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Summary Review of the Aquatic Toxicology of Munitions Constituents

Guilherme R. Lotufo, Gunther Rosen, William Wild, and Geoffrey Carton

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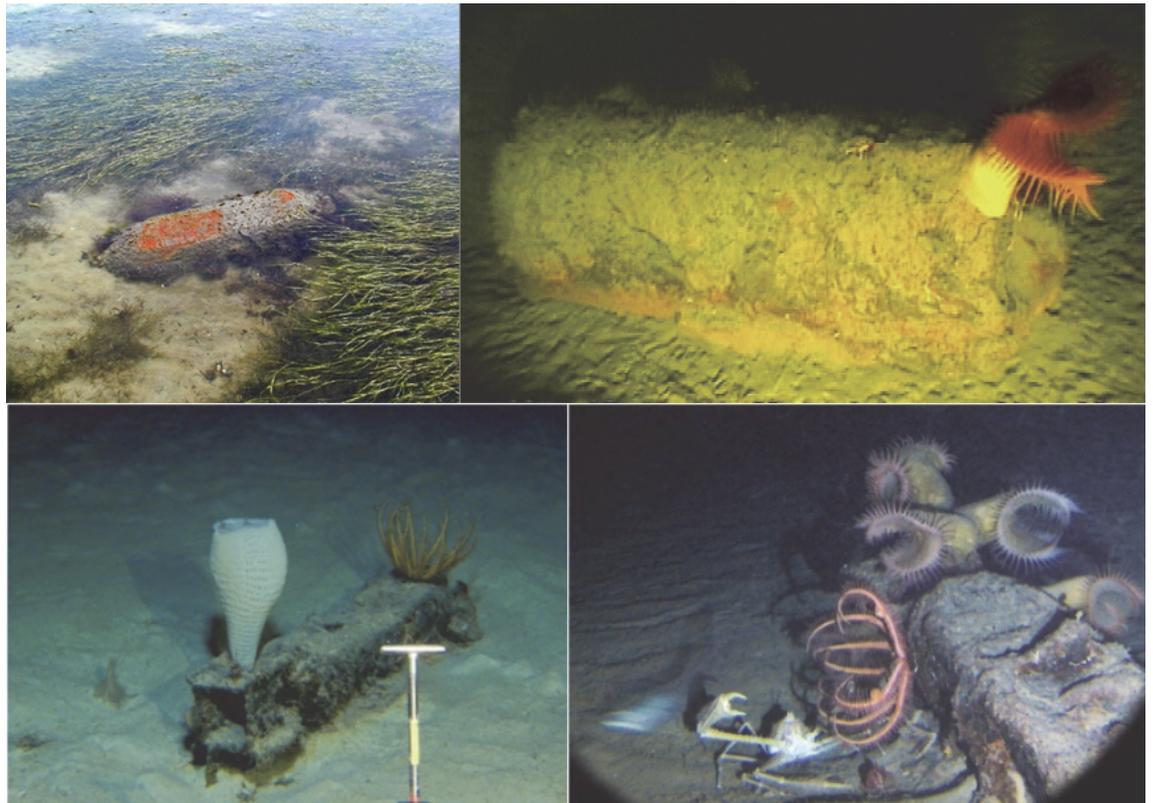
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Summary Review of the Aquatic Toxicology of Munitions Constituents

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Final report

Abstract

Military munitions are present in waters around the world, including those waters located at current and former Department of Defense sites. This report provides a review of the aquatic ecotoxicology of munitions constituents (MC), including nitroaromatics (2,4,6-trinitrotoluene (TNT), dinitrotoluenes (DNTs), 1,3,5-trinitrobenzene (TNB), 2,4,6-trinitrophenylmethylnitramine (tetryl) and 2,4,6-trinitrophenol (picric acid)); nitrate esters (nitrocellulose (NC), pentaerythritoltetranitrate (PETN), nitroglycerine (NG)); and nitramines (hexahydro-1,3,5- trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)). The major focus of this report is on the fate and effects of MC in the marine environment. Most MC observed in this study rapidly degraded in aqueous exposure systems and nitroaromatics showed a significant binding affinity with organic matter. To support the assessment of risk from MC in aquatic environments, laboratory-based toxicity data have been derived for a variety of aquatic species for both lethal and sublethal exposure endpoints using spiked water or sediment. Frequently, unrealistically high concentrations were used to derive toxicity benchmarks. In general, nitramines were less toxic than nitroaromatics, with a wide range of sensitivity among species. MC are weakly hydrophobic and bioaccumulative potential was low, as expected. High elimination rates for MC resulted in a virtually complete loss of body residue within hours to days following transfer to clean water. Uptake of TNT resulted in the substantial formation of bound residues. For fish, aqueous exposure was the dominant route of exposure to explosive compounds, with dietary uptake providing only minimal contribution. More realistic exposures using Composition B and multiple species found the presence of munitions in aquatic environments unlikely to result in biological effects. Verification of this conclusion should be pursued by determining site-specific exposure risk.

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Preface

1 Introduction

As a result of military training and weapons testing activities, munitions are present at numerous current and former Department of Defense (DoD) sites. Many active and former military installations have ranges and training areas that include aquatic environments, such as ponds, lakes, rivers, estuaries and coastal ocean areas. In addition, until 1970, it was accepted practice to dispose of wastes — including excess, obsolete and unserviceable munitions — in deepwater areas. Munitions are also present in waters around the world due to combat and training operations.

Munitions constituents (MC) are defined in 10 U.S.C. 2710 (e) (4) as "Any materials originating from unexploded ordnance, discarded military munitions, or other military munitions, including explosive and nonexplosive materials, and emission, degradation, or breakdown elements of such ordnance or munitions." Additional definitions of terms used herein are provided in Appendix A. This report addresses the aquatic ecotoxicology of explosives, propellants, and related compounds, with a focus on the marine environment. This report does not address the aquatic ecotoxicology of other MC, such as insensitive munitions, perchlorate, white phosphorus, and metals released from both initiating primers and corrosion of munitions casings.

The following sections provide a review of the scientific literature concerning the investigation of the aquatic toxicity and bioaccumulation potential of MC parent compounds and transformation products in sediment and aqueous exposures. Those studies contain valuable information about the use in the assessment of ecological risks potentially associated with releases of these compounds into the aquatic environment. Each section summarizes the results of studies published in peer-reviewed literature as well as in a few unpublished studies conducted by the authors of this report. More detailed information associated with these studies can be obtained from the cited articles.

- Section 2 provides an overview of munitions found in the environment.
- Section 3 provides an overview of MC and some background information on the explosives and their degradation products addressed in this report.

- Section 4 summarizes aqueous toxicity data for major explosive compounds and their transformation products to marine fish and marine invertebrates. The exposures used in the studies described in this section involved the addition of pure compounds to water at high enough concentrations to induce effects and calculate toxicity metrics (both aqueous and tissue-based) using standard laboratory methods.
- Section 5 summarizes sediment toxicity exposures of major explosive compounds to marine invertebrates. Toxicity exposures involved the addition of single compounds to sediment at high enough concentrations to induce effects and calculate toxicity metrics. This section provides information on the fate of MC during the storage and exposure period following spiking for several different sediment types, as well as the effects of sediment-associated MC to a variety of invertebrates and fish.
- Section 6 describes results from the bioaccumulation potential investigations of 2,4,6-trinitrotoluene (TNT) (and its major transformation products), Royal Demolition Explosive (RDX), and High Melting Explosive (HMX) in marine fish and invertebrates. Uptake rates, elimination rates, half lives, and bioconcentration factors (BCF) are reported. Body-part-specific bioaccumulation in marine fish following exposure to TNT, RDX, and HMX are also presented. Section 6 also provides information about the formation and elimination kinetics of nonextractable tissue-bound TNT and its metabolites in fish and mussels. A summary of body-part-specific bioconcentrations of MC and of the potential for dietary uptake of the explosive TNT to fish are also presented.
- Section 7 addresses the fate and effects of TNT and RDX released from Composition B fragments in microcosm exposures. These experiments involved a variety of endpoints for marine fish and invertebrates to characterize dissolution and potential biological exposure and effects under "realistic" scenarios using different exposure scenarios with multiple species.
- Section 8 summarizes marine toxicity and bioaccumulation data of MC for which research has been conducted.
- Section 9 provides the references cited in this report.

2   Munitions in the Environment

Munitions in the environment may release explosive residuals due to corrosion and breaching in terrestrial and aquatic sites or due to blow-in-place (BIP) detonation (Pennington et al. 2008; Lewis et al. 2009; Pascoe et al. 2010). Munitions also pose risks associated with the physical impacts of accidental detonation (MacDonald et al. 2009). Areas where explosives impact soil or sediment quality are sometimes extensive; some artillery ranges are several square miles in area. The MC residues found in these areas are typically heterogeneous in distribution (Jenkins et al. 2001).

Manufacturing of explosives and their loading, assembling, packing into munitions for use in testing, training, and combat has impacted terrestrial and aquatic systems (Monteil-Rivera et al. 2009). Thousands of sites throughout the world are potentially contaminated, with MC in the soil, sediment, groundwater or the surface water of inland habitats, at concentrations that span several orders of magnitude (Talmage et al. 1999; Jenkins et al. 2001). Explosives loaded into munitions as well as fragments of explosives and munitions remaining following incomplete detonations may be present in surface soils and in aquatic habits.

U.S. Military

In the United States, unexploded ordnance (UXO) and discarded military munitions (DMM) are present at sites designated for Base Realignment and Closure (BRAC), at Formerly Used Defense Sites (FUDS), and at operational military ranges. Within the FUDS program, the U.S. Army Corps of Engineers (USACE) has identified more than 400 sites, totaling more than 10 million acres that potentially contain munitions in underwater environments. The U.S. Navy and U.S. Marine Corps' munitions response program (MRP) has identified an additional 33 sites containing munitions. The inventory includes sites that date back to the 18th century and some that were used as recently as the 1990s (SERDP 2010).

Underwater Munitions

Military testing and training ranges may have both terrestrial and aquatic components. Although many aquatic environments encumbered with munitions have resulted from overshoots of land ranges, offshore areas

also have been used as ranges. This is evident in many areas by simple inspection of the shoreline adjacent to target and practice ranges (McDonald 2008). Munitions present at these sites include inert and training rounds and also munitions that failed to function as designed and either remain unexploded (e.g., UXO) or that did not fully consume their explosive fill (e.g., low order rounds). Munitions and explosives of concern (MEC) may also be present in the underwater environment due to disposal, accidents and combat.

Underwater munitions sites encompass a variety of environmental conditions with complex characteristics and chemistry, such as depth, temperature, salinity, bathymetry, and sediment type, all of which can vary with time (SERDP 2010).

Munitions in the Marine Environment

The United States military has conducted open water disposal of a portion of both U.S. and foreign (captured) excess, obsolete, or unserviceable conventional and chemical munitions from at least as early as 1919 until 1970, at which time the DoD prohibited the practice. In 1972, Congress enacted the Marine Protection, Research, and Sanctuaries Act (Public Law 92-532) that generally prohibits sea disposal of all such material (Davis 2009).

Although the extent of biological receptors' exposure to MC in marine environments is largely unknown, the presence of munitions in aquatic environments is well-documented (Darrach et al. 1998; Dave 2003; Cusson et al. 2003; van Ham et al. 2007) and low concentrations of some explosive MC were measured in marine sediments (Darrach et al. 1998, Pascoe et al. 2010). It is anticipated that commonly used explosive fillers such as TNT, RDX and ammonium picrate may leak from underwater corroded, or breached, munitions as well as from fragments of explosives formulations that remain following low-order (incomplete) detonations (Wang et al. 2011).

Other Stressors

Other stressors, such as mechanical disturbance or unusual water quality parameters, can potentially contribute to adverse ecological effects. These other stressors need to be considered when evaluating the effects of MC on the aquatic environment.

3 Overview of Munitions Constituents

Modern military explosives have an extensive history over the past 100 years, and terrestrial sites have been contaminated by (1) explosive manufacturing processes, (2) DMM, or (3) UXO. The U.S. Environmental Protection Agency (USEPA 1993) loosely classifies all "explosive waste" as energetic material, which can be further categorized as propellants, explosives, or pyrotechnics (PEP), based on typical use in military munitions.

Explosives are further classified as primary or secondary based on their susceptibility to initiation. Primary explosives, which include lead azide and lead styphnate, are highly susceptible to initiation. Primary explosives often are referred to as "initiating explosives" because they can be used to ignite secondary explosives (USEPA 1993).

Secondary explosives, which include TNT, RDX (cyclonite or Royal Demolition Explosive), and High Melting Explosive (HMX), are more prevalent at MEC sites than are primary explosives. Since they are formulated to detonate only under specific circumstances, secondary explosives often are used as main charge or boostering explosives. Secondary explosives are based on a binder and a crystalline explosive such as RDX. Secondary explosives also can be classified according to their chemical structure as nitroaromatics (e.g., TNT), and nitramines, which would include RDX (USEPA 1993).

This report deals almost exclusively with secondary explosives since they are present in munitions at much greater quantities than primary explosives. The report only addresses the aquatic ecotoxicology of explosives and propellants and related compounds. Most explosive chemicals used by the DoD are classified as nitroaromatics, nitramines or nitrate esters.

Among the nitroaromatics, TNT is widely used as an explosive, and DNT (2,4-dinitrotoluene) as a component of many single-base propellants. RDX and HMX are cyclic nitramines used in various explosives, and nitroglycerin and nitrocellulose are nitrate esters used in gun and some rocket propellants. Table 3.1 summarizes the energetic chemicals present in some military explosives. Many types of fillers used in munitions are composition

explosives, consisting of two or more explosive compounds mixed to produce an explosive with more suitable characteristics for a particular application. Older energetic formulations contain compounds such as tetryl or ammonium picrate.

Table 3.1. Energetic chemicals present in military explosive and propellant formulations (USEPA 2012).

Compound or Explosive Formulations	Uses	Chemical Ingredients
Composition B	Artillery; mortar	60% Military-grade RDX (Contains . 10% HMX) 39% Military-grade TNT (Contains 1% other TNT isomers and DNTs); 1 % wax
Composition C4	Demolition explosive	91% Military-grade RDX
Tritonal	Air Force bombs	Military-grade TNT, aluminum
Composition A4	40-mm grenades	Military-grade RDX
TNT	Artillery	Military-grade TNT
Composition H-6	Navy and Marine bombs	Military-grade RDX and TNT, aluminum
Octol	Antitank rockets	Military-grade HMX and TNT
Explosive D	Naval projectiles	Ammonium Picrate

Information on the content of specific munitions may be found in Army and Navy manuals (e.g., US Army 1990, 1993; U.S. Navy 1987, 1999) and from online sources such as ORDATA (http://ordatamines.maic.jmu.edu/default.aspx). DoD employees can obtain detailed information about munitions through the Defense Ammunition Center's Munitions Items Disposition System (MIDAS) database. Access to MIDAS requires a Common Access Card.

With the exception of nitroglycerine, the major energetic compounds used by the DoD are solids at ambient temperatures. As a group, military-grade explosives have relatively low vapor pressure, low water solubility and hydrophobicity (Table 3.2).

The environmental fate of an MC is determined by its physical properties, such as solubility, density, melting point, vapor pressure, Henry's Law constant, and partitioning coefficients, as well as the physical properties of the aquatic environment. Physical parameters of the aquatic environment that influence the chemical fate of MC include temperature, pH, salinity, dissolved oxygen, and the ionic strength of seawater. All of these factors in combination determine to what extent each compound will react in the environment. Possible chemical reactions in the aquatic environment include hydrolysis, photolysis, thermolysis, and oxidation. A review of the

Table 3.2. Physicochemical properties of munitions constituents addressed in this report (USEPA 2012, unless specified).

Common Name	Chemical Abstract Service Number	Molecular Weight (g mol^{-1})	Water Solubility at 25 °C (mg L^{-1})	Octanol/Water Partition Coeff. (log K_{ow})
TNT (2,4,6-trinitrotoluene)	118-96-7	227.13	130	1.6
1,3,5-TNB (1,3,5-trinitrobenzene)	99-35-4	213.1	385[a]	1.18[a]
2-Am-DNT (2-amino-4,6-dinitrotoluene)	35572-78-2	197.17	42	1.94
4-Am-DNT (4-amino-2,6-dinitrotoluene)	19406-51-0	197.17	42	1.91
2,4-DANT (2,4-diamino-6-nitrotoluene)	6629-29-4	167.17	ND	0.7[b]
2,6-DANT (2,6-diamino-4-nitrotoluene)	59229-75-3	167.17	ND	0.67[b]
2,4-DNT (2,4-dinitrotoluene)	121-14-2	182.15	270	1.98
2,6-DNT (2,6-dinitrotoluene)	606-20-2	182.15	206	2.02
Tetryl (methyl-2,4,6-trinitrophenylnitramine)	479-45-8	287.17	75	2.04
Picric Acid	88-89-1	229.10	12800	1.33
Ammonium Picrate (Explosive D)	131-74-8	246.14	10,200[c]	no data
RDX (Royal Demolition Explosive)[e]	121-82-4	222.26	56.3	0.90
HMX (High Melting Explosive)[f]	2691-41-0	296.16	4.5	0.17
Nitroguanidine (NQ)	556-88-7	104.07	4400	-0.89
Nitroglycerin (NG)	55-63-0	227.11	1800	1.62
Nitrocellulose (NC)	9004-70-0	10^5-10^6	insoluble	no data
PETN (Pentaerythrite tetranitrate)	78-11-5	316.17	43[d]	3.71
DEGDN (Diethyleneglycoldinitrate)	693-21-0	196.12	4000[a]	0.98[a]

ND - not determined, [a] from Rosenblatt et al. (1991), [b] from Elovitz and Weber (1999), [c] from Greenwald (1926), [d] This value is uncertain; range of cited values from 2.1 to 43 mg L^{-1}, [e] hexahydro-1,3,5-trinitro-1,3,5-triazine or cyclo-1,3,5-trimethylene-2,4,6-trinitramine, also known as cyclonite, [f] Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine

physicochemical properties for major explosives and a discussion of their environmental fate in the marine environment are provided in Noblis (2011). Singh et al. (2012) summarized information on the biodegradation and biotransformation pathways of major explosives.

The following subsections provide basic background information on explosives and propellants for which data on toxicity and bioaccumulation in aquatic organisms are available.

Nitroaromatics

2,4,6-trinitrotoluene (TNT)
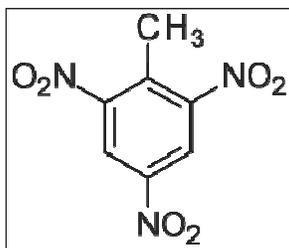
The U.S. military use of TNT began around 1912 typically in TNT only compositions, or mixed with powdered aluminum for enhanced blast effect. It is a stable explosive and is relatively insensitive to shock. Due to production limitations preceding both World War I and early World War II, TNT was blended with other energetics (e.g., Amatol is 50-80 % ammonium nitrate and 50-20% TNT) to stretch existing inventories and satisfy demands for large quantities of wartime explosives. TNT continues to be an explosive component of modern military munitions, although it is commonly mixed with other compounds (e.g., RDX). TNT is used in many explosive mixtures such as pentolite, tetrytol, torpex, tritonal, picratol, and Composition B.

At room temperature, TNT is a yellow solid. It is slightly soluble in water. Photochemical transformation (i.e., photolysis or photooxidation) of TNT in aqueous solutions has been investigated (O'Sullivan et al. 2011). When associated with organic matter in the water column, soil or sediment, TNT typically biotransforms, sometimes extensively, to reduced animated compounds, first to produce aminodinitrotoluene (Am-DNT) and preferentially 4-Am-DNT, with further reduction producing diaminonitrotoluene (DANT) and preferentially 2,4-DANT (Monteil-Rivera et al. 2009). Strong sorption to sediment organic matter not explained by hydrophobic partitioning has been reported for freshwater (Elovitz and Weber 1999) and marine sediment (Pennington et al. 2011).

1,3,5-trinitrobenzene (1,3,5-TNB)
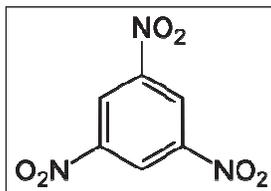
The compound 1,3,5-TNB has been primarily produced for use as an explosive. It can also be used to vulcanize natural rubber and as an acid-base indicator in the pH range of 12. The compound 1,3,5-TNB is also formed as a by-product of TNT manufacture and as a product of TNT environmental transformation, including its photolysis (Talmage et al. 1999).

At room temperature, 1,3,5-TNB is a clear to light yellow solid. It is slightly soluble in water. The compound 1,3,5-TNB appears to be resistant to photolysis; biotransformation of 1,3,5-TNB involves reduction of the nitro groups to form amino groups (Talmage et al. 1999). Extensive transformation of 1,3,5-TNB has been shown to occur in freshwater (Steevens et al. 2002; Lotufo and Farrar 2005) and marine sediment (Lotufo et al. 2001).

2,4-and 2,6- Dinitrotoluene (2,4-DNT and 2,6-DNT)
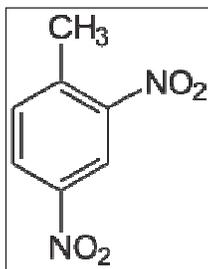
DNTs are a primary product used in propellants and are also formed as a byproduct during the TNT manufacturing process. Like TNT, DNTs can be reduced to the corresponding mono- or diamine derivatives (Monteil-Rivera et al. 2009). DNTs have also been associated, either as precursors or by-products, with the synthesis of polyurethane foams, coatings, and elastomers (USAPHC 2011). There are six isomers of DNT, the most common one is 2,4-DNT. Technical grade DNT is composed of

approximately 75% 2,4-DNT, 20% 2,6-DNT, and 5% other isomers (USAPHC 2011).

The 2,4-DNT and 2,6-DNT isomers are yellow to reddish crystaline solids at room temperature and are moderately soluble in water. Reported low log K_{oc} (1.65 and 1.96 for 4-DNT and 2,6-DNT respectively) suggest low affinity for organic particulate matter (USEPA 2012). However, strong sorption of 2,4-DNT to soil organic matter has been reported (Singh et al. 2010).

Tetryl, Ammonium Picrate (Explosive D), and Picric Acid

Tetryl	Ammonium Picrate	Picric Acid
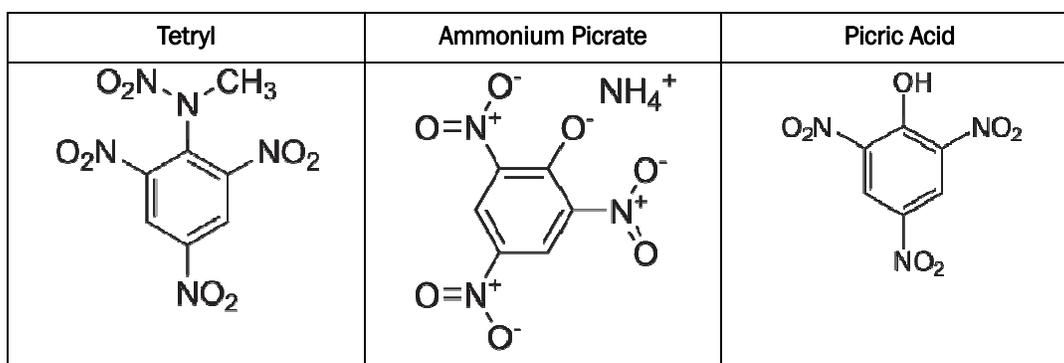
Tetryl (methyl-N-2,4,6-trinitrophenylnitramine-) was first synthesized in 1877 and used since 1906 as an explosive in booster charges, detonator charges, and as a component of explosives mixtures (Tetrytols) during WWII (U.S. Army 1961). Since 1973, the use of tetryl by the US military has been gradually phased out in favor of plastic bonded RDX and HMX formulas (Talmage et al. 1999).

Picric acid (2,4,6-trinitrophenol), a strongly acidic phenol (pK_a = 0.38) was used as a military explosive starting in the 1880s. Due to a tendency to form shock-sensitive picrates in the presence of metals and for its corrosive effects on shell casings, picric acid as an explosive composition was largely discontinued during the first part of the 20th century in favor of other formulations (USEPA 1993).

Ammonium picrate (2,4,6-trinitrophenol ammonium salt or picric acid ammonium salt) is a salt formed by reacting picric acid and ammonia. First prepared in 1841, it was adopted for use as a bursting-charge explosive by the U.S. Navy in 1907. From 1917 to WWII, ammonium picrate, also known by the military formula designation "Explosive D," was a common explosive

in virtually all calibers of armor-piercing ammunition due to its relative insensitivity to shock and friction (U.S. Army 1961). Picratol, a mixture consisting of 48% TNT and 52% ammonium picrate, was developed during WWII to allow faster production of cast bomb and projectiles compared with traditional pressed shell methods required by Explosive D alone. Explosive D was gradually replaced by RDX blends such as Composition A toward the end of WWII and was of limited use in the post-war era.

Tetryl is a yellow crystalline solid powder material with low solubility in water. Picric acid is a colorless, yellow, or yellow-red crystalline powder. Ammonium picrate and picric acid are very soluble in water. When dissolved in water, ammonium picrate and picric acid dissociate to unstable picrate salts such as potassium picrate or picramic acid (2-amino-4,6-dintrophenol).

Photolysis and hydrolysis are major environmental transformation processes for tetryl in aqueous media, as detailed in Talmage et al. (1999). In natural, sunlit waters, tetryl is transformed via photolysis and hydrolysis to several degradation products, including picric acid, which is the primary hydrolysis product of tetryl (Talmage et al. 1999). Information on the fate of tetryl in sediments is limited to a single paper (Nipper et al. 2002) that reported the formation of unknown breakdown products in sandy and fine-grained marine sediments spiked with tetryl.

