TET	T	N	Toxicity of munitions to aquatic organisms	Burrows D, JC Dacre. 1975. Toxicity to aquatic organisms and chemistry of nine selected waterborne pollutants from munitions manufacture - A literature evaluation. NTIS AD-A010 660. Report TR 7503.
DNT TET	T	N	Toxicity of spiked sediments and porewater	Carr RS and M Nipper. Toxicity of marine sediments and pore waters spiked with ordnance compounds. Courter Products, Boyne City, MI.
DNT TET TNP	T	C	Chemical and toxicity tests on spiked sediments	Nipper M, RS Carr, JM Biedenbach, RL Hooten, K Miller. 2002. Toxicological and chemical assessment of ordnance compounds in marine sediments and porewaters. *Mar Poll Bull.* 44(8):789-806.
DNT TNT DNB NB TET DNP RDX	T	C	Database of toxicity data for ordnance compounds	Nipper M, RS Carr, JM Biedenbach, RL Hooten, K Miller, S Saepoff. 2001. Development of marine toxicity data for ordnance compounds. *Arch Environ Contam Toxicol.* 41(3):308-318.

Agent	Category	Status	Notes	Citation
TNT, TNB, RDX, HMX	E	N	Factors affecting solubility of TNT, TNB, RDX, HMX	Amos, J.C., Major, M.A., Checkai, R.T. and Simini, M. 1994. Effects of cosolutes on the aqueous solubility of TNT, TNB, RDX, and HMX. Society of Environmental Toxicology and Chemistry 15th Annual Meeting: Ecological Risk: Science Policy, Law and Policy, Denver, CO.
HMX	T	C	Toxicological profile of HMX	ATSDR. 1997. Toxicological Profile for HMX (Octahydro-1,3,57-tetranitro-1,3,5,7-tetrazocine). Prepared for Agency of Toxic Substances and Disease Registry.
HMX	E	A	Water quality criteria for HMX	Bausum HT. 1989. Recommended Water Quality Criteria for Octahydro-1,3,5,7-Tentranitro-1,3,5,7-Tetrazocin (HMX). Army Biomedical Research and Development Laboratory, Fort Detrick, MD. 27:87.
HMX	T	A	Acute toxicity of HMX to aquatic organisms	Bentley RE, GA LeBlanc, GA Hollister, BH Sleight, III. 1977. Acute Toxicity of 1,3,5,7-Tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) to Aquatic Organisms. EG and G Bionomics, Wareham, MA. Apr 29:29.
HMX	T	A	Acute toxicity of HMX to aquatic organisms	Bentley RE, SR Petrocelli, DC Suprenant. 1984. Determination of the Toxicity to Aquatic Organisms of HMX and Related Wastewater Constituents. Part 3. Toxicity of HMX, TAX, and SEX to Aquatic Organisms. Springborn Bionomics, Inc, Wareham, MA. Oct:94.
HMX	E	A		Boopathy, R. 2001. Enhanced biodegradation of cyclotetramethylenetetranitramine (HMX) under mixed electron-acceptor condition. *Bioresour Technol.* 76(3): 241-244.
HMX	B	C	Toxicokinetics of HMX in rat and mouse	Cameron BD. 1986. HMX: Toxicokinetics of (^{14})C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171600.
HMX	T	A	Acute toxicity of HMX	Cuthbert JA et al. 1985. HMX: Acute Toxicity Tests in Laboratory Animals. US Army Medical Research and Development Command, Ft. Detrick, MD. Report No. 2051.
HMX	T	C	Toxicity of HMX to mice	Everett DJ and SM Maddock. 1985. HMX: 13 Week Toxicity Study in Mice by Dietary Administration. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171602.
HMX	T	C	Toxicity of HMX to mice	Greenough RJ and P McDonald. 1985. HMX: 14 Day Toxicity in Mice by Dietary Administration. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171596.
HMX	E	A	Accumulation of HMX in plants	Groom, C.A., Halasz, A., Paquet, L., Morris, N., Olivier, L., Dubois, C. and Hawari, J. 2002. Accumulation of HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in Indigenous and agricultural plants grown in HMX-contaminated anti-tank firing-range soil. *Environmental Science & Technology.* 36(1): 112-118.
HMX	E	A	Aerobic degradation of HMX	Harkins, V.R., Mollhagen, T., Heintz, C. and Rainwater, K. 1999. Aerobic biodegradation of high explosives, phase I - HMX. *Bioremediation Journal.* 4: 285-290.
HMX	E	A	Degradation of HMX in sludge	Hawari, J., Halasz, A., Beaudet, S., Paquet, L., Ampleman, G. and Thiboutot, S. 2001. Biotransformation routes of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by municipal anaerobic sludge. *Environ Sci Technol.* 35(1): 70-75.
HMX	B	C	Toxicity of HMX to rats and mice	Henderson MS. 1985. HMX: Analysis in Plasma Obtained after 90 day Toxicity Studies with Rats and Mice. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171603.
HMX	T	A	Acute toxicity of HMX to aquatic organisms	LeBlanc GA, DA Schoenfeld, DC Surpenant, RE Bentley. 1983. Determination of the Toxicity to Aquatic Organisms of HMX and Related Wastewater Constituents. Part 1. The Effects of Food Concentration, Animal Interactions, and Water Volume on Survival Growth and Reproduction of *Daphnia magna* under Flow-Through Conditions. EG and G Bionomics, Wareham, MA. Jan:43.
HMX	T	A	Acute toxicity of HMX to aquatic organisms	LeBlanc GA, DA Schoenfeld, DC Surpenant, RE Bentley. 1983. Determination of the Toxicity to Aquatic Organisms of HMX and Related Wastewater Constituents. Part 2. The Acute and Chronic Toxicity of Acetone, Dimethyl Formamide, and Triethylene Glycol to *Daphnia magna*. EG and G Bionomics, Wareham, MA. Jan:47.

Agent	Category	Status	Notes	Citation
HMX	E	A	Drinking water health advisory for HMX	McLellan WL, WR Hartley, ME Brower. 1992. Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX). Drinking Water Health Advisory: Munitions. Lewis Publishers, Boca Raton, FL. 247-273.
HMX	E	A	Fate of HMX in soils	Monteil-Rivera, F., Groom, C. and Hawari, J. 2003. Sorption and degradation of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in soil. *Environ Sci Technol.* 37(17): 3878-3884.
HMX	T	C	Toxicity of HMX to earthworms	Robidoux PY, J Hawari, S Thiboutot, G Ampleman, GI Sunahara. Chronic toxicity of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in soil determined using the earthworm (Eisenia andrei) reproduction test. *Environ Pollut.* 111(2):283-92.
HMX	T	A	Acute and subchronic toxicity of HMX	Wilson AB. 1985. Determination of the Acute and Subchronic Mammalian Toxicity of HMX. US Army Medical Research and Development Command, Ft. Detrick, MD. ADA-A173 743.
HMX	E	A	Uptake and fate of HMX in plants	Yoon, J.M., Oh, B.T., Just, C.L. and Schnoor, J.L. 2002. Uptake and leaching of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by hybrid poplar trees. *Environmental Science & Technology.* 36(21): 4649-4655.
NB	T	C	Toxicological profile of NB	ATSDR. 1990. Toxicological Profile for Nitrobenzene. Prepared for Agency of Toxic Substances and Disease Registry.
NB	T	C	Literature review and summary of NB studies	Beauchamp RO, RD Irons, DE Rickert, DB Couch, TE Hamm Jr. 1982. A critical review of the literature on nitrobenzene toxicity. *Crit Rec Toxicol.* 11(1):33-84.
NB	T	C	Toxicity testing of NB on fish and amphibians	Black JA, WJ Birge, WE McDonnall, AG Westerman, BA Ramey, DM Bruser. 1982. Aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. University of Kentucky Water Resource Research Institute, Lexington, KY. Report No. 133.
NB	T	A	NB toxicity to rats	Bond, J.A., Chism, J.P., Rickert, D.E. and Popp, J.A. 1981. Induction of hepatic and testicular lesions in Fischer-344 rats by single oral doses of nitrobenzene. *Fundam Appl Toxicol.* 1(5): 389-394.
NB	T	A	NB toxicity to mice	Burns, L.A., Bradley, S.G., White, K.L., Jr., McCay, J.A., Fuchs, B.A., Stern, M., Brown, R.D., Musgrove, D.L., Holsapple, M.P., Luster, M.I. and et al. 1994. Immunotoxicity of nitrobenzene in female B6C3F1 mice. *Drug Chem Toxicol.* 17(3): 271-315.
NB	T	C	Inhalation toxicity of NB to mice and rats	Cattley RC, JI Everitt, EA Gross, OR Moss, TE Hamm Jr., JA Popp. 1994. Carcinogenicity and toxicity of inhaled nitrobenzene in B6C3F1 mice and F344 and CD rats. *Fundam Appl Toxicol.* 22(3):328-340.
NB	T	C	Structure-activity toxicity of NB	Cronin, M.T., Gregory, B.W. and Schultz, T.W. 1998. Quantitative structure-activity analyses of nitrobenzene toxicity to Tetrahymena pyriformis. *Chem Res Toxicol.* 11(8): 902-908.
NB	T	A	Reproductive toxicity in rats from NB inhalation	Dodd DE, EH Fowler, WM Snellings, IM Pritts, RW Tyl, JP Lyon, FO O'Neal, G Kimmerle. 1987. Reproduction and fertility evaluation in CD rats following nitrobenzene inhalation. *Fundam Appl Toxicol.* 8(4):493-505.
NB	E	A	Degradation of NB	Haigler, B.E. and Spain, J.C. 1991. Biotransformation of nitrobenzene by bacteria containing toluene degradative pathways. *Appl Environ Microbiol.* 57(11): 3156-3162.
NB	B	A	NB toxicity to rats	Harada, N. and Omura, T. 1980. Participation of cytochrome P-450 in the reduction of nitro compounds by rat liver microsomes. *J Biochem (Tokyo).* 87(5): 1539-1554.
NB	T	A	NB toxicity to rats	Iida, S., Misaka, H. and Naya, M. 1997. A flow cytometric analysis of cytotoxic effects of nitrobenzene on rat spermatogenesis. *J Toxicol Sci.* 22(5): 397-407.
NB	T	N	Effects of NB on rat sperm motility	Kito Y, Y Hamamatsu, M Naya. 1999. Application of Crj:CD(SD)IGS rats to reproduction and developmental toxicity study: effects of nitrobenzene on sperm examination. *Teratology.* 59(5):39-40.
NB	T	A	Testicular toxicity of NB to rats	Levin AA, T Bosakowski, LL Earle, BE Butterworth. 1988. The reversibility of nitrobenzene-induced testicular toxicity: Continuous monitoring of sperm output from vasocystotomized rats. *Toxicology.* 53(2-3):219-230.

Agent	Category	Status	Notes	Citation
NB	E	C	Environmental fate of benzene derivatives	Lu, P.Y. and Metcalf, R.L. 1975. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. *Environ Health Perspect.* 10: 269-284.
NB	T	N	Toxicity of benzene derivatives to fathead minnow	Marchini S, ML Tosato, TJ Norberg-King, DE Hammermeister, MD Hoglund. 1992. Lethal and sublethal toxicity of benzene derivatives to the fathead minnow, using a short-term test. *Environ Toxicol Chem.* 11(2):187-195.
NB	E	A	Uptake and fate of NB in plants	Mc Farlane, C., Pfleeger, T. and Fletcher, J. 1990. Effect, uptake and disposition of nitrobenzene in several terrestrial plants. *Environmental Toxicology and Chemistry.* 9(4): 513-520.
NB	T	N	NB vapors toxicity tests on mice and rats	Medinsky MA and RD Irons. 1985. Sex, strain, and species differences in the response of rodents to nitrobenzene vapors. IN: Toxicity of Nitroaromatic Compounds. Eds: Rickert, DE. Hemisphere Publishing Corporation, New York.
NB	T	A	NB toxicity to rats	Morgan, K.T., Gross, E.A., Lyght, O. and Bond, J.A. 1985. Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. *Neurotoxicology.* 6(1): 105-116.
NB	E	A	Anaerobic degradation of NB	Nielsen, P.H., Bjarnadottir, H., Winter, P.L. and Christensen, T.H. 1995. In situ and laboratory studies on the fate of specific organic compounds in an anaerobic landfill leachate plume, 2. Fate of aromatic and chlorinated aliphatic compounds. *Journal of Contaminant Hydrology.* 20(1-2): 51-66.
NB	T	N	Toxicity of NB and derivatives to rat hepatocytes	O'Brien PJ, WC Wong, J Silva, S Khan. 1990. Toxicity of nitrobenzene compounds towards isolated hepatocytes: dependence on reduction potential. *Xenobiotica.* 20(9):945-955.
NB	T	N	Subchronic toxicity of NB to rats	Reddy TV, FB Daniel, M Robinson, GR Olson, B Wiechman. Subchronic toxicity studies on 1,3,5-trinitrobenzene and tetryl in rats. Environmental Monitoring Systems Lab, Cincinnati, OH.
NB	T	N	Metabolism and excretion of NB by rats and mice	Rickert D, JA Bond, RM Long, JP Chism. 1983. Metabolism and excretion of nitrobenzene by rats and mice. *Toxicol Appl Pharmacol.* 67(2):206-214.
NB	T	N	Toxicity of NB	Rickert, DE, Ed. Toxicity of Nitroaromatic Compounds. Hemisphere Publishing Corporation, Washington, D.C. 1985.
NB	T	A	Toxicity of inhaled NB to rats	Tyl RW, KA France, LC Fisher, DE Dodd, IM Pritts, JP Lyon, FO O'Neal, G Kimmerle. Developmental toxicity evaluation of inhaled nitrobenzene in CD rats. *Fundam Appl Toxicol.* 8(4):482-492.
NB	E	C	Fate and transport of NB in sandy soils	Wilson, J.T., Enfield, C.G., Dunlap, W.J., Cosby, R.L., Foster, D.A. and Baskin, L.B. 1981. Transport and Fate of Selected Organic Pollutants in a Sandy Soil. *Journal of Environmental Quality.* 10(4): 501-506.
NB	T	C	Toxicity of nitrobenzenes to aquatic organisms	Yen JH, KH Lin, YA Wang. 2002. Acute lethal toxicity of environmental pollutants to aquatic organisms. *Ecotoxicol Environ Saf.* 52(2):113-116.
NB DNB	T	A	NB toxicity to Sertoli cell function	Allenby, G., Sharpe, R.M. and Foster, P.M. 1990. Changes in Sertoli cell function in vitro induced by nitrobenzene. *Fundam Appl Toxicol.* 14(2): 364-375.
NB DNB	T	C	Toxicity of NB to rats	Cave, D.A. and Foster, P.M. 1990. Modulation of m-dinitrobenzene and m-nitrosonitrobenzene toxicity in rat Sertoli--germ cell cocultures. *Fundam Appl Toxicol.* 14(1): 199-207.
NB DNB	TB	C	Toxicity and bioconcentration factor of NB to fish	Deneer JW, TL Sinnige, JLM Hermens. 1987. Quantitative structure-activity relationships for the toxicity and bioconcentration factor of nitrobenzene derivatives towards the guppy Poecilia reticulata. *Aquatic Toxicology.* 10(2-3):115-129.
NB DNB	P	A	Physical properties of NB	Hajjar, N.P., Brower, M.E., Turck, P.A., Kruger, C.L. and Hartley, W.R. 1992. 1,3-Dinitrobenzene (DNB). *IN: Drinking Water Health Advisory: Munitions. Lewis Publishers, Boca Raton, FL.* 1992: 49-86.
NB DNB	B	A	Toxicokinetics of NB	Nystrom, D.D. and Rickert, D.E. 1987. Metabolism and excretion of dinitrobenzenes by male Fischer-344 rats. *Drug Metab Dispos.* 15(6): 821-825.
NB DNB	E	A	Degradation of NB	Trapido, M., Dello, A., Goi, A. and Munter, R. 2003. Degradation of nitroaromatics with the Fenton reagent. *Proceedings of the Estonian Academy of Sciences, Chemistry [Proc. Eston. Acad. Sci. Chem.].* 52(1): 38-47.

Agent	Category	Status	Notes	Citation
NB DNT	E	C	Microbial degradation of NB and DNT	Davis, E.M., Murray, H.E., Liehr, J.G. and Powers, E.L. 1981. Basic Microbial Degradation Rates and Chemical Byproducts of Selected Organic Compounds. *Water Research.* 15(9): 1125-1127.
NB DNT	E	C	Partitioning of organic pollutants in aquatic ecosystems	Davis, E.M., Turley, J.E., Casserly, D.M. and Guthrie, R.K. 1983. Partitioning of selected organic pollutants in aquatic ecosystems. 5. International Biodeterioration Symposium, Aberdeen (UK), Sep 1981.
NB DNT	T	N	Mammalian and bacterial metabolism of NB and DNT	Rickert DE. 1985. Mammalian and bacterial metabolism of nitroaromatic compounds. IN: Toxicity of Nitroaromatic Compounds. Rickert DE ed. Hemisphere Publishing Corporation, Washington DC.
NB NP	E	C	Volatilization of organic chemicals from water	Ince, N. and Inel, Y. 1989. Volatilization of Organic Chemicals from Water. *Water, Air and Soil Pollution WAPLAC.* 47: 1-2.
NB NP DNP	T	C	Toxicity of NB to fish	Holcombe GW, GL Phipps, ML Knuth, T Felhaber. 1984. The acute toxicity of selected substituted phenols, benzenes and benzoic acid esters to fathead minnows Pimephales promelas. *Environ Pollut.* 35(4):367-381.
NB TET	T	N	Toxicity of NB and TET to guinea pigs	FitzGerald GB, A Austin, N DiGuilio. Acute toxicity evaluation of nitroaromatic compounds. Toxikon Corp., Woburn, MA.
NB TET	T	N	Subchronic toxicity of NB and TET to rats	Reddy TV. Subchronic toxicity studies on1,3,5-trinitrobenzene, 1,3-dinitrobenzene, and tetryl in rats. Subchronic toxicity evaluation of n-methyl-n,2,4,6-tetranitroaniline tetryl) in Fischer 344 rats. Environmental Monitoring Systems Labs, Cincinnati, OH.
NB TNB	T	N	Toxicity of nitrobenzene and trinitrobenzene mixtures	Lange JH and KW Thomulka. 1998. Evaluation of mixture toxicity for nitrobenzene and trinitrobenzene at various equitoxic concentrations using the Vibrio harveyi bioluminescence toxicity test. *Fresenius Environ Bull.* 7(7-8):444-451.
NB TNB	T	C	Toxicity of NB mixtures to marine bacterium	Thomulka KW and JH Lange. 1997. Mixture toxicity of nitrobenzene and trinitrobenzene using the marine bacterium Vibrio harveyi as the test organism. *Ecotoxicol Environ Saf.* 36(2):189-195.
NB TNB DNB	T	C	Toxicity of NB derivatives	Gough, K.M., Belohorcova, K. and Kaiser, K.L.E. 1994. Quantitative structure-activity relationships (QSARs) of Photobacterium phosphoreum toxicity of nitrobenzene derivatives. *Science of the Total Environment [SCI. TOTAL ENVIRON.].* 142(3): 179-190.
NB TNB DNB	T	C	Toxicity of BV and derivatives to algae	Schmitt H, R Altenburger, B Jastorff, G Schueuermann. 2000. Quantitative structure-activity analysis of the algae toxicity of nitroaromatic compounds. *Chem Res Toxicol.* 13(6):441-450.
NG	E	A	Aerobic degradation of NG	Accashian, J.V., Vinopal, R.T., Kim, B.J. and Smets, B.F. 1998. Aerobic growth on nitroglycerin as the sole carbon, nitrogen, and energy source by a mixed bacterial culture. *Appl Environ Microbiol.* 64(9): 3300-3304.
NG	T	A	Toxicity of NG by skin absorption	Anderson, J.A., McGuire, E.J., Watkins, J.R., Fitzgerald, J.E. and de la Iglesia, F.A. 1983. Toxicology studies with a stable intravenous formulation of nitroglycerin. J Appl Toxicol. 3(3): 161-165.
NG	E	A	Degradation of NG in sludge	Bhaumik, S., Christodoulatos, C., Brodman, B.W. and Pal, N. 1998. Biodegradation of glycerol trinitrate by activated sludge: Cosubstrate requirements, inhibition, and kinetics. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering [J. Environ. Sci. Health, Pt. A: Toxic/Hazard. Subst. Environ. Eng.].*(4): 574-571.
NG	T	N	Toxicity of replacement chemicals for NG in munitions	Brown LD, CR Wheeler, DW Korte. Acute oral toxicity of DIGL-RP solid propellant in Sprague-Dawley rats. Letterman Army Institute of Research.
NG	E	A	Anaerobic degradation of NG	Christodoulatos, C., Bhaumik, S. and Brodman, B.W. 1997. Anaerobic biodegradation of nitroglycerin. *Water Research [WATER RES.].* 31(6): 1462-1470.
NG	E	C	Analysis of combustion products of NG-containing propellant	Cropek, D.M., Kemme, P.A., Day, J.M. and Cochran, J. 2002. Use of Pyrolysis GC/MS for Predicting Emission Byproducts from the Incineration of Double-Base Propellant. *Environmental Science & Technology [Environ. Sci. Technol.].* 36(20): 4346-4351.

Agent	Category	Status	Notes	Citation
NG	T	N	Pharmacokinetics of NG	Curry SH and SM Aburawi. 1985. Analysis, disposition, and pharmacokinetics of nitroglycerin. Division of Pharmacokinetics, College of Pharm., Univ. of Florida, Gainesville, FL.
NG	T	A	Toxicity of NG	Fehrenbach, A., Wittwer, T., Meyer, D., von Vietinghoff, S., Viehover, M., Fehrenbach, H., Richter, J. and Wahlers, T. 2001. Nitroglycerin alters alveolar type II cell ultrastructure after ischemia and reperfusion. *J Heart Lung Transplant.* 20(8): 876-888.
NG	E	A	NG degradation in wastewaters	Hempfling, C. 1997. Ultraviolet/oxidation treatment of explosive wastewaters using a commercial process. *Environmental Progress [Environ. Prog.]. no.* 3(164).
NG	T	A	Chronic toxicity test of NG to rabbits	Imoto S, M Kuramoto, K Iwabuchi, H Nagai, K Shimpo. 1986. Percutaneous chronic toxicity study of 10% nitroglycerin (NT-1 ointment) in rabbits. *J Toxicol Sci.* 11(2):31-57.
NG	T	A	Teratological test of NG to rabbits	Imoto S, M Nakao, M Kuramoto, M Takeuchi, K Shimpo, T Tanabe. 1986. Teratological test of 10% nitroglycerin (NT-1 ointment) in rabbits. *J Toxicol Sci.* 11(2):59-70.
NG	T	C	Penetration and kinetics of NG across mouse skin	Kikkoji T, M Gumbleton, N Higo, RH Guy, LZ Benet. 1991. Percutaneous penetration kinetics of nitroglycerin and its dinitrate metabolites across hairless mouse skin in vitro. *Pharm Res.* 8:1231-1237.
NG	B	A	Degradation of NG by rat excreta	King, S.Y. and Fung, H.L. 1984. Rapid microbial degradation of organic nitrates in rat excreta. Re-examination of the urinary and fecal metabolite profiles of pentaerythritol tetranitrate in the rat. *Drug Metab Dispos.* 12(3): 353-357.
NG	T	N	Toxicity tests of NG based propellant	Lewis CM, LD Brown, DW Korte. Dermal sensitization potential of JA-2 solid propellant in guinea pigs. Letterman Army Institute of Research, Presidio of San Francisco, CA.
NG	T	A	Mutagenicity of NG to bacteria	Maragos CM, AW Andrews, LK Keefer, RK Elespuru. 1993. Mutagenicity of glyceryl trinitrate (nitroglycerin) in Salmonella typhimurium. *Mutat Res.* 298(3):187-195.
NG	T	N	Toxicity tests of NG based propellant	Morgan EW, JD Justus, DW Korte. Primary dermal irritation potential of HA-2 solid propellant in rabbits. Letterman Army Institute of Research, Presidio of San Francisco, CA.
NG	E	A	Phytoremediation of NG	Rosser, S.J., French, C.E. and Bruce, N.C. 2001. Engineering plants for the phytodetoxification of explosives. *In Vitro Cellular & Developmental Biology Plant [In Vitro Cell. Dev. Biol. Plant].* 37(3): 330-333.
NG	T	N	Toxicity of replacement chemicals for NG in munitions	Ryabik JR, LD Brown, CR Wheeler, DW Korte. Acute oral toxicity of diethyleneglycol dinitrate (DEGDN) in ICR mice. Letterman Army Institute of Research.
NG	T	N	Toxicity of cutaneous NG to rats	Sato K, H Taniguchi, T Ohtsuka, K Hoshino, K Uchiyama, M Koide. 1984. Toxicity studies of nitroglycerin. 9. Prenatal and postnatal cutaneous administration in rats. *Kiso to Rinsho.* 18:3564-3586.
NG	T	N	Toxicity of subcutaneous NG to rats	Sato K, H Taniguchi, T Ohtsuka, Y Himeno, K Uchiyama, M Koide. 1984. Toxicity studies of nitroglycerin. 6. Prenatal and postnatal subcutaneous administration in rats. *Kiso to Rinsho.* 18:3511-3524.
NG	T	N	Toxicity of cutaneous NG to rats	Sato K, H Taniguchi, T Ohtsuka, Y Himeno, K Uchiyama, M Koide. 1984. Toxicity studies of nitroglycerin. 7. Cutaneous administration during organogenesis in rats. *Kiso to Rinsho.* 18:3525-3552.
NG	T	N	Toxicity of cutaneous NG to rabbits	Sato K, H Taniguchi, YHimeno, K Hoshino, K Uchiyama, M Koide. 1984. Toxicity studies of nitroglycerin. 8. Cutaneous administration during organogenesis in rabbits. *Kiso to Rinsho.* 18:3553-3563.
NG	T	N	Evaluation of aquatic environmental data for establishing water quality criteria	Sullivan JH Jr., HD Putnam, MA Keirn, BC Pruitt Jr., JC Nichols. A summary and evaluation of aquatic environmental data in relation to establishing water quality criteria for munitions-unique compounds. Part 2. Nitroglycerin. Water and Air Research, Inc., Gainesville, FL.
NG	T	A	Carcinogenicity of NG to rats	Tamano, S., Ward, J.M., Diwan, B.A., Keefer, L.K., Weghorst, C.M., Calvert, R.J., Henneman, J.R., Ramljak, D. and Rice, J.M. 1996. Histogenesis and the role of p53 and K-ras mutations in hepatocarcinogenesis by glyceryl trinitrate (nitroglycerin) in male F344 rats. *Carcinogenesis.* 17(11): 2477-2486.

