APPENDIX A
EXPLOSIVE COMPOUNDS DATASET AND ERDC REPORT

APPENDIX B
WILDLIFE TOXICITY INFORMATION

(Provided on CD)

Table A-3. ERDC
Surface Soil Data for Explosive Compounds
Screening Levels for Explosive Compounds
Ecological Risk Assessment for Small Arms Ranges
Habitat Areas, Impact Area
Former Fort Ord, California

Grid #	TNT	2-Am-DNT	4-Am-DNT	2,4-DNT	1,3,5-TNB	RDX	HMX	NG	Tetryl
ORD-01	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-02	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-03	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-04	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-05	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-06	0.03	<d	<d	<d	<d	<d	<d	<d	<d
ORD-07	0.02	<d	<d	<d	<d	<d	<d	<d	<d
ORD-08	0.02	<d	<d	<d	<d	<d	<d	<d	<d
ORD-09	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-010	<d	<d	<d	<d	<d	<d	<d	<d	0.40
ORD-011	0.02	<d	<d	<d	<d	0.04	<d	<d	0.10
ORD-012	<d	<d	<d	<d	<d	<d	<d	<d	0.13
ORD-013	0.13	<d	<d	<d	<d	0.08	<d	<d	<d
ORD-014	<d	<d	<d	<d	<d	<d	<d	<d	0.02
ORD-015	0.02	<d	<d	<d	<d	<d	<d	<d	<d
ORD-016	0.04	0.08	0.09	<d	<d	<d	0.02	<d	<d
ORD-017	0.02	<d	<d	<d	<d	<d	<d	<d	<d
ORD-018	0.07	<d	<d	<d	<d	<d	<d	<d	<d
ORD-019	0.11	0.03	0.03	<d	<d	<d	<d	<d	<d
ORD-020	0.03	0.06	0.07	<d	<d	<d	<d	<d	<d
ORD-021	0.03	<d	<d	<d	<d	<d	<d	<d	<d
ORD-022	2.50	0.11	0.12	<d	0.02	<d	<d	<d	<d
ORD-023	0.03	0.03	<d	<d	<d	<d	<d	<d	0.14
ORD-024	0.04	<d	<d	<d	<d	<d	<d	<d	<d
ORD-025	0.08	<d	<d	<d	<d	<d	<d	<d	<d
ORD-026	0.03	<d	<d	<d	<d	<d	<d	<d	<d
ORD-027	0.02	<d	<d	<d	<d	<d	<d	<d	<d
ORD-028	0.04	<d	<d	<d	<d	<d	<d	<d	0.16
ORD-029	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-030	0.04	<d	<d	<d	<d	<d	<d	<d	<d
ORD-031	0.10	<d	0.04	<d	<d	<d	<d	0.06	<d
ORD-032	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-033	<d	<d	<d	<d	<d	<d	<d	<d	<d

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Grid #	TNT	2-Am-DNT	4-Am-DNT	2,4-DNT	1,3,5-TNB	RDX	HMX	NG	Tetryl
ORD-034	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-035	0.09	<d	0.05	<d	<d	<d	<d	<d	<d
ORD-036	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-039	0.15	0.01	0.05	<d	<d	<d	<d	<d	<d
ORD-040	0.24	<d	0.10	<d	<d	<d	<d	<d	<d
ORD-041	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-042	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-043	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-044	<d	<d	<d	<d	<d	<d	<d	<d	0.04
ORD-045	<d	<d	<d	<d	<d	<d	<d	<d	0.15
ORD-046	0.09	<d	0.03	<d	<d	<d	<d	<d	0.26
ORD-050	<d	<d	<d	<d	<d	<d	<d	<d	0.25
ORD-051	0.02	<d	<d	<d	<d	<d	<d	<d	0.24
ORD-052	<d	<d	<d	<d	<d	<d	<d	<d	0.22
ORD-053	<d	<d	<d	<d	<d	<d	<d	0.03	0.78
ORD-054	<d	<d	<d	<d	<d	<d	<d	<d	1.97
ORD-055	0.04	<d	0.09	<d	<d	<d	<d	<d	1.99
ORD-056	<d	<d	<d	<d	<d	<d	<d	<d	0.25
ORD-058	<d	<d	<d	<d	<d	<d	<d	0.03	0.25
ORD-061	<d	<d	<d	<d	<d	<d	<d	<d	0.25
ORD-062	0.02	<d	0.02	<d	<d	<d	<d	<d	0.04
ORD-063	1.23	0.32	0.09	<d	<d	<d	<d	<d	<d
ORD-064	0.39	0.16	0.15	<d	<d	<d	<d	<d	0.11
ORD-065	<d	<d	<d	<d	<d	<d	<d	<d	0.08
ORD-066	<d	<d	<d	<d	<d	<d	<d	<d	0.03
ORD-067	<d	0.02	<d	<d	<d	<d	<d	<d	0.05
ORD-068	<d	<d	<d	<d	<d	<d	<d	<d	0.07
ORD-069	<d	<d	<d	<d	<d	<d	<d	<d	0.06
ORD-070	0.09	<d	<d	<d	<d	<d	<d	<d	<d
ORD-071	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-072	0.04	<d	0.02	<d	<d	<d	<d	0.048	<d
ORD-073	0.06	0.02	0.04	<d	<d	<d	<d	<d	<d
ORD-074	0.05	0.02	0.03	<d	<d	<d	<d	<d	<d

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Grid #	TNT	2-Am-DNT	4-Am-DNT	2,4-DNT	1,3,5-TNB	RDX	HMX	NG	Tetryl
ORD-075	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-076	0.04	<d	0.03	<d	<d	<d	<d	<d	<d
ORD-077	145	0.94	1.21	0.07	0.14	<d	<d	<d	<d
ORD-078	0.95	0.02	0.02	<d	<d	<d	<d	<d	0.02
ORD-079	0.02	<d	<d	<d	<d	<d	<d	<d	<d
ORD-080	<d	0.02	<d	<d	<d	<d	<d	<d	<d
ORD-081	0.06	0.15	0.13	<d	<d	<d	<d	<d	<d
ORD-082	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-084	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-085	<d	0.02	<d	<d	<d	<d	<d	<d	<d
ORD-086	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-088	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-089	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-091	0.06	<d	<d	<d	<d	<d	<d	<d	<d
ORD-092	0.05	0.02	0.03	<d	<d	<d	<d	<d	<d
ORD-093	7.26	0.23	0.21	<d	<d	<d	<d	<d	<d
ORD-094	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-095	0.03	<d	<d	<d	<d	<d	<d	<d	<d
ORD-096	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-098	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-099	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-100	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-101	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-102	<d	<d	<d	<d	<d	<d	<d	<d	<d
ORD-103	<d	<d	<d	<d	<d	<d	<d	<d	<d

ERDC U.S. Army Engineer Development and Research Center.

Checked: MP

Approved: EJM

Note: all are surface soil samples reported in milligrams per kilogram (mg/kg).
Replaced old data taken from the same locations for data evaluation.

ERDC TR-05-10

Engineer Research and Development Center

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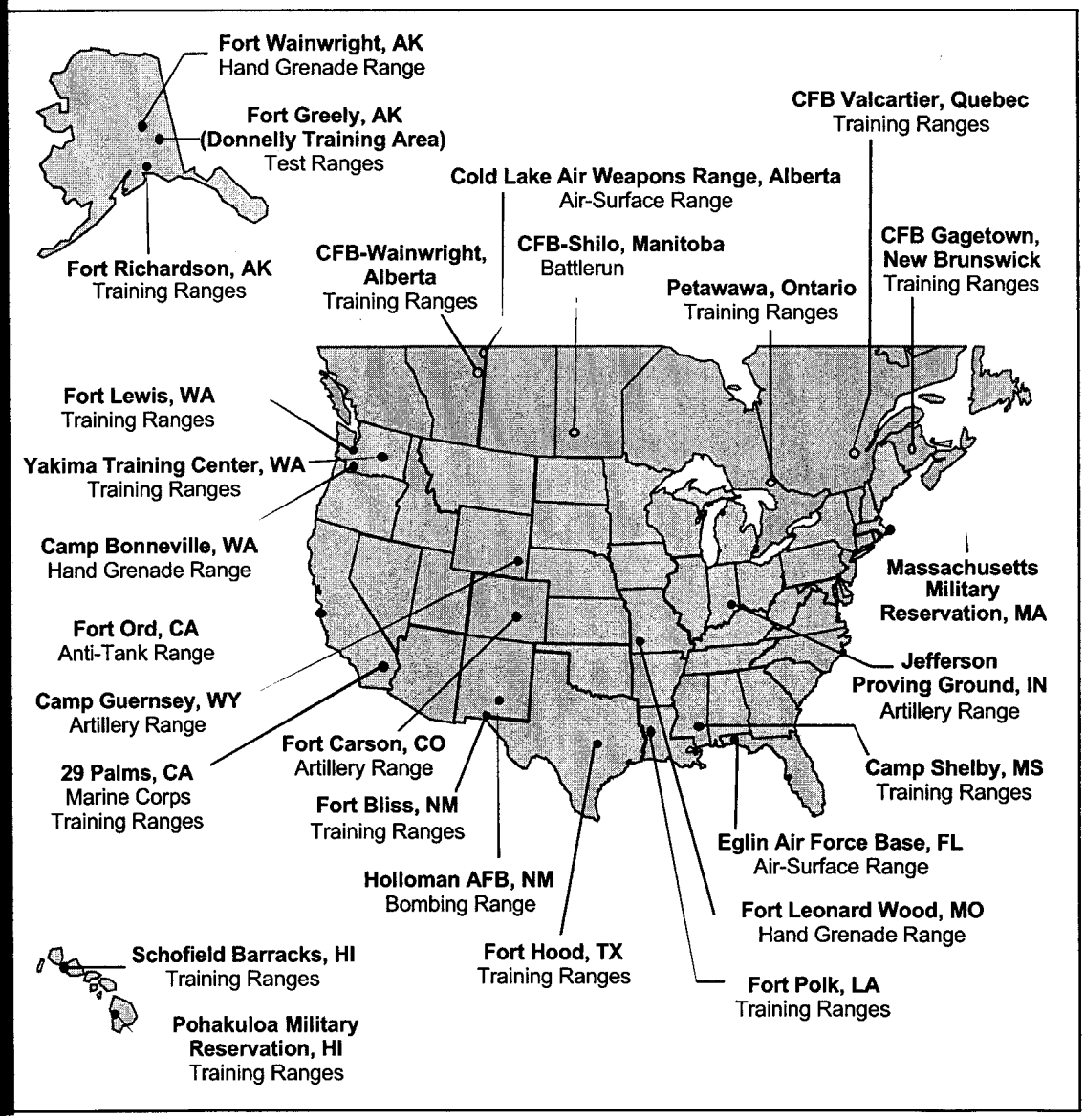
Identity and Distribution of Residues of Energetic Compounds at Military Live-Fire Training Ranges

Thomas F. Jenkins, Sonia Thiboutot, Guy Ampleman, Alan D. Hewitt, Marianne E. Walsh, Thomas A. Ranney, Charles A. Ramsey, Clarence L. Grant, Charles M. Collins, Sylvie Brochu, Susan R. Bigl, and Judith C. Pennington

November 2005



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Prepared for OFFICE OF THE CHIEF OF ENGINEERS and
STRATEGIC ENVIRONMENTAL RESEARCH AND DEVELOPMENT PROGRAM

ABSTRACT

Environmental stewardship of military training ranges is an important objective of the Department of Defense. Therefore, an understanding of the explosives residues resulting from military training with various weapon systems is critical to range managers. A series of field sampling experiments was conducted at 27 military firing ranges in the United States and Canada to provide information on the identity and distribution of energetic munitions constituents. Different types of ranges were studied, including hand grenade, antitank rocket, artillery, bombing, and demolition ranges. Both firing points and impact areas were studied. Energetic compounds (explosives and propellants) were determined and linked to the type of munition used and the major mechanisms of deposition. At impact areas, the largest deposition of residues of energetic compounds is due to low-order detonations, or, in some cases, munitions that split open upon impact. The major residue deposited and its distribution varies for different types of ranges based upon the composition of the high explosive present in the warheads of the rounds fired at that type of range. For antitank range impact areas, the major residue present is HMX from the octol explosive used in the M72 66-mm LAW rockets. At artillery range impact areas, the major residues are TNT and/or RDX from the military-grade TNT and Composition B used in warheads of artillery and mortar rounds. Residues are very heterogeneously distributed at artillery range impact areas and can be described as randomly distributed point sources. RDX and TNT are the major residues at hand grenade ranges and their distribution is less heterogeneous due to the large number of individual detonations in a smaller area that further disperses the residues over the surface and at shallow depths. TNT is the major energetic compound detected at bombing ranges due to its presence in tritonal, the most common explosive used in bombs. RDX is the most common energetic compound at demolition ranges due to its presence as the major component of C4 demolition explosive. NG and 2,4-DNT are also frequently detected at demolition ranges as a result of the disposal of excess propellant. Once dissolved, RDX and HMX are the most mobile of the organic energetic compounds deposited on ranges, both vertically in the soil profile and horizontally across the surface.

Results of these studies demonstrate that the potential for range contamination is specific to range activities. Large areas of training ranges are uncontaminated, while residues in smaller areas, e.g., those around targets, firing points, and low-order detonations, are potentially significant. Range managers can, therefore, limit management practices for residue control to specific areas and specific types of firing activities.

CONTENTS

ILLUSTRATIONS

TABLES

PREFACE

This report was prepared by Dr. Thomas F. Jenkins, Alan D. Hewitt, Marianne E. Walsh, and Charles M. Collins, Environmental Sciences Branch, U.S. Army Engineer Research and Development Center (ERDC), Cold Regions Research and Engineering Laboratory (CRREL), Hanover, New Hampshire; Dr. Sonia Thiboutot, Dr. Guy Ampleman, and Dr. Sylvie Brochu, Defence R&D Canada-Valcartier, Val-Belair, Quebec, Canada; Thomas A. Ranney, Science and Technology Corporation, Hanover, New Hampshire; Charles A. Ramsey, EnviroStat, Inc., Fort Collins, Colorado; Dr. Clarence L. Grant, Professor Emeritus, University of New Hampshire, Durham, New Hampshire; Susan R. Bigl, Geophysical Sciences Branch, ERDC-CRREL; and Dr. Judith C. Pennington, ERDC Environmental Laboratory (EL), Vicksburg, Mississippi.

Financial support for this work was provided by the Strategic Environmental Research and Development Program (SERDP), Mr. Bradley Smith, Executive Director, Dr. Jeffrey Marqusee, Technical Director, and Dr. Robert Holst and Dr. Andrea Leeson, project monitors. Dr. Judith C. Pennington, ERDC-EL, was the Principal Investigator for SERDP Project ER1155 (CP1155). Additional funding was received from U.S. Army Corps of Engineers program "Characterization, Evaluation, and Remediation of Distributed Source Compounds (UXO-C) on Army Ranges." The Technical Director and Program Manager for this work is Dr. John M. Cullinane, ERDC-EL. Funding also was obtained from Director Land Environment and Director General Environment of the Department of National Defence (Canada).

The authors acknowledge John Buck, U.S. Army Environmental Center (USAEC), Aberdeen Proving Ground (APG), Maryland, and Barrett Borrey, U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM), APG, for allowing the authors to join them at Fort Bliss, Jefferson Proving Ground, Fort Polk, Fort Hood, and Fort Carson during their Regional Range Studies at these installations. The USAEC/CHPPM team provided logistics and EOD support and developed the safety plan that allowed for the conduct of research. This study could not have been conducted without their support and collaboration. Charles M. Collins, ERDC-CRREL, is also acknowledged for coordinating and participating in the field sampling at the Donnelly Training Area.

This report was reviewed by Martin H. Stutz, USAEC, and Louise V. Parker, ERDC-CRREL.

Finally, the authors acknowledge the many explosives ordnance technicians in both the United States and Canada who kept the authors safe while conducting field-sampling activities in areas with unexploded ordnance, as well as the soil

sampling teams from ERDC-CRREL: Dennis Lambert, Kevin Bjella, and Jeff Stark; from ERDC-EL, Dr. Jeffery Davis and Tommy Berry, Jr.; from DRDC, Annie Gagnon and Andre Marois; and from CHPPM, Ken Mioduski. Ms. Nancy Perron (CRREL), Ms. Susan Bigl, Mr. Lambert, Mr. Stark, and Mr. Bjella are acknowledged for their assistance in laboratory analysis of soil samples from these ranges, and Ms. Bigl is acknowledged for her help in preparing this report.

This report was prepared under the general supervision of CRREL's Environmental Sciences Branch Chief; CRREL's Geophysical Sciences Branch Chief; Dr. Lance D. Hansen, Deputy Director, CRREL; and James L. Wuebben, Acting Director, CRREL.

The Commander and Executive Director of the Engineer Research and Development Center is Colonel James R. Rowan, EN. The Director is Dr. James R. Houston.

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1 INTRODUCTION

Over the past few years a series of field experiments has been conducted at 27 military installations in the United States and Canada (Fig. 1). The objectives of these studies have been to identify the types of energetic residues present in the surface soils at various types of military live-fire training ranges and to estimate concentrations and distributions of these residues. The concern is that these surface residues could serve as sources for off-site migration of various compounds in groundwater or surface water. Until now most of the results from these studies have been available only in U.S. and Canadian government reports for individual (occasionally several) installations. It is the objective of this report to summarize and synthesize the huge body of knowledge that has been gained from these studies. Also, research to develop approaches to remediate ranges is underway, often with an incomplete understanding of the nature of the problems to be addressed.

For the purposes of this discussion we define energetic compounds as those chemicals used in military explosives and propellants. These include 2,4,6-trinitrotoluene (TNT), 1,3,5-hexahydro-1,3,5-trinitrotriazine (RDX), and 1,3,5,7-tetrahydro-1,3,5,7-tetranitrotetrazocine (HMX), which are used as high explosives, and nitrocellulose (NC), 2,4-dinitrotoluene (DNT), nitroglycerin (NG), and nitroguanidine (NQ), which are used in gun and rocket propellants. Residues of these compounds are deposited onto surface soils, generally as particles (high explosives and propellants) or fibers and slivers (propellants) as troops conduct live-fire training.

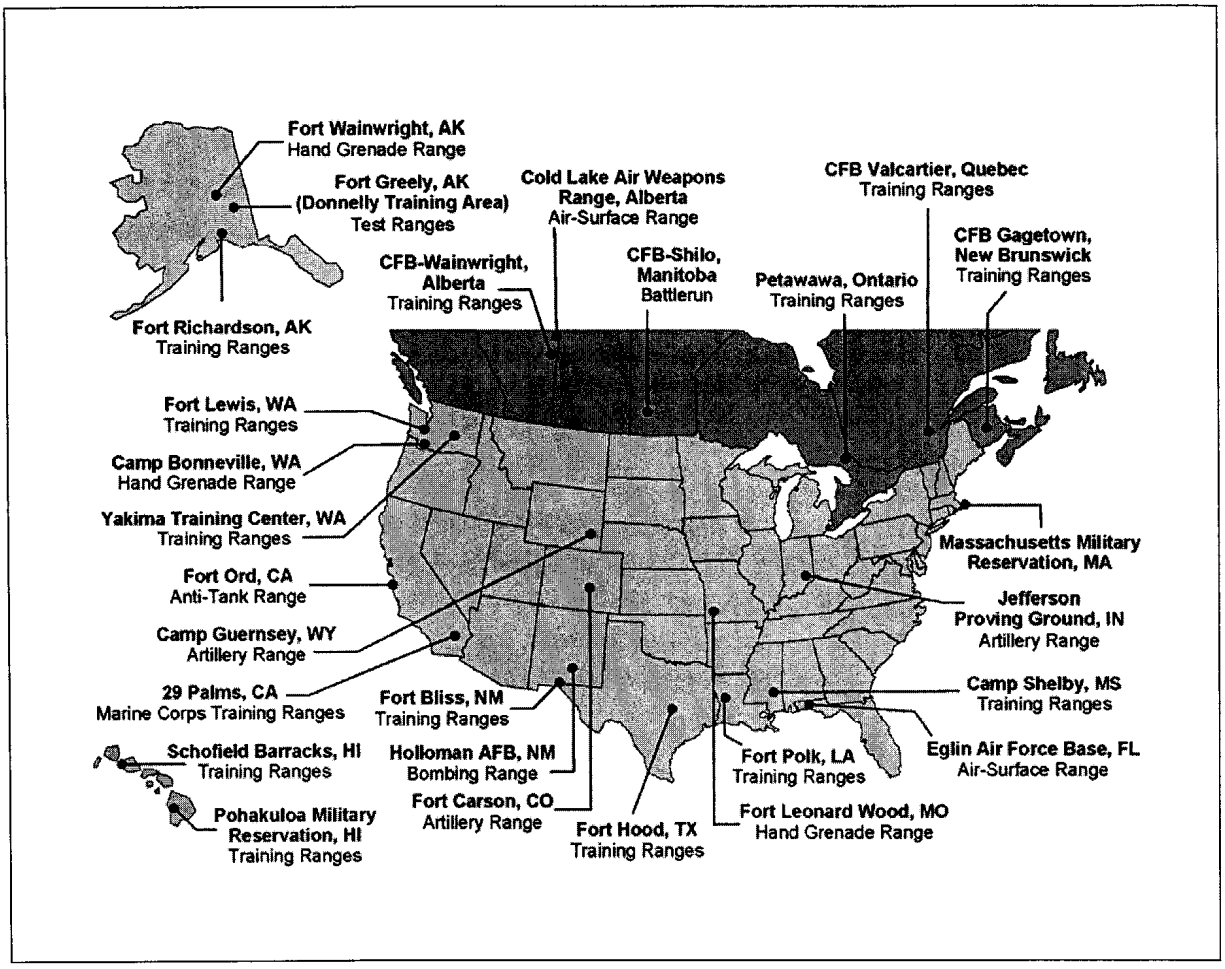


Figure 1. U.S. and Canadian installations where field experiments were conducted.

We have studied a number of different types of live-fire and demolition ranges at U.S. and Canadian military bases. These include hand grenade, rifle grenade, antitank rocket, demolition, tank firing, mortar, artillery, C-130 gunship, and bombing ranges. Training at these ranges is conducted with different types of munitions that contain a variety of energetic formulations. At many ranges, there is an area where the weapon is fired and a separate impact area where detonations occur. Generally, energetic residues at the firing points are composed of compounds used in propellant formulations, whereas residues at the impact areas are compounds used as high explosives in the munition warheads, or white phosphorus (WP) from smoke rounds.

2 METHODS

Soil sampling

Soil sampling methods for the various types of ranges have evolved as our understanding of the nature of the deposition and distribution of energetic compounds has improved. Generally, stainless steel scoops were used to sample non-cohesive soils such as sands and gravels, and specially designed corers were used in more cohesive soils such as silts and clays, and where vegetation is present (M.R. Walsh 2004). Because of the presence of unexploded ordnance (UXO) at many of these ranges, soil sampling often was limited to surface and near-surface depths. Because deposition of residues occurs as particles and fibers at the surface, this was not considered a serious limitation; furthermore, soil-profiling data indicate that the major residue concentrations are nearly always in the top few centimeters of soil.

When soil sampling was conducted to estimate the mean concentration of a compound for a given area, multi-increment composite samples were found to be essential for obtaining representative samples. This was necessitated by the high degree of spatial heterogeneity found for residues of all types of energetic compounds (M.E. Walsh et al. 1997, 2004; Jenkins et al. 1999, 2004a,b, 2005; Hewitt et al. 2005) and the excessive cost associated with analyzing very large numbers of discrete samples. The numbers of increments or mass in the sample needed to provide a reliable estimate of the mean concentration for various ranges differs depending on the nature of the residue deposition (Jenkins et al. 2004a,b, 2005; Hewitt et al. 2005; M.E. Walsh et al. 2005) and the size of the area being characterized. Generally 30 to 50 increments were found to be adequate for 10-m × 10-m (100-m^2) areas, and 50 to 100 increments were adequate for 100-m × 100-m (10,000-m^2) areas. Discrete samples were used to characterize residues near ruptured items and in areas where solid explosives were observed on the surface, or when doing near-surface depth profiling near high-concentration sources.

Sample processing and subsampling

As with sample collection, various methods of sample processing and subsampling were used during these studies as we gained more knowledge of the nature of these residue-containing soils. Because of the particulate nature of the residues, compositional heterogeneity can be a significant component of overall uncertainty (Pitard 1993). Compositional heterogeneity has been defined by Pitard (1993) as the heterogeneity that is inherent to the composition of each particle making up the sample. As a result, the sample processing methodology specified in SW846 Method 8330 (EPA 1994) was found to be inadequate in

several respects for quantitative analysis of energetic compounds in soils from training ranges. Two major changes proved necessary. The first change was to increase the sieve size used during sample processing from #30 (0.595 mm) to #10 (2 mm). For example, we found that in soils from the Fort Lewis hand grenade range, about half of the RDX mass and more than half of the TNT mass was in the size fraction greater than 0.595 mm and less than 2 mm (Table 1). Hewitt et al. (2004) and M.E. Walsh et al. (2005) reported similar findings for soils containing propellant residues. Thus, in our most recent work, we used 2-mm (10-mesh) sieves to separate oversized material from the air-dried soil (Jenkins et al. 2004a, b).

Secondly, Walsh et al. (2002) demonstrated that mechanical grinding prior to subsampling was effective at significantly reducing the subsampling relative standard deviation, sometimes by as much as two orders of magnitude. After sieving, we grind soils from impact areas for 60 sec on a Lab TechEssa LM2 (LabTech Essa Pty. Ltd., Bassendean, WA, Australia) puck mill grinder. For soils from firing points where the residues are often present as fibers, it is necessary to grind for five minutes in one-minute increments, allowing a short cooling period between grinds (M.E. Walsh et al. 2005). After grinding, samples are mixed thoroughly and spread to form a 1-cm-thick layer, and subsamples are obtained by collecting at least 30 increments randomly from the ground material to obtain a subsample mass of about 10 g (Jenkins et al. 2005).

Sample analysis

The 10-g portions of soil are extracted with 20 mL of acetonitrile using either an ultrasonic bath or shaker table for 18 hours. The extracts are then analyzed using either reversed-phase high-pressure liquid chromatography (RP-HPLC) Method 8330 (EPA 1994) or gas chromatography with electron capture detector (GC-ECD) Method 8095 (EPA 1999). Many samples were analyzed using both methods to provide increased confidence in the identity of detected analytes and to provide analytical results for various energetic compounds that can differ in concentration by several orders of magnitude within the same sample.

A few samples were analyzed by other methods such as GC/MS to identify the presence of other organics, but the main objective of this work was to determine concentrations of the energetic compounds and their major environmental transformation products. Thus, the suite of target analytes included the major nitroaromatic and nitramine high explosives used by the Army (TNT, RDX, HMX, tetryl, pentaerythritol tetranitrate [PETN], the major monomeric propellant-related compounds (NG, 2,4-DNT, 2,6-DNT), and the major environmental transformation products that are known to form in aerobic surface and

near-surface soils (1,3,5-trinitrobenzene [TNB], 2-amino-4,6-dinitrotolene [2ADNT], and 4-amino-2,6-dinitrotoluene [4ADNT]). The mono nitro compounds (nitrobenzene [NB], 2-nitrotoluene [2NT], 3-nitrotoluene [3NT], and 4-nitrotoluene [4NT]) are target analytes of Method 8330 and Method 8095 and would have been detected if present, but none were detected in soils from these ranges. In samples containing percent levels of TNT, unsymmetrical isomers of trinitrotoluene and other isomers of DNT are detectable, but no attempt was made to quantify these trace manufacturing impurities. In a recent study by Clausen et al. (2004), in which more than 15,000 soil samples from the Massachusetts Military Reservation (MMR) were analyzed, these target analytes constituted the major detectable organic compounds.

Table 1. Comparison of energetic residues in various particle size ranges for soil samples from the Fort Lewis, Washington, hand grenade range.

Sample	TNT concentration (mg/kg)			RDX concentration (mg/kg)		
	>2 mm	<2 to >0.595 mm	<0.595 mm	>2 mm	<2 to >0.595 mm	<0.595 mm
1	0.19	1.36	0.81	0.02	0.05	0.13
2	0.21	21.0	2.71	0.02	6.36	1.11
3	0.36	3.28	0.55	0.02	0.71	0.29
4	0.18	0.42	2.41	0.01	0.71	0.29
5	0.30	5.72	1.65	0.02	0.04	0.35
6	0.03	16.0	0.04	0.03	0.07	0.38
7	0.11	3.25	0.34	0.03	6.73	1.86
8	0.10	0.05	0.08	0.02	0.05	0.15
9	0.29	3.08	0.06	0.03	6.62	0.68
10	0.05	0.05	0.03	0.02	0.07	0.13
	Mass of TNT (mg)*			Mass of RDX (mg)*		
Sample	>2 mm	<2 to >0.595 mm	<0.595 mm	>2 mm	<2 to >0.595 mm	<0.595 mm
1	0.05	0.31	0.65	0.01	0.01	0.01
2	0.05	5.11	1.94	0.01	1.54	0.79
3	0.07	0.70	0.39	0.004	0.15	0.20
4	0.04	0.10	1.53	0.003	0.01	0.18
5	0.05	1.23	1.19	0.004	0.01	0.26
6	0.01	4.03	0.03	0.01	0.02	0.27
7	0.03	0.98	0.04	0.01	2.03	2.20
8	0.03	0.01	0.078	0.005	0.01	0.13
9	0.06	0.77	0.06	0.01	1.65	0.59
10	0.02	0.02	0.03	0.01	0.03	0.13

* Calculated from the concentrations of TNT and RDX times the mass of soil in the various particle size ranges.

3 RESULTS AND DISCUSSION

The types of ranges studied for each installation where field experiments were conducted are discussed in the following sections and are organized by range type. This was done because different munitions containing different energetic compounds are used at the various types of ranges, and the nature of the deposition and the resulting distribution patterns differ as well.

Hand grenade ranges

Hand grenade ranges are generally only a few hectares in size and, because of the large number of individual detonations in a small area, are poorly vegetated. These ranges often have several training bays from which soldiers throw grenades. Most of the detonation craters lie at distances between 15 and 35 m from the throwing pits. The surfaces of these ranges vary from gravels and sands to clays depending on the location. The management practices used at the various installations also vary significantly. At some ranges craters are filled in and the surface is leveled frequently; at others, the craters are left intact.

The majority of training at hand grenade ranges in the United States is with M67 fragmentation grenades, in which the explosive charge is 185 g of Composition B. In Canada, training is generally with C-13 fragmentation grenades that have the same specifications as the M67. Composition B is 60% military-grade RDX, 39% military-grade TNT, and 1% wax. Military-grade RDX contains about 90% RDX and 10% HMX. Military-grade TNT contains about 99% 2,4,6-TNT and a few tenths of a percent of other isomers of TNT and DNT (Leggett et al. 1977).

Because discrete samples in close proximity from these ranges varied by several orders of magnitude (Jenkins et al. 2001), we conducted two types of studies to improve reproducibility. First we studied the use of multi-increment samples as a means of overcoming the contribution of distributional heterogeneity. Distributional heterogeneity has been defined by Pitard (1993) as the heterogeneity that is inherent in the manner in which the particles are scattered. We conducted a study at the Fort Wainwright hand grenade range where we collected five discrete samples and five sets of replicate multi-increment samples of 5, 10, 20, and 40 increments each within a 10-m × 10-m area. The results for RDX, HMX, TNT, and TNB are presented in Table 2.

RDX concentrations for the five discrete RDX values ranged from 0.78 to 24 mg/kg whereas concentration for the 5, 10, 20, and 40 multi-increment samples ranged from 6.0 to 14, 10 to 28, 7.1 to 14, and 6.5 to 13 mg/kg, respectively. This

reduction in the range of values as the number of increments increased was observed for the three other analytes as well. Subsequent sampling at hand grenade ranges utilized multi-increment samples with increments ranging from 20 to 100, and this approach resulted in a great improvement in reproducibility of replicate samples compared with characterization using discrete samples. At CFB-Petawawa, triplicate replicate 100-increment samples of the entire range resulted in mean concentrations of RDX, HMX, and TNT of 0.63 ± 0.25, 0.22 ± 0.07, and 0.14 ± 0.08 mg/kg, respectively. For purposes of estimating the mass of residues present at hand grenade ranges, these multi-increment sample estimates should be adequate in most cases.

Table 2. Concentrations of energetic residues for discrete and multi-increment surface soil samples at Fort Wainwright, Alaska, hand grenade range.

Sample type	N	Concentration range (mg/kg)			
		HMX	RDX	TNT	TNB
Discrete	5	0.38–3.5	0.78–24	0.02–3.7	0.02–0.41
5-increment	5	1.3–2.1	6.0–14	0.80–1.8	0.09–0.21
10-increment	5	2.0–4.5	11–28	0.68–2.7	0.14–0.29
20-increment	5	1.7–2.3	7.1–14	0.54–2.5	0.10–0.19
40-increment	5	1.5–2.1	6.5–13	0.35–1.9	0.09–0.18

A total of eleven active and two closed hand grenade ranges was sampled (Table 3). The old Castle range at CFB-Gagetown was active when it was first sampled in 1998 (Dube et al. 1999), but was inactive when sampled in 2002 and 2003 (Thiboutot et al. 2003). The target analytes detected at these ranges include RDX, TNT, HMX, TNB, 2ADNT, and 4ADNT. Of the analytes found, RDX usually is present at the highest concentration, with mean surface concentrations ranging from <0.01 to 51 mg/kg.

Table 3. Summary of results for energetic compounds detected in surface soils at hand grenade ranges.

Installation	Year sampled	Samples analyzed	Mean concentration (mg/kg)					
			HMX	RDX	TNT	TNB	4ADNT	2ADNT
Fort Lewis, WA[1,3]	2000	23*	1.8	7.5	9.3	0.05	0.15	0.13
	2001	5† (50)	1.0	4.4	1.5	ND**	ND	ND
Fort Richardson, AK[1,3]	2000	27*	0.02	0.08	0.03	ND	0.01	0.01
Camp Bonneville, WA[2]	2000	48*	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fort Leonard Wood, MO[1]	2001	18† (30)	0.19	0.45	<0.01	<0.01	<0.01	<0.01
CFB-Shilo, Manitoba[1,4]	2001	15† (20)	0.05	0.71	0.06	<0.01	0.02	0.02
Fort Wainwright, AK[1]	2002	25† (1, 5, 10, 20, 40)	2	11	1.2	0.15	ND	ND
Schofield Barracks, HI[1]	2002	3† (30)	9.1	51	36	0.28	0.40	0.03
Pohakuloa Training Center, HI[1]	2002	7† (30)	0.53	5.6	0.78	<0.01	<0.01	<0.01
CFB Gagetown, New Brunswick	2002	5† (30)	0.02	0.12	0.12	<0.01	<0.01	<0.01
Old Castle Range[2,5]	2002	5† (30)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
New Castle Range[1,6] / New Castle Range[1,7]	2003	15† (25)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fort Polk, LA[1]	2003	2† (30)	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
CFB-Petawawa, Ontario[1]	2004	9† (25, 100)	0.18	0.65	0.16	<0.01	<0.01	<0.01

* Discrete samples

† Multi-increment samples with (n) increments per sample

** Not determined

[1] Active ranges

[2] Closed ranges

[3] Jenkins et al. 2001

[4] Ampleman et al. 2003b

[5] Dube et al. 1999

[6] Thiboutot et al. 2003

[7] Thiboutot et al. 2004

The hand grenade ranges appear to fall into two groups; one group of six ranges had concentrations of RDX less than 0.12 mg/kg and the other group of seven ranges had concentrations between 0.45 and 51 mg/kg (Table 3). Studies conducted by Hewitt et al. (2003) have estimated that about 25 μg of RDX and less than 1 μg of TNT are deposited on the soil surface when a hand grenade detonates as designed.

The relatively high concentrations of RDX, HMX, and TNT in the surface soils at Fort Lewis, Fort Wainwright, Schofield Barracks, and Pohakuloa (and probably those at Fort Leonard Wood, Canadian Force Base [CFB]-Shilo and CFB-Petawawa as well) cannot be explained by fragmentation grenades that detonated as designed. At all of these locations we found partially detonated carcasses of M67 (or C13) grenades (Fig. 2). In several instances, chunks of the high-explosive fill were observed next to these carcasses and the inside surfaces of these grenades were coated with high explosive. We are not certain whether these partial detonations occurred when the rounds were thrown or occurred when duds (grenades that did not detonate due to malfunction) were blown in place by explosives ordnance disposal (EOD) technicians using C4 explosive (91% RDX). In either case, we believe the high concentrations of residues observed at these seven ranges were due to these partial detonation events.

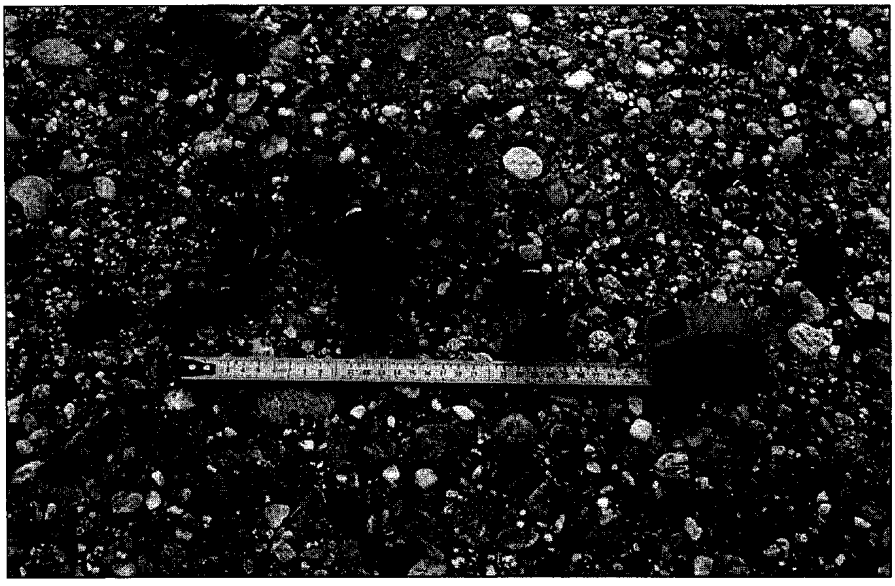
Once a partial detonation takes place, the multitude of normal high order detonations tends to disperse these residues across the range as usage continues. These partial detonations must be rare because about half of the ranges we studied had mean surface concentrations of less than 0.12 mg/kg; residue concentrations in this concentration range could have originated from the thousands of high-order detonations that occur annually at these ranges.

It is interesting that the mean concentration of RDX at the old Castle range at CFB-Gagetown was 5.6 mg/kg when it was sampled as an active range in 1998 (Dube et al. 1999), but the mean concentration was only 0.12 mg/kg after it had been closed and was resampled in 2002 (Thiboutot et al. 2003).

In most cases the highest concentrations of energetic compounds reside in the top few centimeters of soil. For example, at Fort Lewis where the surface is left undisturbed, 16 discrete sample pairs of surface soil and soil from a 10-cm depth were collected at locations ranging from 15 to 25 m from the throwing pit in July 2001 (Jenkins et al. 2001). The mean concentrations were 10.8 and 12.5 times greater in surface soils than at the 10-cm depth for RDX and HMX, respectively, and about 49 times greater for TNT in the surface relative to the 10-cm depth (Table 4). Depending on the management practices for a given range, however, residues can be deeper in the soil profile. For example at Fort Leonard Wood, the surface of the range is disked periodically and the concentrations of RDX, TNT,

and HMX were similar from the surface to a depth of 11 cm (Table 4). Soil samples were not collected at greater depths at these sites because of the fear of encountering live, undetonated hand grenades that had become buried by subsequent detonations.

a. Low-order hand grenade carcass.



b. Pieces from a low-order grenade.

Figure 2. Partially detonated grenade carcass and pieces from a low-order grenade found at Fort Lewis hand grenade range.

Table 4. Concentrations of energetic residues at various depths at the Fort Lewis and Fort Leonard Wood hand grenade ranges.

Soil depth (cm)	Mean concentration (mg/kg)					
	HMX	RDX	TNT	TNB	4ADNT	2ADNT
Fort Lewis, Washington						
0–1.5	1.8	7.5	9.3	0.05	0.15	0.13
10.0–12.5	0.14	0.69	0.19	0.02	0.06	0.08
Fort Leonard Wood, Missouri						
0–1.5	0.52	0.31	<0.01	0.02	<0.01	<0.01
1.5–3.0	0.83	0.81	<0.01	0.04	<0.01	<0.01
3.0–5.0	1.0	0.42	<0.01	0.03	<0.01	<0.01
5.0–7.0	0.54	0.57	<0.01	0.02	<0.01	<0.01
7.0–11.0	0.29	0.36	0.05	0.02	<0.01	<0.01

Antitank rocket range impact areas

Antitank rocket ranges are several hundred hectares in size and covered with low growing vegetation due to the necessity of maintaining a line-of-sight for training. Targets are usually derelict armored vehicles that are placed downrange at distances of 100 meters or more from the firing points. The weapon most often fired at these ranges is the 66-mm M72 light anti-armor weapon (LAW). This item (Fig. 3) contains M7 double-base propellant and the warhead contains 0.3 kg of the melt-cast explosive octol with either a tetryl or RDX booster, depending on the date of manufacture. M7 propellant for the LAW rocket contains 54.6% NC, 35.5% NG, 7.8% potassium perchlorate, 0.9% ethyl centralite, and 1.2% carbon black. Octol is composed of 70% HMX and 30% TNT.

At some ranges practice rounds are fired that contain propellant but do not contain octol. Field experiments were conducted at one closed and seven active antitank rocket ranges, including Fort Ord, California; CFB-Valcartier, Quebec; Yakima Training Center, Washington; Western Area Training Center (WATC)-Wainwright, Alberta; Fort Bliss, New Mexico; CFB-Gagetown, New Brunswick; Pohakuloa Training Center, Hawaii; and CFB-Petawawa, Ontario (Table 5).

The primary residue detected at antitank rocket range impact areas is HMX with concentrations in surface soils adjacent to targets generally in the hundreds of mg/kg (Table 5). TNT, RDX, 4ADNT, and 2ADNT are often detectable as well, but the concentrations are at least several orders of magnitude lower. HMX concentrations decline as the distance from the target increases (Fig. 4).

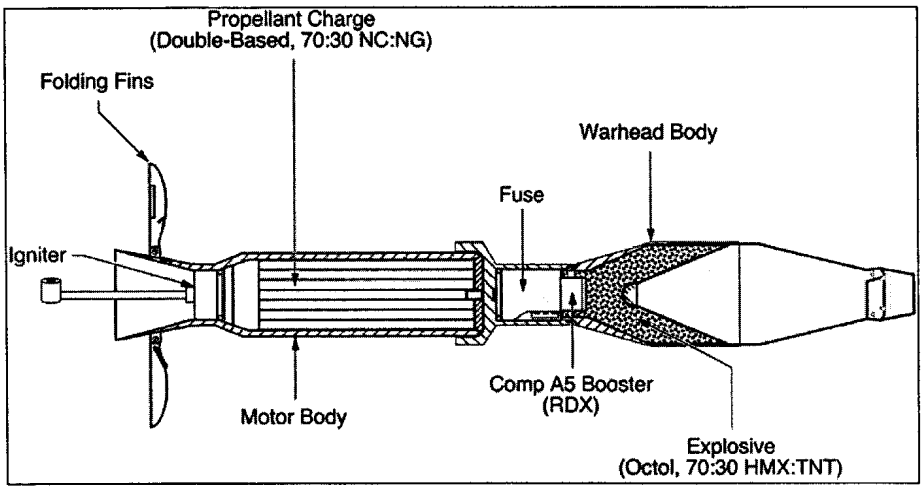


Figure 3. Diagram of 66-mm M72 LAW rocket.

Observations from site inspections indicate that LAW rockets frequently rupture upon impact without detonating, thereby depositing crystalline explosive over the soil surface. This deposition is thought to be the major source of explosives residues at the impact areas of these ranges. For example, soil samples were collected next to a ruptured M72 rocket at 0- to 0.5-cm, 2- to 6-cm, and 6- to 10-cm depths at Yakima Training Center, Washington. The concentration of HMX, TNT, and RDX declined from 10400, 358, and 46 mg/kg at the 0- to 0.5-cm depth, respectively, to 49, 1.7, and 1.5 mg/kg at 6- to 10-cm depth (Pennington et al. 2002).

Because the aqueous solubility of HMX is small (about 4–5 mg/L at 25°C), HMX tends to accumulate on the surface while the more soluble TNT (about 150 mg/L) dissolves, becomes associated with soil cation exchange sites, and undergoes environmental transformations (McCormick et al. 1976). The amino transformation products of TNT can covalently bind to soil organic matter, thereby becoming immobilized (Thorn et al. 2002). The HMX that slowly dissolves does not strongly interact with soils and can be carried through the vadose zone to underlying groundwater aquifers (Mailloux et al. 2000, in press). In most cases concern over the possible presence of buried unexploded ordnance has limited the collection of deep soil cores; however, soil samples were collected at the Fort Ord antitank rocket range to a depth of 120 cm (Fig. 5). In this case HMX was detectable at concentrations generally less than 1 mg/kg as deep as 120 cm whereas TNT, RDX, and amino transformation products of TNT were not detected at depths below 15 cm (Jenkins et al. 1998). Similar results were obtained for depth samples at other sites, although samples usually were not collected at depths below 15 cm.

Table 5. Concentrations of energetic compounds detected in surface soils adjacent to targets at antitank rocket ranges.

Installation[7]	Year sampled	Samples analyzed	Mean concentration (mg/kg)				
			HMX	RDX	TNT	4ADNT	2ADNT
CFB-Valcartier, Quebec[1,3,4]	1995	16*	803	4.6	24	<0.1	<0.1
	1995	5*	399	0.76	3	<0.1	<0.1
	1996	20*	662	<0.1	4	<0.1	<0.1
	2003	4† (30)	898	2.8	7	<0.1	<0.1
WATC-Wainwright, British Columbia[1,3]	1997	11*	987	5.3	126	<0.1	<0.1
Fort Ord, CA[2,5]	1997	8**	307	0.25	0.2	0.69	0.55
CFB-Gagetown, New Brunswick[1,4]	1998	10	680	<1	4	<0.1	<0.1
	2002	5†	874	0.5	6	0.8	0.7
	2003	8†	489	0.5	2	0.4	0.5
Yakima Training Center, WA[1,6]	2001	6† (30)	23	0.8	0.04	0.05	0.12
CFB-Petawawa, Ontario[1]	2004	3† (50)	745	0.32	73	<0.1	<0.1

* Composite samples

† Multi-increment samples with (n) increments per sample

** Discrete samples

[1] Active ranges

[2] Closed range

[3] Thiboutot et al. 1998

[4] Jenkins et al. 2004a

[5] Jenkins et al. 1998

[6] Pennington et al. 2002

[7] Impact areas at Pohakuloa and Fort Bliss anti-tank ranges were not sampled.

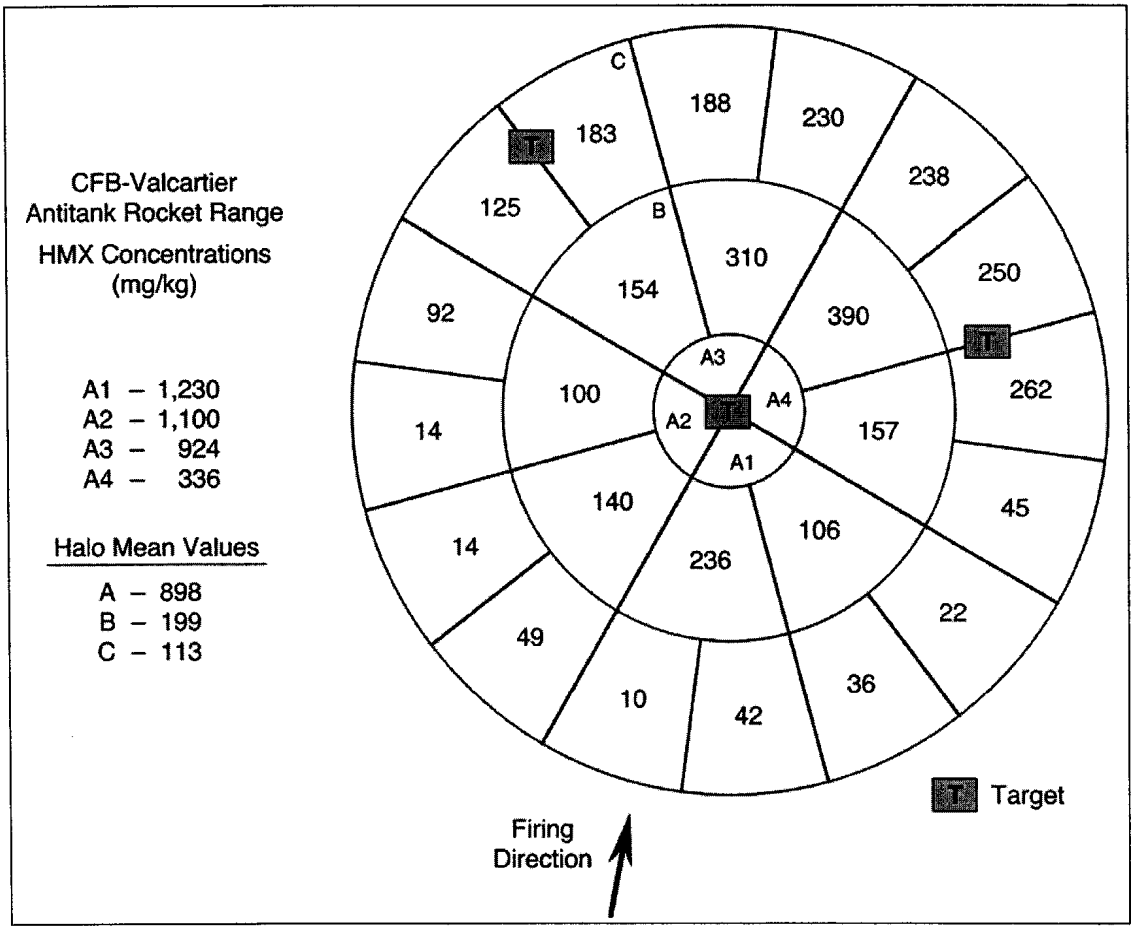
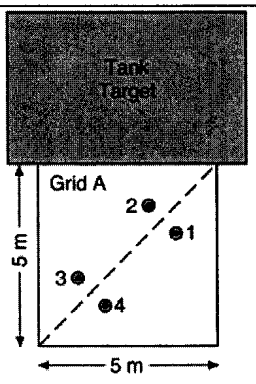


Figure 4. HMX concentrations at the target area of CFB-Valcartier antitank rocket range. (The position of the target is shown with a T.)

Because antitank rockets are propelled all the way to the target, propellants can still be present when these rockets detonate upon impact. Small pieces of propellant are thereby spread over the soil surface in the area around the targets. These residues can be seen visually and NG has been detected at the impact areas at concentrations as high as 23 mg/kg.