When dissolved in water, both ammonium picrate and picric acid dissociate to the picrate ion (Thorne and Jenkins 1997). Similar to TNT, picric acid is also degraded through reduction, and the microbial biodegradation of picric acid results in the formation of 2A-dinitrophenol (Monteil-Rivera 2009). Under aerobic conditions, the mono- or dinitrophenol can then be oxygenated with subsequent ring cleavage (Monteil-Rivera 2009). The fate of picric acid in marine sediments was investigated by Nipper et al. (2005). Transformation rates were highest in incubated, fine-grained sediment and major transformation products included 4-dinitrophenol, aminodinitrophenols (including picramic acid), 3,4-diaminophenol, amino nitrophenol, and nitrodiaminophenol. In the study by Dave et al. (2000), picric acid bound to sediment and resisted hydrolysis, biodegradation and photolysis.

Cyclic Nitramines
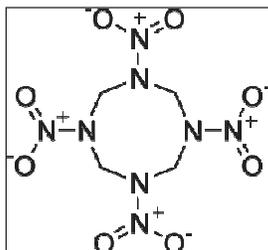
RDX   **HMX**
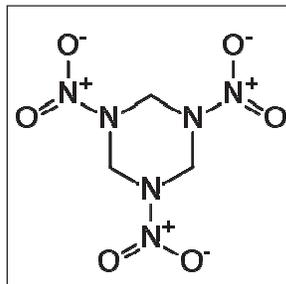
Commonly referred to as Royal Demolition Explosive, RDX (cyclo-1,3,5-trimethylene-2,4,6-trinitramine, hexahydro-1,3,5-trinitro-1,3,5-triazine or cyclonite) was first synthesized for medicinal use in 1899. Although its value as an explosive was soon recognized, it was not until the early to mid-1940s that mass production techniques improved sufficiently enough to supply the necessary quantities for use in military formulations.

Commonly referred to as High Melting Explosive, HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) is a component of plastic explosives, solid fuel rocket propellants, and military munitions, as well as being an impurity of RDX.

At room temperature, both RDX and HMX are crystalline solids. Military-grade RDX generally contains up to 10% HMX as an impurity of the manufacturing process (Talmage et al. 1999). RDX has low solubility in water, and HMX is even less soluble. Their low K_{ow} indicates their hydrophobicity.

The biodegradation of RDX occurs under both aerobic and anaerobic conditions but the anaerobic process is significantly faster (Monteil-Rivera et al. 2009). Unlike nitroaromatic explosives, RDX undergoes ring cleavage and mineralization under both aerobic and anaerobic conditions (Monteil-Rivera et al. 2009). Aerobically, it has been found that microbial cleavage of one of the $N–NO_2$ bonds produces unstable intermediates (Monteil-Rivera et al. 2009). Subsequent degradation reactions are largely governed by the interactions of the newly formed intermediates with water and eventually produce NO_2^-, N_2O, NH_3, HCHO, and HCOOH (Monteil-Rivera et al. 2009). Anaerobically, sequential reduction of the nitro groups

to produce mono-, di- and trinitroso derivatives (MNX, DNX and TNX, respectively) is most frequently observed. The nitroso derivatives may be further transformed to produce the unstable hydroxylamine derivatives, leading to ring cleavage (Crocker et al. 2006). Degradation of HMX does not appear to occur in the field under aerobic conditions, with half-lives in the environment spanning decades (Clausen and Korte 2011). However, anaerobic degradation of HMX has been observed and described (Monteil-Rivera et al. 2009).

RDX and HMX have lower water solubility and significantly less binding affinity with soil organic matter than TNT; RDX and HMX are only poorly immobilized by soils (Monteil-Rivera et al. 2009). Extensive transformation of RDX occurred following spiking to fine-grained sediment (Lotufo et al. 2001; Pennington et al. 2011), but the transformation products were not identified. Different from the fate of RDX in organically-rich sediment, transformation was minimal in sandy sediment (Rosen and Lotufo 2005).

Other Energetic Compounds

Nitroguanidine
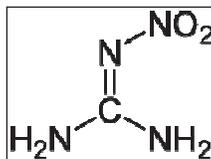
Nitroguanidine is also known by its military designation – NQ. It is a nitroimino compound that exists in two tautomeric forms; the tautomer form depicted above is predominant. It is a stable explosive compound, and when combined with nitrocellulose and nitroglycerin, forms the so-called triple-based propellant mixture.

Nitroguanidine is water soluble and may enter the environment via discharge streams from handling facilities (Kaplan et al. 1982). In addition, it has been found at army ranges as a result of live-fire training (Clausen 2011). Degradation of nitroguanidine was shown to be negligible in activated sludge under aerobic conditions and in sterile sludge under reducing conditions (Kaplan et al. 1982).

Nitrate Esters: nitroglycerin, nitrocellulose, PETN and DEGDN (diethylene glycol dinitrate)

Nitroglycerine	Nitrocellulose	PETN	DEGDN
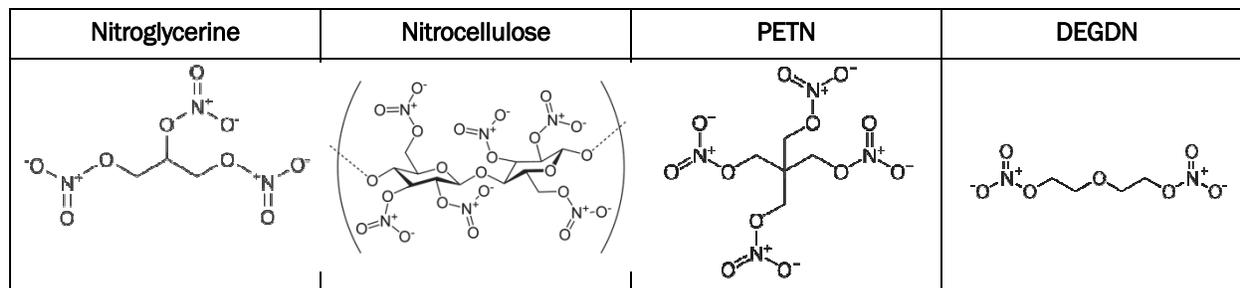
Nitrocellulose is a highly flammable compound formed by nitrating cellulose through exposure to nitric acid or another powerful nitrating agent. When used as a propellant or low-order explosive, it is also known as guncotton. Nitrocellulose occurs as a particulate that is essentially insoluble in water (Bentley et al. 1977d) and very recalcitrant to microbial degradation (Podlipná et al. 2008).

Nitroglycerin or glycerol trinitrate (propane-1,2,3-triyl trinitrate) is used in manufacture of dynamite, gunpowder, and rocket propellants, and as a therapeutic agent (Rocheleau et al. 2011). Double-base propellants used within newer small-arms ammunition typically contain up to 84% nitrocellulose, with 10% nitroglycerin, a stabilizer, and up to 6% filler compounds. The combination of nitroglycerin, nitrocellulose and nitroguanidine forms a triple-base propellant mixture. Nitroglycerin can be released into the environment at firing positions, and in the target areas due to low-order detonation of propellant (Rocheleau et al. 2011). Nitroglycerin is water soluble, and may enter and persist in freshwater environments (USACHPPM 2001). Microbial degradation of nitroglycerin has been reported (Podlipná et al. 2008).

PETN, or pentyl (1,3-dinitrato-2,2-bis(nitratomethyl)propane , or pentaerythritol tetranitrate) is used in some booster charges, demolition explosives, and initiating explosives such as primer cord. PETN is considered practically insoluble in water, not prone to hydrolyze at ambient temperature and recalcitrant to microbial degradation (Podlipná et al. 2008).

DEGDN is an explosive ingredient (60-80%) in dynamite along with nitroglycerine (40-20%) and has been used in propellant mixtures as a plasticizer (Fisher et al. 1989). DEGDN is a colorless, odorless, viscous, oily liquid. Environmental fate studies indicate that DEGDN is a stable compound once dissolved in water (Spanggord et al. 1985).

4   Aqueous Toxicity of Munitions Constituents to Fish and Aquatic Invertebrates

This section summarizes the available data on the toxicity of MC to fish and aquatic invertebrates. The data were generated through laboratory exposures of test organisms to water spiked with energetic MC.

Overview

To support the assessment of risk associated with the presence of explosives in aquatic environments, contaminant-spiked water is often used in laboratory experiments to derive toxicity data for a variety of freshwater and marine species and endpoints. In order to bracket the toxic range and derive toxicity benchmarks, most studies involved the use of exposure concentrations significantly higher than those expected in the environment. Broad overviews of the effects of explosive compounds to aquatic organisms are provided by Talmage et al. (1999), Juhasz and Naidu (2007) and more recently by Nipper et al. (2009) who provided a compilation of available aquatic toxicity data expanded to include nitroaromatic and nitrophenolic compounds that are not considered MC or their transformation products. Although most investigations of the aqueous toxicity of explosives and related compounds were performed using freshwater organisms, toxicity data for a variety of energetic compounds were generated using marine species.

Several studies reported the toxicity of MC to a variety of freshwater fish, including fathead minnows, channel catfish, and zebrafish (reviewed in Talmage et al. 1999 and Nipper et al. 2009; see also Mukhi et al. 2005; Mukhi and Patino 2008; Gust et al. 2011), as well as to frog larvae (Saka et al. 2004; Paden et al. 2011). In contrast, adverse biological effects of MC were reported for only two marine fish species (Nipper et al. 2001; Lotufo et al. 2010a,b,c). In addition, cellular-level effects of TNT on a catadromous eel were also reported (Della Torre et al. 2008a,b,c).

The aqueous toxicity of MC to freshwater invertebrates has been reported for a variety of freshwater species, including amphipods, midges, rotifers, cladocerans and oligochaetes (reviewed in Talmage et al. 1999 and Nipper

et al. 2009). Compared to studies with freshwater organisms, fewer studies addressed the effects of MC to marine invertebrates. Organisms used as test species included larval sea urchins, mysid shrimp (Nipper et al. 2001), larval and adult bivalves (Won et al. 1976; Goodfellow et al., 1983; Rosen and Lotufo 2007a), copepods (Won et al. 1976; Ek et al. 2006, 2007, 2008; Dave 2003; Dave et al. 2000), meiobenthic polychaete (Nipper et al. 2001) and macroalga spores (Nipper et al. 2001). The National Oceanic and Atmospheric Administration is currently investigating toxicity of several explosive compounds to corals.

Most of the studies cited above reported lethal effects. Reproductive effects of MC were reported for several species of freshwater fish (Bailey et al. 1985; Burton et al. 1994; Mukhi and Patino 2008) and invertebrates (Bailey et al. 1985; Peters et al. 1991; Snell and Moffat 1992; Conder et al. 2004a). Reports of sublethal effects for marine invertebrates included impacts to larval development of bivalves and sea urchins (Nipper et al. 2001; Rosen and Lotufo. 2007a) and polychaete reproduction (Nipper et al. 2001). Comparisons of lethal and sublethal endpoints for various MC suggest that the use of sublethal endpoints could be useful for the interpretation of potential long-term environmental effects of those chemicals, as concentra-tions associated with reproductive effects were lower, sometimes substantially, than those promoting mortality (Nipper et al. 2009).

Fate of MC in exposure water during toxicity testing

When fish and adult mussels were exposed to TNT using a static-renewal method in which the TNT-spiked water was exchanged daily, a large fraction of the TNT transformed to Am-DNTs (Lotufo et al. 2010a; Rosen and Lotufo 2007a). In addition, loss of nitroaromatic compounds also occurred in those studies, as the sum concentration of TNT, Am-DNTs and DANTs (referred to as SumTNT) decreased over the 24-h period between TNT-spiked water exchanges. As an example, the temporal pattern of change in SumTNT concentrations for different treatments of a 10-d sheepshead minnow static-renewal and exposure is shown in Figure 4.1. The percent of loss of the SumTNT tended to increase with decreasing concentrations. A similar pattern of temporal changes was observed in the adult mussel experiment. Loss of compounds was likely enhanced by the presence of the organisms themselves and the accumulation of food in the beaker (fish exposure only) and likely occurred as a result of volatilization, binding to food, as well as transformation to unidentified products. Transformation of TNT and decline of SumTNT concentrations between

water exchanges in static-renewal toxicity tests were also observed in other studies (Carr and Nipper 2000; Nipper et al. 2001; Conder et al. 2004b; Ownby et al. 2005; Yoo et al. 2006). In contrast, virtually no transformation or losses of TNT occurred when exposures were conducted with no feeding, such as mussel embryo exposure to TNT (Rosen et al. 2007a).
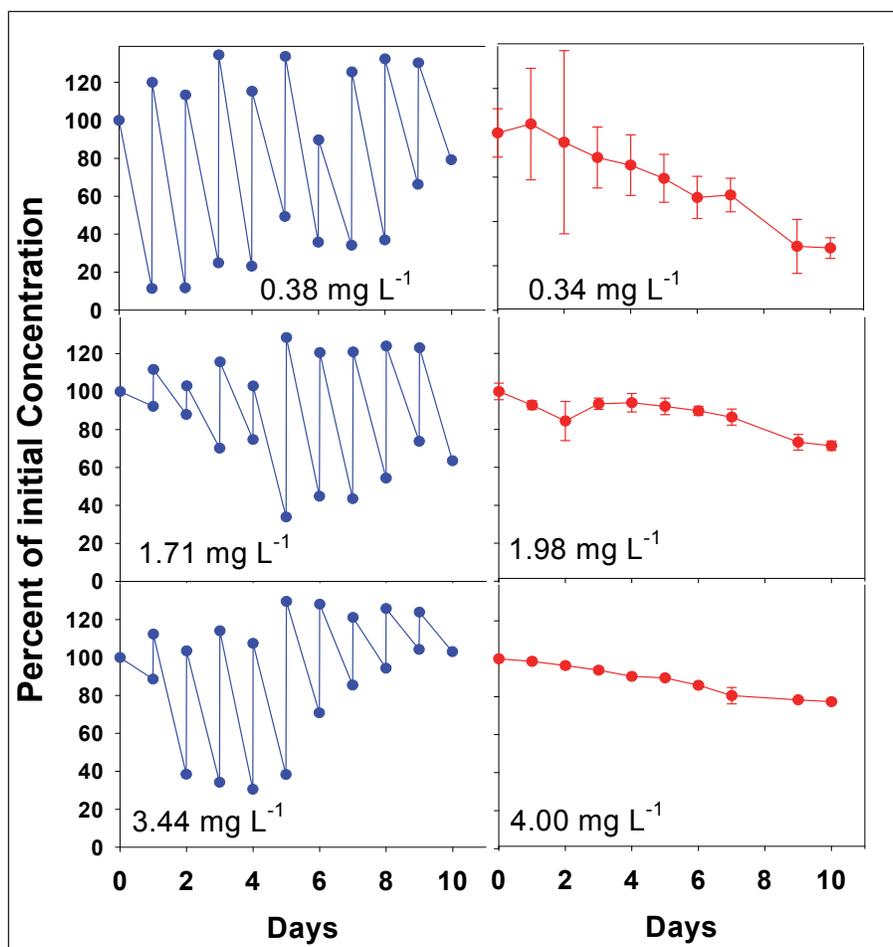
Figure 4.1. Temporal change in aqueous concentrations of SumTNT expressed as a percent of the initial concentration during the TNT 10-d static renewal experiment (left) and the TNT 10-d constant exposure experiment (right) (from Lotufo et al. 2010a).

In contrast with the static-renewal experiment, TNT exposures conducted using a diluter system that delivered spiked water every hour maintained stable concentrations over 24 h time intervals in experiments with sheepshead minnows (Lotufo et al. 2010a). However, the exposure water concentration declined gradually during the course of the 10-d experiment (Figure 4.1). Despite frequent turnover, enhancement of the microbial population due to the build-up of food and fish fecal detritus in the tanks likely promoted transformation of the TNT delivered to the exposure

chambers. The use of a diluter system is recommended and feeding should be avoided whenever possible in aqueous toxicity evaluations of TNT.

During toxicity tests using fathead minnows, transformation of 1,3,5-TNB dinitroaniles (or amino-dinitrobenzenes) occurred, leading to the some-times dramatic decrease in the parent compound concentration during the 24-h period between water exchange events (Lotufo et al. 2010a). However, the concentration decrease for 2-Am-DNT and 2,4-DANT between water exchange events was minimal in similar experiments (Lotufo et al. 2010a). Therefore, any transformation or loss of those compounds was negligible. Persistence of Am-DNTs and DANTs in experimental systems has also been reported for sediment (Lotufo and Farrar 2005), soils (Lachance et al. 2004) and water (Conder et al. 2004b). The extensive transformation of 1,3,5-TNB was surprising since 1,3,5-TNB is considered environmentally persistent, not readily biodegradable, and with a high propensity to contaminate groundwater near production waste disposal sites (Reddy et al. 1997). While static-renewal exposures appear appropriate for toxicity evaluation of Am-DNTs and DANTs, the use of a diluter system is recommended for toxicity evaluation of 1,3,5-TNB and the feeding of test organisms should be avoided whenever possible.

During aqueous exposures of sheepshead minnows to RDX (Lotufo et al. 2010b), the concentration of HMX in the exposure solutions represented approximately 8% of the sum concentration of RDX and HMX, corresponding to the reported concentration of HMX in the military grade RDX used in this study. No RDX or HMX transformation products were detected in the exposure solutions. Only negligible (< 5%) changes in RDX and HMX concentrations occurred between water exchanges in all experiments. Static-renewal exposures seem appropriate for the toxicity evaluation of RDX and HMX and feeding does not seem to promote the breakdown of parent compound as observed for TNT and 1,3,5-TNB.

Nipper et al. (2001) investigated the toxicity of eight MC to five marine species from different phyla. Chemical analysis for the MC at the beginning and end of each test indicated that some compounds were very stable, whereas others were lost in large amounts during the test exposure periods. The percent of loss of the most degradable chemicals tended to increase with decreasing concentrations. Overall, the compounds with three and four nitro groups (TNT, 1,3,5-TNB, and tetryl) exhibited higher loss than those with two nitro groups (1,3-DNB and 2,4- and 2,6-DNT). Picric acid and

RDX exhibited little or no loss. The use of a diluter system is recommended and feeding should be avoided whenever possible in aqueous toxicity evaluations of TNT, 1,3,5-TNB, and tetryl.

	RDX	Picric Acid	1,3-DNB	2,4-DNT	2,6-DNT	2,4,6-TNT	1,3,5-TNB	Tetryl
Mysid	2	14	39	22	14	99	82	100
Alga	0	1	8	5	12	36	90	84
Polychaete	0	0	18	12	14	69	51	100
Redfish	8	0	5	0	12	37	56	73
Urchin emb.	NM	0	2	0	0	0	0	66
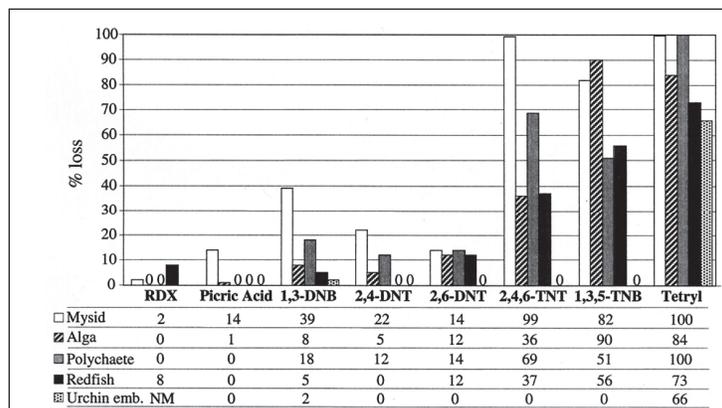
Figure 4.2. Mean percent MC lost in all test concentrations during aqueous exposures (from Nipper et al. 2001). NM - not measured at test termination.

Toxicity of nitroaromatic MC

Numerous aquatic toxicity studies have reported that TNT causes decreased survival in a number of aquatic invertebrate and fish species, typically at concentrations ranging from 1 to 10 mg L^{-1} (Nipper et al. 2009). The lethal toxicity of TNT to marine fish and invertebrates is summarized and compared using median lethal concentration (LC_{50}) values (Table 4.1).

Exposure of the marine species *Sciaenops ocellatus* (redfish) to TNT for 2 days during the hatching period (Nipper et al. 2001) generated an LC_{50} value higher than that reported for *Cyprinodon variegatus* (sheepshead minnow) exposed for 5 days. Toxicity data for longer exposure duration is not available for redfish.

Most invertebrates investigated exhibited similar sensitivities to TNT and overall less sensitivity than fish (Table 4.1; Figure 4.3). Mysid shrimp were the most sensitive species, while adult mussels were the most tolerant among the invertebrate species investigated. The relative high tolerance of adult mussels has also been observed for other contaminant classes. For example, 96 h LC_{50} values for several organophosphorus pesticides ranged from 23 to >56 mg L^{-1} for *M. galloprovincialis*, while LC_{50}s were generally 3-4 orders of magnitude lower in comparable exposures with fish (Serrano et al. 1995).

Table 4.1. Lethal toxicity data for marine fish and invertebrates exposed to nitroaromatic MC.

Species	MC	Duration (d)	LC$_{50}$ (mg L^{-1})	Reference
Sheepshead minnow *Cyprinodon variegatus*	TNT	10	2.3	Lotufo et al. 2010a
	TNT	5	1.7	Lotufo et al. 2010a
	2-Am-DNT	5	8.6	Lotufo et al. 2010a
	2,4-DANT	5	No lethal effects at 50.3 mg L^{-1} [a]	Lotufo et al. 2010a
	1,3,5-TNB	5	1.2	Lotufo et al. 2010a
Redfish *Sciaenops ocellatus*	TNT	2	7.6	Nipper et al. 2001
	2,4-DNT	2	48	Nipper et al. 2001
	2,6-DNT	2	28	Nipper et al. 2001
	1,3,5-TNB	2	1.2	Nipper et al. 2001
Oyster (larvae) *Crassostrea gigas*	TNT	4	8.2	Won *et al.* 1976
Mussel (adult) *M. galloprovincialis*	TNT	4	19.5	Rosen and Lotufo 2007a
Mysid shrimp *Americamysis bahia*	TNT	4	0.98	Nipper et al. 2001
	2,4-DNT	4	4.4	Nipper et al. 2001
	2,6-DNT	4	5.0	Nipper et al. 2001
	1,3,5-TNB	4	0.74	Nipper et al. 2001
Copepod *Tigriopus californicus*	TNT	3	5.3	Won *et al.* 1976
	2-Am-DNT	3	No lethal effects at 50 mg L^{-1} [a]	Won *et al.* 1976
	2,4-DANT	3	No lethal effects at 50 mg L^{-1} [a]	Won *et al.* 1976
	2,6-DANT	3	No lethal effects at 50 mg L^{-1} [a]	Won *et al.* 1976
Copepod *Nitocra spinipes*	TNT	4	7.6	Dave et al. 2000
	2,4-DNT	4	17	Dave et al. 2000
Polychaete *Dinophilus gyrociliatus*	TNT	7	5.6	Nipper et al. 2001
	2,4-DNT	7	20	Nipper et al. 2001
	2,6-DNT	7	13	Nipper et al. 2001
	1,3,5-TNB	7	1.6	Nipper et al. 2001
Amphipod *Eohaustorius estuarius*	TNT	4	4.5	Rosen and Lotufo, unpublished
Amphipod *Leptocheirus plumulosus*	TNT	4	3.6	Rosen and Lotufo, unpublished

LC$_{50}$ - Median lethal concentration, [a] Highest concentration tested.
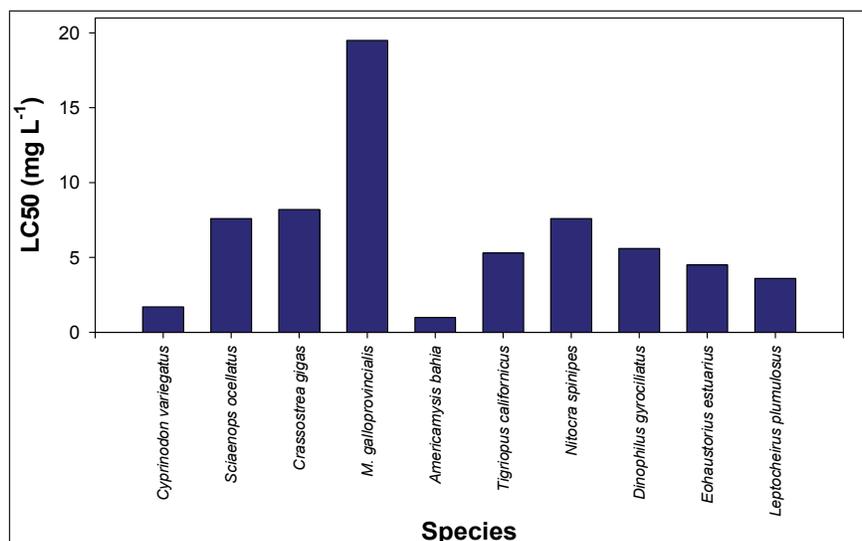
Figure 4.3. Lethal toxicity data for marine fish and invertebrates exposed to TNT. LC$_{50}$ - median lethal concentration. See Table 4.1 for references.

The TNT acute toxicity values derived for marine fish and invertebrates are comparable to those reported for freshwater organisms (Table 4.2), suggesting relatively similar sensitivity among all aquatic fish and invertebrates.

The toxicity of TNT was compared to that of its major transformation products 1,3,5-TNB, 2-Am-DNT and 2,4-DANT using sheepshead minnows (Figure 4.4) (Lotufo et al. 2010a). Nitro-reduction appears to decrease the toxicity of TNT to sheepshead minnows, as the LC$_{50}$ for the mono-aminated compound 2-Am-DNT was approximately 4 times higher than that for TNT. Further amination appears to decrease toxicity even more dramatically, as the highest tested concentration for 2,4-DANT was 20 times higher than the LC$_{50}$ for TNT and yet that concentration failed to promote significant mortality (Table 4.1).