Agent	Category	Status	Notes	Citation
NG	T	A	Toxicity of NG to rats	Taniguchi, Y., Shimada, K., Kohyama, H., Hamai, Y., Hirao, R., Nishimori, T., Kikumori, M. and Yamamoto, H. 1986. [Percutaneous subacute toxicity study of 10% nitroglycerin (NT-1 ointment) in rabbits]. *J Toxicol Sci.* 11 Suppl 2: 11-29.
NG	T	A	Wildlife toxicity assessment for NG	USA CHPPM. 2001. Wildlife Toxicity Assessment for Nitroglycerin (NG). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01F.
NG	E	A	Degradation of NG	Williams, R.E., Rathbone, D.A., Moody, P.C., Scrutton, N.S. and Bruce, N.C. 2001. Degradation of explosives by nitrate ester reductases. *Biochemical Society symposium.* 68: 143-153.
NG DNT WP TNT	T	N	Mammalian toxicity of ordnance compounds	Ellis HV, JR Hodgson, SW Hwang, LM Halpap, DO Helton. Mammalian toxicity of munitions compounds. Phase I. Acute oral toxicity, primary skin and eye irritation, dermal sensitization, disposition and metabolism, and Ames tests of additional compounds. Midwest Research Institute, Kansas City, MO.
NG RDX TNT	T	C	Toxicity of NG, RDX, and TNT	Burton DT, SD Turley, GT Peters. 1993. Toxicity of Nitroguanidine, Nitroglycerin, Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX), and 2,4,6-Trinitrotoluene (TNT) to Selected Freshwater Aquatic Organisms. Maryland University, College Park. Agriculture Experiment Station. AD-A267 467.
NG WP TNT DNT	T	A	Toxicity of munitions factory water pollution	Dacre JC and DH Rosenblatt. 1974. Mammalian toxicology and toxicity to aquatic organisms of four important types of waterborne munitions pollutants - an extensive literature evaluation. US Army Medical Bioengineering Research and Development Laboratory, Aberdeen Proving Ground, Maryland. Technical Report 7403, NTIS AS778-725.
NP	T	C	Toxicological profile of NP	ATSDR. 1992. Toxicological Profile for Nitrophenols: 2-Nitrophenol, 4-Nitrophenol. Prepared for Agency of Toxic Substances and Disease Registry.
NP	T	C	Toxicity of NP to zebra fish	Braunbeck T, V Storch, R Nagel. 1989. Sex-specific reaction of liver ultrastructure in zebra fish (Brachydanio rerio) after prolonged sublethal exposure to 4-nitrophenol. Aquat Toxicol. 14(3):185-202.
NP	T	C	Intestinal metabolism and transport of NP in rats	Fischer E, A Rafiel, S Bojcsev. 1995. Intestinal elimination of p-nitrophenol in the rat. *Acta Physiologica Hungarica.* 83(4):355-362.
NP	TB	C	Toxicity and bioconcentration of NP in crayfish	Foster, G.D. and Crosby, D.G. 1059. Xenobiotic Metabolism of p-Nitrophenol Derivatives by the Rice Field Crayfish (Procambarus Clarkii). *Environmental Toxicology and Chemistry ETOCDK.* 5(12): 1059-1070.
NP	T	C	Chronic toxicity tests of NP to Daphnia	Francis PC, DW Grothe, JC Scheuring. 1986. Chronic toxicity of 4-nitrophenol to Daphnia magna Straus under static-renewal and flow-through conditions. *Bull Environ Contam Toxicol.* 36(5):730-737.
NP	T	C	Metabolism of NP by frogs	Frank, G. and Beyer, J. 1986. Metabolism of 3-nitrophenol by the frog Rana temporaria. *Xenobiotica.* 16(4): 291-294.
NP	B	A	Toxicokinetics of NP	Gorge, G., Beyer, J. and Urich, K. 1987. Excretion and metabolism of phenol, 4-nitrophenol and 2-methylphenol by the frogs Rana temporaria and Xenopus laevis. *Xenobiotica.* 17(11): 1293-1298.
NP	T	C	Toxicity and metabolism of NP by sheep	Gow, P.J., Ghabrial, H., Treepongkaruna, S., Shulkes, A., Smallwood, R.A., Morgan, D.J. and Ching, M.S. 2000. Conjugation of para-nitrophenol by the isolated perfused neonatal sheep liver. *J Pharm Sci.* 89(1): 36-44.
NP	E	A	Fate of NP in lab sediment-water systems	Heim K, I Schuphan, B Schmidt. 1994. Behavior of (^{14}C)-4-nitrophenol and (^{14}C)-3,4-dichloroaniline in lab sediment-water systems: 1. Metabolic fate and partitioning of radioactivity. *Env Toxicol Chem.* 13(6):879-888.
NP	T	A	QSAR study of NP	Hodson, P.V., Parisella, R., Blunt, B.R., Gray, B. and Kaiser, K.L.E. 1991. Quantitative structure-activity relationships for chronic toxicity of phenol, p-chlorophenol, 2,4-dichlorophenol, pentachlorophenol, p-nitrophenol and 1,2,4-trichlorobenzene to early life stages of rainbow trout (Oncorhynchus mykiss). Department of Fisheries and Oceans, Mont-Joli, Que. (Canada). Physical and Chemical Sciences Branch.

Agent	Category	Status	Notes	Citation
NP	T	A	Factors affecting toxicity of NP	Linton, T.K., Mayer, F.L., Simon, T.L., Malone, J.A. and Marking, L.L. 1994. Salinity and temperature effects on chronic toxicity of 2,4-dinitrophenol and 4-nitrophenol to sheepshead minnows (Cyprinodon variegatus). *Environmental Toxicology and Chemistry.* 13(1): 85-92.
NP	E	A	Factors affecting toxicity of NP	Megharaj, M., Rao, A.S. and Venkateswarlu, K. 1993. Influence of rice straw amendment on persistence and algal toxicity of p-nitrophenol in soil. *Soil Biology and Biochemistry.* 25(9): 1185-1188.
NP	E	C	Biodegradation of NP	Nyholm, N., Lindgaard-Jorgensen, P. and Hansen, N. 1984. Biodegradation of 4-nitrophenol in standardized aquatic degradation tests. *Ecotoxicol Environ Saf.* 8(5): 451-470.
NP	B	A	Toxicokinetics of NP	Pena-Egido, M.J., Marino-Hernandez, E.L., Santos-Buelga, C. and Rivas-Gonzalo, J.C. 1988. Urinary excretion kinetics of p-nitrophenol following oral administration of parathion in the rabbit. *Arch Toxicol.* 62(5): 351-354.
NP	T	C	Kinetics of NP in swine	Qiao GL, SK Chang, JD Brooks, JE Riviere. Dermatoxicokinetic modeling of p-nitrophenol and its conjugation metabolite in swine following topical and intravenous administration. *Toxicol Sci.* 54(2):284-294.
NP	E	C	Accumulation and transport of NP in plants	Shafer WE and J Schonherr. 1985. Accumulation and transport of phenol, 2-nitrophenol, and 4-nitrophenol in plant cuticles. *Ecotoxicol Environ Saf.* 10(2):239-252.
NP	ET	C	Toxicokinetics and biotransformations of NP in red abalone	TenBrook, P.L., Kendall, S.M., Viant, M.R. and Tjeerdema, R.S. 2003. Toxicokinetics and biotransformation of p-nitrophenol in red abalone (Haliotis rufescens). *Aquat Toxicol.* 62(4): 329-336.
NP DNP	T	C	Toxicity of NP derivatives to aquatic organisms	Brecken-Folse JA, FL Mayer, LE Pedigo, LL Marking. 1994. Acute toxicity of 4-nitrophenol and 2,4-dinitrophenol, terbufos, and trichloron to grass shrimp (Palaemonetes spp.) and sheepshead minnow (Cyprinodon variegatus) as affected by salinity and temperature. *Environmental Toxicology and Chemistry.* 13(1):67-77.
NP DNP	ET	A	Fate and toxic effects of NP on methanogenic bacteria	Haghighi-Podeh MR and SK Bhattacharya. Fate and toxic effects of nitrophenols on anaerobic treatment systems. *Water Science and Technology.* 34(5-6):345-350.
NP DNP	T	A	Toxicity of NP derivatives to aquatic organisms	Howe GE, LL Marking, TD Bills, JJ Rach, FL Mayer Jr. 1994. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol, and 2,4-dinitrophenol to the amphipod Gammarus pseudolimnaeus and rainbow trout (Orcorhynchus mykiss). *Environmental Toxicology and Chemistry.* 13(1):51-66.
NP DNP	T	A	Factors affecting toxicity of NP	Howe, G.E., Marking, L.L., Bills, T.D., Boogaard, M.A. and Mayer, F.L., Jr. 1994. Effects of water temperature on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to developing rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry.* 13(1): 79-84.
NP DNP	T	C	Toxicity of NP derivatives to rats	Koizumi M, Y Yamamoto, Y Ito, M Takano, T Enami, E Kamata, R Hasegawa. 2002. Comparative study of the toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats. *The Journal of Toxicological Sciences.* 26(5):299-311.
NP DNP	ET	C	Toxicity and degradability of NP in anaerobic systems	Uberoi, V. and Bhattacharya, S.K. 1997. Toxicity and degradability of nitrophenols in anaerobic systems. *Water Environment Research.* 69(2): 146-156.
PETN	T	C	Long-term toxicity of PETN to rats and mice	Bucher JR, J Huff, SL Eustis, HS Lilja, AS Murthy. 1990. No evidence of toxicity or carinogenicity of pentaerythritol tetranitrate given in the diet to F344 rats and B6C3F1 mice for up to two years. *J Appl Toxicol.* 10(5):353-357.
PETN	T	N	Long-term toxicity of PETN to rats and mice	Bucher, J.R., Huff, J., Haseman, J.K., Eustis, S.L., Lilja, H.S. and Murthy, A.S. 1990. No evidence of toxicity or carcinogenicity of pentaerythritol tetranitrate given in the diet to F344 rats and B6C3F1 mice for up to two years. J Appl Toxicol. 10(5): 353-357.
PETN	E	N	Phytoremediation of PETN	French, C.E., Nicklin, S. and Bruce, N.C. 1998. Aerobic degradation of 2,4,6-trinitrotoluene by Enterobacter cloacae PB2 and by pentaerythritol tetranitrate reductase. Applied and Environmental Microbiology [Appl. Environ. Microbiol.]. 64(8): 2864-2868.

Agent	Category	Status	Notes	Citation
PETN	T	N	Metabolism of PETN by human liver, intestine, kidney, and blood serum	Posadas del Rio FA, FJ Juarez, RC Garcia. 1988. Biotransformation of organic nitrate esters in vitro by human liver, kidney, intestine, and blood serum. *Drug Metab Disposition.* 16(3):477-481.
PETN	E	N	Phytoremediation of PETN	Rosser SJ, CE French, NC Bruce. 2001. Engineering plants for the phytodetoxification of explosives. *In Vitro Cellular and Developmental Biology.* 37(3):330-333.
PETN	T	C	Wildlife toxicity assessment for PETN	USA CHPPM. 2001. Wildlife Toxicity Assessment for Pentaerythritol Tetranitrate (PETN). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01G.
PETN	T	N	Acute toxicity of PETN and NG to dogs	van Oettingen WF and DD Donahue. Acute toxic manifestations of PETN. Toxicity and Potential Dangers of Penta-Erythriton-Tetranitrate, Federal Security Agency, US Public Health Service, Public Health Bulletin No. 282.
PETN	E	N	Degradation of PETN by microbial enzyme	Williams RE, DA Rathbone, PC Moody, NS Scrutton, NC Bruce. 2001. Degradation of explosives by nitrate ester reductase. *Biochem Soc Symp.* 68:143-153.
RDX	E	C	Degradation and degradation products of RDX	Adrian, N.R. and Chow, T. 2001. Identification of hydroxylamino-dinitroso-1,3,5-triazine as a transient intermediate formed during the anaerobic biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Environ Toxicol Chem.* 20(9): 1874-1877.
RDX	P	C	Physical properties of RDX	Anon. 1992. RDX. *Dangerous Properties of Industrial Materials Report.* 12(2): 248-256.
RDX	T	C	Toxicological profile of RDX	ATSDR. 1995. Toxicological Profile for RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine). Prepared for Agency of Toxic Substances and Disease Registry.
RDX	E	A	Anaerobic degradation of RDX	Beller, H.R. 2002. Anaerobic biotransformation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by aquifer bacteria using hydrogen as the sole electron donor. *Water Res.* 36(10): 2533-2540.
RDX	T	A	Toxicity of RDX to aquatic organisms	Best, E.P., Miller, J.L. and Larson, S.L. 2001. Tolerance towards explosives, and explosives removal from groundwater in treatment wetland mesocosms. *Water Sci Technol.* 44(11-12): 515-521.
RDX	E	A	Degradation of RDX	Bhushan, B., Halasz, A., Spain, J., Thiboutot, S., Ampleman, G. and Hawari, J. 2002. Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-tiazine catalyzed by a NAD(P)H: nitrate oxidoreductase from Aspergillus niger. *Environ Sci Technol.* 36(14): 3104-3108.
RDX	T	C	Photolysis effects on RDX toxicity	Burton DT and SD Turley. 1995. Reduction of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) Toxicity to the Cladoceran *Ceriodaphnia dubia* Following Photolysis in Sunlight. *Bull Environ Contam Toxicol.* 55:89-95.
RDX	T	C	Acute and chronic toxicity of RDX	Burton DT, SD Turlet, GT Peters. Acute and Chronic Toxicity of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the fathead Minnow (*Pimephale promelas*). *Chemosphere.* 29(3):567-579..
RDX	T	C	Toxicity of RDX to freshwater algae	Burton DT, SD Turley, GT Peters. 1994. The toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the freshwater green alga *Selenastrum capricornutum. Water, Air and Soil Pollution.* 76:449-457.
RDX	E	C	Environmental fate of RDX	Cataldo DA, SD Harvey, RJ Fellows. 1990. An Evaluation of the Environmental Fate and Behavior of Munitions Material (TNT, RDX) in Soil and Plant Systems. Environmental Fate and Behavior of RDX. Pacific Northwest Laboratory, Richland Washington. 88-PP-8853.
RDX	T	C	Mammalian toxicity of RDX	Cholakis JM, LCK Wong, DL Van Goethem, J Minor, R Short, H Sprins, HV Ellis III. Mammalian Toxicological Evaluation of RDX. Midwest Research Institute, Kansas City, Missouri. ADA-092531.
RDX	E	A	Water quality criteria for RDX	Etnier EL. 1986. Water Quality Criteria for Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Oak Ridge National Laboratory, TN. Jun:118.
RDX	E	A	Degradation products of RDX	Fournier, D., Halasz, A., Spain, J., Fiurasek, P. and Hawari, J. 2002. Determination of key metabolites during biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine with Rhodococcus sp. strain DN22. *Appl Environ Microbiol.* 68(1): 166-172.

Agent	Category	Status	Notes	Citation
RDX	T	C	Chronic toxicity of RDX	Furedi-Machacek M, BS Levine, PM Lish. 1984. Determination of the Chronic Mammalian Toxicological Effects of RDX. Acute Dermal Toxicity Test of hexaydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Rabbits. IIT Research Institute, Chicago, Illinois. Project #L6121.
RDX	T	A	Toxicity of RDX to bobwhite quail	Gogal, O.M., Jr., Johnson, M.S., Larsen, C.T., Prater, M.R., Duncan, R.B., Ward, D.L., Lee, R.B., Salice, C.J., Jortner, B. and Holladay, S.D. 2003. Dietary oral exposure to l,3,5-trinitro-1,3,5-triazine in the northern bobwhite (Colinus virginianus). *Environ Toxicol Chem.* 22(2): 381-387.
RDX	T	C	Effects of RDX on microbial activity	Gong P, J Hawari, S Thiboutot, G Ampleman, GI Sunahara. 2001. Ecotoxicological effects of hexahydro-1,3,5-trinitro-1,3,5-triazine on soil microbial activities. *ET&C.* 20(5):947-951.
RDX	EB	C	Fate of RDX in soils and bioaccumulation by plants	Harvey SD, RJ Fellows, DA Cataldo, RM Bean. 1991. Fate of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants. *ET&C.* 10:845-855.
RDX	E	A	Degradation of RDX	Hawari, J., Beaudet, S., Halasz, A., Thiboutot, S. and Ampleman, G. 2000. Microbial degradation of explosives: biotransformation versus mineralization. *Appl Microbiol Biotechnol.* 54(5): 605-618.
RDX	T	A	Degradation of RDX	Jarvis, A.S., McFarland, V.A. and Honeycutt, M.E. 1998. Assessment of the effectiveness of composting for the reduction of toxicity and mutagenicity of explosive-contaminated soil. *Ecotoxicol Environ Saf.* 39(2): 131-135.
RDX	T	C	Toxicity of RDX	Juck, D., Driscoll, B.T., Charles, T.C. and Greer, C.W. 2003. Effect of experimental contamination with the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine on soil bacterial communities. *FEMS Microbiology Ecology.* 43(2): 255-262.
RDX	T	C	Toxicity of RDX to rats	Levine BS, EM Furedi, DE Gordon, JH Burns, PM Lish. 1981. Thirteen week toxicity study of hexahyro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fischer 344 rats. *Toxicol Lett.* 8:241-245.
RDX	E	A	Drinking water health advisory for RDX	McLellan WL, WR Hartley, ME Brower. 1992. Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX). Drinking Water Health Advisory: Munitions. Lewis Publishers, Boca Raton, FL. 133-180.
RDX	T	C	Acute and subchronic toxicity of RDX	Peters GT, DT Burton, RL Paulson, SD Turley. 1991. The acute and subchronic toxicity of hexahydro-1,3,5-trinitro-1,3,50triazine (RDX) to three freshwater invertebrates. *Environ Toxicol Chem.* 10(8):1073-81.
RDX	E	A	Transport of RDX through soils	Ringelberg, D.B., Reynolds, C.M., Walsh, M.E. and Jenkins, T.F. 2003. RDX loss in a surface soil under saturated and well drained conditions. *J Environ Qual.* 32(4): 1244-1249.
RDX	T	C	Toxicity of RDX to mice and swine	Schneider NR et al. 1978. Toxicology of Cyclotrimethylenetrinitramine: Distribution and Metabolism in the Rat and the Miniature Swine. Toxicol Appl Pharmacol. 39:531-541.
RDX	E	A	Transport of RDX through soils	Selim, H.M., Xue, S.K. and Iskandar, I.K. 1995. Transport of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science.* 160(5): 328-339.
RDX	E	A	Fate of RDX in soils	Sheremata, T.W., Halasz, A., Paquet, L., Thiboutot, S., Ampleman, G. and Hawari, J. 2001. The fate of the cyclic nitramine explosive RDX in natural soil. *Environmental science & technology.* 35(6): 1037-1040.
RDX	E	A	Fate of RDX in soils	Singh, J., Comfort, S.D., Hundal, L.S. and Shea, P.J. 1998. Long-term RDX sorption and fate in soil. *Journal of Environmental Quality.* 27(3): 572-577.
RDX	E	A	Transport of RDX in trees	Thompson, P.L., Ramer, L.A. and Schnoor, J.L. 1999. Hexahydro-1,3,5-trinitro-1,3,5-triazine translocation in poplar trees. *Environmental Toxicology and Chemistry.* 18(2): 279-284.
RDX	E	A	Fate of RDX in soils	Tucker, W.A., Murphy, G.J. and Arenberg, E.D. 2002. Adsorption of RDX to soil with low organic carbon: Laboratory results, field observations, remedial implications. *Soil and Sediment Contamination.* 11(6).
RDX	E	A	Degradation of RDX	Zhang, C. and Hughes, J.B. 2003. Biodegradation pathways of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Clostridium acetobutylicum cell-free extract. *Chemosphere.* 50(5): 665-671.

Agent	Category	Status	Notes	Citation
RDX	B	C	Metabolism of RDX by rats	Schneider NR, SL Bradley, ME Anderson. 1978. The distribution and metabolism of cyclotrimethylenetrinitramine (RDX) in the rat after subchronic administration. *Toxicol Appl Pharmacol.* 46(1):163-71.
RDX HMX	E	C	Environmental fate of RDX and HMX	Bhadra R, DG Wayment, RK Williams, SN Barman, MB Stone, JB Hughes, JV Shanks. 2001. Studies on plant-mediated fate of the explosives RDX and HMX. *Chemosphere.* 44:1259-1264.
RDX HMX	E	A	Phytoremediation of HMX and TNT	Bhadra, R., Wayment, D.G., Williams, R.K., Barman, S.N., Stone, M.B., Hughes, J.B. and Shanks, J.V. 2001. Studies on plant-mediated fate of the explosives RDX and HMX. *Chemosphere.* 44(5): 1259-1264.
RDX HMX	E	C	Detection of explosives and degradation products in soil	Groom CA, S Beaudet, A Halasz, L Paquet, J Hawari. 2001. Detection of the cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX) and their degradation products in soil environments. *J Chromat.* 909:53-60.
RDX HMX	E	C	Transformations of RDX and HMX	Price CB, JM Brannon, SL Yost. 1998. Transformations of RDX and HMX Under Controlled Eh/pH Conditions. US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi. IRRP-98-2.
RDX HMX	E	A	Review of data for establishing water quality criteria	Sullivan JH Jr., HD Putnam, MA Keirn, BC Pruitt Jr., JC Nichols. 1979. A Summary and Evaluation of Aquatic Environmental Data in Relation to Establishing Water Quality Criteria for Munitions Unique Compounds. Part 4: RDX and HMX. Water and Air Research, Inc., Gainesville, FL. Mar:71.
RDX HMX TNB	T	C	Toxicity of RDX to aquatic organisms	Lotufo GR, JD Farrar, LS Inouye, TS Bridges, DB Ringelberg. Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic invertebrates. *Environ Toxicol Chem.* 2001. 20(8):1762-71.
TA	PT	C	Properties of TA	Anon. 1988. Terephthalic acid. *Dangerous Properties of Industrial Materials Report.* 8(4): 68-71.
TA	P	A	Characterization of smoke from military smoke grenade	Anthony JS, CL Crouse, WT Muse, SA Thompson. Characterization of pyrotechnically disseminated terephthalic acid as released from the M8 smoke pot. Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, MD.
TA	T	A	Toxicity of colored smoke dyes	Brooks AL, FA Seiler, RL Hanson, RF Henderson. 1989. In vitro genotoxicity of dyes present in colored smoke munitions. *Environ Mol Mutagen.* 13(4):304-313.
TA	T	A	Toxicity of TA to rats and mice	Hall IH, OT Wong, DJ Reynolds, R Simlot, JJ Chang. 1993. Terephthalic acid in Sprague-Dawley rats as a hypolipidemic agent. *Arch Pharm.* 326:5-13.
TA	E	A	Biodegradation of TA	He X, Z Zhang, S MA. 1992. Study on biodegradability of terephthalic acid. *China Journal of Environmental Science.* 13(3):18-24.
TA	T	C	Toxicity of TA	Hoshi A, R Yanai, K Kuretani. 1968. Toxicity of terephthalic acid. *Chem Pharm Bull.* 16(9):1655-1660.
TA	T	A	Toxicity and biodegradation of TA	Kim M-N, B-Y Lee, I-M Lee, H-S Lee, J-S Yoon. 2001. Toxicity and biodegradation of products from polyester hydrolysis. *Journal of Science and Health Part A: Toxic/Hazardous Substances and Environmental Engineering.* A36(4):447-463.
TA	E	A	Anaerobic treatment of TA contaminated wastewaters	Kleerebezem, R., Mortier, J., Hulshoff Pol, L.W. and Lettinga, G. 1997. Anaerobic pre-treatment of petrochemical effluents: Terephthalic acid wastewater. 2. IAWQ International Conference on Pretreatment of Industrial Wastewaters, Athens (Greece), 16-18 Oct 1996.
TA	E	A	Anaerobic treatment of TA contaminated wastewaters	Macarie, H., Noyola, A. and Guyot, J.P. 1992. Anaerobic treatment of a petrochemical wastewater from a terephthalic acid plant. *Water Science and Technology.* 25(7): 223-235.

Agent	Category	Status	Notes	Citation
TA	T	C	Distribution, absorption, and excretion of TA in rats and rabbits	Moffitt AE Jr., JJ Clary, TR Lewis, MD Blanck, VB Perone. 1975. Absorption, distribution, and excretion of terephthalic acid and dimethyl terephthalate. *American Industrial Hygiene Association Journal.* 36(8):633-641.
TA	T	C	Toxicity of TA smoke grenades to rats	Muse, W.T. Jr., J.S. Anthony, J.D. Bergmann, D.C. Burnett, C.L. Crouse, B.P. Gaviola and S.A. Thompson. 1997. Chemical and toxicological evaluation of pyrotechnically disseminated terepthalic acid smoke. *Drug and Chemical Toxicology.* 20(4):293-302.
TA	T	A	Teratology study of TA in rats	Ryan BM, NS Hatoum, JD Jernigan. 1990. A segment II inhalation teratology study of terephthalic acid in rats. *Toxicologist.* 10(1):40.
TA	T	A	Inhalation toxicity of TA to rats	Shi A, D Wang, X Wang, X Xu. 2000. Changes of pulmonary surfactant in the rat lung exposed to terephthalic acid. *Wei Sheng Yan Jiu.* 29(2):71-72.
TA	TB	C	Uptake, metabolism, and accumulation of TA by chicken liver	Tremaine LM and AJ Quebbemann. 1985. The Renal Handling of Terephthalic Acid. *Toxical Appl Phamacol.* 77:165-174.
TA	P	C		United States Environmental Protection Agency (USEPA). 1983. AP-42, Fifth Edition, Volume. Chapter 6: Organic Chemical Process Industry.
TA	T	A	Toxicokinetics of TA in rats	Yao H, X Wang, D Wang, J Dai. 2001. Toxicokinetics of terephthalic acid. *Wei Sheng Yan Jiu.* 30(1):23-24.
TA	T	A	Toxicity of TA to rat liver	Yao H, X Wang, X Xu, A Shi. 2002. Study on the injury of liver induced by terephthalic acid ethylene glycol and/or dowtherm A in rats. *Wei Sheng Yan Jiu.* 31(1):12-14.
TET	B	A	Toxicokinetics of tetryl	Anusevicius, Z., Sarlauskas, J., Nivinskas, H., Segura-Aguilar, J. and Cenas, N. 1998. DT-diaphorase catalyzes N-denitration and redox cycling of tetryl. *FEBS Lett.* 436(2): 144-148.
TET	T	C	Toxicological profile of tetryl	ATSDR. 1995. Toxicological Profile for Tetryl (2,4,6-Trinitrophenyl-N-methylnitramine. Prepared for Agency of Toxic Substances and Disease Registry.
TET	E	C	Degradation of Tetryl by anaerobic processes	Boopathy, R. 2000. Formation of aniline as a transient metabolite during the metabolism of tetryl by a sulfate-reducing bacterial consortium. *Curr Microbiol.* 40(3): 190-193.
TET	T	C	Toxicity summary for tetryl	Daugherty ML. 1991. Toxicity summary for trinitrophenylmethylnitramine. Oak Ridge Reservation Environmental Restoration Program, US Department of Energy Contract No. DE-AC05-84OR21400.
TNT, HMX, RDX,TET	E	A	Remediation of explosives contaminated groundwaters	Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. *Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).* Weston Roy F., Inc., West Chester, PA (USA).
TET	E	A	Degradation of tetryl by bioslurry	Fuller ME, J Kruczek, RL Schuster, PL Sheehan, PM Arienti. 2003. Bioslurry treatment for soils contaminated with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl). J Hazard Mater. 100(1-3):245-257.
TET	T	A	Toxicity of tetryl to rats	Reddy, T.V., Olson, G.R., Wiechman, B., Reddy, G., Torsella, J., Daniel, F.B. and Leach, G.J. 1999. Toxicity of tetryl (N-methyl-N,2,4,6-tetranitroaniline) in F344 rats. *International Journal of Toxicology [Int. J. Toxicol.].* 18(2): 97-107.
TET	E	A	Toxicokinetics of tetryl	Shah, M.M. and Spain, J.C. 1996. Elimination of nitrite from the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) catalyzed by ferredoxin NADP oxidoreductase from spinach. *Biochem Biophys Res Commun.* 220(3): 563-568.
TET	T	N	Mutagenicity of tetryl	Whong W-Z, ND Speciner, GS Edwards. 1980. Mutagenic activity of tetryl, a nitroaromatic explosive, in 3 microbial test systems. Toxicol Lett (AMST). 5(1):11-18.

Agent	Category	Status	Notes	Citation
TET TNT	T	N	Allergic reactions to explosives and war gasses	Foussereau J, C Benezra, HI Maibach, N Hjorth. 1982. Army Arsenals. IN: Occupational Contact Dermatitis, Clinical and Chemical Aspects. WB Saunders Company, Philadelphia, PA. Pages 171-176.
TET TNT	E	N	Analysis of vapors from mines	Jenkins TF, WF O'Reilly, RP Murrmann, DC Leggett, CI Collins. 1973. Analysis of vapors emitted from military mines. Cold Regions Research and Engineering Laboratory, US Army, Hanover, NH. Report No. AD-768-709.
TNB	T	C	Toxicity of DNT	Deneer JW, CJ van Leeuwen, W Seinen, JL Maas-Diepeveen, JLM Hermens. QSAR study of the toxicity of nitrobenzene derivatives towards *Daphnia magna, Chlorella pyrenoidosa,* and *Photobacterium phosphoreum. Aquatic Toxicol.* 15:83-98.
TNB	T	C	Toxicity of NB	Reddy, G., Reddy, T.V., Choudhury, H., Daniel, F.B. and Leach, G.J. 1997. Assessment of environmental hazards of 1,3,5-trinitrobenzene. *Journal of Toxicology and Environmental Health.* 52(5): 447-460.
TNB	T	C	Toxicity of NB to fish	van der Schalie, W.H., Shedd, T.R. and Zeeman, M.G. 1988. Ventilatory and movement responses of Bluegills exposed to 1,3,5-trinitrobenzene. Aquatic Toxicology and Hazard Assessment. Philadelphia PA, American Society for Testing and Materials. 10: 307-315.
TNT	T	N	Carcinogenic risk of TNT	1996. 2,4,6-Trinitrotolunene. *IARC Monographs on the Evaluation of the Carcinogenic risk of Chemicals to Humans*. 65:449-475.
TNT	T	A	Light-enhanced toxicity of TNT	Arfsten, D.P., Davenport, R. and Schaeffer, D.J. 1994. UV-A coexposure enhances the toxicity of aromatic hydrocarbons, munitions, and metals to Photobacterium phosphoreum. *Biomed Environ Sci.* 7(2): 101-108.
TNT	T	C	Toxicological profile of TNT	ATSDR. 1995. Toxicological Profile for 2,4,6-Trinitrotoluene. Prepared for Agency of Toxic Substances and Disease Registry.
TNT	T	A	Toxicity of TNT wastewaters	Bailey HC, RJ Spanggord, RJ Javitz, DHW Liu. 1984. Toxicity of TNT (Trinitrotoluene) Wastewaters to Aquatic Organisms. Chronic Toxicity of 2,4-dinitrotoluene and Condensate Water. SRI International, Menlo Park, CA. 4:91.
TNT	T	A	Toxicity of TNT wastewaters	Bailey HC, RJ Spanggord, RJ Javitz, DHW Liu. 1985. Toxicity of TNT Wastewaters to Aquatic Organisms. Volume 3. Chronic Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene. SRI International, Menlo Park, CA. May:80.
TNT	B	C	Toxicity of TNT and metabolites	Banerjee HN, M Verma, L-H Hoa, M Ashraf, SK Dutta. 1999. Cytotoxicity of TNT and its metabolites. Y*ale J Biol Med.* 72(1):1-4.
TNT	E	A	Remediation of TNT in soils	Breitung, J., Bruns-Nagel, D., Steinbach, K., Kaminski, L., Gemsa, D. and von Low, E. 1996. Bioremediation of 2,4,6-trinitrotoluene-contaminated soils by two different aerated compost systems. *Appl Microbiol Biotechnol.* 44(6): 795-800.
TNT	E	A	Biodegradation of TNT	Bruns-Nagel, D., Brettung, J., von Loew, E., Steinbach, K., Gorontzy, T., Kahl, M., Blotevogel, K.H. and Gemsa, D. 1996. Microbial transformation of 2,4,6-trinitrotoluene in aerobic soil columns. *Applied and Environmental Microbiology.* 62(7): 2651-2656.
TNT	E	A	Biodegradation of TNT	Carpenter, D.F., McCormick, N.G., Cornell, J.H. and Kaplan, A.M. 1978. Microbial transformation of ^{14}C-labeled 2,4,6-trinitrotoluene in an activated-sludge system. *Appl Environ Microbiol.* 35(5): 949-954.
TNT	E	A	Light-enhanced toxicity of TNT	Cui, H., Hwang, H.M., Cook, S. and Zeng, K. 2001. Effect of photosensitizer riboflavin on the fate of 2,4,6-trinitrotoluene in a freshwater environment. *Chemosphere.* 44(4): 621-625.
TNT	T	C	Toxicity of TNT	Davenport R, LR Johnson, DJ Shaeffer, H Balbach. 1994. Light-Enhanced Toxicity of TNT and Some Related Compounds to *Daphnia magna* and *Lytechinus variagatus* Embryos. *Ecotox Environ Saf.* 27:14-22.
TNT	T	C	Toxicity of TNT	Dilley JV, CA Tyson, J Spanggord, DP Sasmore, GW Newell, JC Dacre. 1982. Short-term oral toxicity of 2,4,6-trinitrotoluene in mice, rats, and dogs. *J Toxicol Environ Health.* 9(4):565-85.
TNT	T	C	Toxicity of TNT	Dodard SG, AY Renoux, J Powlowski, GI Sunahara. Lethal and subchronic effects of 2,4,6-trinitrotoluene (TNT) on Enchytraeus albidus in spiked artificial soil. *Ecotoxicol Environ Saf.* 2003. 54(2):131-8.