As with hand grenade ranges, collection of reproducible samples at antitank ranges has been problematic (Jenkins et al. 1999). At CFB-Valcartier, a 10-m × 10-m area just in front of a target was divided into one hundred 1-m × 1-m cells and a discrete sample was collected from the top 1.5 cm in each. The concentrations of HMX in these samples varied from 8 to 1520 mg/kg, demonstrating the futility in trying to represent the mean concentration for decision units using

discrete samples (Jenkins et al. 2004a, 2005). Multi-increment samples have been shown to provide more representative samples for characterizing the impact areas at these ranges (Jenkins et al. 2005). Here again, the use of machine grinding to reduce the soil particle size and an increase in sample size to 10 g were effective at minimizing the error due to compositional heterogeneity for samples collected at antitank range impact areas where HMX is the major contaminant (Walsh et al. 2002).



Sample 3

Sample Depth	Concentrations (mg/kg) HMX	RDX	TNT	4ADNT	2ADNT
0 – 15 cm:	479	0.2	0.2	0.4	0.2
15 – 30 cm:	4.3	<0.1	<0.1	<0.1	<0.1
30 – 45 cm:	1.3	<0.1	<0.1	<0.1	<0.1
45 – 60 cm:	0.2	<0.1	<0.1	<0.1	<0.1
105 –120 cm:	2.0	<0.1	<0.1	<0.1	<0.1

Sample 2

Sample Depth	Concentrations (mg/kg) HMX	RDX	TNT	4ADNT	2ADNT
0 – 15 cm:	302	0.6	0.1	1.2	1.0
15 – 30 cm:	1.0	0.1	<0.1	<0.1	<0.1
30 – 45 cm:	0.4	<0.1	<0.1	<0.1	<0.1
45 – 60 cm:	0.2	<0.1	<0.1	<0.1	<0.1
105 –120 cm:	0.4	<0.1	<0.1	<0.1	<0.1

Sample 4

Sample Depth	Concentrations (mg/kg) HMX	RDX	TNT	4ADNT	2ADNT
0 – 15 cm:	136	0.1	0.1	0.7	0.6
15 – 30 cm:	0.6	<0.1	<0.1	<0.1	<0.1
30 – 45 cm:	0.4	<0.1	<0.1	<0.1	<0.1
45 – 60 cm:	0.3	<0.1	<0.1	<0.1	<0.1
105 –120 cm:	0.7	<0.1	<0.1	<0.1	<0.1

Sample 1

Sample Depth	Concentrations (mg/kg) HMX	RDX	TNT	4ADNT	2ADNT
0 – 15 cm:	273	0.5	0.1	1.1	0.9
15 – 30 cm:	0.7	<0.1	<0.1	<0.1	<0.1
30 – 45 cm:	0.7	<0.1	<0.1	<0.1	<0.1
45 – 60 cm:	0.7	<0.1	<0.1	<0.1	<0.1
105 –120 cm:	7.0	<0.1	<0.1	0.1	0.1

Figure 5. Concentrations of energetic compounds with depth at Fort Ord antitank rocket range impact area.

Table 6. Summary of results for nitroglycerin (NG) near firing points at active anti-tank rocket ranges.

Installation	Year sampled	Samples analyzed	Mean NG concentration (mg/kg)								
			In front					Behind			
			0–10 m	10–20 m	20–30 m	30–40 m	40–50 m	0–10 m	10–20 m	20–30 m	30–40 m
Yakima Training Center, WA[1]	2001	2 (30)*	3	NS**	NS	NS	NS	NS	NS	NS	NS
Schofield Barracks, HI[2]	2002	4 (30)*	NS	NS	NS	NS	NS	1200	9.4	NS	NS
CFB-Gagetown, New Brunswick[3,4]	2002	4 (30)*	176	65	NS	NS	14	1130	NS	NS	NS
	2003	15 (30)*	160	160	87	55	12	4700	2320	380	84
Fort Bliss, NM[5]	2002	10 (30)*	1	0.5	<0.1	NS	NS	1	NS	NS	NS
CFB-Valcartier, Quebec[6]	2003	13 (30)*	NS	4.2	0.8	0.1	0.4	910	490	104	NS
CFB-Petawawa, Ontario	2004	8 (40)*	NS	NS	NS	NS	NS	2240	380	NS	NS

* Multi-increment samples with (n) increments

** No sample collected

[1] Pennington et al. 2002

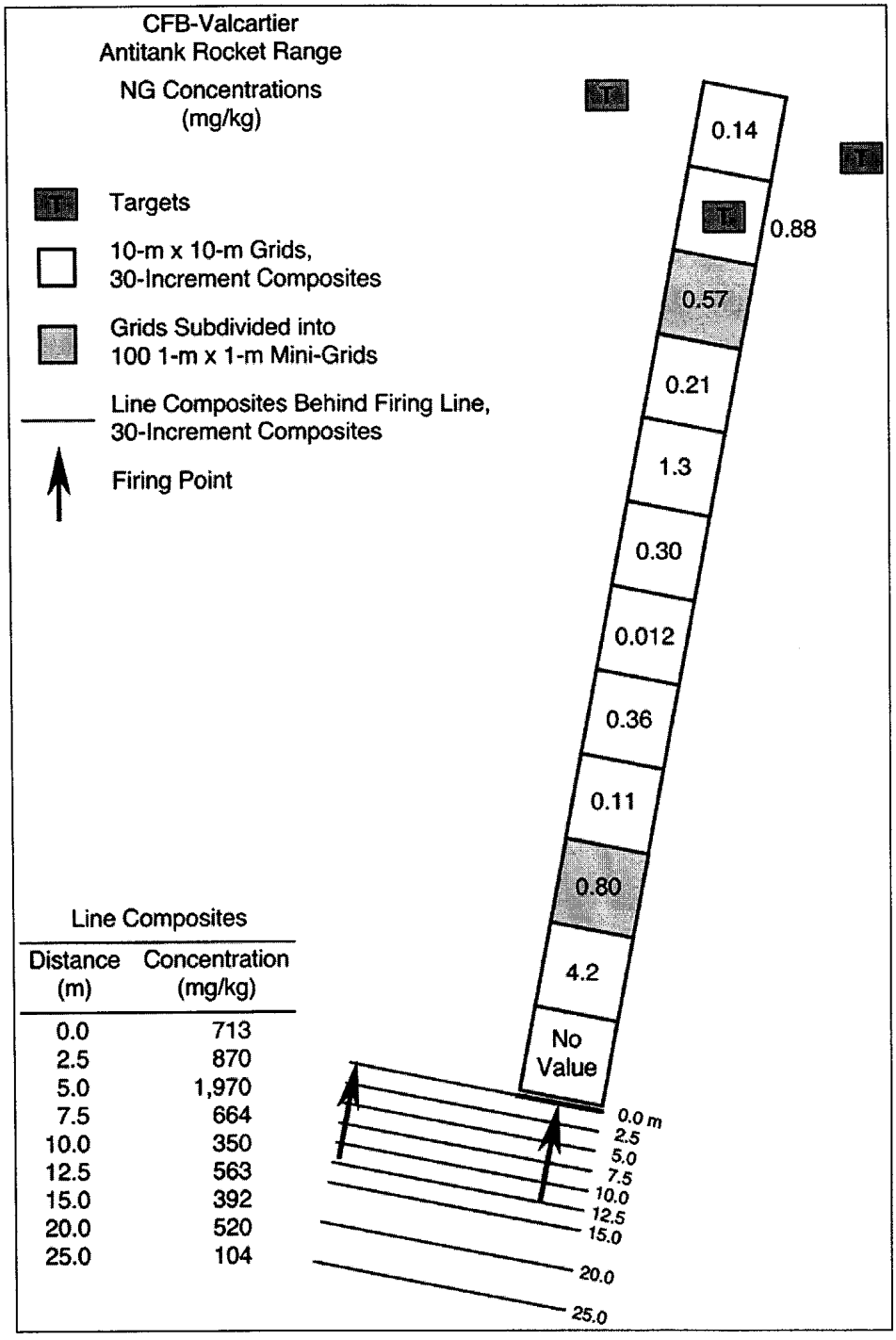
[2] Hewitt et al. 2004

[3] Thiboutot et al. 2003

[4] Thiboutot et al. 2004

[5] Pennington et al. 2003

[6] Jenkins et al. 2004a



Line Composites

Distance (m)	Concentration (mg/kg)
0.0	713
2.5	870
5.0	1,970
7.5	664
10.0	350
12.5	563
15.0	392
20.0	520
25.0	104

Figure 6. Concentrations of NG in multi-increment soil samples in front of and behind the rocket firing line at CFB-Valcartier antitank rocket range.

Antitank range firing points

Sampling has been conducted at six antitank rocket range firing points (Table 6). In all cases NG was the primary energetic compound detected, although only a few samples were analyzed for perchlorate. NG concentrations in surface soil samples from 0 to 25 m behind the firing line at CFB-Valcartier were generally in the hundreds of mg/kg, whereas concentrations between the firing line and the target were generally much lower (Fig. 6). Often a gravelly parking area is located behind the firing line at antitank rocket ranges and we sampled the soil at depths as great as 63 cm in this area at CFB-Gagetown in 2003 (Thiboutot et al. 2004). In one soil profile, NG concentrations declined from 20 mg/kg in the surface 0- to 5-cm depth to 6.4 mg/kg at the 20- to 27-cm depth, and to a concentration of about 0.2 mg/kg from the 40-cm depth to as deep as 60 cm (Table 7). Surface concentrations as high as 11,300 mg/kg were found at this site (Thiboutot et al. 2003).

At CFB-Valcartier we subdivided a 10-m × 10-m area 20–30 m in front of the firing line into one-hundred 1-m × 1-m cells and collected a discrete surface sample in each (0–2.5 cm). NG concentrations ranged from 0.02 to 3.4 mg/kg, indicating once again that discrete samples should not be used to estimate energetic concentrations for areas (decision units) near firing points (Jenkins et al. 2004a). A set of 50 30-increment samples was simulated using random numbers from this set of 100 discrete samples. The values obtained ranged from 0.34 to 0.93 mg/kg (Jenkins et al. 2004a). The value for the 30-increment sample actually collected within this 10-m × 10-m area was 0.80 mg/kg, well within the range simulated. Clearly the use of a 30-increment sample to estimate the mean concentration within this area provides a much more reproducible estimate than one or a small set of discrete samples.

Artillery ranges

Artillery ranges are the largest training ranges in the army inventory, covering an area of hundreds of square kilometers. Firing positions are often arranged around the circumference of the range with firing fans leading into the impact areas, generally positioned near the center of the range (Fig. 7). In the past, fixed firing points were established, but with more modern mobile artillery, firing activities have become more diffuse as training has evolved to support a "shoot and scoot" strategy. Once fired, artillery and mortar rounds travel several kilometers before impacting in the general vicinity of targets. The flight path takes these rounds over an area referred to as the firing safety fan, where only a very few defective rounds impact. Often, this is the largest area of the range. Once the rounds arrive near targets, most rounds are set to detonate upon impact.

Table 7. Nitroglycerin concentrations in depth profile samples collected in front of and behind the firing point at Wellington Antitank Range at CFB-Gagetown in 2003.

Location	Soil concentration (mg/kg)
Front, center of firing point, 10 m	**NG**
0–5 cm	11*
5–7 cm	15
7–11.5 cm	6.5
11.5–13 cm	0.06
13–18 cm	<d
18–22 cm	0.01
22–27 cm	<d
27–31 cm	0.02
31–35 cm	0.02
35–39 cm	0.01
39–42 cm	0.00
42–47 cm	0.00
47–52 cm	0.01
52–57 cm	0.01
Behind, center of firing point, 10 m	
0–5 cm	20
5–10 cm	14
10–20 cm	0.50
20–27 cm	6.4
27–35 cm	5.8
35–39 cm	0.32
39–42 cm	0.23
42–47 cm	0.15
47–50 cm	<d
50–56 cm	0.03
56–59 cm	0.22
59–63 cm	0.34

* Analysis by RP-HPLC (unshaded) and GC-ECD (shaded)

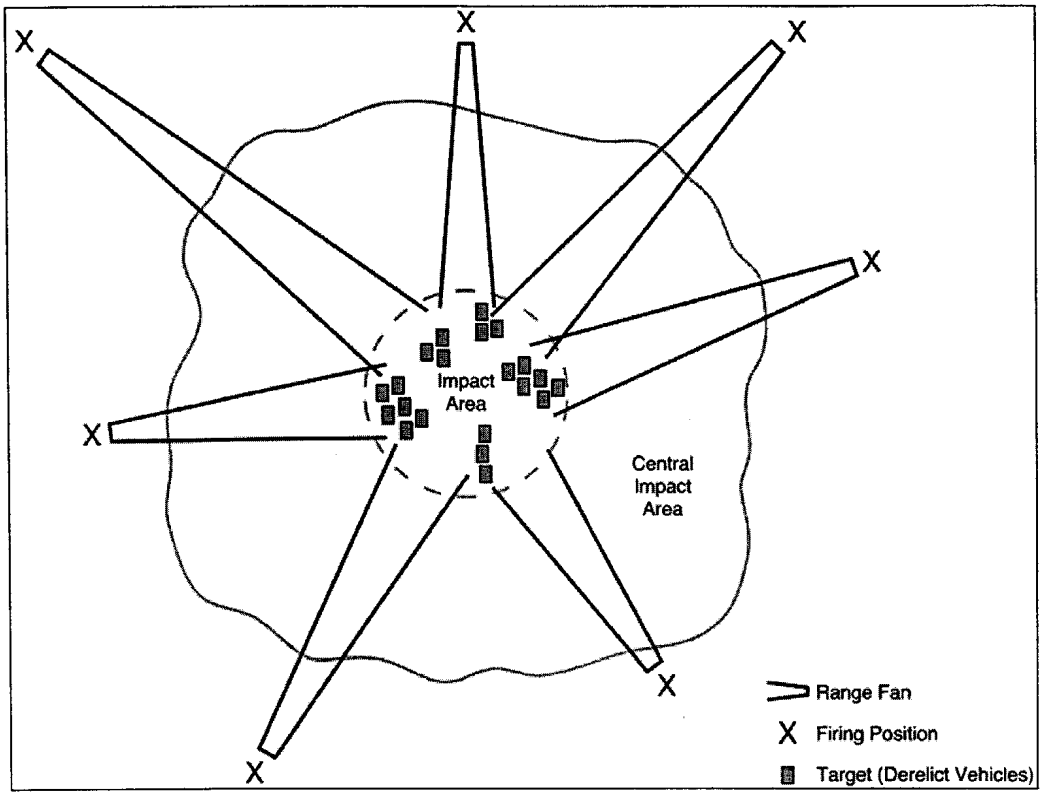


Figure 7. Schematic diagram of an artillery range showing firing points, range safety fan, and impact areas.

When the rounds perform as designed, these detonations result in the formation of a crater in the soil; the size is based on the type of round and the physical properties of the soil. Occasionally a round will impact without detonating, resulting in either a surface or subsurface UXO. For ranges where the soil is rocky or very hard, many of these UXO items can be seen on the surface. In a relatively small number of cases, a round will partially detonate upon impact, resulting in what is called a low-order detonation. In this case, only a portion of the explosive fill is consumed, sometimes leaving a substantial fraction of the explosive fill in or near the ruptured casing. Sometimes a high-order detonation will occur near enough to a surface UXO item that the item will be ruptured without detonation or with a low-order detonation. Here again, a substantial portion of the explosive fill will remain.

Climatic conditions and vegetative cover varies widely for the artillery ranges we have sampled in different parts of North America. For example, we have sampled ranges located in hot arid portions of the western United States (Pennington et al. 2003, Hewitt et al. 2005), ranges in subarctic Alaska and

Canada (Walsh et al. 2001, 2004; Ampleman et al. 2003a), a range located in a salt marsh in coastal Alaska (Walsh et al. 1995), ranges in moist southeastern United States (Jenkins et al. 2004b, Hewitt et al. 2005), ranges in a tropical setting in Hawaii (Hewitt et al. 2004), and ranges in cool, moist areas of eastern Canada (Thiboutot et al. 2003, 2004). Some ranges are sparsely vegetated, some heavily forested, some are open plains, and others are located in wetlands.

Many of the artillery ranges have been used for training for many decades. The munitions that have been fired include ordnance currently in the inventory as well as ordnance that was used pre- and post World War II, the Korean Conflict, and Vietnam. Because there has been no uniform management strategy in the past, UXO of a wide array of munitions are present on these ranges and many of these items are still live. For this reason access is tightly controlled and the length of time that we had access to the various ranges varied considerably.

The munitions fired to the greatest extent into these ranges are artillery and mortars, although various rockets, missiles, and Air Force and Navy bombs have been used as well. Currently the major munition systems being fired into these ranges include 155-mm howitzers, 105-mm howitzers, 120-mm main tank guns, 81-mm mortars, 60-mm mortars, and 120-mm mortars. Munitions including 90-mm recoilless rifle rounds, 4.2-in. mortar rounds, 8-in. artillery rounds, bombs of various sizes, 40-mm grenades, 106-mm high-explosive plastic (HEP) rounds, 2.75-in. LAW rockets, and TOW missiles also have been fired into some of these ranges. These munitions are delivered using single-, double-, triple-base gun propellants and rocket and missile propellants. Single-base propellant is composed of NC and 2,4-DNT, double-base propellant is composed of NC and NG, and triple-base propellant is composed of NC, NG, and nitroguanidine (NQ). The high explosives used in artillery and mortar warheads are generally either TNT or Composition B, although some older rounds also contained tetryl (methyl-2,4,6-trinitrophenyl nitramines). Some smoke-generating munitions contain white phosphorus (WP). Bombs that have been dropped in some of these ranges contain TNT or tritonal (TNT and aluminum), 40-mm grenades contain Composition A5 (RDX), and LAW rockets contain octol (HMX and TNT).

A listing of the 16 artillery ranges where we have collected samples is shown in Table 8. After each installation, we have indicated the types of areas sampled. Because at the beginning of this work there was very little information about energetic residues on these ranges, we sampled a variety of areas, including firing points, target areas, areas in and near detonation craters, areas adjacent to UXO items, areas where chunks of explosive were observed on the surface, areas where a round had undergone a low-order detonation, and areas that were away from the firing points or targets but were within the firing safety fans.

Table 8. Installations at which artillery ranges have been sampled for energetic residues.

Installation	Year sampled	Types of areas sampled						
		Firing points	Target areas	Areas with partial detonations	Firing fan areas*	Craters	Near-UXO items	Areas with chunk explosives
Fort Richardson, AK[1]	1992	x	x					
Fort Greely (Donnelly Training Area), AK[2]	2000	x						
Fort Lewis, WA[3]	2000	x	x			x		
Yakima Training Area, WA[3]	2001	x	x			x	x	
Camp Guernsey, WY[3]	2001		x	x				x
CFB-Shilo, Manitoba[4]	2001		x		x			
Fort Bliss, NM[5]	2002	x	x	x	x			x
Jefferson Proving Ground, IN	2002		x		x			
Schofield Barracks, HI[6]	2002	x	x		x			
CFB-Gagetown, New Brunswick[7, 8]	2002 2003		x	x	x			x
Fort Polk, LA[9]	2003		x	x	x			x
Fort Hood, TX[10]	2004		x	x			x	x
Fort Carson, CO[10]	2004	x	x					
29 Palms, CA[10]	2004		x	x				x
Massachusetts Military Reservation, MA	2004		x					
CFB-Petawawa, Ontario	2004	x						

* Areas away from any known firing activity or detonations

1 Walsh et al. 1995
2 Walsh et al. 2001
3 Jenkins et al. 2001
4 Thiboutot et al. 2003
5 Pennington et al. 2003
6 Hewitt et al. 2004
7 Ampleman et al. 2003b
8 Thiboutot et al. 2004
9 Jenkins et al. 2004b
10 Hewitt et al. 2005

Table 9. Summary of sampling results for surface soils at artillery firing points.					
			Mean surface soil concentration (mg/kg)		
Installation	**Weapon fired**	**Propellant type**	**2,4-DNT**	**2,6-DNT**	**NG**
Fort Greely (Donnely Training Area), AK	105-mm howitzer	single base			
FP BoWhale			4.3	NA	<0.01
FP Big Lake			9.1	0.35	<0.01
FP Mark			1.1	NA	NA
FP Sally			0.66	NA	NA
Yakima Training Center, WA	120-mm tank gun	single, triple base			
MPRC: 10 m from fixed firing point			24	0.40	4.6
MPRC: 20 m from fixed firing point			8.2	0.13	1.3
MPRC: 30 m from fixed firing point			2.2	<0.01	0.64
MPRC: 50 m from fixed firing point			0.68	<0.01	0.33
MPRC: 75 m from fixed firing point			0.19	<0.01	0.50
Yakima Training Center, WA	155-mm howitzer	single, triple base			
7 m from firing point			<0.03	<0.02	26
12 m from firing point			<0.03	<0.02	3.0
22 m from firing point			3.2	0.05	6
32 m from firing point			0.27	<0.02	1.85
Fort Bliss, NM (14 composite samples)	155-mm howitzer	single, triple base			
Non detects: 12 samples			<0.002	<0.001	<0.001
Maximum value found			0.97	<0.001	<0.001

Table 9 (cont'd).

Installation	Weapon fired	Propellant type	Mean surface soil concentration (mg/kg)		
			2,4-DNT	2,6-DNT	NG
Fort Lewis, WA (600 rounds fired)*	105-mm howitzer	single base			
At muzzle of 105-mm howitzer			63	<0.01	<0.01
5 m from muzzle			84	<0.01	<0.01
10 m from muzzle			57	<0.01	<0.01
15 m from muzzle			15	<0.01	<0.01
20 m from muzzle			4.0	<0.01	<0.01
CFB-Petawawa, Ontario	various mortars	single, double base	0.91	<0.01	3.58
Schofield Barracks, HI					
Max in seven composite samples	105-mm and 155-mm	single, triple base	0.04	<0.01	0.35
Fort Richardson, AK	105-mm howitzer	single base			
surface 0- to 3-cm depth			9.6	<0.01	<0.01
3- to 6-cm depth†			2.2	<0.01	<0.01
6- to 10-cm depth†			0.063	<0.01	<0.01
10- to 20-cm depth†			0.56	<0.01	<0.01
Fort Carson, CO	Mostly mortars	mostly double base	0.11	<0.01	12

* Surface samples collected from top 0.5 cm of surface soil

† Soils collected at specified depths below surface

Artillery range firing points

A number of firing point areas have been sampled at various artillery ranges (Table 8). These have included areas where 105-mm and 155-mm howitzers have been fired, an area where various mortars were fired, and an area where 120-mm tank guns were fired (Table 9). The largest amount of sampling was conducted in areas where 105-mm howitzers were fired. The propellant used for these guns is single base and 2,4-DNT was found to be the residue present at the highest concentration in all cases. We did not attempt to determine the concentration of NC because it is polymeric and does not present a problem for off-site migration, which is the major concern for energetic residues. Also, there are no validated methods for this compound when dispersed in a soil matrix.

The highest concentrations of 2,4-DNT are for samples from Fort Lewis (Jenkins et al. 2001), but these were collected from an area just in front of 105-mm howitzers where 600 rounds had been fired in the preceding month, and the samples were collected from only the top 0.5 cm of soil. When the concentration of 2,4-DNT in a sample was above 3 mg/kg, we sometimes detected much lower concentrations of 2,6-DNT as well. 2,6-DNT is an impurity in military-grade 2,4-DNT. Soil samples were collected primarily in surface soils, except at Fort Richardson, where soils were sampled as deep as 20 cm. In this case the concentration of 2,4-DNT declined from 9.6 mg/kg in the surface 0- to 3-cm sample to 0.56 mg/kg in the sample from 10 to 20 cm. To investigate the physical nature of these propellant residues, metal trays were placed in front of 105-mm howitzers during a firing event at Fort Richardson, Alaska. Microscopic analysis of the residues indicated that at least a portion of the residues was unburned or partially burned propellant fibers with fiber lengths ranging from 0.4 to 7.5 mm (Taylor 2004 and personal communication*). The unburned fibers contained much higher concentrations of 2,4-DNT than did the partially burned ones.

At Yakima Training Center we were able to collect surface soil samples at a multi-purpose range complex in front of a fixed firing point for 120-mm tank firing (Pennington et al. 2002). Both 2,4-DNT and NG were detected at 75 m, the farthest distance from the firing point sampled (Table 9). At Yakima we also sampled an area where a 155-mm howitzer had recently been fired. In this case, the residue was largely NG although some 2,4-DNT was also detected. The propellants used with 155-mm howitzers can be either single base for short range target practice or a combination of single base and triple base for longer range firing activities.

* Personal communication, Susan Taylor, CRREL, 2004.

Samples from areas at artillery ranges away from impact areas and firing points

At Camp Shelby, Mississippi; Fort Bliss, New Mexico; Fort Polk, Louisiana; Fort Carson, Colorado; and Jefferson Proving Ground, Indiana, the U.S. Army Environmental Center (USAEC) and the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) conducted Regional Range Studies to assess the overall environmental impacts of residues from firing activities on artillery ranges. The USAEC/CHPPM group used a stratified random sampling strategy unbiased by any judgmental observations, and collected 5-point composite samples from 10-m × 10-m grids established at various points across these areas. Because target areas represent only a small fraction of the total area of artillery ranges and their sampling area selection was unbiased, most of the areas that they sampled were quite a distance from any recognizable activity. We accompanied the USAEC/CHPPM sampling teams at all of these sites with the exception of Camp Shelby, and we collected random 30-increment samples within some of the same 10-m × 10- m grids that they sampled. Most of these samples collected by both the USAEC/CHPPM and CRREL protocols for these sampling locations did not contain detectable energetic residues using either RP-HPLC or GC-ECD methods, indicating that most of the total area at these ranges is virtually uncontaminated (Table 10).

At CFB-Shilo, Manitoba, and CFB-Gagetown, New Brunswick, Thiboutot et al. (2003, 2004) collected sets of multi-increment samples at various distances between the firing points and targets. Here again, the concentrations of energetic compounds were generally near or below analytical detection limits (Table 10), indicating that the largest portion of the range has very low concentrations of energetic residues. We also collected a set of 77 50-increment samples and a set of 16 discrete samples using a grid node sampling approach from the Washington Range at Fort Greely. This range is used to test artillery, mortar, TOW missiles, and a variety of other weapons under very low temperature conditions and has been used for many years. Of the 77 multi-increment samples, 74 had no detectable residues of energetic compounds, and the maximum concentrations for the other three samples were 0.61 mg/kg for HMX, 0.62 mg/kg for 2,4-DNT, and 0.27 mg/kg for RDX. Of the 16 discrete samples, 10 had no detectable residues and the maximum concentrations for RDX, TNT, HMX, 2,4-DNT, 2,6-DNT, 4ADNT, and 2ADNT were 0.036, 0.012, 0.004, 9.5, <0.03, 0.016, and 0.018 mg/kg, respectively (Walsh et al. 2001). Similar sets of 50-increment samples were collected on the west side of the Washington range and at the Georgia Island range using the grid-node approach. No energetic residues were detected in any of the 68 samples analyzed.

Table 10. Results for unbiased samples collected at artillery range areas that were within the firing fan but away from firing points and targets.

Installation	Number of samples analyzed	Number of samples with no detectable energetic compounds	Maximum concentration (mg/kg)						
			RDX	TNT	HMX	2,4-DNT	NG	4ADNT	2ADNT
Camp Shelby, MS (AEC/CHPPM)[1]	54	53	<0.23	<0.23	0.33	<0.23	<0.48	<0.23	<0.23
Fort Bliss, NM (AEC/CHPPM)[2]	161	151	8	0.20	2.7	<0.001	0.35	0.27	0.19
Fort Bliss, NM (ERDC)[3]	23	14	0.009	0.049	0.066	0.011	0.97	0.011	0.012
Jefferson Proving Ground, IN (AEC/CHPPM)[4]	170	138/RDX 167/TNT 169/DNT	0.098	0.06	<0.05	0.58	<0.05	<0.05	<0.02
Jefferson Proving Ground, IN (ERDC)	105	103/RDX 100/TNT	0.036	0.232	<0.05	<0.001	<0.05	<0.001	<0.001
Fort Polk, LA (AEC/CHPPM)[5]									
CFB-Shilo, MN (DRDC/ERDC)[6]	26	16	0.022	1.6	<0.01	0.046	0.015	<0.001	<0.001
CFB-Gagetown, New Brunswick (DRDC/ERDC)[7]	18	7	<0.05	0.21	<0.05	0.02	0.49	<0.01	<0.01
Fort Greely (Donnelly Training Area), AK (ERDC)									
WA Range: 50-increment samples	77	74	0.27	<0.001	0.61	0.62	<0.02	<0.002	<0.002
WA Range: discrete samples[8]	16	10	0.036	0.012	0.004	9.5	<0.03	0.016	0.018
Georgia Island Range: 50-increment samples[9]	44	44	<0.002	<0.001	<0.004	<0.001	<0.02	<0.002	<0.002
West side of WA Range: 50-increment samples[9]	24	24	<0.002	<0.001	<0.004	<0.001	<0.02	<0.002	<0.002
Total	718	631 Maximum	8	1.6	2.7	9.5	0.97	0.27	0.19

[1] USACHPPM 2001
[2] USACHPPM 2004
[3] Pennington et al. 2003
[4] USACHPPM 2003
[5] USACHPPM 2005
[6] Ampleman et al. 2003b
[7] Thiboutot et al. 2003
[8] Walsh et al. 2001
[9] Walsh et al. 2004

Table 11. Analytical results for individual soil samples collected near artillery targets.								
Installation	# of increments per sample	Distance from target (m)	Mean concentration (mg/kg)					
			HMX	RDX	TNT	4ADNT	2ADNT	TNB
Camp Guernsey, WY[1]	30	1	0.14	<0.03	<0.02	<0.03	<0.04	<0.02
	30	5	<0.03	0.003	0.003	0.02	0.01	<0.003
	30	10	<0.03	<0.003	0.013	0.04	0.03	<0.003
	30	15	<0.03	<0.003	<0.001	0.01	0.007	<0.003
Fort Bliss, NM: (Target 1)[2]	30	2	3.1	2.1	0.69	0.1	<0.01	<0.01
Fort Bliss, NM: (Target 1)	30	5	0.03	0.01	0.57	0.08	<0.01	<0.01
Fort Bliss, NM: (Target 2)	30	2	<0.03	<0.003	<0.001	<0.002	<0.01	<0.01
Fort Bliss, NM: (Target 3)	30	2	<0.03	<0.003	<0.001	0.04	<0.01	<0.01
Fort Bliss, NM: (Target 4)	30	2	0.02	0.01	0.02	0.02	<0.01	<0.01
Fort Bliss, NM: (Target 5)	30	2	0.08	0.37	<0.01	0.002	<0.01	<0.01
Fort Bliss, NM: (Target 5)	30	5	0.04	0.03	<0.001	<0.01	<0.01	<0.01
Fort Hood, TX[3]	10	0–2	0.010	0.016	0.006	<0.01	<0.01	<0.01
	10	0–2	<0.01	0.003	<0.01	<0.01	<0.01	<0.01
	10	0–2	<0.01	0.013	0.008	0.004	0.007	<0.01
	10	2–5	<0.01	0.008	<0.01	<0.01	<0.01	<0.01
	10	2–5	<0.01	<0.01	0.021	0.004	0.004	<0.01
	10	2–5	<0.01	0.010	0.059	0.040	0.040	<0.01
	10	2–5	<0.01	0.007	0.007	0.004	0.007	<0.01
	10	5–10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	10	5–10	<0.01	<0.01	0.013	<0.01	<0.01	<0.01
	10	10–20	0.092	0.14	<0.01	<0.01	<0.01	<0.01
	10	10–20	0.011	0.037	<0.01	0.009	0.009	<0.01
	10	10–20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	10	10–20	<0.01	<0.01	0.005	0.007	0.007	<0.01

Table 11 (cont'd). Analytical results for individual soil samples collected near artillery targets.								
Installation	# of increments per sample	Distance from target (m)	Mean concentration (mg/kg)					
			HMX	RDX	TNT	4ADNT	2ADNT	TNB
Fort Polk, LA[4]	10	0–2	15	16	1.2	0.25	0.31	<0.01
	10	0–2	1.40	1.20	0.14	0.17	0.21	<0.01
	10	0–2	0.42	2.2	0.52	0.28	0.36	<0.01
	10	2–5	0.36	0.50	19	0.91	1.20	0.082
	10	2–5	0.88	0.45	0.44	0.17	0.23	<0.01
	10	2–5	0.24	0.72	0.076	0.074	0.096	<0.01
	10	2–5	0.22	1.8	14	0.27	0.25	<0.01
	10	2–5	0.12	0.42	0.23	0.18	0.27	<0.01
	10	2–5	1.9	13	4.4	0.53	0.73	<0.01
	10	2–5	0.23	1.2	2.2	0.61	0.88	<0.01
	10	2–5	0.13	0.29	9.5	1.1	1.4	<0.01
	10	2–5	0.064	0.11	0.78	0.30	0.40	<0.01
Fort Greely, AK[5,6]	7	5	<0.01	0.002	<0.001	<0.001	<0.001	<0.001
	7	5	<0.01	0.004	<0.001	<0.001	<0.001	<0.001
	7	10	0.11	0.002	0.002	<0.001	<0.001	<0.001
	7	10	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	15	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	15	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	20	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	20	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	25	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	25	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	30	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	30	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001

Table 11 (cont'd).

Installation	# of increments per sample	Distance from target (m)	Mean concentration (mg/kg)					
			HMX	RDX	TNT	4ADNT	2ADNT	TNB
Fort Greely, AK[5, 6] (cont'd)	7	35	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	35	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	40	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	40	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	45	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	45	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	50	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	7	50	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001

[1] Pennington et al. 2002
[2] Pennington et al. 2003
[3] Hewitt et al. 2005
[4] Jenkins et al. 2004b
[5] Walsh et al. 2001
[6] Wire-guided missile target

Artillery range target areas

Because target areas receive the largest numbers of detonations per unit area, we collected samples systematically around targets at many of the artillery ranges that we visited. These targets are generally derelict trucks, tanks, and armored personnel carriers, and many have sustained enormous damage after years of target practice. Because of the danger of encountering buried UXO items and the fact that most detonations scatter residue over the surface, most of the soil samples from these areas were collected from surface soil.

Table 11 presents a series of results from the analysis of surface soils collected near targets at five artillery impact areas. At Camp Guernsey, Wyoming, we collected a series of duplicate 30-increment samples at distances of 1, 5, 10, and 15-m from the perimeter of a truck target. HMX, RDX, TNT, 4ADNT, 2ADNT, and TNB were detected in at least one of these samples, but except for one HMX value at 0.14 mg/kg, concentrations were less than 0.05 mg/kg. At Fort Bliss, New Mexico, we collected 30-increment samples at distances of 2 and 5 meters from the target perimeter. Concentrations of HMX, RDX, TNT, 4ADNT, 2ADNT, and TNB were always less than 1 mg/kg, except for one 2-m sample at Target Number 1 where HMX and RDX were 3.1 and 2.1 mg/kg, respectively. At Fort Hood, 10-increment samples were collected at distance intervals of 0–2 m, 2–5 m, 5–10 m, and 10–20 m. Concentrations for the same six analytes were always less than 0.14 mg/kg. Soil samples collected from 0–2 and 2–5 m around a target area at Fort Polk had the highest concentrations of these target analytes, with maximum values for HMX, RDX, TNT, 4ADNT, 2ADNT, and TNB of 15, 16, 19, 1.1, 1.4, and 0.082 mg/kg, respectively. At Fort Greely, Alaska, we collected 20 seven-increment samples at distances ranging from 5 to 50 m from a target used for testing TOW missiles. In only three of these samples were energetic compounds detected and the maximum concentration was 0.11 mg/kg for HMX.

We also collected a set of six systematic 100-increment samples in a 100-m × 100-m area next to a target at Fort Hood, Texas. This area had over 600 craters within the 10,000-m^2 area, 55 of which were considered to be recent (within the last several months). The mean and range (r) of values obtained for these six samples were RDX (mean = 1.2 mg/kg, r = 0.12 to 3.68 mg/kg), TNT (mean = 0.30 mg/kg, r = <0.001 to 0.81 mg/kg), and HMX (mean = 0.21 mg/kg, r = 0.035 to 0.63 mg/kg). A set of 36 discrete samples was also collected within this area. RDX was detected in only seven of these samples, HMX was detected in eight, and TNT was not detected in any. However, the TNT transformation products (4ADNT and 2ADNT) were detected in two of these samples. It should be noted, though, that a small area that had visible chunks of Composition B was found

within this 10,000-m^2 area, and this may account for the low levels of residues detected in the multi-increment samples, and the low frequency of detections in the set of discrete samples.

Overall, the concentrations of energetic compounds near artillery targets are low and there does not appear to be a defined concentration gradient. Surface soil samples from some targets can have concentrations in excess of one mg/kg, but the concentrations at most targets are less, sometimes below the detections limits of the analytical methods used. In many cases we used SW846 Method 8095 (EPA 1999) for samples from artillery range impact areas because the concentrations of energetic compounds were less than the detection limits of the RP-HPLC method, SW846 Method 8330 (EPA 1994).

Artillery ranges near low-order (partial) detonations

By far the highest concentrations of energetic residues that we encountered at artillery ranges were associated with rounds that had undergone a low-order detonation (Table 12). One example of these partial detonations is shown in Figure 8. In most cases chunks of pure explosive were observed on the soil surface near these items and concentrations of energetic compounds in the surface soil (particles <2 mm) were at the percent levels in a few cases (Table 12). The highest concentration that we encountered for a soil sample was from Fort Hood where the TNT concentration beneath a low-order 4.2-in. mortar was 143,000 mg/kg (14.3%). The areas influenced by these low-order detonations were explored in several cases. At Fort Polk we collected a set of 100 discrete samples in a 10-m × 10-m area that was subdivided into 100 1-m × 1-m cells (Jenkins et al. 2004b). The visible mass of Composition B on the surface of each cell was collected and weighed separately from the soil samples. The RDX concentrations in these soil samples varied from 0.037 to 2390 mg/kg (Fig. 9) and the highest concentrations, i.e., those >100 mg/kg, were isolated in two small areas near where chunks of pure explosive were observed on the surface. About two-thirds of the total RDX present within this area was in the soil-sized fraction (<2 mm) and only about one-third in the visible chunks found on the surface. Some of the locations of these low-order detonations were near targets, but many others were found as we traversed the range in areas away from any recognizable targets. We believe that these low-order detonations and UXO items that have been ruptured by subsequent detonations represent the main source of residues on artillery ranges.

Table 12. Concentration of energetic compounds in surface soil samples near low-order detonations at artillery ranges.

Installation	Description of surface soil samples	Concentration (mg/kg)						
		HMX	RDX	TNT	4ADNT	2ADNT	TNB	2,4-DNT
Fort Greely, AK[1]	Beneath a low-order 2.75-in. rocket warhead	40	340	130	1	0.8	0.2	0.04
Fort Lewis, WA[2]	Beneath a low-order 155-mm round	<10	<10	15,100	110	102	15	40
Camp Guernsey, WY[3]	Beneath a ruptured 500-lb bomb	<10	<10	9,440	<10	<10	50	<10
Yakima Training Center, WA[3]	Near a low-order 155-mm round	5.2	54	<1	<1	<1	<1	<1
Fort Bliss, NM[4]	Beneath a low-order 2.75-in. rocket warhead	302	1,130	14	3.3	2.8	<1	<1
Fort Bliss, NM[4]	Beneath a low-order 155-mm round	<10	<10	2,520	<10	<10	148	<10
Fort Bliss, NM[4]	Beneath a 90-mm round	149	678	1,110	12	18	9	1.3
29 Palms, CA[5]	Beneath a chunk of Composition B from low-order 155-mm	94	825	537	0.05	0.11	4	<0.1
CFB-Gagetown, NB[6]	Within a crater from a low-order 500-lb bomb	<10	<10	42,200	<10	<10	<10	<10
Fort Carson, CO[5]	Beneath a low-order 106-mm HEP round	59	<1	336	<1	<1	<1	<1
Fort Carson, CO[5]	Beneath ruptured 8-in. round	53	308	451	6	5	0.3	1
Fort Hood, TX[5]	Beneath a low-order 4.2-in. mortar	59	323	143,000	<10	20	26	26

[1] Walsh et al. 2001
[2] Jenkins et al. 2001
[3] Pennington et al. 2002
[4] Pennington et al. 2003
[5] Hewitt et al. 2005
[6] Thiboutot et al. 2003

Figure 8. Low-order 155-mm artillery round found at Fort Bliss.

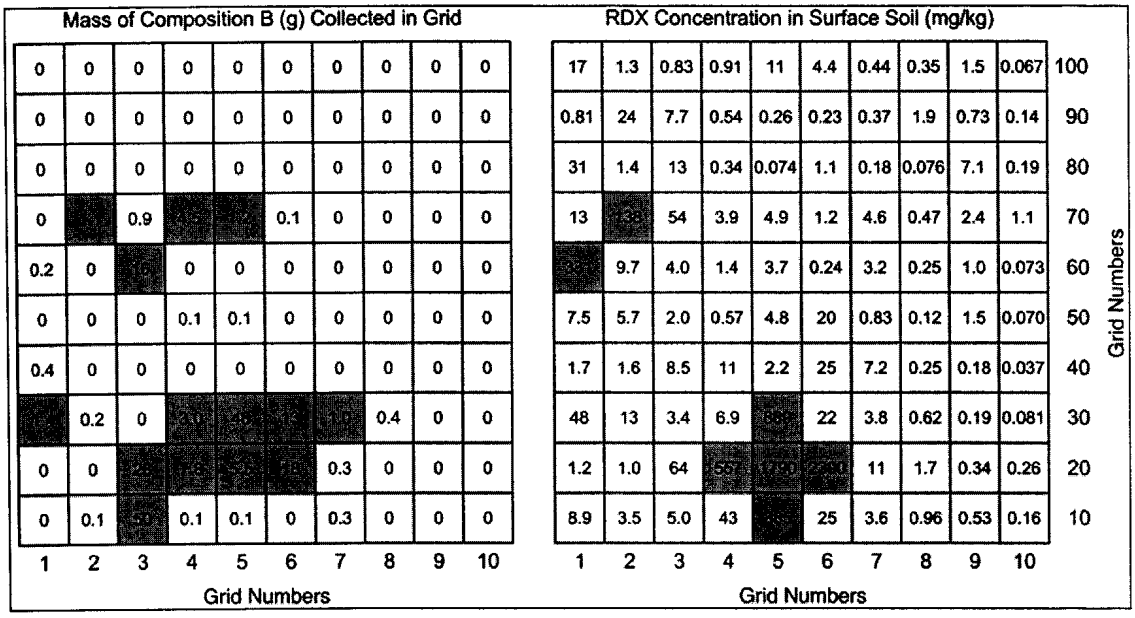


Figure 9. Mass of Composition B and soil RDX concentrations and their relative position in the 10-m × 10-m sampling grid near a low-order 81-mm mortar round at Fort Polk's artillery range impact area.

Bombing ranges

Air Force ranges are very large, generally hundreds of square kilometers in size, but the areas currently used for training with high-explosives-containing bombs are much smaller, generally only tens of hectares. We sampled two live-fire bombing ranges: Cold Lake Air Weapons Range (CLAWR) in Alberta (Ampleman et al. 2003a, 2004) and Holloman Air Force Range (HAFB) in New Mexico (Jenkins et al. in press). We also sampled several other ranges where bombing with HE-containing bombs had been conducted (Donnelly Training Area, Alaska; Camp Guernsey, Wyoming; Fort Polk, Louisiana; CFB-Gagetown, New Brunswick; 29 Palms, California; and Fort Carson, Colorado). The Air Force conducts regularly scheduled range maintenance activities where duds and chunks of high explosive (larger than golf-ball size) observed on the surface are gathered up and destroyed by detonating with C4, and craters are often filled in.

The high explosive present in U.S. and Canadian Air Force bombs is usually either tritonal (TNT, aluminum powder) or H-6 (TNT, RDX, aluminum powder). Some older bombs contained TNT. Although experiments documenting the residue deposited when a bomb detonates as designed have not been conducted, experimental results for large artillery rounds indicate that large mass HE

detonations are very efficient, dispersing only microgram-to-milligram quantities of residue when they detonate high order (M.R. Walsh et al. 2005). As with other ordnance items, low-order detonations are the major source of residues from bombs.

Figure 10. Reddish-colored crater formed from a low-order 500-lb bomb at CFB-Gagetown.

Figure 10 shows a low-order bomb crater at CFB-Gagetown where the TNT dissolving from chunks of tritonal in the bottom of the crater turned red as a result of photodegradation. Communication with range personnel at CLAWR indicates that low-order bomb detonations generally occur several times per year at their range. A low-order bomb can deposit kilogram quantities of residues as chunks and soil size particles. We observed low-order bombs at Camp Guernsey (Fig. 11) and at HAFB.

Figure 11. Low-order bomb found on the impact range at Camp Guernsey, Wyoming.

Because of the very large amount of explosive that remains after a low-order detonation, we believe it is these occurrences that produce the largest mass of residue at bombing ranges. Some of these low-order events probably occur during the bombing exercise, but the one we observed at HAFB was apparently caused by a bomb detonation occurring in close proximity to a subsurface 2000-lb dud (Fig. 12). Bomb detonations produce many sharp metal fragments, as designed, and these high-velocity fragments can rupture UXOs present nearby. This phenomena is believed to happen on a frequent basis on training ranges where intense live-fire training is conducted in areas where many UXO have accumulated over the years. This has been simulated in a PhD study (Lewis 2004) where munitions were easily broken by fragments from detonations of other rounds nearby and the fate of explosive from broken shells was measured in soil columns (Pennington et al. 2004).

Figure 12. Soil sampling being conducted near a low-order 2000-lb bomb at Holloman Air Force Base, New Mexico.

Results for soil samples collected at CLAWR, HAFB, near a low-order bomb at Camp Guernsey, at the bombing areas at Fort Polk, and near some low-order bomb craters at CFB-Gagetown are presented in Table 13. The concentration of TNT in these samples from the single bombing target at CLAWR ranged from 3 to 408 mg/kg, with a mean value of 86 mg/kg for a 50-m-radius circle. The mean concentrations of RDX, HMX, 4ADNT, 2ADNT, 2,4-DNT, and TNB in these samples were 0.27, 0.21, 0.71, 1.2, 0.20, and 0.13 mg/kg, respectively. Because the TNT concentrations were two orders of magnitude higher than RDX, and we observed several small chunks of tritonal present in the sampled area, we believe that these residues were from a tritonal-containing bomb. Because the soil around the target at CLAWR is tilled to reduce the chance of a wild fire, residue concentrations for different samples are less heterogeneous than those encountered at some other ranges.

Table 13. Concentrations of energetic residues at live-fire bombing range impact areas.								
		Concentration (mg/kg)						
Installation	Distance from target	TNT	RDX	HMX	4ADNT	2ADNT	2,4-DNT	TNB
Cold Lake Air Weapons Range, AB 2003a	0–10 m (mean n = 2)	32.2	<0.01	<0.01	1.14	1.78	0.17	0.08
	10–30 m (mean n = 8)	83.3	0.56	0.14	0.91	1.39	0.20	0.06
	30–50 m (mean n = 16)	94.1	0.1	0.23	0.62	1.04	0.1	0.17
Cold Lake Air Weapons Range, AB 2004	0–10 m (mean n = 2)	41.1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	10–30 m (mean n = 8)	44.4	0.05	<0.01	0.12	0.19	<0.01	<0.01
	30–50 m (mean n = 16)	41.6	0.38	0.10	<0.01	<0.01	<0.01	<0.01
Holloman AFB, NM (d)	**Area sampled**							
	within low-order bomb crater	60.0	<0.1	<0.1	0.19	0.19	0.20	0.69
	100 m x 100 m*	5.94	0.09	0.03	0.10	0.12	0.03	0.02
	100 m x 100 m†	0.58	0.01	<0.01	0.01	0.07	<0.01	0.04
	10 m x 10 m*	16.1	<0.01	<0.01	0.60	0.61	0.09	0.051
	10 m x 10 m†	0.28	<0.01	<0.01	0.01	0.02	<0.01	<0.01
Camp Guernsey, WY (c)	**Low-order bomb**							
	3 m from bomb	13.0	0.09	0.03	1.86	1.44	0.03	0.16
	5 m from bomb	0.26	<0.03	<0.03	0.30	0.23	<0.03	<0.01
	10 m from bomb	0.30	<0.03	<0.03	0.06	0.04	<0.03	<0.01
Fort Polk, LA	**Area near large bombing craters**							
	inside/toe crater #1	0.01	<0.01	<0.01	0.02	0.02	<0.01	<0.01
	rim crater #1	<0.01	<0.01	<0.01	0.02	0.02	<0.01	<0.01
	bottom crater #2	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	toe to rim crater #2	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	sides crater #2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	sides crater #2	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 13 (cont'd).

Installation	Distance from Target	Concentration (mg/kg)						
		TNT	RDX	HMX	4ADNT	2ADNT	2,4-DNT	TNB
CFB-Gagetown, NB	**Low-order bomb crater**							
	Crater #2 at 1 m	276	0.08	0.25	2.8	4.5	0.57	0.59
	crater #2 at 2 m	334	<0.01	<0.10	1.2	1.8	0.20	0.45
	Crater #3 at 1 m	17.6	<0.01	<0.10	<0.10	<0.10	<0.10	<0.10
	Crater #3 at 2 m	24.6	<0.01	<0.10	<0.10	<0.10	<0.10	<0.10
	Crater #4 at 1 m	1860	<0.01	<1.0	<1.0	<1.0	<1.0	<1.0
	Crater #4 at 2 m	3720	<0.01	<1.0	<1.0	<1.0	<1.0	<1.0
	Crater #4 at 5 m	2540	<0.01	<1.0	<1.0	<1.0	<1.0	<1.0
Fort Carson, CO	**25-m × 25-m area with HE chunks**	15.3	<0.01	<0.01	1.8	1.7	0.04	0.14
29 Palms, CA	**100-m × 100-m area with H-6 chunks**	1.4	9.4	1.3	<0.10	<0.10	<0.10	<0.10

* Area with chunks from 2000-lb low order

† Area away from 2000-lb low order

a Ampleman et al. 2003

b Ampleman et al. 2004

c Pennington et al. 2002

d Jenkins et al. in press

Similarly, concentrations of TNT ranged from 0.58 to 5.94 mg/kg in two 100-m × 100-m grids at HAFB, one containing an area with a low-order 2000-lb bomb and one about 50 m from the bomb. Concentrations of RDX were less than 0.1 mg/kg in most samples from this range. The concentration of TNT within a crater containing a low-order bomb averaged 60 mg/kg and the concentration within a 10-m × 10-m grid located just uphill from the crater averaged 16.1 mg/kg. Very different results were found for a 500-lb bomb crater that we sampled at Fort Polk. No energetic residues were detectable in soil samples from this crater, indicating that it was formed by a high-order detonation.