Freshwater species varied in their relative sensitivity to lethal toxicity of TNT and its major aminated transformation products (Table 4.3). The toxicity of 2-Am-DNT was lower than that of TNT for fathead minnows. For larvae of the frog *Xenopus laevis*, the toxicity of 2-Am-DNT and 4-Am-DNT were also lower than that of TNT. For invertebrates, 2-Am-DNT exhibited toxicity similar to that of TNT. The compound 2,4-DANT was more toxic than TNT for *Hyalella azteca* but substantially less toxic to *Chironomus dilutus* (formerly known as *C. tentans*). The compound 1,3,5-TNB was more toxic than TNT to sheepshead minnows and fathead minnows, as well as for the invertebrates *Daphnia magna* and *Hyalella azteca*, but was almost identically lethal as TNT to *Chironomus dilutus*.

Table 4.2. Lethal toxicity data for freshwater fish, amphibians and invertebrates exposed to TNT.

Species	Duration (d)	LC$_{50}$ (mg L^{-1})	Reference
Bullfrog (tadpole) *Rana catesbeiana*	4	40.3	Paden et al. 2011
Frog (tadpole) *Xenopus laevis*	4	3.8	Saka et al. 2004
Fathead minnow *Pimephales promelas*	10	2.2	Yoo et al. 2006
Pimephales promelas	4	2.9	Liu et al. 1983a
Bluegill *Lepomis macrochirus*	4	2.6	Liu et al. 1983a
Rainbow trout *Oncorhynchus mykiss*	4	0.8	Liu et al. 1983b
Channel catfish *Ictalurus punctatus*	4	2.4	Liu et al. 1983a
Amphipod *Hyalella azteca*	4	3.6	Sims and Steevens 2008
Oligochaete *Lumbriculus variegatus*	2	5.2	Liu et al. 1983b
Oligochaete *Tubifex tubifex*	4	7.7 [a]	Conder et al. 2004c
Cladoceran *Daphnia magna*	4	5.1	Johnson et al. 1994
Cladoceran *Ceriodaphnia dubia*	7	No lethal effects at 6.1 mg L^{-1} [b]	Griest et al. 1998
Midge (larva) *Chironomus tentans*	4	1.9	Conder et al. 2004c
Rotifer *Branchionus calyciflorus*	1	5.6	Toussaint et al. 1995

LC$_{50}$ - median lethal concentration, [a] Based on nominal concentrations, [b] Highest concentration tested.
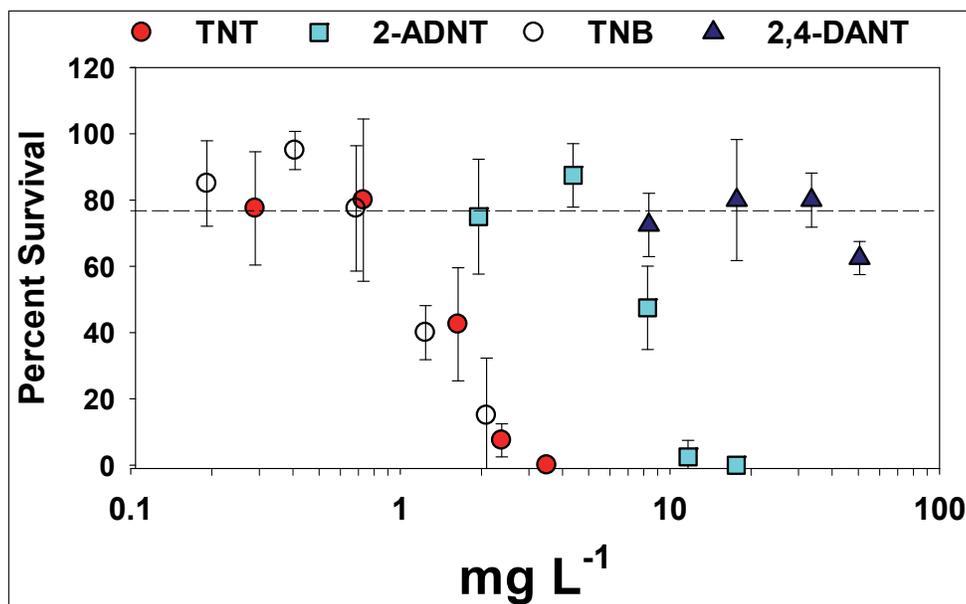
Figure 4.4. Mean percent survival of juvenile sheepshead minnows (*Cyprinodon variegatus*) at termination of the 5-d exposure to aqueous exposures to TNT, 2-Am-DNT, 1,3,5-TNB and 2,4-DANT (on log scale) (from Lotufo et al. 2010a). Error bars are ± 1 standard deviation. The dashed line indicates mean control percent survival.

Table 4.3. Lethal toxicity data for fish, a tadpole and aquatic invertebrates exposed to TNT, Am-DNTs, DANTs, and 1,3,5-TNB.

Species	Duration (d)	LC$_{50}$ (mg L^{-1})						Reference
		TNT	2-Am-DNT	4-Am-DNT	2,4-DANT	2,6-DANT	1,3,5-TNB	
Marine species								
Sheepshead minnow *Cyprinodon variegatus*	5	1.7	8.6	ND	No effect at 50.3 mg L$^{-1 a}$	ND	1.2	Lotufo et al. 2010a
Freshwater species								
Frog tadpole *Xenopus laevis*	4	3.8	32.7	22.7	ND	ND	ND	Saka et al. 2004
Fathead minnow *Pimephales promelas*	4	2.4	14.8	6.9	ND	ND	1.0	Pearson et al. 1979
Cladoceran *Daphnia magna*	2	11.9	4.5	5.2	ND	ND	2.7	Pearson et. al. 1979
	4	5.1	1.1	5.1	ND	ND	ND	Johnson et al. 1994
Cladoceran *Ceriodaphnia dubia*	7	No effect at 6.0 mg L$^{-1 a}$	4.9	6.6	No effect at 0.2 mg L$^{-1 a}$	No effect at 2.0 mg L$^{-1 a}$	No effect at 2.0 mg L$^{-1 a}$	Griest et al. 1998

Species	Duration (d)	LC$_{50}$ (mg L^{-1})						Reference
		TNT	2-Am-DNT	4-Am-DNT	2,4-DANT	2,6-DANT	1,3,5-TNB	
Amphipod *Hyalella azteca*	4	3.6	3.8	9.2	1.7	ND	2.3	Sims and Steevens 2008
Midge (larva) *Chironomus dilutus*	4	1.9	3.3	ND	33.3	ND	2.2	Lotufo, unpublished

LC$_{50}$ - median lethal concentration, ND - not determined, [a] Highest concentration tested, [b] The No observed effect concentration was 3.0 and lowest observed effect concentration was 6.0 mg L^{-1}.

The sublethal effects of TNT, examined as mussel larval development, occurred at a concentration substantially lower than the lethal median concentration determined for adult mussels (Rosen and Lotufo 2007a) (Table 4.4). Mussel larvae were also more sensitive to the developmental effects of TNT than sea urchin embryos (Davenport et al. 1994, Nipper et al. 2001, Rosen and Lotufo 2007a) (Table 4.4). Embryo-larval development involves critical phases of differentiation (e.g., gastrulation), which are often more sensitive than other life stages. The development of functional mussel byssal threads has been previously investigated as a sublethal endpoint (Serrano et al. 1995). After 48 h of TNT exposure, the byssal thread development endpoint was more sensitive than survival by a factor of 3, indicating that functional byssi is a relatively sensitive sublethal endpoint for TNT (Rosen and Lotufo 2007a). Sublethal effects, as a decrease in offspring production in freshwater cladoceran *C. dubia*, occurred at a concentration substantially lower than the lethal median concentration for 2,4-DANT and 2,6-DANT (Table 4.5). For TNT, 2-Am-DNT and 4-Am-DNT, sublethal effects occurred at concentrations similar to those promoting lethal effects (Table 4.5).

Reports on sublethal effects of TNT on growth or reproduction of marine fish have not been found. For freshwater fish, TNT concentration of 0.014 mg L^{-1} was reported during a 9-month TNT life cycle test as promoting a reduction in total length of the parental females after spawning was completed (Burton et al. 1993).

The toxicity of 2,4-DNT and 2,6-DNT to marine organisms was investigated by Nipper et al. (2001). Comparison of toxicity values (Tables 4.1 and 4.4) reveal that these compounds have a lower potency than TNT for the few species investigated. Neither 2,6-DNT nor its main biotransformation

Table 4.4. Sublethal toxicity data for marine invertebrates and alga exposed to nitroaromatic MC, and comparison with lethal toxicity data, when available.

Species	MC	Duration (d)	Sublethal Effect (mg L^{-1})	Duration (d)	LC$_{50}$ (mg L^{-1})	Reference
Mussel *Mytilus galloprovincialis*	TNT	2	0.75 Embryo development EC$_{50}$	4	19.5 Adult	Rosen and Lotufo 2007a
	TNT	4	6.6 Adult byssal thread formation EC$_{50}$			
Sea Urchin *Arbacia punctulata*	TNT	2	12 Embryo development EC$_{50}$		ND	Nipper et al. 2001
	2,4-DNT	2	51 Embryo development EC$_{50}$		ND	Nipper et al. 2001
	2,6-DNT	2	6.7 Embryo development EC$_{50}$		ND	Nipper et al. 2001
	1,3,5-TNB	2	1.3 Embryo development EC$_{50}$		ND	Nipper et al. 2001
Sea Urchin *Lytechinus variegatus*	TNT	NR[a]	10 Embryo development rerported effect		ND	Davenport et al. 1994
	2,4-DNT	NR	10 Embryo development rerported effect		ND	Davenport et al. 1994
	2,6-DNT	NR	10 Embryo development rerported effect		ND	Davenport et al. 1994
Polychaete *Dinophilus gyrociliatus*	TNT	7	1.1 Reproduction EC$_{50}$	7	5.6	Nipper et al. 2001
	2,4-DNT	7	5.2 Reproduction EC$_{50}$	7	20	Nipper et al. 2001
	2,6-DNT	7	2.1 Reproduction EC$_{50}$	7	13	Nipper et al. 2001
	1,3,5-TNB	7	0.4 Reproduction EC$_{50}$	7	1.6	Nipper et al. 2001
Copepod *Schizopera knabeni*	2,6-DNT	4	55 Reproduction EC$_{50}$	4	65	Nipper et al. 2005
	2-A-6-NT	4	8 Reproduction EC$_{50}$	4	No lethal effects at 42 mg L^{-1} [b]	Nipper et al. 2005
Alga *Ulva fasciata*	TNT	4	0.61 Germling length EC$_{50}$		ND	Nipper et al. 2001
	2,4-DNT	4	1.7 Germling length EC$_{50}$		ND	Nipper et al. 2001
	2,6-DNT	4	2.7 Germling length EC50		ND	Nipper et al. 2001

Species	MC	Duration (d)	Sublethal Effect (mg L^{-1})	Duration (d)	LC$_{50}$ (mg L^{-1})	Reference
	2,6-DNT	4	4 Germling length EC$_{50}$		ND	Nipper et al. 2005
	2-A-6-NT	4	20 Germling length EC$_{50}$		ND	Nipper et al. 2005
	1,3,5-TNB	4	0.03 Germling length EC$_{50}$		ND	Nipper et al. 2001

EC$_{50}$ - median effective concentration, LC$_{50}$ - median lethal concentration, ND - Not determined, [a] Not reported (typical duration for this assay is 2 d) , [b] Highest concentration tested.

Table 4.5. Sublethal toxicity data for freshwater cladoceran *Ceriodaphnia dubia* exposed to nitroaromatic MC, and comparison with lethal toxicity data, when available. Exposur e duration was 7 d for all exposures.

MC	Sublethal Effect (mg L^{-1})	Lethal Effect (mg L^{-1})	Reference
TNT	2.7 Reproduction LOEC	No effects at 2.7 [a]	Burton et al. 1993
TNT	3.3 Reproduction EC$_{50}$	No effects at 6.0 [a]	Griest et al. 1998
2-Am-DNT	3.1 Reproduction EC$_{50}$	4.9 LC$_{50}$	Griest et al. 1998
4-Am-DNT	5.1 Reproduction EC$_{50}$	6.6 LC$_{50}$	Griest et al. 1998
2,4-DANT	0.05 Reproduction EC$_{50}$	No effects at 0.2 [a]	Griest et al. 1998
2,6-DANT	0.33 Reproduction EC$_{50}$	No effects at 2.0 [a]	Griest et al. 1998
1,3,5-TNB	1.41 Reproduction EC$_{50}$	No effects at 2.0 [a]	Griest et al. 1998

EC$_{50}$ - median effective concentration, LC$_{50}$ - median lethal concentration, LOEC - Lowest observed effects concentration, [a] Highest concentration tested.

product, 2-A-6-NT, were particularly toxic to copepod, *S. knabeni*, adult female survival. The reproduction endpoint, however, indicated significantly higher toxicity of the biotransformation product (Table 4.4). The opposite happened in *U. fasciata* zoospore germination tests, where the toxicity of the parent compound, 2,6- DNT, was markedly higher than that of 2-A-6-NT (Table 4.4). Because toxicity data for only one marine fish species was found in the available literature (Nipper et al. 2001), 2,4-DNT and 2,6-DNT toxicity data for freshwater fish, an amphibian, as well as for a freshwater cladoceran, are presented in Table 4.6. For the species of fish investigated, the toxicity of 2,4-DNT and 2,6-DNT was similar among

species and between compounds. Tadpoles were more tolerant than fish. *Daphnia magna* (immobilization endpoint) was more sensitive than fish (survival endpoint) and 2,4-DNT was substantially more potent than 2,6-DNT to that species (Deneer et al. 1989).

Table 4.6. Toxicity data for freshwater animals exposed to DNTs.

Species	MC	Duration (d)	Toxic Effect (mg L^{-1})	Reference
Bullfrog tadpole *Rana catesbeiana*	2,4-DNT	4	79.3 (LC$_{50}$)	Paden et al. 2011
	2,6-DNT	4	92.4 (LC$_{50}$)	Paden et al. 2011
Fathead minnow *Pimephales promelas*	2,4-DNT	4 4	24.3 (LC$_{50}$) 32.8 (LC$_{50}$)	Bailey and Spanggord 1983 Broderius et al. 2005
	2,6-DNT	4	18.5 (LC$_{50}$)	Bailey and Spanggord 1983
Common carp *Cyprinus carpio*	2,4-DNT	4	26.9 (LC$_{50}$)	Zhao et al. 1997
	2,6-DNT	4	20.0 (LC$_{50}$)	Zhao et al. 1997
Bluegill *Lepomis macrochirus*	2,4-DNT	4	13.5 (LC$_{50}$)	Liu et al. 1983b
Rainbow trout *Oncorhynchus mykiss*	2,4-DNT	4	16.3 (LC$_{50}$)	Liu et al. 1983b
Catfish Ictalurus punctatus	2,4-DNT	4	24.8 (LC$_{50}$)	Liu et al. 1983b
Cladoceran	2,4-DNT	21	0.6 (EC$_{50}$) [b]	Deneer et al. 1989
Daphnia magna	2,6-DNT	21	9.6 (EC$_{50}$) [b]	Deneer et al. 1989
EC$_{50}$ - median effective concentration, LC$_{50}$ - median lethal concentration, [a] Highest concentration tested, b Immobilization endpoint.				

When the common carp (*Cyprinus carpio*) was exposed to 2,4-DNT for 7 or 15 d, significant sublethal effects on the hepatosomatic index (at 0.5 mg L^{-1} after 7 d), enzyme activity (at 0.1 mg L^{-1} after 7d), energy reserves (at 1.0 mg L^{-1} after 4 d) and relative condition factor (at 0.1 mg L^{-1} after 15 d) were reported (Xu and Jing 2012).

Toxicity of nitrophenolic MC

When comparing the toxicity of MC using eight compounds of concern and marine toxicity tests with five species of marine organisms, Nipper et al. (2001), reported that tetryl, along with 1,3,5-TNB, was the most toxic compound overall (Table 4.7). It was also the most degradable compound, often being reduced to very low or below-detection levels at the end of the

test exposure. Tetryl has not been manufactured in the United States for several decades and may no longer have any environmental relevance. In study of Nipper et al. (2001), picric acid and RDX were the least toxic chemicals tested overall. The concentrations of picric acid causing mortality to redfish and sheepshead minnows were very similar. Oysters were also tolerant to the lethal effects of picric acid (Goodfellow et al. 1983). The reduction of picric acid to picramic acid promoted substantial toxicity increase as the main breakdown products of picric acid, 2,4-DNP and picramic acid, were more toxic to copepods, oysters, and macroalga zoospores than the parent compound (Nipper et al. 2005; Goodfellow et al. 1983). The toxicity of 2,4-DNP was higher than that of picramic acid for macroalga zoospores, but both chemicals exhibited similar toxicity to copepod for reproduction (Nipper et al. 2005). The potential reasons for higher toxicity of picric acid to macroalga zoospores observed in Nipper et al. (2001) when compared to results reported in Nipper et al. (2005) were not discussed in the more recent study. The concentrations of picric acid eliciting mortality to freshwater fish (Table 4.8) were similar to concentrations reported as toxic to marine fish. Similarly, the picric acid LC_{50} for the marine copepod *N. spinipes* and the freshwater cladoceran *D. magna* (Table 4.8) were similar. The transformation product 2,4-DNP was more toxic than the parent compound to both marine and freshwater fish and invertebrates, most substantially so for bluegills (Tables 4.7 and 4.8).

Table 4.7. Lethal and sublethal effects concentrations for marine fish, invertebrates and alga exposed to nitrophenolic MC.

Species	MC	Duration (d)	Sublethal Effects (mg L^{-1})	Lethal Effects (mg L^{-1})	Reference
Redfish *Sciaenops ocellatus*	Tetryl	2	ND	1.1 LC_{50}	Nipper et al. 2001
	Picric acid	2	ND	127 LC_{50}	Nipper et al. 2001
Sheepshead minnow *Cyprinodon variegatus*	Picric acid	4	ND	130 LC_{50}	Heitmuller et al. 1981
	2,4-DNP	4	ND	29 LC_{50}	Heitmuller et al. 1981
Mysid shrimp *Americamysis bahia*	Tetryl	4	ND	0.37 LC_{50}	Nipper et al. 2001
	Picric acid	4	ND	13 LC_{50}	Nipper et al. 2001

Species	MC	Duration (d)	Sublethal Effects (mg L^{-1})	Lethal Effects (mg L^{-1})	Reference
Sea Urchin *Arbacia punctulata*	Tetryl	2	0.05 Embryo development EC$_{50}$	ND	Nipper et al. 2001
	Picric acid	2	281 Embryo development EC$_{50}$	ND	Nipper et al. 2001
Polychaete *Dinophilus gyrociliatus*	Tetryl	7	0.01 Reproduction EC$_{50}$	0.03 LC$_{50}$	Nipper et al. 2001
	Picric acid	7	155 Reproduction EC$_{50}$	265 LC$_{50}$	Nipper et al. 2001
Copepod *Schizopera knabeni*	Picric acid	4	60 Reproduction EC$_{50}$	No lethal effects at 136 [a]	Nipper et al. 2005
	Picramic acid	4	8.6 Reproduction EC$_{50}$	No lethal effects at 15.4 [a]	Nipper et al. 2005
	2,4-DNP	4	9.4 Reproduction EC$_{50}$	14 LC$_{50}$	Nipper et al. 2005
Copepod *Nitocra spinipes*	Picric acid	4	ND	92 LC$_{50}$	Dave et al. 2000
Oyster *Crassostrea virginica*	Picric acid	6	28 Shell deposition EC$_{50}$	255 LC$_{50}$	Goodfellow et al. 1983
	Picramic acid	6	6 Shell deposition EC$_{50}$	70 LC$_{50}$	Goodfellow et al. 1983
Alga *Ulva fasciata*	Tetryl	4	0.20 Germling length EC$_{50}$	ND	Nipper et al. 2001
	Picric acid	4	94 Germling length EC$_{50}$	ND	Nipper et al. 2001
	Picric acid	4	472 Germling length EC$_{50}$	ND	Nipper et al. 2005
	Picramic acid	4	88 Germling length EC$_{50}$	ND	Nipper et al. 2005
	2,4-DNP	4	20 Germling length EC$_{50}$	ND	Nipper et al. 2005

EC$_{50}$ - Median effective concentration, LC$_{50}$ - Median lethal concentration, ND – Not determined, [a] Highest concentration tested.

Table 4.8. Lethal concentrations for freshwater fish and invertebrates exposed to nitrophenolic MC.

Species	Duration (d)	LC$_{50}$ (mg L^{-1})			Reference
		Picric acid	2,4-DNP	Picramic acid	
Rainbow trout *Oncorhynchus mykiss*	4	110	ND	46	Goodfellow et al. 1983
Bluegill *Lepomis macrochirus*	5	170	0.62	ND	Buccafusco et al. 1981
Cladoceran *Daphnia magna*	2	85	4.1	ND	LeBlanc 1980

LC$_{50}$ - Median lethal concentration, ND - Not determined.

Toxicity of RDX and HMX

RDX caused decreased survival of some fish and aquatic invertebrate species at concentrations similar to those reported as acutely toxic for TNT. Other fish species and aquatic invertebrates exhibited decreased survival in exposure to concentrations approaching or exceeding 40 mg L^{-1} (Table 4.9), the approximate solubility limit for this compound in water. Lotufo et al. (2010b) and ENSR International (2005) reported similar toxicity values for RDX to sheepshead minnows (Table 4.9). Test-organisms in both studies were approximately two-week-old juvenile fish. ENSR International (2005) reported 96-h LC$_{50}$ values for two species of marine fish that were lower than that for sheepshead minnows (Table 4.9). A saturated aqueous solution of RDX did not promote any effects on redfish during a 48-h embryo hatching and larval survival test (Nipper et al. 2001). The toxicity of RDX to 5 species of freshwater fish suggest they are similarly sensitive to the lethal effects of RDX as marine species (reviewed in Talmage et al. 1999 and Nipper et al. 2009; see also Muhki et al. 2005, Muhki and Patino 2008, and Warner et al. 2012).

A variety of aquatic invertebrate species, both marine and freshwater, were tolerant to the lethal effects of RDX (Peters et al. 1991; Burton et al. 1993; Dave et al. 2000; Nipper et al. 2001; ENSR International 2005; Rosen and Lotufo 2007a; Gust et al. submitted). Exposure to RDX at 28 mg L^{-1} or higher failed to elicit mortality in adult mussels, Dungeness crabs, mysid shrimp, polychaetes, amphipods and copepods, and failed to impair normal development of embryonic mussels and echinoderms (e.g., urchins) and exposure to RDX at 7.2 mg L^{-1} failed to elicit mortality of the coral *Acropora formosa* (Table 4.9).

Table 4.9. Lethal and sublethal toxicity data for marine fish and invertebrates exposed to RDX or HMX.

Species	MC	Duration (d)	Effects Concentration (mg L^{-1})	Reference
Sheepshead minnow *Cyprinodon variegatus*	RDX	10	9.9 (LC_{50})	Lotufo et al. 2010b
	RDX	4	9.8 (LC_{50})	ENSR International 2005
	HMX	5	No lethal effects at 2 [a]	Lotufo et al. 2010b
Inland silverside *Menidia beryllina*	RDX	4	7.1 (LC_{50})	ENSR International 2005
Speckled sand dab *Citharichthys stigmaeus*	RDX	4	2.4 (LC_{50})	ENSR International 2005
Redfish *Sciaenops ocellatus*	RDX	2	No lethal effects at 68 [a, b]	Nipper et al. 2001
Mussel *Mytilus galloprovincialis* (adult)	RDX	4	No lethal effects at 28 [a]	Rosen and Lotufo 2007a
(embryo)	RDX	2	No developmental effects at 28 [a]	Rosen and Lotufo 2007a
(embryo)	RDX	2	No developmental effects at 47 [a]	ENSR International 2005
(adult)	HMX	4	No lethal effects at 2 [a]	Rosen and Lotufo 2007a
(embryo)	HMX	2	No developmental effects at 2 [a]	Rosen and Lotufo 2007a
Mysid shrimp *Americamysis bahia*	RDX	4	No lethal effects at 47 [a]	Nipper et al. 2001
	RDX	4	No lethal effects at 53 [a]	ENSR International 2005
Dungeness crab *Cancer magister*	RDX	4	No lethal effects at 41 [a]	ENSR International 2005
Sand dollar (embryo) *Dendraster excentricus*	RDX	3	No lethal effects at 41 [a]	ENSR International 2005
Sea Urchin *Arbacia punctulata*	RDX	2	No developmental effects at 75 [a,b]	Nipper et al. 2001
Polychaete *Dinophilus gyrociliatus*	RDX	7	No lethal effects at 49 [a]	Nipper et al. 2001
	RDX	7	26 Reproduction (EC_{50})	Nipper et al. 2001
Polychaete *Neanthes arenaceadentata*	RDX	4	No lethal effects at 43 [a]	ENSR International 2005
Copepod *Nitocra spinipes*	RDX	4	No lethal effects at 36 [a]	Dave et al. 2000
Amphipod *Eohaustorius estuarius*	RDX	4	No lethal effects at 39 [a]	Rosen and Lotufo, unpublished data

Species	MC	Duration (d)	Effects Concentration (mg L^{-1})	Reference
Amphipod *Leptocheirus plumulosus*	RDX	4	No lethal effects at 39 [a]	Rosen and Lotufo, unpublished
Coral *Acropora formosa*	RDX	g	No lethal effects at 7.2 [a]	Gust et al. (in preparation)
Alga *Ulva fasciata*	RDX	4	8.1 Germling length (EC$_{50}$)	Nipper et al. 2001

EC$_{50}$ - Median effective concentration, a - Highest concentration tested, [b] Reported as target concentrations exceeding the solubility limit of 56.3 mg L^{-1} (Table 3.1).