Agent	Category	Status	Notes	Citation
TNT	B	A	Metabolism of TNT by rats	El-Hawari AM, JR Hodgson, RN Shiotsuka, CC Lee. 1978. Disposition and metabolism of 2,4,6-trinitrotoluene after oral, dermal, and intratracheal dosing to rats. *Pharmacologist.* 20(3):255.
TNT	E	C	Fate of TNT in soils and binding to organic matter	Eriksson, J. and Skyllberg, U. 2001. Binding of 2,4,6-trinitrotoluene and its degradation products in a soil organic matter two-phase system. *J Environ Qual.* 30(6): 2053-2061.
TNT	E	A	Biodegradation of TNT	Esteve-Nunez, A., Lucchesi, G., Philipp, B., Schink, B. and Ramos, J.L. 2000. Respiration of 2,4,6-trinitrotoluene by Pseudomonas sp. strain JLR11. *J Bacteriol.* 182(5): 1352-1355.
TNT	E	N	Kinetics of TNT transformations	Felt DR, SL Larson, LD Hansen. Kinetics of base-catalyzed 2,4,6-trinitrotoluene transformations. Army Engineer Waterways Experiment Station, Vicksburg, MS.
TNT	E	N	Transformations of TNT in soil and water	Felt DR, SL Larson, LD Hansen. Molecular weight distribution of the final production of TNT-hydroxide reaction. Army Engineer Waterways Experiment Station, Vicksburg, MS.
TNT	E	N	Degradation of TNT by PETN reductase	French CE, S Nicklin, NC Bruce. 1998. Aerobic degradation of 2,4,6-trinitrotoluene by Enterobacter cloacae PB2 and by pertaerythritol tetranitrate. Applied and Environmental Microbiology. 64(8):2864-2868.
TNT	T	C	Toxicity of TNT	Furedi EM, BS Levine, DE Gordon, VS Rac, PM Lish. Determination of the Chronic Mammalian Toxicological Effects of TNT. Twenty-Four Month Chronic Toxicity/Carcinogenicity Study of Trinitrotoluene (TNT) in the Fischer 344 Rat. Volume 1. IIT Research Institute, Chicago, Illinois. ADA-168637.
TNT	T	C	Toxicity of TNT	Gogal RM Jr., MS Johnson, CT Larsen, MR Prater, RB Duncan, DL Ward, SD Holladay. Influence of dietary 2,4,6-trinitrotoluene exposure in the northern bobwhite (*Colinus virginianus*). *Environ Toxicol Chem.* 2002. 21(1):81-6.
TNT	T	C	Toxicity of TNT to plants	Gong, P., Siciliano, S.D., Greer, C.W., Paquet, L., Hawari, J. and Sunahara, G.I. 1999. Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field-contaminated soils to indigenous microorganisms. Environmental Toxicology and Chemistry. 12: 2681-2688.
TNT	T	C	Toxicity of TNT	Green A, D Moore, D Farrar. 1999. Chronic toxicity of 2,4,6-trinitrotoluene to a marine polychaete and an estuarian amphipod. *Env Toxicol and Chem.* 18(8):1783-1790.
TNT	T	A	Toxicity of TNT	Hankenson, K. and Schaeffer, D. 1991. Microtox Assay of Trinitrotoluene, Diaminonitrotoluene, Dinitromethylaniline Mixtures. *Bulletin of Environmental Contamination and Toxicology BECTA6.* 46(4): 550-553.
TNT	E	A	Phytoremediation of TNT	Hannink, N., Rosser, S.J., French, C.E., Basran, A., Murray, J.A., Nicklin, S. and Bruce, N.C. 2001. Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. *Nat Biotechnol.* 19(12): 1168-1172.
TNT	E	A	Phytoremediation of TNT	Hitchcock, D.R., McCutcheon, S.C. and Smith, M.C. 2003. Using rotifer population demographic parameters to assess impacts of the degradation products from trinitrotoluene phytoremediation. *Ecotoxicol Environ Saf.* 55(2): 143-151.
TNT	E	A	Anaerobic degradation of TNT	Hwang, P., Chow, T. and Adrian, N.R. 2000. Transformation of trinitrotoluene to triaminotoluene by mixed cultures incubated under methanogenic conditions. *Environmental Toxicology and Chemistry [Environ. Toxicol. Chem.]. no.* 4: 836-841.
TNT	B	C	Bioaccumulation of TNT	Johnson MS, LS Franke, RB Lee, SD Holladay. Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: is the dermal route significant? *Environ Toxicol Chem.* 1999. 18(5):873-878.
TNT	T	C	Toxicity of TNT	Johnson MS, SD Holladay, KS Lippenholz, JL Jenkins, WC McCain. 2000. Effects of 2,4,6-trinitotoluene in a holistic environmental exposure regime on a terrestrial salamander, Ambystoma tigrinum. Toxicol Pathol. 28(2):334-41.
TNT	T	A	TNT combustion products	Johnson, L.R., Davenport, R., Balbach, H. and Schaeffer, D.J. 1994. Comparative toxicity of trinitrotoluene and aminodinitrotoluenes to Daphnia magna, Dugesia dorotocephala, and sheep erythrocytes. Ecotoxicology and Environmental Safety. 27(1): 34-49.

Agent	Category	Status	Notes	Citation
TNT	T	A	Toxicity of TNT	Johnson, M.S., Ferguson, J.W. and Holladay, S.D. 2000. Immune effects of oral 2,4,6-trinitrotoluene (TNT) exposure to the white-footed mouse, Peromyscus leucopus. *International Journal of Toxicology.* 1: 5-11.
TNT	E	A	Aerobic degradation of TNT	Kalafut, T., Wales, M.E., Rastogi, V.K., Naumova, R.P., Zaripova, S.K. and Wild, J.R. 1998. Biotransformation patterns of 2,4,6-trinitrotoluene by aerobic bacteria. *Curr Microbiol.* 36(1): 45-54.
TNT	E	A	Degradation of TNT in bioslurry	Krumholz, L.R., Li, J., Clarkson, W.W., Wilber, G.G. and Suflita, J.M. 1997. Transformations of TNT and related aminotoluenes in groundwater aquifer slurries under different electron-accepting conditions. *J Ind Microbiol Biotechnol.* 18(2-3): 161-169.
TNT	E	N	Environmental fate of explosives in soils	Larson SL, CA Weiss, MR Martino, JW Adams. Role of expandable clays in the environmental fate of trinitrotoluene contamination. Army Engineer Waterways Experiment Station, Vicksburg, MS.
TNT	T	C	Toxicity of TNT	Levine BS, EM Furedi, DE Gordon, PM Lish, JJ Barkley. 1984. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. *Toxicology.* 32(3):253-65.
TNT	T	C	Toxicity of TNT	Levine BS, JH Rust, JJ Barkley, EM Furedi, PM Lish. 1990. Six month oral toxicity study of trinitrotoluene in beagle dogs. *Toxicology.* 63(2):233-44.
TNT	E	A	Degradation of TNT	Lucero, M.E., Mueller, W., Hubstenberger, J., Phillips, G.C. and O'Connell, M.A. 1999. Tolerance to nitrogenous explosives and metabolism of TNT by cell suspensions of Datura innoxia. *In Vitro Cellular & Developmental Biology Plant.* 6: 480-486.
TNT	P	N	Analysis of TNT vapors	Murrmann RP, TF Jenkins, DC Leggett. 1971. Composition and mass spectra of impurities in military grade TNT vapor. Cold Regions Research and Engineering Laboratory, US Army Corps of Engineers, Hanover, NH. AD-725-474.
TNT	E	C	Bioavailability of TNT from contaminated soils	Palmer, W.G., Beaman, J.R., Walters, D.M. and Creasia, D.A. 1997. Bioavailability of TNT residues in composts of TNT-contaminated soil. *J Toxicol Environ Health.* 51(2): 97-108.
TNT	E	A	Degradation of TNT	Pasti-Grigsby, M.B., Lewis, T.A., Crawford, D.L. and Crawford, R.L. 1996. Transformation of 2,4,6-trinitrotoluene (TNT) by actinomycetes isolated from TNT-contaminated and uncontaminated environments. *Appl Environ Microbiol.* 62(3): 1120-1123.
TNT	T	A	Toxicity of TNT	Pederson, G.L. 1970. *Evaluation of toxicity of selected TNT wastes on fish, phase i-acute toxicity of alpha-TNT to Bluegills.* ARMY ENVIRONMENTAL HYGIENE AGENCY, EDGEWOOD ARSENAL, MD. 1 january 1970-31 october 1970.
TNT	T	C	Toxicity of TNT to plants	Peterson, M.M., Horst, G.L., Shea, P.J. and Comfort, S.D. 1998. Germination and seedling development of switchgrass and smooth bromegrass exposed to 2,4,6-trinitrotoluene. *Environmental Pollution.* 99(1): 53-59.
TNT	E	A	Fate of TNT in soils and binding to organic matter	Qaisi, K.M., Ro, K.S., Constant, W.D. and Smith, M.L. 1996. Soil - water partitioning and mass transfer kinetics of 2,4,6 trinitrotoluene in highly contaminated soil. *Environmental Sciences and Pollution Mgmt.* A31(9): 2079-2085.
TNT	E	A	Biodegradation of TNT	Renoux, A.Y., Sarrazin, M., Hawari, J. and Sunahara, G.I. 2000. Transformation of 2,4,6-trinitrotoluene in soil in the presence of the earthworm Eisenia andrei. *Environmental Toxicology and Chemistry.* 6: 1473-1480.
TNT	E	A	Biodegradation of TNT	Rho, D., Hodgson, J., Thiboutot, S., Ampleman, G. and Hawari, J. 2001. Transformation of 2,4,6-trinitrotoluene (TNT) by immobilized Phanerochaete chrysosporium under fed-batch and continuous TNT feeding conditions. *Biotechnol Bioeng.* 73(4): 271-281.
TNT	T	C	Toxicity of TNT	Robidoux PY, J Hawari, S Thiboutot, G Ampleman, GI Sunahara. Acute toxicity of 2,4,6-trinitrotoluene in earthworm *Eisenia andrei. Ecotoxicol Environ Saf.* 1999. 44(3):311-21.
TNT	E	A	Water quality criteria for TNT	Ryon MG. 1987. Water Quality Criteria for 2,4,6-Trinitrotoluene (TNT): Final Report. Department of Energy, Washington, DC. Aug:140.
TNT	E	N	Fate of TNT in several soils	Sheremata TW, S Thiboutot, G Ampleman, L Paquet, A Halasz, J Hawari. 1999. Fate of 2,4,6-trinitrotoluene and its metabolites in natural and model soil systems. *Environ Sci Technol.* 22:4002-4008.

Agent	Category	Status	Notes	Citation
TNT	E	A	Phytoremediation of TNT	Siciliano, S.D. and Greer, C.W. 2000. Plant-Bacterial Combinations to Phytoremediate Soil Contaminated with High Concentrations of 2,4,6-Trinitrotoluene. *Journal of Environmental Quality [J. Environ. Qual.]. no.* 1: 311-316.
TNT	T	C	Toxicity of TNT and metabolites	Smock LA, DL Stoneburner, JR Clark. 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. *Water Research.* 10:537-543.
TNT	E	A	Biodegradation of TNT	Snellinx, Z., Nepovim, A., Taghavi, S., Vangronsveld, J., Vanek, T. and van der Lelie, D. 2002. Biological remediation of explosives and related nitroaromatic compounds. *Environ Sci Pollut Res Int.* 9(1): 48-61.
TNT	E	A	Biodegradation of TNT	Stahl, J.D. and Aust, S.D. 1993. Metabolism and detoxification of TNT by Phanerochaete chrysosporium. *Biochem Biophys Res Commun.* 192(2): 477-482.
TNT	E	A	Uptake and fate of TNT in plants	Thompson, P.L., Ramer, L.A. and Schnoor, J.L. 1998. Uptake and transformation of TNT by hybrid poplar trees. *Environmental Science & Technology.* 32(7): 975-980.
TNT	E	N	Fate and transport of TNT	Townsend DM, TE Myers, DD Adrian. 2,4,6-Trinitrotoluene (TNT) transformations and absorption in thin-disk soil columns. Army Engineer Waterways Experiment Stations, Vicksburg, MS.
TNT	E	A	Biodegradation of TNT	Van Aken, B., Hofrichter, M., Scheibner, K., Hatakka, A.I., Naveau, H. and Agathos, S.N. 1999. Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete Phlebia radiata. *Biodegradation.* 10(2): 83-91.
TNT	E	A	Biodegradation of TNT	Vanderberg, L.A., Perry, J.J. and Unkefer, P.J. 1995. Catabolism of 2,4,6-trinitrotoluene by Mycobacterium vaccae. *Appl Microbiol Biotechnol.* 43(5): 937-945.
TNT	E	A	Binding of TNT to activated carbon	Vasilyeva, G.K., Kreslavski, V.D., Oh, B.T. and Shea, P.J. 2001. Potential of activated carbon to decrease 2,4,6-trinitrotoluene toxicity and accelerate soil decontamination. *Environ Toxicol Chem.* 20(5): 965-971.
TNT	E	N	Environmental degradation of munitions contamination	Walsh ME. Environmental transformations products of nitroaromatics and nitramines: literature review and recommendations for analytical method development. Cold Regions Research and Engineering Lab, Hanover, NH.
TNT	T	C	Toxicity of TNT and metabolites	Won WD, LH DiSalvo, NG James. 1976. Toxicity and Mutagenicity of 2,4,6-Trinitrotoluene and its Microbial Metabolites. *Appl Environ Toxicol.* 31(4):576-580.
TNT	E	A	Phytoremediation of TNT	Zoh, K.-D. and Horne, A.J. 1999. Removal of TNT using plants in constructed wetlands. Wetlands and Remediation: An International Conference, Salt Lake City, UT, Battelle Press, 505 King Ave Columbus OH 43201.
TNT DNT	T	N	Toxicity of TNT, DNT and isomers	Ellis HV, C Hong, C Lee. 1980. Mammalian toxicity of munitions compounds. Summary of toxicity of nitrotoluenes. Report No. 11. Midwest Research Institute, Kansas City, MO. DAMD17-74-C-4073.
TNT DNT	E	N	Study on munitions disposal lagoon	Fuchs JS, ML Oneto, NB Casabe, OG Segura, R Tarulla, M Vaccarezza, C Sanchez-Rivas, EM Kesten, EJ Woods. 2001. Ecotoxicological characterization of a disposal lagoon from a munitions plant. *Bull Environ Contam Toxicol.* 67(5):696-703.
TNT DNT	T	N	Toxicity of TNT and DNT metabolites after composting	Honeycutt ME, AS Jarvis, VA McFarland. 1996. Cytotoxicity and mutagenicity of 2,4,6-trinitrotoluene and its metabolites. *Ecotoxicol Environ Saf.* 35(3):282-287.
TNT DNT	T	N	Worker exposure to DNT and TNT and effects	Letzel S, T Goen, M Bader, J Angerer, T Kraus. 2003. Exposure to nitroaromatic explosives and health effects during disposal of military wastes. *Occup Environ Med.* 60(7):483-488.
TNT DNT	T	N	Toxicity of waste waters from TNT production facilities	Liu DHW, RJ Spanggord, HC Bailey, HS Javitz, DCL Jones. Toxicity of TNT wastewaters to aquatic organisms. Volume 2. Acute toxicity of condensate wastewater and 2,4-dinitrotoluene. SRI International, Menlo Park, CA.
TNT DNT HMX	TE	N	Toxicity and UV activation of ordnance compounds	Dave G, E Nilsson, A-S Wernersson. 200. Sediment and water phase toxicity and UV-activation of six chemicals used in military explosives. *Aquat Ecosyst Health.* 3(3):291-299.

Agent	Category	Status	Notes	Citation
TNT DNT NG WP	T	N	Mammalian toxicity of ordnance compounds	Lee CC, JV Dilley, JR Hodgson, DO Helton, WJ Wiegand, DN Roberts, BS Andersen, LM Halfpap, LD Kurtz, N West. 1975. Mammalian toxicity of munitions compounds: Phase I. Acute oral toxicity, primary skin and eye irritation, dermal sensitization and disposition and metabolism. Environmental Protection Agency Research Division, US Army Medical Research and Development Command, Washington, DC.
TNT DNT RDX HMX	E	A	Fate and transport of explosives	Layton D, B Mallon, W Mitchell, L Hall, R Fish. 1987. Conventional Weapons Demilitarization: A Health and Environmental Effects Base Assessment. Phase 2. Explosives and Their Cocontaminants. Lawrence Livermore National Laboratory, CA. Environmental Sciences Div. Dec:427.
TNT DNT RDX HMX	T	C	Toxicity of munitions to earthworms	Phillips CT, RT Checkai, RS Wentsel. 1993. Toxicity of Selected Munitions and Munition-Contaminated Soil on the Earthworm *Eisenia foetida.* Edgewood Research, Development, and Engineering Center. Aberdeen Proving Ground, Maryland. ERDEC-TR-037.
TNT DNT TET NB	P	N	Calculation of PPLV from Army ammunition plant	Rosenblatt DH and MJ Small. Preliminary pollution limit values for Alabama Army Ammunition Plant. Army Medical Bioengineering Research and Development Lab, Fort Detrick, MD.
TNT DNT TET NB PETN	T	C	Toxicity of munitions compounds to aquatic organisms	Drzyzga O, T Gorontzy, A Schmidt, KH Blotevogel. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology.* 28(2):229-235.
TNT HMX	E	C	Modeling fate and transport of TNT and HMX	Lynch, J.C., Brannon, J.M., Hatfield, K. and Delfino, J.J. 2003. An exploratory approach to modeling explosive compound persistence and flux using dissolution kinetics. *J Contam Hydrol.* 66(3-4): 147-159.
TNT HMX RDX PETN NP	E	C	Environmental fate of explosives in soils	French, C.E., Rosser, S.J. and Bruce, N.C. 2001. Biotransformations of explosives. *Biotechnol Genet Eng Rev.* 18: 171-217.
TNT HMX RDX TET	E	A	Composting effects on explosive contaminated soils	Williams, R.T., Ziegenfuss, P.S., Mohrman, G.B. and Sisk, W.E. 1989. Composting of Explosives and Propellant Contaminated Sediments. *IN: Hazardous and Industrial Wastes: Proceedings of the 21st Mid Atlantic Industrial Waste Conference. Technomic Publishing Co., Inc., Lancaster, Pennsylvania.* 1989: 599-611.
TNT RDX	E	C	Fate of explosives in groundwater	Best EPH, SL Sprechner, SL Larson, HL Fredrickson, DF Bader. 1999. Environmental behavior of explosives in groundwater from the Milan Army ammunition plant in aquatic and wetland plant treatments. Uptake and Fate of TNT and RDX in plants. *Chemosphere.* 39(12):2057-2072.
TNT RDX	T	C	Toxicity of TNT and RDX to rats	Dilley JV, CA Tyson, RJ Spanggord, DP Sasmore, GW Newell, JC Dacre. 1982. Short-term oral toxicity of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine mixture in mice, rats, and dogs. *J Toxicol Environ Health.* 1982. 9(4):587-610.
TNT RDX	ET	N	Effects of composting on toxicity of munitions contaminated soils	Jarvis AS, VA McFarland, ME Honeycutt. 1998. Assessment of the effectiveness of composting for the reduction of toxicity and mutagenicity of explosives-contaminated soil. *Ecotoxicol Environ Saf.* 39(2):131-135.

Agent	Category	Status	Notes	Citation
TNT RDX	T	C	Toxicity of TNT and RDX in rats	Levine BS, EM Furedi, DE Gordon, JJ Barkley, PM Lish. 1990. Toxic interaction of the munitions compounds TNT and RDX in F344 rats. *Fund Appl Toxicol.* 15:373-380.
TNT RDX	B	C	Plant uptake of explosives from soils	Price RA, JC Pennington, SL Larson, D Neumann, CA Hayes. 1997. Plant Uptake of Explosives from Contaminated Soil and Irrigation Water at the Former Nebraska Ordinance Plant, Mead, Nebraska. US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi. EL-97-11
TNT RDX	E	A	Uptake and fate of explosives in plants	Price, R.A., Pennington, J.C., Larson, S.L., Neumann, D. and Hayes, C.A. 2002. Uptake of RDX and TNT by agronomic plants. *Journal of Soil Contamination.* 11(3): 307-326.
TNT RDX HMX	E	C	Effect of composting on explosives degradation	Griest, W.H., Stewart, A.J., Tyndall, R.L., Caton, J.E., Ho, C.H., Ironside, K.S., Caldwell, W.M. and Tan, E. 1993. Chemical and toxicological testing of composted explosives-contaminated soil. *Environmental Toxicology and Chemistry.* 12(6): 1105-1116.
TNT RDX HMX	ET	C	Toxicity of nitroaromatics	Hovatter PS, SS Talmage, DM Opresko, RH Ross. 1997. Ecotoxicity of nitroaromatics to aquatic and terrestrial species at Army superfund sites. *Environmental Toxicology and Rish Assessment: Modeling and Risk Assessment, 6th Volume.* ASTM STP 1317.
TNT RDX HMX	E	N	Site characterization of military firing range	Jenkins TF, ME Walsh, PG Thorne, PH Miyares, TA Ranney. Site characterization for explosives contamination at a military firing range impact area. Cold Regions Research and Engineering Lab, Hanover, NH.
TNT RDX HMX	E	A		Lynch, J.C., Brannon, J.M. and Delfino, J.J. 2002. Dissolution rates of three high explosive compounds: TNT, RDX, and HMX. *Chemosphere.* 47(7): 725-734.
TNT RDX HMX	T	C	Chronic toxicity of TNT, RDX, and HMX	Robidoux PY, C Svendsen, J Caumartin, J Hawari, G Ampleman, S Thiboutot, JM Weeks, GI Sunahara. 2000. Chronic toxicity of energetic compounds in soil determined using the earthworm *Eisenia andrei* reproductive test. *ET&C.* 19(7):1764-1773.
TNT RDX HMX	T	C	Toxicity of TNT, RDX, and HMX to earthworms	Robidoux PY, J Hawari, G Bardai, L Paquet, G Ampleman, S Thiboutot, GI Sunahara.. TNT, RDX, and HMX decrease earthworm (Eisenia andrei) life cycle responses in a spiked natural forest soil. *Arch Environ Contam Toxicol.* 2002. 43(4):379-88.
TNT RDX HMX	E	N	Fate and transport evaluation of explosives in soils at Fort Greely, AK	Shaw RB, WW Doe, S Houston. Ecological soil characterization of the Delta Creek and Washington Impact Areas, Fort Greely, Alaska. Colorado State University, Fort Collins. Center for Environmental Management of Military Lands.
TNT RDX HMX	T	C	Toxicity of TNT, RDX, and HMX	Steevens JA, BM Duke, GR Lotufo, TS Bridges. Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3.5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to Chironomus tentans and Hyalella azteca: low-dose hormesis and high-dose mortality. *Environ Toxicol Chem.* 2002. 21(7):1475-82.
TNT RDX HMX NB	E	N	Evaluation of remediation process for removal of explosives from groundwater	Fleming EC, ME Zappi, E Toro, R Hernandez, K Myers. Laboratory assessment of advanced oxidation processes for treatment of explosives and chlorinated solvents in groundwater from the former Nebraska Ordnance Plant. Army Engineer Waterways Experiment Station, Vicksburg, MS.
TNT RDX HMX NB TNB	T	C	Toxicity of explosives to bacteria and mammalian cells	Lachance, B., Robidoux, P.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. *Mutation Research Genetic Toxicology and Environmental Mutagenesis.* 444(1): 25-39.

Agent	Category	Status	Notes	Citation
TNT RDX HMX TET	E	C	Environmental effects of munitions compounds	Talmage SS, SM Opresko, CJ Maxwell, CJE Welsh, FM Cretella, PH Reno, FB Daniel. 1999. Nitroaromatic Munition Compounds: Environmental Effects and Screening Values. *Rev Environ Contam Toxicol.* 161:1-156.
TNT RDX HMX TET	T	N	Mutagenicity of ordnance and metabolites	Tan EL, CH Ho, WH Griest, RL Tyndall. 1992. Mutagenicity of trinitrotoluene and its metabolites formed during composting. *J Toxicol Environ Health.* 36(3):165-75.
TNT RDX HMX TET NB NP	ET	N	Analysis of areas on or around ammunition plants	Levsen K, P Mussmann, E Berger-Preiss, A Preiss, D Volmer, G Wuensch. 1993. Analysis of nitroaromatics and nitramines in ammunition waste water an in aqueous samples from former ammunition plants and other military sites. *Acta Hydrochimica et Hydrobiologica.* 21(3):153-166
TNT RDX HMX TET TNB PETN	T	A	Toxicity of explosives to luminescent bacteria	Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology.* 28(2): 229-235.
TNT RDX NB	E	N	Kinetics of explosives in groundwater	Hildenbrand M and V Neumann. 1995. REV-reactor and soil column studies of sorption and migration of explosives in Elsnig sandy aquifers. *Acta Universitatis Carolinae Geologica.* 39(1):131-135.
TNT RDX TET	E	C	Fate of TNT, RDX, and TET in soils and plants	Cataldo DA, SD Harvey, RJ Fellows. 1993. The Environmental Behavior and Chemical Fate of Energetic Compounds (TNT, RDX, Tetryl) in Soil and Plant Systems. Pacific Northwest Laboratory, Richland, Washington. PNL-SA-22362.
TNT RDX TET	E	C	Migration of TNT, RDX, and TET in soil	Kayser EG and NE Burlinson. 1988. Migration of Explosives in Soil: Analysis of RDX, TNT, and Tetryl from a ^{14}C Lysimeter Study. *Journal of Energetic Materials.* 6:45-71.
TNT RDX TET	E	N	Migration of explosives in soils	Kayser EG and NE Burlinson. Migration of explosives in soil. Naval Surface Weapons Center, Silver Spring, MD.
TNT TET	E	N	Environmental fate of TNT, TET, and metabolites	Fellows RJ, SD Harvey, DA Cataldo. Evaluation of the environmental fate and behavior of munitions materiel (tetryl and polar metabolites of TNT) in soil and plant systems. Battelle Pacific Northwest Labs, Richland, WA.
TNT TNB	T	A	Toxicity of explosives to microbial communities	Fuller, M.E. and Manning, J.F., Jr. 1998. Evidence for differential effects of 2,4,6-trinitrotoluene and other munitions compounds on specific subpopulations of soil microbial communities. *Environmental Toxicology and Chemistry.* 17(11): 2185-2195.