Explosives residues were detected in all of the samples collected near the target array located 2 km downstream from the Delta Creek Impact Area at Donnelly Training Area, Alaska (Walsh et al. 2004). In the composite samples, the following residues were determined: TNT (<1–314,000 μg/kg); RDX (7–1,400 μg/kg); HMX (<25–110 μg/kg); 2,4-DNT (1–33 μg/kg),and NG (<15–51 μg/kg). Only four of the samples had TNT above 1,000 μg/kg, and the median concentration was 80 μg/kg. The amino-DNT reduction products were detected in each sample as well, but concentrations were low (<200 μg/kg). One of the discrete samples collected near a 500-lb bomb partial detonation had a TNT concentration of 17,300,000 μg/kg, a concentration far exceeding any other sample we collected.

At Fort Carson, soil samples were collected in a 25-m × 25-m area where a large number of chunks of tritonal were observed on the surface. These chunks were probably deposited from a low-order bomb. The mean TNT concentration within this area was 15.3 mg/kg; TNT transformation products TNB, 2ADNT, and 4ADNT were detectable at low concentrations as well. Chunks of explosive were not included in the soil samples. Here again, the concentration of RDX was less than 0.1 mg/kg.

The H-6 explosive from a low-order bomb was detected only at 29 Palms. In this area we observed chunks of H-6 and the mean concentrations of RDX, TNT, and HMX in a 100-m × 100-m area just downslope of where the largest mass of explosive was observed were 9.4, 1.4, and 1.3 mg/kg, respectively. RDX was detected on a bombing range only where H-6 bombs were detonated, or when blow-in-place with C4 had occurred. TNT was the major energetic residue present at live-fire bombing ranges.

Demolition ranges

Demolition ranges at active DoD training facilities are used by the military explosive ordnance disposal (EOD) technicians to destroy duds of various munitions that are considered safe to move. Sometimes chunks of high explosive or

unused propellants are also destroyed at these ranges, either by demolition or burning. Demolition ranges are generally only a few hectares in size and sparsely vegetated near demolition craters. Demolition craters are often used many times before being filled in. At active installations, a quantity of C4 explosive is usually placed on the item, and it is detonated using a blasting cap, eliminating any detonation hazards from these items. Results from studies reported by Pennington et al. (2004) indicate that substantial residues of energetic compounds can sometimes be deposited during demolition events, particularly if they result in a low-order detonation for the item being destroyed or if the C4 doesn't detonate completely.

At some Air Force demolition ranges, C4 explosive is used to blow a hole in practice bombs to ensure that they contain no high explosives before these items can be removed from the range for recycling. We sampled two areas where this practice was employed, one at Eglin Air Force Base (AFB), Florida, and the other at Holloman AFB, New Mexico. Surface soil samples from both demolition ranges contain detectable concentrations of RDX and HMX (Table 14). At Eglin, the mean concentrations for six discrete samples were 8.84 and 0.54 mg/kg for RDX and HMX, respectively. At Holloman, the mean concentrations of RDX and HMX for three 30-increment composite samples collected within a 25-m circle around the demolition crater were 11.4 and 1.84 mg/kg, respectively. Because the items being detonated do not contain any explosives or propellants, the residues deposited originate from the C4 demolition explosive. The C4 demolition explosive is unconfined and this may lead to lower destruction efficiencies than for detonation of confined charges. Unconfined charges lead to detonations of lower pressure and temperature, two parameters that influence strongly the efficiency of the transformation processes in the detonation fire ball. Lower pressure and temperature cause incomplete oxidation processes and result in spreading of higher levels of unaltered energetic compounds in the environment.

Surface soil sampling was also conducted at a number of other demolition ranges at Fort Polk, Louisiana; Schofield Barracks and Pohakuloa, Hawaii; CFB-Petawawa, Ontario; Cold Lake Air Weapons Range, Alberta; and Camp Shelby, Mississippi (Table 15). These ranges were used to destroy high-explosives-containing munition items containing a variety of explosives. With the exception of two samples from CLAWR, RDX and HMX were detected in all samples from these ranges, probably from the C4 demolition explosive. Concentrations of these two explosives varied significantly from <0.03 at CLAWR to 60.2 mg/kg at Pohakuloa. At several ranges we observed pieces of C4 on the surface. During a blow-in-place test at Redstone Arsenal, small pieces of undetonated C4 were deposited over a small area when one of the two blasting caps failed and the secondary did not completely detonate the C4 block (Pennington et al. 2005).

These events probably occur infrequently, but they are probably a source of the RDX residues in some cases.

Table 14. Concentrations of explosives residues in soils at ranges where C4 was used to demonstrate that practice bombs contain no high explosive prior to metals recycling.

Installation	Date sampled	Sample#	Concentration (mg/kg)	
			HMX	RDX
Eglin AFB	Feb 03	1	0.18	1.81
		2	<0.01	0.48
		3	0.52	1.60
		4	<0.01	0.58
		5	0.61	13.9
		6	1.94	34.6
		mean	0.54	8.84
Holloman AFB	May 05	1	0.59	2.04
		2	3.98	27.8
		3	0.96	4.39
		mean	1.84	11.4

TNT was also detected in some samples from these demolition ranges, but except for two high-concentration samples from Pohakuloa, the concentrations of TNT were less than 0.6 mg/kg. NG and 2,4-DNT were detected in samples from several of these ranges. These compounds are generally components of propellant formulations where excess propellant is supposed to be destroyed by burning. Sometimes, however, these propellants are detonated instead, spreading propellant grains across the surface. It is not possible to determine whether the residues of NG and 2,4-DNT found at these ranges were from burned propellant or propellant that was incorrectly detonated.

Residue mobility

To investigate the mobility of energetic residues in the soil, we collected soil samples at depth below several low-order detonations at a variety of ranges (Table 16). The highest concentrations of TNT, RDX, or HMX were in the surface soil. Sometimes the highest concentrations for 4ADNT and 2ADNT were found in subsurface samples because these compounds are formed as dissolved TNT moves through the soil. In several of these data sets, HMX and RDX penetrated deeper into the soil profile than TNT. This is consistent with the lower soil/water partition coefficients for HMX and RDX relative to TNT (Pennington

and Brannon 2002), and the susceptibility of TNT to attenuation reactions with soil components (Haderlein et al. 1996, Thorn et al. 2002). RDX and HMX have been found in groundwater below several training ranges (Jenkins et al. 2001, Clausen et al. 2004, Mailloux et al. in press), but TNT has not.

At Fort Bliss (Pennington et al. 2003) we took a series of surface soil samples downslope from low-order detonations of a 90-mm and a 155-mm round (Table 17). In both cases some migration of energetic compounds was observed. Residues of HMX and RDX were considerably more mobile than TNT downslope of the 90-mm round. Residues of TNT were higher than RDX downslope of the 155-mm round because this round contained TNT.

Detonation craters and UXO presence

We collected a series of samples at several installations to determine the residual concentrations of energetic compounds within impact craters and around their perimeter (Table 18). RDX, HMX, TNT, 2ADNT, and NG were detected in only 46, 6, 30, 48, and 6 of the 126 samples analyzed, respectively. Except for two samples, concentrations were always less than 1 mg/kg. Similarly, we collected samples next to intact UXO items at Camp Guernsey (Table 18). Here again, residue concentrations were always below 1 mg/kg. When these UXO items at Camp Guernsey were detonated with C4 and soil samples collected in the area where the UXO item had been prior to its destruction, much higher residue concentrations were found in two of the three cases (Table 18). Overall, areas near detonation craters and intact UXO items are not heavily contaminated with residues of energetic compounds, but the destruction of UXO items with C4 (BIP) can sometimes result in a substantial increase of energetic compound concentrations in the near vicinity where the detonations occur. The use of C4 for blow-in-place detonations eliminates the safety issues associated with the presence of the UXO at training ranges; however, it can contribute to the environmental impact by distributing RDX in the environment.

Table 15. Concentrations of explosives residues in surface soils at demolition ranges where C4 was used to detonate high-explosives-containing munition items.

Installation	Date	Type*	Concentration (mg/kg)							
			HMX	RDX	TNT	4ADNT	2ADNT	2,4-DNT	NG	TNB
Cold Lake Air Weapons Range, AB	Aug 02	MI-30	<0.03	<0.03	0.01	<0.01	<0.01	<0.01	0.02	<0.01
		MI-30	<0.03	<0.03	0.52	<0.01	<0.01	<0.01	0.01	<0.01
		MI-30	<0.03	0.82	0.07	<0.01	<0.01	<0.01	<0.02	<0.01
Schofield Barrracks, HI	Nov 02	MI-30	0.70	3.94	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		MI-30	0.68	4.38	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pohakuloa, HI	Nov 02	MI-30	7.12	39.6	0.20	0.12	0.11	<0.01	<0.01	0.06
		MI-30	7.12	45.6	9.22	0.17	0.20	<0.01	1.23	0.30
		MI-30	11.1	60.2	0.58	0.15	0.14	0.34	0.10	0.19
		MI-30	7.8	36.0	11.6	0.25	0.35	0.64	10.5	0.23
Fort Polk, LA	Jun 03	MI-30	0.03	0.17	0.03	0.02	<0.01	1.51	0.13	<0.01
		MI-30	0.07	0.33	0.09	0.08	0.13	2.4	<0.01	<0.01
CFB-Petawawa, ON	Oct 04	MI-?	1.06	30.5	<0.01	<0.01	<0.01	12.0	1.15	<0.01
		MI-?	0.08	0.72	0.05	<0.01	<0.01	2.05	0.44	<0.01
		MI-?	0.55	2.45	0.05	<0.01	<0.01	1.00	<0.01	<0.01
Camp Shelby, MS	Apr 05	MI-65	0.32	1.1	<0.04	<0.08	<0.08	<0.04	<0.1	<0.01
		MI-65	0.27	0.59	<0.04	<0.08	<0.08	<0.04	0.33	<0.01
		MI-90	0.10	0.32	<0.04	<0.08	<0.08	0.66	<0.1	<0.01

* MI: Multi-increment sample—number of increments

Table 16. Concentrations with depth samples collected below low-order (partial) detonations or chunks of explosive at artillery ranges.

Installation (location of samples)	Mean concentration (mg/kg)							
	Depth (cm)	HMX	RDX	TNT	4ADNT	2ADNT	TNB	2,4-DNT
Fort Greely, AK (under 2.75-in. warhead)	surface	40	340	130	1.0	0.84	0.17	0.036
	2–5	0.61	2.4	0.28	0.065	0.084	<0.001	<0.001
	5–7	0.06	0.38	0.013	0.015	0.024	<0.001	<0.001
	10	0.03	0.03	<0.001	0.003	0.007	<0.001	<0.001
Fort Bliss, NM (under chunk of TNT)	Surface	<1	<1	2100	<1	<1	42	<1
	1–2	<1	<1	194	<1	<1	21	<1
	2–3	<1	<1	103	<1	<1	5.4	<1
Fort Bliss, NM (under 2.75-in. warhead)	Surface	302	1,130	13.5	3.3	2.8	0.09	<0.01
	3–4	17	111	1.5	1.2	1.9	<0.01	<0.01
Fort Lewis, WA (under 155-mm round)	Surface	<0.01	<0.01	15,100	110	102	15	40
	5	<0.01	<0.01	710	146	153	<0.01	10
	10	<0.01	<0.01	46	20	30	0.14	20
	15	<0.01	<0.01	2.5	0.19	0.19	0.06	0.01
Camp Guernsey, WY (under ruptured bomb)	Surface	<10	<10	9,440	<10	<10	<10	<10
	1–3	4.2	0.6	240	<10	<10	3.2	<1
	4–7	1.3	<1	42	14.9	19	0.96	2.0
Fort Hood, TX (under low-order 81-mm mortar)	Surface	52	212	5.0	6.5	8	<0.01	0.76
	1–3	6.3	26	0.48	2.2	3	<0.01	0.23
	3–7.5	6.7	26	1.6	1.7	3.2	<0.01	0.18
	7.5–10	4.2	13	0.30	1.1	2.0	<0.01	0.14
Fort Hood, TX (under chunk of Composition B)	Surface	129	861	459	14	9.8	<0.01	<0.01
	1–4	31	173	31	8.7	5.1	<0.01	<0.01
	9–14	127	832	331	2.8	1.9	<0.01	<0.01
	16–20	12	56	9.5	2.2	1.4	<0.01	<0.01

Table 16 (cont'd). Concentrations with depth samples collected below low-order (partial) detonations or chunks of explosive at artillery ranges.

Installation (location of samples)	Mean concentration (mg/kg)							
	Depth (cm)	HMX	RDX	TNT	4ADNT	2ADNT	TNB	2,4-DNT
Fort Hood, TX (area with Composition B)*	Surface	0.95	2.2	0.064	0.21	0.24	<0.001	<0.001
	2–6	0.40	3.7	<0.001	<0.001	<0.001	<0.001	<0.001
	6–9	0.12	0.33	<0.001	<0.001	<0.001	<0.001	<0.001
	9–12	0.13	0.25	<0.001	<0.001	<0.001	<0.001	<0.001
	12–16	0.10	0.22	<0.001	<0.001	<0.001	<0.001	<0.001
Fort Carson, CO (under 106-mm HEP round)	Surface	59	336	<0.01	<0.01	<0.01	<0.01	<0.01
	3–4	19	97	<0.01	<0.01	<0.01	<0.01	<0.01
	4–5	8.9	49	<0.01	<0.01	<0.01	<0.01	<0.01
	5–6	1.3	5.8	<0.01	<0.01	<0.01	<0.01	<0.01
	6–7	1.1	4.6	<0.01	<0.01	<0.01	<0.01	<0.01
	7–8	1.4	6.0	<0.01	<0.01	<0.01	<0.01	<0.01

* No chunks present at surface

Table 17. Concentration of energetic compounds for soil samples collected downslope of low-order (partial) detonations or chunks of explosive at Fort Bliss.

Installation (location of samples)	Down-slope (m)	Mean concentration (mg/kg)						
		HMX	RDX	TNT	4ADNT	2ADNT	TNB	2,4-DNT
Fort Bliss, NM (low-order 155-mm with chunks of TNT)*	0.2	<0.03	<0.03	6,270	<0.03	<0.04	98	<0.003
	1	<0.03	<0.03	1.3	0.2	0.17	<0.02	<0.003
	2	<0.03	<0.03	38	0.8	0.07	<0.02	<0.003
	3	<0.03	0.05	0.01	<0.002	<0.003	<0.003	<0.003
	4	<0.03	0.10	0.03	0.003	<0.003	<0.003	<0.003
	5	<0.03	0.02	348	0.007	0.004	<0.003	<0.003
	12	0.04	0.03	0.04	<0.002	<0.003	<0.003	<0.003
	30	<0.03	<0.003	<0.001	<0.002	<0.003	<0.003	<0.003
Fort Bliss, NM (low-order 90-mm)	0	149	678	1,100	12	18	9.0	1.3
	2	50	110	0.38	0.15	0.10	0.09	0.04
	3.7	41	39	0.21	0.12	0.06	<0.001	<0.001
	6	3.3	0.67	<0.001	<0.001	<0.001	<0.001	<0.001

* 155-mm round located in an arroyo

Table 18. Summary of concentrations for energetic compounds (mg/kg) for crater samples and samples next to intact UXO items at artillery ranges in the United States and Canada.

Installation	Year sampled	Crater samples analyzed	Type of craters	HMX		RDX		TNT		2ADNT		NG	
				Number <d[†]	Max value	Number <d	Max value	Number <d	Max value	Number <d	Max value	Number <d	Max value
Fort Greely (Donnelly Training Area), AK	2000	3 craters (13 samples)	BIP* mortar, tow missile, SADARM	13	<0.026	5	0.016	6	0.008	12	0.003	12	0.37
Fort Lewis, WA	2000	12 craters (47 samples)	Live-fire , mortars, artillery	47	<0.026	30	0.093	28	1.75	16	0.031	ND*	ND
Yakima Training Center, WA	2001	5 craters (31 samples)	Live fire, artillery	31	<0.026	26	0.017	31	<0.001	30	0.003	31	<0.022
CFB-Gagetown, New Brunswick	2002	8 craters (15 samples)	artillery	13	1.1	11	6.4	11	1.9	8	0.14	11	0.12
Fort Polk, LA	2003	5 craters (15 samples)	105-mm, 155-mm, bombs	14	0.060	4	0.061	12	0.27	11	0.46	13	0.005
Schofield Barracks, HI	2003	5 craters (8 samples)		8	<0.026	4	0.015	8	<0.001	1	0.013	8	<0.022
Camp Guernsey, WY	2001	36	three 155-mm rounds	21	0.53	18	0.33	13	0.550	4	0.45	36	<0.022
Yakima Training Center, WA	2001	10	105-mm, 155-mm, illumination	9	0.026	7	0.72	10	<0.001	8	0.049	10	<0.022
Camp Guernsey, WY	2001	49	three 155-mm rounds	11	83	11	541	7	294	26	0.59	49	<0.022

* BIP: Blow-in-place detonation crater

† Number of samples where concentrations were below analytical detection limits

4 SUMMARY AND CONCLUSIONS

The types of residues, their concentrations, and distributions differ depending on the type of range and munition used. In general, the largest residue concentrations for all impact areas appear to be due to low-order detonations spreading particles and larger chunks of high explosive over the soil surface.

For hand grenade ranges, low-order detonations occur either when grenades are thrown during training or when duds are blown in place using C4 explosive. The C4 explosive used for detonating duds contains 91% military-grade RDX, of which about 10% is HMX. The major energetic residues on hand grenade ranges are RDX and TNT from Composition B, the explosive charge in M67 and C13 fragmentation grenades. For ranges where a recent partial detonation has occurred, concentrations are generally in the low mg/kg range and the distributions are more spatially homogeneous than at other types of impact ranges due to the thousands of individual detonations that continually redistribute the residue. Because grenade ranges are small in size, composite samples consisting of 30 increments have been found to be adequate for obtaining representative samples of surface soils.

At antitank rocket ranges the major residue present in surface soils at the target area is HMX from the octol used as the high explosive in the warhead of 66-mm M72 LAW rockets. A concentration gradient is present in surface soils relative to the distance from targets. HMX concentrations in surface soils near targets are generally in the hundreds to low thousands of mg/kg, with TNT concentrations about one-hundredth that of HMX. The high levels of HMX in the soil at antitank rocket ranges can be attributed to the high dud- and rupture rate of the M72 rockets. For sample collection, the impact area should be stratified into areas near targets, and areas in front of and in back of targets. Short-range spatial heterogeneity in residue concentrations at these sites is high, and in order to get representative samples, it is necessary to take multi-increment samples with a minimum of 30 increments.

At the firing points of antitank rocket ranges, NG is present from the double-base propellant used in the 66-mm M72 rockets. The major deposition of residue is behind the firing line due to the back blast from this weapon. Concentrations as high as the low percent level are sometimes found in soil up to 25 m behind the firing line. NG is also found between the firing line and the target, but the concentrations are generally several orders of magnitude lower than behind the firing line. Multi-increment samples have been found to provide adequate characterization for samples from impact areas and firing points at antitank rocket ranges.

Because the residues in these samples are largely present as particles of propellant, samples must be processed using larger sieves (10 mesh, 2 mm) than recommended in SW846 Methods 8330 and 8095. We also recommend thorough grinding of samples using a mechanical grinder prior to subsampling to preserve the representativeness of the portion of the sample to be used for extraction and analysis.

Most of the total acreage at artillery ranges that is remote to firing points and targets is uncontaminated with residues of energetic compounds. At artillery and mortar firing points, the energetic residues are usually either 2,4-DNT or NG, depending on the type of propellant used for the specific firing platform. Residues can be deposited at distances up to 100 meters in front of the muzzle. For 105-mm howitzers, the major detectable residue is 2,4-DNT, which can accumulate into the mg/kg range for fixed firing points. The residues from the single-base propellant used with this weapon are distributed primarily as burnt or unburnt propellant fibers. Residue deposition from 155-mm howitzers and mortars is primarily NG from double- or triple-base propellants. The NG does not seem to accumulate to concentrations as high as those for 2,4-DNT from single-base propellants. Propellant residues are deposited at the soil surface and the highest concentrations remain at the surface unless the soil is disturbed. Both NG and 2,4-DNT are deposited in an NC fiber matrix, thereby probably limiting their bioavailability and leachability.

Near targets at impact ranges, the majority of detonations of munitions are high-order detonations, and, as found by Hewitt et al. (2003), they appear to deposit very little residue. The major energetic residue deposition is due to low-order (partial) detonations that can deposit chunks of pure explosive. Residue concentrations of hundreds or thousands of mg/kg are often found in the surface soils next to these detonations. The major residues are TNT and RDX from military-grade TNT and Composition B, the major explosives used in mortar and artillery rounds. The distribution of residues in the area of the range where detonations occur is best described as randomly distributed point sources. Some of these point sources may be due to low-order detonations that are from blow-in-place of surface UXO items. At present the detection of these point source areas has been visual, but research is underway to try to develop a near-real time detection capability. The collection of representative samples in areas subject to these partial detonations is a major challenge and approaches utilizing multi-increment sampling have not been adequate.

The major residue present at bombing ranges is generally TNT from the tritonal used as the high explosive in most bombs. Concentrations can be in the tens to hundreds of ppm in and near bomb craters where low-order detonations

have occurred. RDX concentrations are generally low at these ranges unless a bomb containing H-6 explosive had undergone a low-order detonation.

RDX and HMX from C4 are generally the residues present at highest concentrations in demolition ranges where C4 explosive is used to blast small holes in practice bombs to ensure that they contain no high explosive prior to recycling activities. RDX is generally the residue present at the highest concentration at EOD demolition ranges due to use of C4 to destroy duds and other explosives-containing items. Concentrations can sometimes be in the low mg/kg in surface soils at these sites.

RDX and HMX appear to be the most mobile of the energetic compounds present at training ranges. This is true for both downward migration through the soil profile and also overland in runoff. This agrees with results reported for energetic compounds in groundwater (Clausen et al. 2004, Jenkins et al. 2001).

Results of these studies demonstrate that the potential for range contamination is specific to the type of range and the type of activity. Large areas of training ranges are uncontaminated, while residues in smaller areas, e.g., those around targets, firing points, and low-order detonations, are potentially significant. Range managers can, therefore, limit management practices for residue control to specific areas and specific types of firing activities.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
November 2005	Technical Report	

4. TITLE AND SUBTITLE

Identity and Distribution of Residues of Energetic Compounds at Military Live-Fire Training Ranges

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Engineer Research and Development Center
Cold Regions Research and Engineering Laboratory
72 Lyme Road
Hanover, NH 03755-1290

8. PERFORMING ORGANIZATION REPORT

ERDC TR-05-10

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of the Chief of Engineers
Washington, D.C. 20314-1000

Strategic Environmental Research and Development Program
Arlington, VA 22203

10. SPONSOR / MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

Available from NTIS, Springfield, Virginia 22161.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Environmental stewardship of military training ranges is an important objective of the Department of Defense. Therefore, an understanding of the explosives residues resulting from military training with various weapon systems is critical to range managers. A series of field sampling experiments was conducted at 27 military firing ranges in the United States and Canada to provide information on the identity and distribution of energetic munitions constituents. Different types of ranges were studied, including hand grenade, antitank rocket, artillery, bombing, and demolition ranges. Both firing points and impact areas were studied. Energetic compounds (explosives and propellants) were determined and linked to the type of munition used and the major mechanisms of deposition. At impact areas, the largest deposition of residues of energetic compounds is due to low-order detonations, or, in some cases, munitions that split open upon impact. The major residue deposited and its distribution varies for different types of ranges based upon the composition of the high explosive present in the warheads of the rounds fired at that type of range. For antitank range impact areas, the major residue present is HMX from the octol explosive used in the M72 66-mm LAW rockets. At artillery range impact areas, the major residues are TNT and/or RDX from the military-grade TNT and Composition B used in warheads of artillery and mortar rounds. Residues are very heterogeneously distributed at artillery range impact areas

(continued)

15. SUBJECT TERMS

2,4-DNT	Demolition ranges	Hand grenade ranges	Nitroglycerin	Training ranges
Antitank ranges	Energetic compounds	HMX	Propellants	
Artillery ranges	Explosives	Impact areas	RDX	
Bombing ranges	Firing points	Live fire	TNT	

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	U	64	

ABSTRACT *(continued)*

and can be described as randomly distributed point sources. RDX and TNT are the major residues at hand grenade ranges and their distribution is less heterogeneous due to the large number of individual detonations in a smaller area that further disperses the residues over the surface and at shallow depths. TNT is the major energetic compound detected at bombing ranges due to its presence in tritonal, the most common explosive used in bombs. RDX is the most common energetic compound at demolition ranges due to its presence as the major component of C4 demolition explosive. NG and 2,4-DNT are also frequently detected at demolition ranges as a result of the disposal of excess propellant. Once dissolved, RDX and HMX are the most mobile of the organic energetic compounds deposited on ranges, both vertically in the soil profile and horizontally across the surface.

Results of these studies demonstrate that the potential for range contamination is specific to range activities. Large areas of training ranges are uncontaminated, while residues in smaller areas, e.g., those around targets, firing points, and low-order detonations, are potentially significant. Range managers can, therefore, limit management practices for residue control to specific areas and specific types of firing activities.

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

USCHPPM

Wildlife Toxicity Assessment for 1,3,5-Trinitrobenzene (1,3,5-TNB)

NOVEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 37-EJ-1138-01B
Approved for public release; distribution unlimited.

Readiness Thru Health

Wildlife Toxicity Assessment for 1,3,5-Trinitrobenzene (1,3,5-TNB)

FINAL REPORT
NOVEMBER 2001

Prepared by
Health Effects Research Program
Environmental Risk Assessment Program

USACHPPM Document No: 39-EJ1138-01B

Acknowledgements

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When referencing this document use the following citation
Salice, C.J. and G.Holdsworth. 2001. Wildlife Toxicity Assessment for 1,3,5-Trinitrobenzene. U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Project Number 39-EJ1138-01B, Aberdeen Proving Ground, Maryland, November 2001.

Table of Contents

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

Wildlife Toxicity Assessment for 1,3,5-TNB

CAS No. 99-35-4 **November 2001**

1. INTRODUCTION

1,3,5-Trinitrobenzene (1,3,5-TNB) is one of several compounds that have been released to the environment during the manufacture of explosives and in load, assembly and pack (LAP) activities at U.S. Army ammunition plants (AAPs) and other military installations. The compound has a close structural relationship with the most widely produced military explosive, trinitrotoluene (TNT), of which it is a manufacturing by-product and an environmental degradation product. The importance of 1,3,5-TNB as an environmental contaminant is related to its widespread distribution at and around military sites and to its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of 1,3,5-TNB on wildlife, with emphasis on identifying levels at which wildlife species may be adversely effected. Evaluating the toxicity of the compound is intended to contribute to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for wildlife ranging in the vicinity of 1,3,5-TNB contaminated sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological, and ecological databases were electronically searched May 5, 2000, using Dialog to identify primary reports of studies and reviews on the toxicity of 1,3,5-TNB. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined), and wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and "key words in context." All available abstracts of those articles that were selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For 1,3,5-TNB, 42 articles were marked for retrieval from 259 initial hits, a disparity arising because the initial sweep captured a substantial number of reports that featured the use of trinitrobenzene sulfonic acid to study colitis in laboratory rodents.

These were eliminated in tier 2 of the selection process. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to searching Dialog, a number of U.S. Army reports were identified in the Defense Technical Information Center (DTIC). Secondary references and sources of information on 1,3,5-TNB included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for 1,3-Dinitrobenzene/1,3,5-Trinitrobenzene* (ATSDR, 1995), the National Library of Medicine's Hazardous Substances Database (HSDB, 2000), the U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997).

2.2 Environmental Fate and Transport

The synthetic compound 1,3,5-TNB is used as a high explosive for commercial mining and military use, as a narrow-range pH indicator and as an agent to vulcanize natural rubber (HSDB, 2000). The compound is a manufacturing by-product of the explosive, TNT, and is released to the environment in discharged wastewater. Additionally, any TNT itself that is present in the waste stream may be degraded to 1,3,5-TNB by photolysis under certain conditions of pH and organic matter content (Talmage et al., 1999). 1,3,5-TNB has been released to the environment during LAP operations performed at AAPs and from open-pit incineration of waste explosives (Simini et al., 1995, Wentsel et al., 1979). Military firing ranges may be contaminated by nitroaromatic compounds from ruptured, but unexploded ordinance. Soil concentrations of up to 67,000 mg/kg have been detected on military reservations (Hovatter et al., 1997).

1,3,5-TNB has an estimated vapor pressure of 3.2×10^{-6} mm Hg at 25°C (Spanggord et al., 1980), a low value implying that partitioning to air is unlikely. 1,3,5-TNB is readily soluble in a variety of organic solvents at ambient temperature, and has a water solubility of 340–385 mg/L at 20–25°C. The compound has been identified in both surface water and groundwater. Furthermore, 1,3,5-TNB has been identified in stream sediments . Hovatter et al. (1997) and Talmage et al. (1999) reported 1,3,5-TNB soil concentration data for certain AAPs, depots and arsenals.

As noted above, TNT will undergo photolysis to produce 1,3,5-TNB in aqueous solution, which is resistant to further photolytic degradation. Nitroaromatic compounds, in general, resist hydrolysis under environmental conditions, with 1,3,5-TNB conforming to this pattern. The various values derived for a K_{oc} (organic carbon/water partition coefficients) for 1,3,5-TNB are estimates, since experimentally determined values are unavailable (Table 1). Talmage et al. (1999) reported K_{oc} values within the range of 76–520 ml/g, indicating a moderate degree of adsorption of 1,3,5-TNB to suspended sediments, and high to moderate soil mobility.

Table 1. Summary of Physical-Chemical Properties of 1,3,5-Trinitrobenzene

CAS No.	99-35-4
Molecular weight	213.11
Color	yellow-white
Physical state	orthorhombic crystals/rhombic plates
Melting point	122.5–125.5 °C
Boiling point	315 °C
Odor	no data
Solubility in water	340–385 mg/L at 20-25 °C: soluble in benzene, methanol, ethanol, ether, and carbon disulfide
Partition coefficients:	
Log K_{ow}	1.18
Log K_{oc}	1.88
Vapor pressure at 25 ^{o}C	3.2×10^{-6} mm Hg
Henry's Law constant at 25 ^{o}C	3.08×10^{-9} atm.m3/mole
Conversion factors	1 ppm = 8.7 mg/m^3
	1 mg/m^3 = 0.115 ppm

Sources: ATSDR (1995), Talmage et al. (1999), HSDB (2000), Burrows et al. (1989)

1,3,5-TNB is subject to microbial degradation, but the process appears to be limited to the compound's nitrogenous moieties. Thus, aerobic metabolism of 1,3,5-TNB by *Pseudomonas* sp. produces 1,5-dinitroaniline, dinitrobenzene, 5-nitrobenzene, and ammonia. This indicates that 1,3,5-TNB is probably not used as a carbon source by this organism, since no further breakdown was observed (Talmage et al., 1999).

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Toxicity - Oral

2.3.1.1 Mammalian Oral Toxicity - Acute

The review by Wentsel et al (1979) cited the Korolev et al. (1977) study on the acute oral toxicity of 1,3,5-TNB in which respective LD_{50} values of 600, 450 and 730 mg/kg were reported for white mice, white rats and guinea pigs (strains unknown). Earlier, Fogelman et al. (1955) determined an LD_{50} of 505 mg/kg for male albino rats (strain unknown) receiving a single gavage dose of 5% weight per volume "TNB" in 0.5% methylcellulose. Moreover, clear-cut indications of cyanosis were described in addition to clinical and neurological effects. Necropsy of study animals that died during the seven-day observation period revealed hemorrhagic lungs, stained kidneys, and a darkening of the blood that was likely indicative of methemoglobin formation. Some of these features were also evident in survivors (number unstated) that were sacrificed at term. More recently, FitzGerald et al. (1991, 1992) conducted a number

of acute toxicological tests on military related compounds including 1,3,5-TNB. Five male and female Fischer 344 (F344) rats were exposed to 185, 260 and 335 mg/kg 1,3,5,-TNB in corn oil. Five male and female Swiss mice were similarly exposed to 500, 700 and 900 mg/kg. In addition to results from primary eye and dermal irritation tests, the average acute oral LD_{50} of 1,3,5-TNB for combined sexes was 284 mg/kg in rats and 804 mg/kg in mice (FitzGerald et al., 1991, 1992).

Some acute studies have been conducted with 1,3,5-TNB to investigate endpoints other than lethality. Watanabe et al. (1976) injected nine male Wistar rats intraperitoneally with 100 µmoles/kg 1,3,5-TNB in 2 ml/kg propylene glycol and found formation of methemoglobin in blood samples obtained five hours after dosing. In another study, four male F344 rats per group were given a single oral dose of 1,3,5-TNB (Chandra et al., 1995a). Blood was collected at 5 hours and 24 hours after dosing. The compound was administered in corn oil via oral gavage at doses of 35.5 or 71 mg/Kg. Methemoglobenemia was increased in blood collected 5 hours after administration of 1,3,5-TNB, although other hematological parameters were unchanged. Also, they found significant anemia with reduced red blood cells (RBC), hemoglobin, and hematocrit in rats receiving 1,3,5-TNB for four or ten days. Myers et al. (1999) administered a single oral dose of 50 mg/kg 1,3,5-TNB in corn oil to four male shrews (*Cryptotis parva*) and used gas chromatography/mass spectrometry to demonstrate the formation of hemoglobin adducts in blood samples obtained 24 hours after dosing. *In vitro* incubation of blood with 1,3,5-TNB also resulted in adduct formation; the data suggested a role for cysteine residues in hemoglobin-1,3,5-TNB binding.

Chandra et al. (1995b, 1997) administered a single dose of 1,3,5-TNB in corn oil by gavage to male F344 rats at 35.5 and 71 mg/kg to examine the acute toxicological effects of 1,3,5-TNB on brain and testicular morphology and histopathology. No effects of 1,3,5-TNB on these endpoints were seen in the single dose phase of these studies.

2.3.1.2 Mammalian Oral Toxicity – Subacute

Subacute studies involve repeated dosing of animals and parameters are measured at the end of a14-day study duration. Reddy et al. (1994a, 1996b), using five F344 rats/sex/group, conducted a 14-day oral toxicity study on 1,3,5-TNB to establish suitable dosing levels for longer-term studies. The compound was added to feed to obtain dietary concentrations of 0, 50, 200, 400, 800 and 1200 mg/kg. The respective doses, as calculated by the authors, were 0, 4.52, 16.85, 33.67, 55.76 and 91.93 mg/kg-day in males, and 0, 4.54, 17.5, 34.14, 59.08 and 79.35 mg/kg-day in females. Clinical signs were monitored twice daily, food and water consumption twice weekly, while body weights were recorded at the beginning, at termination, and weekly during the in-life phase of the study. A full suite of hematological and clinical chemistry parameters was assessed in blood samples obtained at necropsy. All tissues and major organs were observed for gross morphological lesions, and the weights of certain key organs were recorded. Samples from numerous internal organs and tissues were fixed and processed for

histopathological examination. Sections of sampled tissues from high-dose and control rats were examined under a light microscope, along with sections from all dose groups for potential target organs such as the spleen and kidney.

After treatment the body weights of both sexes of high-dose rats were reduced compared to controls, probably associated with a comparative reduction in food consumption in animals receiving 1,3,5-TNB. Some dose-related changes in organ weight/body weight ratio were evident, including comparative weight increases in brain, spleen and kidneys, and reductions in thymus and testes. The lowest dose at which effects were seen was 16.85 mg/kg-day, a level associated with relative increases in kidney weights in male rats. Though there were no obvious, treatment-related changes in clinical chemistry parameters, most hematological indices were altered compared to controls after 14 days of treatment. For example, a statistically significant and dose-dependent reduction in hematocrit and red blood cell count was observed in all female groups and in males that received at least 55.76 mg/kg-day (RBC decrease) and 33.67 mg/kg-day (hematocrit decrease). Reduced hemoglobin concentrations were observed in female rats that received 59.08 and 79.35 mg/kg-day 1,3,5-TNB. Conversely, methemoglobin increased in a dose dependent manner in both sexes at a dose of approximately 34 mg/kg-day and higher.

There were gross pathological signs in the testes of male rats dosed at 55.76 mg/kg-day and higher, effects that were probably associated with the overall reduction in testicular size. The lesions were marked by moderate to severe seminiferous tubular degeneration, fewer mature spermatids and an apparent reduction in spermatogenic cells. Cell debris and some multinucleate cells were observed in a generally restricted tubular lumen. Another consistent histopathological response to 1,3,5-TNB was the appearance of hyaline droplets in the cortical tubule cells in the kidneys of male rats receiving 16.85 mg/kg-day and above. As described by the authors, many of these large, irregular-shaped droplets were associated with the onset of tubular degeneration (Reddy et al., 1994a, 1996b).

A number of no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were evident from the results of this study. For example, a NOAEL of 4.52 mg/kg-day and a LOAEL of 16.85 mg/kg-day corresponded to the histopathological changes in the kidney in male rats (cortical tubular hyaline droplet formation) that were described as occurring in those animals receiving 200 mg 1,3,5-TNB/kg diet and above. However, the most sensitive indices of toxicity were the hematological changes evident in female rats at the lowest dose, which suggests a LOAEL of 4.54 mg/kg-day for these responses (see Table 2).

In another study, four male F344 rats/group received gavage doses of 35.5 and 71 mg/kg 1,3,5-TNB in corn oil for either 4 or 10 days (Chandra et al., 1995a). Blood was collected daily and showed hematological changes similar to those described by Reddy et al. (1994a, 1996b). Using the same protocol, Chandra et al. (1995b) also observed a range of histopathological lesions in the brain of high-dose rats, including gliosis, vacuole formation, malacia, demyelination, edema and hemorrhages, and the

formation of petechial hemorrhages in the cerebellar peduncles. No such responses were observed in rats receiving 1,3,5-TNB at 35.5 mg/kg-day. Also, histopathological changes and reductions in testis weights were apparent in male F344 rats, with complete cessation of spermatogenesis in those receiving 71 mg/kg for 10 days (Chandra et al., 1997).

Twelve male and female F344 rats/group and 12 male NCI-Black Reiter (NBR) rats/group were gavaged for 10 days with 1,3,5-TNB in corn oil at 0, 35.5 and 71 mg/kg. Other groups of male F344 rats received 35.5 mg/kg for 20 or 30 days (Kim et al., 1997). The endpoints of the study were the relative incidence of hyaline droplets in the kidney among the groups and an evaluation of the role of the $\alpha 2_{\mu}$-globulin protein in droplet formation. There was dose-dependent hyaline droplet formation in the kidneys of male F344 rats but hyaline droplets were absent from kidney sections of NBR and female F344 rats that had received the same treatments. Immunohistochemical staining with anti-$\alpha 2_{\mu}$-globulin protein anti-serum was apparent in droplet-accumulating regions of the kidneys of affected animals, though mostly confined to the renal proximal convoluted tubular epithelial cells. Hyaline droplets and $\alpha 2_{\mu}$-globulin-positive staining areas were concomitantly absent from reference groups such as treatment-negative controls and female F344 rats and male NBR rats receiving 1,3,5-TNB-treatment negative controls. These observations suggest the possible importance of $\alpha 2_{\mu}$-globulin-related hyaline droplet formation in tubular necrosis and subsequent cellular proliferation of kidneys in male F344 rats dosed with 1,3,5-TNB. They do not, however, address the mechanism by which 1,3,5-TNB induces an increase in kidney weight observed in female F344 and male NBR rats.

Immunohistochemical staining of excised brains was used to evaluate the integrity of the blood-brain barrier in F344 rats challenged with 1,3,5-TNB (Chandra et al., 1999). Five male F344 rats/group were gavaged with 1,3,5-TNB in corn oil at 71 mg/kg for 4, 5, 6, 7, 8 or 10 days with one dose per day. The experiments were carried out to determine whether the vascular bed mediates the pathogenesis of 1,3,5-TNB-induced encephalopathy by comparing the extent of extravasated plasma albumin between treated and control groups. Animals receiving 10 daily doses of 1,3,5-TNB showed an increased intensity and extravascular distribution of immunoreactive albumin in the cerebellum and other parts of the brain, while controls and treated animals granted a recovery period were comparatively unaffected. The authors considered the incidence of vasogenic brain edema to be a critical event in 1,3,5-TNB neurotoxicity.

A fourteen day toxicity evaluation of TNB was conducted in the shrew, *Cryptotis parva*, by feeding 10 animals/group/sex a diet containing 0, 5, 10, 20, and 40 mg/Kg of 1,3,5-TNB (Reddy et al., 2000). The calculated average consumed doses were 0, 10.68, 22.24, 37.79 and 98.27 mg/kg/day for males and 0, 10.75, 21.61, 45.26 and 98.72 mg/kg/day for females. Food and water consumption, body weight and organ weights were measured and hematological and histopathological changes were studied. There were no significant differences in food and water consumption and hematological parameters between control and 1,3,5-TNB fed shrews. The NOAEL for both sexes

of shrews was 10.68 mg/kg/day for the oral 14-day study. The LOAEL was 21.6 mg/kg/day for the decreased body and liver weights in males and increased spleen weight in females. (Reddy et al., 2000).

2.3.1.3 Mammalian Oral Toxicity – Subchronic

Reddy et al. (1994b, 1998) described a 90-day toxicological study in 15 F344 rats/sex/group receiving 0, 66.7, 400, or 800 mg 1,3,5-TNB/kg diet, which corresponded to doses, as calculated by the authors, of 0, 3.91, 22.73, and 44.16 mg/kg-day in males and 0, 4.29, 24.7, and 49.28 mg/kg-day in females. A full range of in-life, clinical chemistry/hematological, gross pathological and histopathological evaluations were carried out as previously described for the 14-day study by Reddy et al.(1994a, 1996b).

Critical findings included the formation of kidney lesions characterized by hyaline droplet formation, cortical tubular degeneration and respondent regeneration of tubular cells. These effects were evident in males at all doses. Other responses included methemoglobinemia and erythroid cell hyperplasia in the spleen of high-dose and mid-dose groups (both sexes) and decreased testicular weight with accompanying seminiferous tubular degeneration. Body weight was also significantly less than corresponding controls in males and females at the two highest doses of 1,3,5-TNB. Based on their data the authors considered the most sensitive endpoint to be the kidney lesions evident in male F344 rats and assigned the 3.91 mg/kg-day dose as the subchronic LOAEL for 1,3,5-TNB.

Narayan et al. (1995) investigated the effects of subchronic administration of 1,3,5-TNB on the levels of neurotransmitters and their metabolites in the brain of Sprague-Dawley rats. In a complex protocol, six animals/sex/group received various dietary amounts of 1,3,5-TNB between 0 and 800 mg/kg, equivalent to daily doses of 0, 3, 23 and 51 mg/kg-day in males and 0, 4, 30 and 60 mg/kg-day in females, as calculated by the authors. Exposure duration was 90 days in females and 28 days in males. At necropsy the authors dissected the excised brains into discrete regions, including brain stem, frontal cortex, cerebral cortex, caudate nucleus, septum, hypothalamus, thalamus, hippocampus and cerebellum. Among the neurotransmitters measured were norepinephrine, epinephrine, dopamine, 5-hydroxytryptomine, homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoacetic acid.

Compared to controls, levels of some neurotransmitters changed dramatically with treatment. For example, the specific concentration of norepinephrine in the septum increased at least tenfold in both sexes at the lowest dose. Effects such as this suggested a LOAEL of 3 mg/kg-day.

As discussed by Reddy et al. (1997) and on IRIS (U.S. EPA, 2000), Reddy et al. (1995) published an abstract of a meeting presentation in which a 90-day study of 1,3,5-TNB in the White-footed mouse (*Peromyscus leucopus*) was described. Ten animals/sex/group were fed diets of 0, 150, 375 and 750 mg 1,3,5-TNB/kg, with corresponding dose levels calculated to be 0, 23.5, 67.4 and 113.5 mg/kg-day in males and 0, 20, 65 and 108 mg/kg-day in females.

Results from this study included erythroid hyperplasia of the spleen, increased reticulocytes in mid- and high-dose males, and testicular degeneration in the high-dose males, among other effects. Based on the statistically significant change in reticulocyte count in the mid- and high-dose males, 23.5 mg/kg-day is a subchronic NOAEL for 1,3,5-TNB in *Peromyscus*, as suggested in the review by Reddy et al. (1997). This choice differs from a subchronic NOAEL of 67.4 mg/kg-day that was chosen by Talmage et al. (1999) from data on the 1,3,5-TNB-induced testicular effects observed in high-dose animals in the same study. However, both levels of response suggest that the White-footed mouse is less sensitive to the toxic effects of 1,3,5-TNB than F344 or Sprague-Dawley rats.

2.3.1.4 Mammalian Oral Toxicity – Chronic

Reddy et al. (1996a, 2001) used the 14-day and 90-day studies on the toxicity of 1,3,5-TNB in F344 rats to determine suitable dose levels of the compound for a 2-year, chronic toxicity study. Using 60 rats/sex for control groups and 75 rats/sex for test groups, the authors carried out a full range of in-life, clinical chemistry/hematological, gross pathological and histopathological evaluations similar to those described above for their 14-day and 90-day studies (Reddy et al., 1994a, 1994b, 1996b, 1998). However, 10 animals/sex/group were sacrificed after 3, 6, and 12 months for a complete interim histopathological examination of target organs. The levels of 1,3,5-TNB in diet were 0, 5, 60 and 300 mg/kg, which corresponded to calculated dose levels of 0, 0.22, 2.64, and 13.44 in males and 0, 0.23, 2.68, and 13.31 mg/kg-day in females.

Among the key findings, Reddy et al. (1996a, 2001) observed an increased formation of methemoglobin compared to controls in the high-dose groups of either sex. Mid- and high-dose females showed reduced mean corpuscular hemoglobin (MCH), among other hematological fluctuations. While there were no gross necropsy findings related to treatment, histopathological examination of the kidney revealed a myriad of lesions, some probably related to changes in the organ that occur normally in aged F344 rats and some probably related to treatment. For example, necrosis of the cortical tubular cells and formation of tubular cytoplasmic droplets were evident in the mid- and high-dose groups of both sexes. Notwithstanding their appearance in both sexes of F344 rats, these lesions were described by the authors as being "hyaline," large and mostly spheroid in shape and rendered visible with Mallory's Heidenbain protein stain. Immunohistochemical binding to the $\alpha 2_{\mu}$-globulin protein revealed a positive pattern of staining that had a diffuse pattern across the field of view and not necessarily associated with the droplets. The authors concluded that a diagnosis of $\alpha 2_{\mu}$-globulin-related nephropathy would probably be incorrect since there appeared to be no significant increase in tubular cell necrosis. In addition, there were no signs of the granular casts, linear papillary mineralization and tubular hyperplasia that are typical manifestations of this condition.

Increased splenic erythroid cell hyperplasia and pigment deposition were present in mid- and high-dose groups in the interim sacrifices but were restricted to high-dose groups in the 2-year survivors. This suggested that, after 2 years, animals had compensated for the regenerative anemia previously noted in the results from interim sacrifices.

In addition to the effects of 1,3,5-TNB on hematological and histopathological parameters, adults of both sexes at the highest dose were significantly smaller than controls. Body weight at two years was significantly affected by 1,3,5-TNB in both males and females. Decreased body weight was likely the result of decreased food consumption seen in both sexes for all doses of 1,3,5-TNB. However, the effect of the compound on food consumption was significant only for males at the intermediate and high dose levels. It is also possible that smaller body size in 1,3,5-TNB dosed rats may have been the result of alterations in the energy budget of rats resulting from compound-induced toxicity.

The authors considered the methemoglobinemia and splenic erythroid cell hyperplasia with pigment deposition to be the critical effects of 1,3,5-TNB and suggested that a NOAEL of 2.68 mg/kg-day would be appropriate (Reddy et al., 1996a, 2001). This conclusion was set forth in a short review by Reddy et al. (1997) and adopted by the IRIS compilers in their derivation of a oral toxicity reference dose (human health) for 1,3,5-TNB of 3×10^{-2} mg/kg-day (U.S. EPA, 2000).

Reddy et al. (1997) and U.S. EPA (2000) cite a developmental study in Sprague-Dawley rats in which 1,3,5-TNB was administered at 0, 11.25, 22.5, 45 and 90 mg/kg-day by gavage in 1% agar to an unstated number of pregnant females during the major period of organogenesis (gestation days 6–15) (Cooper and Caldwell, 1995). Adverse clinical signs were apparent among the dams, and a number of developmental effects were noted among the pups of dams receiving the highest dose. These included reduced mean fetal weight and crown-rump length, with skeletal variations apparently noted in one animal. These results suggest a developmental NOAEL of 45 mg/kg-day.

2.3.1.5 Mammalian Oral Toxicity – Other

Sprague Dawley rats were used to examine the reproductive impacts of dietary exposure to 1,3,5-TNB (Kinkead et al., 1994a). Ten animals/sex/group received 0, 70, 400 or 800 mg 1,3,5-TNB/kg diet, levels calculated by the authors to result in average doses of 0, 3, 23 and 51 mg/kg-day in males and 0, 4, 30 and 60 mg/kg-day in females (0, 8, 55 and 110 mg/kg-day during lactation). Male rats were dosed for 14 days prior to mating, and then throughout the mating period for a total of 35 days. Female rats were dosed for 14 days prior to mating, and then throughout mating, gestation and lactation, with an additional week post-weaning for a total of 70 days. Pups were maintained on the group-specific treated diet for a total of 7–14 days post-weaning. F_0 male rats were necropsied at termination with sperm count and morphology evaluated in two or three males/group at sacrifice. Testes and epididymides were weighed and examined

histopathologically. F_0 female rats were necropsied at termination with pieces of spleen, kidney and liver processed for histopathological evaluation. Blood samples were taken from all groups at termination for methemoglobin analysis. Measures of reproductive performance were noted, including the copulation and fertilization indices and the number of live and dead pups at birth.

A number of the effects of 1,3,5-TNB in this experiment were consistent with those observed in earlier studies described above. For example, there was evidence of enlarged and discolored spleens with hemisiderosis in high- and mid-dose females, testicular atrophy and spermatogenic deficits in high-dose males, and signs of encephalitis in high- and mid-dose females. Neurological signs, for example, head tilt and loss of equilibrium, were observed in lactating dams. Perhaps the most novel findings of the study related to reproductive performance, which, for copulation and fertility indices, were normal, notwithstanding the spermatogenic deficits referred to above. Compared to controls, there were no differences in the F_1 sex ratios and offspring/litter among the groups, although the number of surviving pups after 4 and 21 days were lower than controls in the high-dose group. More than one potential NOAEL can be derived from the results described by Kinkead et al. (1994a). For example, a NOAEL of 4 mg/kg-day may be protective against 1,3,5-TNB-induced encephalitis in female rats (an effect that was evident in mid- and high-dose groups, only), while a NOAEL of 23 mg/kg-day may protect against the testicular atrophy that was evident in high-dose males.