The results of the chronic toxicity of RDX to fish and aquatic invertebrates (both freshwater and marine) are presented in Table 4.10. Surprisingly, sheepshead minnows in a 28-d exposure (ENSR International 2005) were more tolerant to RDX than in a 10-d exposure (Lotufo et al. 2010b) and *Daphnia magna* was substantially more tolerant to RDX in the study by ENSR International (2005) than reported in Bentley et al. (1977a).

Table 4.10. Chronic toxicity data for fish and aquatic invertebrates exposed to RDX.

Species	Duration (d)	Effects	NOEC (mg L^{-1})	LOEC (mg L^{-1})	Reference
Marine species					
Sheepshead minnow *Cyprinodon variegatus*	28	Survival/growth	9.7	21.4	ENSR International 2005
Freshwater species					
Fathead minnow *Pimephales promelas*	30	Growth	3.0	5.8	Bentley et al. 1977b
	240	Survival	3.0	6.3	Bentley et al. 1977b
	28	Growth	1.4	2.4	Burton et al. 1994
Zebrafish *Danio regio*	56	Survival	1.4	9.6	Mukhi and Patino 2008
Cladoceran *Ceriodaphnia dubia*	7	Survival/reproduction	3.6	6.0	Peters et al. 1991
Cladoceran *Daphnia magna*	21	Reproduction	2.2	4.8	Bentley et al. 1977a
	21	Reproduction	41	No effects at 41 [a]	ENSR International 2005

NOEC - No observed effect concentration, LOEC - lowest observed effect concentration, [a] Highest concentration tested.

RDX at sublethal concentrations caused short-term negative effects on growth, but no negative effects on reproductive endpoints for zebrafish (Mukhi and Patino 2008). Maternal exposure to RDX did not affect egg fertilization and embryo hatching rates in zebrafish (Mukhi and Patino 2008). These observations indicated that RDX at water concentrations that do not cause significant parental mortality (up to 3.2 mg L^{-1}) also do not have any deleterious effects on egg quality. Feeding behavior in zebrafish was affected during the first several weeks of exposure to RDX at 9.6 mg L^{-1}, as fish were reported as relatively lethargic compared to fish in the control. These differences in behavior eventually disappeared towards the end of the 12-week exposure (Mukhi and Patino 2008). Incidence of vertebral deformity was observed at 1.8 mg L^{-1} for fathead minnows and at 13.8 mg L^{-1} for zebrafish after 96 h of exposure, approximately 2x lower than concentrations associated with significant lethal effects, 3.5 mg L^{-1} for fathead minnows and 27.7 mg L^{-1} for zebrafish (Warner et al. 2012). Decreased expression of collagen-coding transcripts was associated with spinal deformity and were likely involved in sensitivity to RDX. That study also found that the number and magnitude of differentially expressed transcripts increased with increasing RDX concentration for both species. Differentially expressed genes were enriched in functions related to neurological disease, oxidative-stress, acute-phase response, vitamin/mineral metabolism and skeletal/muscular disorders. Gust et al. (2011) assessed the impacts of 0.83 mg L^{-1} of RDX on fathead minnows in one-year and multigenerational bioassays. RDX had no significant effect on gonadosomatic index or condition factor assayed after up to 12 months. In addition, RDX had no significant effect on live-prey capture rates, egg production, or fertilization. Considering both marine and freshwater fish and invertebrates, the lowest sublethal effects concentration reported for RDX was 2.4 mg L^{-1} for the fathead minnow growth endpoint by Burton et al. (1994).

Exposure to HMX at concentrations reported as its maximum achieved concentration in seawater failed to elicit mortality of exposed sheepshead minnows (Lotufo et al. 2010b) and mussels (Rosen and Lotufo 2007a), both for survival and embryonic development endpoints (Table 4.6). Studies with freshwater fish (Bentely et al. 1977) demonstrated that fathead minnows were susceptible to the effects of HMX at 7 d post hatch, but reported a 4-d LC$_{50}$ value (15 mg L^{-1}) based on target concentrations that exceeded the solubility limit of 4.5 mg L^{-1} for that compound. All other life stages for fathead minnows as well as other fish species and invertebrates were unaffected by even the highest concentrations of HMX tested (Bentley et al.

1977c; 1984; summarized in Talmage et al. 1999 and Nipper et al. 2009) (Table 4.11). The effect and highest no-effect concentration reported in those studies were above the reported solubility limit of HMX (Table 3.1).

Table 4.11. Toxicity data for freshwater fish and invertebrates exposed to HMX.

Species	Duration (d)	Effects Concentration (mg L^{-1})	Reference
Fathead minnow *Pimephales promelas*	4	15^b (LC$_{50}$)	Bentley et al. 1977c
	32	No effects on embryo hatching, survival or growth at 3.3	Bentley et al. 1984
Bluegill *Lepomis macrochirus*	4	No lethal effects at 32 [a,b]	Bentley et al. 1977c
Rainbow trout *Oncorhynchus mykiss*	4	No lethal effects at 32 mg L^{-1} [a,b]	Bentley et al. 1977c
Channel catfish *Ictalurus punctatus*	4	No lethal effects at 32 [a,b]	Bentley et al. 1977c
Cladoceran *Daphnia magna*	2	No lethal effects at 32 [a,b]	Bentley et al. 1977c
	28	No effects on reproduction at 3.9	Bentley et al. 1984
Amphipod *Gammarus fasciatus*	2	No lethal effects at 32 [a,b]	Bentley et al. 1977c
Isopod *Asellus militaris*	2	No lethal effects at 32[a,b]	Bentley et al. 1977c
Midge (larva) *Chironomus dilutus*	2	No lethal effects at 32 [a,b]	Bentley et al. 1977c

LC$_{50}$ - median lethal concentration, [a] Highest concentration tested, [b] - Reported as target concentrations exceeding the solubility limit of 4.5 mg L^{-1} (Table 2.1).

Toxicity of nitroguanadine, nitrogyclerin, nitrocellulose, PETN, and DEGDN

The only marine receptor investigated for the toxicity of nitroguanidine (NQ) was the copepod *N. spinipes* (Dave et al. 2000). No lethal effect to *N. spinipes* was reported even at the highest concentration tested (683 mg L^{-1}). Similarly, exposure of freshwater fish and aquatic invertebrates to exceedingly high concentrations of that compound also failed to result in measurable toxicity (Table 4.12). In the test with the copepod *N. spinepis,* the only marine receptor investigated for the toxicity of PETN, no effect was reported even at the highest concentration tested (32 mg L^{-1}) (Dave et al. 2000). For the freshwater cladoceran *Daphnia magna,* immobilization was

Table 4.12. Lethal and sublethal toxicity data for marine and freshwater fish and invertebrates exposed to propellant related energetic compounds.

Species	MC	Duration (d)	Effects Concentration (mg L^{-1})	Reference
Marine species				
Copepod *Nitocra spinipes*	NQ	4	No lethal effects at 683 [a]	Dave et al. 2000
	PETN	4	No lethal effects at 32 [a]	Dave et al. 2000
Freshwater species				
Fathead minnow *Pimephales promelas*	NG	4	2 (LC$_{50}$)	Bentley 1977d
	NQ	4	No lethal effects at 2714 [1][a]	van der Schalie 1985
	DEGDN	4	491.4 (LC$_{50}$)	Fisher et al. 1989
Rainbow Trout *Oncorhynchus mykiss*	NQ	4	No lethal effects at 1703 [a]	van der Schalie 1985
	NG	4	0.7 (LC$_{50}$)	Burton 1983
	DEGDN	4	284.1 (LC$_{50}$)	Fisher et al. 1989
Bluegill *Lepomis macrochirus*	DEGDN	4	258.0 (LC$_{50}$)	Fisher et al. 1989
Channel catfish *Ictalurus punctatus*	DEGDN	4	278.3 (LC$_{50}$)	Fisher et al. 1989
Cladoceran *Daphnia magna*	NG	7	16 Lethal LOEC	Burton 1993
	NG	7	5 Reproduction LOEC	Burton 1993
	NQ	2	No lethal effects at 1024 [a]	Dave et al. 2000
	PETN	2	No lethal effects at 49 [a]	Dave et al. 2000
	DEGDN	2	90.1 (LC$_{50}$)	Fisher et al. 1989
Midge (larva) *Paratanytarsus parthenogeneticus*	DEGDN	2	159.8 (LC$_{50}$)	Fisher et al. 1989
Mayfly *Hexagenia bilinata*	DEGDN	2	342.6 (LC$_{50}$)	Fisher et al. 1989

[a] Highest concentration tested.

reported only for the highest test concentration (49 mg L^{-1}) after 48 h. No marine toxicity data was found for nitroglycerin, nitrocellulose and DEGDN. Toxicity (Table 4.12) by DEGN to freshwater fish and invertebrates only occurred at concentrations above 150 mg L^{-1} (Table 4.12) while freshwater toxicity data (Table 4.12) for nitroglycerin indicate toxic effects at a much lower range when compared to nitroguanidine, PETN and DEGDN (Table 4.12). Studies with nitrocellulose indicated no toxicity at concentrations up to 1000 mg L^{-1} when tested with several species of freshwater fish

and invertebrates (Bentley et al. 1977c). The concentrations, however, were derived from a target concentration of an active ingredient in static toxicity tests using "a slurry of poacher pit fines" collected at an army ammunition plant, with nitrocellulose at 11.8% active ingredient. The overall lack of toxicity of nitrocellulose is likely a result of its insolubility in water.

Toxicity of insenstive muntions constituents

The military services are developing and evaluating several insensitive munitions to replace conventional organic explosives in future weapon systems. The new explosives are chemically stable enough to withstand mechanical shocks without loss of energetic performance. Among these are 3-nitro-1,2,4-trizole-5-one (NTO) as a replacement for RDX and 2,4-dinitroanisol (DNAN) as a TNT substitute (Badgujar et al. 2008). The insensitive munition formulation (IMX)-101 was recently approved as the main fill ingredient in M795 155-mm artillery munitions with over 20,000 lb manufactured (Fung et al. 2009). This relatively new insensitive munition mixture consists of DNAN, NTO, and NQ. Information on the toxicity of NTO (S&ME 2007; Haley et al. 2009; Sayers 2009a,b) and DNAN (Kennedy et al. 2013) is limited and was not included in this summary review.

Sublethal biochemical effects

Sublethal biochemical effects can also occur due to exposure of organisms to explosive MC. Extensive investigations of biochemical-level effects on the European eel (*Anguilla anguilla*) were conducted by Della Torre et al. (2008a,b,c) using aqueous exposures. Their results seem to indicate an inhibitory effect of TNT on ethoxyresorufin-*O*-deethylase (EROD)-dependent catalytic activities and a possible involvement of phase II enzymes as well as the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome C reductase in TNT metabolism in eels (Della Torre et al. 2008a). TNT effects on the eel's neurosteroidogenic pathways was also reported (Della Torre et al. 2008b), as well as an indication that TNT is a potential competitive inhibitor of cytochrome P4501A activities. A metabolic pathway for TNT involving NADPH, NADPH cytochrome C reductase and phase II enzymes is also suggested (Della Torre et al. 2008c).

Significant inhibition of EROD activity was also observed in juvenile rainbow trout (*Onchorhynchus mykiss*) dosed intraperitoneally with TNT at

different concentrations (Ek et al. 2003). In a similar study, trout treated with TNT had increased glutathione *S*-transferase and glutathione reductase activities, and a decreased percentage of oxidized glutathione compared to control fish (Ek et al. 2005), indicating that TNT oxidizes macromolecules and activates antioxidant defense systems. The compounds TNT, 2-Am-DNT and 4-Am-DNT were found in the hydrolyzed bile of the TNT-treated trout, indicating that this fish species can detoxify and excrete TNT. The presence of TNT and its transformation products in the bile may be suitable as a direct marker of TNT exposure in fish at contaminated sites (Ek et al. 2005).

Gust et al. (in preparation) has demonstrated increased expression of cytochrome P450 in coral (*Acropora formosa*) exposed to RDX as a mechanism to detoxify the chemical insult. In addition, potential insult to coral health was evidenced by the significant increase of coral mucocyte densities at the maximum RDX exposure (7.2 mg L^{-1}), but the zooxanthellae tissue densities were not affected.

Advances in molecular biology are extending the use of biomarkers to the level of the genes, which, when coupled with bioinformatics, are progressing towards the development of powerful new tools to ecotoxicologists. Environmental genomics (using gene expression profiles after exposure to toxicants, also called toxicogenomics) allow insight into modes of action and may be used to assess the causal agents and potential toxicity (Garcia-Reyero et al. 2009). Recent research aims the development of robust methods for using gene expression analysis for identifying gene expression biomarkers of chemical exposure that can be further used to monitor specific chemical exposures in the environment (Garcia-Reyero et al. 2009, 2012).

Gust et al. (2011) conducted a comprehensive investigation of the effects that RDX exposure elicited on transcript expression in fathead minnow brain tissue. Significant mortality occurred a 10 mg L^{-1} after 10 days of exposure to spiked water. Conversely, microarray data indicated significant changes in transcript expression at concentrations as low as 0.625 mg L^{-1}. Individual gene functions affected by RDX exposures indicated changes in metabolic processes involved in: oxygen transport, neurological function, calcium binding/signaling, energy metabolism, cell growth/division, oxidative stress and ubiquitination.

Using a custom complementary DNA (cDNA) microarray for *Daphnia magna*, Garcia-Reyero et al. (2009) identified distinct expression finger-prints in response to exposure at sublethal concentrations of MC. RDX exhibited a robust gene expression pattern affecting diverse pathways involved in exoskeletal maintenance such as protein catabolism, cell structure, and cellular transport. An induction of oxidative stress response genes was seen after exposure to 1,3,5-TNB, DNB, and TNT. Both 4-Am-DNT and 2,4-DNT affected genes (e.g., vitellogenin) directly involved in reproduction and development. TNT caused up-regulation of ferritin, indicating possible interference with iron metabolism or oxidative stress. In a follow-up study, Garcia-Reyero et al. (2012) have demonstrated that, to a limited extent, it is possible to predict gene expression changes in the *D. magna* given the chemical mixture to which it has been exposed.

Relationship between bioaccumulation of MC and toxicity

This subsection describes the relationship between bioaccumulation and toxic effects. Section 5 discusses bioaccumulation of TNT, RDX, and HMX in marine fish and aquatic invertebrates and reports uptake rates, elimina-tion rates, half lives, and bioconcentration factors for those compounds.

Three of the studies cited in this chapter (Rosen and Lotufo 2007a; Lotufo et al. 2010a, and Rosen and Lotufo, unpublished data) measured the whole body tissue concentration (or whole body burden) of toxicity test organisms. Exposure to TNT resulted in the bioaccumulation of the parent compound, as well as of the transformation products 2-Am-DNT and 4-Am-DNT in sheepshead minnows (Lotufo et al. 2010a), adult mussels (Rosen and Lotufo 2007a) and amphipods (Rosen and Lotufo, unpublished). The sum concentration of 2-Am-DNT and 4-Am-DNT formed by biotransformation exceeded that of TNT in adult mussels and in the amphipod *L. plumulosus*. Exposure to 2-Am-DNT and 2,4-DANT resulted in detectable accumulation of those compounds in fish (Lotufo et al. 2010a) and no apparent biotrans-formation. However, fish exposed to 1,3,5-TNB failed to bioaccumulate parent and transformation products above detection levels (Lotufo et al. 2010a). Aqueous exposure to RDX and HMX resulted in measurable bioaccumulation of parent compounds in fish (Lotufo et al. 2010b; Muhki and Patino 2008) and in invertebrates and no apparent biotransformation (Rosen and Lotufo 2007a, 2007b; Rosen and Lotufo, unpublished).

When the whole body residue can be linked to a specific biological effect, the body residue is termed the critical body residue (CBR) (McCarty and

Mackay, 1993). Typically, tissue residue concentrations are more directly relatable to toxic effects than water-based concentrations and provide an integrated assessment of the exposure an organism receives over time and space (Meador et al. 2006).

Critical residues associated with significant mortality of organisms exposed to MC are presented in Table 4.13 as median lethal residues (LR_{50}). For exposures that did not result in significant mortality, the body residues in organisms surviving the highest exposure concentrations are reported as no-observed-effect residue (NOER).

Table 4.13. Body residue toxicity metrics for freshwater and marine invertebrates exposed to nitroaromatic MC.

Species	Habitat	Endpoint	Body Residue		Lethal Concentration (mg L^{-1})[a]	Reference
			nmol g^{-1} wet wt	mg kg^{-1} wet wt		
SumTNT						
Sheepshead minnow *Cyprinodon variegatus*	Marine	10-d LR_{50}	26	5.4	2.3 10-d LC_{50}	Lotufo et al. 2010a
Fathead minnow *Pimephales promelas*	Freshwater	mean residue associated with 10-d 50% mortality	15	3.4	2.2 10-d LC_{50}	Yoo at al. 2006
Mussel (adult) *Mytilus galloprovincialis*	Marine	4-d LR_{50}	51	14.0	19.5 4-d LC_{50}	Rosen and Lotufo 2007a
Amphipod *Eohaustorious estuarius*	Marine	4-d LR_{50}	162	36.8	4.5 4-d LC_{50}	Rosen and Lotufo, unpublished
Amphipod *Leptocheirus plumulosus*	Marine	4-d LR_{50}	77	17.5	3.6 4-d LC_{50}	Rosen and Lotufo, unpublished
Oligochaete *Tubifex tubifex*	Freshwater	4-d LR_{50}	172	39.0	7.7 4-d LC_{50}	Conder et al. 2004c
Midge (larva) *Chironomus dilutus*	Freshwater	4-d LR_{50}	118	8.9	1.9 4-d LC_{50}	Conder et al. 2004c
2-Am-DNT						
Cyprinodon variegatus	Marine	5-d LR_{50}	275	54.2	8.6 5-d LC_{50}	Lotufo et al. 2010a
DANT						
Cyprinodon variegatus	Marine	5-d NOER	284	47.4	No lethal effects at 50.3 mg L^{-1} [b]	Lotufo et al. 2010a

Species	Habitat	Endpoint	Body Residue		Lethal Concentration (mg L^{-1})[a]	Reference
			nmol g^{-1} wet wt	mg kg^{-1} wet wt		
RDX						
Cyprinodon variegatus	Marine	10-d LR50	135	30.0	9.9 10-d LC$_{50}$	Lotufo et al. 2010b
Zebrafish *Danio regio*	Freshwater	Mortality (33% after 28 d)	40	9	9.6 33% mortality after 28 d	Mukhi and Patino 2008
Mytilus galloprovincialis	Marine	4-d NOER	86	19.6	No lethal effects at 28 mg L^{-1} [b]	Rosen and Lotufo 2007a
Eohaustorious estuarius	Marine	4-d NOER	268	59.5	No lethal effects at 39 mg L^{-1} [b]	Rosen and Lotufo, unpublished
Leptocheirus plumulosus	Marine	4-d NOER	191	42.4	No lethal effects at 39 mg L^{-1} [b]	Rosen and Lotufo, unpublished
HMX						
Cyprinodon variegatus	Marine	10-d NOER	14	4.1	No lethal effects at 2 mg L^{-1} [b]	Lotufo et al. 2010b
Mytilus galloprovincialis	Marine	4-d NOER	3	0.9	No lethal effects at 2 mg L^{-1} [b]	Rosen and Lotufo 2007a

LR$_{50}$ - Median lethal residue, NOER - No-observed-effect residue, [a] Concentration in the exposure water associated with observed effects, [b] Highest concentration tested.

For SumTNT, the CBR was similar for sheepshead and fathead minnows (Lotufo et al. 2010b; Yoo et al. 2006), suggesting similar sensitivity among fish species. For invertebrates, the SumTNT CBR was lowest for mussels and highest for the amphipod *E. estuarius*. The CBRs from SumTNT fall within a range that is substantially lower than the range considered typical for compounds causing mortality by general narcosis (McCarty and Mackay, 1993), suggesting that lethal effects of TNT are associated with specific modes of action.

The lowest CBR for RDX was reported for zebrafish exposed to 9.6 mg L^{-1} for 28 d by Mukhi and Patino (2008), who reported 33% mortality of fish exposed for 28 d. A higher CBR was obtained for sheepshead minnows exposed to 9.9 mg L^{-1} for 10 d by Lotufo et al. (2010a). Water-only exposure of adult mussels and amphipods to even higher concentrations of RDX in water failed to result in significant decrease in survival (Rosen and Lotufo 2007a; Rosen and Lotufo in preparation). Body residues were measured in studies exposing sheepshead minnows (Lotufo et al. 2010a) or mussels

(Rosen and Lotufo 2007a) to concentrations of HMX in water approaching its solubility limit. CBRs could not be determinded in those studies due to lack of significant mortality.

If the CBR of a chemical is known, or can be estimated, and confidence about its application to the species of concern exists, the CBR approach can be directly used for assessment of potential risk to the population using organisms collected in the field. When the tissue concentration of a given chemical is well below its CBR (e.g., by order of magnitude) in field-collected specimens, that chemical would not be considered a toxicity driver. An example of the use of this approach in field assessment is provided in Redman et al. (2012). Most MC are efficiently biotransformed and eliminated by fish and aquatic invertebrates (Section 6). The exposure concentration of MC may vary over time depending on the source of contamination to the water column. Therefore, aquatic organisms, especially mobile species, may have body residue that vary widely overtime, complicating the use of the CBR approach in field evaluations.

5 Toxicity of Munitions Constituents to Aquatic Invertebrates and Fish in Sediment Exposures

This section summarizes the available data on the toxicity of sediment-associated explosives to marine and freshwater fish and invertebrates. Laboratory studies used sediment amended with explosives which were used in toxicity tests using whole-sediment or extracted porewater.

Overview

Major explosive contaminants such as 2,4,6-trinitrotoluene TNT, 1,3,5-TNB, RDX, HMX, and tetryl and their transformation products were detected in freshwater sediment samples collected from military installations at concentrations ranging from low (e.g., less than 6.7 mg kg^{-1}) to high percent levels (up to 711,000 mg kg^{-1}) (Talmage et al. 1999). Those extremely high concentrations ("hot spots") are indicative of large amounts of non-dissolved and non-sorbed explosives in some contaminated sites. Low concentrations (< 0.003 mg kg^{-1}) of 2,6-dinitrotoluene (2,6-DNT), tetryl, and 2,4,6-trinitrophenol (picric acid) acid were detected in marine sediments near a naval facility in Ostrich Bay, Puget Sound, WA (Pascoe et al. 2010). Field experiments using cleaved shells containing TNT demonstrated that leakage from breached munitions can serve as a source of sediment contamination (Dave 2003).

The fate of explosive compounds in soils has been well characterized (Pennington and Brannon 2002, Monteil-Rivera et al. 2009). In contrast, fewer studies addressed the fate of explosives and related compounds in sediments, despite the potential ecological risk associated with their presence in this compartment of aquatic environments. Recent studies characterizing the processes that occur following the initial contact of explosives and related compounds with sediments revealed rapid rates of transformation for a variety of nitroaromatic compounds (Elovitz and Weber 1999; Lotufo et al. 2010c; Pennington et al. 2011).

Compared to investigations addressing the soil ecotoxicity of MC (reviewed by Kuperman et al. 2009), few studies addressed the toxicity of sediment-associated MC to marine invertebrates (Green et al. 1999; Dave

et al. 2000; Lotufo et al. 2001; Nipper et al. 2002; Nipper et al. 2005; Rosen and Lotufo 2005), as well to freshwater invertebrates (Steevens et al. 2002; Conder et al. 2004a; Lotufo and Farrar 2005), as reviewed in Lotufo et al. (2009a). Infaunal marine invertebrates used in those investigations burrow in and ingest sediment, and are, therefore, potentially exposed to sediment contaminants via sediment and food particles, interstitial (pore) water, and overlying water. Bottom-dwelling fish come into contact with sediment-associated contaminants through direct uptake from benthic prey, directly from sediment bed particles through ingestion or dermal contact, and from the overlying water via suspended sediment particles, or the dissolved phase. The evaluation of toxicity of MC to fish resulting from exposure to contaminated sediment was also investigated (Lotufo et al. 2010c).

Fate of nitroaromatic MC amended to sediment for toxicity testing

Fate kinetics and mass balance studies conclusively demonstrate that the fate of TNT in wet sediment is dependent on a combination of reductive transformation and sorption processes (Elovitz and Weber 1999; Pennington et al. 2011).