Agent	Category	Status	Notes	Citation
TNT TNB DNT RDX HMX	ET	N	Study of soils at Joliet Army Ammunition Plant	Simini M, RS Wentsel, RT Checkai, CT Phillips, NA Chester, MA Major, JC Amos. Evaluation of soil toxicity at Joliet Army Ammunition Plant. *Environ Toxicol Chem.* 14(4):623-630.
UG	E	N	Evaluation of soils and surface water at military ranges	Ampleman G, S Thiboutot, S Desilets, A Gagnon, A Marois. Evaluation of the soils contamination by explosives at CFB Hillwack and CFAD Rocky Point. Defense Research Establishment Valcartier, Courcellette, Quebec.
UG	T	N	Occupational exposure to explosives	Bodeau DT. 1993. Military energetic materials: Explosives and propellants. IN: Occupational Health: The Soldier and the Industrial Base. Textbook of Military Medicine. Part III. Preventative Medicine and the Environment, Volume 2. Deeter DP and JC Gaydos, Eds.
UG	E	N	Literature review of fate and transport of explosives	Brannon JM and JC Pennington. Environmental fate and transport process descriptors for explosives. Army Engineer Waterways Experiment Station.
UG	T	N	Toxicity of JA-2 solid propellant to rats	Brown LD, JD Justus, CR Wheeler, DW Korte. Acute oral toxicity of JA-2 solid propellant in Sprague-Dawley rats. Letterman Army Institute of Research, Presidio of San Francisco, CA.
UG	E	N	Fate of explosives in soils from military sites	Checkai RT, MA Major, RO Nwanguma, JC Amos. Transport and fate of nitroaromatic and nitramine explosives in soils from open burning pen detonation areas. Edgewood Research, Development, and Engineering Center, Aberdeen Proving Grounds, MD.
UG	PETB	C	Literature review	Dacre, J.C., W.D. Burrows, C.W.R. Wade, A.F. Hegyeli, T.A. Miller and D.R. Cogley. 1979. Problem definition studies on potential environmental pollutants V. Physical, chemical, toxicological and biological properties of seven chemicals used in pyrotechnic compositions. Prepared by US Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD for US Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD.
UG	E	N	Analysis of airborne explosives combustion products	Einfield W, RA Rasmussen, CO Eckard, MB Johnson. Airborne gaseous and particulate products from large-scale bulk explosive detonations and propellant burns. Sandia National Labs, Albuquerque, NM.
UG	T	N	Toxicity of explosives leachate to Daphnia	Haley MV, RT Checkai, CW Kurnas, RS Wentsel. Toxicity determination of explosives contaminated soil leachates to Daphnia magna using an adapted toxicity characteristics leaching procedure. Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, MD.
UG	PETB	C	Database of toxicity, environmental fate, accumulation and properties of contaminants	HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. Onlineat:http://toxnet.nlm.nih.gov. Accessed December 17, 2003.
UG	E	N	Decomposition of nitroaromatics	Larson RA, PL Miller, TO Crowley. 1996. Borohydride photoreduction of nitroaromatic compounds related to military ordnance constituents. *Environ Sci Technol.* 30(4):1192-1197.
UG	T	N	Toxicity of JA-2 solid propellant to rabbits	LeTellier YC, CM Lewis. Acute oral toxicity of JA-2 solid propellant in rabbits. Letterman Army Institute of Research, Presidio of San Francisco, CA.
UG	T	N	Toxicity of JA-2 solid propellant to mice	Morgan EW, DF Frost, CR Wheeler, DW Korte. Acute oral toxicity of JA-2 solid propellant in ICR mice. Letterman Army Institute of Research, Presidio of San Francisco, CA.
UG	E	N	Guide for explosives contamination characterization	Thiboutot S, G Ampleman, AD Hewitt. Guide for characterization of sites contaminated with energetic materials. Engineering Research and Development Center, Hanover, NH. Cold Regions Research and Engineering Lab.

Table 2. Physical and chemical properties of military unique compounds.

Constituent	Appearance	CAS Number	Molecular Weight (g)	Melting Point Deg C	Boiling Point Deg C	Vapor Pressure		Henry's Law Constant (H) atm m^3/mole	Water Solubility		BCF L/kg	Log K_{ow}	Log K_{oc}
						mm Hg	Temp Deg C		mg/L	Temp Deg C			
Terephthalic Acid	White crystals or powder	100-21-0	166.13	140.6	288	9.20E-06	25	[3.88E-03]	15	20	[19]	2	[290]
Dinitrotoluene Isomers		25321-14-6											
2,4-Dinitrotoluene	Yellow to orange crystals	121-14-2	182.14	71	300	1.47E-04	22	1.30E-07	2.70E+02	22	[7]	1.98	[360]
2,6-Dinitrotoluene	Yellow to red solid	606-20-2	182.14	66	285	5.67E-04	25	9.26E-08	180	20	11	2.1	19 ; 72
2,3-Dinitrotoluene	Yellow crystalline solid	602-01-7	182.14	59 - 61	NR	[4.00E-04]	25	[9.3-E-08]	NR	NR	[9.4]	[2.2]	[370]
2,5-Dinitrotoluene	Yellow needles	619-15-8	182.14	52.5	NR	[4.00E-04]	25	[9.3-E-08]	NR	NR	[9.4]	[2.2]	[360]
3,4-Dinitrotoluene	Yellow needles	610-39-9	182.14	58.3	NR	[4.00E-04]	25	[9.3-E-08]	100	25	<2.7	2.08	[360]
3,5-Dinitrotoluene	Yellow to red crystals	618-85-9	182.14	93	NR	1.90E-03	25	[9.3-E-08]	NR	NR	[9.4]	[2.28]	[360]
Nitrobenzene	Yellow liquid	98-95-3	123.11	5.7	210.8	0.245	25	2.40E-05	1.80E+03	25	1.6 - 15	1.85	30.6 - 370
Nitrophenol Isomers		25154-55-6											
2-Nitrophenol	Light yellow crystalline solid	88-75-5	139.11	44 - 45	216	0.113	25	1.30E-05	2100	[20]	2.2 - 22	1.79	32 - 266
3-Nitrophenol	Colorless to light yellow crystalline solid	554-84-7	139.11	97	194	0.1	25	2.00E-09	13550	25	20	2	1.68
4-Nitrophenol	Colorless to light yellow crystalline solid	100-02-7	139.11	113 - 114	279	9.79E-05	20	1.30E-08	16000	25	2 - 79	1.91	1.7
2,4-Dinitrophenol	Yellow crystalline solid	51-28-5	184.11	112 - 114	Sub-limes	3.90E-04	20	8.60E-08	2790	20	0.4 - 4	1.67	[200]
1,3-Dinitrobenzene (1,3-DNB)	Colorless to light yellow crystalline solid	99-65-0	168.11	89 - 90	302.8	2.00E-04	25	4.90E-08	533	25	2 - 75	1.49	[150]

Constituent	Appearance	CAS Number	Molecular Weight (g)	Melting Point Deg C	Boiling Point Deg C	Vapor Pressure		Henry's Law Constant (H) atm m^3/mole	Water Solubility		BCF L/kg	Log K_{ow}	Log K_{oc}
						mm Hg	Temp Deg C		mg/L	Temp Deg C			
Pentaerythritol Tetranitrate (PETN)	White crystals or powder	78-11-5	316.15	140	180	1.04E-10	25	[1.20E-11]	43	25	[74]	1.61	[179 - 1720]
Cyclotrimethyl-enetrinitramine (RDX)	White crystalline solid	121-82-4	222.26	205 - 206	de-compos es	4.10E-09	NR	[6.30E-08]	59.8	25	4 - 5.9	0.87	42 - 167
2,4,6-Trinitrotoluene (TNT)	Colorless to pale yellow solid	118-96-7	227.13	80.1	240 ex-plodes	1.99E-04	20	4.57E-07	1.30E+02	20	[40]	1.6	1600
High Melting Point Explosive (HMX)	Colorless solid	2691-41-0	296.2	276 - 286	NR	3.33E-14	25	2.60E-15	6.63	20	NR	.26 ; .06	0.54
1,3,5-Trinitrobenzene (1,3,5-TNB)	Yellow solid	99-35-4	213.11	122.5	315	3.20E-06	20	[3.08E-09]	340	20	[5 ; 23]	1.1	[104 ; 178]
Nitroglycerin (NG)	Colorless to yellow viscous liquid or solid	55-63-0	227.09	2.8 ; 13.5	218 ex-plodes	2.00E-04	20	[4.30E-08]	1800	25	[4]	1.62	180
Trinitrophenyl-methylnitramine (Tetryl)	Colorless to yellow crystalline solid	479-45-8	287.15	130 - 132	187 ex-plodes	4.00E-10	20	[1.00E-11]	75	20	[54]	2.4	[406]

Notes:

Physical-chemical properties taken from Hazardous Substances Data Base (HSDB) and EPI v.3.0 (EPA chemical estimation software)

Value in brackets are estimated

NR - Not Reported

4 Database Adequacy

The physical and chemical properties of the chemicals of concern are reasonably well understood and documented. This section focuses on the adequacy of information on environmental fate and transport, bioaccumulation, and toxicity, particularly as they relate to conducting ecological risk assessments. Each chemical and category combination is evaluated for adequacy and has been rated as excellent, adequate, inadequate, or no data available (Table 3).

1,3,5-Trinitrobenzene

Environmental Fate and Transport

TNB is expected to be highly mobile in soils (HSDB 2003). TNB will be degraded by photolysis on soil surfaces, and volatilization from moist soils is not expected to occur (HSDB 2003).

TNB is expected to adsorb to suspended solids or sediment in aquatic systems at moderate to low levels (HSDB 2003). Direct photolysis is the primary degradation mechanism for TNB in aquatic systems (HSDB 2003).

Based on an extrapolated vapor pressure of $3.2X10^{-6}$ mm Hg at 20º C, atmospheric TNB is expected to exist partly in the vapor phase and partly adsorbed to atmospheric particulate matter (HSDB 2003). Wet deposition is a possible fate of atmospheric TNB based on its water solubility of 340 mg/l at 20º C (HSDB 2003).

No other information for the environmental fate and transport of TNB was found. Due to the similar structure of TNB to other nitrobenzenes, the environmental fate and transport may be similar to other nitrobenzene compounds.

Bioaccumulation and Trophic Transfer

No information was found on the bioaccumulation and trophic transfer of TNB. Therefore, the database is evaluated as inadequate.

Table 3. Summary of adequacy of information available for Ecological Risk Assessment.

Constituent	Environmental Fate and Transport			Bioaccumulation and Trophic Transfer		Toxicity (Ingestion Studies)						
	Air	Aquatic	Terrestrial	Aquatic	Terrestrial	Aquatic Invertebrates	Fish	Soil Fauna	Plants	Birds	Mammals	Reptiles and Amphibians
1,3,5-Trinitrobenzene (1,3,5-TNB)	Inadequate	Adequate	Adequate	No Data	No Data	Adequate	Adequate	Adequate	No Data	No Data	Adequate	No Data
1,3-Dinitrobenzene (1,3-DNB)	Inadequate	Adequate	Adequate	Inadequate	Inadequate	Adequate	Adequate	Adequate	No Data	No Data	Adequate	No Data
2,4 - Dinitrophenol (2,4-DNP)	Adequate	Adequate	Adequate	Inadequate	Inadequate	Adequate	Adequate	No Data	No Data	No Data	Inadequate	No Data
Dinitrotoluene isomers	Adequate	Adequate	Inadequate	Inadequate	Inadequate	Adequate	Adequate	Inadequate	No Data	No Data	Adequate	No Data
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	Inadequate	Adequate	Excellent	Inadequate	Inadequate	Adequate	Adequate	Adequate	Inadequate	No Data	Adequate	No Data
Nitrobenzene (NB)	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Inadequate	Inadequate	No Data	Adequate	No Data
Nitroglycerin (NG)	Adequate	Adequate	Adequate	Inadequate	Inadequate	Adequate	Adequate	Inadequate	Inadequate	No Data	Inadequate	No Data
Nitrophenol isomers (e.g. 2 - Nitrophenol; 4 - Nitrophenol)	Inadequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Inadequate	No Data	No Data	Inadequate	No Data
Pentaerythritol Tetranitrate (PETN)	Adequate	Inadequate	Inadequate	Inadequate	Inadequate	Inadequate	Inadequate	Inadequate	No Data	No Data	Inadequate	No Data
Hexahydro-1,3,5-trinitro-1,2,5-triazine (RDX)	Adequate	Adequate	Excellent	Adequate	Adequate	Excellent	Adequate	Inadequate	Adequate	No Data	Adequate	No Data
Trinitrophenylmethyl-nitramine (Tetryl)	Inadequate	Inadequate	Adequate	Inadequate	Inadequate	Inadequate	Inadequate	No Data	Adequate	No Data	Adequate	No Data
2,4,6-Trinitrotoluene (TNT)	Inadequate	Adequate	Excellent	Adequate	Adequate	Adequate	Adequate	Adequate	Excellent	Adequate	Excellent	Amphibian[1]

Inadequate = some data are available but inadequate for modeling.
Adequate = data are adequate for modeling but only limited data from one to three studies are available.
Excellent = high quality data are available from four or more laboratory and/or field studies.

[1] One study on dermal absorption in salamanders is available and is adequate for deriving a TRV.

Toxicity

Little toxicological information was found for TNB. Quantitative Structure-Activity Relationship (QSAR) studies have found that the toxicity of nitroaromatic compounds is related to the number of constituents and their relative positions, indicating that TNB will be more toxic than other nitroaromatics (Deneer et al. 1989, Gough et al. 1994, Schmitt et al. 2000). Thomulka and Lange (1997) found that TNB is more toxic than nitrobenzene based on comparison of calculated EC_{50} values of 15.3 and 239, respectively. Nipper et al. (2001) determined NOEC and LOEC values for TNB to several marine organisms. NOEC and LOEC values for sea urchin fertilization and embryo development tests were 35, 48, 0.48, and 1.1 mg/l, respectively. Algae NOEC and LOEC values for germination were 0.046 and 0.093 mg/l, respectively. Polychaete survival NOEC and LOEC values were 1.2 and 2.4 mg/l, respectively. Redfish larvae survival NOEC and LOEC values were 0.99 and 2.00 mg/l, respectively. Mysid survival NOEC and LOEC values were 0.96 and 1.88 mg/l, respectively (Nipper et al. 2001). All NOEC and LOEC values were less than those for other nitrobenzenes. Indigenous soil microbial communities exhibited negative correlations of basal respiration rates and phospholipid fatty acid production when exposed to TNB (Fuller and Manning 1998).

TNB was found to be mutagenic using the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay (Lachance et al. 1999).

The range between the highest TNB concentration causing no responses and the lowest concentration causing short-term ventilatory responses to bluegill was 0.06 to 0.13 mg/l. This is comparable to reported no effect/effect ranges for TNB in early life stage tests of 0.08 to 0.12 mg/l for fathead minnows and 0.08 to 0.17 mg/l for rainbow trout (van der Schalie et al. 1988). Lotufo et al. (2001) found TNB reduced survival of amphipods in spiked sediments, and significantly decreased reproduction at low levels.

USACHPPM (2001) developed a wildlife toxicity assessment to determine the adequacy of data for deriving toxicity reference values for wildlife receptors. That report identified no data for avian, reptile, and amphibian receptors.

The database is adequate for evaluating potential toxicity to benthic invertebrates, soil fauna, and mammalian receptors, but inadequate for all other ecological receptors.

1,3-Dinitrobenzene

Environmental Fate and Transport

DNB is expected to be highly mobile in sandy and silty soils based on the estimated K_{oc} of 150, but the mobility of DNB in clay soils is expected to be low (HSDB, 2003). Biodegradation in soils is expected to occur very slowly as volatilization is not expected to occur (HSDB 2003).

DNB is not expected to adsorb to sediments or suspended solids in the water column, or volatilize from water (HSDB 2003). A study of 1,3-dinitrobenzene degradation from Tennessee River water collected downstream from an Army Ammunition Plant (AAP) found that microorganisms could be grown on 1,3-dinitrobenzene as the sole carbon source (Mitchell and Dennis 1982). The half-lives of 1,3-dinitrobenzene in plain river water and in enrichment samples were found to be 1 and 9.7 days, respectively. River water taken further downstream showed significantly less degradation ability, indicating that microbial population in the proximity of the AAP had adapted to the elevated concentrations of 1,3-dinitrobenze and were better suited to degrade that compound (Mitchell and Dennis 1982). Trapido et al. (2003) found the Fenton reagent enhanced degradation of DNB.

Based on the vapor pressure of $2X10^{-4}$ mm Hg at 25º C, atmospheric DNB is expected to exist in the vapor phase (HSDB 2003). Atmospheric vapor phase DNB will be degraded by photolysis and by reacting with hydroxyl radicals (HSDB 2003).

DNB can be metabolized by microbes in sewage effluent under both aerobic and anaerobic conditions (Hallas and Alexander 1983). Hajjar et al. (1992) reported that 58% of DNB was degraded aerobically in sewage effluent after 28 days by *Candida pucherrima*. The major degradation product was carbon dioxide.

Bioaccumulation and Trophic Transfer

Measured BCF values of 2 – 75 for DNB indicate that potential for bioconcentration in aquatic organisms is low to moderate (HSDB 2003). Deneer et al. (1987) determined the log BCF for DNB to be <0.70 micro-mol/l.

Nystrom and Rickert (1987) studied the metabolism and excretion of DNB after oral dosing to rats, finding that 60% of DNB was eliminated in 24 hrs. The primary route of elimination was in the urine, with the primary metabolites being identified as 3-aminoacetanilide, 4-acetamidophenylsulfate, 1,3-diacetamidobenzene, and 3-nitroaniline-N-glucuronide.

Toxicity

Little data was found for the toxicity of DNB. Quantitative Structure-Activity Relationship (QSAR) studies have found that the toxicity of nitroaromatic compounds is related to the number of constituents and their relative positions, indicating that DNB will be more toxic than other mono-nitroaromatics (Deneer et al. 1989, Gough et al. 1994, Schmitt et al. 2000).

DNB is a known Sertoli cell toxicant (Allenby et al. 1990, Cave and Foster 1990). Cave and Foster (1990) concluded that DNB requires metabolic activation before it can exert its toxicity to Sertoli cells.

Nipper et al. (2001) determined NOEC and LOEC values for DNB to several marine organisms. NOEC and LOEC values for sea urchin fertilization and embryo development tests were 110, 315, <84, and 84 mg/l respectively. Algae NOEC and LOEC values for germination were 0.3 and 0.65 mg/l respectively. Polychaete survival NOEC and LOEC values were 9.7 and 19.6 mg/l respectively. Redfish larvae survival NOEC and LOEC values were 25.2 and 49.6 mg/l respectively. Mysid survival NOEC and LOEC values were 5.2 and 9.7 mg/l respectively (Nipper et al. 2001).

USACHPPM (2001) developed a wildlife toxicity assessment to determine the adequacy of data for deriving toxicity reference values for wildlife receptors. That report identified no data for avian, reptile, and amphibian receptors.

The database is adequate for evaluating potential toxicity to benthic invertebrates, soil fauna, and mammalian receptors, but inadequate for all other ecological receptors.

2,4-Dinitrophenol

Environmental Fate and Transport

If released to air, a vapor pressure of $3.9X10^{-4}$ mm Hg at 20 deg C indicates 2,4-dinitrophenol will exist solely as a vapor in the ambient atmosphere. Vapor-phase 2,4-DNP will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 28 days.

If released in water, vapor pressure, water solubility, and presence predominantly in ionic forms in most natural waters (pKa = 4.09 for 2,4-DNP), suggest that volatilization of dinitrohpneols from water to air is not expected to be a significant fate proc-

ess. Photodegredation, however, may be an important fate process based on evidence suggesting that 2,4-dinitrophenol absorbs light at wavelengths greater than 290 nm, although the kinetics of this reaction require more research. The biodegradation half-life of 2,4-dinitrophenol was reported as 68 and 2.8 days in aerobic and anaerobic waters, respectively.

If released to soil, 2,4-dinitrophenol is expected to have moderate mobility based upon an estimated K_{oc} value of 200. However, as mentioned above, the pKa of 2,4-dinitrophenol is 4.09, which indicates that this compound will exist primarily as an anion on moist soil surfaces and anions are expected to have very high mobility in moist soils. Similar to water, volatilization of 2,4-dinitrophenol from moist soil surfaces is not expected to be an important fate process since the anion will not volatilize and the neutral species has a Henry's Law constant of $8.6X10^{-8}$ atm-cu m/mole at 20º C. Volatilization of 2,4-DNP is not expected to volatilize from dry soil surfaces either based upon its vapor pressure. The transport of DNP from soil to adjacent land or surface water may occur as a result of lateral movement via runoff and/or hydrogeological movement of contaminated groundwater to surface water.

If released in soils containing goethite, 2,4-DNP adsorption is due to surface complex formation at protonated mineral surfaces. The adsorption of 2,4-DNP on goethite was shown to be highest near pH 4.5 and negligible at neutral and alkaline pH. While the adsorption in calcareous soils low in goethite, organic carbon, and clay content was low at a pH >7. The leaching of dinitrophenols is high in such soils. Conversely, the leaching of dinitrophenols from soils will decrease when the organic carbon, clay or goethite content of soil increases and the pH of soil-water attains a pH of <6.

Bioaccumulation and Trophic Transfer

BCF values in the range of 0.4 to 0.7 were measured in carp exposed to 50 µg/l of 2,4-dinitrophenol over 6 weeks. A BCF value of 4 was measured for carp exposed to 5 ug/l of 2,4-dinitrophenol over 6 weeks. These values suggest that bioconcentration of 2,4-dinitrophenol in aquatic organisms is low.

Bioaccumulation in plants would not be a significant process. At concentrations likely to cause maximum bioaccumulation (10 mg/kg), the bioaccumulation factor (concentration in plant over concentration in soil) in lettuce, carrot (tops, peels, and root), hot pepper foliage, and fruits was <0.01 at a soil pH of 6.7-7.2. In addition to this low bioaccumulation, dinitrophenol compounds undergo metabolism in plants suggesting that plant accumulation of dinitrophenols due to uptake would not be significant.

The database for bioaccumulation is inadequate for evaluating potential exposures to ecological receptors. The literature summary did not identify any studies evaluating potential bioaccumulation to higher order ecological receptors in either terrestrial or aquatic environments.

Toxicity

Haghighi-Podeh et al. (1995) examined the toxic effects of nitrophenols on acetate enrichment, methanogenic systems and determined that toxicity decreases in the following order: 2,4-dinitrophenol > 4-nitrophenol > 2-nitrophenol > 3-nitrophenol. Uberoi and Bhattacharya (1997a,b) confirmed these findings when examining the toxic effects and degradability nitrophenols in anaerobic acetate and propionate enrichment systems. The toxicity to both systems decreased in the following order: 2,4-dinitrophenol > 4-nitrophenol > 2-nitrophenol. Furthermore, Uberoi et al. (1997) found that under anaerobic conditions 2,4-dinitrophenol were transformed both abiotically and biotically to 2-amino,4-nitrophenol.

LD_{50} values for animals treated once with 2,4-dinitrphenol by gavage were 30 mg/kg for white rats 71 mg/kg for weanling male rats, and 72 mg/kg for weanling male CFl mice. In a fairly reliable study on mature rats of each sex treated once by gavage, a dose-related increase in mortality was observed, with no mortality at doses of l0-27 mg/kg, 37% mortality at 30 mg/kg, and 100% mortality at 100 mg/kg. A "100% survival dose" of 20 mg/kg and a "100% lethal dose" of 60 mg/kg were reported for white rats treated once by gavage. A "100% survival dose" of 20 mg/kg and a "100% lethal dose" of 30 mg/kg were reported in dogs treated once by gavage.

The cause of death in these acute studies was generally attributed to the pyretic effect of 2,4-dinitrophenol, produced by an increase in metabolic rate. 2,4-Dinitrophenol is an uncoupler of oxidative phosphorylation. In humans or animals exposed to 2,4-DNP, the energy produced from the Krebs cycle is not stored in adenosine triphosphate (ATP), but is released as heat. This short-circuiting of metabolism results in the characteristic clinical signs of increased basal metabolic rate, oxygen consumption, perspiration, and body temperature. Elevated environmental temperatures may compromise the body's ability to dissipate the heat.

Koizumi et al. (2001) examined the toxicity of 2,4-dinitrophenol in newborn and young rats. Based on the results, it can be concluded that the toxic response in newborn rats is at most 4 times higher than that in young rats at least in the case of 2,4-dinitrophenol.

Brecken-Folse et al. (1994) studying juvenile grass shrimp and sheepshead minnows determined that the 96 hr LC_{50}s for 2,4-dinitrophenol ranged from 13 to 50 mg/l.

Temperature and salinity is also said to affect the toxicity of 2,4-dinitrophenol. Toxicity decreased as salinity increased for 2,4-dinitrophenol and sheepshead minnows, but toxicity to grass shrimp increased as salinity increased (Brecken-Folse et al. 1994). Toxicity decreased with increased temperature for grass shrimp exposed to 2,4-dinitrophenol and increased with temperature for sheepshead minnows exposed to 2,4-dinitrophenol (Brecken-Folse et al. 1994).

Howe et al. (1994a) examined the effects of selected water temperatures on the toxicity of 2,4-dinitrophenol to rainbow trout (*Oncorhynchus mykiss*). For 2,4-dinitrophenol, time-independent NOEC values at 7, 12, and 17º C, respectively, were 1.07, 0.50, and 0.80 mg/l for growth and 1.30, 1.89, and 1.60 mg/l for mortality. Temperature did, however, affect the rate at which time-independent NOECs were reached. More time was required to reach time-independent NOECs as temperature decreased.

Howe et al. (1994b) also examined the effects of pH and temperature on the toxicity of 2,4-dinitrophenol to rainbow trout (*Oncorhynchus mykiss*) and the amphipod *Gammarus pseudolimnaeus* and found that the toxicity of the compound was significantly affected by pH. The toxicity of nitrophenols decreased as pH increased. Temperature, also significantly affected the toxicity of 2,4-DNP to both species (Howe et al. 1994b). Toxicity increased with temperature in the amphipod, while toxicity decreased as temperature increased for rainbow trout (Howe et al. 1994b). Chemical bioconcentration was also significantly affected by temperature and pH and was directly related to toxicity in most tests (Howe et al. 1994b).

Phipps et al. (1981) determined 96 hr LC_{50}s for fathead minnow (*Pimephales promelas*). The mean of duplicate 96 hr LC_{50}s (mg/l) in ascending order of toxicity were: pentachlorophenol, 0.22; 2,4,6-tribromophenol, 6.6; 2,4-dichlorophenol, 8.2; 2-chlorophenol, 12; 2,4-dinitrophenol, 17; 2,4-dimethylphenol, 17; 2,6-dimethylphenol, greater than 27; phenol, 29; 4-nitrophenol, 61; and 3-methoxyphenol, 76 (Phipps et al.1981).

The database is inadequate for deriving toxicity reference values for any ecological receptors except for aquatic invertebrates.

Dinitrotoluene

Environmental Fate and Transport

DNT is expected to have moderate mobility in soils and adsorb to suspended solids and sediments in aquatic systems based on an estimated K_{oc} of 370 (HSDB 2003).

Degradation of DNT in aquatic systems will occur by photolysis. Volatilization is not expected to occur (HSDB 2003).

Aerobic degradation of DNT is accelerated by addition of ethanol and phosphate as nutrient and cosubstrates to munitions plant wastewaters (Christopher et al. 2000). 2,4-DNT was rapidly degraded by a bacterial mixture of *Acinetobacter*, *Pseudomonas alcaligenes*, *Flavobacterium*, and *Rhodotorula* from levels of 50 mg/l to 12 mg/l and 10 mg/l at 2 and 7 days, respectively. 2,6-DNT was more resistant to degradation by the same bacterial mixture, which only successfully degraded 25 mg/l of the original 50 mg/l load after both 2 and 7 days (Davis et al. 1981). In a model waste stabilization pond, 92.2% of 2,6-DNT was degraded by an algae and bacteria mixture with addition of a waste feedstock (dry powdered milk) to mimic nutrient loading. Volatilization, sedimentation, water column residuals, and effluent loss accounted for 0.3, 3.6, 1.2, and 2.7 percent respectively of und-degraded DNT (Davis et al. 1983). A laboratory-scale upward-flow anaerobic sludge bed reactor supplied with a mixture of volatile fatty acids and/or glucose as electron donors transformed 2,4-DNT to a nonidentified and nondegradable metabolite under anaerobic conditions (Razo-Flores et al. 1999). Riefler and Smets (2000) determined the redox potential for 2,4-DNT to be -0.397 V, and suggested that transfer of the first electron is the rate-limiting step in nitroreduction. Hallas and Alexander (1983) found that 2,6-dinitrotoluene was degraded slowly anaerobically in sewage effluent, but no loss was evident in 28 days in aerated sewage effluent.

Based on an estimated vapor pressure of 4.0×10^{-4} mm Hg at 25° C, atmospheric DNT is expect to exist solely in the vapor phase, which will be degraded photochemically (HSDB 2003).