In another reproductive study conducted by the same research group, dietary amounts of 0, 30, 150 and 300 mg 1,3,5-TNB/kg were administered to 18 male and 12 female Sprague-Dawley rats/group for up to 90 days (Kinkead et al., 1994b, 1995). Animals were divided into subgroups to accommodate a number of pre-mating, post-mating and recovery dosing regimen. The doses, as calculated by the authors, were 0, 2, 9 and 19 mg/kg-day for males and 0, 3, 14 and 29 mg/kg-day for females. Subsets of animals were subjected to Opto-Varimex open-field activity evaluation tests, though with no evidence of compound-related depletions in motor skills. At necropsy, samples of blood were taken for clinical chemistry/hematological evaluation. Organ weights were monitored and histopathological evaluations were made of the pituitary, spleen, liver, kidneys, bone marrow and reproductive organs. Sperm counts and motility were measured in comparison to various indices of reproductive performance.

Among the key findings were decreases in sperm motility and degeneration of the seminiferous tubules of the testes in mid- and high-dose males and nephropathy/hyaline droplet formation in males at all dose levels. Methemoglobin was formed in the mid- and high-dose groups for both sexes, although these levels returned to normal in a subset of males allowed to recover after treatment. Splenic hemosiderosis was noted in mid- and high-dose groups of both sexes. By analogy to the findings of Kinkead et al. (1994a), there were no adverse effects of 1,3,5-TNB on mating or fertility, gestation length, sex ratio, or the number of offspring/litter. However, during lactation the body weights of pups borne to high-dose parents were reduced compared to controls. From these data a discriminating index of toxicity

appeared to be the changes in sperm morphology and motility to which a NOAEL of 2 mg/kg-day and a LOAEL of 9 mg/kg-day could be assigned. However, 2 mg/kg-day would be a LOAEL for the hyaline droplet-related nephropathy that was evident in male rats at all 1,3,5-TNB dose levels.

2.3.1.6 Studies Relevant to Mammalian TRV Development for Oral Exposures

In the experimental studies described in this review, the overwhelming majority were carried out in laboratory rats, predominantly F344 and Sprague-Dawley strains. Less frequently employed species included the white-footed mouse and the shrew, with no studies identified in birds, reptiles or amphibians. The principal toxicological effects of 1,3,5-TNB are displayed in Table 2 and Figure 1. Responses to 1,3,5-TNB emerging from experimental studies in laboratory animals included (1) nephropathy associated with $\alpha 2_{\mu}$-globulin-associated hyaline droplet formation in male rats (2) atrophy of the testis with associated degeneration of the seminiferous tubules and sperm deficits, (3) structural and functional impairment of the brain, (4) methemoglobin formation, and (5) reduced body weight. These effects are discussed below, in the context of relevancy to TRV derivation.

Nephropathic changes associated with hyaline droplet formation and $\alpha 2_{\mu}$-globulin formation in male rats appear to be a sensitive indicator of 1,3,5-TNB toxicity since no clear-cut NOAEL could be identified for this response in any of the evaluated studies. For example, in Sprague-Dawley rats exposed to the compound for up to 90 days, the lowest dose tested (2.0 mg/kg-day) was identified as a subchronic LOAEL for this response (Kinkead et al., 1994b, 1995). This is a lower value than the chronic NOAEL of 2.68 mg/kg-day emerging from the 2-year study on 1,3,5-TNB with methemoglobinemia specified as the principal effect (Reddy et al., 1996a, 1997, 2001; U.S. EPA, 2000). Unfortunately, data from the 2-year study on 1,3,5-TNB may be unsuitable to shed light on the issue of a chronic NOAEL for $\alpha 2_{\mu}$-globulin-associated hyaline droplet formation and related nephropathy in males, since Reddy et al. (1996a, 2001) described incipient nephropathy and droplet formation in the kidneys of *both* sexes of mid- and high-dose F344 rats. They considered this effect to be inappropriate for a diagnosis of $\alpha 2_{\mu}$-globulin-associated nephropathy in this study, even though gender-specific hyaline droplet formation had been a consistent feature of the toxicological impacts of 1,3,5-TNB in F344 rats (Reddy et al., 1994a,1994b, 1996b, 1998). Since the precise nature and biological significance of the droplets remains uncertain, this endpoint cannot be used for the derivation of a TRV for 1,3,5-TNB.

Impairment of the male reproductive organs with associated decreases in sperm production and motility is a consistent response of experimental animals to nitroaromatic compounds including 1,3,5-TNB. However, similar to the methemoglobinemia, this response appears to be at least partially reversible on cessation of exposure. Moreover, there was no significant effect of 1,3,5-TNB on reproductive performance (Kinkead et al., 1994a,b, 1995). This suggests that these responses may not be critical when deriving quantitative benchmarks for wildlife protection.

Methemoglobinemia can create a functional hypoxic blood condition. Carbon monoxide poisoning also creates a functionally similar condition. Humans with levels of COHb above 10% have reported symptoms of headache while other adverse effects, such as decreased psychomotor performance, were reported when COHb concentrations exceeded 2% (ACGIH 1997). Chronic congenital methemoglobinemia in humans has been found where 10-50% of circulating blood pigment is in the form of methemoglobin with subjects exhibiting no overt signs of toxicity (Smith 1996). In chronic toxicity study, Reddy et al. (1996a, 2001) report differences between control and high dose rats of less than 2%. Given the uncertainty associated with the reported methemoglobin increase in these investigations, increased methemoglobinemia due to 1,3,5-TNB was not considered biologically significant.

Histopathological lesions in various parts of the brain are a consistent feature of responses to 1,3,5-TNB on the part of experimental animals. However, the mechanisms by which such responses are brought about are poorly understood, although the induction of an imbalance in neurotransmitters and their metabolites may play at least a partial role in bringing about these effects (Narayan et al., 1995). No clear links between these histopathological lesions and impaired health or performance were established. Hence, the TRV cannot be based on this response.

Reduced body weight was found in 1,3,5-TNB exposed F344 rats under both subchronic (Reddy et al., 1994b, 1998) and chronic study regimes (Reddy et al., 1996a, 2001). Both studies were high quality with respect to experimental design and execution. A diminution in growth rate may have ecological ramifications by, for example, causing a delay in time to maturity or increasing risk of predation. Reduced somatic growth may not be a direct effect of exposure to 1,3,5-TNB as decreased food consumption was a common response. However, it can be argued that decreased food consumption, as a result of avoidance or direct toxicity, will compromise an organisms function and hence will likely result in altered health or performance. Given the prevalence of reduced body size in 1,3,5-TNB exposed mammals and the likely ecological ramifications of this effect, this endpoint was selected for derivation of the TRV based on data from the chronic study by Reddy et al. (1996a, 2001).

Taken together, the experimental findings summarized in this report present a consistent picture of the overall toxicity of 1,3,5-TNB in experimental animals, with dose levels for toxic effects in the 2–5 mg/kg-day region. Available evidence suggests that the White-footed mouse may be less sensitive to the toxicological effects of 1,3,5-TNB than F344 or Sprague-Dawley rats, although Talmage et al. (1999) used the experimentally derived NOAELs from F344 rats (Reddy et al., 1996a, 2001) and White-footed mice (Reddy et al., 1995) to derive reasonably consistent chronic screening criteria for several wildlife species based on feeding pattern estimates and size comparisons.

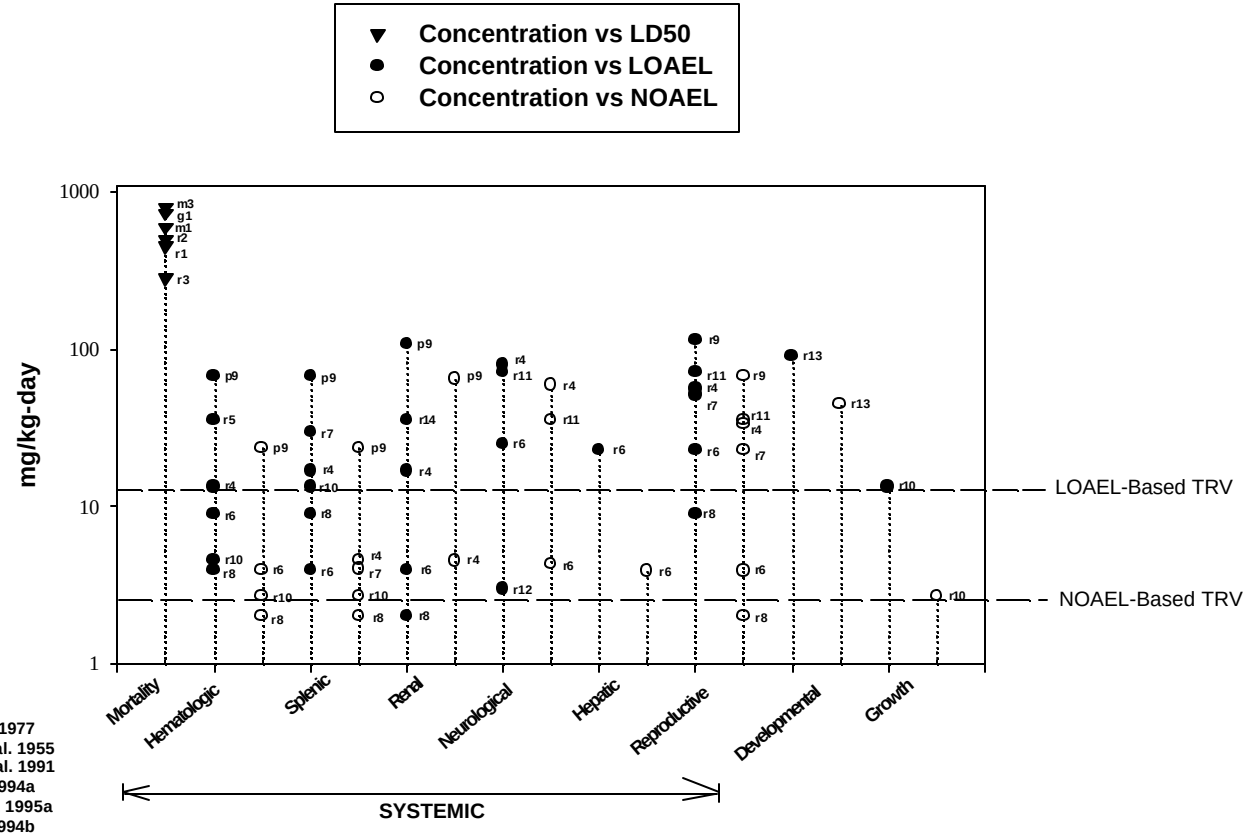
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Reddy et al. (1996a ; 2001)	Rat (Fischer 344)	2-y	2.68	13.31	Methemoglobinemia, spleen erythroid cell hyperplasia. Decreased body weight.
Reddy et al. (1994b; 1998)	Rat (Fischer 344)	90-d	NA	3.91 (m)	Nephropathy/$\alpha 2_{\mu}$-globulin-associated hyaline droplet formation in males at all doses.
			4.29	22.73	Methemoglobinemia, spleen erythroid cell hyperplasia in high-dose and mid-dose groups (both sexes).
Reddy et al. (1994a)	Rat (Fischer 344)	14-d	NA	4.54 (f)	Reduced erythrocyte count and hematocrit in all female groups.
			4.52 (m)	16.85 (m)	Histopathological changes to the kidney in males.
Kinkead et al. (1994b; 1995)	Rat (Sprague-Dawley)	90-d	2.0 (m)	9.0 (m)	Sperm motility/seminiferous tubular degeneration of the testes.
			NA	2.0 (m)	Nephropathy/hyaline droplet formation in males at all doses.
Kim et al. (1997)	Rat (Fischer 344)	10-, 20- and 30-d	NA	35.5 (m)	Nephropathy/$\alpha 2_{\mu}$-globulin-associated hyaline droplet formation in males at all doses.
Narayan et al. (1995)	Rat (Sprague-Dawley)	90-d	NA	3.0	Increase in tissue concentration of various neurotransmitters in several regions of the brain, potentially associated with neurological disorders and histopathological lesions.
Kinkead et al. (1994a)	Rat (Sprague-Dawley)	7-w (m)	23	51	Testicular degeneration and sperm depletion in males.
		10-w (f)	4	23	Encephalitis in females.
Chandra et al. (1995a)	Rat (Fischer 344)	10-d	NA	35.5 (m)	Hematological deficits and methemoglobin formation.
Chandra et al. (1995b)	Rat (Fischer 344)	10-d	35.5 (m)	71 (m)	Histopathological lesions in the brain.
Chandra et al. (1997)	Rat (Fischer 344)	10-d	NA	35.5 (m)	Degenerative changes to the testes.
Cooper and Caldwell (1995)	Rat (Sprague-Dawley)	GDs 6–15	45 (f)	90 (f)	Developmental deficits among the pups.
Reddy et al. (1995)	Mouse (*Peromyscus leucopus)*	90-d	67.4 (m)	113.5 (m)	Testicular degeneration in high-dose males.
			23.5 (m)	67.4 (m)	Erythroid hyperplasia, increase in reticulocyte count in mid- and high-dose males.
Reddy et al. (2000)	Shrew *(Cryptotis parva)*	14-d	10.75 (m)	21.60 (m)	Decrease in liver and body weight.
			10.68 (f)	22.24 (f)	Increase in spleen weight.

NA = not applicable
d = day
w = week
f = female
m = male

Figure 1.

1,3,5-TNB HEALTH EFFECTS TO MAMMALS



1 = Korolev et al. 1977
2 = Fogelman et al. 1955
3 = FitzGerald et al. 1991
4 = Reddy et al. 1994a
5 = Chandra et al. 1995a
6 = Reddy et al. 1994b
7 = Kinkead et al. 1994a
8 = Kinkead et al. 1994b
9 = Reddy et al. 1995
10 = Reddy et al. 1996, 2001
11 = Chandra et al. 1995b
12 = Narayan et al. 1995
13 = Cooper and Caldwell, 1995
14 = Kim et al. 1997

RAT (*Rattus*) = r
MOUSE (*Mus*) = m
GUINEA PIG (*Cavia*) = g
New World Mouse (*Peromyscus*) = p

2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using mammals were found.

2.3.3 Mammalian Dermal Toxicity

In vitro percutaneous absorption of 14C-TNB was studied using viable skin from hairless guinea pigs (HGP), F344 rats and human skin in assembled flow-through diffusion cells (Kraeling et al 1998). Results showed that absorption of 1,3,5-TNB in an acetone or water vehicle was rapid, occurring within 24 hrs. The absorption of 1,3,5-TNB in HGP skin was 72% in acetone and 82 % in water. The absorption of 1,3,5-TNB in rat skin was 61% in acetone and 66% in water. However the absorption of 1,3,5-TNB in human skin was 38% in acetone and 75% in water. These results show the absorption of 1,3,5-TNB in acetone was reduced in human skin when compared to rats and HGP. Although this study provided some insight into the percutaneous absorption of 1,3,5-TNB, it did not provide any data on toxicity of this compound when exposure occurs via the dermal route.

2.4 Summary of Avian Toxicology

Toxicological data for the effects of 1,3,5-TNB in avian species was not located. Ecotoxicological research on the effects of this compound in birds is recommended.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of 1,3,5-TNB in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of 1,3,5-TNB in reptilian species was not located. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES[1]

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Decreased body weight, an indication of a lower growth rate, was used to determine the TRV. Growth is an ecologically relevant parameter, which when altered, may affect future fitness. Data on female rats was used because these data were protective of males since females were exposed to slightly lower doses. Moreover, growth as an endpoint is also protective of adverse reproductive effects (Kinkead et al. 1994a,b). Growth data, as indicated by body size, also meet the minimum data requirements of the Standard Practice, Section 2.2 (USACHPPM 2000) and therefore no uncertainty factors are required in the derivation of the TRV. Derivation of the TRV was attempted using the Benchmark dose approach, however, model fit was unacceptable (Appendix B) due primarily to non-homogeneous variance. The NOAEL/LOAEL approach was used and the resulting TRVs are shown in Table 4. This TRV is given a medium confidence rating since there was only one chronic study in F344 rats. Moreover, limited mammalian wildlife toxicity data were available.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	2.68 mg/kg/d	Medium
LOAEL-based	13.31 mg/kg-d	Medium

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not Available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time

[1] TRVs are for screening purposes only and are not intended to be predictors of effects in field situations. Site specific conditions may justify adjustments of these values based on toxicity information relevant to specific assessment endpoints.

3.2 Toxicity Reference Values for Birds

Not available at this time

3.3 Toxicity Reference Values for Amphibians

Not Available at this time.

3.4 Toxicity Reference Values for Reptiles

Not Available at this time.

4. IMPORTANT RESEARCH NEEDS

Data regarding the toxicity of 1,3,5-TNB are from laboratory rodents, primarily rats, with a few studies on mammalian wildlife species. Hence, the most obvious research need is for studies on the toxicity of 1,3,5-TNB to avian, amphibian and reptile species. Currently, there are no data on these groups of organisms although they are likely to be present at contaminated sites and important components of local habitats. Toxicological testing on these types of organisms would provide data needed to develop TRVs for non-mammalian, ecological receptors.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

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

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

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

The search strategy for **Birds:**

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

The search strategy for **Laboratory Mammals:**

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

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers
- And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD

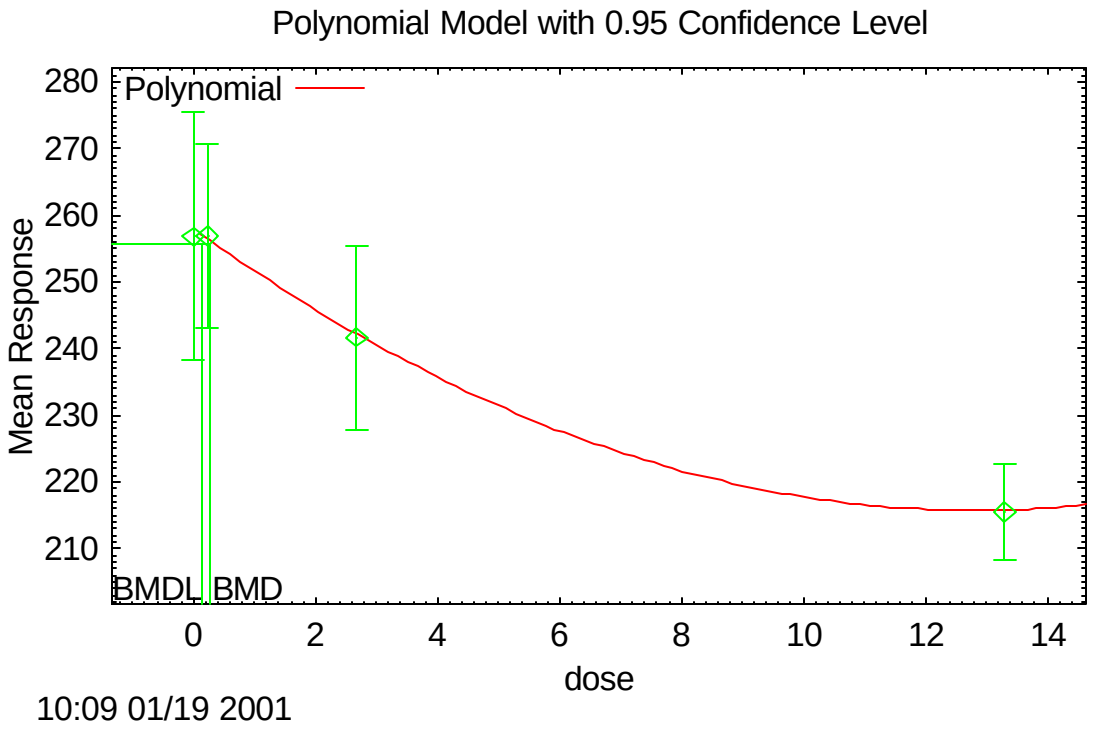
All abstracts from the Dialog search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 259 hits on 1,3,5-TNB were obtained in the initial search, of which 65 were selected for abstract evaluation. Forty-two of these articles and reviews were retrieved for this survey.

APPENDIX B

Benchmark Dose Calculation for Mammals

The data presented below are from Reddy et al. (1996a, 2001) with mean body weight at two years in Fischer 344 rats as the mean response. Data from females were used since the doses used for females were slightly lower than for males and there was a clear dose response. Although the model fit appears adequate, statistically, the model does not fit the data well enough to warrant use of this approach. Test 2 for the constant variance model indicated that variances were not homogenous and suggested using a non-constant variance model. However, the non-constant variance model indicated that the data did not fit the model (Test 3 rejected) and that the variances were inadequately modeled (Test 4 rejected). Both variance models were attempted with various distributions. Hence, it was concluded that the Benchmark Dose approach could not be satisfactorily used. Simulations using the Benchmark Dose Software indicate that another data point (i.e., dose) would likely permit an adequate fit of the data.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1

Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	380.894
rho =	0
beta_0 =	257.586
beta_1 =	-6.56575
beta_2 =	0.256109

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	176.196	0.0252302
rho	0.121769	24.6244
beta_0	257.624	0.341104
beta_1	-6.58872	2.33176
beta_2	0.25723	30.4537

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	beta_0	beta_1	beta_2
alpha	1	1	0.00026	0.0027	0.0027
rho	1	1	0.00022	0.0026	0.0026
beta_0	0.00026	0.00022	1	0.6	0.52
beta_1	0.0027	0.0026	0.6	1	0.99
beta_2	0.0027	0.0026	0.52	0.99	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	10	257	26	258	18.6	-0.0427
0.23	10	257	19.3	256	18.6	0.0434
2.68	10	242	19.3	242	18.5	-0.00398
13.31	10	216	10.1	215	18.4	0.00391

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)

Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-136.743	5	283.486
A2	-132.792	8	281.585
A3	-477.62	6	967.241
fitted	-136.682	5	283.364
R	-149.344	2	302.687

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.1024	6	<.0001
Test 2	7.90151	3	0.04809
Test 3	689.656	2	<.0001
Test 4	-681.877	1	<.0001

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .05. You may want to consider a different variance model

The p-value for Test 4 is less than .05. You may want to try a different model

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.950000

BMD = 0.285666

BMDL = 0.151617

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted

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

USCHPPM

Wildlife Toxicity Assessment for 1,3,5-Trinitrohexahydro-1,3,5-Triazine (RDX)

JULY 2002

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 37-EJ-1138-01H

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FINAL REPORT
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Approved for Public Release; Distribution Unlimited

Acknowledgements

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When referencing this document use the following citation
Salice, C.J. and G.Holdsworth. 2001. Wildlife Toxicity Assessment for 1,3,5-Trinitrohexahydro-1,3,5-Triazine (RDX). U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Project Number 39-EJ1138-01B, Aberdeen Proving Ground, Maryland, May 2002.

Table of Contents

Wildlife Toxicity Assessment for RDX

CAS No.121-82-4 **DRAFT**

1. INTRODUCTION

The explosive 1,3,5-Trinitrohexahydro-1,3,5-Triazine (CAS No. 121-82-4) is more frequently known as RDX (Royal Demolition Explosive). RDX is an explosive chemical that has found widespread application in detonators, grenades, bombs and a variety of other military ordnance. Structurally, the compound is a trinitro-substituted triazine with the empirical formula, $C_3H_6N_6O_6$. In addition to RDX, other synonyms include: 1,3,5-triaza-1,3,5-trinitrocyclohexane, hexahydro-1,3,5-trinitro-1,3,5-triazine, cyclotrimethylenenitramine, hexogen, cyclonite, among others (ATSDR, 1995). The importance of RDX as an environmental contaminant is related to its widespread distribution at and around military sites and its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of RDX on wildlife and reports toxicity reference values (TRVs) for RDX. The TRVs are intended to serve as protective exposure standards for wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254 (TG254), the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were searched electronically August 23, 2000, using Dialog® to identify primary reports of studies and reviews on the toxicology of RDX. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. All available abstracts of articles selected as potentially relevant to TRV development were further evaluated using criteria outlined in TG254 (USACHPPM, 2000). For RDX, 19 articles were marked for retrieval from 31 initial hits. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to searching the Dialog Inc. database, a number of U.S. Army reports were identified in the Defense Technical Information Center (DTIC). Secondary references and sources of information on RDX included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for RDX* (ATSDR, 1995), the National Library of Medicine's Hazardous Substances Databank (HSDB,

2000), the U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997).

2.2 Environmental Fate and Transport

Military grade RDX (containing about 10% high melting explosive (HMX) by weight) has been a widely used explosive since the early years of World War II, when it began to either replace or supplement trinitrotoluene (TNT) as the primary ingredient in shells, bombs and detonators. Although the compound is currently manufactured only at the Holston Army Ammunition Plant (AAP) in Kingsport, Tennessee, a pattern of manufacturing and assembling practices has resulted in its release to the environment in considerable amounts at this and other sites, either as a single compound or mixed with other explosives. Talmage et al. (1999) reported that concentrations of up to 30 mg/L RDX had been detected in groundwater at Milan AAP, while surface water impoundments and associated sediments at this facility also displayed concentrations in the ppm range. Soil concentrations of up to 13,900 mg RDX/kg are listed by Talmage et al. (1999) for this and other military sites. Physicochemical properties of RDX relevant to its environmental fate and transport are listed in Table 1.

Table 1. Summary of Physical-Chemical Properties of RDX

Molecular weight	222.26
Color	White
Physical state	crystalline solid
Melting point	205–206 °C
Boiling point	decomposes
Odor	no data
Solubility Water	38.4 mg/L; slightly soluble in methanol, ether, ethyl acetate, glacial acetic acid
Partition coefficients:	
Log K_{ow}	0.87
Log K_{oc}	0.84–2.2
Vapor pressure at 20 °C	1.0×10^{-9}, 4.0×10^{-9} mm Hg
Henry's Law constant	1.2×10^{-5} atm.m3/mole
Conversion factors	1 ppm = 9.1 mg/m^3
	1 mg/m^3 = 0.11 ppm

Sources: ATSDR, 1995; Talmage et al., 1999; HSDB, 2000

RDX has an estimated vapor pressure of $1–4 \times 10^{-9}$ mm Hg at 25°C, a low value implying that partitioning to air is unlikely. Furthermore, the compound is soluble only to a limited extent in a number of common organic solvents and in water (38.4 mg/L at 20–25°C). However, despite its limited

solubility, the compound has been detected in both surface water and groundwater (see Talmage et al. 1999 for review). Hovatter et al. (1997) and Talmage et al. (1999) also present RDX soil concentration data from other studies for a number of AAPs, depots and arsenals.

Photolysis is a potentially important process for degrading RDX, since the compound can absorb ultraviolet light strongly at wavelengths between 240 and 250 nm. In addition, biodegradation of RDX has been demonstrated under anaerobic conditions in the presence of a number of microbial isolates and mixed cultures, with total degredation in 5 days or less. Thus, when RDX was incubated in an anaerobic test system containing sewage sludge and mixed cultures in nutrient broth, the disappearance of RDX was accompanied by the formation of a range of metabolites including hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hydrazine, 1,1-dimethyl-hydrazine, 1,2-dimethyl-hydrazine, formaldehyde and methanol (McCormick et al., 1981, 1984).

Studies have shown that plants are able to absorb RDX from soil and to a lesser degree from irrigation water. Radio-labeled RDX accumulated in bush bean grown on RDX amended soil with the highest concentration of RDX in the seeds followed, in order of decreasing concentration, by leaves, stems, roots and pods (Cataldo et al., 1990). Concentration ratios were on the order of 20 to 60 for seeds and leaves, which suggests an efficient uptake mechanism and high plant mobility. Analysis of bush bean grown in RDX amended hydroponic solution showed that approximately 23 and 50% of the rabio-label present in the root and leaves, respectively, was parent RDX after a 7-day exposure (Harvey et al., 1991). The efficiency of RDX absorption varies with species and is inversely proportional to organic matter content of the soil. Studies on uptake of RDX from spiked irrigation water showed a lower uptake of RDX by tomato, bush bean, corn, soybean, alfalfa, lettuce and radish (Checkai and Simini, 1996). Concentrations of RDX in the plants were less than that of the irrigation water. Thus, research to date indicates that plant uptake of RDX is highest from RDX contaminated soils and, importantly, that RDX in plants can be a potential exposure route for herbivorous terrestrial wildlife.

2.3 Mammalian Toxicity

2.3.1 Mammalian Toxicity - Oral

2.3.1.1 Mammalian Oral Toxicity – Acute

Dilley et al. (1978) conducted a number of toxicological tests on TNT, RDX, and a mixture of the two compounds styled “LAP” (for load, assembly and pack wastewater), employing mixtures of each compound in corn oil that were administered to the test animals by gavage. Mortality and clinical parameters were observed on all survivors for 14 days prior to termination. The acute oral LD_{50} for RDX in male Sprague-Dawley rats was 71 mg/kg-day compared to values of 1320 and 574 for TNT and LAP,

respectively. Dilley et al. (1978) reported respective acute oral LD_{50}s of <75 and 86 mg/kg-day for RDX in male and female Swiss-Webster mice, compared to 660 mg/kg-day for TNT in either sex of mouse and 947 and 1131 mg/kg-day for LAP in male and female mice, respectively.

Data on the acute oral lethality of RDX in experimental animals were provided also by Cholakis et al. (1980), who determined LD_{50}s in male and female F344 rats (10/sex/group) and B6C3F1 mice (5/sex/group) given single doses of the compound by gavage in either 1% aqueous methylcellulose (rats) or a mixture of 1% methylcellulose and 1% polysorbate 80 (mice). Broadly consistent with the findings of Dilley et al. (1978), Cholakis et al. (1980) derived a combined (male/female) acute oral LD_{50} of 118.1 mg RDX/kg-day in F344 rats and 80.3 mg/kg-day in the mice. Overall, the acute lethality data on RDX of Dilley et al. (1978) and Cholakis et al. (1980) have yielded lower values than the LD_{50} of 200 mg/kg-day obtained for the compound in the earlier studies of von Oettingen et al. (1949).

In addition to acute oral lethality, single dose experiments with RDX have been used to determine the toxicokinetic behavior of the compound in experimental animals. For example, Schneider et al. (1977) administered 100 mg RDX/kg by gavage to Sprague-Dawley rats (n = 70, sex not specified) and Pittman-Moore miniature swine (n = 10 female) and monitored the partitioning of the compound between feces, urine and the major organs and tissues. Only a small amount of RDX (less than 3%) was recovered in the feces of the rats, suggesting that the bulk of the material had been transported across the gastrointestinal absorption barrier. When 50 mg/kg ^{14}C RDX was administered to the rats, most of the radioactivity was found in the liver and urine after 24 hours, with further partitioning to other parts of the body during the next three days. Overall, 43% of the radioactivity was expired as $^{14}CO_2$.

Inferential support for the concept of the liver as a major catabolic site for RDX is provided by French et al. (1976) who, in a meeting abstract, reported profound ultrastructural changes in the liver of rats (strain, sex, number unstated) as a result of oral administration of a single dose of 100 mg RDX/kg. Among other membrane perturbations, the smooth endoplasmic reticulum was highly proliferated after 48 hours, possibly indicating the induction of the mixed function oxidase system. By contrast, ultrastructural changes to the kidney due to RDX were minor and inconsistent.

Although the liver appears likely to be the primary site of RDX catabolism, the compound or another pharmacologically active metabolite of RDX has the capacity to induce neurotoxicological responses in male and female Sprague-Dawley rats. Thus, in the acute section of a multiphase study, MacPhail et al. (1985) administered single gavage doses of up to 50 mg RDX/kg in 2% carboxymethylcellulose and observed overall decreases in such responses as startle-response amplitude, startle-response latency, figure-8 maize motor activity, conditioned flavor aversions and schedule-controlled responses.

2.3.1.2 Mammalian Toxicity – Subacute

Ferguson and McCain (1999) conducted a 14-day, subacute study on the oral toxicity of RDX to the white-footed mouse, *Peromyscus leucopus*. Ten male and ten female mice were in each of five RDX exposure groups. RDX was mixed with feed in concentrations of 0.00, 0.05, 0.10, 0.20, 0.40 and 0.80 mg RDX/g feed which corresponded to oral doses of 0, 8, 16, 31 and 59 mg RDX/kg body weight/day in males and 0, 8, 15, 32 and 68 mg RDX/kg body weight/day in females. Exposure continued until day 14 at which point, mice were euthanized by carbon dioxide asphyxiation. Data on feed consumption, body weight, organ weight, organ-to-body weight ratio and organ-to-brain weight ratios were collected and statistically analyzed. Blood samples were obtained and used for hematological and clinical chemistry analyses, however, the analyses could not be conducted so the data were unavailable. After examining tissues for gross pathological lesions, the liver, kidney, spleen, brain, thymus, and testes were collected and weighed from half of the animals in each group and submitted for histological examination. The same tissues, minus the spleen, thymus, and brain from the remaining animals were frozen and analyzed for biochemical parameters.

Results indicated very little compound-induced toxicity. In part, the authors attributed the lack of anticipated toxicity of RDX to *P. leucopus* to a higher metabolic rate and faster food transit time, which may increase the resiliency of this species compared to other *Mus* species. Mice exposed to RDX did show increased ovary, ovary-to-brain and ovary-to-body weights for the groups fed 0.05, 0.20 and 0.40 mg RDX/g feed. The effect, however, was not considered biologically significant since there was not a dose-response and the findings were unsupported by histological analyses. Similarly, liver weights were increased for females from the 0.05 and 0.20 exposure groups. Again, the finding was not considered biologically significant or compound related. Females in the two high dose groups showed an increase in spleen weight and spleen-to-brain weight ratios. Histopathological analyses did not reveal any treatment-related effects although the significant weight changes in the high dose group suggest possible RDX-induced toxicity. A potential NOAEL based on the weight change in the spleen is 16 mg RDX/ kg bw/day while a potential LOAEL is 31 mg RDX/kg bw/day.

2.3.1.3 Mammalian Toxicity – Subchronic

Mammalian species that have been used as models for testing the subchronic toxicological impact of RDX include beagle dogs, cyanomolgus (rhesus) monkeys, Sprague-Dawley and F344 rats and B6C3F1 and Swiss-Webster mice. Litton Bionetics (1974a) exposed three beagles/sex/group to 0.1, 1 or 10 mg RDX/kg-day as a dietary additive for 90 days. Urinalysis was carried out after four weeks, eight weeks and at term, along with clinical chemistry and hematological determinations in blood samples collected at the same intervals. All survivors were subjected to a gross necropsy at term, organ weights were recorded, and histopathological comparisons of the brain, thyroid, lungs, heart, liver, spleen, kidney,

adrenals, stomach, small intestine, and bone marrow were made between the high-dose and control groups. However, no abnormal findings in any measured parameter were noted at the doses chosen for the study.

Similarly, in another study by Litton Bionetics (1974b), the same exposure duration and dose levels (by gavage) as for the dogs showed the subchronic effects of RDX to be comparatively benign in rhesus monkeys. The appearance of increased numbers of degenerate or necrotic megakaryocytes in sections of bone marrow from some high-dose monkeys led to a dose of 1.0 mg/kg-day as a no observed adverse effect level (NOAEL) for this study. However, elevated numbers of megakaryocytes appeared in one of the three control animals examined for this feature, thereby suggesting the effect may not be compound-related.

Brown (1975) reported a study on rats (number and strain unstated) in which RDX was administered in the diet at doses of 0, 0.3, 2.5, 6.5, 15, 50 or 100 mg/kg-day for 12 weeks. Increased levels of RDX in the blood in response to all doses except the lowest were associated with increases in the specific activities of brain monoamine oxidase and cholinesterase and in the capacity of excised brain tissue to take up oxygen. Since these effects were negligible at the lowest dose, 0.3 mg/kg-day was chosen as a subchronic NOAEL for RDX.

The toxicological studies on RDX reported by Cholakis et al. (1980) featured 90-day studies in F344 rats and B6C3F1 mice in which 10 animals/sex/group were exposed to RDX in feed at doses of 0, 10, 14, 20, 28 and 40 mg/kg-day. In a supplemental study, additional mice were exposed to 0, 40, 60 and 80 mg/kg-day for 2 weeks and then to 0, 320, 160 and 80 mg/kg-day, respectively, for the final 11 weeks of the investigation. A suite of toxicological endpoints were monitored, including clinical signs, body weights and food consumption, clinical chemistry and hematological parameters, gross pathology and histopathology.

In the rats, there was a reduction in body weight gain in the high-dose males concomitant with a reduction in food consumption. In addition, sporadic though possibly compound-related hematological changes were noted, including a reduction in hemoglobin and hematocrit in high-dose males and males receiving 28 mg/kg-day after 30 and 60 days. Reticulocytes and platelets were increased in high-dose males after 90 days. There were few if any changes in clinical chemistry parameters, gross pathology or histopathology in the rats receiving RDX, findings that, taken together, suggest a NOAEL of 20 mg/kg-day based on the hematological changes.

The absence of any compound-related toxicological consequences of the same doses of RDX in exposed mice led to a supplemental study in which a number of sporadic responses were observed. For example, a number of clinical signs were evident across the groups, with marked hyperactivity among the males. Four of 10 high-dose males and 2/12 high-dose females died during week 11 of the study. Perhaps the most consistent treatment-related changes were observed at gross necropsy where dose-

dependent and statistically significant increases in absolute and relative liver weights were observed in both sexes of mice. These changes appeared to be associated with the onset of hepatocellular vacuolization and other histopathological liver lesions, supporting the designation of a NOAEL at the time-weighted average mid-dose of 145 mg/kg-day.

Levine et al. (1981) conducted a study similar to the 90-day study in F344 rats reported by Cholakis et al. (1980), but with dose levels extending to 600 mg/kg-day. At 600 mg/kg-day, most of the subjects developed tremors and convulsions followed by death. Less severe toxicological responses were evident at the lower dose levels, including a concomitant reduction in body weight gain and food consumption in males receiving 100 mg/kg-day. Among the compound-related clinical chemistry changes was a dose-dependent reduction in plasma triglycerides that was statistically significant at 30 mg/kg-day and above. Increased relative liver weight in females receiving 100 mg/kg-day justified the choice of 30 mg/kg-day as a subchronic NOAEL for RDX in this strain of rat.

The report of acute neurological effects of RDX in male Sprague-Dawley rats had a subchronic component in which animals were gavaged for 30 days with 0, 1, 3 or 10 mg RDX/kg-day in 2% aqueous carboxymethylcellulose (MacPhail et al., 1985). Neurotoxicological tests were carried out before the onset of dosing and then on days 16 and 31. However, no significant effects of RDX were observed at any of the dose levels.

Dilley et al. (1978) investigated the subchronic oral toxicity of a 1.6:1 mixture of TNT and RDX (LAP) in dogs, rats and mice. The subchronic toxicity of TNT, but not RDX was evaluated in the study as well. Generally, the authors concluded that the results suggested that TNT dominated the toxicity of the LAP mixture. Similar to the studies of Dilley et al. (1978) on LAP, Levine et al. (1990) reported a 90-day dietary study in 10 F344 rats/sex/group in which the toxicological effects of mixtures of TNT and RDX ("composition B") were evaluated. In this study, the authors concluded that many of the toxicological effects of each explosive individually were actually antagonized by the presence of the other compound.

2.3.1.4 Mammalian Oral Toxicity – Chronic

The first study to examine the chronic toxicity of RDX in experimental animals was that of Hart (1976), who administered the compound as a dietary supplement to 100 Sprague-Dawley rats/sex/group for 104 weeks. The stated RDX levels of 0, 1.0, 3.1 and 10 mg/kg have been interpreted by the IRIS compilers (U.S. EPA, 2000) and other reviewers (Talmage et al., 1999) as referring to doses in mg/kg (body weight)-day, though ambiguities in the study report suggest possibly that the above values might refer to the concentrations of RDX in feed. If this were the case, the actual dose levels would have been at least an order of magnitude lower than those normally assumed for this study, and possibly explain why, out of a full suite of clinical chemistry, hematology, urinalysis, gross pathology and

histopathological examinations, few if any compound-related changes were observed. However, as it stands, the data point to a NOAEL of 10 mg/kg-day for RDX, the highest dose tested.

Levine et al. (1983) reported on the chronic toxicity of RDX in 75 F344 rats/sex/group exposed to the compound in feed in amounts equivalent to doses of 0, 0.3, 1.5, 8 or 40 mg/kg-day for a total of 2 years. Clinical signs were observed twice daily and food consumption and body weights were monitored weekly up to test week 14 after which, they were monitored biweekly. Ophthalmic examinations were carried out on subjects during weeks 2, 25, 51, 76 and 103. Blood samples were taken at weeks 13, 26, 52, 78 and 104 for clinical chemistry and hematological determinations. Interim sacrifices of 10 rats/sex/group were carried out at weeks 27 and 52. At these points and at term, animals were subjected to a gross pathological examination. Samples of a wide range of organs and tissues were preserved by chemical fixation. Tissues from animals in control and high-dose groups were examined histopathologically, along with sections of brain, gonads, heart, liver, kidney, spleen, and spinal cord from all dosed groups.

Most rats receiving 40 mg RDX/kg-day died during the treatment period, many displaying profound clinical signs such as tremors, convulsions, hyperactivity, and discolored/opaque eyes. Body weight gain was also reduced in this and the intermediate-dose group, a change potentially associated with reduced food consumption. High-dosed rats had reduced RBC counts, hemoglobin concentration, and hematocrit, while the platelet count was increased in intermediate-dose males, however, these hematological parameters fell within normal ranges (Wolford et al., 1986). There were some fluctuations in clinical chemistry parameters, including relative decreases in plasma cholesterol and triglycerides and in the activity of serum glutamate-pyruvate transaminase. High-dose females displayed an increased incidence of cataracts at week 78 and week 104. Organ weight changes were noted, in particular, an increase in the relative weights of liver and kidneys in both sexes of high-dose rats and a reduction in testis weights of high-dose males. Also, observations indicated toxic effects in the spleen as early as 6 months into the study. After 2 years, the appearance of a hemosiderin-like pigment in the spleen was evident in all dose groups from 1.5 mg/kg-day and up. This finding points to a NOAEL of 0.3 mg/kg-day, a value that was used as such by the IRIS compilers to derive a human health reference dose of 3×10^{-3} mg/kg-day (U.S. EPA, 2000).

A similar study to that described above was conducted by the same researchers on B6C3F1 mice in an experiment in which 85 animals/sex/group were exposed via diet to RDX at concentrations approximating doses of 0, 1.5, 7, 35 and 100 mg/kg-day (Lish et al., 1984). The high-dose level, 175 mg/kg-day, had been lowered during the course of the experiment due to high mortality. Reduced body weight gain was noted in both sexes of high-dose mice, although food consumption was comparatively unaffected. Hematological parameters showed little change, although hematocrit and hemoglobin concentrations were reduced in high-dose females at an interim time point. Hypercholesterolemic and hypertriglyceridemic effects of RDX were observed, the former parameter displaying marked dose-response. A number of

gross pathological and histopathological effects of RDX were evident in the mice, including increased relative liver and kidney weights in high- and intermediate-dose animals. Histopathological changes at the 2-year time point included degeneration of the testes in high- and intermediate-dose males, suggesting a NOAEL of 7 mg/kg-day for this response. Other important histopathological effects of RDX included a dose-dependent increase in the incidence of hepatocellular adenomas and carcinomas in the liver of females.

2.3.1.5 Mammalian Oral Toxicity – Other

Schneider et al. (1978) followed their acute studies on the toxicokinetics of RDX in Sprague-Dawley rats with subchronic studies in which the compound was administered either in drinking water or by gavage at 20 mg/kg-day for up to 90 days. Some animals were also exposed via drinking water to saturated unlabeled or ^{14}C-labeled RDX. The results pointed consistently to the relative inability of the compound to accumulate in the plasma or tissues. Overwhelmingly, the compound was released to the urine or as $^{14}CO_2$, with lesser amounts in the feces and carcass.

Angerhofer et al. (1986) investigated the teratological potential of RDX in pregnant Sprague-Dawley rats. In a pilot study, six pregnant rats/group were given 0, 10, 20, 40, 80 or 120 mg/kg by gavage in gum acacia on gestation days (GD) 6–15, and the parameters measured at GD 20 included the numbers of viable fetuses, nonviable fetuses, resorptions, implantations, and corpora lutea. Fetal parameters included weight, size, sex, and the incidence of external malformations and visceral abnormalities. The lowest dose inducing maternal toxicity in the pilot study (20 mg/kg-day) was chosen as the highest dose in the main part of the study. In the main study, 25 pregnant rats/group were given 0, 2, 6 or 20 mg/kg by gavage in gum acacia on gestation days (GD) 6–15. 31% of females receiving 20 mgRDX/kg died in the main study. For the survivors, there were few changes in reproductive parameters compared to controls and no compound-related anomalies among the teratological findings. The authors suggested a dose of 2 mg/kg-day as a lowest observed adverse effect level (LOAEL) for the reductions in fetal size that were evident at the lowest dose tested. Inspection of the statistical results suggested that the original analyses may have been suspect. Statistical reanalysis of the data indicated that fetal size was significantly affected only at the highest dose, 20 mg/kg-day. Hence, the revised LOAEL is 20 mg/kg-day and the NOAEL is 6 mg/kg-day.

Reproductive toxicity and teratological studies have also been conducted by Cholakis et al. (1980), who administered 0.2, 2 or 20 mg RDX/kg-day by gavage to pregnant female F344 rats between GDs 6–19 and to New Zealand white rabbits between GDs 7–29. At sacrifice, the uteri were examined for live fetuses and resorptions, while the fetuses themselves were examined for skeletal abnormalities and visceral perturbations. Food consumption was reduced in high-dose rats through the first three days

of dosing, though with subsequent recovery. In addition, this group displayed a reduction in body weight, marked neurological signs and 24% (6/25) lethality. However, no changes in reproductive parameters were noted; there were no soft tissue or skeletal anomalies due to RDX exposure. Dosing pregnant New Zealand white rabbits at the same levels resulted in few changes in reproductive parameters but a catalogue of teratological responses that were essentially sporadic and therefore of uncertain significance. These responses included spina bifida, misshapen cranium, meningocele, misshapen and enlarged eye bulges, abdominal wall defects, gastroschisis, appendicular reduction anomalies and "tail problems."

Cholakis et al. (1980) also reported a two-generational reproductive study in which male and female CD rats were fed diets adjusted to nominal daily doses of 0, 5, 16 or 50 mg RDX/kg for 13 weeks. F_o adults were then mated within the groups with 26 of the resulting F_1 progeny maintained on the same diets for another 13 weeks. After a further round of mating, the F_2 progeny were necropsied and processed for histopathological examination.

There was a reduction in body weight gain in all generations of high-dose rats, which may have been related to a concomitant depletion in food consumption. Mortality reached 18% in high-dose rats of the F_0 generation with 17% and 52% stillbirths in the F_1 and F_2 high-dose progeny, respectively. Reductions in the number of fertile high-dose male and female rats were observed during the F_0 mating, although these differences were statistically insignificant. Notwithstanding these changes, there appeared to be no specific reproductive or developmental changes due to treatment in this experiment, since feeding 16 mg/kg-day produced no apparent effects.

2.3.1.6 Studies Relevant to Mammalian TRV Development: RDX Ingestion Exposures

The range of animal models in which responses to acute, subacute and subchronic RDX administration have been monitored includes beagle dogs, cyanomolgus (rhesus) monkeys, Sprague Dawley and F344 rats, Swiss-Webster and B6C3F1 mice, miniature swine and New Zealand white rabbits.

There is a striking contrast between the acute lethality of RDX in experimental animals and those of other explosive/energetic compounds such as TNT and HMX. For example, acute oral LD_{50} values for the latter compounds may be found in the 500–1000 mg/kg-day range, suggesting low-to-moderate lethality, whereas the LD_{50} for RDX is in the 50–200 mg/kg-day range, with a median value closer to 100 mg/kg-day. This suggests that RDX has a higher acute toxicity than other explosive compounds. However, if RDX is characterized by comparatively high acute toxicity, the precise targets for these toxic effects remain to be fully identified. Toxicokinetic evidence indicates that the compound is readily absorbed at the gastrointestinal brush border but has a transitory existence in the body with rapid breakdown into a range of metabolic products including single carbon compounds occurring in the liver in some animals. The importance of the liver in response to RDX is underscored by the histological changes that take place when a receptor is challenged with the compound. Perturbations of clinical

chemistry parameters potentially related to liver function, such as plasma lipid levels and enzyme activities such as serum glutamate-pyruvate transaminase, lend further weight to the concept that the liver is one of the primary sites of RDX toxicity.

Liver effects are also evident in a number of subchronic and chronic studies on RDX, the responses manifesting in dose-dependent increases in organ to body weight ratios and in changes to the cellular architecture revealed histologically. In the 24-month dietary study of RDX in B6C3F1 mice, histopathological evidence of compound-related hepatocellular adenoma and carcinoma formation was obtained in females. However, no effects were seen in male mice or either sex rats suggesting the response is not generally associated with rodent exposure to RDX. The ecological relevance of RDX-induced liver toxicity is questionable.

Other reasonably consistent responses that have been elicited in experimental animals exposed to RDX include changes in the levels of some hematological parameters associated with anemia and changes to the size and histopathology of the spleen. Although increased pigmentation of the spleen was used as the basis for a NOAEL of 0.30 mg/kg-day (Levine et al., 1983), associated hematological parameters, although significantly different than controls for the high dose group, fell within normal ranges (Wolford et al., 1986). This indicates that the increased pigmentation of the spleen was not associated with any hematological changes that would cause functional impairment. Given the lack of biological significance in this effect, increased pigmentation of the spleen is of questionable relevance.

As outlined in Technical Guide 254 (USACHPPM, 2000), TRVs are derived from toxicological effects likely to be ecologically relevant. Decreased growth is regarded as an ecologically relevant parameter and was common to two studies on chronic ingestion of RDX, one on F344 rats (Levine et al, 1983) and one on B6C3F1 mice (Lush et al., 1984) and two studies on subchronic ingestion of RDX, one on F344 rats (Cholakis et al., 1980) and one on Swiss Webster mice (Dilley et al., 1978). These data suggest that reduced growth is a consistent feature of RDX-exposed rodents. From an ecological perspective, reduced growth and /or associated reductions in food consumption can affect the ecological performance of individuals by causing alterations in energy allocation patterns that could ultimately result in altered reproductive performance (Calow, 1991; Congdon et al., 2001). All three studies showing reduced growth in RDX-exposed rodents were well designed and well executed and can be considered high quality. For derivation of the TRV, the data on chronic toxicity in F344 rats (Levine et al., 1983) is most appropriate as these data meet the requirements of TG 254 (USACHPPM, 2000) and as such, require no uncertainty factors. Moreover, these data are protective of the data on B6C3F1 mice (Lush et al., 1984).