When TNT was spiked into marine or freshwater sediments for toxicity and bioavailability investigations, aminodinitrotoluenes (Am-DNTs) and diaminonitrotoluenes (DANTs) were detected following the short (4 h) post-spiking mixing periods, with transformation continuing during storage or toxicological testing for both marine (Rosen and Lotufo 2005; Lotufo et al. 2010c) and freshwater sediments (Steevens et al. 2002; Conder et al. 2004a; Lotufo and Farrar 2005). An inverse relationship between the TNT spiking concentration and the transformation rate has been reported on a percentage basis (Rosen and Lotufo 2005; Lotufo et al. 2010c) (Figures 5.1 and 5.2). That is, the lower the concentration of TNT added to sediment, the higher the observance of transformation to Am-DNTs, and to their daughter product, DANTs on a percent of total TNT basis. The rate of transformation appears to decrease with increasing spiking concentration, as observed for sediment in Lotufo et al. (2010c) (Figure 5.2). Decreasing TNT transformation at high spiking concentrations likely resulted from increasing saturation of binding sites in the sediment or soil particles and inhibition of microbial activity.
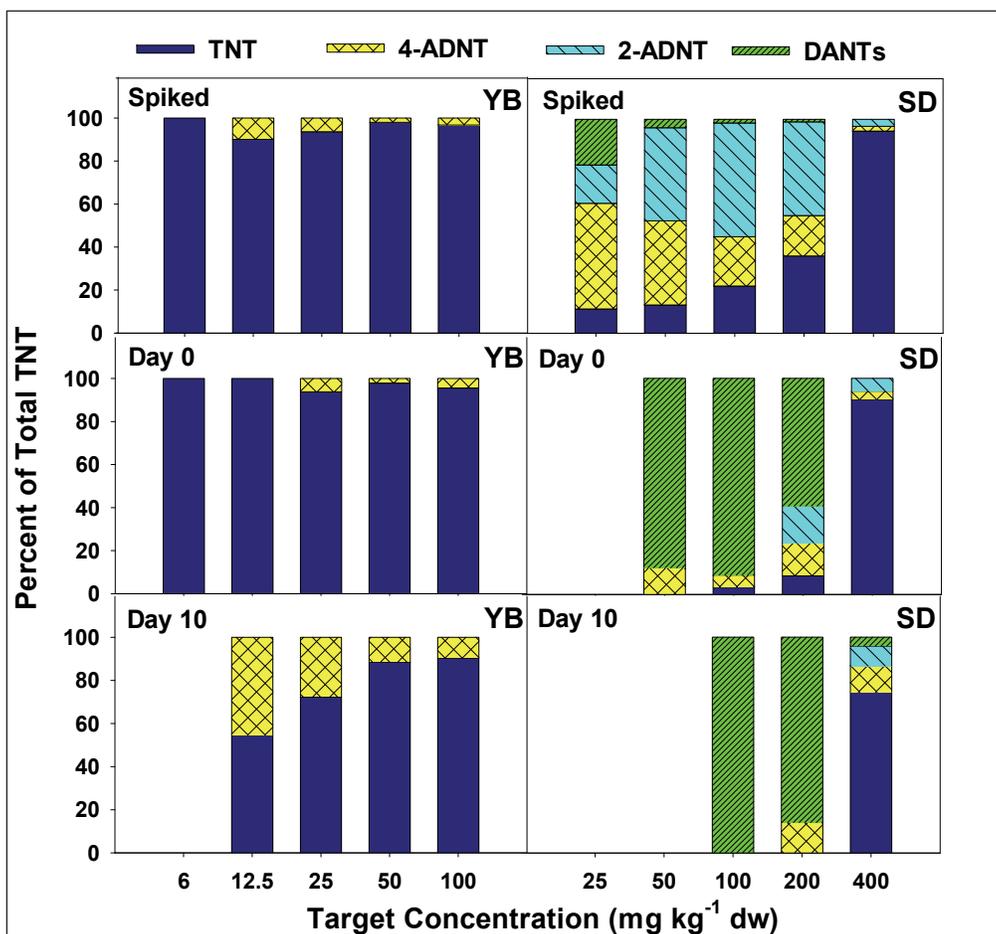
Figure 5.1. Percent of total concentration in sediment samples corresponding to the concentration of trinitrotoluene (TNT), 2-aminodinitrotoluene (2-Am-DNT), 4-aminodinitrotoluene (4-Am-DNT), and diaminonitrotoluenes (DANTs) in solvent extracts of ^{14}C-TNT-spiked Yaquina Bay (YB) and San Diego Bay (SD) toxicity test sediments (from Rosen and Lotufo 2005). Absence of bars indicates neither that TNT nor its transformation products were detectable.

The transformation of TNT to aminated products was substantially lower in marine coarse-grained than in fine-grained sediment when both were spiked with the same initial organic-carbon-normalized concentrations (Rosen and Lotufo 2005). When sand with non-detectable total organic carbon (TOC) was spiked with TNT, only a small degree of transformation to Am-DNTs occurred over time, while spiking fine-grained sediment (TOC = 1.1%) with TNT resulted in a large degree of transformation (Figure 5.2). The stimulatory effect of enhanced sediment TOC on the transformation of TNT in water overlying the contaminated sediment was demonstrated by Harrison and Vane (2010).
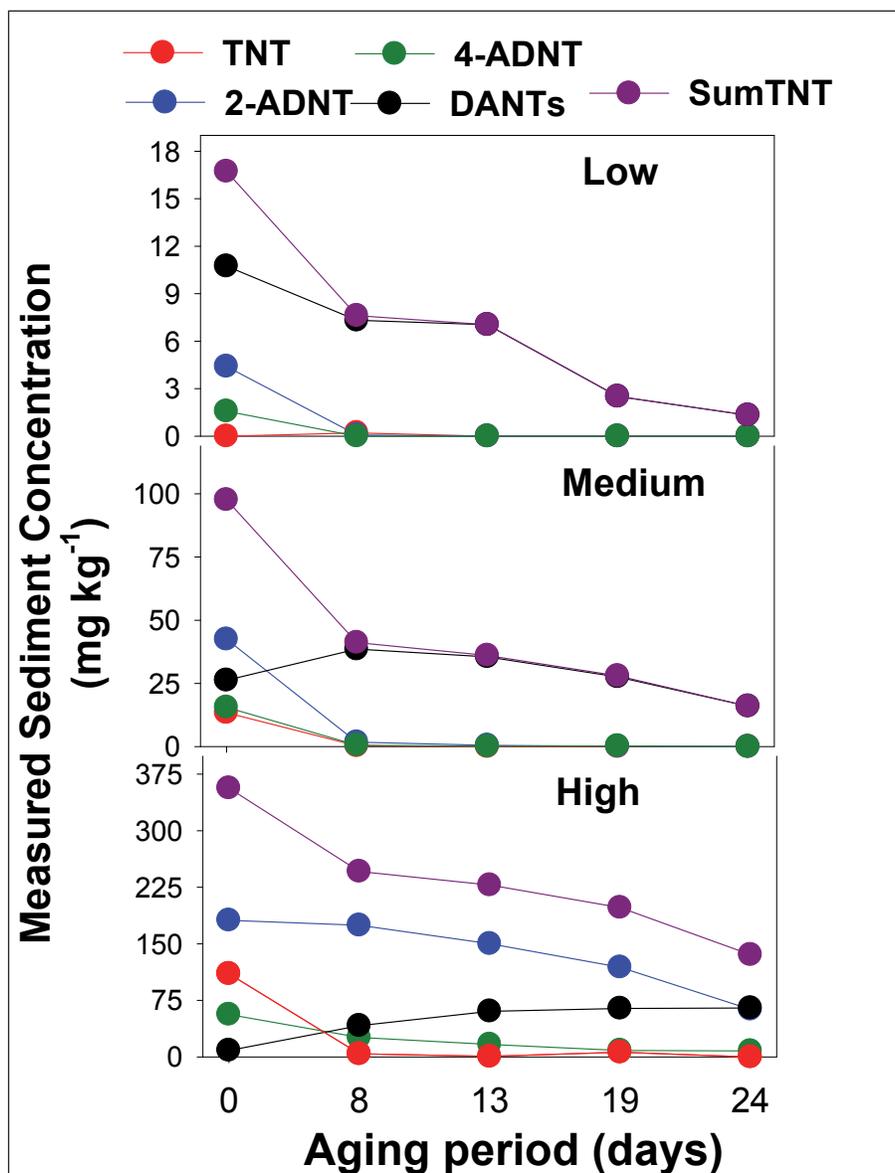
Figure 5.2. TNT and transformation product concentrations over time in fine-grained marine sediment (1.1% total organic carbon) (from Lotufo et al. 2010c).

When TNT was spiked into marine sediments, as the contact time with sediment ("aging period") increased, the proportion of solvent-extractable TNT in the sediment decreased (Steevens et al. 2002, Conder et al. 2004a; reviewed in Lotufo et al. 2009a). Such a decrease of solvent-extractable TNT and TNT transformation products in marine sediments following spiking was attributed to covalent binding to sediment organic matter (Pennington et al. 2011) and has been extensively reported for soils (Eriksson et al. 2004, Thorn et al. 2004, Singh et al. 2010) and freshwater sediments (Elovitz and Weber 1999). A description of the different types of mechanisms

responsible for binding of TNT and its degradation products to soil organic matter is provided in Singh et al. (2010).

The fraction of solvent-resistant transformation products of TNT, likely associated with the sediment through covalent binding to sediment particles was higher in spiked fine-grained sediment compared to sandy sediment (Rosen and Lotufo 2005) (Figure 5.3).
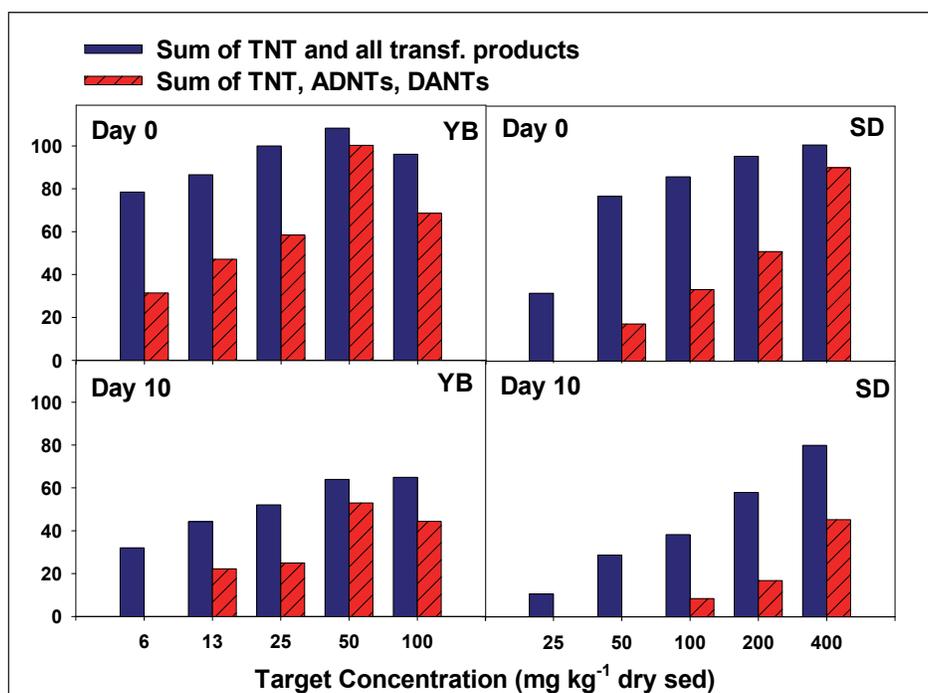
Figure 5.3. Percent of total radioactivity in sediment corresponding to the sum of solvent-extracted TNT and all its transformation products, including unknown compounds, as determined by radioactivity (blue), and to the sum of solvent-extracted TNT, Am-DNTs and DANTs, as determined by chemical analysis (red) in sandy sediment (Yaquina Bay, YB) and fine-grained sediment (San Diego Bay, SD) at initiation (Day 0) and termination (Day 10) of a toxicity test (from Rosen and Lotufo 2005). Absence of bars indicates neither that TNT nor its transformation products were detectable.

The measured sum concentration of TNT and its major transformation products, Am-DNTs and DANTs (SumTNT), in marine and freshwater sediments spiked with TNT was typically lower than the target (or nominal) concentration following a short mixing period (up to 4 h) (Table 5.1), indicating that rapid transformation of spiked TNT to compounds that sorb strongly to sediment and resisted organic solvent extraction occurs in a variety of sediment types.

Table 5.1. Target concentrations and concentrations measured after spiking with TNT and mixing for sediments of different types and total organic carbon (TOC) content used in toxicity tests.

Sediment type and source	TOC (%)	SumTNT Concentration (mg kg-1)		Ratio Meas/Tgt	Mixing period (h)	Moisture condition[a]	Reference
		Target	Measured				
Fine-grained Freshwater	0.65	12.5	< 0.1[b]	0.004	0.5	Dry	Steevens et al. 2002
	0.65	25	< 0.1[b]	0.002	0.5	Dry	Steevens et al. 2002
	0.65	50	0.1	0.002	0.5	Dry	Steevens et al. 2002
	0.65	100	4.3	0.04	0.5	Dry	Steevens et al. 2002
	0.65	200	66.5	0.33	0.5	Dry	Steevens et al. 2002
	0.65	400	153.3	0.38	0.5	Dry	Steevens et al. 2002
	0.65	127	28	0.22	1	Wet	Lotufo and Farrar 2005
	0.65	238	53	0.22	1	Wet	Lotufo and Farrar 2005
	0.65	605	135	0.22	1	Wet	Lotufo and Farrar 2005
	0.65	1365	304	0.22	1	Wet	Lotufo and Farrar 2005
Fine-grained Marine	0.36	25	23	0.94	4	Wet	Rosen and Lotufo 2005
	0.36	50	52	1.03	4	Wet	Rosen and Lotufo 2005
	0.36	100	92	0.92	4	Wet	Rosen and Lotufo 2005
	0.36	200	148	0.74	4	Wet	Rosen and Lotufo 2005
	0.36	400	350	0.87	4	Wet	Rosen and Lotufo 2005
Sandy Marine	0.09	6	3	0.46	4	Wet	Rosen and Lotufo 2005
	0.09	12.5	76	0.62	4	Wet	Rosen and Lotufo 2005
	0.09	25	18	0.72	4	Wet	Rosen and Lotufo 2005
	0.09	50	37	0.73	4	Wet	Rosen and Lotufo 2005
	0.09	100	63	0.63	4	Wet	Rosen and Lotufo 2005

[a] Analysis followed solvent extraction of dry or wet substrate, [b] Numerical value represents the detection limit. Assumed as 0.05 mg kg-1 for calculating the measured/target ratio.

Exposure chambers containing sediment amended with TNT and uncontaminated overlying water were used for toxicity testing (Rosen and Lotufo 2005). A substantial portion (up to 50%) of the ^{14}C from the radiolabeled TNT originally associated with the sediment particles was present in the porewater and in the non-renewed overlying water (contained the greater fraction of the C^{14}) at the end of the 10 d exposures (Figure 5.4). Such high partitioning was expected given the reported relatively high aqueous solubility of TNT and its transformation products (Table 3.1). Renewal of overlying waters is typically performed during chronic sediment exposures using benthic invertebrates to maintain adequate water quality, especially when feeding is required. However, exchange of overlying water during invertebrate exposures to TNT-spiked sediment is expected to rapidly deplete contaminants from the exposure system. Therefore, it is recommended that toxicity evaluations of nitroaromatic compounds spiked to sediments should use exposure conditions (e.g., duration, water quality requirements) that do not require water changes during the experimental period (Conder et al. 2004a).
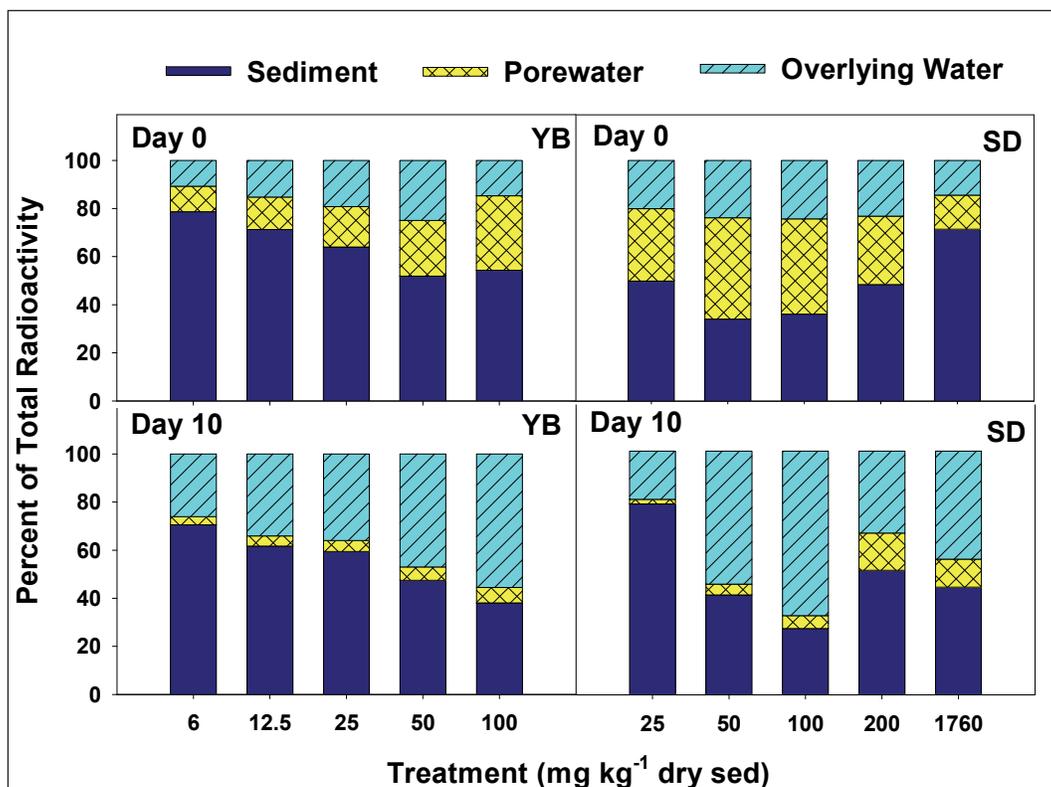
Figure 5.4. Percentage of total radioactivity from radiolabeled-TNT in the exposure chamber that was associated with various media at toxicity test initiation (Day 0) and termination (Day 10) for sandy sediment (Yaquina Bay, YB) or fine-grained sediment (San Diego Bay, SD) (from Rosen and Lotufo 2005).

Because TNT is rapidly transformed in sediment and the concentration of sumTNT decreases during the toxicity exposures, establishing accurate concentration-response relationships for TNT and related compounds using data from sediment toxicity testing is challenging. In addition, partitioning of TNT and its transformation products to the overlying water renders the latter as a potentially significant source of contaminant uptake to test organisms, relative to the sediment compartment, confounding the extrapolation of sediment toxicity test results to realistic field scenarios.

The fate of 2,6-DNT in spiked marine sediments has been investigated using fine-grained and sandy marine sediments (Nipper et al. 2004). These studies showed that biotransformation of 2,6-DNT started soon after the initial contact of the chemical with the sediments and proceeded for several months. Transformation rates were greater in fine-grained than in sandy sediment. The transformation product 2-amino-6-nitrotolune (2-A-6-NT) was formed by reduction and the compound 2-nitrotoune (2-NT) was formed by loss of a nitro group. After prolonged incubation, these chemicals tended to be replaced by high molecular weight polymers, possibly derived from polymerization of 2,6-DNT or its transformation products. Complete disappearance of the parent compound and known transformation products from fine-grained and sandy sediments occurred during a 56-d incubation at 20°C. The possibility of mineralization of at least part of the 2,6-DNT was speculated. Although the majority of 2,6-DNT in spiked sediments is biotransformed, abiotic transformation took place in sterilized sediments.

Analytically determined concentrations of 2,6-DNT and 2-A-6-NT, as well as picric acid, in marine sediments were much lower when samples were dried at room temperature and extracted with acetonitrile (Nipper et al. 2004), according to standard procedures for soils and sediments than when sediments were not dried prior to extraction. A portion of the parent compounds could be transformed during the drying procedure, and the authors suggest that sediments contaminated with explosives should not be air-dried prior to extraction for chemical measurements.

Toxicity of nitroaromatic MC amended to sediment

Due to the rapid transformation of MC spiked to sediment described above, target (or nominal) concentrations were higher, sometimes by orders of magnitude, than the actual, measured concentrations. Therefore, analytically determined concentrations measured at exposure initiation more accurately represent the effects concentrations than target concen-

trations, especially when toxic response occurs shortly after exposure initiation. Rapid manifestation of toxicity is expected for TNT and its major transformation products as these chemicals reach steady state in small organisms very quickly (Section 6). Lethal effects of TNT were observed during the initial few days of exposure in freshwater sediment toxicity tests (Conder et al. 2004a; Lotufo and Farrar 2005). Therefore, the use of short exposure duration for the evaluation of the effects of sediment-associated explosives is desirable.

Results of toxicity investigations using sediments spiked with TNT and related compounds as well as other explosive compounds are summarized in Table 5.2. Due to the paucity of data for the marine environment, freshwater toxicity data was also included. For each study, no observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) are provided as initial concentrations expressed as SumTNT concentrations. The earliest study of the toxicity of TNT in sediment (Green et al. 1999) employed ^{14}C-labeled TNT and used radioactivity measurements as a surrogate for the sediment concentrations. Most of the radioactivity detected in the sediments in that study likely corresponded to non-solvent-extractable transformation products of TNT. This was later confirmed in similar spiked-sediment studies performed using sediments from the same site (Lotufo, unpublished study). Therefore, the effects concentrations reported in that study are likely substantially higher than the concentration of SumTNT, explaining the apparent high relative tolerance of the amphipod *Leptocheirus plumulosus* and polychaete *Neanthes arenaceodentata* to sediments spiked with this chemical. In the other studies in Table 5.2, sediment concentrations of SumTNT at initiation of the toxicity exposure were chemically measured, therefore providing more accurate determination of the exposure concentration suitable for expressing lethal toxicity.

Table 5.2. Lethal toxicity data for nitroaromatic MC determined for aquatic invertebrates using spiked sediment exposures.

Spiked MC	Species	Habitat	TOC (%)	Duration (d)	NOEC mg kg^{-1}	LOEC mg kg^{-1}	Reference
TNT	Amphipod *Leptocheirus plumulosus*	Marine	2.70	28	116	228	Green et al. 1999
	Polychaete *Neanthes arenaceodentata*	Marine	2.70	28	275	508	Green et al. 1999

Spiked MC	Species	Habitat	TOC (%)	Duration (d)	NOEC mg kg⁻¹	LOEC mg kg⁻¹	Reference
	Amphipod *Hyalella azteca*	Freshwater	0.65	10	< 0.1[a] (25)	0.1 (50)	Steevens et al. 2002
	Midge (larva)	Freshwater	0.65	10	4 (100)	67 (200)	Steevens et al. 2002
	Chironomus dilutes	Freshwater	0.65	10	ND	37 (43)	Lotufo and Farrar 2005
	Oligochaete *Tubifex tubifex*	Freshwater	0.96	28	25 (100)	69 (320)	Conder et al. 2004a
	Amphipod	Marine	0.09	10	24 (50)	44 (100)	Rosen and Lotufo 2005
	Eohaustorius estuarius	Marine	0.36	10	20 (100)	38 (200)	Rosen and Lotufo 2005
2,4-DANT	*Leptocheirus plumulosus*	Marine	2.70	28	< 0.1[a] (57)	3.0 (113)	Lotufo et al. 2001
	Neanthes arenaceodentata	Marine	2.70	28	187 (444)	574 (1,185)	Lotufo et al. 2001
	Hyalella azteca	Freshwater	0.65	10	< 0.1[a] (25)	0.3 (50)	Steevens et al. 2002
	Chironomus dilutes	Freshwater	0.65	10	110 (400)	ND	Steevens et al. 2002
		Freshwater	0.65	10	33 (120)	59 (243)	Lotufo and Farrar 2005
2-Am-DNT	*Chironomus dilutes*	Freshwater	0.65	10	23 (51)	59 (72)	Lotufo and Farrar 2005
1,3,5-TNB	*Leptocheirus plumulosus*	Marine	2.70	28	7 (119)	13 (237)	Lotufo et al. 2001
	Neanthes arenaceodentata	Marine	2.70	28	107 (468)	ND	Lotufo et al. 2001
	Hyalella azteca	Freshwater	0.65	10	< 0.1[a] (50)	1 (100)	Steevens et al. 2002
	Chironomus dilutes	Freshwater	0.65	10	7.6 (400)	ND	Steevens et al. 2002
		Freshwater	0.65	10	59 (140)	131 (289)	Lotufo and Farrar 2005
2,6-DNT	Amphipod *Ampelisca abdita*	Marine	0.10	10	5	ND	Nipper et al. 2004
		Marine	1.10	10	0.5	ND	Nipper et al. 2004

TOC - total organic carbon, NOEC - No observed effect concentration, LOEC - lowest observed effect concentration, ND - not determined due to insufficient mortality , NOEC and LOEC reported as sum concentrations of parent and transformation products measured at experiment initiation (when available, target concentrations are shown in parenthesis) , [a] Numerical value represents detection limit .

However, concentrations measured using solvent extraction of air-dried sediments (Lotufo et al. 2001; Steevens et al. 2002) likely underestimated the actual concentration of extractable compounds in the sediments and consequently resulted in the determination of lower than actual effects concentrations.

Except for *H. azteca*, lethality was observed remarkably similar SumTNT concentrations for a variety of experimental species and sediment organic carbon content. However, comparisons of the toxicity of TNT-spiked sediments using different sediments and species should take into account the variable relative concentration of parent and transformation products in the different exposure sediments, since there are potential toxicity differences among compounds and non-additive mixture interactions.

Complete mortality of juvenile sheepshead minnows (*Cyprinodon variegatus*) was observed after only 24 h of exposure to moderately organic-rich sediment (TOC=1.1%) that had an initial Sum-TNT concentration of 348 mg kg^{-1}, while no mortality was observed at an initial sediment concentration of 6.9 mg kg^{-1} (Lotufo et al. 2010b). Therefore, an LC$_{50}$ would likely occur somewhere between these two concentrations. The study concluded that toxicity was unlikely to be caused by dermal uptake from sediment; rather, it was due to exposure to the overlying water which was not renewed during the experimental period and therefore contained lethal concentrations of TNT and its transformation products. No significant mortality of freckled blennies (*Hypsoblennius ionthas*) occurred in exposure to sediment Sum-TNT concentrations as high as 198 mg kg^{-1} (the highest concentration studied) for up to 7 days (Lotufo et al. 2010b).