Bioaccumulation and Trophic Transfer

The relatively low K_{ow} values of 1.98 for 2,4-DNT and 1.72 for 2,6-DNT indicate that DNT will not bioaccumulate (ATSDR 1998). A measured BCF of 11 also indicates potential for bioaccumulation is low (HSDB 2003).

BCF values of 2,4-DNT of 9.15 and 4.15 were measured in carp (Lang et al,1997). Metabolites in the carp liver were identified as 4-amino-2-nitrotoluene and 2,4-diamino-toluene, indicating that the low BCF values were a result of metabolism. Major urinary metabolites of rats dosed with DNT were 2,4-dinitrobenzyl alcohol and 2,6-dinitrobenzyl alcohol (Mori et al. 1996). Mori et al. (1997) found that 2,4-dinitrobenzaldehyde and 2,6-dinitrobenzaldehyde, which are potent mutagens, are formed by the hepatic metabolism of 2,4-dinitrobenzene and 2,6- dinitrobenzene, which is a biliary hepatic metabolite of 2,4-DNT and 2,6-DNT.

The data on DNT metabolism and bioaccumulation indicate that DNT will not bioaccumulate.

The database for determining the potential for bioaccumulation in aquatic environments is adequate, but is inadequate for evaluating bioaccumulation in higher order receptors and through terrestrial food webs.

Toxicity

Drzyzga et al. (1995) categorized DNT as "very toxic to aquatic organisms". DNT toxicity in water has been found to increase when photolytically when exposed to sunlight (Dave et al. 2000, Davenport et al. 1994). Marine sediment and porewater toxicity tests were performed using 2,6-DNT using sandy and fine-grained sediment substrates (Nipper et al. 2002). NOEC values of 4.63 and >0.549 were determined for amphipods in sandy and fine-grained sediments, respectively. Sea urchin embryo development tests gave EC_{50} values of 36.9 and 0.043 mg/l for pore water from sandy and fine-grained sediments, respectively. Polychaete survival tests gave EC_{50} values of 21.1 and 0.046 for porewater from sandy and fine-grained sediments, respectively. Macro-algae zoospore germination tests gave EC_{50} values of 5.68 and 0.092 mg/l for porewater from sandy and fine-grained sediments, respectively (Nipper et al. 2002). These data suggest that DNT is more toxic in fine-grained sediment substrates. Nipper et al. (2001) developed marine toxicity data for 2,4- and 2,6-DNT. 2,4-DNT NOEC and LOEC values were generally higher that 2,6-DNT NOEC and LOEC values for all tests, indicating 2,4-DNT to be more toxic.

Dodard et al. (1999) evaluated the ecotoxicity of 2,4- and 2,6-DNT using the 15-minute Microtox luminescent bacteria and 96 hr freshwater alga growth inhibition tests. IC_{50} and IC_{20} values for 2,4-DNT using the Microtox tests were 269 and 44.1 uM, respectively. IC_{50} and IC_{20} values for 2,6-DNT using the Microtox tests were 16.4 and 16.4.2 uM, respectively. EC_{50} and EC_{20} values for 2,4-DNT using the 96-hour green alga tests were 14.3 and 8.91 uM, respectively. EC_{50} and EC_{20} values for 2,6-DNT using the 96 hr green alga tests were 90.3 and 66.9.2 uM, respectively. In other studies, neither 2,4- or 2,6-DNT or technical DNT, a mixture of DNT isomers with 2,4-DNT as the major constituent, induced an increase in morphological transformation of Syrian hamster embryo cells.

The toxicity information database for DNT is inadequate for deriving toxicity reference values for any ecological receptors except aquatic invertebrates and mammals. It should be noted that the mammalian studies are all on laboratory rats and mice or dogs.

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)

Environmental Fate and Transport

HMX is persistent in the environment, with volatilization to air, sorption to soils and sediments, and bioconcentration levels being minimal (ATSDR 1997). Based on the calculated K_{oc} of 0.54, HMX will be highly mobile in soils (ATSDR 1997). Monteil-Rivera et al. (2003) studied the sorption and long-term fate of HMX in soils of varying TOC levels. Organic content did not significantly affect the sorption of HMX to soils, which was degraded by 60% over 20 wks. Williams et al. (1989) found composting soils contaminated with explosive compounds including HMX with a mixture of straw/horse manure, alfalfa, horse feed, and sediment reduced total explosives from 17,872 to 74 ppm over 22 wks.

In waters, photolysis will be the primary degradation pathway, with a half-life of about 17 days (ATSDR 1997). Dennis et al. (1990) found that continuous flow granular activated carbon is a feasibly technique for removing HMX from groundwater. Lynch et al. (2003) developed several models to estimate the persistence and dissolution rates of explosive compounds in water, finding that HMX residues will persist in water for months to years before being fully dissolved. Harkins et al. (1999) aerobically degraded HMX in contaminated groundwater from 6 to 1 mg/l in 5.2 days using microbial consortia and livestock manure as a supplemental carbon source.

Since HMX will not volatilize, atmospheric HMX will be solely in the particulate phase, which will be removed by dry deposition.

There have been various studies performed to study the degradation of HMX in wastewater effluents by microbial communities. Boopathy (2001) found that HMX was biodegraded under sulfate reducing, nitrate reducing, fermenting, methanogenic, and mixed electron accepting conditions using enrichment cultures developed from the anaerobic digester sludge of a sewage treatment plant. The primary degradation products of HMX in a municipal anaerobic sludge were nitrous oxide and formaldehyde, which was subsequently biotransformed further to produce carbon dioxide and nitrogen (Hawari et al. 2001).

Studies on the plant-mediated fate of HMX have shown that there is minimal uptake and transformations of HMX by several plant classes. Groom et al. (2002) evaluated the HMX accumulation by alfalfa, bush bean, canola, wheat, and perennial ryegrass, wild bergamot, western wheat grass, brome grass, koeleria, goldenrod, blueberry, anemone, common thistle, waxberry, western sage, and Drummond's

milk vetch. No evidence of plant-mediated HMX chemical transformations was observed with these species.

HMX was resistant to transformation by aquatic plants after 16 days of exposure (Bhadra et al. 2001). Poplar trees were able to take up 70% of spiked HMX from soils over 65 day, which was accumulated in the leaves (Yoon et al. 2002). Of the accumulated HMX, 57% was leached from the leaves by deionized water over 5 days, indicating that the uptake and storage process did not metabolize HMX. Yoon et al. (2002) concluded that phytoremediation of HMX would not be a feasible remediation technique.

The fate and transport of HMX in the environment is reasonably well understood and the database is adequate for risk assessment purposes.

Bioaccumulation and Trophic Transport

The literature summary did not identify any BCF values for HMX.

Henderson (1985) studied HMX levels in the plasma of rats after 90 day toxicity studies. The results of this study indicated that systematic absorption of HMX is very low, probably due to the low aqueous solubility of HMX. The bulk of the administered dose was excreted unchanged.

Radio labeled HMX was administered orally and intravenously to rats and mice, and the toxicokinetics of HMX were studied (Cameron 1986). HMX was dosed orally at 500 mg/kg. Rats eliminated 85% and 4% of the original dose in the feces and urine respectively after 4 days. Mice eliminated 70% and 3% of the original dose in the feces and urine respectively after 4 days. Following intravenous dosing of 2 mg/kg of HMX to rats, 61% and 3% of the original dose was eliminated in the urine and feces, respectively. The toxicokinetic studies indicate that HMX is rapidly excreted unchanged after administration, which indicates that the potential for bioaccumulation of HMX is low.

The database on the potential for bioaccumulation in aquatic and terrestrial food webs is not sufficiently developed to be considered adequate for the purposes of ecological risk assessment, although bioaccumulation and transformation in terrestrial plants has been characterized.

Toxicity

Everett and Maddock (1985) performed 13-week dietary toxicity studies of HMX to mice. Male and mice were dosed at 5, 12, 30, 75, or 200 mg/kg/day and female mice

were dosed at 10, 30, 90, 250, or 750 mg/kg/day. Increased number of deaths occurred at 200mg/Kg/day for male mice and 250 or 750 mg/kg/day for female mice. Everett and Maddock (1985) could not conclude what had caused death in these mice, as only minor changes of doubtful significance were observed in mice following terminal studies. Greenough and McDonald (1985) performed a 14 day dietary toxicity study of HMX to rats to determine the suitable doses for further dietary studies. Mortality occurred at HMX concentrations of 900 and 2700 mg/kg/day for males, and slightly higher concentrations for females. Robidoux et al. (2001) evaluated the sublethal and chronic effects of HMX in artificial soils to earthworms. Nominal concentrations of HMX in soils had significant effects to earthworm growth and reproduction, including number of hatched and total cocoons, number of juveniles, and juvenile biomass. The determined EC values for reproduction and developmental effects of HMX to earthworms was determined to be <280 mg/kg, which was the lowest concentration tested. In a similar study, Robidoux et al. (2002a) found significant effects to earthworm reproduction parameters after exposure to HMX in a spiked natural forest soil. The EC_{10} and EC_{20} values for earthworm reproduction parameters ranged from <15.6 (number of cocoons, number of juveniles per hatched cocoon, juvenile biomass) to >711 (mean juvenile biomass, hatchability) mg/Kg.

Lachance et al. (1999) evaluated HMX for mutagenicity using the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay. HMX did not show mutagenicity at its maximum aqueous solubility limit.

Drzyzga et al. (1995) determined the ecotoxicological potential for HMX using the luminescent bacterium *Vibrio fischeri*. HXM was classified as "toxic to aquatic organisms". Effects of HMX were evaluated in spiked sediment with two freshwater invertebrates: the midge *Chironomus tentans* and the amphipod *Hyalella azteca* (Steevens et al. 2002). Survival of the midge and the amphipod was unaffected after exposure to HMX at the highest concentrations of 1,000 and 400 mg/kg, respectively. Growth of the midge, measured as total weight, significantly increased growth was observed after exposure to sublethal concentrations of HMX.

The effects of HMX on lettuce and barley in an artificial silica soil and natural forest soil (Robidoux et al. 2003). Plants were not affected by an HMX exposure up to 3,320 mg/kg dry soil using silica or 1,866 mg/kg dry soil using a forest soil.

Talmage et al. (1999) considered the literature on HMX toxicity to several aquatic species. The chronic toxicity NOAEC value for water flea survival and offspring production was 3.9 mg/l. The chronic toxicity NOAEC value for fathead minnow percent hatch for embryos, percent survival, mean total length, and average weight for larvae was 3.3 mg/l. The NOAEC value for toxicity to algae was 32 mg/l.

NOAEL and LOAEL values for 13 wk dietary exposure to male rats were 50 and 150 mg/kg/day, respectively. NOAEL and LOAEL values for 13 wk dietary exposure to female rats were 115 and 270 mg/kg/day, respectively. NOAEL and LOAEL values for 13 wk dietary exposure to male mice were 30 and 75 mg/kg/day, respectively. NOAEL and LOAEL values for 13 wk dietary exposure to female mice were 30 and 250 mg/kg/day, respectively.

USACHPPM (2001) developed a wildlife toxicity assessment to determine the adequacy of data for deriving toxicity reference values for wildlife receptors. That report identified no data for avian, reptile, and amphibian receptors.

The database is adequate for evaluating potential toxicity to benthic invertebrates, soil fauna, and mammalian receptors, but inadequate for all other ecological receptors.

Nitrobenzene

Environmental Fate and Transport

Measured K_{oc} values from 30.6 to 370 indicate nitrobenzene will have moderate to very high mobility in soils (HSDB 2003). The Henry's Law constant for nitrobenzene is $40x10^{-5}$ atm-m^3/mole, which allows for volatilization from moist soil surfaces (HSDB 2003). Wilson et al. (1981) found 60 – 80% of nitrobenzene applied to a sandy soil laboratory column was not volatilized or degraded, and exhibited rapid leaching through the soil column. Nitrobenzene can be degraded both aerobically and anaerobically (Hallas and Alexander 1983, HSDB 2003). Nielsen et al. (1995) found that nitrobenzene in a leachate plume from a landfill could be degraded under methanogenic, Fe(III) and NO_3 reducing conditions. Nitrobenzene was degraded by a mixture of five bacteria (*Acinetobacter*, *Pseudomonas alcaligenes*, *Flavobacterium*, and *Rhodotorula*) at load of 10, 50, and 100 mg/l, but inhibited activities of the same bacteria mixture at 200 mg/l (Davis et al. 1981). Davis et al. (1983) tested the degradation of several organic pollutants from model waste stabilization ponds. Nitrobenzene was degraded by 89.5% over 12 days. Volatile loss accounted for 4.9% of nitrobenzene loss, while sedimentation, water column residuals, and loss in effluent accounted for 2.3, 1.0, and 2.3% respectively of un-degraded nitrobenzene from the model waste stabilization pond. Algal uptake accounted for the removal of nitrobenzene from water. The model waste stabilization pond used by Davis et al. (1983) included the addition of waste feedstock (powdered dry milk) to mimic wastewater organic loading and only included phytoplankton, algae and bacteria as inoculums. Lu and Metcalf (1975) ran a similar study using a model aquatic ecosystem that did not include input of supplemental organics and included phtyo- and zooplanktons,

algae, snails, water flea, mosquito larvae, and mosquito fish. In this model aquatic ecosystem, nitrobenzene was degraded significantly less than in the model waste stabilization pond. The majority of nitrobenzene was retained as the parent compound (Lu and Metcalf, 1975). A comparison of these two models suggests supplemental organic loading enhances nitrobenzene degradation. Haigler and Spain (1991) identified the mono- and dioxygenase enzymes to be the first to attack and split the nitrobenzene ring for initial degradation. Trapido et al. (2003) found that the Fenton reagent successfully degraded nitrobenzene and *m*-nitrobenzene.

Nitrobenzene is not expected to adsorb to suspended solids or sediments in water based on K_{oc} values of 30.6 to 370 for soils and 89 for sediments (HSDB 2003). Nitrobenzene is expected to volatilize from water surfaces or be degraded by photolysis (HSDB 2003). Dennis et al. (1990) found that nitrobenzene can feasibly be removed from groundwater using continuous flow granular activated carbon treatment. In a study of organic chemical volatilization from water, Ince and Inel (1989) found the ratio of the mass transfer coefficient of the chemical to that of oxygen to be 0.10 for nitrobenzene. Degradation of nitrobenzene occurs both aerobically and anaerobically in supercritical waters (Lee and Park 1996). Anaerobic degradation yields primarily benzene and nitrite, while aerobic degradation yields aniline, phenol, 2-(2-pyridinyl)-benzonitrile, and dibenzofuran (Lee and Park 1996). A study of 1,3-dinitrobenzene degradation from Tennessee River water collected downstream from an Army Ammunition Plant (AAP) found that microorganisms could be grown on 1,3-dinitrobenzene as the sole carbon source (Mitchell and Dennis 1982). The half-lives of 1,3-dinitrobenzene in plain river water and in enrichment samples were found to be 1 and 9.7 days, respectively. River water taken further downstream showed significantly less degradation ability, indicating that microbial population in the proximity of the AAP had adapted to the elevated concentrations of 1,3-dinitrobenze and were better suited to degrade that compound (Mitchell and Dennis 1982).

McFarlane et al. (1990) studied the potential for nitrobenzene uptake by hydroponic plants, finding that all eight species tested displayed a capacity to chemically alter nonpolar nitrobenzene into both polar and insoluble products. Shah and Campbell (1997) found nitrobenzene was reduced in a solution containing ferredoxin NADP oxidoreductase from spinach leaves and NADPH generating system, producing phenylhydroxylamine on 1:1 basis.

Based on a vapor pressure of 0.245 mm Hg at 25º C, atmospheric nitrobenzene is expected to exist solely in the vapor-phase, and be degraded primary by photolysis (HSDB 2003).

The fate and transport of nitrobenzenes reasonably well understood and the database is adequate for ecological risk assessment purposes.

Bioaccumulation and Trophic Transfer

Based on BCF values ranging from 1.6 to 15, the potential for bioconcentration in aquatic organisms is low (HSDB 2003).

Nitrobenzene isomers are primarily metabolized by nitro group reduction and conjugation with glutathione in rats (Nystrom and Rickert 1987). Eliminations of 85%, 60%, and 75% of the radiolabeled 1,2-, 1,3-, and 1,4-Dinitrobenzene doses were measured over 24 hours from rat excreta (Nystrom and Rickert 1987). Cytochrome P-450 assists in the reduction of nitrobenzene in rat liver to aniline (Harada and Omura 1980).

The ability for metabolism of nitrobenzene and its low BCF values indicate that nitrobenzene will not significantly bioaccumulate in organisms.

Bioaccumulation in aquatic environments and terrestrial plants is well understood, but potential trophic transfer to higher order ecological receptors is less well understood. Therefore, the database is adequate for evaluating lower trophic levels but inadequate for higher trophic levels.

Toxicity

Dodd et al. (1987) determined the NOEL level for reproductive effects in rats exposed to nitrobenzene vapors to be 10 ppm. Inhalation tests of nitrobenzene to rats and mice indicate that inhaled nitrobenzene is carcinogenic and toxic in mice and rats, and that the spectrum of these responses in animals is dependent on species, sex, and genetic background (Cattley et al. 1994). No fetus developmental toxicity was observed to in rats during organogenesis after pregnant rats were exposed to nitrobenzene concentrations of 1, 10, and 40 ppm (Tyl et al. 1987). Administration of single oral doses (550 mg/kg body wt) of nitrobenzene to rats induced petechial hemorrhages in the brain stem and cerebellum, and bilaterally symmetric degeneration in the cerebellum and cerebellar peduncles within 48 hrs of treatment (Morgan et al. 1985). Burns et al. (1994) found the liver and spleen to be the primary organs affected by nitrobenzene dosed by gavage, leading to alterations in bone marrow activity.

Allenby et al (1990) concluded that nitrobenzene is probably a Sertoli cell toxicant in view of its similar disruptive effects on various parameters of Sertoli cell function. Cave and Foster (1990) concluded that *m*-dinitrobenzene requires metabolic

activation before it can exert its toxicity to Sertoli cells. A single oral dose of nitrobenzene induced testicular degeneration and approximately a 17 day period of aspermia resulted. After 100 days post-treatment, there was greater than 90% regeneration of the seminiferous epithelium (Levin et al. 1988). Bond et al. (1981) found orally dosed rats developed histopathological changes induced by a single oral dose of nitrobenzene, consistently involved only the liver and testes. Hepatocellular nucleolar enlargement was consistently detected in rats given doses of nitrobenzene as low as 110 mg/kg, suggesting that nucleolar enlargement was independent of cell death and subsequent regeneration. The author concluded that the observed liver and testicular damage are probably due to a direct effect of nitrobenzene or its metabolites.

Black et al. (1982) determined the LC_{50} ranges for combined frequencies for mortality and teratogenesis at 4 days posthatching of fish and amphibian species. The LC_{50} range for nitrobenzene was determined to be 0.002 to 0.64 mg/l.

Cytotoxic effects of nitrobenzene on spermatogenesis of mature Sprague-Dawley rats were analyzed by measuring the DNA content distribution and testicular weight at 1, 2, and 3 wks of daily oral dose of nitrobenzene (60 mg/kg/day). The data indicated that 1C cells were destroyed, and meiosis of secondary spermatocytes was suppressed, but NB had little effect on the spermatocytes prior to the early pachytene stage (Iida et al. 1997).

The toxicity database for nitrobenzene is adequate for aquatic invertebrates and mammals, but inadequate for the remaining ecological receptors.

Nitroglycerin

Environmental Fate and Transport

Nitroglycerin has an estimated K_{oc} of 180, indicating moderate mobility is soils (HSDB 2003). Hydrolosis is expected to occur in alkaline soils (HSDB 2003). Nitroglycerin will undergo aerobic biodegradation readily by removal of nitrate (HSDB 2003). Aerobic degradation of nitroglycerin proceeds via a set of successive denitration reactions to form the isomers 1,2-nitroglycerin, 1,3-nitroglycerin and glycerol mononitrate (Bhaumik et al. 1998). Bacterial cultures are able to degrade nitroglycerin under both aerobic and anaerobic conditions, with anaerobic conditions occurring ten times faster (Accashian et al. 1998). Christodoulatos et al. (1997) found anaerobic biodegradation occurred via successive denitration of the parent molecule and production of glycerol dinitrate, and glycerol mononitrate.

Phytoremdiation studies of explosives-contaminated soils have isolated the bacterium *Enterobacter cloacae* PB2, which is capable of utilizing nitrate ester explosives such as pentaerythritol tetranitrate (PETN) and nitroglycerin as the sole source of nitrogen for growth (Rosser et al. 2001, Williams et al. 2001).

Nitroglycerin has a water solubility of 1800 mg/l at 20º C, indicating that it will readily dissolve into the water column (USACHPPM 2001). Photolysis is expected to occur at a slow rate, but some commercially available UV treatments have been utilized to treat wastewaters containing nitroglycerin (Hempfling 1997, HSDB 2003). The half-life of nitroglycerin hydrolysis is estimated at 1 year under between pH 3-8 and significantly shorter under alkaline conditions (USACHPPM 2001).

Atmospheric nitroglycerin is expected to occur in both the vapor and particulate phases (HSDB 2003). Vapor-phase nitroglycerin will be degraded by hydroxyl radicals and have an estimated half-life of 15 days (HSDB 2003). Particulate phase nitroglycerin will be deposited by wet and dry deposition. Cropek et al. (2002) identified formaldehyde, ethylene oxide, ethyl acetate, and ethylenediamine as primary byproducts of nitroglycerin pyrolosis.

Bioaccumulation and Trophic Transfer

Nitroglycerin has an estimated BCF of 4, which indicated low potential for bioaccumulation in aquatic organisms (HSDB 2003). King and Fung (1984) found that rat excreta rapidly degraded nitroglycerin in vitro, most likely due to gut flora. The limited data on nitroglycerin suggests that bioconcentration will not occur but the database in adequate to quantify bioaccumulation potential in terrestrial and aquatic environments.

Toxicity

Toxicological studies of nitroglycerin typically apply to its use as a pharmaceutical agent. Multiple formulations have been developed using nitroglycerin as a parent compound. For this review, it is assumed that nitroglycerin is the primary active ingredient for these pharmaceutical agents and that the observations and conclusions apply to nitroglycerin toxicity. Single dose I.V. studies in rats and mice produced LC_{50} values of 17.3 and 18.2 mg/kg in male and female mice, and 24.4 and 23.2 mg/kg in male and female rats, respectively (Anderson et al. 1983). Minimal reactions were observed from two-week subacute i.v. doses to rats at 2.5, 5.0 and 10.0 mg/kg/day, and to dogs at 1.0 and 3.0 mg/kg/day (Anderson et al. 1983). Fehrenbach et al. (2001) studied cytotoxic side effects from use of nitroglycerin during lung transplant, finding pathologic effects on alveolar epithelial integrity. Studies of nitroglycerin ointments on rabbits gave no-effect doses of 240 mg/kg/day for re-

productive performance of dams and fetal development and, no-toxic effect dose of 15 mg/kg/day for the skin and 60 mg/kg/day for the general somatic system (Imoto et al. 1986a, Imoto et al. 1986b). Topically applied nitroglycerin ointment produced systemic effect in rabbits, either in behavior, hematologic and electrocardiographic examination, food consumption, body weight change or urinalysis (Taniguchi et al. 1986). Recent studies have found that nitroglycerin may act as a mutagen, which when tested on *Salmonella typhimurium* strain TA1535, caused observed mutations caused by the release of intracellular nitric oxide (Maragos et al. 1993). Hepatocellular carcinomas were reported in rats after prolonged feeding of nitroglycerin in diet at 78 wks of age (Tamano et al. 1996).

Burton et al. (1993) determined the toxicity of nitroglycerin to several freshwater aquatic organisms, including green alga, hydra, midge, cladoceran, rainbow trout, and fathead minnow. Chronic toxicity tests yielded LOEC and NOEC values of 0.59 and 0.37 mg/l for green alga, respectively. Cladoceran neonate production 7 day tests gave LOEC and NOEC values of 5.48 and 3.23 mg/l, respectively. Rainbow trout 60 day ELS tests found LOEC and NOEC values of 0.06 and 0.03 mg/l, respectively based on a reduction in dry weight. Fathead minnow 28 day ELS tests gave LOEC and NOEC values of 0.20 and 0.12 mg/l respectively based on reduction in hatching success.

USACHPPM (2001) developed a wildlife toxicity assessment to determine the adequacy of data for deriving toxicity reference values for wildlife receptors. That report identified no data for avian, reptile, and amphibian receptors.

The database is adequate for evaluating potential toxicity to benthic invertebrates, soil fauna, and mammalian receptors, but inadequate for all other ecological receptors.

Nitrophenol

Environmental Fate and Transport

A nonsteady-state equilibrium model predicts the following distribution of 4-nitrophenol: air 0.0006%; water 94.6%; soil 0.95%; sediment 4.44%; and biota 0.00009%. The atmospheric concentration of 2-nitrophenol is expected to be higher than the 4-isomer because it has a Henry's law constant value that is higher: 2-nitrophenol 1.3x10-5 atm-cu m/mole; 3- and 4-nitrophenol $2x10^{-9}$ atm-cu m/mole and 1.310^{-8} atm-cu m/mole). Based upon these Henry's Law constants, volatilization from moist soil surfaces and water surfaces may occur for the 2-isomer, while it is not likely for the 3- and 4-isomers. This was confirmed in a laboratory investigation

examining the persistence of p-nitrophenol with or without rice straw amendment to soil under non-flooded and flooded conditions (Megharaj et al. 1993). Nitrophenol (p-) disappeared more rapidly in flooded soil than in non-flooded soil (Megharaj et al. 1993).

Nitrophenols are reported to have vapor pressures of 0.005 to 0.11 mm Hg. This data suggests that both the 2- and 4- isomers are expected to be present predominantly in the vapor-phase in the atmosphere. Vapor-phase nitrophenols will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be in the range of 4 to 18 days. Nitrophenols are not expected to volatilize from dry soil surfaces based upon this vapor pressure data.

Based on K_{oc} values of 32-266, nitrophenols are expected to have high mobility in soils. K_{oc} values also suggest that if released into water, nitrophenols are not expected to adsorb to suspended solids and sediment in the water column. Heim et al. (1994) found that on the whole, 4-nitrophenol shows low persistence and accumulation in sediment-water systems.

Uberoi and Bhattacharya (1997a, b) examined the degradability of three selected nitrophenols in anaerobic acetate and propionate enrichment systems. Under anaerobic conditions 2-nitrophenol and 2,4-dinitrophenol were transformed both abiotically and biotically to 2-aminophenol and 2-amino,4-nitrophenol, respectively.

The fate and transport database for nitrophenols is adequate in soil and water, but inadequate for evaluating potential transport in air.

Bioaccumulation and Trophic Transfer

A number of aquatic species have been examined for their ability to bioconcentrate or bioaccumulate nitrophenols. The bioconcentration factor (BCF) (wet-weight basis) for 4-nitrophenol in a species of green algae (*Chlorella fusca*) was 30. In golden orfe fish (*Leuciscus idus melanotus*), the whole-body BCF after 3 days of exposure was 57. With ^{14}C radiolabeled test compound, the mean plateau whole-body ^{14}C BCF for 4-nitrophenol in the fathead minnow (*Pimephales promelas*) was 180. Based on available BCFs, 4-nitrophenol would biomagnify from lower to higher tropic levels in both aquatic and terrestrial organisms.

Studies have also been conducted on the ability of organisms to metabolize and excrete nitrophenol compounds. Fischer et al. (1995) found that the jejunum of the rat was able to metabolize nitrophenol (p-) rapidly and to transport the metabolites efficiently back into the luminal solution. These results indicate that the intestinal me-

tabolism and the excretion of metabolites may play a role in the elimination of xenobiotics containing phenolic hydroxyl groups and that the small intestine of the rat forms predominantly nitrophenol (p-)-G after luminal administration of nitrophenol (p-) (Fischer et al. 1995).

Pena-Egido et al. (1988) examined the urinary excretion kinetics of p-nitrophenol in rabbits following oral administration of parathion at a dose of 3 mg/kg. Elimination of p-nitrophenol began rapidly, and of the total amount excreted during the study period, 46% was excreted in the first 3 hrs; 85% was excreted at 6 hrs after administration of the pesticide. The mean maximum excretion rate of p-nitrophenol was 111.15 +/- 61.02 micrograms/h reached in a time of 0.77 +/- 0.26 hr. The formation and disappearance rate constants of the metabolite were 2.85 +/- 2.80 hr-1 and 0.80 +/- 0.28 hr-1, respectively. A linear relationship was observed between the plasma concentrations of parathion and the urinary excretion rate of p-nitrophenol (Pena-Egido et al. 1988).

Frank and Beyer (1986) also found that nitrophenols were metabolized efficiently by organisms. Frogs injected with 3-nitrophenol excreted 85-93% of the administered dose within 17 hrs; 70-90% dose was metabolized (Frank and Beyer 1986). Gorge et al. (1987) also examined the metabolism of these compounds in frogs and found that *Rana temporaria* and *Xenopus laevis* excrete 90-95% of the given dose, and metabolize 50-65% dose of phenol, 4-nitrophenol and 2-methylphenol within 24 hrs, to about the same extent.