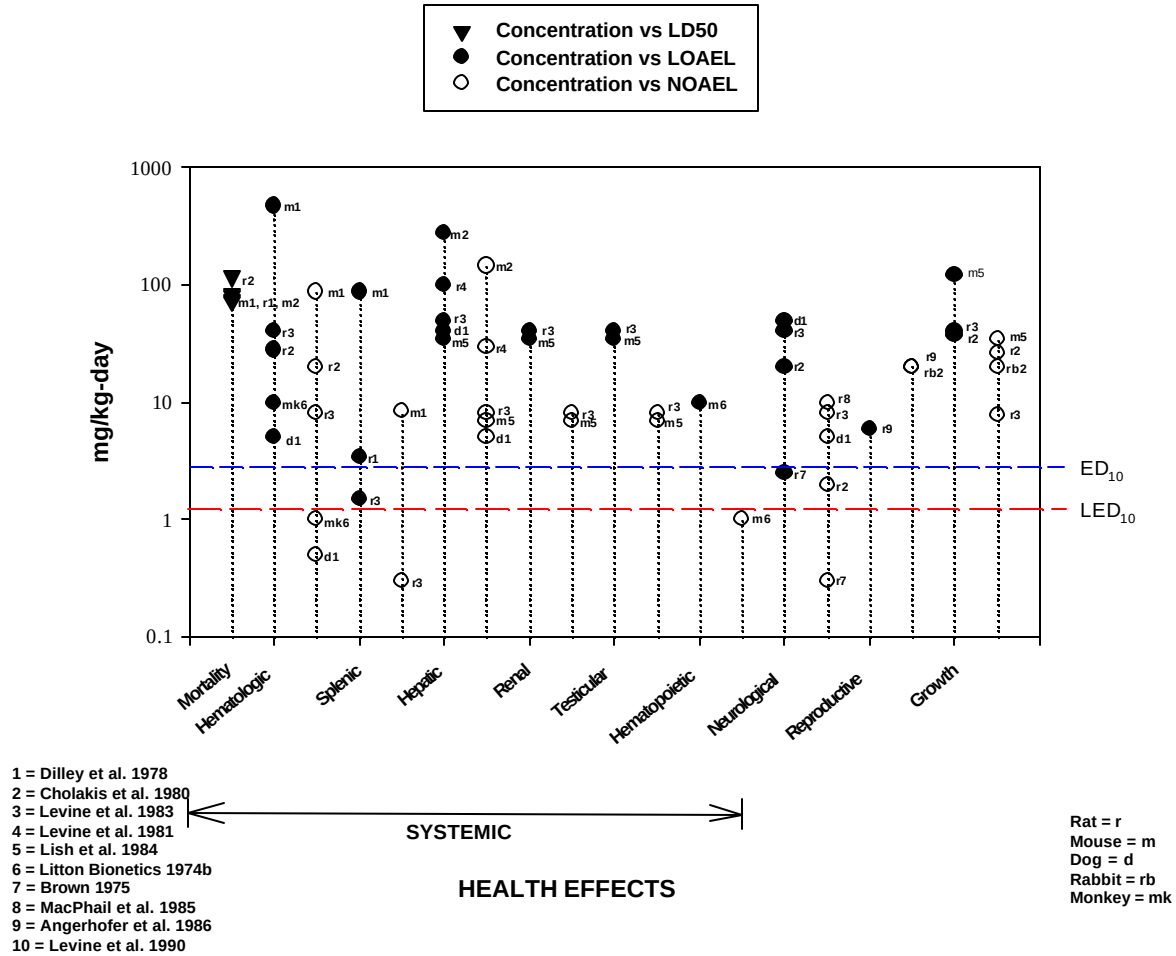
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Angerhofer et al. (1986)	Rat (f) (Sprague-Dawley)	GD 6–15	NA	2	Comparative reductions in fetal size
Cholakis et al. (1980)	Rat (f) F344)	GD 6–19	2	20	Neurological signs/lethality
	Rabbit (f) (NZ white)	GD 7–29	20	NA	Reproductive/Developmental toxicity
Litton Bionetics (1974a)	Dog (Beagle)	90-d	10	NA	NA
	Monkey (rhesus)	90-d	1	10	Elevated megakaryocyte count
Brown (1975)	Rat (strain unstated)	12-w	0.3	2.5	Increased brain monoamine oxidase and cholinesterase activity
Levine et al. (1981)	Rat (F344)	90-d	30	100	Increased liver weight
McPhail et al. (1983)	Rat (m) (Sprague-Dawley)	30-d	10	NA	Neurological testing
Cholakis et al. (1980)	Rat (F344)	90-d	26.4	37.7	Reduced hemoglobin and hematocrit. Reduced body weight.
	Mice (B6C3F1)	90-d	145 (TWA)	277 (TWA)	Lethality and neurological signs, enlarged liver and hepatocellular lesions
Levine et al. (1990)	Rat (F344)	90-d	NA	5.0/29.8*	Reduced body weight gain in males
Hart (1976)	Rat (Sprague-Dawley)	104-w	10	NA	NA
Levine et al. (1983)	Rat (F344)	104-w	8	39.8	Decreased body weight
Lish et al. (1984)	Mouse (B6C3F1)	104-w	7	35	Atrophy of the testis in males, increases in relative and absolute kidney and liver weights, decreased body weight
			NA**	NA**	Hepatocellular adenoma and carcinoma in females

* Doses are those of TNT/RDX mixed in various proportions
** Identifying a NOAEL for tumorigenic responses may be unsafe, in line with existing U.S. EPA understandings on the identification of a subthreshold dose for a carcinogenic effect
GD = gestation day
TWA = time-weighted average
NA = not applicable
RBC = red blood cell

Figure 1.

RDX HEALTH EFFECTS TO MAMMALS



2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using mammals were found.

2.3.3 Mammalian Dermal Toxicity

No dermal studies conducted using mammals were found.

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity - Oral

2.4.1.1 Avian Oral Toxicity - Acute

One study was located on the toxicity of RDX to an avian species. Gogal et al. (2001) studied the acute, subacute, and subchronic toxicity of RDX to the Northern Bobwhite (*Colinus virginianus*). For the acute study, the objective was to determine the approximate lethal dose (ALD). RDX was administered to birds orally in a water vehicle. One male and one female per group were dosed with one of the following, 125, 187, 280, 420, 630, 945, 1417, or 2125 mg RDX/kg. Birds were observed for 14 days after administration of RDX and on day 14, surviving birds were weighed, bled, euthanized by electrocution and necropsied. The ALD values 14 days after the exposure were 280 mg/kg for male and 187 mg/kg for female Northern Bobwhite.

2.4.1.2 Avian Oral Toxicity - Subacute

Groups of six male and six female birds were exposed to RDX in the feed at concentrations of 0, 83, 125, 187, 280 and 420 ppm RDX for 14 days (Gogal et al., 2001). Daily doses of RDX were calculated to be 10.8, 13.4, 22.3 and 26.3 mg RDX/kg body weight, respectively. Feed consumption, body weight, spleen weight/body weight ratio, liver weight/body weight ratio and egg production were measured. Hematological analyses included whole blood cellularity, packed cell volume (PCV), total protein and mean corpuscular volume (MCV). Histological analyses were conducted on liver, kidney, spleen, brain, spinal cord, intestine, heart, lung, pancreas and gonad tissues.

Results showed that there was a significant, linear decrease in feed consumed with increasing levels of dietary RDX and a concomitant decrease in body weight with increasing levels of RDX in the diet. The ratios of spleen weight/body weight in females and liver weight/body weight in both sexes were also significantly affected by dose and generally decreased with increasing RDX. Hematological effects of RDX exposure included an increase in packed cell volume in females, a decline in total plasma protein in females, an increase in heterophils and an increase in the heterophil/lymphocyte ratio in blood. Egg production showed a significant, linear decrease with increasing RDX for both week one and week two.

The authors report a NOAEL of 8.7 mg RDX/kg/day and a LOAEL of 10.6 mg RDX/kg/day based on a dose-related decrease in body weight and egg production.

2.4.1.3 Avian Oral Toxicity - Subchronic

Five groups of 10 male and female Northern Bobwhite were provided with 0, 125, 187, 280 or 420 ppm RDX in the feed for 90 days (Gogal et al. 2001). The calculated daily oral doses were reported to be 0, 10.8, 13.4, 22.3 and 26.3 mg/kg for the 0, 125, 187, 280 and 420 ppm, respectively. Feed was weighed and replaced on a weekly basis. Parameters measured included those mentioned in the 14-day study including 5-part leukocyte differentials, lymphocyte mitogen-induced proliferation and leukocyte apoptosis/necrosis assays. Histological analyses were as above with the addition of bone marrow. Changes in egg production were also evaluated. Although the same doses used in the subacute were identical to those in the subchronic study, no significant effects of RDX were seen after exposure for 90 days. These data suggest that Northern Bobwhite develop a tolerance from exposure to RDX in the feed. However, although no significant effects were seen, there were dose-dependent trends apparent for several parameters including, a decrease in feed consumption, decrease in total protein, a decrease in PCV and a decrease in egg production. No severe effects were noted. Since no significant effects of RDX were seen after 90 days of exposure, a LOAEL was not reported.

2.4.1.4 Avian Oral Toxicity - Chronic

No data are available.

2.4.1.5 Avian Oral Toxicity - Other

No data are available.

2.4.1.6 Studies Relevant for Avian TRV Development for Ingestion Exposures

Only one study was located on the effects of RDX on an avian species. Gogal et al. (2001) investigated acute, subacute, and subchronic effects of orally administered RDX in Northern Bobwhite (*Colinus virginianus*). In the 14-day study, there were significant effects of RDX on both body weight and egg production. In the 90-day study, the same doses of RDX were used as in the 14-day study, however, no significant effects of RDX were seen, although there were dose-dependent decreases in body weight and total egg production. These data suggest that Northern Bobwhite develop a tolerance to prolonged dietary exposure to RDX. Although data from long-term exposures (i.e., subchronic and chronic) are preferred, in this case the subacute data on egg production is especially relevant. A rationale is provided below.

Birds are highly vagile animals and thus often experience the environment in patchily distributions. Under these realistic exposure scenarios, birds are most likely to experience short-term exposures on the order of days as opposed to weeks. Therefore, a 14-day exposure to RDX may be more ecologically relevant than longer exposure scenarios. Moreover, these data are protective of longer exposure scenarios tested to date. Although these changes in egg production and other parameters (e.g., body weight gain) may be due to the reduction in consumed feed, food avoidance may also be an ecologically relevant parameter. Since the primary endpoint chosen is a reproductive one, under TG 254 and consistent with Sample et al. (1996), data on egg production in quail exposed to RDX for 14 days can be considered equivalent to a long-term investigation since the exposure occurred during a sensitive life cycle stage. Hence, the avian TRV for RDX was derived from the 14-day oral exposure in Northern Bobwhite (Gogal et al., 2001).

Table 3. Summary of Relevant Avian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL mg/kg/d	LOAEL mg/kg/d	Effects at LOAEL
Gogal et al. (2001)	Northern Bobwhite (*Colinus virginianus*)	ALD	NA	NA	187 mg/kg for female 280 mg/kg for male
		14 d	8.7	10.6	Decreased body weight in males and females and decreased egg production.
		90 d	26.3	NA	No statistically significant effects however, there were several dose-related trends; decreased egg production, feed consumption, total plasma protein and packed cell volume.

ALD = approximate lethal dose
NA = not applicable

2.4.2 Avian Inhalation Toxicity

No data are available.

2.4.3 Avian Dermal Toxicity

No data are available.

2.5 Amphibian Toxicology

Toxicological studies on the effects of RDX in amphibian species were not located. Ecotoxicological research on the effects of RDX on amphibians is recommended.

2.6 Reptilian Toxicology

Toxicological studies on the effects of RDX in reptilian species were not located. Ecotoxicological research on the effects of RDX on reptiles is recommended.

3 RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Decreased body weight was reported for both F344 rats (Levine et al., 1983) and B6C3F1 mice (Lish et al., 1984) after two years of oral dosing with RDX. Decreased body weight, an indication of a lower growth rate or a decrement in energy allocation, was used to determine the TRV because this endpoint may be ecologically relevant through effects on fitness. For example, indicated alterations in energy allocation patterns may impair reproductive function and/or schedules (Calow, 1991; Congdon et al., 2001). In addition, sustaining a smaller body size for longer time periods may increase risk of predation. Both chronic studies (Levine et al., 1983; Lish et al., 1984) indicated decreased growth in rats and mice fed RDX, and hence the effect may be a consistent feature of RDX exposure. For TRV determination, data on female F344 rats was used because these data were protective of males and exhibited a clear dose response relationship (Levine et al., 1983). In addition, the TRV based on the F344 rat data was protective of B6C3F1 mice. Growth, as indicated by body size, also meets the minimum data requirements of the Standard Practice, Section 2.2 (USACHPPM 2000) and therefore no uncertainty factors were required in the derivation of the TRV. The TRV was derived using the Benchmark dose approach (Appendix B) and the values presented in Table 4. This TRV is given a medium confidence rating since there were only two chronic studies and no wildlife toxicity data were available.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
LED_{10}	1.19 mg/kg/d	Medium
ED_{10}	2.73 mg/kg-d	Medium

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not Available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

The ecologically relevant parameter for RDX toxicity in birds was decreased fecundity (i.e. egg production) reported for Northern Bobwhite exposed to dietary concentrations of RDX for 14 days (Gogal et al., 2001). For this endpoint, the effect was significant and dose dependent and the study was of high quality. Decreased egg production was used to determine the TRV because it is an ecologically relevant parameter indicative of impaired reproductive performance, which can have direct impacts on population dynamics, particularly for this species.

Exposure to RDX in this study occurred during a sensitive life cycle stage, and therefore can be considered equivalent in value to a chronic exposure evaluation. Given the data quality, the dose dependent nature of the effect, and the ecological relevance of effect, the Benchmark Dose approach was used. The TRVs derived using the Benchmark dose approach (Appendix C) are presented in Table 5. It should be noted that although there was not a significant effect of RDX on egg production in quail for the 90-day exposure, there was a trend; egg production decreased with increasing concentrations of RDX. The Benchmark Dose approach was applied to these data as well and are presented and Appendix D. A benchmark dose (BMD or ED_{10}) of 8.14 mg/kg-d was calculated from the model fit of the mean response at the 10% response level. A lower-bound on the benchmark dose (BMDL or LED_{10}) was calculated to be 3.65 mg/kg-d from the lower 95% confidence interval (CI) of the modeled curve. Comparison of Benchmark Doses for the 14-day and 90-day studies indicate that TRVs derived from the 14-day study

are protective of TRVs derived from the 90-day study. Since data from only one study was located, the TRVs presented below are given a low confidence rating.

Table 5. Selected Ingestion TRVs for the Class Aves

TRV	Dose	Confidence
LED_{10}	3.65 mg/kg/d	Low
ED_{10}	8.14 mg/kg-d	Low

3.3 Toxicity Reference Values for Amphibians

Not Available at this time.

3.4 Toxicity Reference Values for Reptiles

Not Available at this time.

4. IMPORTANT RESEARCH NEEDS

The limited availability of data on the toxicity of RDX to wildlife species precludes the development of a high-confidence TRV. Hence, more studies on the toxicity of RDX to wildlife species are needed. In particular, long-term toxicity studies on mammals and additional studies on non-mammalian wildlife such as birds, reptiles and amphibians are particularly warranted. More information regarding the toxicity of RDX to wildlife would likely allow the derivation of a high confidence TRV.

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Whong, W.Z., N.D. Speciner, and G.S. Edwards. 1980. Mutagenic activity of tetryl, a nitroaromatic explosive in three microbial test systems. Toxicol. Lett. 5:11-17 (as cited in U.S. EPA, 2000).

Wolford, S.T., R.A. Schroer, F.X. Gohs, P.P. Gallo, M. Brodeck, H.B. Falk, and R. Ruhren. 1986. Reference range data base for serum chemistry and hematology values in laboratory animals. J. Toxicol. Environ. Health, 18:161-188.

APPENDIX A
LITERATURE REVIEW

The following files were searched in Dialog:

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

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

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

The search strategy for **Birds:**

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

The search strategy for **Laboratory Mammals:**

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

The search strategy for **Wild Mammals:**

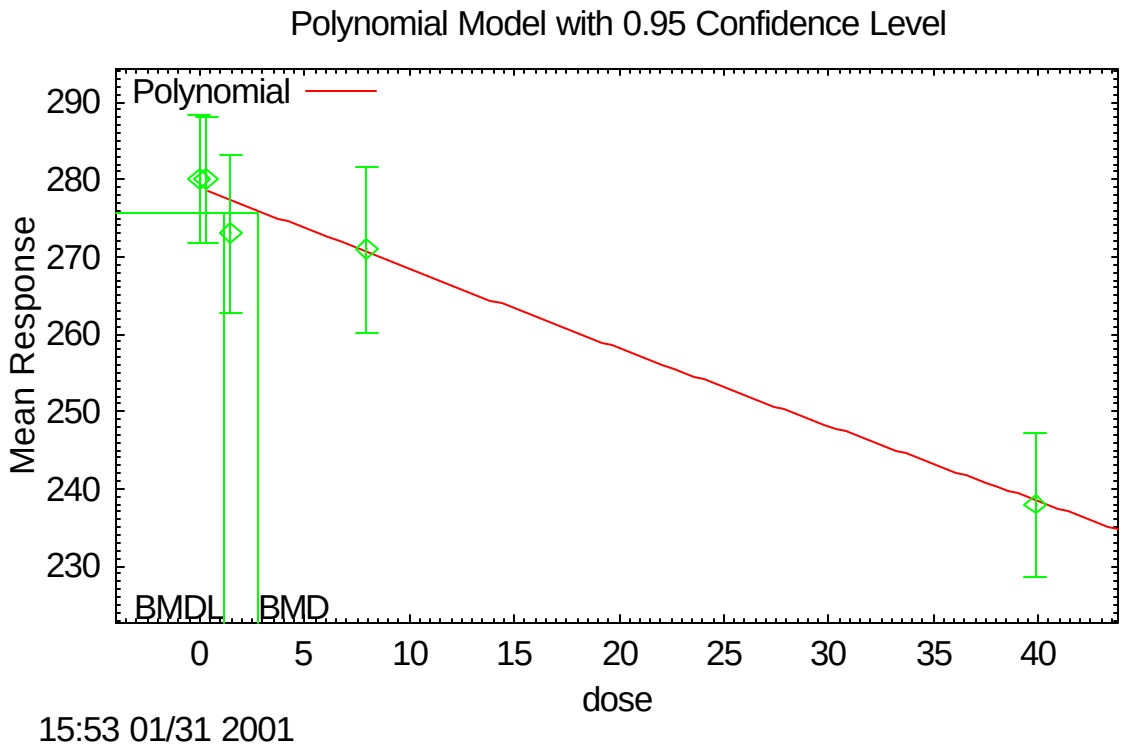
- Chemical name, synonyms, CAS numbers
- And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 31 hits on RDX were obtained in the initial search, all of which were selected for abstract evaluation. Nineteen of these articles and reviews were retrieved for this survey.

APPENDIX B
Benchmark Dose Calculation for Mammals

The data presented below are from Levine et al. (1983) with mean body weight at two years in Fischer 344 rats as the response. Data from females was used since it showed a clear dose response and was protective of males. The model fit was adequate, and a benchmark dose (BMD) and benchmark dose low (BMDL) were obtained from this analysis.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha = 868.792
beta_0 = 278.559
beta_1 = -1.05871
beta_2 = 0.00104466

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	851.428	0.0116566
beta_0	278.62	0.481016
beta_1	-1.07224	7.18802
beta_2	0.00135624	277.868

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	5.8e-007	4e-007	-5.8e-007
beta_0	5.8e-007	1	0.49	0.39
beta_1	4e-007	0.49	1	0.98
beta_2	-5.8e-007	0.39	0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	43	280	27	279	29.2	0.0473
0.302	45	280	27	278	29.2	0.0584
1.486	42	273	33	277	29.2	-0.138
7.969	41	271	34	270	29.2	0.0287
39.85	26	238	23	238	29.2	-0.00151

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-762.527	6	1537.05
A2	-758.957	10	1537.91
fitted	-763.071	4	1534.14
R	-781.587	2	1567.17

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	45.2597	8	<.0001
Test 2	7.14095	4	0.1286
Test 3	1.0875	2	0.5806

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.950000

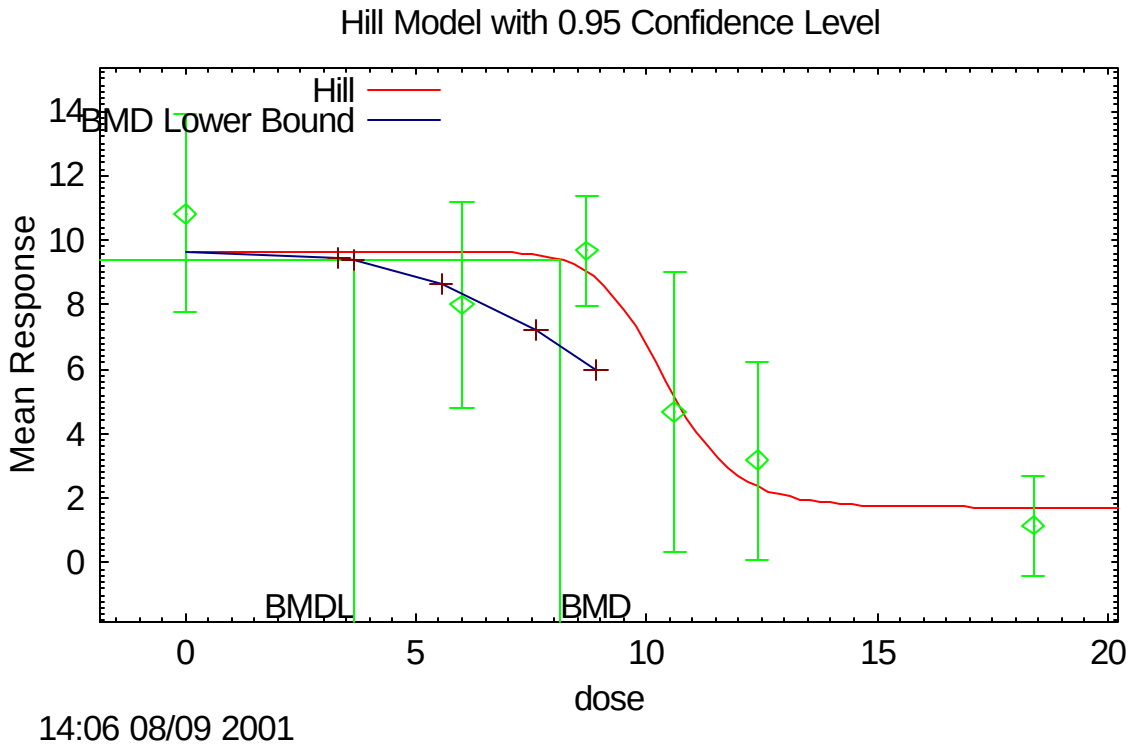
BMD = 2.73077

BMDL = 1.18567

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted

APPENDIX C
Benchmark Dose Calculation for Bobwhite Quail

The data presented below are total egg production from quail exposed to RDX in the feed for 14 days from Gog al et al. (2001). These data were considered for the TRV because they represent a sensitive stage in the life cycle and are protective of 90-day effects. The model fit was adequate, and a benchmark dose (BMD) and benchmark dose low (BMDL) were obtained from this analysis.



The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 6
Total number of records with missing values = 0

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha = 6.36633
rho = 0 Specified
intercept = 10.83
v = -9.663
n = 8.29994
k = 10.0943

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	0	0	0	0	0
rho	0	1	0	0	0	0
intercept	0	0	1	0	0	0
v	0	0	0	1	0	0
n	0	0	0	0	1	0
k	0	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	7.65144	1
rho	0	1
intercept	9.6589	1
v	-7.94406	1
n	13.7149	1
k	10.3768	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	6	10.8	2.93	9.66	2.77	0.423
6	6	8	3.03	9.65	2.77	-0.598
8.7	6	9.67	1.63	9.01	2.77	0.239

10.6	6	4.67	4.13	5.11	2.77	-0.161
12.4	6	3.17	2.93	2.35	2.77	0.295
18.4	6	1.17	1.47	1.72	2.77	-0.199

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-52.235092	7	118.470185
A2	-48.036623	12	120.073247
fitted	-54.628079	5	119.256157
R	-71.471672	2	146.943344

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	46.8701	10	<.0001
Test 2	8.39694	5	0.1357
Test 3	4.78597	2	0.09136

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

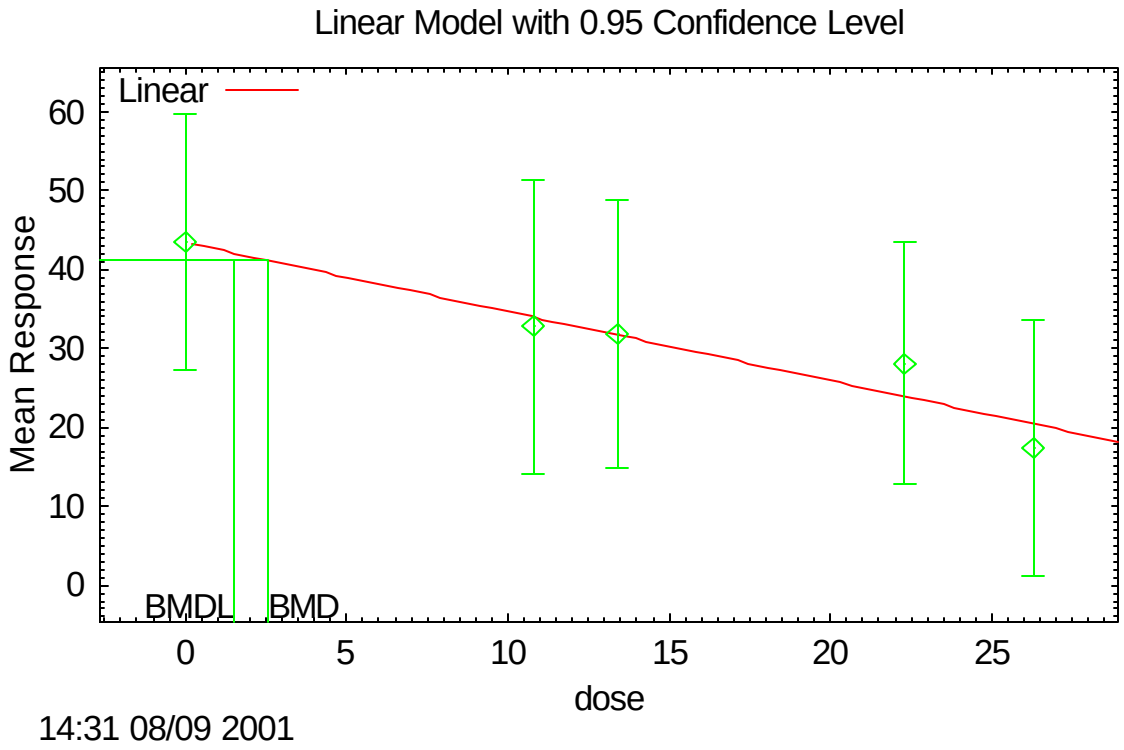
Confidence level = 0.95

BMD = 8.14449

BMDL = 3.6501

APPENDIX D
Benchmark Dose Calculation for Bobwhite Quail

The data presented below are total egg production from quail exposed to RDX in the feed for 90 days from Gogal et al. (2001). These data were not significant, however, a dose-related trend is readily apparent. The model fit was adequate, and a benchmark dose (BMD) and benchmark dose low (BMDL) were obtained from this analysis, although the approximations are suspect due to the lack of significance in the effect.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha = 544.388
rho = 0 Specified
beta_0 = 43.4465
beta_1 = -0.874347

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	495.548	99.1096
beta_0	43.4465	5.88362
beta_1	-0.874347	0.341382

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	-2.4e-007	-2.5e-008
beta_0	-2.4e-007	1	-0.84
beta_1	-2.5e-008	-0.84	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
26.3	10	17.4	22.5	20.5	22.3	-1.37
22.3	10	28.1	21.4	23.9	22.3	1.86
13.4	10	31.8	23.8	31.7	22.3	0.0224
10.8	10	32.8	26.1	34	22.3	-0.541
0	10	43.5	22.6	43.4	22.3	0.024

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2
Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-179.857527	6	371.715054
A2	-179.628138	10	379.256275
fitted	-180.141630	2	364.283260
R	-183.728560	2	371.457121

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	8.20085	8	0.08449
Test 2	0.458779	4	0.9774
Test 3	0.568206	3	0.9037

The p-value for Test 1 is greater than .05. There may not be a diffence between responses and/or variances among the dose levels. Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2.546

BMDL = 1.53076

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted

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

USACHPPM

Wildlife Toxicity Assessment for 1,3-Dinitrobenzene (m-DNB)

DECEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 37-EJ-1138-01A

Readiness Thru Health

Wildlife Toxicity Assessment for 1,3-Dinitrobenzene (m-DNB)

FINAL REPORT
DECEMBER 2001

Prepared by
Health Effects Research Program
Environmental Risk Assessment Program

USACHPPM Document No: 39-EJ1138-01A
Approved for Public Release; Distribution Unlimited

Acknowledgements

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Table of Contents

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

Wildlife Toxicity Assessment for m-DNB

CAS No. 99-65-0 **December 2001**

1. INTRODUCTION

1,3-Dinitrobenzene (m-DNB) is one of several compounds that have been released to the environment during the manufacture of explosives and in load, assembly and pack (LAP) activities at U.S. Army ammunition plants (AAPs) and other military installations. The compound has a close structural relationship with the important military explosive trinitrotoluene (TNT), of which m-DNB is a manufacturing by-product and an environmental degradation product. The importance of m-DNB as an environmental contaminant is related to its widespread distribution at and around military sites and to its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of m-DNB on wildlife, with emphasis on identifying levels at which wildlife species may be adversely effected. Evaluating the toxicity of the compound is intended to contribute to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for wildlife ranging in the vicinity of m-DNB contaminated sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched May 17, 2000, using Dialog to identify primary reports of studies and reviews on the toxicology of m-DNB. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and "key words in context." All available abstracts of those articles that were selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For m-DNB, 30 articles were marked for

retrieval from 62 initial hits. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to literature searches using Dialog, a number of U.S. Army reports were identified in the Defense Technical Information Center (DTIC). Secondary references and sources of information on 1,3-dinitrobenzene (m-DNB) included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for 1,3-Dinitrobenzene 1,3,5-Trinitrobenzene* (ATSDR, 1995), the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000), the U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997).

2.2 Environmental Fate and Transport

m-DNB has been used as a component in the chemical synthesis of (1) m-nitroaniline, an intermediate in the production of aniline dyes and (2) m-phenylenediamine, a compound used in the synthesis of aramid fibers and spandex (ATSDR, 1995). As with its structural analog 1,3,5-trinitrobenzene (1,3,5-TNB), m-DNB is a manufacturing by-product of the explosive TNT, with the potential for release to the environment in discharged wastewater. Additionally, any 2,4-dinitrotoluene (2,4-DNT) present in the waste stream may be degraded to m-DNB by photolysis under certain pH conditions and organic matter content (as reported in Talmage et al., 1999). Incidental release to the environment of m-DNB might be as a result of any or all of the manufacturing processes referred to above. Soil concentrations of up to 45.2 mg m-DNB/kg soil have been reported at contaminated sites such as AAPs (Talmage et al., 1999).

A list of key physico-chemical properties of m-DNB that pertain to the environmental fate and transport of the compound is provided in Table 1.

m-DNB has an estimated vapor pressure of 5.13×10^{-6} mm Hg at 25°C (ATSDR, 1995), a low value implying that partitioning to air is unlikely. Although readily soluble in a variety of organic solvents at ambient temperature, m-DNB is sparingly soluble in water (370–500 mg/L at 20–25°C). However, despite its limited solubility, the compound has been identified in both surface water and groundwater. Furthermore, significant concentrations in sediments have been identified in streams contaminated with m-DNB. Talmage et al. (1999) present m-DNB soil concentration data for a selection of AAPs, depots and arsenals.

As noted in ATSDR (1995), there are no experimental data on the photolysis of m-DNB in aqueous solution but, on theoretical grounds, the process may be expected to occur, given the potential for the compound to absorb light at wavelengths > 290 nm. Log K_{oc} values have been estimated within the range 1.39 – 2.3, which indicate a moderate degree of adsorption of m-DNB to suspended sediments, and high to moderate mobility in soil.

Table 1. Summary of Physical-Chemical Properties of 1,3-Dinitrobenzene

Molecular weight	168.11
Color	yellow-white
Physical state	crystals/rhombohedral plates
Melting point	89–90 °C
Boiling point	300–303 °C
Odor	no data
Solubility	370–500 mg/L in water at 20–25 °C; soluble in chloroform, acetone and ether
Partition coefficients:	
Log K_{ow}	1.49 to 1.62
Log K_{oc}	1.39 to 2.3
Vapor pressure at 25 °C	5.13×10^{-6} mm Hg
Henry's Law constant at 25 °C	2.33×10^{-6} atm.m3/mole
Conversion factors	1 ppm = 6.88 mg/m^3 1 mg/m^3 = 0.145 ppm

Sources: ATSDR, 1995; Talmage et al., 1999; HSDB, 2000; Wentsel et al., 1979

m-DNB is subject to microbial degradation by a variety of microbes that use the compound as a carbon as well as a nitrogen source. This capacity for microbial degredation differs from that of 1,3,5-TNB in which the aromatic six-carbon ring structure is conserved. Various mixed cultures of microorganisms obtained from rivers or sewage sludge effluents have been shown to break down m-DNB. Aerobic degradation of m-DNB to carbon dioxide has been demonstrated with the microbial strain *Candida pucherrima* (Dey and Godbole, 1986). ATSDR (1995) documents a wide range of microbial genera that can break down m-DNB.

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Toxicity - Oral

2.3.1.1 Mammalian Oral Toxicity - Acute

The review by Wentsel et al. (1979) contains a summary of earlier studies on the acute oral toxicity of m-DNB in experimental animals. For example, Kiese (1949) derived an oral LD_{50} of approximately 10 mg m-DNB/kg in dogs. Cody et al. (1981) reported an average (male/female) oral LD_{50} for m-DNB of 83 mg/kg. In this study, six male and female Carworth Farms rats were administered one of six doses of m-DNB ranging from 36 to 180 mg/kg. The compound was administered as a 1% solution in corn oil via intubation. In another study, FitzGerald et al. (1991) developed average oral LD_{50}s for m-DNB of 59.5 mg/kg in Fischer 344 (F344) rats and 80.4 mg/kg in Swiss mice. Five rats and mice of both sexes were

dosed with one of three levels of m-DNB suspended in corn oil. In rats the doses were 62, 68 and 74 mg/kg while in mice the doses were 50, 70 and 90 mg/kg.

Some studies on acute toxicity of m-DNB have focused on endpoints other than lethality. For example, Watanabe et al. (1976) demonstrated the formation of methemoglobin in blood samples obtained five hours after intraperitoneal injections of male Wistar rats with 100 µmoles/kg m-DNB in 2 ml/kg propylene glycol (PEG). The primary focus of this study was the methemoglobin-forming potential of 1,3-diamino-2,4,6-trinitrobenzene (DATNB), the parallel use of other nitrogen-substituted aromatic compounds, such as m-DNB, serving as reference compounds. Of the compounds employed in this survey, m-DNB was considered one of the most potent inducers of methemoglobin. Myers et al. (1999) administered a single oral dose of 50 mg/kg m-DNB by gavage in corn oil to four male shrews (*Cryptotis parva*) and demonstrated the formation of hemoglobin adducts in blood samples obtained 24 hours after dosing. *In vitro* incubation of blood with m-DNB also resulted in adduct formation, suggesting a role for cysteine residues in hemoglobin-m-DNB binding. This finding was consistent with an earlier *in vitro* demonstration that m-DNB and other DNB isomers can bind irreversibly to red blood cell macromolecules (Cossum and Rickert, 1987).

A number of single, oral dose studies of m-DNB have provided information regarding the biochemistry and toxicokinetics of neurotoxicological and testicular impairment induced by the compound. For example, Philbert et al. (1987a) used germ-free (GF) and conventional male F344 rats to demonstrate the ability of intestinal microflora to moderate the neuropathological effects of m-DNB. After a single oral dose of 20 or 25 mg/kg m-DNB in PEG, GF male rats (n = 15) displayed a marked ataxic response, in contrast to conventional rats (n = 12) or to GF rats that had been reseeded with a "cocktail" of intestinal microflora. Conventional rats did show ataxia when dosed with 20 mg/kg m-DNB each day for five days. GF rats accumulated approximately 20 and 13 times more radio-labeled m-DNB into the liver and brain, respectively, compared to conventional rats. As described by the authors, light and electron microscopy revealed that histopathological lesions of the brain were limited to the brain stem and inferior colliculus, although animals displaying these features were not necessarily those that had displayed the pronounced clinical signs. The histopathological lesions were considered similar to those typically brought about by thiamine deficiency, a well-documented feature of GF rats. The authors speculated that an observed rise in lactate in the damaged regions of the brains of treated GF rats might reflect interference by m-DNB of oxidative metabolism and pyruvate utilization. Subsequent research by the same group indicated that m-DNB interfered with intracellular redox mechanisms resulting in impaired glucose utilization (Philbert et al., 1987b). The main body of the report contained a detailed histopathological analysis of neurological lesions induced by an experimental protocol similar to that employed in their previous report (Philbert et al., 1987a). Light and electron microscopy revealed the formation of "bilaterally symmetrical vacuolated lesions (that) involve cerebellar roof, vestibular and

superior olivary nuclei and the inferior colliculi." The authors considered the primary cellular targets to be astrocytes, oligodendrocytes and vascular elements, with secondary neuronal involvement. However, the precise mechanism of action of the compound has remained obscure (Philbert et al., 2000).

2.3.1.2 Mammalian Oral Toxicity – Subacute

Subacute studies involve repeated dosing of animals and parameters are measured at the end of a 14-day study duration. Reddy et al. (1994a) reported a 14-day study on the toxicity of m-DNB in five F344 rats/sex/group that was conducted to establish dosing levels suitable for longer-term studies. The compound was added to the diet to concentrations of 0, 2.5, 10, 25, 75, and 150 mg/kg, yielding respective doses that were calculated by the authors to be 0, 0.21, 0.8, 1.98, 5.77 and 10.56 mg/kg-day in males and 0, 0.22, 0.87, 2.02, 6.28 and 11.82 mg/kg-day in females. Clinical observations were made twice daily, food and water consumption twice weekly, while body weights were recorded at the beginning, weekly during the in-life phase of the study, and at termination. A complete profile of hematological and clinical chemistry parameters was assessed in blood samples obtained at necropsy. All tissues and major organs were observed for gross morphological lesions, and the weights of certain organs were recorded. Tissues were sampled, fixed and processed for histopathological examination. Slides of sections cut from high-dose and control tissues were examined under a light microscope. In addition, sections of certain potential target organs such as spleen and kidney (male only) were similarly examined for all groups.

There were no significant decreases in body weight in any of the treatment groups compared to control, although there was a comparative reduction in food consumption in both sexes of animals receiving m-DNB at the highest dose. Some dose-related changes in organ weight/body weight ratio were seen, including comparative increases in liver, spleen and kidneys, and reductions in testis. The lowest effective dose for responses such as these was 1.98 mg/kg-day, a level associated with relative increases in kidney weights in male rats, although this particular response may have been incidental to treatment since it did not appear to be part of a dose-response relationship. Though there were no obvious treatment-related changes in clinical chemistry parameters, most hematological indices were reduced compared to controls after 14 days of treatment. For example, there was a statistically significant reduction in hematocrit and erythrocyte count in female groups at a dose level of 2.02 mg/kg-day that appeared to be part of a dose-related response. Methemoglobin was significantly increased in females at a dose of 1.98 mg/kg-day and higher, and in males as 0.87 mg/kg-day and higher. Gross pathological signs were seen in the testes of male rats dosed at 5.77 mg/kg-day and higher and in the spleen of females at 2.02 mg/kg-day and males at 1.98 mg/kg-day. There were treatment-related histopathological changes in the bone marrow, spleen and brain of high-dose rats. In males, histopathological changes were noted in the spleen and kidneys of rats ingesting 1.98 mg m-DNB/kg/day and above and in the testes of rats

ingesting 5.77 mg m-DNB/kg/day. These lesions were characterized in the kidneys by the occurrence of tubular degeneration and associated hyaline droplet formation and in the testis by seminiferous tubular degeneration, the appearance of cell debris, and the formation of multinucleate cells.

A number of possible NOAELs and LOAELs could be derived from this study. For example, a NOAEL of 0.8 and a LOAEL of 1.98 mg/kg-day would be appropriate, based on the histopathological changes in the kidney in male rats. However, the most sensitive endpoint in the study appeared to be the hematological changes and the formation of methemoglobin that were evident in male rats at a dose of 0.8 mg/kg-day which, yielded a NOAEL of 0.21 mg/kg-day (Table 2).

2.3.1.3 Mammalian Oral Toxicity – Subchronic

Reddy et al. (1995) conducted a 90-day toxicological study in which 15 F344 rats/sex/group received 0, 1, 6 or 30 mg m-DNB/kg in their diet, amounts calculated by the authors to be equivalent to doses of 0, 0.06, 0.35 and 1.73 mg/kg-day in males and 0, 0.07, 0.39 and 1.93 mg/kg-day in females. A full range of in-life, clinical chemistry/hematological (at 45 and 90 days), gross pathological and histopathological evaluations were carried out as described above for the 14-day study (Reddy et al., 1994a).

Critical findings included an increase in the average relative spleen weight and a reduction in relative testis weights, a reduction in the absolute testis weight and the onset of profound histopathological changes in the spleen, kidney and testes of high-dose groups. While sporadic changes in clinical chemistry parameters appeared not to be related to treatment, marked dose-dependent changes in hematological parameters were observed, including reductions in hemoglobin, hematocrit and erythrocyte counts in both sexes of high- and mid-dose rats, increases in platelet counts in high-dose females and in reticulocytes and methemoglobin formation in mid- and high-dose groups. These results indicated a NOAEL of 0.06 mg/kg-day for the hematological responses, with an associated LOAEL of 0.35 mg/kg-day.

The earlier report of Cody et al. (1981) described subchronic experiments in which m-DNB was administered to both sexes of Carworth Farms rats in drinking water. In one experiment, concentrations of 0, 50, 100 or 200 mg/L were provided to 6 animals/sex/group for 8 weeks, while in the second, 0, 3, 8 or 20 mg m-DNB/L were provided to 20/sex/group for 16 weeks. Average dose levels of 0, 4.72, 7.26 and 12.45 mg/kg-day in males and 0, 5.97, 9.0 and 24.43 mg/kg-day in females was calculated for rats in the 8-week study, based on the data in the report.

In general, dose levels of the 8 week study were associated with profound toxic responses, including four of six fatalities in high-dose males, two of six fatalities in high-dose females, reductions in body weight gain compared to controls that were probably over and above any anticipated reduction due to curtailed food and water intake. Reduced hemoglobin concentrations, enlarged spleen (except in high dose females) and atrophy of testes were in evidence at all m-DNB dose levels.

Average doses of 0, 0.4, 1.13 and 2.64 mg/kg-day in males and 0, 0.48, 1.32 and 3.1 mg/kg-day in females could be determined for rats in the 16-week study, based on the data in the report (Cody et al., 1981). Among the compound-related findings were reduced testicular weights with depleted spermatogenesis in high-dose males and enlarged spleens associated with a number of histopathological manifestations including hemosiderosis in all dose groups for males and in the mid- and high-dose groups for females. These findings suggested that a NOAEL of 0.48 mg/kg-day (with a related LOAEL of 1.32 mg/kg-day) would be appropriate for the spleen effects, which, appeared to be the most sensitive response to m-DNB. A NOAEL from this study (0.4 mg/kg-day) was used by the IRIS compilers to derive a reference dose (RfD) (human health) of 1×10^{-4} mg/kg-day for m-DNB (U.S. EPA, 2000).

Testicular impairment and indicators of reproductive success were the subject of a subchronic study on the toxicity of m-DNB in Sprague-Dawley rats (Linder et al., 1986; Perreault et al., 1989). Twelve male Sprague-Dawley rats/group were gavaged 5 days/week for 12 weeks with 0, 0.75, 1.5, 3.0 or 6.0 mg m-DNB/kg-day in a corn oil/acetone mixture. As a functional assessment of male reproduction, each male was mated with two virgin females after 10 weeks and pregnant females were then terminated on gestation day 21 and evaluated for reproductive parameters. All males were sacrificed after 12 weeks and a battery of sperm parameters were evaluated according to dose, including spermatid count, cauda reserves, percentage motility, morphology, histopathology and fertility. There was severe toxicity in those animals receiving 6 mg/kg-day m-DNB, as evidenced by ataxia, loss of balance, muscular rigidity, and a lower body weight. These effects became especially marked during the first week of breeding, to such an extent that breeding and m-DNB treatment were discontinued in this group after 4 days. However, there were no clinical signs of toxicity in subjects receiving 3 mg/kg-day m-DNB or less. In male rats sacrificed after 12 weeks, there were decreased testicular and epididymal weights at 3.0 mg/kg-day and increased spleen weights in animals receiving 1.5 and 3.0 mg/kg-day. The most sensitive parameter of testicular toxicity appeared to be the spermatid count, which showed a significant difference (72%) to controls at 1.5 mg/kg-day and above, indicating a NOAEL of 0.75 mg/kg-day and a LOAEL of 1.5 mg/kg-day. In addition to testicular toxicity, male reproductive success was deleteriously impacted by m-DNB. No litters were produced in females mated with males receiving 3.0 or 6.0 mg 2,3-DNB/kg-day, and significantly fewer pups compared to controls were produced as a result of matings of females with males receiving 0.75 and 1.5 mg/kg-day. This data indicates that m-DNB disrupts normal reproductive function in males and suggests a LOAEL of 0.75 mg/kg-day.

2.3.1.4 Mammalian Oral Toxicity – Chronic

No studies were identified that explored the toxicity of m-DNB through chronic exposure.

2.3.1.5 Mammalian Oral Toxicity – Other

A large number of experimental studies have demonstrated that m-DNB is a potent testicular toxicant. In fact, a single dose of m-DNB to male laboratory rodents has served as a model for the investigation of testicular impairment. For example, Linder et al. (1988) administered a single oral dose of 48 mg m-DNB/kg in acetone/corn oil (2% v/v) to eight male Sprague-Dawley rats/group and demonstrated a marked seminiferous tubular degeneration, with reduced testicular and epididymal weights, reduced numbers of sperm heads, degeneration of sperm tails, and the appearance of dead and "decapitated" cells. Subsets of treated animals were terminated after different time intervals up to 175 days, throughout which treated males were mated intermittently with untreated females. In line with the reduction in numbers and integrity of the sperm, some of the matings produced no fertilized eggs, for example, at 34–38 days post-treatment. However, some but not all treated males recovered their fertility in subsequent matings. A companion paper examined the detailed histopathological consequences of a single oral dose of 48 mg m-DNB/kg as used in Linder et al. (1988), showing the testis to be severely damaged at 24 hours post-treatment, with the appearance of "increased numbers of regressive seminiferous tubules that exhibited degenerating pachytene spermatocytes, chromatin margination in spermatids, deformed spermatic heads, retained spermatids and reduced numbers of meiotic figures" (Hess et al., 1988). The ability of spermatogenesis to recover from this chemical insult was variable, since only three of seven treated males were morphologically and functionally normal at 175 days post-treatment.

Blackburn et al. (1988) compared the ability of 1,2-, 1,3- and 1,4-DNB to induce testicular lesions in four male Wistar rats/group receiving a single dose of 50 mg/kg by gavage in PEG. Histopathological lesions of the testis were noted in those animals receiving m-DNB but not 1,2- or 1,4-DNB. This suggests a stereospecificity of the mechanism by which m-DNB induces testicular atrophy and sperm deficits, a feature not shared with the mechanism of methemoglobin induction in which 1,4-DNB was just as effective as m-DNB. In the dose response phase of their study, Blackburn et al. (1988) found single doses of 5 and 10 mg m-DNB/kg to be ineffective in inducing testicular lesions in Wistar rats, although testicular lesions limited to Stages VIII through XI of the spermatogenic cycle were evident 12 hours after a single oral dose of 25 mg m-DNB/kg and similarly, 48 hours after a single oral dose of 15 mg 1,3,-DNB/kg.. The authors considered the ultrastructural evidence to implicate the Sertoli cells as the prime targets for the toxic action of m-DNB with germ cell damage a secondary event. This conclusion was endorsed by Rehnberg et al. (1988) who measured a range of testicular and serum hormone concentrations in male Sprague-Dawley rats (receiving a single gavage dose of 32 mg m-DNB/kg in corn oil) and found little if any evidence that the m-DNB-induced testicular lesions might be secondary to changes in testicular, brain or pituitary hormone levels.

A report by Evenson et al. (1989a) demonstrated that the testicular effects of m-DNB were also apparent in male adult but not prepubertal B6C3F1 mice exposed to the compound. They administered a

single gavage dose of 0, 8, 16, 32, 40 or 48 mg m-DNB/kg in corn oil/acetone and measured testicular responses at various time intervals for the next 25 days. The 48 mg/kg dose appeared to be the critical dose level for the onset of testicular effects in adult B6C3F1 mice, resulting in abnormal spermatogenesis, reduced testicular weights, altered germ-cell type ratios, abnormal chromatin structures and an increase in abnormal sperm head morphology. The same researchers exposed 16 male Sprague-Dawley rats/group to single doses of either 0, 8, 16, 32 or 48 mg m-DNB/kg and examined the compound's effects on spermatogenesis using flow cytometry, 1, 4, 16 or 32 days post-dosing (Evenson et al., 1989b). An increase in the incidence of unusual cell types was observed on day 1 after 48 mg/kg and on day 4 after 16 mg/kg or greater. The presence of haploid, diploid and tetraploid cells signaled a dose-dependent increase of germinal cell types as a consequence of treatment, elevated numbers of which persisted to post-treatment day 32 and beyond, often accompanied by aberrant sperm effects. Using Sprague-Dawley rats as well, Linder et al. (1990) observed a single dose no observed adverse effect level (NOAEL) of 8 mg/kg and a lowest observed adverse effect level (LOAEL) of 16 mg/kg for changes in epididymis weight, sperm head counts, caudal sperm reserves and sperm morphology.

Holloway et al. (1990) used *in vitro* fertilization to investigate the influence of m-DNB on Sertoli cells and thus the functional capacity of developing germ cells. Male Wistar (AP/ALPK) rats were given a single oral dose of either 0, 5, 15 or 25 mg m-DNB/kg in PEG, and sperm were collected from the cauda epididymis at various time points thereafter. The sperm was used in an *in vitro* fertilization technique through which reduced sperm fertilizing capacity was observed from 1.5 to 5 weeks and from 7.5 to 8.5 weeks after treatment with 15 and 25 mg m-DNB/kg and for 3, 5.5, 7.5 and 8.5 weeks after treatment with 5 mg m-DNB/kg. The authors interpreted their data to indicate that m-DNB did not affect all Sertoli cells equally but acted in a stage-specific manner, with Stages III, IX, XII and XIV appearing to be especially vulnerable to the toxicant. This stage-specific mechanism of m-DNB on the Sertoli cells was reported in a review by Nolte et al. (1995) and by McEuen et al. (1995) who obtained similar testicular damage in Sprague-Dawley rats irrespective of the route of administration of the compound (oral or intraperitoneal). Given the different concentrations of parent compound and metabolites that resulted from intraperitoneal versus oral dosing, McEuen et al (1995) speculated that only the duration of testicular exposure to the toxicant may govern susceptibility to toxicity once a threshold blood level of m-DNB is reached.