Lotufo et al. (2001) suggested that with respect to lethality, *L. plumulosus* was more sensitive to 1,3,5-TNB and 2,4-DANT than to TNT, whereas *N. arenaceodentata* was more sensitive to TNT than to 1,3,5-TNB or 2,4-DANT when comparing LC$_{50}$ from this study and the study by Green et al. (2001), which also utilized the sediment for spiking. The TNT LC$_{50}$ (203 mg kg^{-1} for *L. plumulosus* and 320 mg kg^{-1} for *N. arenaceodentata*) were calculated using the sum concentration of TNT and all its transformation products in the sediment (Green et al. 1999). The 1,3,5-TNB and 2,4-DANT 28-d LC$_{50}$ for *L. plumulosus* calculated on the basis of the measured amount of compound added to the sediment were 90.4 mg kg^{-1} for 2,4-DANT and 95.4 mg kg^{-1} for 1,3,5-TNB; while a substantially higher 28-d LC$_{50}$ value,

450.9 mg kg^{-1}, was calculated for 2,4-DANT using *N. arenaceodentata* (Lotufo et al. 2001).

The toxicity of TNT, 2-Am-DNT, 2,4-DANT spiked to freshwater sediment was compared for the midge *Chironomus dilutus* (Lotufo and Farrar 2005) using the same sediment matrix and exposure conditions. Lethal toxicity was similar for all compounds, with differences in LOEC values never exceeding a factor of 4 (Table 5.2). Overall, reduced transformation products of TNT appear to be less lethal than TNT to marine and freshwater invertebrates (Table 5.2). Toxic interaction among TNT and its major transformation products in spiked sediment exposures was additive in the single study addressing the mixture toxicity of nitroaromatic compounds in sediment, suggesting the toxicity of a nitroaromatic mixture would be reasonably predicted using single-compound toxicity data (Lotufo and Farrar 2005).

The broad range of toxicity of TNT and related compounds in spiked-sediment exposures likely resulted from differences in species sensitivity, compound bioavailability and the relative concentration of parent and transformation compounds in the exposure sediments. Inaccuracies in quantifying exposure concentrations likely contributed to the observed differences among studies.

The toxicity of fine-grained and sandy sediments spiked with 2,6-DNT was examined using the amphipod *Ampelisca abdita* in 10-d exposures (Nipper et al. 2004). No significant lethal effects were observed in any treatment (Table 5.2). Since the highest 2,6-DNT measured concentration in sediments used in toxicity evaluations was relatively low (5 mg kg^{-1}), the effects of 2,6-DNT at concentrations representative of the lethal range for other nitroaromatic compounds are unknown. Additional reports of the toxicity of sediments spiked with 2,6-DNT transformation products have not been found in the available literature.

Fate and toxicity of nitrophenolic MC in sediment

The fate of picric acid in marine sediments was investigated by Nipper et al. (2004) using the same sediments and experimental conditions described above for 2,6-DNT. Transformation rates were highest in fine-grained sediment incubated at 20°C. Major transformation products included 4-dinitrophenol, aminodinitrophenols (including picramic acid), 3,4-diaminophenol, amino nitrophenol, and nitrodiaminophenol. High

performance liquid chromatography analysis of the sediment extracts revealed unknown compounds speculated to correspond to aminonitro-toluene isomers, diaminotoluenes, dinitrotoluenes, and nitrotoluenes. Complete degradation of the parent compound and known transformation products occurred during the 56-d storage period, but unidentified trans-formation products remained in the sandy and fine-grained sediments. Similar to the fate of 2,6-DNT, picric acid disappeared from sterilized spiked marine sediments, suggesting a certain degree of abiotic transformation.

The toxicity of both sediment types spiked with picric acid was examined using *A. abdita* in 10-d exposures (Nipper et al. 2002). Significant amphipod mortality was observed in the sandy sediment at picric acid concentrations relatively similar to lethal concentrations reported for TNT and its major breakdown products (Table 5.3). In the fine-grained sample, mortality was highest at an intermediate concentration in which picric acid was below the detection limit, with greater survival in the two higher and lower concentrations, thus generating a U-shaped concentration-response relationship. The toxicity in treatments with picric acid below detection limit was attributed to unknown transformation products. Inhibition of microbial transformation at the highest concentrations tested in that study could have contributed to the lower mortality at higher concentrations compared to mortality at the intermediate concentrations.

Table 5.3. Toxicity of nitrophenolic MC was determined for the marine amphipod *Ampelisca abdita* using spiked sediment exposures (Nipper et al. 2002).

MC	Sediment	TOC (%)	Duration (d)	NOEC mg kg^{-1}	LOEC mg kg^{-1}
Picric acid	Sand	0.10	10	73	162
Picric acid	Fine-grained	1.10	10	BDL	BDL
Tetryl	Sand	0.10	10	0.5	4
Tetryl	Fine-grained	1.10	10	0.1	ND

NOEC - No observed effect concentration , LOEC - lowest observed effect concentration , NOEC and LOEC reported as sum concentrations of parent and transformation products measured at experiment initiation (target concentration not reported), BDL - Significant mortality occurred but concentrations of picric acid and its transformation products were below the detection limit, ND - not determined due to insufficient mortality in all treatments.

Information on the fate of tetryl in sediments is limited to a study by Nipper et al. (2002). Unknown breakdown products were formed in sandy and fine-grained marine sediments spiked with tetryl. Picric acid, a primary hydrolysis product of tetryl in seawater, was detected in the sandy sediment

only. Tetryl-spiked sandy sediments produced significant amphipod mortality at relatively low concentrations in a 10-d exposure using *A. abdita* (Table 5.3). Significant lethal effects were not observed in the tetryl-spiked fine-grained sediment (Table 5.3), in which the highest concentration (0.1 mg kg^{-1}) was much lower than lethal levels in the sandy sediment. Since tetryl has not been produced in the United States since the early 1970s and the quantities in individual munitions were small, recent research efforts to understand its fate and effects in sediments were minimal. However, given its elevated toxicity to a variety of species (Section 4), the need for additional studies of its fate and biological effects in different types of sediment should be considered.

Toxicity and fate of nitramine MC in sediment

The cyclic nitramine explosives RDX and HMX have lower water solubility and significantly less binding affinity with soil organic matter than TNT (Table 3.1). Therefore, major differences in the fate of those compounds in aquatic systems are expected. Studies using ^{14}C-labeled compounds suggested, but did not conclusively demonstrate by chemical analysis of transformation products, that RDX and HMX spiked into fine-grained, organically rich sediments were likely degraded during the course of toxicity experiment (Lotufo et al. 2001). A substantial portion of the radioactivity originally associated with RDX- or HMX-spiked sediments partitioned to the overlying water in exposure chambers during the course of toxicity tests using fine-grained sediment and was present in that compartment at exposure termination (Figure 5.5). That radioactivity was likely associated with transformation products more soluble in water than the parent compounds. Radiolabel mass balance revealed substantial losses of radioactivity from exposure chambers in static exposures to RDX, which may have resulted from volatilization of RDX transformation products or loss via mineralization.

Different from the fate of RDX in organically rich sediment, transformation was minimal in sandy sediment, and a much smaller fraction of the spiked radioactivity was associated with the overlying water at termination of a 10-d toxicity exposure (Figure 5.6) (Rosen and Lotufo 2005). In the latter study, RDX was added to sediments in excessive amounts, up to 0.24% of the sediment dry mass, to attain concentrations expected to promote toxicity to an amphipod. In that study, most of the spiked RDX remained in the sediment/porewater compartment of the exposure chamber and likely corresponded to undissolved crystals rather than to compound bound to

sediment particles. RDX has demonstrated low sorption affinity to soil particles, with a concentration of 80 mg kg^{-1} or greater creating a saturated porewater (Savard et al. 2010).
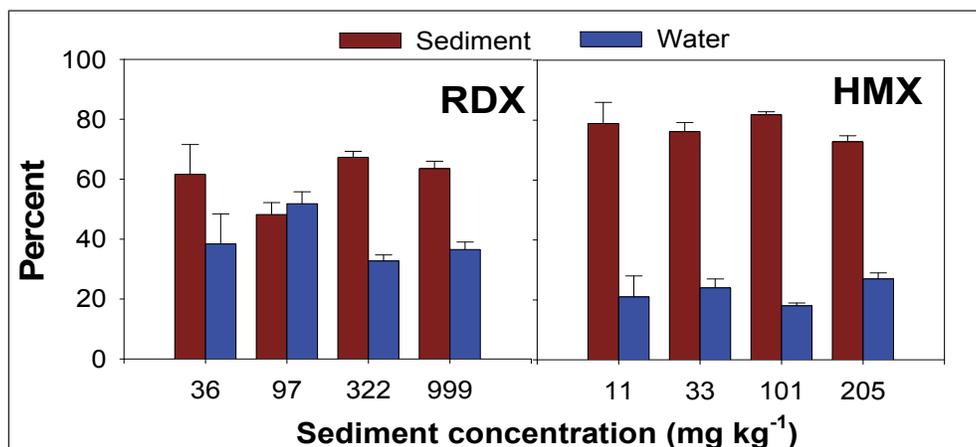
Figure 5.5. Percentage of the total radioactivity from RDX or HMX in the exposure chamber that was associated with sediment and overlying water at termination of a toxicity test (from Lotufo et al. 2001). Error bars represent± 1 standard deviation.
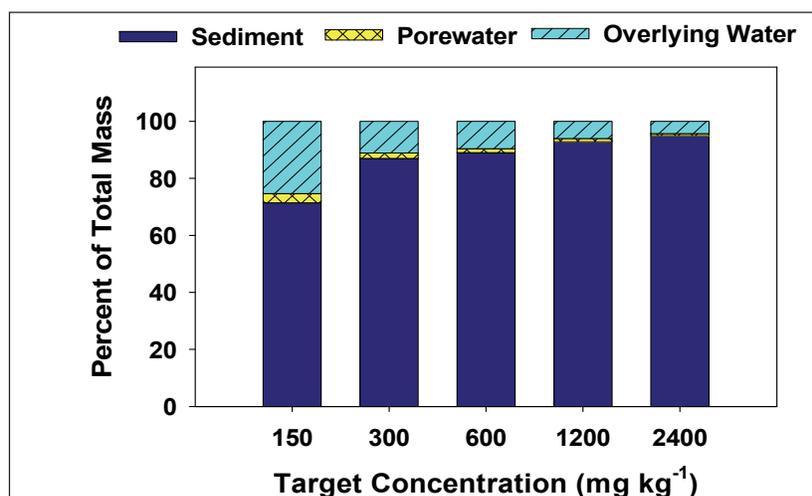
Figure 5.6. Percentage of the total mass of RDX in the exposure chamber that was associated with various media at toxicity test termination for a sandy sediment (from Rosen and Lotufo 2005).

The toxicities of RDX or HMX were investigated using spiked fine-grained marine sediments, with *L. plumulosus* and *N. arenaceodentata* 10-d exposures (Lotufo et al. 2001). The toxicity of RDX was also investigated using sandy sediment (Rosen and Lotufo 2005). No significant mortality was observed in any exposure, even when the measured concentration approached or exceeded 1 g kg^{-1} (Table 5.4). Reports of the toxicity of RDX or HMX transformation products in sediment exposures have not been found in the available literature.

Table 5.4. Toxicity of RDX and HMX determined for aquatic invertebrates using spiked sediment exposures.

Spiked chemical	Species	Habitat	TOC (%)	Duration (d)	NOEC mg kg^{-1}	LOEC mg kg^{-1}	Reference
RDX	Amphipod *Leptocheirus plumulosus*	Marine	2.70	10	891	ND	Lotufo et al. 2001
	Polychaete *Neanthes arenaceodentata*	Marine	2.70	10	891	ND	Lotufo et al. 2001
	Amphipod *Eohaustorius estuarius*	Marine	0.09	10	2229	ND	Rosen and Lotufo 2005
	Amphipod *Hyalella azteca*	Freshwater	0.65	10	102	ND	Steevens et al 2002
	Midge (larva) *Chironomus dilutus*	Freshwater	0.65	10	711	ND	Steevens et al 2002
HMX	*Leptocheirus plumulosus*	Marine	2.70	10	115	ND	Lotufo et al. 2001
	Neanthes arenaceodentata	Marine	2.70	10	353	ND	Lotufo et al. 2001
	Hyalella azteca	Freshwater	0.65	10	126	ND	Steevens et al 2002
	Chironomus dilutus	Freshwater	0.65	10	146	ND	Steevens et al 2002

TOC – Total organic carbon, NOEC - No observed effect concentration, LOEC - lowest observed effect concentration, NOEC and LOEC reported as concentrations measured at experiment initiation, ND - not determined due to insufficient mortality in all treatments. See Table 4.2 for species habitat information.

Sublethal toxicity and stimulatory effects of MC in sediment exposures

Few records of biological effects other than lethality are available for sediment exposures to explosives. In studies where effects other than mortality were investigated, non-lethal effects (i.e., significant decreases in growth (Steevens et al. 2002) or reproduction (Conder et al. 2004a, Green et al. 1999) were reported only in treatments where survival was also significantly decreased and therefore were not truly sublethal manifestations of sediment exposure to explosives. However, a sublethal decrease in offspring production was observed in *L. plumulosus* and a sublethal decrease in biomass was exhibited by juvenile *N. arenoceodentata* at concentrations a few fold lower than lethal concentrations (Lotufo et al. 2001). Lethal effects of RDX to *L. plumulosus* and *N. arenoceodentata* were not observed (Lotufo et al. 2001) (Table 5.4). To further elucidate lethal and sublethal effects on growth and reproduction, additional studies using a variety of benthic invertebrates are warranted.

Exposure to explosive-spiked sediments has promoted stimulatory effects on growth of a few species of freshwater invertebrates. Significantly increased biomass was observed for *C. dilutus* exposed to RDX, HMX (Steevens et al. 2002), TNT, and 1,3,5-TNB (Lotufo and Farrar 2005) in sediment treatments that did not cause significant lethality. Similar effects were observed for *H. azteca* exposed to 1,3,5-TNB or RDX (Steevens et al. 2002) and for *N. arenaceodentata* exposed to TNT (Green et al. 1999). The mechanisms responsible for hormetic-like concentration-response relationships reported in these studies are unknown but were speculated to be related to explosives-induced changes in the nutritional status of the sediment and biochemical and physiological alterations in invertebrates (Green et al. 1999).

Toxicity of porewater extracted from sediments spiked with explosives

The toxicity of porewaters from fine-grained and sandy marine sediments spiked individually with 2,6-DNT, tetryl, or picric acid, and allowed to equilibrate at 4 °C for 5-10 days, varied with both the compound and the nature of the spiked sediment (Nipper et al. 2002). Toxicity was assessed using sea urchin (*Arbacia punctulata*) embryological development, macroalga (*Ulva fasciata*) zoospore germination and germling growth, and polychaete (*Dinophilus gyrociliatus*) survival and reproduction tests. Tetryl and picric acid were the most and least toxic of the three chemicals, respectively, in porewaters from both kinds of sediments (Nipper et al. 2002). Samples spiked with 2,6-DNT contained a transformation product identified as 2-A-6-NT. Unidentified peaks, possibly transformation products, were also seen in some of the picric acid- and tetryl-spiked samples. Porewater from the fine-grained sediment spiked with 2,6-DNT and tetryl was up to three orders of magnitude more toxic than porewater from the sandy sediment (Table 5.5), possibly due to the presence of relatively toxic non-identified transformation products.

Further assessments were performed of the toxicity of transformation products of 2,6-DNT and picric acid in porewaters from spiked sandy and fine-grained marine sediments over a 6-month sediment incubation period at 10 and 20 °C. During these assessments, incremental transformation was reported (Nipper et al. 2005). Toxicity of porewater from sediments spiked with 2,6-DNT decreased for the macroalga, *U. fasciata* zoospores, with sediment incubation time as transformation progressed, but increased for the copepod, *Schizopera knabeni*, nauplii. The porewater of fine-grained sediment spiked with either 2,6-DNT or picric acid had lower toxicity than

Table 5.5. Toxicity of marine porewater extracted from sandy or fine-grained sediments spiked with 2,6-DNT, tetryl or picric acid (from Nipper et al. 2002).

Sediment type	MC	Urchin Embryo development EC_{50}	Polychaete LC_{50}	Reproduction EC_{50}	macroalga zoospore Germling length EC_{50}
		mg L^{-1}			
Sandy	2,6-DNT	36.9	21.1	8.16	3.28
Fine grained	2,6-DNT	0.043	0.046	0.023	No effects at 0.087 [a]
Sand	Tetryl	0.27	0.055	0.66	0.48
Fine grained	Tetryl	0.00056	0.0019	0.0019	No effects at 0.003[a]
Sand	Picric acid	592.8	127.5	83.4	83.1
Fine grained	Picric acid	190.2	170.4	64.8	11.22

EC_{50} - median effective concentration, LC_{50} - median lethal concentration, [a] Highest concentration tested.

its sandy counterpart after 6 months, suggesting faster microbial transformation in fine-grained sediments, resulting in further transformation of picramic acid and 2,4-DNP into less toxic products (Nipper et al. 2005). Overall, transformation products of picric acid and 2,6-DNT were more toxic than their parent compounds, but that transformation, primarily of biotic nature, proceeds towards the formation of less toxic products given sufficient time.

Preliminary sediment quality benchmarks

Sediment screening criteria for many MC are not available in sources typically used in regulatory-driven ecological risk assessments for contaminated sediment sites. Preliminary sediment quality benchmarks (SQBs) for MC were developed for screening potential risks to marine benthic invertebrates at an MC-contaminated sediment site in Puget Sound, WA by Pascoe et al. (2010). Those SQBs were developed using freshwater and limited marine water-only toxicity values and equilibrium partitioning theory to relate water concentrations of the compounds to sediment concentrations. Pascoe et al. (2010) presented SQBs for 25 MC as organic carbon-normalized values and as ranges of dry weight values for various levels of organic carbon content of sediments. Comparison of the preliminary SQBs with method detection limits and sample detection

limits achieved at the contaminated sediment site demonstrates their utility in risk screening of benthic invertebrates.

Sediment toxicity values, expressed as NOECs, for nine MC based on sediment spiking experiments compiled in Lotufo et al. (2009a), and the dry weight SQB values derived Pascoe et al. (2010) are compared in Table 5.6. The spiked sediment NOECs for all MC except 2,6-DNT are substantially higher than the SQB values. The differences between equilibrium partitioning-based SQBs and spiked sediment NOECs reported in Lotufo et al. (2009a) are related to the uncertainties in quantifying exposures in spiked sediment due to chemical and biological transformation , as discussed in Lotufo et al. (2009a) and in the uncertainty associated with assuming equilibrium between sediment and porewater (Pascoe et al. 2010).

Table 5.6. Marine sediment quality benchmarks (SQB) and no observed effect concentration (NOEC) from sediment toxicity tests for nine MC.

MC	SQB[a] ($mg\ kg^{-1}$)		Spiked Sediment NOEC[b] ($mg\ kg^{-1}$)
	Low	High	
TNT	0.0055	0.064	<0.1
2-Am-DNT	0.0065	0.0075	23
2,4-DANT	0.00045	--	<0.1
2,6-DNT	2.1	2.7	0.500
1,3,5-TNB	0.0008	0.0043	<0.1
Picric acid	1.7	--	73
Tetryl	0.003	0.031	0.1
HMX	0.0019	0.215	126
RDX	0.006	0.039	102

[a] values derived Pascoe et al. (2010), [b] sediment toxicity data compiled in Lotufo et al. (2009a)

6 Toxicokinetics and Bioconcentration of Munitions Constituents

This section summarizes and discusses the bioconcentration, bioaccumulation, biotransformation and toxicokinetic processes of nitroaromatic and cyclic nitramine MC in aquatic organisms. Due to the paucity of information on this subject from studies investigating marine organisms, information from freshwater studies are also included in this overview. Investigations of the bioconcentration and biotransformation of nitrophenolic MC and nitrate esters have not been found in the available literature.

Overview

Bioaccumulation is the process that causes an increased chemical concentration in an organism compared to that in its ambient environment, through all exposure routes, including dietary absorption and transport across body surfaces (Borga et al. 2012). Bioaccumulation includes uptake by respiration of chemical from the environment surrounding the organism and dietary exposures. Major routes of chemical elimination include loss by respiration, fecal egestion, urinary excretion, metabolic biotransformation, and growth dilution (Arnot 2009).

Bioconcentration is the process by which a chemical is absorbed by an organism from the water only through its respiratory and dermal surfaces. It is the net result of competing rates of uptake and elimination, including biotransformation (Arnot and Gobas 2006). MC compounds are weakly hydrophobic, and therefore, according to predictive models (e.g., Meylan et al. 1999; Arnot and Gobas 2003), their potential to bioconcentrate is low. Reports on the bioconcentration potential of MC in aquatic animals (reviewed in Lotufo et al. 2009b; Lotufo 2011) are scarce. In addition, studies examining the uptake from water, as well as elimination and biotransformation potential were conducted only for a few compounds using a small number of aquatic species (reviewed in Lotufo et al. 2009b).

Biomagnification can be regarded as a special case of bioaccumulation in which the chemical concentration in the organism exceeds that in its prey due to dietary absorption occurring faster than elimination (Gobas and

Morrison 2000). The octanol-water partition coefficient, K_{OW}, represents the hydrophobicity of a chemical and how it thermodynamically distributes between aqueous and organic phases (Arnot 2009). In aquatic food webs, poorly metabolized hydrophobic chemicals with log $K_{OW} > 5$ generally biomagnify, whereas chemicals with log $K_{OW} < 5$, even if poorly metabolized, do not (Gobas et al. 1999). Most explosive MC have log K_{OW} less than 2 and all of the common explosive MC have a log K_{OW} less than 4 (Table 3.1). Consequently, the chemicals are not expected to transfer efficiently through the dietary route and subsequently magnify. This expectation was confirmed in laboratory studies showing that the dietary uptake of MC in fish was minimal (reviewed in Lotufo et al. 2009b).

Bioconcentration of MC

A bioconcentration factor (BCF) is the ratio of a chemical concentration in an aquatic organism to that in the surrounding water, and is the most frequently used indicator of a compound's propensity to bioconcentrate in aquatic organisms (Meylan et al. 1999). Bioconcentration factors are determined primarily using steady state concentrations, or by using kinetically derived uptake and elimination rates (Lotufo and Lydy 2005). Bioconcentration factors reported for explosives and related compounds in invertebrate and fish species are provided in Table 6.1.

The BCFs for TNT ranged widely from 0.3 to 9.7 ml g^{-1} for a variety of marine and freshwater invertebrates and fish species (Conder et al. 2004b; Lotufo and Lydy 2005, Belden et al. 2005a; Ownby et al. 2005; Yoo et al. 2006; Rosen and Lotufo 2007b). Comparison of the bioconcentration of TNT and its major biotransformation products in the oligochaete *Tubifex tubifex* yielded lower BCFs for TNT and 2,4-DANT than for 2-Am-DNT and 4-Am-DNT (Conder et al. 2004b). A similar comparative study with sheepshead minnows (*Cyprinodon variegatus*) revealed similar BCFs for TNT and 2-Am-DNT, and a lower BCF value for 2,4-DANT (Lotufo and Lydy 2005).

The RDX BCFs were similar for channel catfish (Belden et al. 2005b), zebrafish (Muhki and Patino 2008), sheepshead minnows (Lotufo and Lydy 2005) and in the oligochaete *Lumbriculus variegatus* (Belden et al. 2005b). The RDX BCFs were lower for the mussel *Mytilus galloprovincialis* (Rosen and Lotufo 2007b), ranging from 0.8 to 2.1 ml g^{-1}. The HMX bioconcentration in aquatic organisms was low, with a reported BCF of 0.51 ml g^{-1} for juvenile sheepshead minnows (Lotufo and Lydy 2005) and 0.31 ml g^{-1} for adult mussels (Rosen and Lotufo 2007b).

Table 6.1. Empirically determined bioconcentration factor (BCF) values for fish and aquatic invertebrates.