Shafer and Schonherr (1985) determined partition coefficients for phenol, 2-nitrophenol, and 4-nitrophenol with isolated cuticles from mature tomato and green pepper fruits and from the adaxial surface of rubber (*Ficus*) leaves. Partition coefficients of the phenols (pH 3.0) for the system insoluble polymer matrix/buffer (MX/b) ranged from 43.6 to 164.9 and could be predicted from n-octanol/buffer (o/b) partition coefficients using the equation log KMX/b = 0.363 + 0.952 log $K_{o/b}$ where (r = 0.986). In plant cuticular membranes the partition coefficient values were lower, especially for 4-nitrophenol, ranging from 32.4 to 110.8. The transport-limiting layer in plant cuticles acts as a diffusion and solubility barrier.

Information is available to model uptake into aquatic organisms and terrestrial plants, but the database is inadequate for modeling potential bioaccumulation in higher order ecological receptors.

Toxicity

Brecken-Folse et al. (1994) studying juvenile grass shrimp and sheepshead minnows determined that the 96 hr LC_{50}s for 4-nitrophenol ranged from 12 to 31 mg/l and for

2,4-dinitrophenol from 13 to 50 mg/l. Nipper et al. (2002) determined the 2,4,6-trinitrophenol (picric acid) 10 day LC_{50} value to the amphipod *Ampelisca abdita* in sandy sediment to be 144 mg/kg dry weight. Fine-grained sediment spiked with 2,4,6-trinitrophenol (picric acid) generated a U-shaped concentration-response curve in the amphipod test, with increased survival both in the lowest and highest concentration (Nipper et al. 2002). Phipps et al. (1981) determined 96 hr LC_{50}s for fathead minnow (*Pimephales promelas*). The mean of duplicate 96 hr LC_{50}s (mg/L) in ascending order of toxicity were: pentachlorophenol, 0.22; 2,4,6-tribromophenol, 6.6; 2,4-dichlorophenol, 8.2; 2-chlorophenol, 12; 2,4-dinitrophenol, 17; 2,4-dimethylphenol, 17; 2,6-dimethylphenol, greater than 27; phenol, 29; 4-nitrophenol, 61; and 3-methoxyphenol, 76 (Phipps et al.1981).

Braunbeck et al. (1989) examined morphological alterations of the liver of zebra fish (*Brachydanio rerio*) following prolonged exposure to 0.1, 1 and 5 mg/l 4-nitrophenol (4-NP) and discovered that liver reactions were both sex- and dose-dependent. Numerous structural modifications were seen at 1 mg/l and male fish primarily react with a proliferation of smooth endoplasmic reticulum, female fish display a high degree of fenestration within cisternae of the rough endoplasmic reticulum (Braunbeck et al. 1989). At 5 mg/l, deformations of the nuclear membrane and partial lysis of mitochondria could be observed (Braunbeck et al. 1989). At 1 and 5 mg/l 4-NP in about 25% of the animals investigated showed symptoms of degenerative transformations of the liver tissue into huge multinucleate cell masses with completely different ultrastructure (Braunbeck et al. 1989). Hodson et al. (1991) examined rainbow trout (*Oncorhynchus mykiss*) exposed to waterborne phenol, p-chlorophenol, 2,4-dichlorophenol, p-nitrophenol or 1,2,4-trichlorobenzene for 85 days discovering that these compounds primarily acted to reduce growth rate and to increase mortality rate.

Some discrepancy exists over the effect of salinity on the toxicity of nitrophenols to sheepshead minnows. In 96 hr LC_{50} tests, Brecken-Folse et al. (1994) concluded that an inverse relationship existed between toxicity and salinity. However, Linton et al. (1994) concluded that variations of salinity and/or temperature do not change the NOEC. Howe et al. (1994a, b) weighed in on this issue also, finding that the toxicity of nitrophenols was significantly affected by both temperature and pH.

Koizumi et al. (2001) concluded that the toxic response in newborn rats is at most 4 times higher than that in young rats, at least in the cases of 4-nitrophenol and 2,4-dinitrophenol.

In the 28 day young rat study, clear toxic signs followed by death occurred at 80 mg/kg (Koizumi et al. 2001). NOAELs were 110 mg/kg/day in newborn rats and 400 mg/kg/day in young rats (Koizumi et al. 2001).

There are no chronic toxicity tests available for mammals (ATSDR 1992) but there are subchronic oral studies in laboratory rats and mice. The literature summary did not identify any toxicity information for avian receptors, reptiles and amphibians, or higher order mammalian receptors. Therefore, the database is inadequate for developing toxicity reference values for ecological receptors.

Pentaerythritol Tetranitrate (PETN)

Environmental Fate and Transport

PETN has an estimated K_{oc} range of 179 to 1,720, which suggests low to medium mobility in soil and the tendency to adsorb to soil particles (HSDB 2003). Aerobic degradation of PETN has been enhanced by the isolation of a strain of *Enterobacter cloacae*, designated PB2, which is capable of utilizing PETN as a sole carbon source, resulting in rapid mineralization (Binks et al. 1996, French et al. 1998, Nicklin et al. 1999, Williams et al. 2001). Rosser et al. (2001) successfully isolated PETN reductase, the enzyme responsible for initiating the degradation of PETN in PB2, and introduced this enzyme into plants for phytoremediation uses.

Based on the estimated K_{oc} range of 179 to 1,720, PETN is expected to have low to medium mobility in sediment (HSDB 2003). PETN has relatively low water solubility of 43 mg/l at 25º C which indicates that PETN will remain adsorbed to sediments (HSDB 2003). PETN has a low estimated Henry's Law Constant of $1.2x10^{-11}$ atm-m^3/mole at 25º C, indicating that volatilization from water is not likely (HSDB 2003). Hydrolysis of PETN is more likely to be the predominant degradation pathway for PETN in water (HSDB 2003).

PETN has a vapor pressure of $1.035x10^{-10}$ mm Hg at 25º C indicating atmospheric PETN will be predominantly in the particulate phase, which will be removed by wet and dry deposition (HSDB 2003). Vapor phase PETN will be degraded by reaction with hydroxyl radicals with an estimated half-life of 21 days (HSDB 2003). Significant atmospheric movement of PETN is not expected to occur.

There are no experimentally derived values with which to model the fate and transport of PETN. Therefore, the database is evaluated as inadequate for estimating environmental fate and transport for ecological risk assessment purposes.

Bioaccumulation and Trophic Transfer

PETN has an estimated BCF of 74, indicating bioaccumulation is not likely (HSDB 2003). Human studies have found that PETN can be absorbed through the gastro-

intestinal tract and through the lungs, but not dermally (HSDB 2003). In these studies, approximately 50% of administered PETN was eliminated as reduced metabolites pentaerythritol and mononitrate in urine and feces within 48 hrs (HSDB 2003). Posadas del Rio et al. (1988) found PETN to be metabolized by human kidney, liver, small intestine, and blood serum. King and Fung (1985) found that PETN was rapidly degraded in rat feces and urine, likely due to metabolism by intestinal microflora. While data on PETN bioaccumulation is limited, the estimated BCF and toxicokinetic studies suggest that PETN will not bioaccumulate.

The database is inadequate for determining the potential for bioaccumulation in aquatic and terrestrial environments.

Toxicity

There is little information available regarding the toxicity of PETN to ecological receptors. A Wildlife Toxicity Assessment was performed by USACHPPM (2001) which had similar shortcomings finding relevant animal toxicity studies for developing TRVs for PETN. USACHPPM (2001) determined the ingestion NOAEL for mammals to be 170 mg/kg/day with medium confidence, and the ingestion LOAEL for mammals to be 1700 mg/kg-day with low confidence. No other TRVs were set due to insufficient toxicological studies. Bucher et al. (1990) conducted 14 day, 13 wk, and 2 yr studies on effects of a 1:4 PETN and D-lactose monohydrate mixture (PETN, NF) fed to rats and mice. PETN, NF was found to be essentially non-toxic to rats and mice in all study periods, with no adverse effects clearly related to PETN being observed. In a comparative toxicity survey, Drzyzga et al. (1995) classified PETN as toxic to aquatic organisms.

The limited data available on the toxicity of PETN indicates that risk of toxicity is low.

The database is inadequate with which to develop toxicity reference values for any ecological receptors.

Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX)

Environmental Fate and Transport

RDX is expected to have high to moderate mobility in soils based on the estimated K_{oc} of 70 (HSDB 2003). Laboratory studies have found only limited retention and minimal breakdown of RDX in soils (Selim et al. 1995, Singh et al. 1998, Sheremata et al. 2001). Following an evaluation of RDX transport and sorption in soils from an

ammunition plant, Tucker et al. (2002) found that crystalline RDX will remain in soils and continually be leached to subsurface soils by rainwater. Degradation of RDX in soils is a slow process both aerobically and anaerobically (HSDB 2003). Ringelberg et al. (2003) tested loss of RDX from contaminated soils under saturated and unsaturated conditions, finding RDX concentration fell below the limit of detection within 3 weeks under saturated conditions, while under unsaturated conditions, 42% of the original RDX was still present after 5 wks. Formaldehyde, carbon dioxide, and nitrous oxide are the primary degradation products (Bhushan et al. 2002, Fournier et al. 2002, Hawari et al. 2000). Transient metabolites include hydroxylamino-dinitroso-1,3,5-triazine, mononitroso-, monohydroxylamino-, mononitrosomonohydroxylamino-, monoamino-, diamino-, and triamino-compounds (Adrian and Chow 2001, Zhang and Hughes 2003). Beller (2002) found that nitrate may inhibit RDX metabolism in groundwaters, but RDX reduction will proceed regardless of whether or not a more favorable nitrogen source is present. Several studies have focused on phytoremediation of soils and groundwater contaminated with RDX. Both aquatic and wetland plants were able to uptake RDX into plant biomass, and exhibited some biotransformations to unknown metabolites (Best et al. 1999, Best et al. 2001, Bhadra et al. 2001). Poplar trees grown in contaminated soils took up RDX, translocating up to 60% to the leaves (Thompson et al. 1999). Harvey et al. (1991) recovered 86 (+/-3)% of RDX in soil through hydroponic bush bean biomass after 2 months. Price et al. (2002) found corn, tomato, lettuce, and radish plants would uptake RDX from contaminated irrigation waters, concluding that human health hazards from ingestion of vegetables growing in soils contaminated with low levels of RDX be carefully considered in plans for future use of remediated sites.

The primary degradation process of RDX in water is expected to be direct photochemical degradation (HSDB 2003). Anaerobic degradation occurs slowly, and is only enhanced slightly with addition of supplemental carbon (HSDB 2003). Volatilization from water in not expected to be significant based on an estimated Henry's Law constant of 6.3×10^{-8} atm-cu m/mole (HSDB 2003). Dennis et al. (1990) found that continuous flow granular activated carbon is a feasibly technique for removing tetryl from groundwater.

RDX has an estimated vapor pressure of 4.1×10^{-9} mm Hg at 25º C, indicating that it will exist solely in the particulate phase in the atmosphere, which will be removed by wet and dry deposition (HSDB 2003).

The fate and transport of RDX has been well-studied and the database is adequate for modeling environmental fate and transport in air and water and excellent for modeling in soils.

Bioaccumulation and Trophic Transfer

Little information was found regarding the bioaccumulation or trophic transfer of RDX. Several studies in have indicated found that RDX would accumulate in plant tissues. Measured BCF values of 4.0 (catfish) and 5.9 (fathead minnow), indicate that bioconcentration in aquatic organisms is low (HSDB 2003). Talmage et al. (1999) found measured BCF values ranging from 1.5 (unspecified fish) to 11 (fathead minnow), also indicating potential for bioaccumulation in aquatic organisms is low.

The database for modeling bioaccumulation is adequate in aquatic environments and in terrestrial plants, but no data is available for modeling bioaccumulation potential in higher order terrestrial ecological receptors.

Toxicity

Soils contaminated with nitroaromatic compounds, such as RDX, have been found to be toxic to several classes of organisms. Due to the persistence of RDX in soils, earthworms and benthic invertebrates are heavily affected by RDX contamination (Lotufo et al. 2001, Nipper et al. 2001, Simini et al 1995). Soil microbe activity, including potential nitrification, nitrogen fixation, dehydrogenase, basal respiration, and substrate-induced respiration, was reduced up to 36% above control levels in RDX contaminated soils (Gong et al. 2001). Juck et al. (2003) found no observable effects of RDX to soil bacteria diversity at levels of 1000 mg/kg RDX in natural soils. Reproductive toxicity tests to earthworms determined a LOEC value of 95 mg/kg soil contaminated with RDX (Robidoux et al. 2000). Various reproduction parameters of fecundity, including total and hatched number of cocoons, number of juveniles, and their biomass, were significantly decreased by RDX (Robidoux et al. 2002a). Jarvis et al. (1998) evaluated the effects of composting on RDX-contaminated soils, finding a reduction in lethality to earthworms due to composting, and an increased mutagenicity resulting from metabolites of explosives formed during composting. Steevens et al. (2002) found that soil organisms exposed to explosives at contaminated sites may be affected at concentrations less than 25 mg/kg through hormetic growth enhancement and at higher concentrations through increased mortality.

Gogal et al. (2003) determined acute lethal doses for females and males Northern Bobwhite Quail fed dietary RDX to be 187 and 280 mg/kg respectively. Levine et al. (1981) dosed RDX to rats in the diet for up to 13 weeks at doses of 0, 1, 10, 30, 100, 300 or 600 mg/kg/day. Toxicological responses included slight reductions of food intake and body weight gain, hyperreactivity to approach, decreased serum triglyceride levels, marginal leukocytosis and mortality. A similar study produced hy-

potriglyceridemia, behavioral changes, and mortality in rats dosed at 30, 100, or 300 mg/kg/day (Levine et al. 1990). Talmage et al. (1999) identified several toxicity analyses on the effects of RDX to mammalian species. NOAEL concentrations ranged from 2 – 16 mg/kg/day for rats, and were 7 mg/kg/d and 2 mg/kg/d for mice and rabbits, respectively. LOAEL concentration ranged from 16 mg/kg/day (reduced pup weight) to 50 mg/kg/day (mortality) for rats. Testicular damage occurred at 35 mg/kg/day in mice, and toxicity was observed for rabbit dams and embryos at 20 mg/kg/day.

Drzyzga et al. (1995) classified RDX as "toxic to aquatic organisms". Toxicity tests using fathead minnows produced 96 -hr LC_{50}, LOEC, and NOEC concentrations of 12.7, 2.4, and 1.4 mg/l respectively (Burton et al. 1994a). Toxicity tests using freshwater algae produced LOEC and NOEC concentrations of 4.8 and 0.5 mg/l (Burton et al. 1994b). Talmage et al. (1999) identified several acute and chronic analyses of RDX to aquatic organisms. Acute EC_{50}/LC_{50} concentrations ranged from 3.8 mg/l (7-day post-hatch fathead minnow) to >100 mg/l (water flea, scud, midge, sowbug). Continuous flow-through tests were more toxic to fish and invertebrates than static tests. Chronic toxicity was not noted in minnows or catfish at concentration up to 6.3 mg/l RDX. A significant decrease in the reproduction of water fleas was observed at concentrations ranging from 4.8 – 20 mg/l.

Lachance et al. (1999) found that RDX was not cytotoxic or genotoxic to bacterial and mammalian cells in vitro at maximum solubility concentration.

The database is adequate for deriving toxicity reference values for aquatic invertebrates, plants, and mammals, but inadequate for avian receptors, reptiles and amphibians, and soil fauna.

Trinitrophenylmethylnitramine (Tetryl)

Environmental Fate and Transport

Based on an estimated K_{oc} of 406 and water solubility of 75 mg/l at 20º C, tetryl will have medium mobility in soil, indicating the possibility of tetryl leaching into groundwater (HSDB 2003). No data is available for aerobic degradation of tetryl in soils, but tetryl is expected to undergo slow hydrolysis in acidic and neutral soils. Hydrolysis is expected to occur rapidly in highly alkaline soils (HSDB 2003). Studies on the anaerobic degradation of tetryl have found that anaerobic degradation will occur. Boopathy (2000) found that tetryl was degraded by a sulfate-reducing bacterial consortium by a co-metabolizing process when pyruvate served as the growth substrate. In cultures where tetryl was the sole carbon source, tetryl degra-

dation was minimal (15%) as compared to 100% tetryl degradation over 7 days with pyruvate present (Boopathy 2000). Boopathy (2000) identified aniline as degradation product of tetryl. Dennis et al. (1990) found that continuous flow granular activated carbon is a feasibly technique for removing tetryl from groundwater. Fuller et al. (2003) found that addition of a bioslurry to tetryl-contaminated soils resulted in a 99.9% (100,000 mg/kg to <100 mg/kg) reduction in tetryl concentrations in 180 – 200 days. Shah and Spain (1996) found tetryl is enzymatically reduced to the nitroanion radical under both aerobic and anaerobic conditions by the ferredoxin NADP oxidoreductase of spinach plants. Williams et al. (1989) conducted composting experiments on explosive-contaminated soils containing a mixture of explosive, including tetryl. Levels of explosives were reduced by 99% after 22 wks at 55º C, showing that composting would result in complete degradation of explosives material.

In aquatic systems, hydrolysis and photolysis are the primary processes for degradation of tetryl. The hydrolysis half-life of tetryl has been estimated to be 305 days, and the photolysis of tetryl has been observed to be at least an order of magnitude faster that hydrolysis (HSDB 2003).

Tetryl has an estimated vapor pressure of less than $1x10^{-8}$ mm Hg (HSDB 2003). This vapor pressure suggests that tetryl will exist primarily in the particulate-phase in the atmosphere, and will undergo both wet and dry deposition. The half-life of atmospheric vapor-phase tetryl is 11 days, but the relatively low amount of tetryl expected in the vapor phase indicates that long-range atmospheric transfer is not significant (HSDB 2003).

ATSDR (1995) states that the physical-chemical properties of tetryl are not sufficiently understood to be able to model fate and transport in the environment. The literature summary indicates that the database is inadequate for air and water, but adequate for soil for modeling fate and transport.

Bioaccumulation and Trophic Transfer

No studies on the bioaccumulation and trophic transfer of tetryl were available. Anusevicius et al. (1998) found that rat liver catalyzed denitrification of tetryl, showing that tetryl can be metabolized and possible eliminated. The estimated BCF of 54 suggests that tetryl will not bioaccumulate in aquatic organisms (HSDB 2003).

The database is inadequate for determining the potential for bioaccumulation in aquatic and terrestrial environments.

Toxicity

Nipper et al. (2001) conducted comparative toxicity tests using several munitions compounds to determine marine toxicity data. Of the compounds tested, tetryl was the most toxic to aquatic organisms. Mean EC_{50} values ranged from 0.01 mg/l to 0.43 mg/l. In a similar study, Nipper et al. (2002) found the porewater toxicity of tetryl was influenced by grain-size and organic content of parent sediments. Tetryl was the most toxic of tested compounds to aquatic organisms, but it was not clear if the observed toxicity was caused by the compounds or their degradation products. Drzyzga et al. (1995) classified tetryl as "very toxic to aquatic organisms" following toxicity testing using luminescent bacteria. Reddy et al. (1999) performed a 14- and 90-day dietary study of tetryl using rats. Hematological and histopathological effects were observed in the 14-day study. Hematological effects included decreased hemoglobin and hematocrit and increased number of reticulocyts, total blood protein, and albumin levels. Histopathological changes were the deposition of cytoplasmic droplets in kidneys. In the 90-day study, a significant decrease in terminal body weights was observed in females and males. Histopathological changes were noted in the spleen and kidneys. A NOAEL for both sexes was determined to be 13 mg/kg body weight/day (Reddy et al. 1999).

Literature on the toxicity of tetryl is limited, but existing studies indicate that tetryl is highly toxic to several classes of organisms. Literature reviews conducted by Hovatter et al. (1997) and Talmage et al. (1999) found that tetryl, as with other nitroaromatic munition compounds, will pose a threat to ecological receptors, but more studies have to be done to completely characterize this threat.

The database is adequate for estimating toxicity reference values for mammals, but inadequate for the remaining ecological receptors.

Trinitrotoluene (TNT)

Environmental Fate and Transport

The average K_{oc} for TNT is 1600, indicating that it will not be mobile in soils (HSDB 2003). Selim et al. (1995) found TNT to be highly mobile in soil columns using bentonite mixed with sand, Norwood, and Kolin class soils as substrates. Eriksson and Skyllberg (2001) studied the bonding of TNT to dissolved and particulate soil organic matter. After 20 hrs of equilibration, 60 – 90% of added TNT was free in the soils, while only 0.5 – 6% and 10 – 30 % was associated with dissolved soil organic matter and particulate soil organic matter, respectively. The authors concluded that TNT would bind more to particulate soil organic matter. Binding to dissolved soil

organic matter was is pH dependant, while binding with particulate soil organic matter is independent of pH. Activated carbon added to TNT contaminated soils has been found to rapidly bind TNT, and promote its oxidation to aminodinitrotoluene metabolites (Vasilyeva et al. 2001).

Degradation in aerobic zones of soil will occur by photolysis on soil surfaces, or slow volatilization from moist soils (HSDB 2003). TNT was percolated through an aerobic soil column, which successfully removed 90% of TNT after 19 days with a glucose plus ammonium sulfate buffer (Bruns-Nagel et al. 1996), indicating the need for cometabolism for aerobic soil degradation.

TNT in soil degrades rapidly under anaerobic conditions (HSDB 2003). Drzyzga et al. (1999) found that there is the possibility of high incorporation levels of TNT metabolites into the soil organic matrix mediated by microbial cometabolism under strictly anoxic conditions.

The effectiveness of composting TNT contaminated soils has been tested by several studies. Breitung et al (1996) compared two soil-composting systems: one using aeration from the beginning of the experiments, and one using aeration after a 65 day anaerobic prephase. The systems with an anaerobic prephase performed better at converting TNT to aminodinitrotoluene during the anaerobic phase, which were completely removed during the aerobic phase of the experiment. Williams et al. (1989) found composting of explosives contaminated soils with a mixture of manure, alfalfa, and horse feed reduced total explosives levels by 99% after 22 wks. Griest et al. (1993) studied the effects of composting on soils contaminated with explosive residues, including TNT. Soils were composted with cow manure, sawdust, alfalfa, potato, and apple waste in both static and mechanically stirred piles. Concentrations of TNT in both compost and leachate were decreased by between 77.5 – 99.9% after 90 days (Griest et al. 1993).

In water, TNT is expected to degrade by photolysis and by biotransformations under anaerobic conditions (HSDB 2003). Hydrolysis, volatilization, bioconcentration, and adsorption to suspended solids or sediments are not expected to be major fates of TNT (HSDB 2003). Dennis et al. (1990) found that continuous flow granular activated carbon is a feasibly technique for removing TNT from groundwater. Cui et al. (2001) found that riboflavin significantly enhanced the degradation of TNT in natural water systems by enhancing the photosensitivity of TNT and metabolites. Lynch et al. (2003) developed models to predict the persistence and dissolution of TNT solids in water. The models show that TNT dissolution is correlated with temperature where the estimated half-lives of TNT at 10º C and 30º C were 431 days and 100 days, respectively. Qaisi et al. (1996) determined the soil to water partitioning ki-

netics for TNT. The average mass transfer coefficient value was $3.4E^{-3}$/hour, and the average soil distribution coefficient was 4.88 l/kg.

Several studies have evaluated the used of activated sludge treatments for TNT-contaminated wastewaters. After 3-5 days incubation, no radio labeled TNT could be detected after anaerobic activated sludge system treatment (Carpenter et al. 1978). A study of TNT transformations in anaerobic sludge identified triaminotoluene as the primary degradation product (Hawari et al. 1998). Anaerobic transformation of TNT by biofilm microorganisms obtained from a wastewater treatment plant receiving explosive manufacturing wastewater resulted in transformation of TNT to a mixture of 2-amino-4,6-dinitrotolune; 4-amino-2,6-dinitrotoluene; 2,4-diamino-6-nitrotoluene; and 2,6-diamino-4-nitrotoluene before culminating in the formation of triaminotoluene (Hwang et al. 2000). Slurries of aquifer sediment and groundwater depleted TNT at rates of 27, 7.7 and 5.9 μμg/day under methanogenic, sulfate-reducing and nitrate-reducing conditions, respectively (Krumholz et al. 1997).

The plant-mediated fate of soils and waters contaminated with TNT has been evaluated by several studies. Phytoremediation has been shown to enhance the degradation of TNT contaminated soils and water (Hitchcock et al. 2003, Snellinx et al. 2002, Zoh and Horne 1999). Hannink et al. (2001) found that expression of nitroreductase in plants could catalyze the reduction of TNT to hydroxyaminodinitrotoluene, which is subsequently reduced to aminodinitrotoluene derivatives. Price et al. (2002) evaluated the uptake of TNT in contaminated soils and irrigation water by vegetable plants, including corn, tomato, lettuce, and radish. TNT was only detected in corn. Siciliano and Greer (2000) studied the interactions of several grasses inoculated with *Pseudomonas sp*. Strain 14, which is capable of transforming TNT into mono- and di-amino metabolites. The results were mixed, with some plants being killed while others were able to reduce TNT levels by 30 – 50%. Hybrid poplar trees grown in water and soil contaminated with TNT were able to take up 75% of TNT in root tissues and up to 10% eventually being translocated to the leaves. TNT was transformed by the tree to 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, and to a number of unidentified compounds (Thompson et al., 1998). Best et al. (1999) found that aquatic and wetland plants were able to uptake TNT rapidly. Biotransformations of TNT were noted in plants after 7 days incubation to amino-dinitrotoluenes and other unidentified compounds.

Studies on organisms capable of degrading TNT have identified several organism classes that effectively degrade or mineralize TNT. Pasti-Grigsby et al. (1996) found actinomycetes were able to transform TNT into reduced intermediates. The PETN reductase *E. cloacae* PB2 was found to be capable of slow aerobic growth with TNT as the sole nitrogen source (French et al. 1998). Kalafut et al. (1998) found the *Ba-*

cillus sp. bacteria strain has the most diverse bioremediation potential for degrading TNT, owing to its growth in the presence of TNT, high level of reductive ability, and capability of removing-NO_2 from the nitroaromatic ring. Immobilized *Phanerochaete chrysosporium* cultures were able to mineralize TNT to a level of 15.3% following a 41-day incubation period in a microcosm (Rho et al. 2001). Under anoxic conditions *Pseudomonas sp*. strain JLR11 can use 2,4, 6-trinitrotoluene (TNT) as the sole N source (Esteve-Nunez et al. 2000). Incubated suspension cultures of *Datura innoxia* degraded TNT within 12 hours, leaving less than 1% of the initial TNT in the growth medium (Lucero et al. 1999). The fungus *Phanerochaete chrysosporium* has been found to reduce and detoxify TNT in activated sludge systems (Snellinx et al. 2002, Stahl and Aust 1993). The white-rot fungus *Phlebia radiata* has also been identified as a candidate for the biodegradation of TNT as well as its reduction metabolites (Van Aken et al. 1999). Mycobacterium vaccae strain JOB-5 cometabolized TNT in the presence of propane as a carbon and energy source (Vanderberg et al. 1995). Earthworms transformed TNT by a reductive pathway to 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene, and traces of 2,6-diamino-4-nitrotoluene, which were found in earthworm tissues (Renoux et al. 2000). Boopathy and Kulpa (1994) found a *Methanococcus sp*. methanogenic bacteria strain, isolated from lake sediment, was able to transform 100 ppm of TNT within 40-60 days of incubation at 30º C. The soil bacterial has an optimal pH range of 6-7 and maximum metabolic activity at 20 to 22º C (Boopathy et al. 1997).

Based on a vapor pressure of 1.99×10^{-4} mm Hg at 20º, atmospheric TNT will exist almost entirely in the vapor phase and be degraded by direct photolysis (HSDB 2003).

Bioaccumulation and Trophic Transport

The estimated BCF for TNT is 40, based on a water solubility of 130 ppm at 20º C, which indicates that bioconcentration will not be significant (HSDB 2003). Talmage et al. (1999) identified BCF values of 20.5 for fish, 453 for green algae, 209 for water flea, 202 for oligochaetes, and 9.5 and 338 for bluegill (*Lepomas macrochirus*) muscle and viscera, respectively. These data indicate that there is a potential for bioaccumulation in aquatic organisms.

Johnson et al. (1999) found that salamanders exposed to TNT contaminated soils accumulate levels of aminodinitrotoluenes. A study on the mechanism of bioactivation and covalent binding of TNT using rat liver found that TNT was rapidly reduced to yield 4-hydroxylamino-2,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene as intermediates which were further metabolized to form 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene (Leung et al. 1995).

The database for evaluating bioaccumulation is evaluated as adequate for terrestrial and aquatic environments.