A number of studies have searched for compounds that might serve as plasma biomarkers of the incipient testicular damage induced by chemical toxicants such as m-DNB. Of a number of hormones and enzymes evaluated, the lactate dehydrogenase-C_4 (LDH-C_4) isoenzyme and the androgen binding protein (ABP) were shown to be viable candidates when single doses of 0, 10, 20 and 30 mg m-DNB/kg were administered to male Wistar (Alpk APfSD) rats and 25 mg m-DNB/kg was administered in a time course experimental protocol (Reader et al., 1991). Using the same experimental animal, Suter et al.

(1998) correlated histopathological lesions and ABP production to further implicate Sertoli cell dysfunction as the etiologically significant event in germ cell depletion.

Two research groups have employed morphometry to visualize the toxicological effects of m-DNB. Davis et al. (1994) administered a single oral dose of 0, 15 or 25 mg m-DNB/kg to male Sprague-Dawley rats, which then were sacrificed after 22 to 24 days. Collected sperm were measured under the light microscope for total width, interior width, and symmetry using automated sperm morphometry analysis, the studies revealing three subpopulations of m-DNB-affected sperm, two of which were abnormal to varying degrees. Ninety-three percent of sperm harvested from the untreated controls had a normal appearance, with dose-dependently lower proportions in m-DNB-treated groups (78% and 66% respectively, for the low- and high-dose groups, respectively). Matsui and Takahashi (1999) gavaged male Sprague-Dawley rats with a single dose of 0 or 25 mg m-DNB/kg, with groups of four rats being sacrificed 1, 2, 4 or 7 days after administration. Sertoli cells were examined under the microscope, and the toxicity of m-DNB was evaluated by counting the proportion of germ cells without abnormalities in each treatment group, according to four user-defined clusters of stages of the spermatogenic process. As described by the authors, a large number of vacuoles were seen in Sertoli cell cytoplasm one day after m-DNB administration. These changes were considered instrumental in the apoptotic cell death of both pachytene and diplotene spermatocytes, round spermatids and other germ cells associated with Sertoli cells.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Acute, subacute, and subchronic experimental studies have delineated a distinct and well-defined range of toxicological effects of m-DNB. However, the range of animal models employed in these studies, predominantly F344 and Sprague-Dawley rats, may lend a degree of uncertainty to quantitative estimates of dose levels potentially protective of mammalian wildlife. In general, the use of a narrow range of experimental animals reduces confidence in whether the resulting NOAELs and LOAELs have necessarily captured the sub-threshold for mammalian wildlife.

While the IRIS compilers chose splenic enlargement and the formation of hemosiderin deposits as the principal effect of m-DNB for RfD derivation (U.S. EPA, 2000), a clear spectrum of other toxicological responses to m-DNB has emerged including (1) hematological effects including methemoglobinemia (2) nephropathy associated with cytoplasmic and/or hyaline droplet formation, (3) structural and functional impairment of the brain deficits, and (4) atrophy of the testis with associated degeneration of the seminiferous tubules and sperm.

Methemoglobinemia is reversible and can create a functional hypoxic blood condition. Carbon monoxide poisoning also creates a functionally similar condition. Humans with levels of COHb above 10% have reported symptoms of headache, yet the preponderance of adverse effects occur when COHb

concentrations exceed 2% (ACGIH 1997). Chronic congenital methemoglobinemia in humans has been found where 10-50% of circulating blood pigment is in the form of methemoglobin with subjects exhibiting no overt signs of toxicity (Smith 1996). Reddy et al. (1995) report differences between control and high dose rats of approximately 3% or less from m-DNB exposure. Given the uncertainty associated with the reported methemoglobin increase in these investigations, increased methemoglobinemia due to m-DNB was not considered biologically significant.

m-DNB also caused increased erythroid cell hyperplasia and pigmentation in the spleen. In addition, the data showed decreased hematocrit, red blood cell count, hemoglobin and an increase in mean cell volume in primarily the high dose groups, however, these values were within ranges considered normal for rats of that age group (Wolford et al., 1986). The combined effects of m-DNB on spleen histopathology and hematological parameters are indicative of anemia although the biological significance of these effects in these models are questionable.

There is uncertainty regarding whether the nephropathic changes and cytoplasmic droplet formation in the kidney in response to m-DNB represent the same phenomenon as the well described $\alpha 2_{\mu}$-globulin-mediated hyaline droplet formation that is typical of male F344 rats and those of other strains. For example, in the 90-day study on m-DNB, Reddy et al. (1995) found 9 out of 10 high-dose females but only 1 out of 10 high-dose males displaying cytoplasmic kidney droplets on histopathological examination, a finding that contrasts with the current understanding of the $\alpha 2_{\mu}$-globulin-mediated hyaline droplet phenomenon as a characteristic of male rats. For m-DNB, this weakens the analogy with the nephropathy displayed by 1,3,5-TNB in the subacute (Reddy et al., 1994b) and subchronic studies (Reddy et al., 1994c), which appears to have the "classical" $\alpha 2_{\mu}$-globulin-mediated hyaline droplet etiology. However, the cytoplasmic droplets identified in the kidney of F344 males in the 14-day study of m-DNB toxicity were clearly identified as hyaline (Reddy et al., 1994a). Contrasting these observations with those of 1,3,5-TNB-induced nephropathy in F344 rats in a 2-year study (Reddy et al., 1996) and those of m-DNB-induced nephropathy in the 90-day study (Reddy et al., 1995) (in each case with lesions that apparently did not resemble the typical $\alpha 2_{\mu}$-globulin-mediated hyaline droplet histopathology and gender-specific incidence) holds open the possibility that age-related morphological and chemical differences may exist between the kidney droplets formed in response to shorter periods of nitroaromatic dosing compared to those becoming manifest after a longer period of dosing. Although this response is common to nitroaromatic exposure, the impact of increased incidence of hyaline droplets in the kidney is unknown. Hence, given the uncertainty in etiology related to hyaline droplet formation and the lack of known biological/ecological significance, this endpoint cannot be used for derivation of the TRV.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Linder et al. (1986) Perreault et al. (1989)	Rat (Sprague Dawley)	12-w	0.54	1.1	Reduced spermatid head count
			NA	0.54	Significant reduction in reproductive performance (pups/litter)
Philbert et al. (1987)	Rat (F344)	5-d	NA	20	Ataxia in 6/6 male rats.
Reddy et al. (1995)	Rat (F344)	90-d	0.07	0.35	Methemoglobinemia and an increase in reticulocytes
			0.39	1.73	Reduction in RBCs and in other hematological responses, changes in spleen and testicular histopathology
Reddy et al. (1994a)	Rat (F344)	14-d	0.21	0.8	Methemoglobin formation
			0.8	1.98	Enlargement of the spleen
			1.98	5.77	Nephropathy associated with hyaline droplet formation, testicular degeneration
Cody et al. (1981)	Rat (Carworth Farms)	8-w	NA	4.72	Enlarged spleen, fluctuation in hemoglobin levels, atrophy and histopathological lesions of the testes
Cody et al. (1981)	Rat (Carworth Farms)	16-w	0.48	1.32	Enlarged spleen
			1.13	2.64	Depleted spermatogenesis

NA = not applicable
RBC = red blood cell

m-DNB appears to be a neurological toxicant, with pronounced histopathological lesions induced in various regions of the brain as a consequence of acute dosing. While speculation on the mechanism of these effects has centered on (1) the possibility of impairment of oxidative metabolism and a perturbation of intracellular redox mechanisms and/or (2) changes in the concentrations and activities of various neurotransmitters and their metabolites, there is little solid evidence to indicate precisely how the observed effects are brought about (Philbert et al., 2000). The most pertinent study on the neurological effects of m-DNB was Philbert et al. (1987). However, in this study, rats showed signs of ataxia although only 6 conventional (non-germ free) rats were dosed with one concentration of m-DNB. The limited data and study design precludes an adequate assessment of the neurological effects of m-DNB. Although it is reasonable to infer that ataxia will likely result in decreased a lower survival, the lack of a dose-response regime and endpoints consistent with the other data suggest the questionable nature of these data for TRV derivation. Given these uncertainties associated with the whole-organism effects of the histopathological lesions and the limited availability of data, these studies alone cannot be used for derivation of the TRV.

Impairment of the male reproductive organs with associated decreases in sperm production and motility and reproductive performance is a consistent response of experimental animals to m-DNB (Linder et al., 1986; 1988; Blackburn et al., 1988; Evenson et al., 1989a; 1989b; Holloway et al., 1990). As described in Section 2.3.1.1, a single dose of m-DNB of 5 mg/kg-day or more is sufficient to induce testicular effects and sperm deficits in various strains of experimental animals (Holloway, et al., 1990). In the most pertinent study (Linder et al., 1986), the NOAEL was 0.75 mg/kg for rats exposed to m-DNB in the diet for 12 weeks. From and ecological perspective, the most significant effect was decreased reproduction in m-DNB dosed males. In fact, no litters were produced from non-dosed females mated with males that had been dosed with 3 and 6 mg/kg-day and the number of pups per litter was significantly less from females mated with males dosed with 0.75 and 1.5 mg/kg-day compared to females mated with control males. Although the effects of m-DNB on reproduction in males appears to be strong, limited data suggest that this response appears to be somewhat reversible on complete cessation of exposure (Linder et al., 1986). Nevertheless, given the severity of reproductive effects even from a single dose of m-DNB, decreased reproduction in males was chosen as the endpoint for derivation of the TRV.

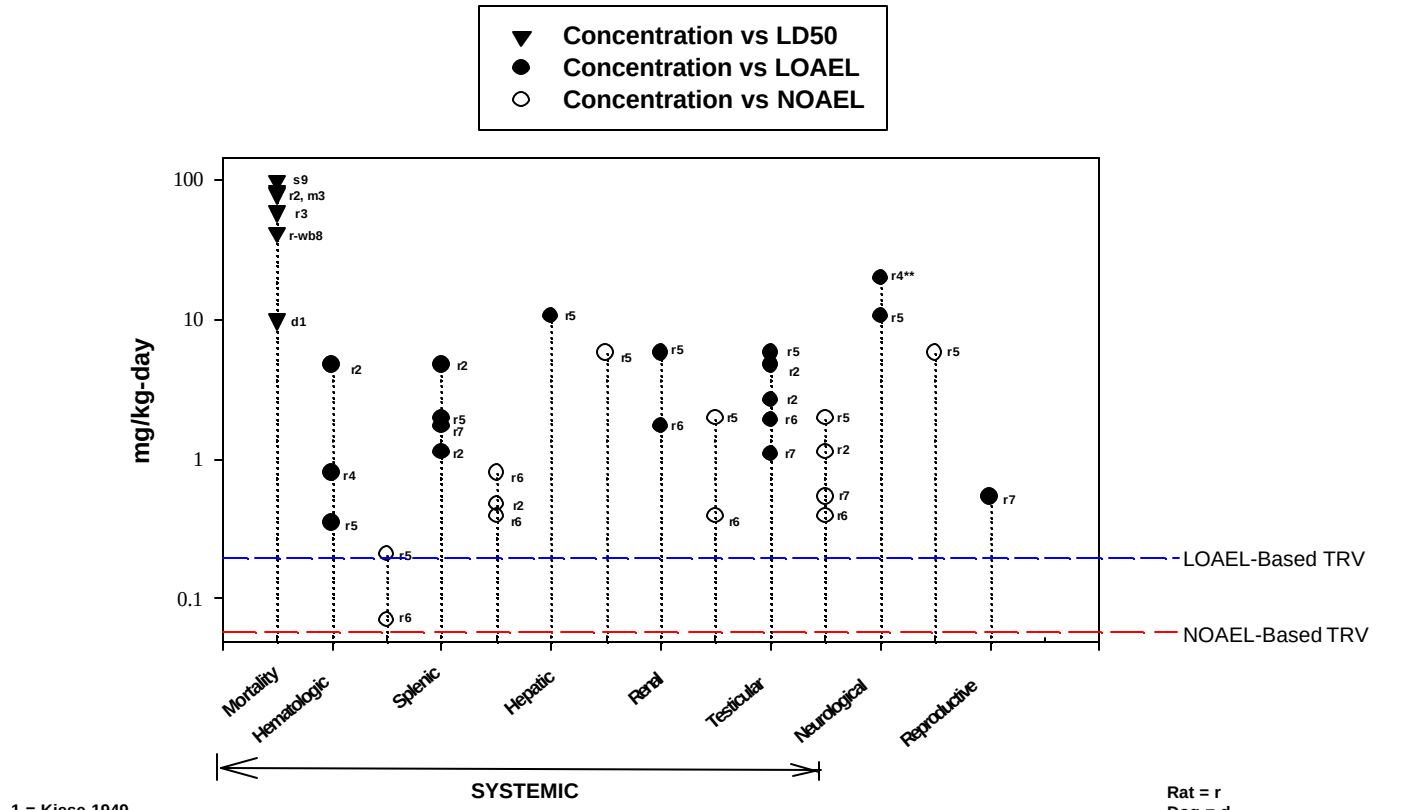
2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using mammals were found.

2.3.3 Mammalian Dermal Toxicity

No dermal studies conducted using mammals were found.

1,3-DNB HEALTH EFFECTS TO MAMMALS*



1 = Kiese 1949
2 = Cody et al. 1981
3 = FitzGerald et al. 1991
4 = Philbert et al. 1987
5 = Reddy et al. 1994a
6 = Reddy et al. 1995
7 = Linder et al. 1986
8 = Schafer 1972
9 = Schafer et al. 1983
** indicates acute exposure

Rat = r
Dog = d
Mouse = m
Red-winged blackbird = r-wb
Starling = s

*Avian lethality data included

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity - Oral

2.4.1.1 Avian Oral Toxicity - Acute

Researchers with the U.S. Fish and Wildlife Service (Schafer, 1972; Schafer et al., 1983) reported LD_{50}s for this compound in Red-winged Blackbirds (*Agelaius phoeniceus*) and European Starlings (*Sturnus vulgaris*) of 42 and >100 mg/kg, respectively. No other information was presented.

2.4.1.2 Avian Oral Toxicity - Subchronic

No data are available.

2.4.1.3 Avian Oral Toxicity - Chronic

No data are available.

2.4.1.4 Avian Oral Toxicity - Other

No data are available.

2.4.2 Avian Inhalation Toxicity

No data are available.

2.4.3 Avian Dermal Toxicity

No data are available.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of m-DNB in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

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

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The parameters relevant to m-DNB toxicity involved changes in testicular histopathology and reproductive performance in male rats (Table 2.). The data relevant for TRV derivation for m-DNB is limited to subchronic oral exposure in rats. No chronic data are available and no repeated dosing studies have been conducted on mammals other than rats. Values used to derive the TRV were obtained from (Linder et al., 1986).

As outlined in Technical Guide 254, toxicity values used for derivation of a TRV should be based on potentially ecologically relevant effects. Effects of m-DNB on male reproduction was chosen as the parameter for TRV derivation based on decreased litter production from females mated with males exposed to m-DNB via oral gavage (LOAEL was 0.75 mg/kg-day, Linder et al., 1986). Also, this level at which reproductive effects occur is protective of the neurological effects noted by Philbert (1987).

Data on the toxicity of m-DNB is limited to two mammalian species, rats and mice, and no chronic toxicity studies were conducted. Although a study on m-DNB toxicity in shrews was available, it was of little use for TRV development because it provided information only on hemoglobin adduct formation, no controls were used and subjects were euthanized 24h after exposure. Given the above data limitations, the approximation approach was the only viable means for deriving the TRV for m-DNB (USACHPPM, 2000). An uncertainty factor of 20 was used to derive the NOAEL-based approximate TRV from a subchronic LOAEL of 0.75 mg/kg-day (Linder et al., 1986). An uncertainty factor of 4 was used to derive the LOAEL-based approximate TRV from a the same subchronic LOAEL. Final values for the TRV were rounded. As stated, there are limited data for multiple species and no chronic toxicity data for DNB. However, the reported studies are of relatively high quality with a broad scope of observations that are consistent with each other and of other nitroaromatic compounds. It is for these reasons the TRV for m-DNB presented below was given a Medium confidence value.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	0.04 mg/kg/d	Medium
LOAEL-based	0.2 mg/kg/d	Medium

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not Available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Only acute LD50 data are available for two species of birds, Red-winged Blackbirds and European Starlings. This information is originally presented in Schafer (1972) and no other supporting information was presented. Although the approximate method could be used to derive a TRV from these data, in the absence of details from the original reports, it is recommended that a TRV not be derived for birds until more information is available.

3.2 Toxicity Reference Values for Amphibians

Not Available at this time.

3.4 Toxicity Reference Values for Reptiles

Not Available at this time.

4. IMPORTANT RESEARCH NEEDS

The limited availability of data on the toxicity of m-DNB to wildlife species precludes the development of a high-confidence TRV. Hence, more studies on the toxicity of m-DNB to wildlife species are required. In particular, chronic toxicity studies on mammals and additional studies on non-mammalian wildlife such as birds, reptiles and amphibians are particularly warranted. More information regarding the toxicity of m-DNB to wildlife would likely allow the derivation of a high confidence TRV.

5. REFERENCES

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APPENDIX A
LITERATURE REVIEW

The following files were searched in Dialog:

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

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

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

The search strategy for **Birds:**

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

The search strategy for **Laboratory Mammals:**

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

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers
- And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 62 hits on m-DNB were obtained in the initial search, of which 45 were selected for abstract evaluation. Thirty of these articles and reviews were retrieved for this survey.

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

USCHPPM

Wildlife Toxicity Assessment for 2,4,6-TRINITROTOLUENE (TNT)

OCTOBER 2000

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Project No: 39-EJ-1138-00

Readiness Thru Health

Acknowledgements

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When referencing this document use the following citation
Johnson, M.S. and M.J. McAtee. 2000. Wildlife Toxicity Assessment for 2,4,6-Trinitrotoluene. U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Project Number 39-EJ-1138-00. Aberdeen Proving Ground, Maryland. October.

Table of Contents

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

Wildlife Toxicity Assessment for 2,4,6-Trinitrotoluene

CAS No. 118-96-7 **October 2000**

1. INTRODUCTION

This Wildlife Toxicity Assessment is the result of a thorough investigation of the scientific literature regarding the toxicological characteristics of 2,4,6-trinitrotoluene (TNT) that may be important for the health of wildlife (mammals, birds, reptiles and amphibians) exposed to the substance. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

This document is designed to support ecological risk assessment activities. The measures of toxicity derived in this document are intended to be used in screening-level assessments. By definition, the measures of toxicity presented herein evaluate the likelihood of effects in *individual* organisms that may be relevant to a *population* of organisms in the wild. This Wildlife Toxicity Assessment does not specifically address how the measures, or any resulting risk estimates, relate to demographic rates or outcomes for any particular population of interest. Assessing risk to populations involves using these methods and other lines of evidence before risk management actions to protect populations can be recommended based upon scientific information. Therefore, the toxicity measures in this document should not be used to demonstrate unacceptable population risks that require remedial action without further site-specific study.

2. TOXICITY PROFILE

2.1 Literature Review

Given the predominant military use of trinitrotoluene, many studies were found from U.S. Army sources. These military sponsored studies, and subsequent reports, were found through TOXLINE and DTIC searches. However, the most appropriate ones were found though traditional cross-referencing techniques and through individual queries to project investigators within the Army. Several databases were searched and Appendix A contains details of this search.

2.2 Environmental Fate and Transport

The distribution of TNT at many U.S. military sites is substantial. At least 17 Army installations have reported soil concentrations ranging from 0.08 to 64,000 micrograms per gram (μg/g) (Hovatter et al. 1997). Of those that had detectable concentrations, 5 installations had samples in which surface soils exceeded 10,000 μg TNT/g soil dry weight (Walsh and Jenkins 1992).

A summary of physical and chemical properties is provided in Table 1. An important route for the contamination of surface water, ground water, and surface soils with TNT has historically been due to large aqueous effluents of rinse water ("pink water," Walsh and Jenkins 1992, ATSDR 1995). Some sources have reported wastewater emissions ranging from 61 – 210 pounds/day (Rosenblatt et al. 1973). Due to its relatively low vapor pressure, and relatively high water solubility, TNT does not actively partition from surface waters to the atmosphere (ATSDR 1995). Photolysis studies, comparing river waters and distilled water, have shown that the rate of TNT photolysis is directly related to increases in pH and organic matter content (Spanggord et al. 1980). Generally, TNT is not expected to hydrolyze or bioconcentrate in aquatic systems under normal environmental conditions (HSDB 1997).

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

CAS No.	118-96-7
Molecular weight	227.13
Color	yellow-white
State	Monoclinic needles
Melting point	80.1°C
Boiling point	240°C
Odor	Odorless
Solubility	130 mg/L in water at 20°C; soluble in acetone, benzene, alcohol and ether
Partition coefficients	
Log K_{OW}	1.60; 2.2 (measured), 2.7 (estimated)
K_{OC}	300 (estimated), 1,100 (measured)
Vapor pressure (at 20°C)	1.99E-04 mm Hg
Henry's Law constant (at 20°C)	4.57E-07 atm m^3/mole
Conversion factors	1 ppm = 9.28 mg/m^3 1 mg/m^3 = 0.108 ppm

Source: ATSDR (1995)

Soil contamination of TNT can result from spills, disposal of solid waste, open incineration and detonation of explosives, or leaching from poorly engineered impoundments (Burrows et al. 1989). Retrieval and subsequent destruction of unexploded ordinance (UXO) can result in soil contamination as well (includes open burning/open detonation, OB/OD areas). Based primarily upon the physical and chemical properties of TNT (i.e., octanol-water partition coefficient (K_{ow}) and water solubility), TNT is not expected to bioaccumulate or biomagnify in terrestrial systems (HSDB 1997).

Based on the measured and estimated soil organic carbon adsorption coefficient (K_{oc}) of 300 – 1100, TNT is not expected to significantly partition to sediment (from surface waters) or sorb to soil particles (HSDB 1997, ATSDR 1995). However, the biotransformation of TNT in soil can be significant, and can be readily reduced under anaerobic conditions. These anaerobic reactions occur through microbial reduction, primarily through successive reduction of the nitro groups (Burrows et al. 1989). Several bacteria have been identified in these reactions. They include species of *Pseudomonas, Escherichia, Bacillus, Citrobacter, Enterobacter, Klebseilla, Veillonella,* and *Clostridium* (Burrows et al. 1989). Fungi are also capable of reducing TNT (Burrows et al. 1989, ATSDR 1995). Microbial transformation of TNT leads to a variety of reduction products, including 2-amino and 4-amino dinitrotoluene and azoxydimers (Burrows et al. 1989, HSDB 1997), though some oxidation products have been identified (Won et al. 1974). Biological transformation by bacterial and fungal species occurs slowly in the environment, with slightly higher rates in the presence of other carbon sources. However, biological degradation may not extend to cleavage of the TNT ring (the successive reductions of each of the nitro groups to amines followed by oxidative deamination to a phenol that releases an ammonia or nitrite has been described (HSDB 1997)). Accurate mass balance without the use of radio-labeled compound is difficult with TNT based on its crystal forming tendencies, low organic solubility, and relatively low water solubility (M. Major, USACHPPM, pers. comm.).

Another process that can affect the fate and transport of TNT in the environment is photolysis. Photolysis has been reported to produce “pink water” from TNT-contaminated surface water (ATSDR 1995). Numerous transformation products have been identified in pink water, the predominant ones including 1,3,5-trinitrobenzene, 4,6-dinitroanthranil, 2,4,6-trinitrobezaldehyde, 2,4,6-trinitrobenzonitrile, in addition to several azo and azoxy derivatives formed by the coupling of nitroso and hydroxyamine products (Jerger et al. 1976, Spanggord et al. 1980).

2.3 Summary of Mammalian Toxicology

2.3.1 Mammalian Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

Oral lethal dose to 50% of the exposed population (LD_{50}) values of 660 milligrams per kilogram (mg/kg) in male and female mice and 1320 and 795 mg/kg in male and female rats, respectively, have been reported (Dilley et al. 1982a). These animals developed seizures (grand mal), followed by mild convulsions 1 – 2 hours after exposure. All deaths occurred within 24 hours after exposure; red urine and lethargy were other signs of exposure (Dilley et al. 1982a). Animals that survived the convulsions were still alive 14 days following the exposure (Dilley et al. 1982b). Variation in response for dogs was considered significant (Voegtlin et al. 1921). Cyanosis was evident 12 hours following administration of 100 mg/kg TNT. Severe incoordination and tremors followed. However, the authors note that some dogs receiving 100% of the 100 mg/kg dose did not exhibit the same symptoms as those receiving 50% or less (Voegtlin et al. 1921). Most species showed signs of ataxia after dosing (Voegtlin et al. 1921, Dilley et al. 1982b).

Cats injected intraperitoneally with 0.10 to 0.15 grams per kilogram (g/kg) TNT died within 5.5 hours (Bredow and Jung 1942). Injections of 0.04 g/kg caused convulsions, paralysis of the hindlimbs, decrease in body temperature, and enhanced saliva secretion. Methemoglobin was also present in the blood. Cats given daily subcutaneous injections of 50 mg/kg TNT died within 4 to 9 days (Lillie 1943). Each showed signs of splenic congestion. Livers had fat accumulation (steatosis) and Kupffer cell hemosiderosis.

White-footed mice (*Peromyscus leucopus*; 10/group/sex) were exposed to one of five treatments of 0, 0.042, 0.083, 0.165, and 0.330% TNT in feed for 14 days (Johnson et al. 2000a). These treatments were calculated by the authors to be equivalent to 66, 145, 275, and 602 mg TNT/kg body weight per day (bw/d) for males and 70, 142, 283, and 550 mg/kg/d for females for the 0.042, 0.083, 0.165, and 0.330% TNT, respectively. Indicators suggesting hemolysis were evident in the 0.330% treatment for both sexes, where only males had suppressed splenic phagocyte hydrogen peroxide production for the 0.165 and the 0.330% treatments, and a reported reduction in phagocytosis for males in all TNT exposures. However, the authors note that the significance of the latter endpoint (i.e., inhibited phagocytosis for males and not females) is questionable.

Oral LD_{50} estimates for cotton rats (*Sigmodon hispidus*) exposed to TNT in corn oil were 607 and 767 mg TNT/kg bw for males and females, respectively (Reddy et al. 2000). Animals exhibited an increased respiratory rate within 90 minutes after dosing. Orange-colored urine and urinary bladder distension was observed in all animals at necropsy. No other abnormal histological observations were reported.

A 7-day gavage exposure representing 1/8, 1/4, and 1/2 the LD_{50} for male (75.9, 151.8, and 303.5 mg TNT/kg bw/d) and female cotton rats (96, 192, and 384 mg TNT/kg bw/d) was conducted using corn oil (Reddy et al. 2000). Histopathology of major organs as well as hematology, hepatic metabolizing enzymes, and clinical chemistry of the sera were evaluated. Splenic weights were increased in the 192 (females only) and the 384 mg/kg/d treatments; and liver weights were increased in the 151.8 (males only) and 303.5 mg/kg/d treatments. These two high dose groups also showed hematological results consistent with erythrolytic anemia. Hemosiderin laden macrophages were noted in the spleen of rats receiving the lowest dose. Subtle testicular lesions were noted in the two high dose groups.

2.3.1.2 Mammalian Oral Toxicity - Subchronic

Subchronic exposures to rats, mice, and dogs have produced consistent hematologic effects (Von Oettingen et al. 1944, Dilley et al. 1982b, Levine et al. 1990a, b). Exposures of 13 weeks were sufficient to produce anemia (consisting of reduced number of red blood cells, reduced hemoglobin and hematocrit) in all of these species. Increases in immature red blood cells (reticulocytes), reduction in blood, hematocrit, and corpuscle volumes were evident after only 15 days in dogs administered TNT in gelatin capsules of dosages ranging 5 – 33 mg (Voegtlin et al. 1921). TNT exposure is reported to result in direct hemolysis within circulating blood, leading to an increase in spleen weight. Dilley et al. (1982a, b) reported similar findings including pathological assessment of the spleen that suggested hemolytic anemia in beagles. Other important effects included increased liver weight (including hepatocytomegaly), intestinal inflammation (and mucoid stools), enlarged kidneys, and splenic congestion in mice, rats, and dogs (Dilley et al. 1982b, Levine et al. 1990a, b). Most animals in the highest dose group of all species displayed some degree of hemosiderosis of the spleen (Dilley et al. 1982b). Rats and dogs had dose-related increased serum cholesterol and lower iron and serum glutamic-pyruvic transaminase (SGPT) levels following the 13-week exposure period; mice seemed to be more resistant to treatment (Dilley et al. 1982b). Increased serum cholesterol was consistent with doses in rats and dogs (Levine et al. 1984, Dilley et al. 1982b). Other endpoints consistent with anemia were decreased erythrocyte numbers, hemoglobin and hematocrit values, and occasionally bone marrow hyperplasia.

Testicular atrophy was most pronounced in rats (Dilley et al. 1982b), and consisted of dose-related degeneration of the germinal epithelium lining the seminiferous tubules and hyperplasia of interstitial Leydig cells (in high dose group, 300 mg/kg/d; Levine et al. 1984). The No Observable Effect Levels (NOELs) for these three species were: dogs, 0.20; rats, 1.42; and mice, 7.76 mg/kg/d, suggesting that dogs were the most sensitive (Dilley et al. 1982b). Dilley et al. (1982b) also mention that the effects appear to be totally reversible (up to a 4-week exposure) following a 4-week recovery period.

A single study investigating the functional response of splenic phagocytes to TNT in NMRI mice was conducted (through chemiluminescent analysis) from exposure TNT metabolites (2,4-diamino-dinitrotoluene, 2,4,6 triaminotoluene, 2-amino-6-nitrotoluene, 4-amino-3,5-dinitrotoluene, and 2-amino-4,6-dinitrotoluene) *in vitro* (Thierfelder and Masihi 1995). This assay quantifies intracellular-activated oxygen species. Relatively high doses of metabolites were associated with reduced response relative to controls. Specifically, > 1 milligram per liter (mg/L) of 2,4-diaminotrinitrotoluene, >50 mg/L for 4-amino-3,5-dinitrotoluene, and > 100 mg/L for 2-amino-4,6-dinitrotoluene caused a plateau of 57 – 65% inhibition (Thierfelder and Masihi 1995).

Results of a 90-day feeding study using white-footed mice (*Peromyscus leucopus*) provided evidence that Nearctic mice may be more resistant than Palearctic (Old-World: *Mus)* species. McCain (1998) exposed 100 male and female *P. leucopus* to concentrations of 660, 1320, and 2640 parts per million (ppm) TNT in feed. The calculated dosage was about 165, 330, and 660 mg/kg/d, respectively. The highest concentration used in this study (2640 ppm; 660 mg/kg/d) was equivalent to the LD_{50} of 660 mg/kg reported by Dilley et al. (1982b) in *Mus*, yet none died during the study. Initial animal weight reduction consistent with reduced palatability was reported, yet all groups gained weight over time. McCain (1998) found only exposures to 1320 and 2640 ppm associated with adverse physiological changes (organ weight, incidence of chromaturia, hemosiderin, etc.), and established a No Observable Adverse Effect Level (NOAEL) of 660 ppm (165 mg/kg/d).

2.3.1.3 Mammalian Oral Toxicity - Chronic

Effects from chronic exposures were consistent with those of sub-chronic exposures. Two studies using Fisher 344 rats (Furedi et al. 1984) and beagle dogs (Levine et al. 1990a) reported dose-dependent indicators suggesting hemolytic anemia (e.g., reduced hemoglobin, hematocrit, and erythrocyte counts, increased quantities of reticulocytes). These effects were different from controls at doses ≥ 8.0 (i.e., and 32 mg/kg/d for dogs; Levine et al. 1990a) and for all TNT treatments for rats (i.e., 0.4, 2.0, 10.0, and 50.0 mg/kg/d; Furedi et al. 1984). Exposures for the rat study lasted 106 weeks and 26 weeks for dogs. Compensatory responses to anemia were minimal in rats (e.g., erythrocytic macrocytosis and reticulocytosis; Furedi et al. 1984). Methemoglobinemia was apparent in both studies in animals of the higher dose groups. Reduction in body weight was apparent in rats exposed to 10 mg/kg/d or greater, and at 8 mg/kg/d or greater for dogs (Furedi et al. 1984, Levine et al. 1990a). Dose-related hepatomegaly (and increased kidney weights) was evident in rats receiving > 2.0 mg/kg/d; this was only evident in the high dose group for dogs. Splenomegaly was evident in rats and dogs in the higher dose groups. Hemosiderosis in Kupfer's cells was seen in various dogs at most dose levels (Levine et al. 1990a). Renal injury was supported by gross and tissue morphological examinations (in high dose groups; Furedi et al.

1984). Increased pigment deposition occurred in the kidneys (as did evidence of bone marrow fibrosis) of rats exposed to 2.0 mg/kg/d or greater (Furedi et al. 1984). It was reported that the observed enteritis of the small intestine was related to TNT treatment in dogs (Levine et al. 1990a). Urinary bladder carcinomas were evident in some rats (2 males and 4 females of 1794 and 1754 rats, respectively) exposed for 106 weeks (Furedi et al. 1984). Given the rate of occurrence for these types of neoplasias, this finding was considered biologically significant. An NOEL was determined to be 0.4 mg/kg/d for rats (Furedi et al. 1984); none was found for dogs (Levine et al. 1990a). TNT was found to be mutagenic (without S9 activation) in *Salmonella typhimurium;* the reduced metabolites were less potent mutagens (Tan et al. 1992).

2.3.1.4 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Primary target organs for TNT include the nervous system (primarily from acute effects) and blood (Table 2, Figure 1). Since TNT causes erythrolysis, the primary blood conditioning organs may also be affected (e.g., liver and kidney). These conditions were found in *Peromyscus* (McCain 1998), beagle dogs (Dilley et al. 1982b, Levine 1990a), rats (Furedi et al. 1984), and laboratory mice (*Mus*; Dilley et al. 1982b). Several studies were found that were current, well designed, and appropriate for the development of Toxicity Reference Values (TRVs) for mammals. The work of Dilley et al. (1982b), Levine et al. (1984, 1990a) and Furedi et al. (1984) are particularly valuable since they include chronic, subchronic and acute exposures, and use several species identified above. Two Orders and three families of *Mammalia* are represented that include: Carnivora: Canidae; Rodentia: Cricetidae, Muridae. Two wildlife species were also evaluated. Effects from exposure are consistent, yet slightly variable in magnitude of effect. Each study identifies several NOAELs and Low Observable Adverse Effect Levels (LOAELs) for various endpoints of effect, and the investigations are inclusive of other potential organ systems. It is for these reasons that this review is sufficient to derive class-specific TRVs for TNT.

With few exceptions, data from acute studies where gavage methods were employed were deemed irrelevant and not used for comparison (TRV derivation) purposes. Exceptions included acute or gavage studies that included other species not previously evaluated (e.g., Reddy et al. 2000). All other reports that evaluated TNT in feed were of sufficient quality and importance to include in this evaluation. These studies were consistent in quality and reporting of the methods.

2.3.2 Mammalian Oral Toxicity – Other

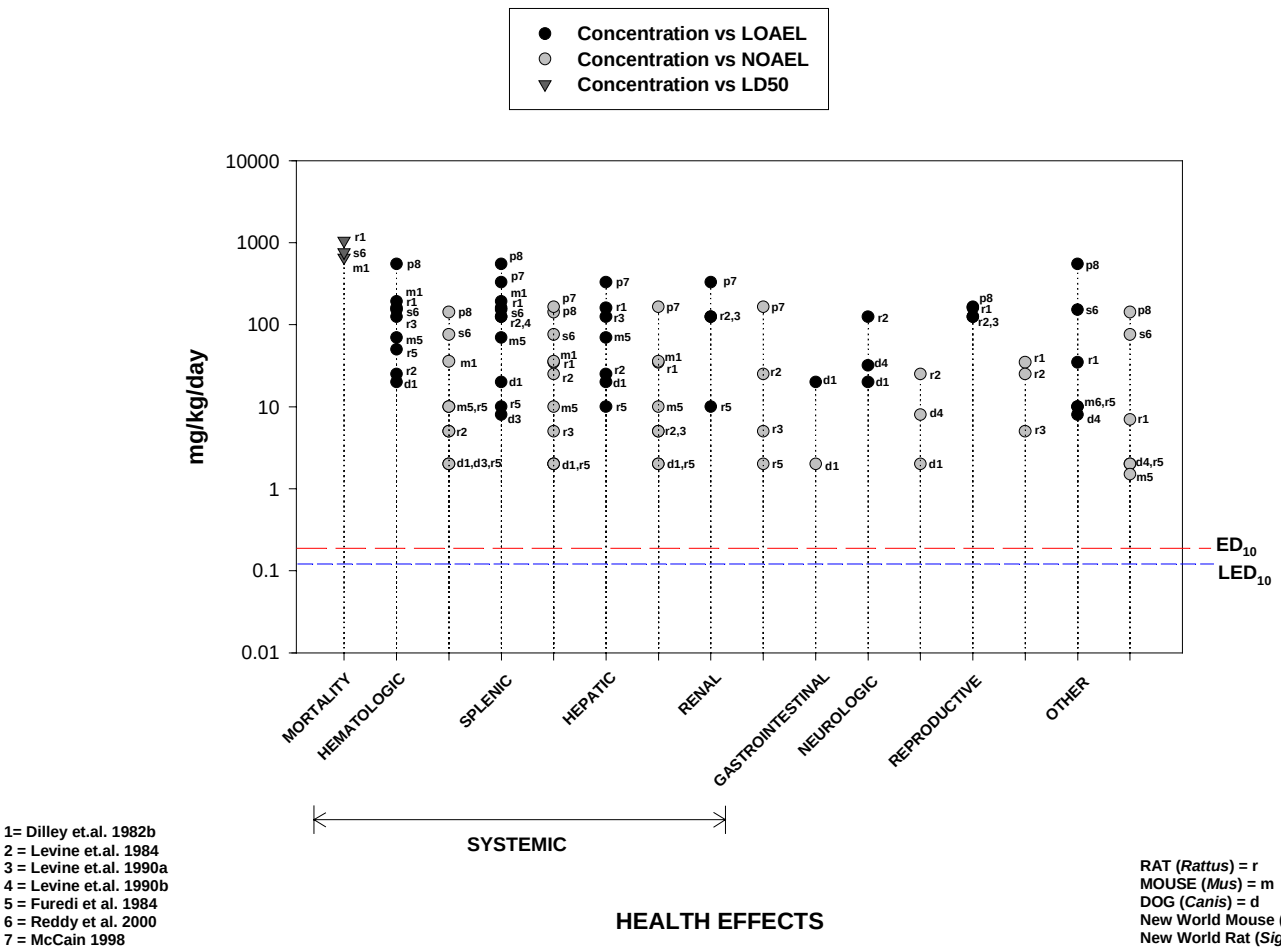
No other data relevant to oral exposures for mammals were found.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
McCain 1998	Mouse (*Peromyscus leucopus*)	90 d	165	330	Increased kidney, liver, spleen weights; presence of hemosiderin in spleen; chromaturia; increased extramedullary hematopoiesis in spleen
Johnson et al. 2000	Mouse (*Peromyscus leucopus*)	14 d	142	550 (♀)	Indicators of erythrolytic anemia (increased spleen weight, histopathology); decreased intracellular hydrogen peroxide of splenic phagocytes); phagocytosis results of uncertain biological significance
Reddy et al. 2000	Cotton rat (*Sigmodon hispidus*)	7 d	76 (♂)	152 (♂)	Erythrolytic anemia; changes in spleen and liver pathology, hematology; changes in hepatic glutathione S-transerfase for females, not males of uncertain biological significance; male dose protective of female dose for all other endpoints.
Dilley et al. 1982b	Rat (Sprague-Dawley)	13 wk	1.4	160	Anemia and leukocytosis
			34.7	160	Increased cholesterol, decreased body weight (10-20%), increased spleen weight, hemosiderosis, lymphocytosis; testicular atrophy
			7	34.7	Decreased food consumption
	Mouse (Wistar)	13 wk	35.7	193	Decreased hematocrit/RBC, liver necrosis
	Dog (Beagle)	13 wk	2	20	Mucoid stools (red), diarrhea, anemia, increased liver weight, bilirubin, and cholesterol; lethargy
Levine et al. 1984	Rat (Fisher 344)	13 wk	5	25 (♂)	Anemia, increased serum cholesterol
			25	125	Lipofuscin-like pigment in renal cortex, splenic enlargement with congestion, slight lethargy and ataxia; reduced food intake and body weight; atrophic seminiferous tubules, degenerated germinal epithelium
Levine et al. 1990a	Dog (Beagle)	6 mos	2 (♂) 8 (♀)	8 (♂) 32 (♀)	Anemia, methemoglobinemia, increased platelets, slight ataxia; chromaturia
			2 (♂)	8 (♂)	Decrease in body weight (16.4%; females at 32)
Levine et al. 1990b	Rat (Fisher 344)	13 wk	5	125	Increased spleen weight with diffuse congestion
Furedi et al. 1984	Rat (Fisher 344)	24 mos	10 (♀)	50 (♀)	Bone marrow fibrosis
			2 (♀)	10 (♀)	Increased cholesterol, enlarged liver; 14% decrease in body weight gain; splenic congestion, extramedullary hematopoiesis
Furedi et al. 1984	Mouse (B6C3F1)	24 mos	10 (♀)	70 (♀)	Mild anemia, increased liver weight, reduced serum globulin levels; 10-15% decrease in body weight gain; enlarged spleen and lymph nodes

Figure 1.

TNT HEALTH EFFECTS TO MAMMALS



1= Dilley et.al. 1982b
2 = Levine et.al. 1984
3 = Levine et.al. 1990a
4 = Levine et.al. 1990b
5 = Furedi et al. 1984
6 = Reddy et al. 2000
7 = McCain 1998
8 = Johnson et al. 2000a

RAT (*Rattus*) = r
MOUSE (*Mus*) = m
DOG (*Canis*) = d
New World Mouse (*Peromyscus*) = p
New World Rat (*Sigmodon*) = s

2.3.3 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found.

2.3.4 Mammalian Dermal Toxicity

No dermal studies conducted using animals were found; however, information suggesting the importance of dermal exposures for humans has been reported (Hathaway 1977, Woollen et al. 1986). In addition, studies investigating the potential for TNT to transverse mammalian skin *in vitro* from a soil matrix have demonstrated that dermal exposures to TNT in soil may add to total systemic dose (Reifenrath 1994).

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity - Oral

2.4.1.1 Avian Oral Toxicity - Acute

Three experimental trials for the acute lethal dose (ALD) were recently performed on Northern Bobwhite (*Colinus virginianus*) (Gogal et al. *in draft*). Both male and female birds were gavaged with single oral doses of 4508, 3005, and 2003 mg TNT/kg bw and observed for 14 days. All birds except one female exposed to 3005 mg/kg died within 5 days. The female dosed at 2003 mg/kg exhibited extreme ataxia, yet survived until necropsy. Reddish-brown stool was observed 24-48 hrs following dosing, characteristic of hematuria seen in mammals. A single oral dose of 2003 mg/kg was determined to be the lowest concentration resulting in death to Northern Bobwhite.

2.4.1.2 Avian Oral Toxicity - Subchronic

Adult male and female Northern Bobwhite (*Colinus virginianus*; N = 50) were provided TNT in feed at concentrations of 3300, 1560, 863, and 160 mg TNT/kg feed for a 90-day exposure (Gogal et al. *in draft.*). Initially, 4/10 birds died from exposure to the 3300 mg/kg treatment, yet none thereafter. Histopathology and sensitive indicators of immune function were evaluated. The effects included a dose-dependant non-significant decreasing trend in: total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, blood lymphocytes, an increase in late apoptotic/necrotic blood leukocytic cells, and slight hemosiderosis in the liver. It was noted by the authors that significant erythrolytic anemia does not seem to be the major target of toxicity in quail, most likely due to the refractory nature of the avian hematological and vascular system. No adverse histopathology was associated with any animal exposed to the 160 mg/kg treatment.

2.4.1.3 Avian Oral Toxicity - Chronic

No data are available for chronic exposures.

2.4.1.4 Avian Oral Toxicity - Other

No other avian studies are available for TNT.

2.4.1.5 Studies Relevant for Avian TRV Development for Ingestion Exposures

The only study found that evaluated the effects of TNT to birds was Gogal et al. (*in draft*). The 90-day results suggest that birds are much less sensitive to the hemolytic mechanisms found in mammals, yet there is evidence of some mild erythrolytic effect. Given the refractory nature of the avian hematopoietic system and the magnitude of these observations, these findings are of uncertain biological significance. Consistent with the mammalian data are the initial central nervous system (CNS)-related effects of exposure where individuals exhibited ataxia and neuromuscular effects. These effects were observed prior to death of the quail in the high dose group (3300 mg/kg). Therefore, an NOAEL of 7 mg/kg/d was suggested by the authors based upon the lack of adverse pathological and immunotoxicological observations for any individual in the low dose group (160 mg/kg). These data are summarized in Table 3 and Figure 2. An LOAEL was identified as 178 mg/kg/d based on the four deaths that occurred in the high dose group, and that possible adverse histopathology was associated with some individuals in the 3300 mg/kg group.

Table 3. Summary of Relevant Avian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL mg/kg/d	LOAEL mg/kg/d	Effects at LOAEL
		Lowest lethal dose detected (LD_{LOW}) 2003 mg/kg	na	na	Male dies during the determination of the approximate lethal dose at 2003 mg/kg; female did not.
Gogal et al. (*in draft*)	Northern Bobwhite (*Colinus virginianus*)	90 d	7	175	4/10 initial deaths in high dose group (3300 mg/kg); dose-dependant non-significant decreasing trend in: total red blood cell counts, packed cell volume, total plasma protein, blood prolymphocytes, blood lymphocytes, an increase in late apoptotic/necrotic blood leukocytic cells, and slight hemosiderosis in the liver.

na – not applicable

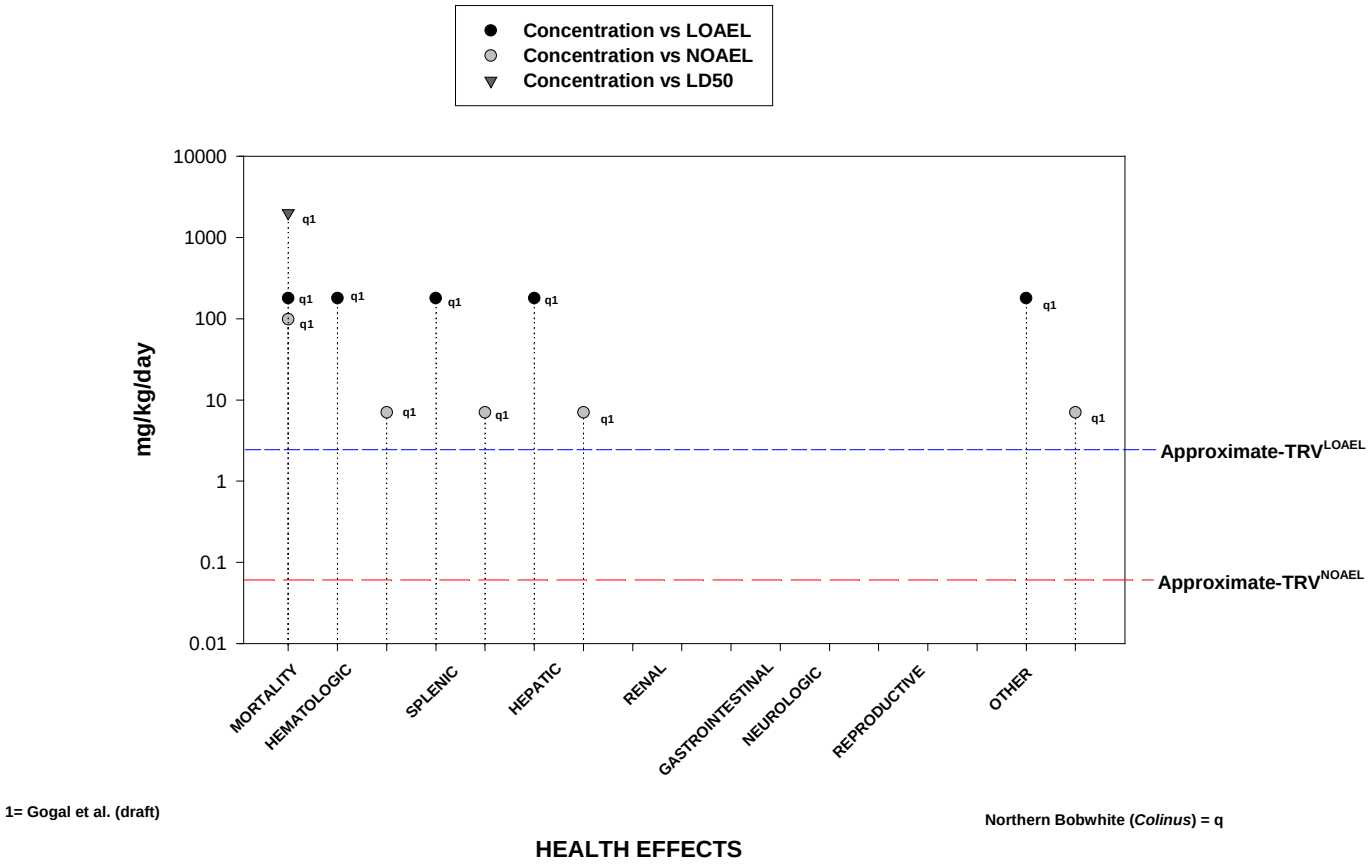
2.4.2 Avian Inhalation Toxicity

No data available.

2.4.3 Avian Dermal Toxicity

No data available.

TNT HEALTH EFFECTS TO BIRDS



1= Gogal et al. (draft)

Northern Bobwhite (*Colinus*) = q

2.5 Amphibian Toxicology

Only one study investigating 14-day exposures to TNT in soil in a terrestrial salamander was located.

2.5.1 Amphibian Microcosm Study

Tiger salamanders (*Ambystoma tigrinum*) were exposed to TNT in a soil matrix and were fed earthworms exposed to TNT in soil using a microcosm design for 14-days (Johnson et al. 2000b). Previous dermal exposures to TNT have been shown to be considerable compared to oral exposures in *Ambystomid* salamanders (Johnson et al. 1999). The TNT concentrations in soil reduced with time, ranging from 280 μg/g at the beginning to 59 μg/g at the conclusion. At which time the primary reduction products of TNT increased (39 and 62 μg/g at the beginning to 58 and 78 μg/g of 2-amino-4,6-dinitrotoluene and of 4-amino-2,6-dintitrotoluene at the conclusion, respectively). Concentrations of TNT in earthworms ranged from 0.25 – 0.62 μg/g, and from 2.1 – 2.6 μg/g of the primary reduction products mentioned previously. Immune function, histopathology, weight changes, and blood parameters were investigated. No adverse health effects were observed and the animals gained weight during exposure.

2.5.2 Relevance for Amphibian TRV Development

This study used a microcosm design that considered all pathways of exposure and potential variation in feeding regimes (Johnson et al. 2000b). Since soil concentrations of TNT were monitored, these data are used to derive a NOAEL for terrestrial salamanders, a soil concentration of 59 mg/kg that reflects all exposure pathways. Since adverse effects were not observed in the study, a LOAEL is not available.