MC	Species	Habitat	Duration (d)	BCF (ml g^{-1})	Reference
TNT	Sheepshead minnow	Marine	0.25	9.71	Lotufo and Lydy 2005
	Cyprinodon variegatus	Marine	1	3.3	Lotufo 2011
	Fathead minnow *Pimephales promelas*	Freshwater	0.25	2.3, 5.0	Yoo et al. 2006
	Channel catfish *Ictalurus punctuatus*	Freshwater	0.3	0.8	Ownby et al. 2005
	Mussel (adult) *Mytilus galloprovincialis*	Marine	0.17	0.31	Rosen and Lotufo 2007b
	Oligochaete *Lumbriculus variegatus*	Freshwater	1	4	Belden et al. 2005a
	Oligochaete *Tubifex tubifex*	Freshwater	2.2	2.5	Conder et al. 2004b
2-Am-DNT	*Cyprinodon variegatus*	Marine	0.25	13.1	Lotufo and Lydy 2005
	Tubifex tubifex	Freshwater	2.2	10.2	Conder et al. 2004b
4-Am-DNT	*Tubifex tubifex*	Freshwater	2.2	12.4	Conder et al. 2004b
2,4-DANT	*Cyprinodon variegatus*	Marine	0.25	0.51	Lotufo and Lydy 2005
	Tubifex tubifex	Freshwater	2.2	2.8	Conder et al. 2004b
2,4-DNT	Common carp *Cyprinus carpio*	Freshwater	3	9.2	Lang et al. 1997
	Goldfish *Carassius auratus*	Freshwater	20	4.5	Wang et al. 1999
2,6-DNT	*Carassius auratus*	Freshwater	20	1.7	Wang et al. 1999
RDX	*Cyprinodon variegatus*	Marine	0.25	1.7	Lotufo and Lydy 2005
		Marine	1	0.7	Lotufo 2011
	Ictalurus punctuatus	Freshwater	0.7	2.0	Belden et al. 2005b
	Zebra fish Danio rerio	Freshwater	28	1.0	Mukhi and Patino 2008
RDX	*Lumbriculus variegatus*	Freshwater	0.7	2.1	Belden et al. 2005b
RDX	*Mytilus galloprovincialis*	Marine	0.17	0.71	Rosen and Lotufo 2007b
HMX	*Cyprinodon variegatus*	Marine	0.25	0.51	Lotufo and Lydy 2005
	Mytilus galloprovincialis	Marine	0.17	0.31	Rosen and Lotufo 2007b

Various predictive models have been proposed to estimate BCFs for organic compounds based on their hydrophobicity as represented by their n-octanol-water partition coefficient (K_{ow}) (Meylan et al. 1999). Explosive compounds are weakly hydrophobic (low K_{ow} values) and therefore BCFs predicted using these models should be low. The equation derived by

Meylan et al. (log BCF = 0.86 log K_{ow} - 0.39) for non-ionic compounds in the log K_{ow} range of 1 to 7 was used to calculate predicted BCFs for explosives and related compounds (Table 6.2) for empirically derived BCF values reported in Table 6.1. A comparison of predicted and empirically derived BCFs revealed that most explosives and related compounds bioconcentrate in aquatic invertebrates and fish at concentrations lower than those predicted using their hydrophobicity (Table 6.2). Most measured BCFs were similar to model-predicted BCFs within a factor of 3. Overall, the bioconcentration of nitroaromatic and nitramine MC appear to be adequately predicted using a generic relationship derived for nonionic compounds suggesting that the bioconcentration of those compounds is a thermodynamically driven partitioning process between the water and the lipid phase of the exposed organisms.

Table 6.2. A comparison of predicted versus measured bioconcentration factor (BCF) values.

MC	log K_{ow} (from Table 2.2)	Predicted BCF (ml g^{-1})	Measured BCF (ml g^{-1})
2,4-DNT	1.98	20.6	4.5 and 9.2
2,6-DNT	2.02	26.1	1.7
TNT	1.60	9.7	0.31 - 9.71
2-Am-DNT	1.94	19.3	10.2 and 13.1
4-Am-DNT	1.91	26.1	12.4
2,4-DANT	0.79	2.0	0.51 and 2.8
RDX	0.90	2.3	0.7 and 2.0
HMX	0.17	0.6	0.31 to 0.51

BCF predicted from log K_{ow} values using a regression model (log BCF = 0.86 log K_{ow} - 0.39) from Meylan et al. (1999) and the range of experimentally-determined values for fish and aquatic invertebrates are from Table 6.1.

MC as bioaccumulative compounds in a regulatory context

Bioaccumulation plays a vital role in understanding the fate of a substance in the environment. Substances having a high bioaccumulation potential can be efficiently taken up by organisms, leading to elevated internal concentrations that can reach critical levels that elicit toxic effects even when the concentration in the environment is low. Bioaccumulative chemicals are typically retained in the organism for a long period after the external concentration has declined. A chemical is considered "bioaccumulative" in the regulatory context of the Stockholm Convention mostly based on its potential to bioconcentrate (i.e., when its bioaccumulation factor in aquatic

species is greater than 5000 ml g^{-1} wet weight or -- in the absence of such data -- when the log K$_{OW}$ is greater than 5) (Gobas et al. 2009). An overview of regulatory bioaccumulation assessment endpoints and criteria is provided in Gobas et al. 2009. Explosive MC compounds are not considered bioaccumulative compounds as their BCF values are < 14 ml g^{-1}, therefore over two orders of magnitude lower than 5000 ml g^{-1}.

Toxicokinetics and biotransformation

Direct uptake from water is usually the major process by which fish and aquatic invertebrates accumulate nonpolar organic compounds with low hydrophobicity, such as TNT and RDX. The bioaccumulation kinetics of TNT, 2-Am-DNT, 4-Am-DNT, and 2,4-DANT were compared in *T. tubifex* (Conder et al. 2004b) with all four compounds reaching steady state concentrations within 1 h of exposure. The uptake clearance rate coefficient (k_u) is defined as the volume or mass of a compartment scavenged of the contaminant per mass of organism per time. Using total radioactivity as a surrogate for whole body concentration, the k_u for TNT into the midge *Chironomus dilutus*, the oligochaete *Lumbriculus variegatus* (Belden et al. 2005a) and juvenile channel catfish (*Ictalurus punctatus*) (Belden et al. 2005a, Ownby et al. 2005) were 26.7, 39.2, and 10.1 ml g^{-1} h^{-1}, respectively. Using analytically measured body residues, the k_u for TNT, 2-Am-DNT and 2,4-DANT into juvenile sheepshead minnows were 7.3, 12.6, and 1.3 ml g^{-1} h^{-1}, respectively (Lotufo and Lydy 2005) and was 0.9 ml g^{-1} h^{-1} into adult mussels (Rosen and Lotufo 2007b). The rate of RDX uptake in sheepshead minnows (Lotufo and Lydy 2005), juvenile channel catfish (Belden et al. 2005b), *L. variegatus* (Belden et al. 2005b), and adult mussels (Rosen and Lotufo 2007b) were more variable (0.15, 1.3, 5.2, and 3.4 ml g^{-1} h^{-1}, respectively), but were consistently less efficient than the uptake for TNT. The uptake clearance rate for 2,4-DNT in carp (*Cyprinus carpio*) (0.41 ml g^{-1}h^{-1}) (Lang et al. 1997) was slower than the rates derived for TNT using juvenile channel catfish or sheepshead minnows and may be due to species-specific differences and/or a decrease in uptake efficiency with increasing organism size, because the carp (5-18 g) were substantially larger than the juvenile minnows and catfish (0.1-0.4 g) used in the latter studies. The k_u for HMX was 0.06 ml g^{-1} h^{-1}for sheepshead minnows (Lotufo and Lydy 2005) and 0.6 ml g^{-1} h^{-1}for for adult mussels (Rosen and Lotufo 2007b) and was therefore slower than the k_u values determined for RDX for those organisms. Table 6.3 summarizes available uptake kinetics data for MC. The overall low efficiency of uptake of MC from water was expected because of the low hydrophobicity of these compounds.

Table 6.3. Toxicokinetic model parameters for fish and aquatic invertebrates exposed to TNT, 2-Am-DNT, 2,4-DANT, 4-DNT, RDX and HMX. See Table 5.2 for species habitat information.

Parameter	Species	Habitat	TNT	SumTNT	2-Am-DNT	2,4-DANT	2,4-DNT	RDX	HMX	Reference
Uptake k_u (ml g^{-1} h^{-1})	Sheepshead minnow *Cyprinodon variegatus*	Marine	7.3	ND	12.6	1.3	ND	0.15	0.06	Lotufo and Lydy 2005
	Fathead minnow *Pimephales promelas*	Freshwater	ND	30.4	ND	ND	ND	ND	ND	Yoo et al. 2006
	Channel catfish *Ictalurus punctuatus*	Freshwater	10.1	ND	ND	ND	ND	1.3	ND	Ownby et al. 2005; Belden et al. 2005b
	Mussel *Mytilus galloprovincialis*	Marine	0.9	2.5	ND	ND	ND	3.4	0.6	Rosen and Lotufo 2007b
	Midge *Chironomus dilutus*	Freshwater	26.7	ND	ND	ND	ND	ND	ND	Belden et al. 2005a
	Oligochaete *Lumbriculus variegatus*	Freshwater	39.2	ND	ND	ND	ND	5.2	ND	Belden et al. 2005a,b
	Common carp *Cyprinus carpio*	Freshwater	ND	ND	ND	ND	0.41	ND	ND	Lang et al. 1997
Elimination k_e (h^{-1})	*Cyprinodon variegatus*	Marine	0.8	ND	1.0	2.7	ND	0.1	0.1	Lotufo and Lydy 2005
	Pimephales promelas	Freshwater	ND	2.2	ND	ND	ND	ND	ND	Yoo et al. 2006
	Ictalurus punctuatus	Freshwater	13.0	ND	ND	ND	ND	0.6	ND	Ownby et al. 2005; Belden et al. 2005b
	Mytilus galloprovincialis	Marine	2.1	2.2	ND	ND	ND	2.5	0.5	Rosen and Lotufo 2007b
	Lumbriculus variegatus	Freshwater	ND	ND	ND	ND	ND	2.5	ND	Belden et al. 2005b
	Oligochaete *Tubifex tubifex*	Freshwater	ND	ND	1.1	ND	ND	ND	ND	Conder et al. 2004c
Half-life $t_{1/2p}$ (h)	*Cyprinodon variegatus*	Marine	0.91	ND	0.72	0.25	ND	7.7	5.8	Lotufo and Lydy 2005
	Pimephales promelas	Freshwater	ND	0.31	ND	ND	ND	ND	ND	Yoo et al. 2006

Parameter	Species	Habitat	TNT	SumTNT	2-Am-DNT	2,4-DANT	2,4-DNT	RDX	HMX	Reference
	Ictalurus punctuatus	Freshwater	0.05	ND	ND	ND	ND	1.2	ND	Ownby et al. 2005; Belden et al. 2005b
	Mytilus galloprovincialis	Marine	0.33	0.32	ND	ND	ND	0.27	1.50	Rosen and Lotufo 2007b

ND – Not determined.

Biotransformation of TNT to Am-DNTs during aqueous exposures occurred very quickly in *T. tubifex* (0.212 and 0.187 h^{-1} for 2-Am-DNT and 4-Am-DNT, respectively) (Conder et al. 2004b), but was markedly slower (0.06 h^{-1} for Am-DNTs) in juvenile sheepshead minnows (Lotufo and Lydy 2005). Substantial biotransformation of TNT to Am-DNTs was also reported for fathead minnows (Yoo et al. 2006) and adult mussels (Rosen and Lotufo 2007b) exposed to TNT for periods as short as 10 minutes. Formation of DANTs resulting from exposure to TNT or Am-DNTs was non-quantifiable or minimal (Lotufo and Lydy 2005, Conder et al. 2004b; Rosen and Lotufo 2007b).

Elimination of TNT and its major biotransformation products was relatively fast for all of the aquatic organisms tested. For example, when *T. tubifex* or sheepshead minnows were transferred to noncontaminated water following exposure to TNT, 2-Am-DNT, 4-Am-DNT (*T. tubifex* only), or 2,4-DANT, measurable amounts of those compounds were completely eliminated from the tissues in less than 3 h (Lotufo and Lydy 2005; Conder et al. 2004b). The tissue sampling schedule used in the *T. tubifex* study precluded the calculation of the exceedingly fast elimination of TNT or 2,4-DANT in that species (Conder et al. 2004c). The elimination rate (k_e) for sumTNT was identical in juvenile fathead minnows and in adult mussels (2.2 h^{-1}), corresponding to a half-life of 0.3 h. The k_e of TNT in juvenile sheepshead minnows was slower, 0.8 h^{-1}, corresponding to a half-life of 0.91 h (Lotufo and Lydy 2005). The k_e values for *T. tubifex* for 2-Am-DNTand 4-Am-DNT were 1.1 h^{-1} and 0.91 h^{-1}, respectively (Conder et al. 2004b). For sheepshead minnows, the k_e for 2-Am-DNT and 2,4-DANT were 1.0 and 2.7 h^{-1}, respectively (Lotufo and Lydy 2005). The rates of elimination of DNTs in goldfish (Wang et al. 1999) were very similar (0.47 and 0.60 h^{-1} for 2,4-DNT and 2,6-DNT, respectively) to the rates determined for TNT and 2-Am-DNT in *T. tubifex* and sheepshead minnows, as expected given the similar hydrophobicity of those nitroaromatic compounds. However, the rate of elimination of 2,4-DNT in carp was substantially slower (0.06 h^{-1}), likely

due to the larger size of the *C. carpio* compared to the organisms used in the other investigations. Elimination of RDX was relatively fast in juvenile channel catfish, adult mussels, and in the oligochaete *L. variegatus* (0.6, 2.5, and 2.5 h^{-1}, respectively) (Belden et al. 2005b; Rosen and Lotufo 2007a), but was substantially slower in juvenile sheepshead minnows (0.1 h^{-1}) (Lotufo and Lydy 2005). The k_e for HMX, reported for sheepshead minnows (0.1 h^{-1}) and adult mussels (0.5 h^{-1}) (Lotufo and Lydy 2005; Rosen and Lotufo 2007b) was very low.

Formation and Elimination of TNT Bound Residues in Marine Invertebrates and Fish

In the studies listed in Table 6.1, BCFs were determined using the parent compound concentrations determined by high performance liquid chroma-tography (HPLC) analysis of solvent extracts of whole-body homogenates. In early studies of the bioconcentration of explosives in aquatic inverte-brates and fish, BCFs were derived using radioactivity measurements of whole-body homogenates as a surrogate for the sum molar concentration of parent and all transformation products. BCFs derived using total radio-activity measurements for body residues were reported for TNT, 2,4-DNT (Liu et al. 1983a), and RDX (Liu et al. 1983c) (Table 6.2). Recent studies of the bioconcentration of TNT parent compound in aquatic invertebrates and fish compared the BCFs calculated for the parent compound with BCFs calculated using the sum concentration of TNT parent compound and its transformation products in the tissues (Table 6.2).

A comparison of BCF for parent compounds, for total extractable compounds (radioactivity in the solvent extract) and for total extractable and non-extractable compounds (total radioactivity in the organism) for the same exposed organisms (Figure 6.1) reveals that the non-extractable (i.e., solvent-resistant and presumably tissue-bound) compounds are accumulated at substantially higher concentrations than extractable transformation products at steady state in fish and aquatic invertebrates. The tissue concentration of parent compounds represented only a fraction, sometimes minimal, of the total extractable concentration in all the species investigated.

Bioconcentration values determined using total radioactivity in the tissue were also reported for 2,4-DNT (Liu et al. 1983a) and RDX (Liu et al. 1983c) (Table 6.4). A comparison of BCFs determined for total radioactivity (Table 6.4) with those determined for a parent compound only (Table 6.1)

for RDX suggests dominant accumulation of parent compound relative to transformation products in aquatic animals. A similar comparison for 2,4-DNT suggests substantial accumulation of transformation products of that compound in aquatic animals.
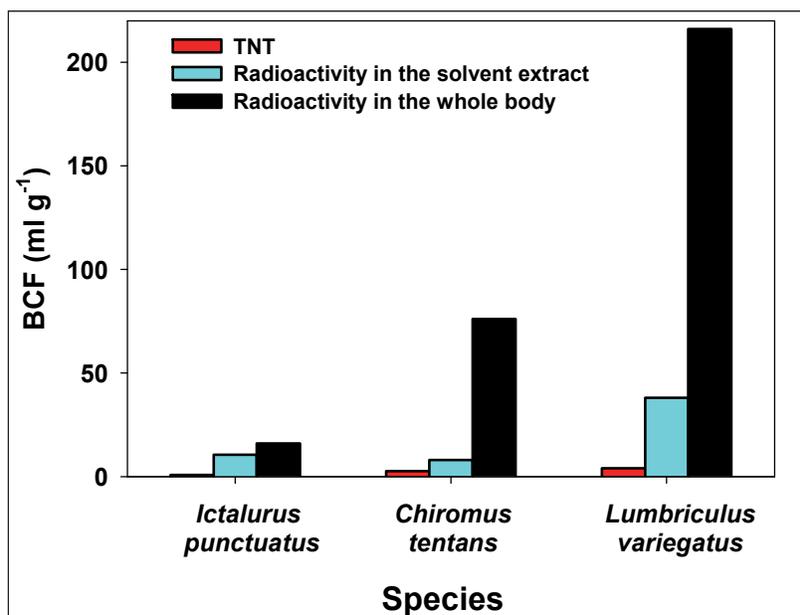
Figure 6.1. Bioconcentration factors (BCF) for freshwater fish and invertebrates exposed to TNT determined for body residues for parent compound, sum concentration of extractable compounds and for the sum concentration of extractable and non-extractable compounds (from Lotufo et al. 2009b).

Table 6.4. Bioconcentration factor (BCF) values for aquatic animals determined using the sum concentration of the parent compound and transformation products obtained from radioactivity in the extract or whole body as surrogate for body residue.

MC	Species	Exposure duration (d)	BCF (ml g^{-1})	Analytical measurement	Reference
TNT	Midge (larva) *Chironomus dilutus*	1	76	Radioactivity in the whole body	Belden et al. 2005b
		1	8	Radioactivity in the solvent extract	Belden et al. 2005b
	Cladoceran *Daphnia magna*	4	209	Radioactivity in the whole body	Liu et al. 1983c
	Oligochaete *Lumbriculus variegatus*	4	202	Radioactivity in the whole body	Liu et al. 1983c
		1	216	Radioactivity in the whole body	Belden et al. 2005b
		1	38	Radioactivity in the solvent extract	Belden et al. 2005b

MC	Species	Exposure duration (d)	BCF (ml g⁻¹)	Analytical measurement	Reference
	Channel catfish *Ictalurus punctuatus*	0.3	16	Radioactivity in the whole body	Ownby et al. 2005
		0.3	10.5	Radioactivity in the solvent extract	Ownby et al. 2005
2,4-DNT	*Daphnia magna*	4	13	Radioactivity in the whole body	Liu et al. 1983a
	Lumbriculus variegatus	4	58	Radioactivity in the whole body	Liu et al. 1983a
RDX	*Daphnia magna*	4	3	Radioactivity in the whole body	Liu et al. 1983c
	Lumbriculus variegatus	4	1.6	Radioactivity in the whole body	Liu et al. 1983c

Bioaccumulation of large fractions of non-identified compounds was also found in the marine amphipod *Eohaustorius estuarius* following exposure to sediment spiked with (^{14}C)TNT (Rosen and Lotufo 2005). For RDX, comparisons of the steady state BCF reported for total radioactivity (3.0 ml g⁻¹) (Liu et al. 1983c) and parent compound (2.4 ml g⁻¹) for *L. variegatus* suggests that minimal biotransformation of RDX occurs in that species.

Non-extractable radioactivity in aquatic invertebrates and fish tissues likely represents covalent conjugates (i.e., macromolecular bound residues) (Conder et al. 2004b, Belden et al. 2005b). However, neither the biological half-life nor the chemical nature of non-extractable transformation products in invertebrates and fish has been investigated to date.

The formation of covalent bonds with proteins in mammalian systems has been reported (Liu et al. 1992; Leung et al. 1995; Bakhtiar et al. 1997). A recent investigation of ^{14}C-TNT toxicokinetics in earthworms (Belden et al. 2011) showed that nonextractable residues accounted for as much as 68% of total body burden. Parent TNT accounted for less than 3% of radioactivity, while Am-DNTs accounted for 7 to 38%. Elimination was much slower for nonextractable residues. The half-lives were 61 to 120 hours for TNT, Am-DNTs, and DANTs, which was significantly lower than the half-lives found for nonextractable residues, which ranged from 201 to 240 hours. Despite resisting organic solvent extraction, 80% of the nonextractable residue in earthworms was solubilized when residual tissue was treated with a dilute aqueous solution of hydrochloric acid (Belden et al. 2011). This extraction behavior is consistent with the assumed transformation pathway shown in Figure 6.2. That is, polar conjugates formed between reactive TNT

metabolites and proteins (e.g., the sulfonamide conjugate shown in Figure 6.2) would not be expected to be solubilized by organic solvents. On the other hand, treatment with diluted acid could feasibly break the S-N bond responsible for conjugation and release the TNT-derived portion of the molecule (i.e., reductive metabolites) into aqueous solution.
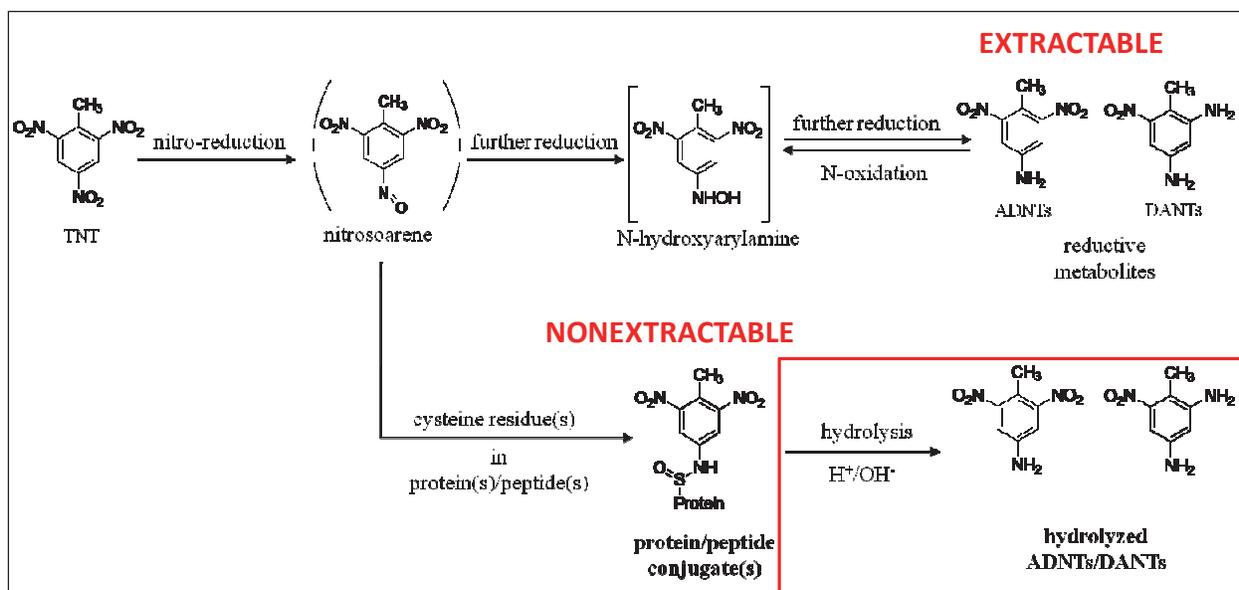
Figure 6.2. Proposed metabolic activation and formation of protein-bound TNT residues in fish and aquatic invertebrates. The red box indicates the identification of Am-DNTs and DANTs as hydrolysable bound residues in tissue of TNT-exposed earthworms (Belden et al. 2011).

Lotufo et al. (unpublished study) examined ^{14}C-TNT toxicokinetics of juvenile sheepshead minnows and adult mussels exposed to 0.3 mg L^{-1} TNT in seawater. After 24 h, mean BCFs were 4 ml g^{-1} and 1.3 ml g^{-1} for fish and mussels, respectively. Those values are similar to those derived from kinetics exposures reported in published studies (Table 6.1).

Based on the concentration of Am-DNTs (present in fish and mussels) and DANTs (present in fish only) in tissues following aqueous exposure, it is apparent that TNT was efficiently biotransformed. The body residues for TNT, Am-DNTs, and DANTs corresponded to 29, 18 and 53% of the total sum TNT residue, respectively, for fish and 18, 82 and 0% for mussels. The use of radiolabeled TNT allowed determination of the concentration of unknown extractable products (i.e., polar transformation products, not Am-DNTs and DANTs), as well as non-extractable residues as sum concentration (i.e., without identification and quantification of individual compounds), as described in Section 6. Considering only the extractable parent and transformation products, polar unknown compounds

accounted for most of the bioaccumulation in TNT-exposed fish and
mussels, while the TNT parent compound was present as only to 12% and
7% of the total accumulation in fish and mussels, respectively (Table 6.5).
When the radioactivity remaining in tissue homogenate following solvent
extraction was considered, non-extractable compounds accounted for 83%
and 75% of the total accumulation in fish and mussels, respectively, while
Sum-TNT corresponded to only 7 and 10%, respectively (Table 6.6).

Table 6.5. Percentage of total body residue for solvent extractable compounds in fish and mussels
(from Lotufo et al. unpublished study).

Species	Compound / Group	Percent of Total Body Residue
Sheepshead minnow *Cyprinodon variegatus*	TNT	11.9
	Am-DNTs	7.4
	DANTs	22.1
	SumTNT	41.3
	Extractable by acetonitrile – unknown	58.7
Mussel *Mytilus galloprovincialis*	TNT	7.3
	Am-DNTs	32.6
	DANTs	0
	SumTNT	39.9
	Extractable by acetonitrile – unknown	60.1

Table 6.6. Percentage of total body residue for solvent-extracted compounds and for bound
(non-extractable) residues in fish and mussels exposed to radiolabeled TNT (from Lotufo et al.
unpublished study).

Species	Compound / Group	Percent of Total Body Residue
Sheepshead minnow *Cyprinodon variegatus*	TNT	2.0
	Am-DNTs	1.2
	DANTs	3.7
	SumTNT	6.8
	Extractable by acetonitrile – unknown	9.7
	Non-extractable	83.4
Mussel *Mytilus galloprovincialis*	TNT	1.8
	Am-DNTs	8.2
	DANTs	0
	SumTNT	10.0
	Extractable by acetonitrile – unknown	15.1
	Non-extractable	74.9

Upon transfer of TNT-exposed fish and mussels to uncontaminated water, the persistence of extractable and nonextractable residues in tissues differed markedly. The half-life of sumTNT in fish and mussels was only 5.5 and 27 h, respectively (Table 6.7). In contrast, the half-lives for non-extractable residues was 80 and 325 h for fish and mussels, respectively (Table 6.7), indicating the substantially higher persistence of bound residues.

Table 6.7. Mean elimination half-lives for SumTNT and for nonextractable residues in fish and mussels (from Lotufo et al. unpublished study).