Toxicity

NOEL values for TNT dosed orally to dog, rats, and mice were determined to be 0.20, 1.42, and 7.76 mg/Kg/day respectively (Dilley et al. 1982). Gogal et al. (2002) performed a range-finding study on the effects of dietary TNT given to northern bobwhite quail at doses of 3,000, 1,500, 750, and 100 mg/kg TNT. Dietary TNT intake caused a dose-dependent decrease in total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, and blood lymphocytes. An increased trend in late apoptotic/necrotic blood leukocytic cells was also observed in TNT-exposed birds, as was hemosiderosis in the liver. The authors concluded that Northern Bobwhites (*Colinus virginianus*) are less sensitive to oral exposures of TNT than mammals (Gogal et al. 2002). Johnson et al. (2000) studied the immune effects of orally dosed TNT to white-footed mice at approximate daily doses of 66, 145, 275, and 601 mg/kg body weight for males and 70, 142, 283, and 550 mg/kg/day for females. Results indicate that TNT primarily affect the spleen. In the highest-dose groups of mice, spleen weight was significantly decreased, and inhibited splenic macrophage phagocytosis, splenic congestion and extramedullary hematopoiesis was also noted. Subchronic toxicity tests on the dietary administration of TNT for 13 wk to mice found toxic effects at 125 mg/kg/day or greater included decreased food intake and body weight gain, elevated serum cholesterol levels, and anemia. Splenomegaly, hepatomegaly/hepatocytomegaly and testicular atrophy with degeneration of the seminiferous tubular epithelium were also seen at 125 and 300 mg/kg/day (Levine et al. 1984). A similar study noted the major toxic effects following the dietary administration of TNT to rats included anemia, hypercholesterolemia, and hepatomegaly, splenomegaly, and testicular atrophy with their accompanying histologic lesions (Levine et al. 1990). The major toxic effects following the oral administration of TNT to dogs included hemolytic anemia, methemoglobinemia, liver injury, splenomegaly with accompanying histologic lesions, and death. Only the highest dose given (32 mg/kg/day) proved to be lethal (Levine et al. 1990). Rats given TNT orally were evaluated for hematological, biochemical, and pathological effects (Reddy et al. 2000). LD_{50} values were determined to be 607 and 767 mg/kg body weight for male and female rats, respectively. Hematological, pathological, and biochemical effects included increased spleen weight, anemia, increased liver weight, and histological changes in liver.

Dodard et al. (2003) studied the effects of TNT exposure in spiked artificial soil on the survival and reproduction rate of the white potworm Enchytraeus albidus. The LC_{50} for survival was 442 mg/kg, and the EC_{50} for fecundity was 111 mg/kg. The data also indicated that TNT was 5-10 times more lethal to juveniles than adults.

The effects of TNT spiked in two garden soils were evaluated on the potential nitrification activity, nitrogen-fixation activity, and dehydrogenase activity of soil microbial communities. The estimated IC_{50} ranged from 39 to 533 mg/kg of the acetonitrile-extractable (AE) TNT (1 week after spiking), depending on indicators and soils. Fuller and Manning (1998) found that the number of culturable heterotrophs, concentrations of phospholipid fatty acid, and basal respiration rates of indigenous microbial soil communities decreased after exposure to TNT. Toxic effects of TNT to earthworms have been evaluated in several studies. Robidoux et al. (1999) evaluated the acute toxicity of TNT to earthworms in spiked natural and artificial soils. LOEC and LC_{50} values in natural soils were 260 and 222.4 mg/kg respectively. LOEC and LC_{50} values in artificial soils were 420 and 364.9 mg/kg, respectively. A study of TNT effects on the reproduction parameters of earthworms (total number of cocoons, number of hatched cocoons, number of juveniles, juvenile biomass) gave LOEC and NOEC values of 110 and 55 mg/kg, respectively (Robidoux et al. 2000). Reproduction parameters of fecundity (total and hatched number of cocoons, number of juveniles, and their biomass) were significantly decreased after exposure to TNT (Robidoux et al. 2002a).

Drzyzga et al. (1995) classified TNT as "very toxic to aquatic organisms". The chronic toxicity of TNT to a marine polychaete and estuarine amphipod were evaluated after 28-days exposure to spiked sediments. Survival of the polychaete and amphipod was decreased at 61 and 6.3 µg/g wet weight in tissue, respectively. Growth and reproduction were significantly reduced at 10.0 and 6.3 µg/g wet weight tissue in the amphipod. Acute toxicity tests on bluegill sunfish gave 96 hr LC_{50} values ranging from 2.3 to 2.8 mg/l (Pederson 1970). Smock et al. (1976) evaluated the toxicity of TNT to algae and the fathead minnow. TNT inhibited the growth of algae at concentrations greater that 9 mg/l and was lethal at 50 mg/l. The 96 hr LC_{50} value for the fathead minnow was 2.58 mg/l. Steevens et al. (2002) found significant toxicity to midge and amphipod after exposure to TNT in sediments. Exposure to TNT resulted in significant effects on amphipod survival at 50 mg/kg. Midge survival was significantly decreased at TNT concentrations of 400 mg/kg. The toxicity of TNT to aquatic organisms is enhanced after exposure to ultraviolet light (Arfsten et al. 1994, Davenport et al. 1994, Johnson et al. 1994).

Hankenson and Schaeffer (1991) found that TNT was 20 to 50 times more toxic than its metabolites using the Microtox test. Lachance et al. (1999) determined the cytotoxic and genotoxic effects of TNT. Mutagenicity was evaluated with the Salmonella fluctuation test and the V79 Chinese hamster lung cell mutagenicity assay, while cytotoxicity was evaluated using V79 and TK6 human lymphoblastic cells. TNT was found to be both cytotoxic and mutagenic. TNT was found to be more toxic than its metabolites.

Talmage et al. (1999) discusses the toxicity of TNT to a variety of aquatic organisms and mammals. Water flea chronic toxicity tests gave NOEC values of 0.03-1.03 mg/l for mortality and reproduction. NOEC values for channel catfish hatchability and fry survival were 0.11-1.35 mg/l. The survival of rainbow trout (*Oncorhynchus mykiss*) fry was decreased at 0.24 mg/l. NOAEL and LOAEL ranges for rats given dietary TNT were 0.4-34.7 and 2-160 mg/kg/day, respectively. NOAEL and LOAEL values for male mice given dietary TNT were 7.5 and 35.7 mg/kg/day, respectively. NOAEL and LOAEL values for female mice given dietary TNT were 8 and 37.8 mg/kg/day, respectively.

The database for deriving toxicity reference values is evaluated as excellent for mammals and plants, and is adequate for the remaining ecological receptors including amphibians. The database is inadequate for reptiles, however.

References

Accashian, J.V., Vinopal, R.T., Kim, B.J. and Smets, B.F. 1998. Aerobic growth on nitroglycerin as the sole carbon, nitrogen, and energy source by a mixed bacterial culture. *Appl Environ Microbiol.* 64(9): 3300-3304.

Adrian, N.R. and Chow, T. 2001. Identification of hydroxylamino-dinitroso-1,3,5-triazine as a transient intermediate formed during the anaerobic biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Environ Toxicol Chem.* 20(9): 1874-1877.

Akhavan J. 2004. The Chemistry of Explosives. The Royal Society of Chemistry. Online at: http://www.rsc.org/is/books/chem_expl.htm. Accessed January 21, 2004.

Allenby, G., Sharpe, R.M. and Foster, P.M. 1990. Changes in Sertoli cell function in vitro induced by nitrobenzene. *Fundam Appl Toxicol.* 14(2): 364-375.

Anderson, J.A., McGuire, E.J., Watkins, J.R., Fitzgerald, J.E. and de la Iglesia, F.A. 1983. Toxicology studies with a stable intravenous formulation of nitroglycerin. *J Appl Toxicol.* 3(3): 161-165.

Anon. 1992. RDX. *Dangerous Properties of Industrial Materials Report.* 12(2): 248-256.

Anusevicius, Z., Sarlauskas, J., Nivinskas, H., Segura-Aguilar, J. and Cenas, N. 1998. DT-diaphorase catalyzes N-denitration and redox cycling of tetryl. *FEBS Lett.* 436(2): 144-148.

Arfsten, D.P., Davenport, R. and Schaeffer, D.J. 1994. UV-A coexposure enhances the toxicity of aromatic hydrocarbons, munitions, and metals to Photobacterium phosphoreum. *Biomed Environ Sci.* 7(2): 101-108.

ATSDR. 1995. Toxicological Profile for 1,3-Dinitrobenzene and 1,3,5-Trinitrobenzene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1998. Toxicological Profile for 2,4- and 2,6-Dinitrotoluene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1995. Toxicological Profile for Dinitrophenols. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1997. Toxicological Profile for HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine). Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1990. Toxicological Profile for Nitrobenzene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1992. Toxicological Profile for Nitrophenols: 2-Nitrophenol, 4-Nitrophenol. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1995. Toxicological Profile for RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine). Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1995. Toxicological Profile for Tetryl (2,4,6-Trinitrophenyl-N-methylnitramine. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR. 1995. Toxicological Profile for 2,4,6-Trinitrotoluene. Prepared by the Agency of Toxic Substances and Disease Registry, Atlanta, GA.

Beller, H.R. 2002. Anaerobic biotransformation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by aquifer bacteria using hydrogen as the sole electron donor. *Water Res.* 36(10): 2533-2540.

Best, E.P., Miller, J.L. and Larson, S.L. 2001. Tolerance towards explosives, and explosives removal from groundwater in treatment wetland mesocosms. *Water Sci Technol.* 44(11-12): 515-521.

Best, E.P., Sprecher, S.L., Larson, S.L., Fredrickson, H.L. and Bader, D.F. 1999. Environmental behavior of explosives in groundwater from the Milan Army Ammunition Plant in aquatic and wetland plant treatments. Uptake and fate of TNT and RDX in plants. *Chemosphere.* 39(12): 2057-2072.

Bhadra, R., Wayment, D.G., Williams, R.K., Barman, S.N., Stone, M.B., Hughes, J.B. and Shanks, J.V. 2001. Studies on plant-mediated fate of the explosives RDX and HMX. *Chemosphere.* 44(5): 1259-1264.

Bhaumik, S., Christodoulatos, C., Brodman, B.W. and Pal, N. 1998. Biodegradation of glycerol trinitrate by activated sludge: Cosubstrate requirements, inhibition, and kinetics. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering [J. Environ. Sci. Health, Pt. A: Toxic/Hazard. Subst. Environ. Eng.J.*(4): 574-571.

Bhushan, B., Halasz, A., Spain, J., Thiboutot, S., Ampleman, G. and Hawari, J. 2002. Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-tiazine catalyzed by a NAD(P)H: nitrate oxidoreductase from Aspergillus niger. *Environ Sci Technol.* 36(14): 3104-3108.

Binks, P.R., French, C.E., Nicklin, S. and Bruce, N.C. 1996. Degradation of pentaerythritol tetranitrate by Enterobacter cloacae PB2. *Applied and Environmental Microbiology.* 62(4): 1214-1219.

Black, J.A., Birge, W.J., McDonnell, W.E., Westerman, A.G. and Ramey, B.A. 1982. The Aquatic Toxicity of Organic Compounds to Embryo-Larval Stages of Fish and Amphibians. University of Kentucky Water Resource Research Institute, Lexington, KY. Report No. 133.

Bond, J.A., Chism, J.P., Rickert, D.E. and Popp, J.A. 1981. Induction of hepatic and testicular lesions in Fischer-344 rats by single oral doses of nitrobenzene. *Fundam Appl Toxicol.* 1(5): 389-394.

Boopathy, R. 2000. Formation of aniline as a transient metabolite during the metabolism of tetryl by a sulfate-reducing bacterial consortium. *Curr Microbiol.* 40(3): 190-193.

Boopathy, R. 2001. Enhanced biodegradation of cyclotetramethylenetetranitramine (HMX) under mixed electron-acceptor condition. *Bioresour Technol.* 76(3): 241-244.

Boopathy, R. and Kulpa, C.F. 1994. Biotransformation of 2,4,6-trinitrotoluene (TNT) by a Methanococcus sp. (strain B) isolated from a lake sediment. *Can J Microbiol.* 40(4): 273-278.

Boopathy, R., Manning, J. and Kulpa, C.F. 1997. Optimization of environmental factors for the biological treatment of trinitrotoluene-contaminated soil. *Arch Environ Contam Toxicol.* 32(1): 94-98.

Braunbeck, T., Storch, V. and Nagel, R. 1989. Sex-specific reaction of liver ultrastructure in zebra fish (Brachydanio rerio) after prolonged sublethal exposure to 4-nitrophenol. *Aquatic Toxicology.* 14(3): 185-202.

Brecken-Folse, J.A., Mayer, F.L., Pedigo, L.E. and Marking, L.L. 1994. Acute toxicity of 4-nitrophenol, 2,4-dinitrophenol, terbufos and trichlorfon to grass shrimp (Palaemonetes spp.) and sheepshead minnows (Cyprinodon variegatus) as affected by salinity and temperature. *Environmental Toxicology and Chemistry.* 13(1): 67-77.

Breitung, J., Bruns-Nagel, D., Steinbach, K., Kaminski, L., Gemsa, D. and von Low, E. 1996. Bioremediation of 2,4,6-trinitrotoluene-contaminated soils by two different aerated compost systems. *Appl Microbiol Biotechnol.* 44(6): 795-800.

Bruns-Nagel, D., Brettung, J., von Loew, E., Steinbach, K., Gorontzy, T., Kahl, M., Blotevogel, K.H. and Gemsa, D. 1996. Microbial transformation of 2,4,6-trinitrotoluene in aerobic soil columns. *Applied and Environmental Microbiology.* 62(7): 2651-2656.

Bucher, J.R., Huff, J., Haseman, J.K., Eustis, S.L., Lilja, H.S. and Murthy, A.S. 1990. No evidence of toxicity or carcinogenicity of pentaerythritol tetranitrate given in the diet to F344 rats and B6C3F1 mice for up to two years. *J Appl Toxicol.* 10(5): 353-357.

Burns, L.A., Bradley, S.G., White, K.L., Jr., McCay, J.A., Fuchs, B.A., Stern, M., Brown, R.D., Musgrove, D.L., Holsapple, M.P., Luster, M.I., et al. 1994. Immunotoxicity of mono-nitrotoluenes in female B6C3F1 mice: I. Para-nitrotoluene. *Drug Chem Toxicol.* 17(3): 317-358.

Burton, D.T. and Turley, S.D. 1995. Reduction of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) toxicity to the cladoceran Ceriodaphnia dubia follow-ing photolysis in sunlight. Bull Environ Contam Toxicol. 55(1): 89-95.

Burton D.T., S.D. Turley, G.T. Peters. 1993. Toxicity of Nitroguanidine, Nitroglycerin, Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX), and 2,4,6-Trinitrotoluene (TNT) to Selected Freshwater Aquatic Organisms. Maryland University, College Park. Agriculture Experiment Station. AD-A267 467.

Burton, D.T., Turley, S.D. and Peters, G.T. 1994a. The toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the freshwater green alga Selenastrum capricornutum. *Water, Air, & Soil Pollution.* 76(3-4): 449-457.

Burton, D.T., Turley, S.D. and Peters, G.T. 1994b. The acute and chronic toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the fathead minnow (Pimephales promelas). *Chemosphere.* 29(3): 567-579.

Cameron BD. 1986. HMX: Toxicokinetics of (^{14})C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171600.

Carpenter, D.F., McCormick, N.G., Cornell, J.H. and Kaplan, A.M. 1978. Microbial transformation of ^{14}C-labeled 2,4,6-trinitrotoluene in an activated-sludge system. *Appl Environ Microbiol.* 35(5): 949-954.

Cattley, R.C., Everitt, J.I., Gross, E.A., Moss, O.R., Hamm, T.E., Jr. and Popp, J.A. 1994. Carcinogenicity and toxicity of inhaled nitrobenzene in B6C3F1 mice and F344 and CD rats. *Fundam Appl Toxicol.* 22(3): 328-340.

Cave, D.A. and Foster, P.M. 1990. Modulation of m-dinitrobenzene and m-nitrosonitrobenzene toxicity in rat Sertoli--germ cell cocultures. *Fundam Appl Toxicol.* 14(1): 199-207.

Christodoulatos, C., Bhaumik, S. and Brodman, B.W. 1997. Anaerobic biodegradation of nitroglycerin. *Water Research [WATER RES.].* 31(6): 1462-1470.

Christopher, H.J., Boardman, G.D. and Freedman, D.L. 2000. Aerobic biological treatment of 2,4-dinitrotoluene in munitions plant wastewater. *Water Research [Water Res.].* 34(5): 1595-1603.

Cropek, D.M., Kemme, P.A., Day, J.M. and Cochran, J. 2002. Use of Pyrolysis GC/MS for Predicting Emission Byproducts from the Incineration of Double-Base Propellant. *Environmental Science & Technology [Environ. Sci. Technol.].* 36(20): 4346-4351.

Cui, H., Hwang, H.M., Cook, S. and Zeng, K. 2001. Effect of photosensitizer riboflavin on the fate of 2,4,6-trinitrotoluene in a freshwater environment. *Chemosphere.* 44(4): 621-625.

Dave, G., Nilsson, E. and Wernersson, A.S. 2000. Sediment and water phase toxicity and UV-activation of six chemicals used in military explosives. *Aquatic Ecosystem Health & Management.* 3(3): 291-299.

Davenport, R., Johnson, L.R., Schaeffer, D.J. and Balbach, H. 1994. Phototoxicology. 1. Light-enhanced toxicity of TNT and some related compounds to Daphnia magna and Lytechinus variagatus embryos. *Ecotoxicol Environ Saf.* 27(1): 14-22.

Davis, E.M., Murray, H.E., Liehr, J.G. and Powers, E.L. 1981. Basic Microbial Degradation Rates and Chemical Byproducts of Selected Organic Compounds. *Water Research.* 15(9): 1125-1127.

Davis, E.M., Turley, J.E., Casserly, D.M. and Guthrie, R.K. 1983. Partitioning of selected organic pollutants in aquatic ecosystems. 5. International Biodeterioration Symposium, Aberdeen (UK), Sep 1981.

Deneer, J.W., Sinnige, T.L., Seinen, W. and Hermens, J.L.M. 1987. Quantitative Structure-activity Relationships for the Toxicity and Bioconcentration Factor of Nitrobenzene Derivatives Towards the Guppy (Poecilia Reticulata). *Aquatic Toxicology AQTODG.* 10(2): 115-129.

Deneer, J.W., van Leeuwen, C.J., Seinen, W., Maas-Diepeveen, J.L. and Hermens, J.L.M. 1989. QSAR Study of the Toxicity of Nitrobenzene Derivatives Towards Daphnia magna, Chlorella pyrenoidosa and Photobacterium phosphoreum. *Aquatic Toxicology AQTODG.* 15(1): 83-98.

Dennis, R.M., Wuicik, W.J., Lowe, W.L. and Marks, P.J. 1990. *Task order 7. Use of activated carbon for treatment of explosives-contaminated groundwater at the Milan Army Ammunition Plant (MAAP).* Weston Roy F., Inc., West Chester, PA (USA).

Dilley, J.V., Tyson, C.A., Spanggord, R.J., Sasmore, D.P., Newell, G.W. and Dacre, J.C. 1982. Short-term oral toxicity of a 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine mixture in mice, rats, and dogs. *J Toxicol Environ Health.* 9(4): 587-610.

Dodard, S.G., Renoux, A.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Ecotoxicity characterization of dinitrotoluenes and some of their reduced metabolites. Chemosphere. 38(9): 2071-2079.

Dodard, S.G., Renoux, A.Y., Powlowski, J. and Sunahara, G.I. 2003. Lethal and subchronic effects of 2,4,6-trinitrotoluene (TNT) on Enchytraeus albidus in spiked artificial soil. *Ecotoxicology and Environmental Safety.* 54(2): 131-138.

Dodd, D.E., Fowler, E.H., Snellings, W.M., Pritts, I.M., Tyl, R.W., Lyon, J.P., O'Neal, F.O. and Kimmerle, G. 1987. Reproduction and fertility evaluations in CD rats following nitrobenzene inhalation. *Fundam Appl Toxicol.* 8(4): 493-505.

Drzyzga, O., Bruns-Nagel, D., Gorontzy, T., Blotevogel, K.H. and von Low, E. 1999. Anaerobic incorporation of the radiolabeled explosive TNT and metabolites into the organic soil matrix of contaminated soil after differ-ent treatment procedures. Chemosphere 38(9): 2081-2095.

Drzyzga, O., Gorontzy, T., Schmidt, A. and Blotevogel, K.H. 1995. Toxicity of explosives and related compounds to the luminescent bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology.* 28(2): 229-235.

Encyclopedia Britannica. 1997. Guide to the Noben Prizes: Nirtroglycerine. Online at: http://www.britannica.com/nobel/micro/426_77.html

Eriksson, J. and Skyllberg, U. 2001. Binding of 2,4,6-trinitrotoluene and its degradation products in a soil organic matter two-phase system. *J Environ Qual.* 30(6): 2053-2061.

Esteve-Nunez, A., Lucchesi, G., Philipp, B., Schink, B. and Ramos, J.L. 2000. Respiration of 2,4,6-trinitrotoluene by Pseudomonas sp. strain JLR11. *J Bacteriol.* 182(5): 1352-1355.

Everett DJ and SM Maddock. 1985. HMX: 13 Week Toxicity Study in Mice by Dietary Administration. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171602.

Fehrenbach, A., Wittwer, T., Meyer, D., von Vietinghoff, S., Viehover, M., Fehrenbach, H., Richter, J. and Wahlers, T. 2001. Nitroglycerin alters alveolar type II cell ultrastructure after ischemia and reperfusion. *J Heart Lung Transplant.* 20(8): 876-888.

Fischer, E., Rafiel, A. and Bojcsev, S. 1995. Intestinal elimination of p-nitrophenol in the rat. *Acta Physiol Hung.* 83(4): 355-362.

Fournier, D., Halasz, A., Spain, J., Fiurasek, P. and Hawari, J. 2002. Determination of key metabolites during biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine with Rhodococcus sp. strain DN22. *Appl Environ Microbiol.* 68(1): 166-172.

Frank, G. and Beyer, J. 1986. Metabolism of 3-nitrophenol by the frog Rana temporaria. *Xenobiotica.* 16(4): 291-294.

French, C.E., Nicklin, S. and Bruce, N.C. 1998. Aerobic degradation of 2,4,6-trinitrotoluene by Enterobacter cloacae PB2 and by pentaerythritol tetranitrate reductase. *Applied and Environmental Microbiology [Appl. Environ. Microbiol.].* 64(8): 2864-2868.

Fuller, M.E., Kruczek, J., Schuster, R.L., Sheehan, P.L. and Arienti, P.M. 2003. Bioslurry treatment for soils contaminated with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl). *J Hazard Mater.* 100(1-3): 245-257.

Fuller, M.E. and Manning, J.F., Jr. 1998. Evidence for differential effects of 2,4,6-trinitrotoluene and other munitions compounds on specific subpopulations of soil microbial communities. *Environmental Toxicology and Chemistry.* 17(11): 2185-2195.

Gogal, O.M., Jr., Johnson, M.S., Larsen, C.T., Prater, M.R., Duncan, R.B., Ward, D.L., Lee, R.B., Salice, C.J., Jortner, B. and Holladay, S.D. 2003. Dietary oral exposure to l,3,5-trinitro-1,3,5-triazine in the northern bobwhite (Colinus virginianus). *Environ Toxicol Chem.* 22(2): 381-387.

Gogal, R.M., Jr., Johnson, M.S., Larsen, C.T., Prater, M.R., Duncan, R.B., Ward, D.L. and Holladay, S.D. 2002. Influence of dietary 2,4,6-trinitrotoluene exposure in the northern bobwhite (Colinus virginianus). *Environmental Toxicology and Chemistry.* 21(1): 81-86.

Gong, P., Hawari, J., Thiboutot, S., Ampleman, G. and Sunahara, G.I. 2001. Ecotoxicological effects of hexahydro-1,3,5-trinitro-1,3,5-triazine on soil microbial activities. *Environmental toxicology and chemistry.* 20(5): 947-951.

Gong, P., Siciliano, S.D., Greer, C.W., Paquet, L., Hawari, J. and Sunahara, G.I. 1999. Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field-contaminated soils to indigenous microorganisms. Environmental Toxicology and Chemistry 12: 2681-2688.

Gorge, G., Beyer, J. and Urich, K. 1987. Excretion and metabolism of phenol, 4-nitrophenol and 2-methylphenol by the frogs Rana temporaria and Xenopus laevis. *Xenobiotica.* 17(11): 1293-1298.

Gough, K.M., Belohorcova, K. and Kaiser, K.L.E. 1994. Quantitative structure-activity relationships (QSARs) of Photobacterium phosphoreum toxicity of nitrobenzene derivatives. *Science of the Total Environment [SCI. TOTAL ENVIRON.].* 142(3): 179-190.

Green, A., Moore, D. and Farrar, D. 1999. Chronic toxicity of 2,4,6-trinitrotoluene to a marine polychaete and an estuarine amphipod. Envi-ronmental Toxicology and Chemistry 8: 1783-1790.

Greenough R.J. and McDonald, P. 1985. HMX: 14 Day Toxicity in Mice by Dietary Administration. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171596.

Griest, W.H., Stewart, A.J., Tyndall, R.L., Caton, J.E., Ho, C.H., Ironside, K.S., Caldwell, W.M. and Tan, E. 1993. Chemical and toxicological testing of composted explosives-contaminated soil. *Environmental Toxicology and Chemistry.* 12(6): 1105-1116.

Groom, C.A., Halasz, A., Paquet, L., Morris, N., Olivier, L., Dubois, C. and Hawari, J. 2002. Accumulation of HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) in Indigenous and agricultural plants grown in HMX-contaminated anti-tank firing-range soil. *Environmental Science & Technology.* 36(1): 112-118.

Haghighi Podeh, M.R., Bhattacharya, S.K. and Qu, M. 1995. Effects of nitrophenols on acetate utilizing methanogenic systems. *Water Research.* 29(2): 391-399.

Haghighi-Podeh, M.R. and Bhattacharya, S.K. 1996. Fate and toxic effects of nitrophenols on anaerobic treatment systems. 18. Biennial Conference of the International Association on Water Quality, (Singapore), 23-28 Jun 1996.

Haigler, B.E. and Spain, J.C. 1991. Biotransformation of nitrobenzene by bacteria containing toluene degradative pathways. *Appl Environ Microbiol.* 57(11): 3156-3162.

Hajjar, N.P., Brower, M.E., Turck, P.A., Kruger, C.L. and Hartley, W.R. 1992. 1,3-Dinitrobenzene (DNB). *IN: Drinking Water Health Advisory: Munitions. Lewis Publishers, Boca Raton, FL.* 1992: 49-86.

Hallas, L.E. and Alexander, M. 1983. Microbial transformation of nitroaromatic compounds in sewage effluent. *Applied and Environmental Microbiology.* 45(4): 1234-1241.

Hankenson, K. and Schaeffer, D. 1991. Microtox Assay of Trinitrotoluene, Diaminonitrotoluene, Dinitromethylaniline Mixtures. *Bulletin of Environmental Contamination and Toxicology BECTA6.* 46(4): 550-553.

Hannink, N., Rosser, S.J., French, C.E., Basran, A., Murray, J.A., Nicklin, S. and Bruce, N.C. 2001. Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. *Nat Biotechnol.* 19(12): 1168-1172.

Harada, N. and Omura, T. 1980. Participation of cytochrome P-450 in the reduction of nitro compounds by rat liver microsomes. *J Biochem (Tokyo).* 87(5): 1539-1554.

Harkins, V.R., Mollhagen, T., Heintz, C. and Rainwater, K. 1999. Aerobic biodegradation of high explosives, phase I - HMX. *Bioremediation Journal.* 4: 285-290.

Harvey, S.D., Fellows, R.J., Cataldo, D.A. and Bean, R.M. 1991. Fate of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants. *Environmental Toxicology and Chemistry.* 10(7): 845-855.

Hawari, J., Beaudet, S., Halasz, A., Thiboutot, S. and Ampleman, G. 2000. Microbial degradation of explosives: biotransformation versus mineralization. *Appl Microbiol Biotechnol.* 54(5): 605-618.

Hawari, J., Halasz, A., Beaudet, S., Paquet, L., Ampleman, G. and Thiboutot, S. 2001. Biotransformation routes of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by municipal anaerobic sludge. *Environ Sci Technol.* 35(1): 70-75.

Hawari, J., Halasz, A., Paquet, L., Zhou, E., Spencer, B., Ampleman, G. and Thiboutot, S. 1998. Characterization of metabolites in the biotransformation of 2,4,6-trinitrotoluene with anaerobic sludge: role of triaminotoluene. *Appl Environ Microbiol.* 64(6): 2200-2206.

Heim, K., Schuphan, I. and Schmidt, B. 1994. Behavior of [^{14}C]-4-nitrophenol and [^{14}C]-3,4-dichloroaniline in lab sediment-water systems. 1. Metabolic fate and partitioning of radioactivity. *Environmental Toxicology and Chemistry.* 13(6): 879-888.

Hempfling, C. 1997. Ultraviolet/oxidation treatment of explosive wastewaters using a commercial process. *Environmental Progress [Environ. Prog.]. no.* 3(164).

Henderson MS. 1985. HMX: Analysis in Plasma Obtained after 90 day Toxicity Studies with Rats and Mice. Inveresk Research International LTD, Edinburgh, Scotland. ADA-171603.