2.6 Reptilian Toxicology

No data for reptiles are available.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Based on the information from five species, as described in Section 2.3.1.4, the dog appears to be the most sensitive mammal from oral exposures to TNT. The lowest LOAEL is 8 mg/kg/d, where Levine et al. (1990a) reported evidence of blood effects and decreased weight gain in dogs receiving 8 mg/kg/d but not at 2 mg/kg/d. The highest NOAEL within the same endpoints and species was the dose of 2 mg/kg/d reported by the same authors. Because decreased weight gain (an indicator of reduced growth and/or energy efficiency) and anemia have the potential to adversely effect future fitness, these endpoints are

considered to be ecologically relevant. In addition, this and the other studies satisfy the minimum data set requirement of the Standard Practice, Section 2.2 (USACHPPM 2000); thus, no uncertainty factors are needed to derive the TRVs. The data were appropriate for a benchmark dose derivation and are presented in Appendix B. A benchmark dose (BMD or ED_{10}) of 0.3 mg/kg/d was calculated from the model fit of the mean response at the 10% response level. A lower-bound on the benchmark dose (BMDL or LED_{10}) was calculated to be 0.2 mg/kg/d from the lower 95% confidence interval (CI) of the modeled curve. These values are selected as the class-specific TRVs (Table 4). Since these studies were well calibrated and the results are consistent with those of others, this TRV is given a high degree of confidence.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
LED_{10}	0.2 mg/kg/d	High
ED_{10}	0.3 mg/kg/d	High

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, since the dog is the most sensitive mammal tested, the class-specific TRVs shown in Table 4 are considered to be protective of non-carnivorous mammals. More specific TRVs may be developed considering the data provided in Table 2.

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

The only study that has evaluated the effects of TNT to birds is Gogal et al. (*in draft.*). These investigations evaluated hematological effects as well as systemic organ and sensitive immune parameters. Given the variation in response, only trends were evident. However, there were no incidences of adverse pathology associated with the low concentration treatment of 160 mg/kg. There were four mortalities in the high concentration treatment of 3300 mg/kg, and a non-significant dose-

related trend was evident in hematological and immune parameters. Though the biological significance concerning the magnitude of the hematological and immune parameters are questionable, the fact that mortality occurred initially in 4/10 animals is significant. The authors calculate an NOAEL at 7 mg TNT/kg bw/d at 160 mg TNT/kg feed dry weight treatment, and an LOAEL (serious) of 178 mg TNT/kg bw/d for the 3300 mg TNT/kg feed treatment. Since this is the only bird study, TRVs based on an approximation of the NOAEL and LOAEL were developed to represent the Class Aves. Given that the 90-d exposure regime represents <10% of the average lifespan of Northern Bobwhite it is considered a subchronic study. Therefore, an uncertainty factor (UF) of 100 was applied to account for interspecific variability (UF of 10) and to extrapolate from a single subchronic study (UF of 10). Table 5 presents the selected TRVs. A low level of confidence has been given to these TRVs because only one study is available, the single study only evaluates one species, and the study has relatively low power in its statistical comparisons.

Table 5. Selected Ingestion TRVs for the Class Aves

TRV	Dose	Confidence
NOAEL-based	0.07 mg/kg/d	Low
LOAEL-based	1.8 mg/kg-d	Low

3.2.2 TRVs for inhalation exposures for the Class Aves

Not available at this time.

3.2.3 TRVs for dermal exposures for the Class Aves

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Since the exposures were relatively brief, considering the average life span of *Ambystomid* salamanders (> 10 years), these were classified as acute exposures and an NOAEL was identified (Johnson et al. 2000b). In addition, since dermal exposures to TNT were reported to be considerable, a pathway-specific (i.e., oral) TRV would not be appropriate. However, since this study used a holistic exposure regime, a media-based value for soil could be derived. The acute (14-d) NOAEL of TNT in soil (59 μg/g) was divided by a UF of 300 to approximate a chronic NOAEL for terrestrial amphibians (a UF of 30 for an acute NOAEL to a chronic NOAEL and a UF of 10 to extrapolate across multiple species).

This resulted in an approximation of an NOAEL-based TRV of 0.2 mg TNT/kg soil dry weight intended to be protective of terrestrial amphibians. However, since an LOAEL was not identified, an approximation of an LOAEL-based TRV could not be derived. Table 6 presents the selected TRVs. A low confidence level has been assigned to the available TRV because a study observing adverse effects was not available, the only study is of limited length of exposure, and no other terrestrial amphibian data is available.

Table 6. Selected Soil TRVs for Terrestrial Amphibians

TRV	Dose	Confidence
NOAEL-based	0.2 mg/kg soil (dry weight)	Low
LOAEL-based	Not available	—

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

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APPENDIX A
LITERATURE REVIEW

The following databases were searched using the following keywords July 13, 1999:

TOXLINE & MEDLINE

Conditions: Two-word search; 1965 to present.

Trinitrotoluene and mammals - Trinitrotoluene = 911
Mammals = 471106
Combination = 158
Of these, 5 were appropriate and included.

Trinitrotoluene and birds - Trinitrotoluene = 911
Birds = 17894
Combination = 1
After review of the title, the single query result was not appropriate for this document.

Trinitrotoluene and wildlife - Trinitrotoluene = 911
Wildlife = 11830
Combination = 7
Of these, none were deemed appropriate for this document.

Trinitrotoluene and salamanders – Trinitrotoluene = 911
Salamanders = 398
Combination = 0

Trinitrotoluene and toads – Trinitrotoluene = 911
Toad = 411
Combination = 0

Trinitrotoluene and reptiles – Trinitrotoluene = 911
Reptiles = 4886
Combination = 0

Trinitrotoluene and snake – Trinitrotoluene = 911
Snake = 5825
Combination = 0

WinSPIRS 2.0

Conditions: Two-word conditional search; 1979-1997.

Trinitrotoluene and amphibian - Trinitrotoluene = 281
Amphibian = 2031
Combination = 0

Trinitrotoluene and salamander - Trinitrotoluene = 281
Salamander = 711
Combination = 0

Trinitrotoluene and frog - Trinitrotoluene = 281
Frog = 4412
Combination = 0

BIOSIS

Conditions: Two-word conditional search; 1984-1997.
Trinitrotoluene and wildlife - Trinitrotoluene = 1182
Wildlife = 17829
Combination = 73
Of these, most concerned the effects of effluent; duplicates with TOXLINE/MEDLINE search.

Trinitrotoluene and mammal - Trinitrotoluene = 1182
Mammal = 44329
Combination = 178
Of these, most concerned the effects of effluent; duplicates with TOXLINE/MEDLINE search.

Trinitrotoluene and bird - Trinitrotoluene = 1182
Bird = 24112
Combination = 3
These were not appropriate (non-laboratory evaluations).

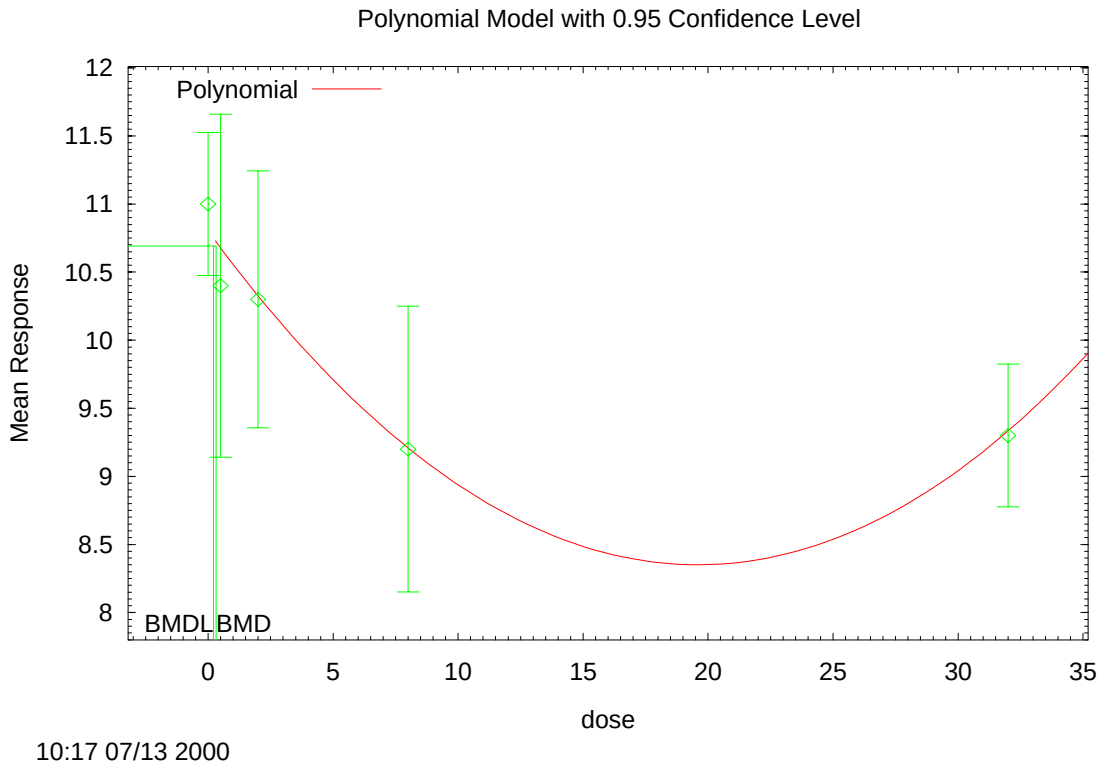
STINET – DTIC

Conditions: Two-word boolean search
Trinitrotoluene and mammal - Combination = 8
Original reports referenced (from which some peer reviewed submissions were based).

Trinitrotoluene and wildlife - Combination = 0
Trinitrotoluene and bird - Combination = 0
Trinitrotoluene and reptile - Combination = 0
Trinitrotoluene and amphibian - Combination = 0

APPENDIX B
Benchmark Dose Calculation for Mammals

The data presented below are from Levine et al. (1990a) where mean body weight (in kg = Mean Response) was measured in dogs from a 6-month feeding study. The data from the most sensitive sex was used in the calculation. Data from changes in hemoglobin and hematocrit followed the same trend and resulted in benchmark dose estimates that were statistically equivalent (One-way ANOVA on Ranks, $P > 0.40$).



Results of the model are presented below:

BMD = 0.324674
BMDL = 0.21622

==========

Polynomial Model. $Revision: 1.1.1.8 $ $Date: 2000/03/22 17:51:39 $
Input Data File: A:\TNT.(d)
Gnuplot Plotting File: A:\TNT.plt

Fri Jul 14 12:23:19 2000

==========

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	0.75
beta_0 =	10.7721
beta_1 =	-0.249975
beta_2 =	0.00637527

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.647838	5.97832
beta_0	10.7721	6.80499
beta_1	-0.249975	100.578
beta_2	0.00637527	3122.43

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-1e-007	-1e-007	-1.2e-007
beta_0	-1e-007	1	0.58	0.48
beta_1	-1e-007	0.58	1	0.98
beta_2	-1.2e-007	0.48	0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	6	11	0.5	10.8	0.805	0.283
0.5	6	10.4	1.2	10.6	0.805	-0.309
2	6	10.3	0.9	10.3	0.805	0.0029
8	6	9.2	1	9.18	0.805	0.0244
32	6	9.3	0.5	9.3	0.805	-0.00146

Model Descriptions for Likelihoods Calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-7.94995	6	27.8999
A2	-4.40918	10	28.8184
fitted	-8.48827	4	24.9765
R	-16.93	2	37.86

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.0416	8	<.0001
Test 2	7.08154	4	0.1316
Test 3	1.07665	2	0.5837

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.950000

BMD = 0.324674
BMDL = 0.21622

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

USCHPPM

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

DECEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 39-EJ-1138-01D

Readiness Thru Health

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

DECEMBER 2001

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Environmental Risk Assessment Program

USACHPPM Project No: 39-EJ1138-01D

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When referencing this document use the following citation
U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM). 2000. Wildlife Toxicity Assessment for 2-Amino-4,6-Dinitrotoluene and 4-Amino-2,6-Dinitrotoluene, Project Number 39-EJ1138-01C, Aberdeen Proving Ground, Maryland, October 2000.

Table of Contents

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

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

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

1. INTRODUCTION

This Wildlife Toxicity Assessment is based on a thorough review of the scientific literature regarding the toxicological characteristics of nitroglycerin that may pertain to the health of wildlife (mammals, birds, reptiles and amphibians) exposed to the substance. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide TG254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000). This document is designed to support ecological risk assessment activities.

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

This Wildlife Toxicity Assessment summarizes the current state of knowledge of the likely harmful impacts of 2A-DNT and 4A-DNT on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of the compound. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for all wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000). Since both 2A- and 4A-DNTs are often found *in vivo* and are the

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

2. TOXICITY PROFILE

2.1 Literature Review

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

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

2.2 Environmental Fate and Transport

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

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

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

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

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

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

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

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

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

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

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

2.3.1.2 Mammalian Oral Toxicity - Subchronic

No data are available.

2.3.1.3 Mammalian Oral Toxicity – Chronic

No data are available.

2.3.1.4 Mammalian Oral Toxicity – Other

No data are available.

2.3.1.5 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

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

2.3.2 Mammalian Inhalation Toxicity

No data are available.

2.3.3 Mammalian Dermal Toxicity

No data are available.

2.4 Summary of Avian Toxicology

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

2.5 Summary of Amphibian Toxicology

Johnson et al. (2000b) exposed 9 tiger salamanders (*Ambystoma tigrinum*) to soil and earthworms containing TNT and 2A-and 4A-DNT. Initial soil concentrations (at time of initial exposure) were 280, 39, and 62 mg/kg soil of TNT, 2A- and 4A-DNT, respectively. Appropriate controls were used. At the termination of exposure (14 days later) soil concentrations were 59, 58, and 78 mg/kg soil of TNT, 2A- and 4A-DNT, respectively. Salamanders were fed earthworms in identical soil preparations. Earthworm

concentrations ranged from 0.25- 0.79 µg/g TNT, 2.1 – 2.6 µg/g 2A-DNT, 2.1-2.5 µg/g 4A-DNT, and had trace amounts of 2,4-diamino-6-nitrotoluene. Salamanders were evaluated for immunological indicators of effects (phagocytosis, radical oxygen intermediate production), blood parameters (5 part differentials, total protein, hematocrit, etc.), and for histopathological indicators of the liver and kidney. No adverse effects were reported for any endpoint, and it was remarked that the animals appeared healthy and maintained an appetite. No adverse effects were reported from exposure to these conditions.

2.5.1 Studies Relevant for Amphibian TRV Development for All Exposures

This study used a microcosm design that considered all pathways of exposure and potential variation in feeding regimes (Johnson et al. 2000b). Since soil concentrations of 2A- and 4A-DNT were monitored, these data are used to derive a NOAEL for terrestrial salamanders. A minimum soil concentration of 40 mg/kg was selected, which reflects all exposure pathways. Since adverse effects were not observed in the study, a LOAEL is not available.

2.6 Summary of Reptilian Toxicology

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

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Only acute data were available for 2A- and 4A-DNT [see Ellis et al (1980)]. The data are fairly consistent and the studies were reported and evaluated properly. Given these data, a TRV may be approximated through the application of uncertainty factors prescribed by TG254 (USACHPPM 2000). Using the lowest LD50 value (for each sex and species) and applying an uncertainty factor of 100 and 20, a NOAEL-based and LOAEL-based TRV can be derived, respectively. These values are presented in Table 3.

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

Table 3. Selected Ingestion TRVs for the Class Mammalia

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

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

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

This assessment is not yet complete pending available data.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

This assessment is not yet complete pending available data.

3.2 Toxicity Reference Values for Birds

No data to derive values for birds were found.

3.3 Toxicity Reference Values for Reptiles

No data to derive values for reptiles were found.

3.4 Toxicity Reference Values for Amphibians

Johnson et al. (2000b) identified values of 2A- and 4A-DNT in soil where no adverse effects were observed in tiger salamanders. Since the exposures were relatively brief, considering the average life span of *Ambystomid* salamanders (> 10 years), these were classified as acute exposures and an NOAEL was identified (Johnson et al. 2000b). In addition, since dermal exposures to TNT were reported to be considerable, a pathway-specific (i.e., oral) TRV would not be appropriate. However, since this study used a holistic exposure regime, a media-based value for soil could be derived. The acute (14-d) NOAEL of TNT in soil (39 μg/g) was divided by a UF of 300 to approximate a chronic NOAEL for terrestrial amphibians (a UF of 30 for an acute NOAEL to a chronic NOAEL and a UF of 10 to extrapolate across multiple species). This resulted in an approximation of a NOAEL-based TRV of 0.13 mg 2A- DNT or 4A-DNT/kg soil dry weight intended to be protective of terrestrial amphibians. However, since an

LOAEL was not identified, an approximation of a LOAEL-based TRV could not be derived. Table 4 presents the selected TRVs. A low confidence level has been assigned to the available TRV because a study observing adverse effects was not available, the only study is of limited length of exposure, and no other terrestrial amphibian data is available.

Table 4. Selected Soil TRVs for Terrestrial Amphibians

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

4. IMPORTANT RESEARCH NEEDS

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

5. REFERENCES

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

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

APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

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

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

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

The search strategy for **Birds:**

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

The search strategy for **Laboratory Mammals:**

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

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers

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

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

- RD

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

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

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

USACHPPM

Wildlife Toxicity Assessment for High Melting Explosive (HMX)

NOVEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No:

Readiness Thru Health

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USACHPPM Document No. 39-EJ-1138-01E

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When referencing this document use the following citation:
U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM). 2001. Wildlife Toxicity Assessment for HMX, Project Number 39-EJ-1138-01E, Aberdeen Proving Ground, Maryland, October 2001.

Table of Contents

Department of the Army

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

Wildlife Toxicity Assessment for HMX

CAS No. 2691-41-0 **October 2001**

1. INTRODUCTION

High Melting Explosive (HMX) is one of several compounds that have been released to the environment during the manufacture of explosives and in load, assembly and pack (LAP) activities at U.S. Army ammunition plants (AAPs) and other military installations. Structurally, the compound (Chemical Abstract Services Registry Number 2691-41-0) is a completely N-nitrated, eight-member heterocyclic ring compound with the empirical formula, $C_4H_8N_8O_8$. In addition to "HMX," it is known by various systematic and trivial names such as cyclotetramethylenetetranitramine, octahydro-1,3,5,7-tetranitro, 1,3,5,7-tetrazocine and octogen, among others. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of HMX on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of HMX. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched May 5, 2000, using DIALOG to identify primary reports of studies and reviews on the toxicology of HMX. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and "key words in context." All available abstracts from articles selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For HMX, 17 articles were marked for retrieval from 32 initial hits.

In addition to DIALOG searching, a number of U.S. Army reports were identified in the Defense Technical Information Center. Secondary references and sources of information on HMX included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for HMX* (ATSDR, 1997), the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000), the U.S. Environmental Protection Agency's (USEPA) Integrated Risk Information System (IRIS) (USEPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (USEPA, 1997). Details concerning the search terms are presented in Appendix A.

2.2 Environmental Fate and Transport

HMX, a more powerful explosive than trinitrotoluene (TNT), has been used as a trigger mechanism for atomic (fission) weapons, as a component in plastic explosives, and in rocket fuels (ATSDR, 1997; USEPA, 1988). The compound's manufacture is limited to a single location in the United States (the Holston Plant at Kingsport, Tennessee), where it has been reported that, typically, up to 45 lb/day will be released to surrounding water bodies in discharged wastewaters from manufacturing and processing. Concentrations of HMX of up to 3.36 mg/L have been detected in effluents from the Holston facility (Talmage et al., 1999). Releases of HMX have also occurred at facilities where munitions are assembled, stored or tested. For example, concentrations of the compound of up to 5700 mg/kg have been reported in soil at some army sites (Talmage et al., 1999). Physicochemical properties of HMX relevant to the environmental fate and transport of the compound are listed in Table 1.

Table 1. Summary of Physical-Chemical Properties of HMX

Molecular weight	296.16
Color	colorless
Physical state	crystalline solid
Melting point	276–280 °C
Boiling point	no data
Odor	no data
Solubility water	5–6.63 mg/L at 20–25 °C; soluble in acetone, cyclohexanone, acetic anhydride, dimethyl sulfoxide
Partition coefficients:	
Log K_{ow}	0.06, 0.26
Log K_{oc}	0.54
Vapor pressure at 25 ^{o}C	3.33×10^{-14} mm Hg
Henry's Law constant at 25 ^{o}C	2.60×10^{-15} atm.m^3/mole
Conversion factors	1 ppm = 12.11 mg/m^3
	1 mg/m^3 = 0.083 ppm

Sources: USEPA, 1988; ATSDR, 1997; Talmage et al., 1999; HSDB, 2000

The vapor pressure and Henry's Law constant are sufficiently low (3.33×10^{-14} mm Hg and 2.60×10^{-15} atm.m^3/mole, respectively) suggesting that HMX is very unlikely to enter the air as a vapor. However, aerial dispersion of the compound while adhering to soil or dust particles has been implicated as a likely mechanism by which the compound can be released to the atmosphere (ATSDR, 1997). With a low log soil organic carbon-water partition coefficient of 0.54, HMX has the potential for high mobility in soil and could leach to ground water. For example, HMX has been detected in ground water at the Louisiana AAP at concentrations up to 4.2 mg/L (Talmage et al., 1999).

Photolysis appears to be the dominant process by which HMX is broken down in the environment, with a reported first order photolytic rate constant of 0.15 days^{-1} (USEPA, 1988). This suggests that an aqueous concentration of 0.5 mg/L HMX will have a half-life of 4–5 days when exposed to natural sunlight. Primary products of this process include nitrate, nitrite, and formaldehyde. By contrast, biodegradation/biotransformational processes involving bacteria or other microflora are extremely slow, though the formation of 1,1-dimethyl hydrazine has been demonstrated as a result of anaerobic degradation (USEPA, 1988).

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Toxicity – Oral

2.3.1.1 Mammalian Oral Toxicity - Acute

In one of a series of studies carried out for the U.S. Army Medical Research and Development Command by Inveresk Research International (IRI), Cuthbert et al. (1985) reported data obtained from various short-term toxicological tests on HMX. These included guinea pig sensitization, eye and skin irritation studies in rabbits, dermal and intravenous lethality in rats and rabbits, and acute oral lethality studies in rats, mice, and rabbits. In the latter, acute oral LD_{50} values of 6.5, 2.0, and between 0.1-0.25 g/kg were found for male Fischer 344 (F344) rats, B6C3F1 mice, and New Zealand white rabbits, respectively. Female oral LD_{50} values for rats and mice were reported as 7.6 and 3.8 g/kg, respectively. The rodent studies were conducted using five animals/sex/group. However, the rabbit investigations used one animal/sex/group at 2000, 1000, 429, 250, 100, and 50 mg/kg. Females died at each dose level. Males died at the 250, 429, 1000, and 2000 mg/kg dose level. While generally indicating that the compound has a low acute toxicity via the oral route, these data suggest the potential for wide interspecies variation.

In a further summary of the IRI data, Wilson (1985) supplemented these findings with toxicokinetic information that had been obtained by administering ^{14}C-labeled HMX by either gavage or intravenous injection to rats and mice. For either experimental animal species, the data indicate a low level of gastrointestinal absorption of unchanged HMX in rodents. For example, in the rats, a total of 85% of the

administered dose had accumulated in the feces 4 days after dosing. Furthermore, the comparative levels of radioactivity released to the urine following intravenous versus oral administration of ^{14}C-HMX suggested that less than 5% of the oral dose of the compound had crossed the gastrointestinal absorption barrier. This result is consistent with the low oral lethality reported by Cuthbert et al. (1985). According to Wilson (1985), the little tissue deposition that had occurred was found in the liver, kidney, and brain.

2.3.1.2 Mammalian Oral Toxicity – Subacute

Greenhough and McDonald (1985a,b) published two reports on behalf of the U.S. Army in which the 14-day oral toxicity of HMX was determined in F344 rats and B6C3F1 mice. These were essentially range-finding studies for subsequent investigations of the subchronic (90-day) toxicity of this compound in these species. In the first study, the authors exposed six rats/sex/group to HMX for 14 days as a dietary addition at target doses of 0, 333, 1000, 3000 and 9000 mg/kg-day (Greenhough and McDonald 1985a). As tabulated by the authors, the actual achieved average doses equivalent to these levels were 0, 335.2, 957.4, 2981, and 8504.3 mg/kg-day in males and 0, 369.2, 1280, 3474.25, and 3055 mg/kg-day in females. In the in-life phase of the study, animals were checked daily for mortality and clinical signs, twice weekly for body weight and once weekly for food and water consumption. At termination, blood samples were taken from all animals and stored frozen. All carcasses were subjected to a gross necropsy, liver and kidney weights were recorded, while excised pieces of brain, heart, kidney, liver, spleen, and thymus were processed for histopathological examination.

Concomitant with the incremental range of HMX doses, the group-specific incidence of compound-related fatalities was 0/6 (controls), 0/6, 0/6, 0/6 and 5/6 (high-dose) in males and 0/6, 0/6, 1/6, 1/6 and 6/6 in females. These deaths were accompanied by the onset of profound clinical signs characteristic of toxicologically challenged animals, most notably in males at the two highest dose levels, but in all groups of female rats. All HMX-treated male rats displayed dose-related suppression of body weight gain, while the two highest groups showed an actual body weight loss after 4 days of exposure. This food consumption-related deficit had partially rebounded by day 7. All females receiving HMX showed an initial body weight loss to levels that stayed depressed compared to initial values for all but those females receiving 333 mg/kg-day (group 2). Some marginal reductions in relative and absolute liver and kidney weights were observed among the treated groups, although it is unclear how much these changes were merely a consequence of dietary fluctuations.

A number of gross pathological findings were described in the report, although some were essentially sporadic in occurrence and, therefore, probably unrelated to dose. However, 4/6 high-dose females displayed smaller than normal spleens and enlarged adrenals, a feature that was also apparent in the single group-4 female that died prematurely. High-dose male rats displayed centrilobular degeneration of the

liver, while hepatocytic hyperplasia and increased cytoplasmic eosinophilia along with lymphocyte depletion in the thymus and spleen were noted in high-dose and other decedent females. However, the extent of these lesions in intermediate groups was not determined, an omission that did not allow a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) based on any observations other than lethality to be established. Based on the findings in the study, these would be (nominally) 3000 and 9000 mg/kg-day, respectively.

A similar protocol to that described above was also used to determine the subacute toxicity of HMX in B6C3F1 mice (Greenhough and McDonald 1985b). Target dietary doses were, in males (groups 1–5), 0, 100, 300, 900 and 2700 mg/kg-day, and, in females (groups 1–5), 0, 320, 800, 2000 and 5000 mg/kg-day. As tabulated by the authors, the actual achieved average doses equivalent to these levels were, for male groups 1–3, 0, 119.5 and 383 mg/kg-day and 0, 344, 882.7 and 2045.6 mg/kg-day for female groups 1–4. The lethality rate for the full sequence of groups was 0/6, 0/6, 5/6, 6/6 and 6/6 in males and 0/6, 0/6, 2/6, 4/6 and 6/6 in females. All HMX-receiving groups displayed clinical signs in response to dosing that were marked by over-excitability in the lower dose groups and by a range of increasingly severe responses leading to death in the higher dose groups. Animals displayed an initial loss of weight that may have been associated with reduced food consumption. For the survivors, these parameters rebounded in parallel during the second week of exposure. This "recovery" was indicated also by similar terminal absolute and relative organ weights between treated and control groups. As described by the authors, the histopathological findings were characterized by a "dose-related increase" in hepatocellular hyperplasia and cytoplasmic eosinophilia, splenic red and white pulp, and thymic cellular depletion. However, by analogy to the 14-day study in rats (Greenhough and McDonald 1985a), the absence of any histopathological examinations of HMX-receiving survivors in the intermediate dose groups has forced the selection of a NOAEL and LOAEL based on lethality. These dose values were approximated as 100 and 300 mg/kg-day, respectively, based on the data for male mice, and to 800 and 2000 mg/kg-day, based on the data for female mice.

2.3.1.3 Mammalian Toxicity – Subchronic

As reported by Everett et al. (1985) and Everett and Maddock (1985), IRI carried out separate 13-week toxicological studies on HMX in F344 rats and B6C3F1 mice. Arising from the range-finding study in rats described earlier (Greenhough and McDonald 1985a), 20 rats/sex/group received dietary doses of 0, 50, 150, 450, 1350 and 4000 mg/kg-day (males) and 0, 50, 115, 270, 620 and 1500 mg/kg-day (females) (Everett et al. 1985). The actual achieved average doses equivalent to these target levels were, in males, 0, 51, 153.5, 461, 1394 and 4101 mg/kg-day, and 0, 50.3, 115.6, 273.3, 627.7 and 1511.9 mg/kg-day in females. In addition to a more extensive range of in-life, necropsy and histopathological observations

than in the 14-day study, all rats received an ophthalmic examination before dosing commenced and during week 13 of dosing. Clinical chemistry and hematological analyses were carried out on blood samples taken from the orbital sinus of 10 males and 10 females during weeks 5 and 12 of treatment. Four-hour urine samples were collected from a subset of subjects among the groups during weeks 5 and 12. These samples were monitored for glucose, blood, protein, ketones, color, pH, specific gravity, etc.

In contrast to the findings of the 14-day study in F344 rats (Greenhough and McDonald 1985a), there were no compound-related deaths and few clinical signs in evidence during the 13 weeks of dosing. All ophthalmological observations were unremarkable before and after treatment. However, body weight gain was reduced in a dose-dependent manner with varying degrees of statistical significance in some groups compared to controls. These changes may have been due, at least in part, to fluctuations in food consumption. Some potentially dose-related hematological changes were observed in both sexes of high-dose rats, including reductions in hemoglobin concentration, packed cell volume and erythrocyte count, and increases in methemoglobin levels. Sporadic, statistically significant differences in plasma enzyme activities (for example, in alkaline phosphatase) were observed in rats exposed to high-dose levels of HMX compared to controls. However, because the extent to which these changes were dose-dependent is uncertain, their relationship to HMX treatment cannot be unequivocally assigned. Although findings from gross necropsy were benign, some apparent dose-dependent histopathological changes were considered by the authors to be compound-related. These included the appearance of enlarged liver cells featuring large nuclei and granular eosinophilic cytoplasm with associated small necrotic foci, which were most evident in male rats.

The designation of a NOAEL for the histopathological effects of HMX in liver may be controversial. Thus, although the effects were most evident in males receiving the compound at the two highest doses, the IRIS compilers (USEPA 2000) and Talmage et al. (1999) chose a nominal dose level of 50 mg/kg-day as the NOAEL, based on an incidence of 2/19 in 150 mg/kg-day-receiving males compared to 0/20 in controls. However, since this difference is statistically insignificant by Fisher's exact test, a viable alternative choice of NOAEL might be the value of 150 mg/kg-day itself, an approach that appears to be more in line with the conclusions of the authors of the study (Everett et al. 1985). Regarding the issue of the precise value of the NOAEL, it could be argued that, if 150 mg/kg-day were adopted as the NOAEL, the next highest dose (450 mg/kg-day) would be unsatisfactorily high for the LOAEL, since the incidence of histopathological liver lesions was 20/20 at this level. Taking all of the incidence data together suggests that the subchronic points-of-departure (NOAEL and LOAEL) for the toxicological effects in F344 rats are likely to exist in a narrow dosimetric region between 100 and 400 mg/kg-day.

Other compound-related histopathological changes were evident in the kidneys of female F344 rats. The incidence of these lesions, characterized by focal atrophy and dilation of the tubules, achieved

statistical significance compared to controls at a dose level of 620 mg/kg-day and above (Fisher's exact test from the data in the study). These changes result in nominal NOAELs and LOAELs of 270 and 620 mg/kg-day, respectively, to protect against the kidney effects.

A 13-week study in B6C3F1 mice featured dietary administration of HMX at target dose levels of, in males, 0, 5, 12, 30, 75 and 200 mg/kg-day, and 0, 10, 30, 90, 250 and 750 mg/kg-day in females (Everett and Maddock 1985). The actual achieved average doses equivalent to these target levels were, in males, 0, 5.2, 12.2, 30.5, 75 and 199.8 mg/kg-day, and 0, 10.5, 30.8, 95.1, 257.1 and 784.5 mg/kg-day in females. A range of toxicological effects was observed similar to those in evidence in the rat study (Everett et al. 1985). However, in contrast to the findings in rats, the apparent toxicological consequences of the compound in the mice were profound, with 65% premature deaths observed in high-dose males and 100% deaths in high-dose females. Lower fatality rates were observed at lower dose levels supporting the conclusion that mortality was likely compound-related. However, other than lethality, few if any obvious HMX-related consequences were apparent among the survivors at any dose level, thereby rendering uncertain the causes of death among the high-dose animals and calling into question the utility of the study to delineate a sufficiently discriminating sub-threshold point of departure for the compound's toxicological consequences. Using mortality as the primary subchronic toxicological effect of HMX from the female mouse data, the nominal NOAEL would be 90 mg/kg-day, with a LOAEL of 250 mg/kg-day. These doses are strikingly similar to those identified for mortality in male B6C3F1 mice in the 14-day subacute toxicity study (Greenhough and McDonald 1985b).

2.3.1.4 Mammalian Oral Toxicity – Chronic

No experimental studies were identified that addressed the chronic toxicity of HMX.

2.3.1.5 Mammalian Oral Toxicity – Other

No other data relevant to oral exposures for mammals were found.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

The toxicological database on HMX is limited (Table 2). The toxicokinetic findings discussed by Wilson (1985) indicate that, typically, only a comparatively small proportion of an orally administered dose of HMX will be absorbed at the gastrointestinal barrier. In addition, Wilson (1985) pointed out that, in IRI experiments, only small amounts of the compound absorbed survived clearance in the urine, where the radioactivity partitioned mostly as highly polar metabolites. Therefore, since mammals clearly have the capacity to metabolize HMX, the fact that the radioactivity eliminated in the feces was overwhelmingly in the form of unchanged HMX supports the suggestion that this component of the load

probably represented unabsorbed substrate rather than HMX that had been absorbed and then undergone hepatobiliary recycling.

As with urinary metabolites, the little amount of compound deposited in the tissues will also have been changed to metabolites of HMX (Wilson 1985). This implies that the toxicological consequences of HMX, including the hepatic and renal changes seen in histopathological specimens and the compound-induced lethality evident at higher doses in either species of test animal (F344 rats and B6C3F1 mice), will probably have resulted from the biochemical activity of one or more metabolites of HMX rather than the parent compound. Unfortunately, examination of the carcasses of the high-dose mice receiving HMX for up to 13 weeks and the results of the necropsy and histopathological findings in animals treated at lower doses and surviving to term failed to offer any clues as to the causes of the premature deaths induced by HMX. In fact, there is little evidence of a single universally applicable mechanism by which HMX induces toxic effects leading to lethality in rodents. To the contrary, histopathological findings in F344 rats were inconsistent since, in the 13-week study (Everett et al. 1985), sublethal microscopic lesions in the liver were observed primarily in exposed males, while kidney effects were largely restricted to the females. This separate and gender-specific pattern of histopathological lesion formation argues against the existence of a single ubiquitous mechanism by which fatalities such as those observed in both sexes of mice from the 13-week study could have been induced.

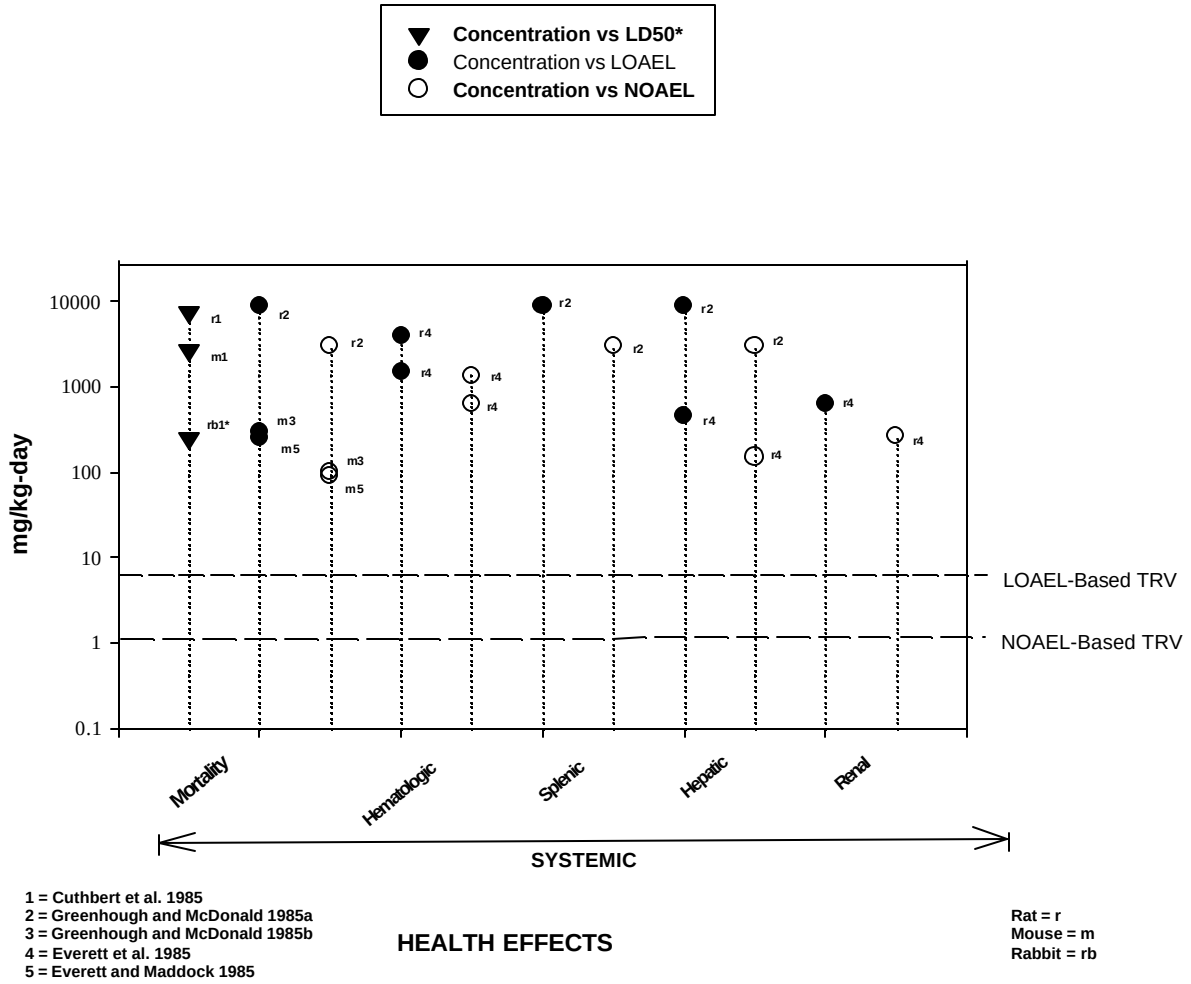
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Cuthbert et al. (1985)	Rabbit	Single acute exposure	100 (m) ? (f)	250 (m) 50 (f)	Mortality, convulsions, miosis, mydriasis, slight hyperkinesias, labored respiration
Cuthbert et al. (1985)	Rat Mouse	LD_{50} LD_{50}	7,360 (m&f) 2,710 (m&f)		
Greenhough and McDonald (1985a)	Rat (F344)	14-d	2981 (m) 1280 (f)	8504 (m) 3055 (f)	Lethality associated with histopathological liver lesions, lymphocyte depletion and spleen effects
Greenhough and McDonald (1985b)	Mice (B6C3F1)	14-d	120 (m)	383 (m)	Lethality associated with histopathological liver lesions, lymphocyte depletion and spleen effects
			883 (f)	2045 (f)	
Everett et al. (1985)	Rat (F344)	13-w	153 (m)	461 (m)	Histopathological lesions of the liver
			273 (f)	628 (f)	Focal atrophy and dilation of the kidney tubules
Everett and Maddock (1985)	Mice (B6C3F1)	13-w	95 (f)	257 (f)	Lethality

Taking the results of all the IRI studies on HMX together suggests that (unknown) metabolites formed from only a very small proportion of the administered load can induce lethality by an unknown mechanism, and that B6C3F1 mice are more susceptible to this effect than F344 rats. However, limited acute toxicity data suggest that the rabbit may be more susceptible to HMX than either B6C3F1 mice or F344 rats, a finding that permits the possibility that the subacute and subchronic NOAELs in rabbits might be even lower than those observed in B6C3F1 mice. Overall, the narrow range of animals employed in the experimental studies described and lack of any wildlife species data limits the confidence in the values selected as class-specific TRVs. These data are graphically presented in Figure 1.

Figure 1.

HMX HEALTH EFFECTS TO MAMMALS



* Indicates lowest lethal dose (LD_{LO})

2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found.

2.3.3 Mammalian Dermal Toxicity

Cuthbert et al. (1985) reported data that included dermal toxicity evaluations in rats and rabbits. For rats, the dermal LD_{50} was determined to be greater than 5.0 g/kg body weight. In rabbits, the percutaneous median lethal dose was determined to be 982.03 (861-1102) mg/kg in abraded/non-abraded skin tests for both sexes combined. HMX was found to be mildly irritating to the skin of rabbits although not an eye irritant. There was no evidence suggesting that HMX has sensitizing effects using the Magnusson-Kligman Maximisation Test in guinea pigs (Cuthbert et al. (1985).

2.3.4 Mammalian Toxicity – Other

Cuthbert et al. (1985) also conducted rat and rabbit intravenous studies using HMX using DMSO as a vehicle. Rat IV LD_{50} was determined to be 25 and 38 mg/kg for males and females, respectively. Rabbit IV LD_{50} was reported as between 10-15 mg/kg for both sexes.

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity – Oral

2.4.1.1 Avian Oral Toxicity - Acute

An Approximate Lethal Dose (ALD) evaluation was conducted using 16 Northern Bobwhite (*Colinus virginianus*; Gogal et al. 2001). Birds were orally gavaged using a water vehicle at eight doses ranging from 125 to 2125 HMX mg/kg body weight. One bird of each sex was used for each dose group. There was only one death (female; 187 mg/kg) 6 days post exposure. There were no marked signs of overt toxicity.

A subsequent ALD was conducted using 8 birds, 4 groups, at doses ranging from 3188 to 10760 mg/kg. One female died at 7173 mg/kg that occurred 12 days post exposure. No dose related remarkable findings were attributed to exposure. The purity of the compounds was determined to be 98.5%. Additional ALDs were conducted where vehicle (e.g., corn oil) and fasting regime was evaluated, each with no predictable patterns in mortality. The authors report that crop contents consisted of impacted HMX in necropsied birds, suggesting the bolus effect from a non-absorbable substance. An on-going subchronic study confirms no adverse effects to birds from exposures as high as 10,000 ppm HMX in feed (Gogal pers. comm..), suggesting that HMX is largely not available for absorption.

2.4.1.2 Avian Oral Toxicity - Subchronic

No data are available.

2.4.1.3 Avian Oral Toxicity – Chronic

No data are available.

2.4.1.4 Avian Oral Toxicity – Other

No data are available.

2.4.2 Avian Inhalation Toxicity

No data are available.

2.4.3 Avian Dermal Toxicity

No data are available.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of HMX in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

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

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The toxicity data for HMX are limited and variable. The acute toxicity information for HMX is limited to mice, rats, and rabbits. The data from the latter were generated from a weak experimental design (i.e., one rabbit of each sex for each dose group). The long-term (90-day and 13-week) oral data were developed using F344 rats and B6C3F1 mice. Mortality was a clearly relevant criterion that occurred in almost every study reviewed (Table 2.).

As outlined in USACHPPM Technical Guide 254, parameters used for derivation of a TRV should be ecologically relevant. Since mortality has a direct impact on the abundance of a particular species, this parameter has clear ecological relevance. All data concerning the toxicity of HMX has been generated from studies on laboratory animals. No wildlife species were used. Although very few animals were used, rabbits appear to be sensitive. Female rabbits died at every dose tested and exhibited symptoms consistent with animals in the high-dose group (Cuthbert et al. 1985).

Due to the limited data available, the approximation approach was used to derive the mammalian oral TRV for HMX (USACHPPM 2000). An uncertainty factor of 50 was used to derive the NOAEL-based approximate TRV from an acute LOAEL for mortality for female rabbits (Cuthbert et al. 1985). An uncertainty factor of 10 was used to derive the LOAEL-based approximate TRV from this same endpoint. These TRVs are consistent with the intravenous studies in rabbits and protective of male mortality that occurred at a far greater dose (250 mg/kg). Hence, these TRVs are consistent with these lines of evidence (Table 3). These TRVs were given a **Low** confidence rating since only one order was sufficiently characterized and there is evidence that suggests that the rodent data may not accurately characterize toxicity of HMX to other species of mammals.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	1 mg/kg/d	Low
LOAEL-based	5 mg/kg/d	Low

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

Since the work conducted in rodents has been well documented, TRVs specific to mammalian omnivores could be derived. Using the information from Everett and Maddock (1985), mice appear to be more sensitive to the effects of oral HMX exposure than rats. They used a 13-week exposure regime where mortality was the only consistent endpoint, possibly due to the low oral bioavailability of HMX. These data are consistent with the findings of other work investigating acute, subchronic, and chronic exposures in rodents (Cuthbert et al. 1985, Everett et al. 1985, Greenhough and McDonald 1985a,b).

An uncertainty factor of 10 was used to derive the NOAEL-based approximate TRV from a subchronic NOAEL. An uncertainty factor of 4 was used to derive the LOAEL-based approximate TRV from a subchronic LOAEL. These TRVs are presented in Table 4. Given that these species have been

studied extensively, yet there are no other omnivore species evaluated, these TRVs are given a **Medium** confidence rating.

Table 4. Selected Ingestion TRVs for Mammalian Omnivores

TRV	Dose	Confidence
NOAEL-based	9 mg/kg/d	Medium
LOAEL-based	62.5 mg/kg/d	Medium

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

No t available at this time.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Not available at this time.

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

The chemical/physical properties of HMX suggest that systemic exposure will be low for many organisms. The metabolic information from studies conducted in rodents show that most of the ingested HMX is excreted unchanged. However, the preliminary data for rabbits suggest differential absorption or biotransformation of HMX in herbivorous animals (e.g., ruminants, hindgut fermenters, etc.). Future work should focus on the possibility of these effects in herbivorous mammals. Additional data should be collected for reptiles and amphibians, though gastrointestinal exposure is likely to be less than that for mammals.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

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

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

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

The search strategy for **Birds:**

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

The search strategy for **Laboratory Mammals:**

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

- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- RD

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers
- AND(didelphidae or opossum? or soricidae or shrew? or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae) or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 32 hits on HMX were obtained in the initial search, all of which were selected for abstract evaluation. Seventeen of these articles and reviews were retrieved for this survey.

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

USCHPPM

Wildlife Toxicity Assessment for NITROGLYCERIN (NG)

NOVEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 37-EJ-1138-01F

Readiness Thru Health

Wildlife Toxicity Assessment for Nitroglycerin (NG)

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When referencing this document use the following citation:
U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM). 2000. Wildlife Toxicity Assessment for Nitroglycerin, Project Number 39-EJ-1138-01F, Aberdeen Proving Ground, Maryland, October 2001.

Table of Contents

Department of the Army

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

Wildlife Toxicity Assessment for Nitroglycerin

CAS No. 2691-41-0 **October 2001**

1. INTRODUCTION

This Wildlife Toxicity Assessment (WTA) is based on a thorough review of the scientific literature regarding the toxicological characteristics of nitroglycerin that may pertain to the health of wildlife (mammals, birds, reptiles and amphibians) exposed to the substance. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000). This document is designed to support ecological risk assessment activities.

2. TOXICITY PROFILE

2.1 Literature Review

Nitroglycerin (also known as trinitroglycerol or TNG) is produced for use as a component of propellants and explosives, and as a pharmaceutical agent. Nitroglycerin is a vasodilator widely used by the medical community in the treatment of angina and other cardiovascular pathology (Gilman et al. 1990, Burrows et al. 1989). There is an extensive body of literature related to the medical uses of nitroglycerin. However, very few of these studies were found relevant to a WTA and the development of wildlife toxicity values.

Given the common military use of NG, several pertinent studies were found in U.S. Army and related sources. These military-related studies, and subsequent reports, were found through TOXLINE, Toxicology, Occupational Medicine Environmental Series (TOMES) and Defense Technical Information Center (DTIC) searches. In addition, relevant studies were found through traditional cross-referencing techniques and through individual queries to project investigators within the Army. Several databases were searched; the details of these searches are found in Appendix A.

Table 1. Table of Physical / Chemical Properties of Nitroglycerin

CAS:	55-63-0
Molecular weight	227.09
Color	Pale yellow
State	Viscous liquid; triclinic or rhombic crystals below melting point
Melting point	13.5°C/2.8°C (labile form)
Boiling point	Apparently boils by decomposing rapidly at temperatures above 145°C; Explodes at 218-256°C
Taste	Sweet, burning taste
Solubility	1800 mg/L @ 25°C; soluble in acetone, benzene, and chloroform.
Partition coefficients	
Log K_{OW}	1.62
Log K_{OC}	1.51 estimated
Vapor pressure (at 20°C)	2.5E-04 mm Hg
Henry's Law constant (at 20°C)	Between 3.3 and 0.06 torr-l/mole
Odor	No information
Conversion factors	1 ppm = 9.29 mg/m^3
	1 mg/m^3 = 0.108 ppm

Sources: USEPA 1992, HSDB 2000, Lyman, W.J. et al. 1985, ACGIH 1991

2.2 Environmental Fate and Transport

Nitroglycerin may be released to the environment from its production and use as a component of propellants and explosives and as a pharmaceutical compound. Wastewater discharges from the manufacture of commercial dynamite preparations, military explosives, and other production sources may contain NG. The relatively high solubility of NG in water (1800 mg/L @ 20°C) suggests that environmentally significant concentrations may be dissolved in waste rinse water and be expected to remain in the water column (USEPA 1992). Neat NG does not polymerize and exists as a stable solid in the form of dipyramidal crystals at temperatures below 13.5°C. Liquid NG begins to decompose at 50 to 60°C, and at 145°C decomposition is so rapid the liquid appears to boil. At 256°C NG explodes spontaneously (USEPA 1992).

Both abiotic and biotic processes influence the fate of NG released to the environment. Physical and chemical degradation of NG is generally slow (Smith 1986). The photolytic half-life of NG in pure water was estimated to be 5 days (Spanggord et al. 1980a), and the hydrolysis half-life of NG at normal environmental temperatures is predicted to be more than 1 year at pH 3 to 8 (Spanggord et al. 1980b). An alkaline environment significantly decreases the hydrolysis half-life of NG (Spanggord et al. 1980b).