Hitchcock, D.R., McCutcheon, S.C. and Smith, M.C. 2003. Using rotifer population demographic parameters to assess impacts of the degradation products from trinitrotoluene phytoremediation. *Ecotoxicol Environ Saf.* 55(2): 143-151.

Hodson, P.V., Parisella, R., Blunt, B.R., Gray, B. and Kaiser, K.L.E. 1991. *Quantitative structure-activity relationships for chronic toxicity of phenol, p-chlorophenol, 2,4-dichlorophenol, pentachlorophenol, p-nitrophenol and 1,2,4-trichlorobenzene to early life stages of rainbow trout (Oncorhynchus mykiss).* Department of Fisheries and Oceans, Mont-Joli, Que. (Canada). Physical and Chemical Sciences Branch.

Holen, I., Mikalsen, S.O. and Sanner, T. 1990. Effects of dinitrotoluenes on morphological cell transformation and intercellular communication in Syrian hamster embryo cells. J Toxicol Environ Health. 29(1): 89-98.

Hovatter, P.S., Talmage, S.S., Opresko, D.M. and Ross, R.H. 1997. Ecotoxicity of nitroaromatics to aquatic and terrestrial species at Army Superfund sites. The 1996 6th Symposium on Environmental Toxicology and Risk Assessment, Orlando, FL, USA, ASTM.

Howe, G.E., Marking, L.L., Bills, T.D., Boogaard, M.A. and Mayer, F.L., Jr. 1994a. Effects of water temperature on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to developing rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry.* 13(1): 79-84.

Howe, G.E., Marking, L.L., Bills, T.D., Rach, J.J. and Mayer, F.L., Jr. 1994b. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol and 2,4-dinitrophenol to the amphipod Gammarus pseudolimnaeus and rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry.* 13(1): 51-66.

HSDB. 2003. Hazardous Substances Databank. A Database of the National Library of Medicine's TOXNET System. http://toxnet.nlm.nih.gov.

Hwang, P., Chow, T. and Adrian, N.R. 2000. Transformation of trinitrotoluene to triaminotoluene by mixed cultures incubated under methanogenic conditions. *Environmental Toxicology and Chemistry [Environ. Toxicol. Chem.]. no.* 4: 836-841.

ICI Explosives, Inc. 2004. Fact Sheet No.55. Online at: http://www.slv2000.qc.ca/bibliotheque/centre_docum/protection/55_98_a.pdf. Accessed January 21, 2004.

Iida, S., Misaka, H. and Naya, M. 1997. A flow cytometric analysis of cyto-toxic effects of nitrobenzene on rat spermatogenesis. J Toxicol Sci. 22(5): 397-407.

Imoto, S., Kuramoto, M., Iwabuchi, K., Nagai, H. and Shimpo, K. 1986. [Percutaneous chronic toxicity study of 10% nitroglycerin (NT-1 ointment) in rabbits]. *J Toxicol Sci.* 11 Suppl 2: 31-57.

Imoto, S., Nakao, M., Kuramoto, M., Takeuchi, M., Shimpo, K. and Tanabe, T. 1986. [Teratological test of 10% nitroglycerin (NT-1 ointment) in rabbits]. *J Toxicol Sci.* 11 Suppl 2: 59-70.

Ince, N. and Inel, Y. 1989. Volatilization of Organic Chemicals from Water. *Water, Air and Soil Pollution WAPLAC.* 47: 1-2.

Jarvis, A.S., McFarland, V.A. and Honeycutt, M.E. 1998. Assessment of the effectiveness of composting for the reduction of toxicity and mutagenicity of explosive-contaminated soil. *Ecotoxicol Environ Saf.* 39(2): 131-135.

Johnson, L.R., Davenport, R., Balbach, H. and Schaeffer, D.J. 1994. Phototoxicology. 2. Near-ultraviolet light enhancement of Microtox assays of trinitrotoluene and aminodinitrotoluenes. *Ecotoxicol Environ Saf.* 27(1): 23-33.

Johnson, M.S., Ferguson, J.W. and Holladay, S.D. 2000. Immune effects of oral 2,4,6-trinitrotoluene (TNT) exposure to the white-footed mouse, Peromyscus leucopus. *International Journal of Toxicology.* 1: 5-11.

Johnson, M.S., Franke, L.S., Lee, R.B. and Holladay, S.D. 1999. Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: Is the dermal route significant? *Environmental Toxicology and Chemistry.* 5: 873-878.

Juck, D., Driscoll, B.T., Charles, T.C. and Greer, C.W. 2003. Effect of experimental contamination with the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine on soil bacterial communities. *FEMS Microbiology Ecology.* 43(2): 255-262.

Kalafut, T., Wales, M.E., Rastogi, V.K., Naumova, R.P., Zaripova, S.K. and Wild, J.R. 1998. Biotransformation patterns of 2,4,6-trinitrotoluene by aerobic bacteria. *Curr Microbiol.* 36(1): 45-54.

King, S.Y. and Fung, H.L. 1984. Rapid microbial degradation of organic nitrates in rat excreta. Re-examination of the urinary and fecal metabolite profiles of pentaerythritol tetranitrate in the rat. *Drug Metab Dispos.* 12(3): 353-357.

King, S.Y. and Fung, H.L. 1985. Examination of the in vitro degradation of [^{14}C] pentaerythritol tetranitrate in rat and human blood with an improved thin-layer radiochromatographic procedure. *J Chromatogr.* 343(1): 129-137.

Koizumi, M., Yamamoto, Y., Ito, Y., Takano, M., Enami, T., Kamata, E. and Hasegawa, R. 2001. Comparative study of toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats. *J Toxicol Sci.* 26(5): 299-311.

Krumholz, L.R., Li, J., Clarkson, W.W., Wilber, G.G. and Suflita, J.M. 1997. Transformations of TNT and related aminotoluenes in groundwater aquifer slurries under different electron-accepting conditions. *J Ind Microbiol Biotechnol.* 18(2-3): 161-169.

Lachance, B., Robidoux, P.Y., Hawari, J., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 1999. Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. *Mutation Research Genetic Toxicology and Environmental Mutagenesis.* 444(1): 25-39.

Lang, P.Z., Wang, Y., Chen, D.B., Wang, N., Zhao, X.M. and Ding, Y.Z. 1997. Bioconcentration, elimination and metabolism of 2,4-dinitrotoluene in carps(Cyprinus Carpio L.). *Chemosphere.* 35(8): 1799-1815.

Lee, D.S. and Park, S.D. 1996. Decomposition of nitrobenzene in supercritical water. *Journal of Hazardous Materials.* 51(1-3): 67-76.

Leung, K.H., Yao, M., Stearns, R. and Chiu, S.H. 1995. Mechanism of bioactivation and covalent binding of 2,4,6-trinitrotoluene. *Chem Biol Interact.* 97(1): 37-51.

Levin, A.A., Bosakowski, T., Earle, L.L. and Butterworth, B.E. 1988. The reversibility of nitrobenzene-induced testicular toxicity: continuous monitoring of sperm output from vasocystotomized rats. *Toxicology.* 53(2-3): 219-230.

Levine, B.S., Furedi, E.M., Gordon, D.E., Barkley, J.J. and Lish, P.M. 1990. Toxic interactions of the munitions compounds TNT and RDX in F344 rats. *Fundamental and Applied Toxicology.* 15(2): 373-380.

Levine, B.S., Furedi, E.M., Gordon, D.E., Burns, J.M. and Lish, P.M. 1981. Thirteen week toxicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fischer 344 Rats. *Toxicology Letters.* 8(4-5): 241-245.

Levine, B.S., Furedi, E.M., Gordon, D.E., Lish, P.M. and Barkley, J.J. 1984. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. *Toxicology.* 32(3): 253-265.

Levine, B.S., Rust, J.H., Barkley, J.J., Furedi, E.M. and Lish, P.M. 1990. Six month oral toxicity study of trinitrotoluene in beagle dogs. *Toxicology.* 63(2): 233-244.

Linton, T.K., Mayer, F.L., Simon, T.L., Malone, J.A. and Marking, L.L. 1994. Salinity and temperature effects on chronic toxicity of 2,4-dinitrophenol and 4-nitrophenol to sheepshead minnows (Cyprinodon variegatus). *Environmental Toxicology and Chemistry.* 13(1): 85-92.

Lotufo, G.R., Farrar, J.D., Inouye, L.S., Bridges, T.S. and Ringelberg, D.B. 2001. Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic invertebrates. *Environmental toxicology and chemistry.* 20(8): 1762-1771.

Lu, P.Y. and Metcalf, R.L. 1975. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. *Environ Health Perspect.* 10: 269-284.

Lucero, M.E., Mueller, W., Hubstenberger, J., Phillips, G.C. and O'Connell, M.A. 1999. Tolerance to nitrogenous explosives and metabolism of TNT by cell suspensions of Datura innoxia. *In Vitro Cellular & Developmental Biology Plant.* 6: 480-486.

Lynch, J.C., Brannon, J.M., Hatfield, K. and Delfino, J.J. 2003. An exploratory approach to modeling explosive compound persistence and flux using dissolution kinetics. *J Contam Hydrol.* 66(3-4): 147-159.

Maragos, C.M., Andrews, A.W., Keefer, L.K. and Elespuru, R.K. 1993. Mutagenicity of glyceryl trinitrate (nitroglycerin) in Salmonella typhimurium. *Mutat Res.* 298(3): 187-195.

Mc Farlane, C., Pfleeger, T. and Fletcher, J. 1990. Effect, uptake and disposition of nitrobenzene in several terrestrial plants. *Environmental Toxicology and Chemistry.* 9(4): 513-520.

Megharaj, M., Rao, A.S. and Venkateswarlu, K. 1993. Influence of rice straw amendment on persistence and algal toxicity of p-nitrophenol in soil. *Soil Biology and Biochemistry.* 25(9): 1185-1188.

Menzie-Cura & Associates, Inc. 2004. Literature Review of Fate and Effects of Several Military Unique Compounds. Prepared for: U.S. Army Corps of Engineers, Construction Engineering Research Laboratory.

Mitchell, W.R. and Dennis, W. 1982. Biodegradation of 1,3-Dinitrobenzene. *Journal of Environmental Science and Health, Part A.* 17(6): 837-853.

Monteil-Rivera, F., Groom, C. and Hawari, J. 2003. Sorption and degradation of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in soil. *Environ Sci Technol.* 37(17): 3878-3884.

Morgan, K.T., Gross, E.A., Lyght, O. and Bond, J.A. 1985. Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. *Neurotoxicology.* 6(1): 105-116.

Mori, M.A., Sayama, M., Shoji, M., Inoue, M., Kawagoshi, T., Maeda, M. and Honda, T. 1997. Biliary excretion and microfloral transformation of major conjugated metabolites of 2,4-dinitrotoluene and 2,6-dinitrotoluene in the male Wistar rat. *Xenobiotica.* 27(12): 1225-1236.

Mori, M.A., Shoji, M., Dohrin, M., Kawagoshi, T., Honda, T. and Kozuka, H. 1996. Further studies on the urinary metabolites of 2,4-dinitrotoluene and 2,6-dinitrotoluene in the male Wistar rat. *Xenobiotica.* 26(1): 79-88.

Nicklin, S., Binks, P.O., Bruce, N.H. and French, C.D. 1999. Detection and biodegradation of explosives, The Secretary of State for Defence in her Britannic Majesty's Government.

Nielsen, P.H., Bjarnadottir, H., Winter, P.L. and Christensen, T.H. 1995. In situ and laboratory studies on the fate of specific organic compounds in an anaerobic landfill leachate plume, 2. Fate of aromatic and chlorinated aliphatic compounds. *Journal of Contaminant Hydrology.* 20(1-2): 51-66.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L. and Miller, K. 2002. Toxicological and chemical assessment of ordinance compounds in marine sediments and porewaters. *Mar. Pollut. Bull.* 44(8): 789-806.

Nipper, M., Carr, R.S., Biedenbach, J.M., Hooten, R.L., Miller, K. and Saepoff, S. 2001. Development of Marine Toxicity Data for Ordnance Compounds. *Archives of Environmental Contamination and Toxicology.* 41(3): 308-318.

Nystrom, D.D. and Rickert, D.E. 1987. Metabolism and excretion of dinitrobenzenes by male Fischer-344 rats. *Drug Metab. Dispos.* 15(6): 821-825.

Pasti-Grigsby, M.B., Lewis, T.A., Crawford, D.L. and Crawford, R.L. 1996. Transformation of 2,4,6-trinitrotoluene (TNT) by actinomycetes isolated from TNT-contaminated and uncontaminated environments. *Appl Environ Microbiol.* 62(3): 1120-1123.

Pederson, G.L. 1970. *Evaluation of toxicity of selected TNT wastes on fish, phase i-acute toxicity of alpha-TNT to Bluegills.* ARMY ENVIRONMENTAL HYGIENE AGENCY, EDGEWOOD ARSENAL, MD. 1 january 1970-31 october 1970

Pena-Egido, M.J., Marino-Hernandez, E.L., Santos-Buelga, C. and Rivas-Gonzalo, J.C. 1988. Urinary excretion kinetics of p-nitrophenol following oral administration of parathion in the rabbit. *Arch Toxicol.* 62(5): 351-354.

Phipps, G.L., Holcombe, G.W. and Fiandt, J.T. 1981. Acute Toxicity of Phenol and Substituted Phenols to the Fathead Minnow. *Bull Environ Contam and Toxicol* 26(5):585-593, NTIS, Springfield, VA.

Posadas del Rio, F.A., Jaramillo Juarez, F. and Camacho Garcia, R. 1988. Biotransformation of organic nitrate esters in vitro by human liver, kidney, intestine, and blood serum. *Drug Metab Dispos.* 16(3): 477-481.

Price, R.A., Pennington, J.C., Larson, S.L., Neumann, D. and Hayes, C.A. 2002. Uptake of RDX and TNT by agronomic plants. *Journal of Soil Contamination.* 11(3): 307-326.

Qaisi, K.M., Ro, K.S., Constant, W.D. and Smith, M.L. 1996. Soil - water partitioning and mass transfer kinetics of 2,4,6 trinitrotoluene in highly contaminated soil. A31(9): 2079-2085.

Razo-Flores, E., Lettinga, G. and Field, J.A. 1999. Biotransformation and biodegradation of selected nitroaromatics under anaerobic conditions. *Biotechnol Prog.* 15(3): 358-365.

Reddy, G., Chandra, S.A.M., Lish, J.W. and Qualls, C.W., Jr. 2000. Toxicity of 2,4,6-trinitrotoluene (TNT) in hispid cotton rats (Sigmodon hispidus): Hematological, biochemical, and pathological effects. *International Journal of Toxicology.* 19(3): 169-177.

Reddy, T.V., Olson, G.R., Wiechman, B., Reddy, G., Torsella, J., Daniel, F.B. and Leach, G.J. 1999. Toxicity of tetryl (N-methyl-N,2,4,6-tetranitroaniline) in F344 rats. *International Journal of Toxicology [Int. J. Toxicol.].* 18(2): 97-107.

Reddy, G., Reddy, T.V., Choudhury, H., Daniel, F.B. and Leach, G.J. 1997. Assessment of environmental hazards of 1,3,5-trinitrobenzene. Journal of Toxicology and Environmental Health. 52(5): 447-460.

Renoux, A.Y., Sarrazin, M., Hawari, J. and Sunahara, G.I. 2000. Transformation of 2,4,6-trinitrotoluene in soil in the presence of the earthworm Eisenia andrei. *Environmental Toxicology and Chemistry.* 6: 1473-1480.

Rho, D., Hodgson, J., Thiboutot, S., Ampleman, G. and Hawari, J. 2001. Transformation of 2,4,6-trinitrotoluene (TNT) by immobilized Phanerochaete chrysosporium under fed-batch and continuous TNT feeding conditions. *Biotechnol Bioeng.* 73(4): 271-281.

Riefler, R.G. and Smets, B.F. 2000. Enzymatic Reduction of 2,4,6-Trinitrotoluene and Related Nitroarenes: Kinetics Linked to One-Electron Redox Potentials. *Environmental Science & Technology [Environ. Sci. Technol.].* 34(18): 3900-3906.

Ringelberg, D.B., Reynolds, C.M., Walsh, M.E. and Jenkins, T.F. 2003. RDX loss in a surface soil under saturated and well drained conditions. *J Environ Qual.* 32(4): 1244-1249.

Robidoux, P.Y., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S., Hawari, J. and Sunahara, G.I. 2003. Phytotoxicity of 2,4,6-trinitrotoluene (TNT) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in spiked artificial and natural forest soils. *Archives of environmental contamination and toxicology.* 44(2): 198-209.

Robidoux, P.Y., Hawari, J., Bardai, G., Paquet, L., Ampleman, G., Thiboutot, S. and Sunahara, G.I. 2002a. TNT, RDX, and HMX decrease earthworm (Eisenia andrei) life-cycle responses in a spiked natural forest soil. *Archives of environmental contamination and toxicology.* 43(4): 379-388.

Robidoux, P.Y., Svendsen, C., Sarrazin, M., Hawari, J., Thiboutot, S., Ampleman, G., Weeks, J.M. and Sunahara, G.I. 2002b. Evaluation of tissue and cellular biomarkers to assess 2,4,6-trinitrotoluene (TNT) exposure in earthworms: effects-based assessment in laboratory studies using Eisenia andrei. *Biomarkers.* 7(4): 306-321.

Robidoux, P.Y., Hawari, J., Thiboutot, S., Ampleman, G. and Sunahara, G.I. 1999. Acute toxicity of 2,4,6-trinitrotoluene in earthworm (Eisenia andrei). *Ecotoxicology and environmental safety.* 44(3): 311-321.

Robidoux, P.Y., Hawari, J., Thiboutot, S., Ampleman, G. and Sunahara, G.I. 2001. Chronic toxicity of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in soil determined using the earthworm (Eisenia andrei) reproduction test. *Environ Pollut.* 111(2): 283-292.

Robidoux, P.Y., Svendsen, C., Caumartin, J., Hawari, J., Ampleman, G., Thiboutot, S., Weeks, J.M. and Sunahara, G.I. 2000. Chronic toxicity of energetic compounds in soil determined using the earthworm (Eisenia andrei) reproduction test. *Environmental Toxicology and Chemistry.* 7: 1764-1773.

Rosser, S.J., French, C.E. and Bruce, N.C. 2001. Engineering plants for the phytodetoxification of explosives. *In Vitro Cellular & Developmental Biology Plant [In Vitro Cell. Dev. Biol. Plant].* 37(3): 330-333.

Schmitt, H., Altenburger, R., Jastorff, B. and Schuurmann, G. 2000. Quantitative structure-activity analysis of the algae toxicity of nitroaromatic compounds. *Chem Res Toxicol.* 13(6): 441-450.

Selim, H.M., Xue, S.K. and Iskandar, I.K. 1995. Transport of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science.* 160(5): 328-339.

Shafer, W.E. and Schonherr, J. 1985. Accumulation and transport of phenol, 2-nitrophenol, and 4-nitrophenol in plant cuticles. *Ecotoxicol Environ Saf.* 10(2): 239-252.

Shah, M.M. and Campbell, J.A. 1997. Transformation of nitrobenzene by ferredoxin NADP oxidoreductase from spinach leaves. *Biochem Biophys Res Commun.* 241(3): 794-796.

Shah, M.M. and Spain, J.C. 1996. Elimination of nitrite from the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) catalyzed by ferredoxin NADP oxidoreductase from spinach. *Biochem Biophys Res Commun.* 220(3): 563-568.

Sheremata, T.W., Halasz, A., Paquet, L., Thiboutot, S., Ampleman, G. and Hawari, J. 2001. The fate of the cyclic nitramine explosive RDX in natural soil. *Environmental science & technology.* 35(6): 1037-1040.

Siciliano, S.D. and Greer, C.W. 2000. Plant-Bacterial Combinations to Phytoremediate Soil Contaminated with High Concentrations of 2,4,6-Trinitrotoluene. *Journal of Environmental Quality [J. Environ. Qual.]. no.* 1: 311-316.

Simini, M., Wentsel, R.S., Checkai, R.T., Phillips, C.T., Chester, N.A., Major, M.A. and Amos, J.C. 1995. Evaluation of soil toxicity at Joliet Army Ammunition Plant. *Environmental Toxicology and Chemistry.* 14(4): 623-630.

Singh, J., Comfort, S.D., Hundal, L.S. and Shea, P.J. 1998. Long-term RDX sorption and fate in soil. *Journal of Environmental Quality.* 27(3): 572-577.

Smock, L.A., Stoneburner, D.L. and Clark, J.R. 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. *Water Res.* 10(6): 539-543.

Snellinx, Z., Nepovim, A., Taghavi, S., Vangronsveld, J., Vanek, T. and van der Lelie, D. 2002. Biological remediation of explosives and related nitroaromatic compounds. *Environ Sci Pollut Res Int.* 9(1): 48-61.

Stahl, J.D. and Aust, S.D. 1993. Metabolism and detoxification of TNT by Phanerochaete chrysosporium. *Biochem Biophys Res Commun.* 192(2): 477-482.

Steevens, J.A., Duke, B.M., Lotufo, G.R. and Bridges, T.S. 2002. Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to Chironomus tentans and Hyalella azteca: low-dose hormesis and high-dose mortality. *Environmental toxicology and chemistry.* 21(7): 1475-1482.

Talmage, S.S., Opresko, D.M., Maxwell, C.J., Welsh, C.J., Cretella, F.M., Reno, P.H. and Daniel, F.B. 1999. Nitroaromatic munition compounds: environmental effects and screening values. *Reviews of Environmental Contamination and Toxicology.* 161: 1-156.

Tamano, S., Ward, J.M., Diwan, B.A., Keefer, L.K., Weghorst, C.M., Calvert, R.J., Henneman, J.R., Ramljak, D. and Rice, J.M. 1996. Histogenesis and the role of p53 and K-ras mutations in hepatocarcinogenesis by glyceryl trinitrate (nitroglycerin) in male F344 rats. *Carcinogenesis.* 17(11): 2477-2486.

Taniguchi, Y., Shimada, K., Kohyama, H., Hamai, Y., Hirao, R., Nishimori, T., Kikumori, M. and Yamamoto, H. 1986. [Percutaneous subacute toxicity study of 10% nitroglycerin (NT-1 ointment) in rabbits]. *J Toxicol Sci.* 11 Suppl 2: 11-29.

Thompson, P.L., Ramer, L.A. and Schnoor, J.L. 1998. Uptake and transformation of TNT by hybrid poplar trees. *Environmental Science & Technology.* 32(7): 975-980.

Thompson, P.L., Ramer, L.A. and Schnoor, J.L. 1999. Hexahydro-1,3,5-trinitro-1,3,5-triazine translocation in poplar trees. *Environmental Toxicology and Chemistry.* 18(2): 279-284.

Thomulka, K.W. and Lange, J.H. 1997. Mixture toxicity of nitrobenzene and trinitrobenzene using the marine bacterium Vibrio harveyi as the test organism. *Ecotoxicol Environ Saf.* 36(2): 189-195.

Trapido, M., Dello, A., Goi, A. and Munter, R. 2003. Degradation of nitroaromatics with the Fenton reagent. *Proceedings of the Estonian Academy of Sciences, Chemistry [Proc. Eston. Acad. Sci. Chem.].* 52(1): 38-47.

Tucker, W.A., Murphy, G.J. and Arenberg, E.D. 2002. Adsorption of RDX to soil with low organic carbon: Laboratory results, field observations, remedial implications. *Soil and Sediment Contamination.* 11(6).

Tyl, R.W., France, K.A., Fisher, L.C., Dodd, D.E., Pritts, I.M., Lyon, J.P., O'Neal, F.O. and Kimmerle, G. 1987. Development toxicity evaluation of inhaled nitrobenzene in CD rats. *Fundam Appl Toxicol.* 8(4): 482-492.

Uberoi, V. and Bhattacharya, S.K. 1997a. Effects of chlorophenols and nitrophenols on the kinetics of propionate degradation in sulfate-reducing anaerobic systems. *Environmental Science & Technology.* 31(6): 1607-1614.

Uberoi, V. and Bhattacharya, S.K. 1997b. Toxicity and degradability of nitrophenols in anaerobic systems. *Water Environment Research.* 69(2): 146-156.

USACHPPM. 2001. Wildlife Toxicity Assessment for Nitroglycerin (NG). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01F.

USACHPPM. 2001. Wildlife Toxicity Assessment for High Melting Explosive (HMX). US Army Center for Health Promotion and Preventative Medicine. Document No. 39-EJ-1138-01G.

USACHPPM. 2001. Wildlife Toxicity Assessment for Pentaerythritol Tetranitrate (PETN). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01G.

USACHPPM. 2001. Wildlife Toxicity Assessment for 1,3-Dinitrobenzene (1,3-DNB). US Army Center for Health Promotion and Preventative Medicine. Document No. 39-EJ-1138-01A.

USACHPPM. 2001. Wildlife Toxicity Assessment for 1,3,5-Trinitrobenzene (1,3,5-TNB). US Army Center for Health Promotion and Preventative Medicine. Document No. 37-EJ-1138-01B.

Van Aken, B., Hofrichter, M., Scheibner, K., Hatakka, A.I., Naveau, H. and Agathos, S.N. 1999. Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete Phlebia radiata. *Biodegradation.* 10(2): 83-91.

van der Schalie, W.H., Shedd, T.R. and Zeeman, M.G. 1988. Ventilatory and movement responses of Bluegills exposed to 1,3,5-trinitrobenzene. Aquatic Toxicology and Hazard Assessment. Philadelphia PA, American Society for Testing and Materials. 10: 307-315.

Vanderberg, L.A., Perry, J.J. and Unkefer, P.J. 1995. Catabolism of 2,4,6-trinitrotoluene by Mycobacterium vaccae. *Appl Microbiol Biotechnol.* 43(5): 937-945.

Vasilyeva, G.K., Kreslavski, V.D., Oh, B.T. and Shea, P.J. 2001. Potential of activated carbon to decrease 2,4,6-trinitrotoluene toxicity and accelerate soil decontamination. *Environ Toxicol Chem.* 20(5): 965-971.

Williams, R.E., Rathbone, D.A., Moody, P.C., Scrutton, N.S. and Bruce, N.C. 2001. Degradation of explosives by nitrate ester reductases. *Biochemical Society symposium.* 68: 143-153.

Williams, R.T., Ziegenfuss, P.S., Mohrman, G.B. and Sisk, W.E. 1989. Composting of Explosives and Propellant Contaminated Sediments. *IN: Hazardous and Industrial Wastes: Proceedings of the 21st Mid Atlantic Industrial Waste Conference. Technomic Publishing Co., Inc., Lancaster, Pennsylvania.* 1989: 599-611.

Wilson, J.T., Enfield, C.G., Dunlap, W.J., Cosby, R.L., Foster, D.A. and Baskin, L.B. 1981. Transport and Fate of Selected Organic Pollutants in a Sandy Soil. *Journal of Environmental Quality.* 10(4): 501-506.

Yoon, J.M., Oh, B.T., Just, C.L. and Schnoor, J.L. 2002. Uptake and leaching of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by hybrid poplar trees. *Environmental Science & Technology.* 36(21): 4649-4655.

Zhang, C. and Hughes, J.B. 2003. Biodegradation pathways of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Clostridium acetobutylicum cell-free extract. *Chemosphere.* 50(5): 665-671.

Zoh, K.D. and Horne, A.J. 1999. Removal of TNT using plants in constructed wetlands. Wetlands and Remediation: An International Conference, Salt Lake City, UT, Battelle Press, 505 King Ave Columbus OH 43201.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
03-2005	Final	

4. TITLE AND SUBTITLE
Military Munitions-Related Compounds Fate and Effects:
A Literature Review Relative to threatened and Endangered Species

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Katherine von Stackleberg, Craig Amos, and Thomas Smith

5d. PROJECT NUMBER
622720A896

5e. TASK NUMBER
T033

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Engineer Research and Development Center (ERDC)
Construction Engineering Research Laboratory (CERL)
PO Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDC/CERL TR-05-10

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Corps of Engineers
441 G Street, NW
Washington, DC 20314-1000

10. SPONSOR/MONITOR'S ACRONYM(S)
DAIM-ED-N

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

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

14. ABSTRACT

As with many anthropogenic substances, there is a general concern about the potential impacts on ecosystems and their component species resulting from military training and testing exercises using various forms of military munitions, which upon impact release or produce various chemical compounds. The biological species present on military training and testing lands may be exposed to these militarily prevalent chemical compounds during soldier training and testing exercises.

This report provides a review and summarization of the literature on the fate and effects of military munitions compounds (MMCs) in the environment. More than 340 reports and other scientific papers were identified that relate directly to primary military munitions constituents and species of high interest (species of high interest include those species considered to be threatened and endangered, as defined in accordance with the Endangered Species Act).

15. SUBJECT TERMS
military training
literature review
threatened and endangered species
environmental management
munitions waste
hazardous waste minimization

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Thomas Smith
Unclassified	Unclassified	Unclassified	SAR	235	19b. TELEPHONE NUMBER (include area code) (217)373-5898