The kinetics and products formed on hydrolysis of NG and each of the isomeric dinitroglycerols (DNGs) and mononitroglycerols (MNGs) have been extensively studied. Chemical hydrolysis does not follow the stepwise successively slower pathway found for microbial systems. Rather, NG hydrolyzes more slowly than the DNGs or MNGs to a complex mixture of products, notably nitrate, nitrite, and oxalate. 1,3-DNG is hydrolyzed to glycidyl nitrate and 1,2-DNG isomerizes to 1,3-DNG to form a similar product on hydrolysis. 2-MNG isomerized to 1-MNG before hydrolysis; the only product identified was nitrate (Capellos et al. 1984).

Nitrate and nitrite are highly mobile in soil and water and microbes can convert nitrate to nitrite, which can potentially cause methemoglobinemia in mammals. Plants may accumulate nitrates and ingestion of plant material by ruminants (rumen microorganisms reduce nitrate to nitrite) may also result in methemoglobinemia (Cockerham 1994). However, the production of hydrolysis products from NG is not expected to be of concern to wildlife because the reaction will not proceed at any appreciable rate outside of an environmentally extreme alkaline environment.

Contrary to some earlier reports that it was recalcitrant to biodegradation, NG proved to be readily biodegradable in batch and continuous tests. Breakdown of NG was found to take place in stages via the isomeric di- and mononitrates, with each successive step proceeding at a slower rate (Wendt et al. 1978, Walker and Kaplan 1992). It was found that NG is not suitable as a source of carbon and nitrogen so nutrients are essential. It was speculated that earlier experiments where NG did not biodegrade were conducted using NG concentrations that were toxic to the microorganisms (Wendt et al. 1978, Smith 1986, Burrows et al. 1989). In the environment, NG would likely be biotransformed through a series of successive denitration steps, and the products mineralized by biological systems and incorporated into the biomass (Walker and Kaplan 1992).

If released on land, NG may readily leach into the soil. Few experimental data are available on its fate in the soil but biodegradation and hydrolysis under alkaline conditions are thought to occur. Few data are available on the fate of NG released in aquatic environments; however, environmental degradation of NG appears to occur primarily through biodegradation and photolysis. If released to the atmosphere, NG will probably be in the form of an aerosol and be subject to gravitational settling and scouring by rain. Photolysis is considered a possibility but data are lacking. The Henry's Law constant calculated from

reported water solubilities and vapor pressures range between 3.3 and 0.06 torr-l/mole, resulting in a half-life for volatilization from water of about 3000 days. Therefore, physical transport from aqueous systems should be relatively unimportant. Nitroglycerin is relatively soluble in water (1800 mg/L at 25°C) and therefore adsorption to soil and sediment and bioconcentration in aquatic organisms should not be appreciable (HSDB 2000, Smith 1986, Spanggord et al. 1980b). Chemical/physical properties of nitroglycerin are presented in Table 1.

2.3 Summary Of Mammalian Toxicology

Nitroglycerin has profound effects on systemic as well as cardiac microcirculation. Its actions are mediated by stimulation of soluble guanylate cyclase in vascular smooth muscle cells. Long-term industrial exposure to NG has been associated with withdrawal symptoms and sudden death from cardiovascular accidents (Klaassen 1996).

Nitroglycerin is rapidly absorbed, rapidly and widely distributed, and rapidly metabolized and eliminated in both laboratory animals and humans. Metabolism appears to occur in both hepatic and extrahepatic tissues via stepwise denitification; elimination is primarily in the urine and expired air. Absorption is somewhat less in mice than other species (Smith et al. 1986).

Urinary metabolites in most species consisted largely of free MNGs, glycerol, and other polar metabolites including glucuronides, while TNG and free DNGs were excreted only in small amounts. Mice excreted only small amounts of free MNG and DNG- and MNG-glucuronides indicating the relatively complete biotransformation in this animal species (USEPA 1992).

Nitroglycerin is absorbed through intact skin in amounts sufficient to cause vasodilation. In humans the most prominent manifestations of NG toxicity are severe headaches and adverse cardiovascular effects, including organic nitrate dependence in the case of chronic exposure (Gilman et al. 1990). In animals, the adverse effect most often observed after administration of NG at high dosage levels is decreased weight gain (related to decreased food consumption); effects were also seen in the liver (lesions), blood (methemoglobinemia), and testes (lesions and aspermatogenesis) (USEPA 1992).

Nitroglycerin has not been shown to be genotoxic in either *in vivo* or *in vitro* studies. Developmental and reproductive studies in animals have failed to demonstrate that NG is a teratogen. However, exposure to high concentrations of NG can result in testicular lesions and male infertility, and delayed development of offspring (Smith 1986).

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity – Acute

The acute toxicity of NG in mammals is moderate. The oral LD_{50} was reported to be approximately 500-900 mg/kg in rats and 500-1200 mg/kg in mice (Lee et al. 1975, Oketani et al. 1982). Differences in the acute toxicity of NG between sexes or among species appeared insignificant or minor.

Charles River CD rats and Albino Swiss mice were administered NG in lactose/peanut oil by oral gavage and survivors observed for 14 days. The oral LD_{50} was determined to be 822 and 884 mg/kg in male and female rats, respectively, and 1188 and 1055 mg/kg in male and female mice, respectively. All the animals became cyanotic and ataxic within 1 hour of dosing, and had very pale extremities and depressed respiration. Animals usually died within 5-6 hours of dosing; no gross pathology was observed in the animals that died. Survivors generally recovered within 24 hours (Lee et al. 1975). The number of male and female animals used in these studies was not provided.

Oketani et al. (1982) administered NG in propylene glycol to S1c:dd mice and S1c:SD rats by oral gavage and observed the surviving animals for 7 days. Ten animals per sex were used in these studies. The LD_{50} was 525 and 540 mg/kg for male and female rats, respectively, and 550 and 500 mg/kg for male and female mice, respectively. Deaths occurred within 48 hours and survivors recovered within 48-72 hours. There were no notable findings at autopsy (Oketani et al. 1982).

Four groups of adult beagle dogs, each consisting of two males and two females were given 25, 50, 100 or 200 mg/kg/day NG in capsules for 5 days. A transient and dose-related severe methemoglobinemia was observed. The 100 and 200 mg/kg/day group animals had cyanosis lasting for several hours. High dose dogs also exhibited decreased activity. Because of the severity of effects and to study any protective effect on methemoglobin formation, the high dose animals were given 3 mg/kg methylene blue intravenously (to induce methemoglobin formation) on the 3rd day and treatment was discontinued thereafter. The two low dose groups had transient methemogobinemia and no adverse clinical effects (Lee et al. 1977, Ellis et al. 1984).

2.3.1.2 Mammalian Oral Toxicity – Subchronic

Thirteen-week oral studies were conducted in dogs, rats, and mice as described in the following paragraphs (Lee et al. 1977, Ellis et al. 1984). Because no adverse effects were observed during the first several weeks of the 13-week studies, doses were increased 5-fold for dogs, rats, and mice starting the 5th, 6th, and 4th week, respectively.

Healthy young adult beagle dogs, 4 males and 4 females per treatment group, were given 0, 0.01, 0.1 or 1 mg/kg/day NG in capsules for 4 weeks, then 0, 0.05, 0.5 and 5 mg/kg/day for 9 more weeks. No

adverse effects were seen at any dose (Lee et al. 1977, Ellis et al. 1984). The no observed adverse effect level (NOAEL) was 5.0 mg/kg/day.

CD-1 mice, 16 male and 16 female per treatment group, were fed 0.001, 0.01 or 0.1% NG in diet (intakes calculated to be 1.3/1.3 m/f, 11.5/10.9 m/f, 107/95 mf mg/kg/day) for 3 weeks, then 0.005, 0.05 and 0.5% (intakes calculated to be 6.4/6.9, 60.2/58.7m/f, 608/561m/f mg/kg/day) for 10 more weeks. Treated mice had mild extramedullary hematopoiesis in the liver and spleen that did not appear dose-related. No other adverse effects were observed at any dose level (Lee et al. 1977, Ellis et al. 1984). The NOAEL was 608 and 561 mg/kg/day in males and females, respectively.

CD rats, 16 males and 16 females per treatment group, were fed 0.001, 0.01, or 0.1% NG in diet (intakes were calculated to be 0.8 and 0.9, 6.0 and 6.4, 59 and 59 mg/kg/day in males and females, respectively) for 5 weeks then 0.005, 0.05 and 0.5% (intakes were calculated to be 2.6 and 3.1, 24.5 and 26.5, 230 and 234 mg/kg/day in males and females, respectively) for 8 more weeks. Reversible decreases in food consumption, weight gain, and increased SGOT (aspartate aminotransferase) levels (enzymes to detect liver damage) were observed after the increase in dosage in the high-dose group; no other toxicologically significant findings were observed. No adverse effects were observed in any other dose groups. The lowest observed adverse effect level (LOAEL) was 230 and 234 mg/kg/day for decreased weight gain and food consumption in males and females, respectively. The NOAEL was 24.5 (males) and 26.5 (females) mg/kg/day.

To assess the effects of higher doses of NG, CD rats, 3 males and 3 females (4 males and 4 female controls) were fed 2.5% NG in diet (overall average intake of 1406 or 1416 mg/kg/day, m/f) for 13 weeks (Lee et al. 1977, Ellis et al. 1984). Dosing for males and females was initiated at 1176 and 1076 mg/kg/day and increased to 1588 or 1773 mg/kg/day, respectively, during the last 5 weeks. A lactose control group was also included with 5 males and 5 females. Animals receiving the NG experienced weight loss (weeks 4-8) and compensated anemia, but resumed gaining weight as feeding continued. Treated rats had altered blood chemistries and relative organ weights, hemosiderosis in the liver and spleen, and moderate to severe testicular degeneration and/or atrophy with severe and complete aspermatogenesis.

2.3.1.3 Mammalian Oral Toxicity – Chronic

Chronic exposure of laboratory animals to high NG concentrations resulted in adverse hematological and liver changes, and decreased body weight gain. Hepatocellular carcinomas as neoplastic nodules, and interstitial cell tumors of the testes were frequently observed in the high-dose group rats after 2-year exposures to NG (Ellis et al. 1978a, Ellis et al. 1984).

Four groups of 38 male and 38 female Charles River CD albino rats were fed diets containing 0, 0.01, 0.1 or 1% (average intakes of 0, 3.04 and 3.99, 31.5 and 38.1; 363 and 436 mg/kg/day in males and females, respectively) NG in their diet for 2 years (Ellis et al. 1978a, Ellis et al. 1984). No adverse effects were observed in any of the low dose rats. Mid-dose rats exhibited decreased weight gain in later months; males and females were about 60 and 30 g lighter than controls, respectively. Some rats fed 0.1% NG (31.0 (males) or 38.1 (females) mg/kg/day) had mild hepatic lesions (areas or foci of hepatocellular alteration that can develop into hepatocellular carcinomas). High-dose rats had decreased food consumption and weight gain, behavioral effects (decreased activity and failure to groom) compensated anemia with reticulocytosis, elevated serum transaminases, and methemoglobinemia, with some excessive pigmentation in the spleens and renal epithelium. After 1 year, 8 high-dose rats had cholangiofibrosis and some had neoplastic foci in the liver. At 2 years, all 13 surviving high-dose rats and 6/16 middle-dose rats had enlarged and grossly abnormal livers with severe cholangiofibrosis and hepatocellular carcinomas, some of which had metastasized to the lung. Interstitial tumors of the testes were observed in one-half the high dose males, in some leading to aspermatogenesis. A decrease in the naturally occurring pituitary chromophobe adenoma and mammary tumors increased the life-span, especially in the females (Ellis et al. 1978a, Dacre et al. 1980, Ellis et al. 1984). The identified NOAEL for this study was 3.04 and 3.99 mg/kg/day and the LOAEL was 31.5 and 38.1 mg/kg/day for decreased weight gain and enlarged, abnormal livers with cholangiofibrosis and hepatocellular carcinomas in males and females, respectively. The LOAEL for male reproductive effects was 363 mg/kg/day. The NOAEL for male reproductive effects was 31.5 mg/kg/day.

Four groups of 58 male and 58 female CD-1 mice were fed diets containing 0, 0.01, 0.1 or 1% (average intake of 0, 11.1 and 9.7, 114.6 and 96.4; 1022 and 1058 mg/kg/day for males and females, respectively) NG for 2 years (Ellis et al. 1978a, 1984, Dacre et al. 1980). No adverse effects were seen in the low- and mid-dose groups during the 24-month study. Decreased feed consumption and weight gain, and behavioral effects (decreased activity and failure to groom) were observed in the high-dose mice. After 1 year, high-dose animals had heme-derived pigment deposits in various organs and liver dysplasia. At 2 years, pigmentation in the liver with a lesser amount in the spleen and/or kidneys was observed in most high-dose and some middle-dose mice (Ellis et al. 1978a, 1984, Dacre et al. 1980). High-dose mice also had decreased weight gain, decreased grooming and methemoglobinemia and its sequelae (Ellis et al. 1978a, 1984). The NOAEL was 11.1 and 9.7 mg/kg/day; the LOAEL was 114.6 and 96.4 mg/kg/day in males and females, respectively, for pigment deposits in liver, spleen and/or kidneys.

Four groups of 6 male and 6 female beagle dogs were given capsules containing 0, 1, 5, or 25-mg/kg/day NG for 1 year. The only effect observed was an occasional dose-related incidence of transient

mild methemoglobinemia (less than 3%) in some dogs at all dose levels treated for 6 months or more (Ellis et al. 1978a, 1984). There were no accompanying effects on body weight, feed consumption, other hematological tests, clinical chemistry and histological examination. The NOAEL was identified as 25.0 mg/kg/day (Ellis et al. 1978a, 1984).

There were quantitative and qualitative differences in effects between species. Oral doses up to 20 mg/kg/day for 5 days or 25 mg/kg/day for 12 months produced only transient methemoglobinemia in dogs. In rats, lifetime feeding of 363 mg/kg/day (males) and 434 mg/kg/day (females) resulted in toxic effects on the liver and blood, and 31.5 or 38.1 mg/kg/day (males and females, respectively) resulted in mild effects on the liver of some animals. Mice were the least affected. Lifetime feeding of 115 mg/kg/day (males) and 96 mg/kg/day (females) resulted in a compensated anemia and some pigment deposits. The only effect common to all three species was transitory methemoglobinemia. Increased interstitial cell tumors of the testes occurred only in rats (Ellis et al. 1978a, 1984).

Suzuki et al. (1975) exposed C57BL/6Jms mice to NG in their drinking water for 18 months at estimated oral administered doses of 0, 1.5, or 6.2 mg/kg/day, and for 12 months at an estimated concentration of 58.1 mg/kg/day. Each treatment group consisted of approximately 50 animals of each sex. No treatment-related adverse effects, including body weight changes, were observed in any dose groups. The NOAEL for the 18-month study was 6.2 mg/kg/day. The NOAEL for the 12-month study was 58.1 mg/kg/day.

2.3.1.4 Mammalian Oral Toxicity – Other

Ellis et al. (1978a) also conducted a three-generation reproductive study in CD rats where the parental generation (F_O) received the same concentrations of NG as the ones in the chronic study for 6 months prior to mating (i.e., 0, 0.01, 0.1 or 1% NG). Matings consisted of 10 males and 20 females from each group for the F_O generation. Twenty to 24 pups from the second litters were randomly chosen in equal numbers from each treatment group and maintained in each respective treatment. At 3 months old, each male was mated with a female from each group and again, only the second-generation offspring were selected for continued treatment. This was repeated until the animals from the 3rd generation (F_{3b}'s) were weaned. All offspring were evaluated for gross physical abnormalities, and the number of live and dead pups were recorded. Survival and body weights were recorded at 0, 4, and 21 days. Fertility in the F_1 and F_2 generation of high-dose males was severely impacted. These effects appeared to result from the decreased feed intake and consequent poor nutritional status of the females and decreased spermatogenesis (due to interstitial tumors) in the males. No other developmental or reproductive effects were identified (Ellis et al. 1978a).

Ellis et al. (1978a) conducted a series of cytogenic studies in dogs and rats in the chronic study. In addition, dominant lethal mutation studies were also conducted. There were no apparent NG-induced mutagenic effects in the cytogenetics analyses of cells from dogs and rats and in the dominant lethal mutation study in rats.

2.3.1.5 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

The relevant studies for development of Toxicity Reference Values (TRVs) are the chronic and subchronic studies performed by Ellis et al. (1978a and 1984) and Lee et al. (1975 and 1977) in dogs, rats, and mice. These studies were conducted properly, containing the information necessary to evaluate the results, and reported results in a clear, logical, concise form. The methods used were consistent with other accepted protocols and contained the proper controls. These studies, therefore, meet or exceed the minimum quality requirements. The chronic studies were considered most relevant both because of the exposure duration and because the dose levels changed during the subchronic studies. This results in NOAEL and LOAEL values that are less certain. Acute studies provided additional information but were not considered applicable to the development of a TRV. The studies by Suzuki et al. (1975) and Oketani et al. (1982) were included as informational data points but were not used to derive a TRV, because these data were from a secondary reference and an English version of the original work could not be obtained. Table 2 summarizes the data from these studies and Figure 1 presents the data in a scatter diagram.

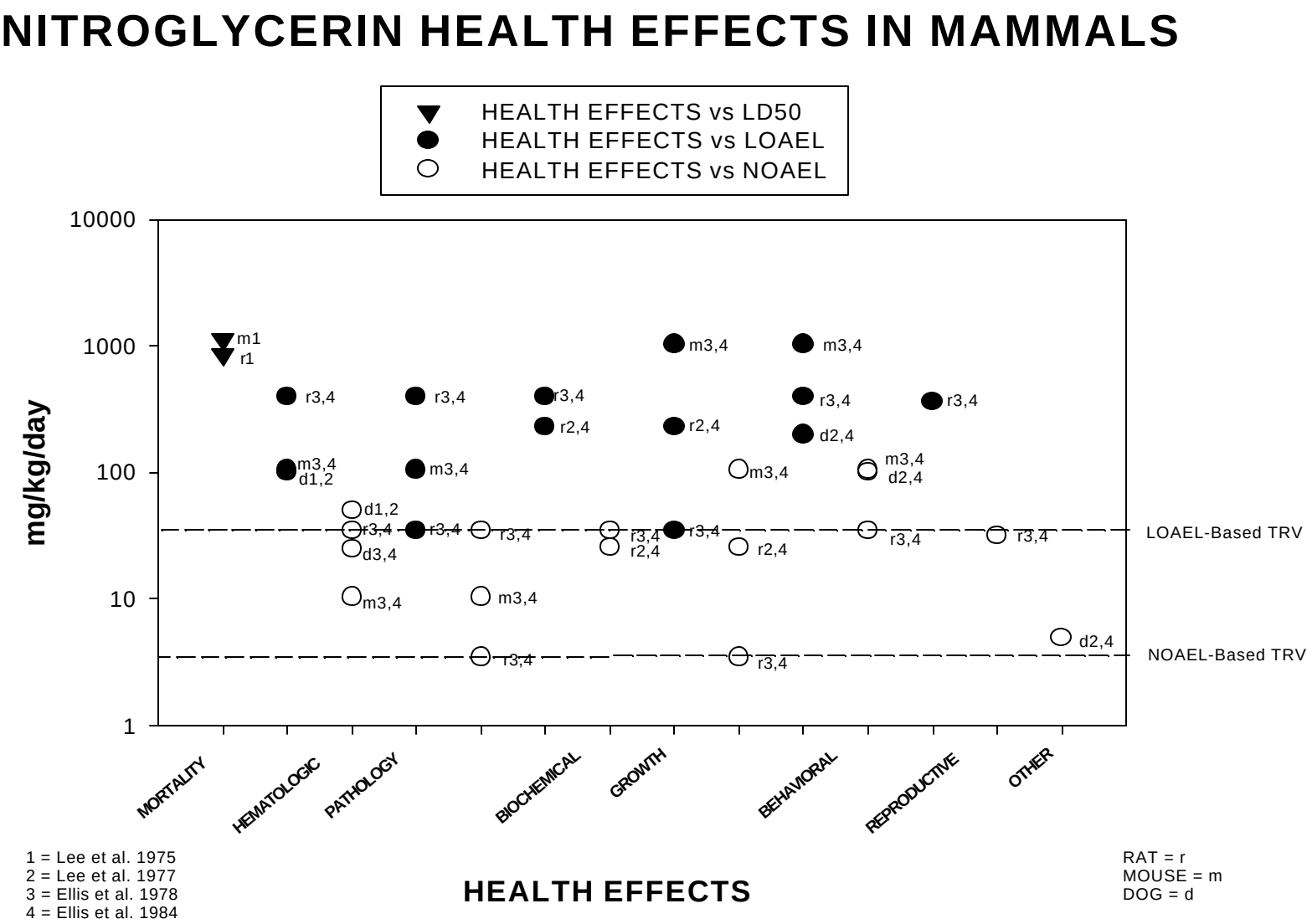
Table 2. Summary of Relevant Mammal Data

Study	Test Organism/Route	Test Duration	Test Results		
			NOAEL	LOAEL	Effects at LOAEL
Lee et al., 1975	Mouse/Albino Swiss/Oral in lactose/peanut oil	Single dose	**LD50** 1188 (M) 1055 (F)		Animals usually died within 5-6 hours; survivors recovered within 24 hours; no gross pathology. N = unknown
Lee et al., 1975	Rat/Charles River/Oral in lactose/peanut oil	Single dose	**LD50** 822 (M) 884(F)		Animals usually died within 5-6 hours; survivors recovered within 24 hours; no gross pathology. N = unknown
Lee et al. 1977 Ellis et al. 1984	Dogs/oral-capsule	5 days	50 mg/kg/day	100 mg/kg/day	Methemoglobinemia; Decreased activity. Cyanosis.
Lee et al. 1977 Ellis et al. 1984	Dog/oral/feed	13 weeks	5.0 g/kg/day	NA	0, 0.01, 0.1 or 1 mg/kg/day for 4 weeks, then 0, 0.05, 0.5 and 5 mg/kg/day for 9 more weeks. No adverse effects seen at any dose. Low number of animals.
Lee et al. 1977 Ellis et al. 1984	Mouse/oral/feed	13 weeks	608 (m) and 561 (f) mg/kg/day	NA	0.001, 0.01 or 0.1% in diet (intake of 1.3/1.3m/f, 11.5/10.9 m/f, 107/95 mf mg/kg/day) for 3 weeks, then 0.005, 0.05 and 0.5% (intake of 6.4/6.9, 60.2/58.7m/f, 608/561m/f mg/kg/day) for 10 more weeks. No treatment-related adverse effects at any dose. USEPA (1992) derived an LOAEL of 6.7 mg/kg/day based on mild hemosiderosis. Informational NOAEL, not considered for derivation of TRVs.

Study	Test Organism/Route	Test Duration	Test Results		
			NOAEL	LOAEL	Effects at LOAEL
Lee et al. 1977 Ellis et al. 1984	Rat/oral/feed	13 weeks	24.5 (m) and 26.5 (f) mg/kg/day	230 (m) and 234 (f) mg/kg/day	Decrease in weight gain and food consumption after increase in dosage in high dose group.
Lee et al. 1977 Ellis et al. 1984	Rat/oral/feed	13 weeks	NA	NA	Only one dose level. (2.5% ;1406 or 1416 mg/kg/day) - initial weight loss, altered blood chemistries, hemosiderosis in liver and spleen; testicular degeneration and aspermatogenesis.
Ellis et al. 1978a Dacre et al. 1980 Ellis et al. 1984	Dog/oral/capsule	12 months	5.0 mg/kg/day	NA	Mild methemoglobinemia (less than 3%, observed at all dose levels). USEPA 1992 did not derive NOAEL. Informational NOAEL, not considered for derivation of TRVs.
Ellis et al. 1978a Dacre et al. 1980 Ellis et al. 1984	Mouse/oral/ feed	2 years	11.10 (m) and 9.72 (f) mg/kg/day	114.6 (m) and 96.4 (f) mg/kg/day	Decreased weight gain, decreased grooming, hemosiderosis.
Ellis et al. 1978a Dacre et al. 1980 Ellis et al. 1984	Rat/Charles River/oral/ feed	2 years	3.04 (m) and 3.99 (f) mg/kg/day	31.5 (m) and 38.1(f) mg/kg/day	Reduced growth (weight loss); mild hepatic lesions like those seen in rats fed higher doses. No adverse effects were observed in the low dose rats.
Ellis et al. 1978a	Rat/Charles River/oral/ feed	3-Generation reproduction study	31.5 (m) and 38.1(f) mg/kg/day	363 (m) and 434 (f) mg/k/day	Infertility in high dose group (No effects in F_O, 1st mating of F_1 produced 3 litters, only 1 F_2 litter). Infertility due to males, testes ¼ size, no sperm in vaginal plugs, severe aspermatogenesis in F_2 males.
Ellis et al. 1978a, 1984	Rat/Charles River/oral/ feed	2 years	31.5 mg/kg/day	363 mg/kg/day	Interstitial cell tumors of the testes with aspermatogenesis.

NA = not applicable

Figure 1


NITROGLYCERIN HEALTH EFFECTS IN MAMMALS
HEALTH EFFECTS vs LD50
HEALTH EFFECTS vs LOAEL
HEALTH EFFECTS vs NOAEL
mg/kg/day
10000
1000
100
10
1
LOAEL-Based TRV
NOAEL-Based TRV
MORTALITY
HEMATOLOGIC
PATHOLOGY
BIOCHEMICAL
GROWTH
BEHAVIORAL
REPRODUCTIVE
OTHER
HEALTH EFFECTS
1 = Lee et al. 1975
2 = Lee et al. 1977
3 = Ellis et al. 1978
4 = Ellis et al. 1984
RAT = r
MOUSE = m
DOG = d

2.3.2 Mammalian Toxicity- Inhalation

No inhalation data for mammals were found.

2.3.3 Mammalian Toxicity- Dermal

Nitroglycerin was a very mild skin irritant but not an eye irritant in rabbits, and is a moderate sensitizer in guinea pigs (Lee et al. 1975). Nitroglycerin was readily absorbed through the skin of rhesus monkeys (Wester et al. 1983). No other relevant dermal toxicity data in mammals were found. However, the potential contribution of the dermal pathway to total exposure dose should be considered in the context of any risk assessment.

2.4 Summary of Avian Toxicology

Toxicological data for the effects of NG in avian species was not located. Ecotoxicological research on the effects of this compound in birds is recommended.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of NG in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended..

2.6 Summary of Reptilian Toxicology

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

3. RECOMMENDED TOXICITY REFERENCE VALUES[1]

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Three species of mammals from two different orders were evaluated using dietary exposures of TNG. The data set contains studies that are comprehensive in the scope of potential effects and in study duration. Moreover, most studies were well documented and contain refined data from all observations. Thus, these studies are considered to be of acceptable quality to derive a TRV.

Most studies have consistent and corroborative findings. Incidences of indicators that suggest liver necrosis, red blood cell lysis, methemoglobinemia, adverse reproductive effects, and weight loss ere reported within the same ranges. Many studies conducted at relatively low levels failed to find adverse effects. Although some findings (e.g., transient and reversible methemoglobinemia) have questionable biological and ecological significance, other effects (e.g., reduced male fertility) are clearly important. Given that most of these data follow a consistent pattern, and that the reproductive data are not appropriate for benchmark dose extrapolation, the NOAEL-LOAEL approach was used.

The NOAEL-based TRV was based on the chronic study by Ellis et al. (1978a) where no adverse effects in weight loss or hepatic lesions were observed. Therefore, the NOAEL was derived from the most sensitive species (rat) and sex tested (males) at 3 mg/kg/d.

The LOAEL-based TRV was derived from the original Ellis et al (1978a) work where at 31.5 mg/kg/d mild hepatic lesions and incidences of weight loss were found in chronically exposed male rats. Therefore, the LOAEL-based TRV of 32 mg/kg/d was selected. This value less than those where other endpoints (e.g., reproductive) were observed and therefore protective of other reported effects.

Because the relevant data, as presented in Section 2.3.1.5, satisfies the minimum data set requirement of the Standard Practice (USACHPPM 2000), no uncertainty factors are needed to select the TRVs.

The studies used to derive the LOAEL-based TRV were chronic and the results are consistent with those of other studies, thus this TRV is given a **High** degree of confidence. The NOAEL-based TRV was

[1]TRVs are for screening purposes only and are not intended to be predictors of effects in field situations. Site specific conditions may justify adjustments of these values based on toxicity information relevant to specific assessment endpoints.

derived from a chronic study that evaluated consistent endpoints of toxicity. However, given the information present in other studies where higher doses were tested in between these of Ellis et al. where no adverse effects were observed, it is likely that the true NOAEL for many species of mammals is likely to be higher. Therefore, the NOAEL-based TRV is given a **Medium** degree of confidence. See Table 3 for a summary of mammalian TRVs.

Table 3. TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	3.0 mg/kg/d	Medium
LOAEL-based	32.0 mg/kg/d	High

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived.

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

At this time, no inhalation TRV can be derived for mammals due to insufficient data.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

At this time, no dermal TRV can be derived for mammals due to insufficient data. However, given that there is evidence that NG has irritant and sensitizing properties, and that dermal exposures have been identified as significant, the dermal route of exposure should be considered for risk assessment purposes.

3.2 Toxicity Reference Values for Birds

At this time no TRVs for birds can be derived due to the lack of data.

3.3 Toxicity Reference Values for Amphibians

At this time no TRVs for reptiles and amphibians can be derived due to the lack of data.

3.4 Toxicity Reference Values for Reptiles

At this time no TRVs for reptiles and reptiles can be derived due to the lack of data.

4. IMPORTANT RESEARCH NEEDS

The effects of NG have not been assessed in avian, amphibian, or reptilian animal systems. It is recommended that these effects be evaluated.

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APPENDIX

LITERATURE REVIEW

The following databases were searched using the following keywords:

TOXLINE & MEDLINE

Conditions: Two-word search; 1965 to present.

Nitroglycerin and mammals - Nitroglycerin = 1938
Mammals = 29485
Combination = 448

Of these, 6 were appropriate and included.

Nitroglycerin and birds - Nitroglycerin = 1938
Birds = 12127
Combination = 1

After review of the title, the single query result was not appropriate for this document.

Nitroglycerin and wildlife - Nitroglycerin = 1938
Wildlife = 12029
Combination = 0

Nitroglycerin and salamanders - Nitroglycerin = 1938
Salamanders = 425
Combination = 0

Nitroglycerine and toads - Nitroglycerin = 911
Toad = 411
Combination = 0

Nitroglycerin and reptiles - Nitroglycerin = 911
Reptiles = 4886
Combination = 0

Nitroglycerin and snake - Nitroglycerin = 911
Snake = 5825
Combination = 0

BIOSIS

(Agricola and Biological Abstracts)

Conditions: One word search; 1984-1999.

Nitroglycerin = 381

Of these, none were appropriate for this document.

WORLD WILDLIFE

Conditions: One word search

Nitroglycerin = 0

STINET – DTIC

Conditions: one word search

Nitroglycerin = *25*

Of these, 2 were considered appropriate and included

In addition, the USEPA Health Advisory for Nitroglycerin (1992) and the Water Quality Criteria for Nitroglycerin (ORNL, 1986) references were consulted and all relevant non-duplicate studies included. Several others, in Japanese, appeared relevant but were not translate due to time and budgetary constraints.

The TOMES database was also searched; most relevant toxicological articles were in Japanese or duplicates from searches above. General chemical and fate and transport information from the HSDB (1999) was included.

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

Wildlife Toxicity Assessment for PENTAERYTHRITOL TETRANITRATE (PETN)

NOVEMBER 2001

Prepared by
Health Effects Research Program
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USACHPPM Document No: 37-EJ1138-01G
Approved for Public Release; Distribution Unlimited

USACHPPM

Readiness Thru Health

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Table of Contents

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

Wildlife Toxicity Assessment for Pentaerythritol Tetranitrate (PETN)

CAS Nos. 78-11-5 **November 2001**

1. INTRODUCTION

Pentaerythritol tetranitrate (PETN) is an explosive chemical that is currently used as the primary ingredient in detonating fuses and as a component (mixed with hexahydro-1,2,5-trinitro-1,3,4-triazine) in "plastic" explosives such as *Semtex*. Structurally, PETN (Chemical Abstract Services Registry Number 78-11-5) resembles nitroglyerin, a compound whose pharmacological as well as explosive properties it shares. Thus, in the human health field, PETN and nitroglycerin are used medicinally in the treatment of angina, through their shared vasodilatory action. However, for either drug, repeated exposure can establish a sequence of tolerance, then dependence as the body adjusts to the presence of either hypotensive agent. Thus, for occupationally exposed subjects, cycles of exposure and withdrawal associated with a 5-day workweek (exposure) followed by a 2-day weekend (withdrawal) can lead to the well-recognized "Monday-morning death" of munitions employees who are exposed to these substances on a regular basis. The phenomenon arises from cardiovascular events that are triggered by unrestrained compensatory vasoconstriction, as the normally high organic nitrate levels in the body become reduced during the weekend (Abrams, 1980). The importance of PETN as an environmental contaminant is related to its distribution at and around military sites and to its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of PETN on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of PETN. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for all wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched August 23, 2000, using DIALOG to identify primary reports of studies and reviews on the toxicology of PETN. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. All available abstracts of those articles that were selected as possibly relevant to TRV development were evaluated for relevancy. For PETN, three articles were marked for retrieval from 47 initial hits. Details of the search strategy and the results of the search are documented in Appendix A. The Defense Technical Information Center was searched for relevant U.S. Army reports. A secondary source was the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000).

2.2 Environmental Fate and Transport

PETN is produced in much smaller quantities than other military explosives. Furthermore, there are few data on the compound's environmental fate or on its site-specific concentrations in environmental media. Physicochemical characteristics of PETN that may be relevant to the environmental fate and transport of the compound are listed in Table 1.

Table 1 Summary of Physical-Chemical Properties of PETN

Molecular weight	316.15
Color	white
Physical state	crystalline solid
Melting point	140 ^{o}C
Boiling point	180 ^{o}C (decomposes above 150 ^{o}C)
Odor	slight
Solubility Water	43 mg/L at 25 ^{o}C; soluble in benzene, sparingly soluble in alcohol, ether, etc.
Partition coefficients:	
Log K_{ow}	1.61
Log K_{oc}	2.25–3.24
Vapor pressure at 25 ^{o}C	1.035×10^{-10} mm Hg
Henry's Law constant at 25 ^{o}C	1.2×10^{-11} atm.m^3/mole
Conversion factors	1 ppm = 12.93 mg/m^3
	1 mg/m^3 = 0.077 ppm

Source: HSDB, 2000

Layton et al. (1987) were unable to find any information about the compound's ability to undergo photolysis but speculated that the process is unlikely to be significant since the compound absorbs little ultraviolet or visible light.

The estimated values for vapor pressure and Henry's Law constants are sufficiently low to indicate that PETN is unlikely to be released to the air as a vapor. However, aerial dispersion of the compound adhering to soil or dust particles is a possible mechanism by which PETN can be released to the atmosphere. The low water solubility coupled with the low Kow suggests that PETN is not very bioavailable to most organisms.

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity – Acute

Von Oettingen et al. (1944) administered a 20 mg/ml PETN solution by gavage to young female albino rats. Following a six-hour period, the entire gastrointestinal tract was removed and the contents analyzed. It was determined that only 13% of the compound was absorbed. Dogs were also given oral doses of 5 mg/kg PETN with only a gradual decrease in blood pressure with a corresponding increase in respiratory rate and minute volume reported. No details were presented. No other studies were identified.

2.3.1.2 Mammalian Oral Toxicity – Subacute

One of the few reports of studies on the toxicity of PETN in laboratory animals was that of NTP (1989), which addressed the toxicity of the compound mixed in a 1:4 ratio with D-lactose monohydrate as a stabilizer, the mixture being designated "PETN, NF." In the various phases of the study, the mixture was administered to F344 rats and B6C3F1 mice in the diet for either 14 days, 13–14 weeks, or 2 years. In the subacute phase of the study, five rats and five mice/sex/group were exposed to 0, 3100, 6200, 12500, 25000 or 50,000 mg PETN, NF/kg diet for 14 days. Based on the data in the study, these concentrations approximated to average daily doses of PETN of 0, 54, 111, 258, 502 and 909 mg/kg-day in male rats, 0, 69, 145, 272, 552 and 1087 mg/kg-day in female rats, 0, 164, 281, 556, 1255 and 2587 mg/kg-day in male mice and 0, 215, 534, 784, 1675 and 3333 mg/kg-day in female mice. However, no clinical signs nor toxicological responses were induced in any of the animals under investigation.

2.3.1.3 Mammalian Oral Toxicity – Subchronic

The same dietary concentrations of PETN, NF that were used in the 14-day study were administered to 10 rats and mice/sex/group for 13 or 14 weeks, resulting in average daily doses of 0, 43, 90, 203, 319 or 625 mg PETN/kg-day to male rats, 0, 54, 105, 240, 463 or 931 mg/kg-day to female rats, 0, 131, 319, 450, 1028 or 2163 mg/kg-day to male mice and 0, 780, 336, 694, 1406 or 3170 mg/kg-day to female

mice, as calculated from the data in the study (NTP 1989). Consistent with the results of the subacute study, few if any overtly PETN-related effects were observed among the responses, although a tendency for higher than normal relative brain and kidney weights was observed in the three high-dose and the highest dose in female rats, respectively. Nitrite was detected sporadically in the urine of some male and female rats, and a Zymbal gland adenoma was detected in a single high-dose female rat. Only female mice exposed to the 50,000 ppm group had lower mean body weights (13%) than controls. No other changes were observed. In general, however, the concept that PETN is comparatively free of toxic effects in F344 rats and B6C3F1 mice at the administered doses is reinforced by the findings of this phase of the study.

Aside from the NTP (1989) report, Kodja et al. (1995) exposed nine female New Zealand white rabbits/group for 15 weeks to 6 mg/kg-day PETN in either regular chow or chow with added cholesterol. The hypercholesterolemic effects of the cholesterol-spiked diet were marked, although the presence of PETN appeared to significantly reduce the incidence and size of consequent atherosclerotic lesions. Twenty-four hours after the completion of the feeding study, excised cross-sectional pieces of the thoracic aorta were tested for endothelial functional integrity, as indicated by their contractile response to KCl, phenylephrine and by their subsequent vasorelaxant response to acetylcholine, in each case measured by a force-displacement transducer. In general, the different diets induced only marginal effects on the contractile response of the thoracic aorta to KCl or to phenylephrine. However, PETN slightly reduced the vasorelaxing potency of acetylcholine.

2.3.1.4 Mammalian Oral Toxicity – Chronic

The 2-year component of the NTP (1989) study on PETN, NF featured the dietary administration of 0, 25,000 and 50,000 mg PETN, NF/kg chow to 50 male F344 rats/group and to both sexes of B6C3F1 mice, and 0, 6200 or 12500 mg PETN, NF/kg chow to 50 female F344 rats/group. The authors report average doses in the rats of 0, 300, and 625 mg PETN/kg-day in males, 0, 100 and 208 mg/kg-day in females, and in the mice 0, 1000 and 2025 mg/kg-day in males and 0, 1275 and 2425 mg/kg-day in females. Clinical signs were monitored twice daily, and body weights and food consumption weekly for 13 weeks, then monthly. At termination, a full suite of organs and tissues were examined at necropsy and preserved by fixation. Histopathological examinations were carried out on sections from all high-dose animals and controls.

In the rats, survival was comparable among the groups and there were no obvious clinical signs. Body weights and food consumption in PETN-receiving groups were similar to controls and there were few noteworthy necropsy findings. Mean body weights of high dose male rats were 2%-9% lower than the controls throughout the study; body weights of all other groups of rats were similar. Histopathologically important findings were restricted to the Zymbal gland, in which a small but an increased incidence in

adenomas and carcinomas was observed compared to controls. However, the observed incidence was within historical data of non-treated rats (NTP 1989). This dose-dependent change, while statistically insignificant when compared to controls (Fisher's exact test), was considered by the NTP study scientists and review board to constitute equivocal evidence of the compound's carcinogenicity in this species. However, no such response was evident among the mice, in which few if any compound-related toxicological effects of PETN, NF were evident. No non-neoplastic lesions were attributable to PETN exposure in either animal model. Mononuclear leukemia rates in male rats were significantly lower relative to treatment. Hepatocellular ademomas or carcinomas also occurred with a significant trend in female mice.

Von Oettingen et al. (1944) also reported a lack of observed effects in a 1-year study in an unspecified strain of young albino rats. Two groups of 45 rats were given either a control diet or one equivalent to 2 mg/kg-d PETN. Food consumption was measured daily and animals were weighed weekly. Blood parameters were evaluated at monthly intervals. Other than a tendency for PETN-exposed animals showing monthly blood parameters having slightly higher hemoglobin and erythrocytes values, no other effects were seen. Additional evaluations included a histopathological examination of the brain, heart, lungs, adrenals, liver, spleen, kidneys, testis, and femur.

2.3.1.5 Studies Relevant to Mammalian TRV Development for Oral Exposures

In general, there is a paucity of experimental data that suggest adverse effects from oral exposure to PETN. However, given the structural similarities corroboration of PETN toxicity with that of nitroglycerin and erythritol-tetranitrate, it is likely that the mode of action is similar. Comparisons of these data also suggest that PETN is less bioavailable (von Oettingen et al. 1944).

The incidences of neoplasms associated with the Zymbal gland were within the bounds of historical results and only apparent in rats from the 104-week study. Moreover, there were no differences in the survival of the rats and mice at the end of the 104-week studies and any ecological impact of cancer in senescent animals is very uncertain and of questionable relevance. In addition, while few reports of studies on the acute lethality of the compound were found, most of the experimental evidence from the NTP (1989) report suggests the benign nature of the compound when administered to experimental animals (Table 2). Thus, when PETN, NF was administered to F344 rats and B6C3F1 mice in dietary concentrations that were at the maximum practical limit (5% w/w), few if any overtly toxicological responses were observed during exposure periods extending up to 104 weeks. Among the few possibly compound-related effects of PETN were the trends toward increased relative kidney weights that achieved statistical significance compared to controls in high-dose female rats after 13 weeks, and the dose-dependent increase in combined adenomas and carcinomas of the Zymbal gland in male and female rats exposed for 2 years. However, the marginal nature of each of these individual responses raises

questions as to whether these findings are incidental or truly compound-related. For example, supporting evidence of the compound's carcinogenicity might be expected to arise from mutagenicity/genotoxicity studies. However, in this instance also, the evidence is equivocal, since the compound has been found to be negative for gene reversion in the Ames test (Mortelmans et al., 1986), negative for the induction of chromosomal aberrations in Chinese hamster ovary cells, but positive for the induction of sister chromatid exchanges in the same experimental system (NTP, 1989).

Taken together these findings speak to the comparatively benign nature of the compound in experimental animals, though within the context of a limited information base. Both NTP studies (14 and 104 week studies in rats and mice) were conducted, reported, and recorded appropriately and are of sufficient quality to be deemed relevant for TRV derivation.

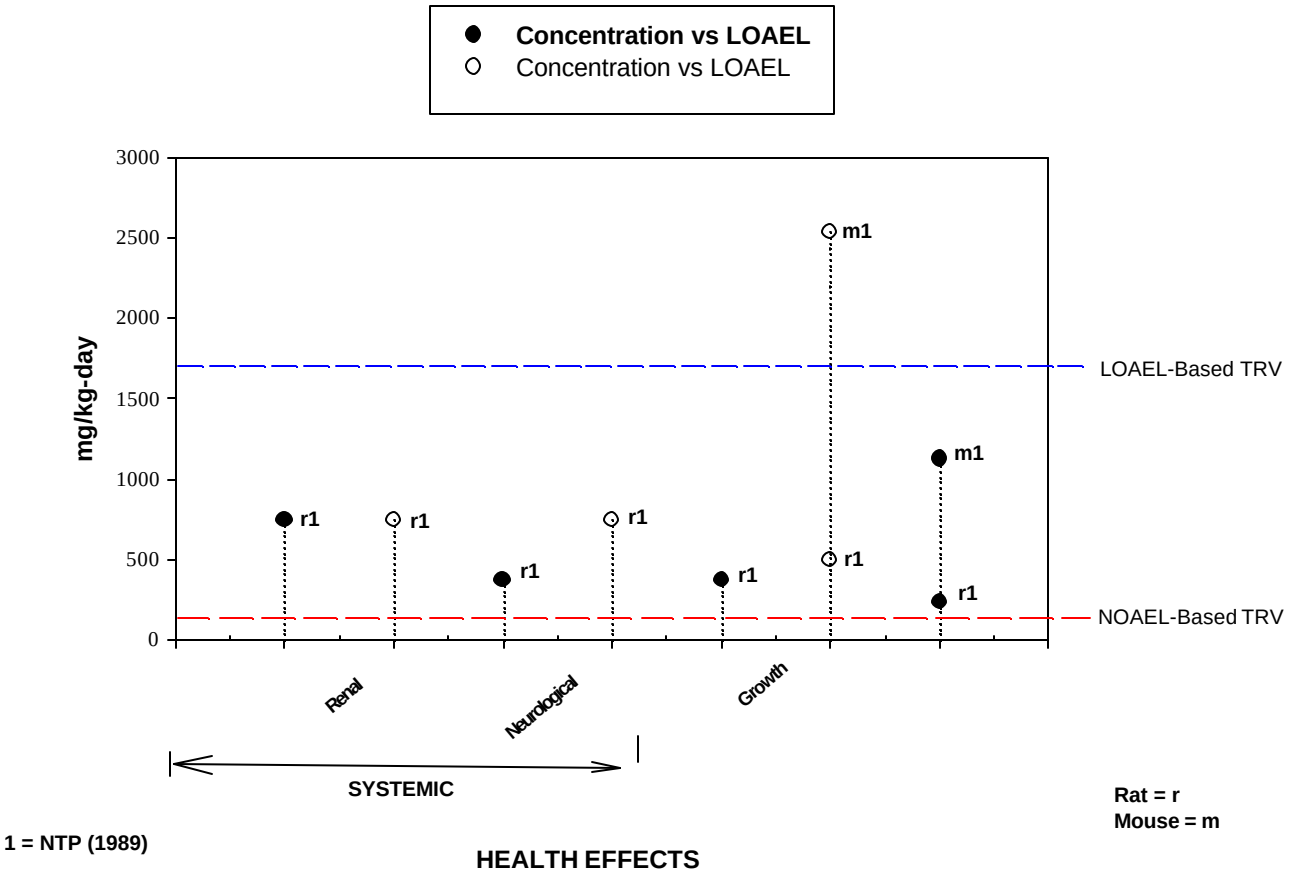
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
NTP (1989)	Rat (F344)	14-w	370 (f) 495 (m)	745 (f) ND (m)	Increase in relative brain and kidney weight in females.
		104-w	240 (m) 166 (f)	500 (m)* ND	Males had 2-9% lower body weight than controls.
NTP (1989)	Mouse (B6C3F1)	14-w	1125 (f) 1730 (m)	2535 (f) ND (m)	Females had 13% lower body weight than controls.
		104-w	1620 (m) 1940 (f)	ND	No adverse effects in mice were observed.
von Oettingen et al (1944)	Dog	Not reported	5	ND	Mild changes in blood pressure and respiratory rate. Secondary source: not used in TRV derivation.
	Rat (strain unspecified)	1-year	2	ND	No effects observed. Secondary source: not used in TRV derivation.

***** The incidences of Zymbal gland neoplasms and non neoplasms were determined to be of "equivocal significance" by the authors on the ability of PETN to be a causitative agent. No differences in survival were seen in any of the dose groups (NTP 1989).

ND = not determined

PETN HEALTH EFFECTS TO MAMMALS



2.3.2 Mammalian Oral Toxicity – Other

No other data relevant to oral exposures for mammals were found.

2.3.3 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found. Von Oettingen et al (1944) reported that PETN is absorbed through the lungs from observations of a fall in blood pressure and a rise in venous and spinal pressures following insufflation of 100 mg PETN in the lower trachea of dogs.

2.3.4 Mammalian Dermal Toxicity

No dermal studies conducted using animals were found. However, a study where PETN was mixed with acetone and was rubbed onto the hands of a human volunteer resulted essentially in the recovery of the entire amount of compound through washing (von Oettingen et al. 1944). This suggests that the probability for dermal absorption is relatively low.

2.4 Summary of Avian Toxicology

Toxicological data for the effects of PETN in avian species was not located. Ecotoxicological research on the effects of this compound in birds is recommended.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of PETN in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of 1,3,5-TNB in reptilian species was not located. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals[1]

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The suite of studies reported by NTP (1989) are comprehensive and of good quality. The NTP (1989) report contains subacute, subchronic, and chronic study information for mice and rats. Extremely high doses were used in an effort to bound the effects data. However, no corroborative effects were observed in either target or magnitude of effect. For example, kidney and brain weights were increased in the high dose for female rats only in the 14-week study, yet there was no other information that suggested that adverse effects were observed relative to the kidney and brain function (i.e., histopathology results revealed no effect), nor did the chronic study show the same effects. Male rats in the high dose group in the 104-week study showed a 2-9% lower body mass than controls, yet these effects were not seen in females or either sex in the 14-week study.

The mouse data were also equivocal. Only the 14-week study showed a significant change in body weight (13%, females only), yet no adverse effects were seen in either sex for the 104-week study. Altogether, these data suggest the likelihood of false positive data rather than a compound-related effect. In addition, the data reported in von Oettingen (1944) also suggest no adverse effects from prolonged oral exposure to PETN.

Information is available from three species and two orders (Carnivora and Rodentia). However, only one chronic LOAEL of questionable relevance is available. Therefore, the NOAEL-LOAEL approach was approximated. Since the highest dose tested in rats approximated 166 and 500 mg/kg-d for female and male rats, respectively, the TRVs were calculated from the female NOAEL (166 mg/kg-d) according to TG254 (USACHPPM 2000). The NOAEL-based TRV for mammals is therefore 170 mg/kg-d and the LOAEL-based TRV for mammals is 1700 mg/kg-d (through the application of a 10-fold uncertainty factor. Since the LOAEL observations do not provide clear indications of an adverse effect, and that only rodents are represented, it is given a LOW confidence rating. However, since no indications of adverse effects were seen at the NOAEL, the NOAEL-based TRV was given a MEDIUM level of confidence. Based on these data it is believed that these levels should be protective of species of mammals.

[1] TRVs are for screening purposes only and are not intended to be predictors of effects in field situations. Site specific conditions may justify adjustments of these values based on toxicity information relevant to specific assessment endpoints.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	170 mg/kg/d	Medium
LOAEL-based	1700 mg/kg-d	Low

3.1.1.1 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, these data are representative of omnivorous rodents, and may be considered as such. More data for other species would be needed to derive values for other foraging guilds.

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

Clear adverse effects from exposure to PETN appear to be lacking. Given the concentrations used in some of the studies reported herein, it is unlikely that further research using traditional laboratory animals would yield different results. However, testing in other mammalian, amphibian, avian, and reptilian species is needed to determine if these observations are different from that of laboratory mammalian models.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

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

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

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

The search strategy for **Birds:**

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

The search strategy for **Laboratory Mammals:**

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

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers

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

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

- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 47 hits on PETN were obtained in the initial search, all of which were sele cted for abstract evaluation. Three of these articles and reviews were retrieved for this survey.

DISTRIBUTION

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Ecological Risk Assessment for Small Arms Ranges
Habitat Areas, Impact Area
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