Species	Compound / Group	Half-life (h)[a]
Sheepshead minnow *Cyprinodon variegatus*	SumTNT	5.5
	Nonextractable	80
Mussel *Mytilus galloprovincialis*	SumTNT	27
	Nonextractable	325
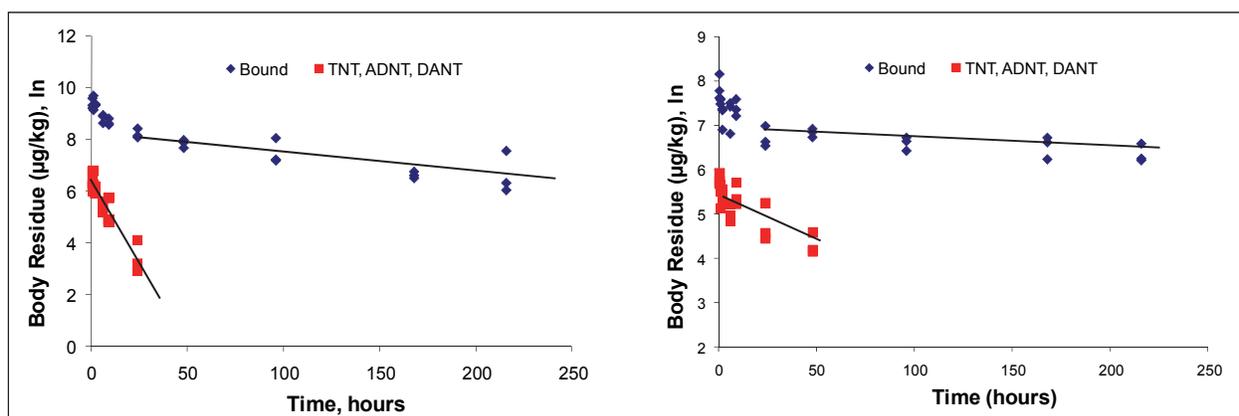
Figure 6.3. Depuration regressions for sheepshead minnows (left) or mussels (right) exposed to ^{14}C-TNT-spiked seawater (Lotufo et al. unpublished study). Red squares are used for sum of TNT and major aminated transformation products and blue diamonds are used for nonextractable (bound) residues. Regression calculations exclude time points prior to 12 h for bound residues.

Bodypart-specific bioaccumulation

Sheepshead minnows were exposed to radiolabeled isotopes of TNT, RDX and HMX to assess the body distribution of those compounds (Lotufo 2011). To allow a comprehensive understanding of the biological fate of those explosives in fish, tissue concentrations were determined for parent compound and major breakdown products. Both solvent-extractable and solvent-nonextractable radiolabeled transformation products were evaluated for mass-balance purposes. The relative contribution of the different body parts assessed in this study to the total fish biomass is

presented in Table 6.8. The contribution of the amount of explosive parent compound or transformation products in a body part relative to the total amount present in the fish is also presented in Table 6.8. Although the liver accounted for a very small fraction of the total biomass, most of the TNT transformation products resided in that organ. Contrastingly, most of the TNT parent compound was present in the viscera, which also accounted for a small fraction of the total biomass (Table 6.8). The remaining tissues (muscles, skin, and subdermal fat) contained a disproportionately small fraction of the accumulated TNT parent compound and transformation products. The contribution of the liver RDX and HMX parent compound and transformation products was disproportionately high, but less so than for TNT; most of the RDX and HMX parent compound and transformation products were present in the body remains (Table 6.8).

Table 6.8. Contribution (as a percent) of different body parts to the total amount of bioaccumulated compounds for different classes of analytes in sheepshead minnows exposed to TNT, RDX and HMX (from Lotufo 2011).

	Liver	Viscera	Gills	Body remains
	3.0 [a]	9.1 [a]	2.1 [a]	85.8 [a]
TNT				
Total	45.6	22.1	1.7	30.6
Solvent-extracted	36.3	32.3	2.0	29.4
TNT	0.5	63.7	2.6	33.3
Am-DNTs	63.1	18.7	4.2	14.0
RDX				
Total	8.4	13.4	5.7	72.5
Solvent-extracted	6.1	15.6	5.2	73.1
RDX	5.0	26.3	8.7	60.1
HMX				
Total	11.5	13.3	6.6	68.6
Extracted	11.9	17.4	7.7	63.0

Total - percent of the total concentration of parent and all transformation products compounds in the body part, Solvent-extracted - acetonitrile-extracted compounds measured using radioactivity, [a] contribution (as percent) of each body part to the total fish biomass is also provided.

Bioaccumulation from diet

The dietary uptake of TNT and RDX was examined using a marine fish, sheepshead minnows (Lotufo and Blackburn et al. 2010), and two freshwater fish, channel catfish (Belden et al. 2005a, 2005b) and fathead

minnow (Houston and Lotufo 2005) using different routes of contaminant delivery.

Juvenile channel catfish force-fed with ^{14}C-TNT-spiked food pellets daily for up to 10 d accumulated radioactivity, mostly as extractable and non-extractable unknown compounds. The ratio of the concentration in fish relative to the TNT concentration in the diet (0.000024 g g^{-1}), to the sum of extractable compounds (0.00048 g g^{-1}), and to the total parent and transformation products (including covalently bound compounds) (0.00091 g g^{-1}) were exceedingly low (Belden et al. 2005a).

Juvenile sheepshead minnows were fed fresh prey (the amphipod *Leptocheirus plumulosus*) pre-exposed to ^{14}C-TNT. Fish received a meal twice daily for up to 7 d (Lotufo and Blackburn 2010). Fathead minnows were fed frozen prey (*Lumbriculus variegatus* worms) pre-exposed to ^{14}C-TNT or ^{14}C-RDX. Fish received a meal twice daily for up to 14 d (Houston and Lotufo 2005). Those two studies used prey pre-exposed to ^{14}C-dichloro-diphenyltrichloroethane (^{14}C-DDT) as a positive control for dietary uptake in the latter study because that compound is substantially more hydro-phobic; hence, the compound will bioaccumulate to a greater extent than explosives. The body residues in the fish remained relatively unchanged over time for ^{14}C-TNT and ^{14}C-RDX. The ratio of the concentration in fish relative to the concentration in the diet, determined using total radioactivity in worms and fathead minnows were 0.018, 0.010, and 0.422 g g^{-1} for ^{14}C-TNT, ^{14}C-RDX, and ^{14}C-DDT, respectively (Houston and Lotufo 2005). Those ratios, determined using total radioactivity in amphipods and sheepshead minnows were 0.068 and 0.388 g g^{-1} for ^{14}C-TNT, and ^{14}C-DDT, respectively (Lotufo and Blackburn et al. 2010). Those low ratios confirm the expected low bioaccumulative potential for TNT and RDX through the dietary route.

To directly assess the relative importance of aqueous and dietary uptake of RDX, juvenile channel catfish were fed *L. variegatus* freshly-exposed to RDX, directly exposed to RDX-spiked water, or exposed to both, for 7 d (Belden et al. 2005b). The same concentration was used for the worm and fish aqueous exposures. The mean bioaccumulation factor (BAF) of 0.04 g g^{-1} obtained from that study for parent RDX was higher than the mean BAF (0.01 g g^{-1}) determined using radioactivity as a surrogate for RDX and its transformation products using fathead minnows (Houston et al. 2005). Results from the comparative exposure scenario study (Belden et al.

2005b) as well as from other dietary uptake investigations (Houston and Lotufo 2005; Belden et al. 2005a) indicated that aqueous exposure is likely the dominant route of exposure for TNT, RDX and similar compounds, with dietary uptake providing only minimal, if any, contribution to the net bioaccumulation of RDX in aquatic systems.

7 Fate, Toxicity, and Bioaccumulation of MC Released from Composition B under Realistic Exposure Conditions

This section is a synopsis of information published in Rosen and Lotufo (2010).

It is anticipated that over time MC will leak from munitions in the underwater environment, resulting in the slow discharge of these chemicals into the surrounding water and sediment on a relatively localized scale. It is also possible that low-order detonations will create fragments of MC (e.g., Composition B) that allow subsequent biological exposure. Although the extent of such exposure is largely unknown, low concentrations of some MC compounds have been measured in marine sediment (Darrach *et* al. 1998; Pascoe et al. 2010). The objective of this study was to evaluate the fate of Composition B (a military formulation containing TNT and RDX) fragments, or "fragments," in aquaria containing seawater and sediment under several different exposure scenarios over a period of approximately one month. The approach was intended to more closely reflect potential real-world exposure scenarios compared to traditional laboratory experiments that involve fortifying water or sediment with pure compounds to create concentrations high enough to elicit effects and develop toxicity or bioaccumulation metrics.

One fish and four different invertebrate species were included in the experimental exposure to more closely simulate real world conditions (i.e., a microcosm) than single species studies would. The authors of this study also intended to more accurately assess any differences in toxicity and bioaccumulative potential among species. Sandy, low organic carbon sediment was used as substrate to minimize binding of compounds to sediment, resulting in a worst-case scenario of MC bioavailability to ecological receptors. The build up of MC released from the fragments into the sediment, porewater and overlying compartment; bioaccumulation; and effects to fish and invertebrates under different treatments were investigated.

Experimental Design

A 3 by 2 factorial experimental design was used to investigate effects of Composition B placement ("Exposed" at sediment surface, "Buried," or no Composition B) and absence or presence of water exchange ("Static" vs. "Flow") on the concentration of MC in the overlying water, porewater, and uptake by organism tissues.

Experimental units were 20-L aquaria, which were replicated three times for each treatment type. An illustration of a typical tank setup is shown in Figure 7.1. Each tank received enough uncontaminated sandy sediment (Yaquina Bay, OR, USA; TOC = <0.1%) to yield a 3.5-cm thick layer. Cylindrical field exposure chambers were filled with 2 cm of sediment and gently pushed approximately 2 cm into the sediment so that animals could be added later through tubes extending above the water surface. Chambers were used for amphipods and polychaetes to ease their recovery from the aquaria, and to strategically control organism exposure. The chambers were positioned so that each would be approximately the same distance away from the Composition B fragments.
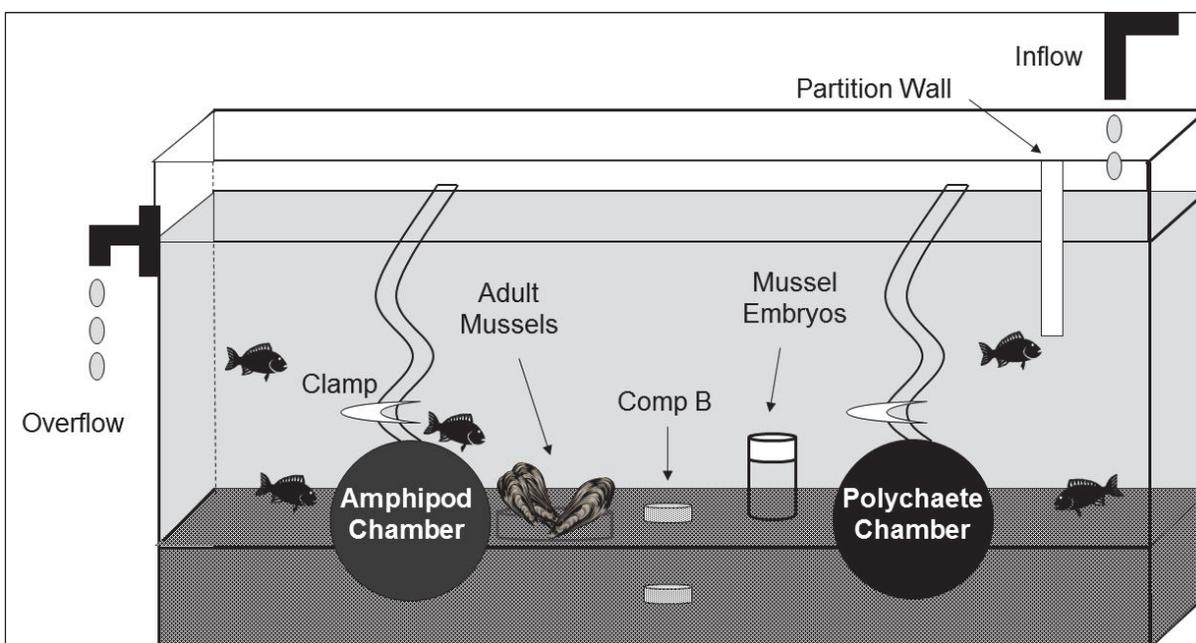
Figure 7.1. Exposure system used to assess fate and effects of Composition B.

Reconstituted seawater (Instant Ocean commercial salt mix in dechlorinated water) was gently added to the aquaria. Three Composition B exposure treatments were used: 1) "Exposed" at the sediment surface;

2) "Buried" at a depth of approximately 2 cm; and 3) "No Composition B" (control). Each Composition B treatment type was held under two different water exchange rates: 1) "Static," or 2) "Flow." The Flow treatment resulted in renewal of each tank with clean seawater at a rate of 0.5 turnovers per day (1 full exchange of overlying water every other day).

Except for control tanks, each tank received two Composition B fragments weighing a total of approximately 0.4 g (range = 0.39-0.41 g). Composition B fragments consisted of 59.5% RDX, 39.5% TNT, and 1% wax by mass and were obtained from the Holston Army Ammunition Plant (Kingsport, TN). Fragments were placed immediately adjacent to one another on (Exposed) or in (Buried) the sediment approximately equidistantly from all sides of the tank. Under this exposure scenario, overlying water samples were removed every other day for a period of 24 days for MC concentration. Following this period, test organisms were added for up to an additional 10 days, making the entire experiment a total of 34 days.

After the 24-d fate characterization period, test organisms were added to the tanks. Amphipods and polychaetes, both sediment dwellers, were added to the cylindrical chambers with mesh screens. Fish were added and allowed to swim freely throughout the tank. Small adult mussels were positioned in a shallow glass dish at the sediment-water interface. In addition, mussel embryos were housed in an open glass scintillation vial placed on the sediment surface. All organisms were exposed for 10 days, except the embryonic mussels, which were exposed for 48 h only. Tissue concentrations for relevant test organisms were measured to determine bioconcentration factors.

The microcosms were maintained at 18 °C with a 16: 8 h light: dark photoperiod. After 2 days, the embryonic mussels were removed from the tanks and assessed for normal development. After 10 days, all other test organisms were removed, assessed for survival and bioaccumulation. Porewater was sampled and analyzed upon experiment termination.

MC in the overlying water

At termination of the 34-d exposure period, approximately 15.5% of the initial Composition B mass had been lost from Exposed Composition B fragments, while only about 1.8% loss was observed from Buried Composition B fragments. Mean loss of fragment mass in both Exposed

and Buried fragments was about 10% greater in the presence of Flow, but this difference was not statistically significant.

The loss of fragment mass was largely attributable to dissolution into the overlying water. The concentration of SumTNT in the overlying water increased linearly in the Static Exposed (SE) treatment tanks for the first 10 days, and then leveled off for the remainder of the exposure (Figure 7.2). SumTNT concentration was highest in the SE treatment compared to any of the other three treatments. This was expected, as this was hypothesized to be the worst-case scenario treatment. Flow Exposed (FE) treatment tank TNT concentrations leveled off more quickly and were 62% lower than SE treatments, due to the loss of compounds through gradual water exchange. SumTNT was composed of 78% parent compound under Flow, but only 46% parent TNT under Static conditions.
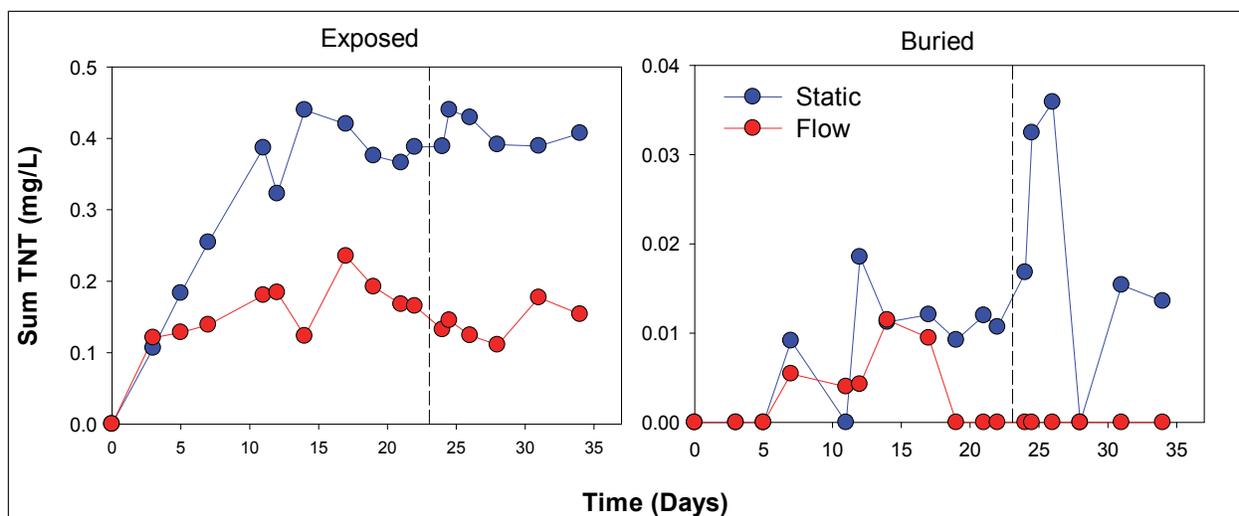
Figure 7.2. TNT concentration in the overlying water from exposures containing Composition B fragments under both static and flow conditions. Fragments were placed either at sediment surface ("Exposed"; left figure) or at a sediment depth of approximately 2 cm ("Buried"; right figure). Note the different scales for the y-axis. The vertical dashed line represents when test organisms were added (day 23).

RDX behaved similarly to TNT in that overlying water concentrations in Exposed tanks increased linearly for the first 10 days followed by stabilization under Static conditions, while steady-state conditions occurred much more quickly (by day 3) under Flow conditions (Figure 7.3). It is interesting to note that unlike TNT, RDX started to increase linearly again in the SE treatment following the addition of the test organisms (Figure 7.3). It is unclear why this occurred, but it could be associated with bioturbation around the fragment by the various test organisms as well as added circulation of water around the fragment associated with the addition of

gentle aeration to the system upon the addition of organisms. As with TNT, RDX did not appreciably partition into the overlying water in treatments in which Composition B was buried.
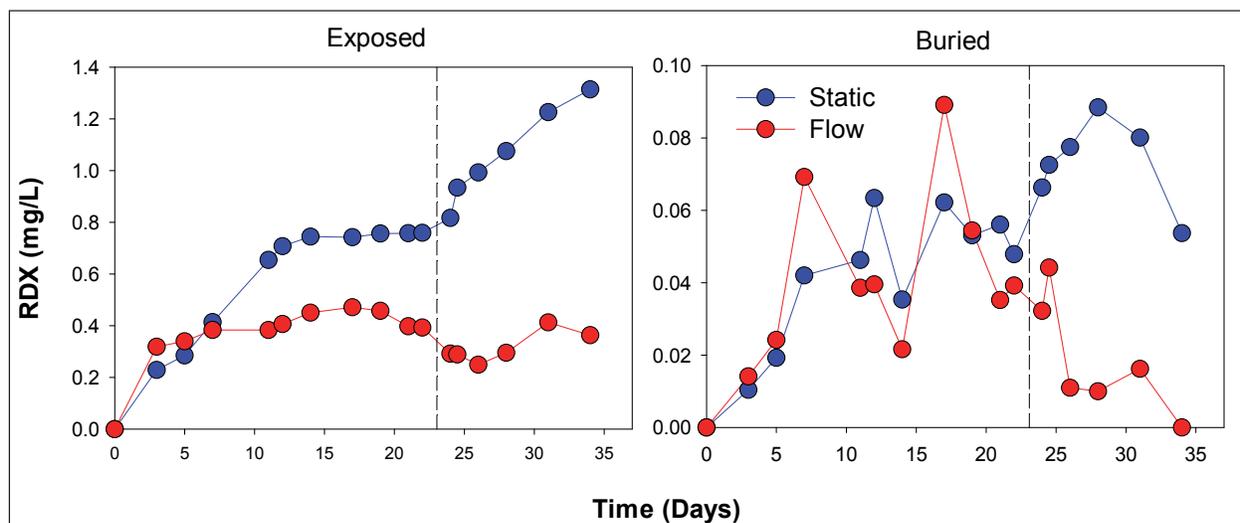
Figure 7.3. The concentration of RDX in the overlying water from exposures containing Composition B fragments, under both static and flow conditions. Fragments were either placed at sediment surface ("Exposed" left figure) or at a sediment depth of approximately 2 cm ("Buried"; right figure). Note the different scales for the y-axis. Vertical dashed line represents when test organisms were added (day 23).

Both TNT and RDX concentrations in the overlying water (up to 0.4 and 1.3 mg L^{-1}, respectively) were substantially lower than the approximate expected solubility limit for those compounds (130 mg L^{-1} and 40 mg L^{-1} for TNT and RDX, respectively). While the Composition B fragment served as the source of RDX and TNT to the overlying water, volatilization, transformation to non-identified compounds, and some binding to sediment likely contributed towards the higher apparent rate of removal of TNT from that compartment. Based on the temporal trend for the compounds, increasing concentrations of RDX but stable concentrations of TNT would be predicted for the days or weeks following the 34-d exposure period used in this study.

MC in the porewater

SumTNT and RDX concentrations were always higher directly under the fragment, as compared to concentrations in the overlying water, but the difference was most dramatic for Static exposures, both when the Composition B fragment was Exposed and Buried (Figure 7.4). Porewater TNT and RDX concentrations under the fragment were as much as 1 order of magnitude higher than the overlying water concentrations in SE
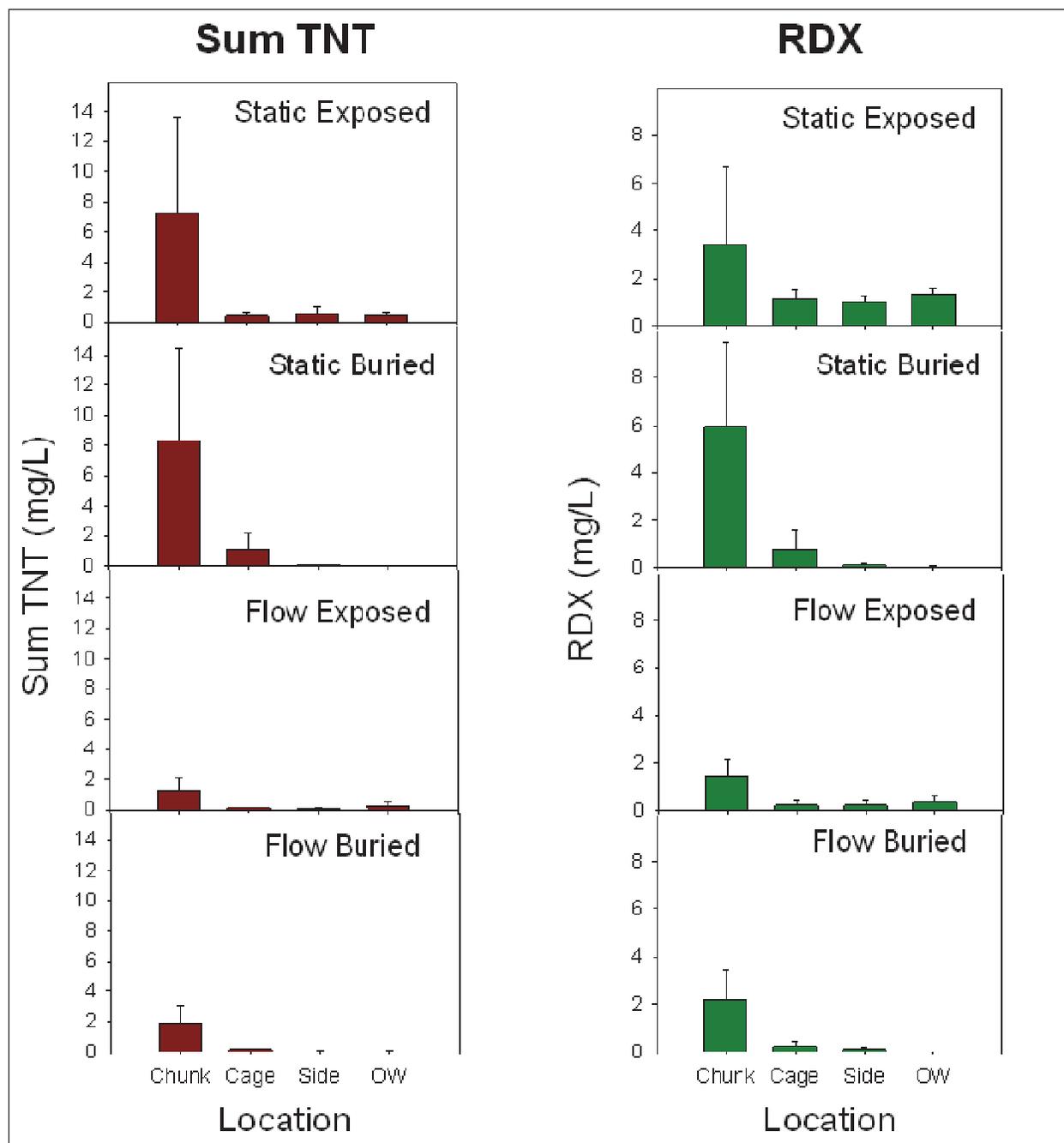
Figure 7.4. Mean (± 1 standard deviation) SumTNT (left) and RDX (right) porewater concentrations at termination of exposures with Composition B fragments. Porewater was sampled directly beneath the Composition B fragment ("Chunk") in the center of each tank, and at increasing distance away from the fragment, next to exposure chamber ("Cage"), and adjacent to edge of glass tank ("Side"). Overlying water (OW) concentrations are also shown for comparison.

treatments and up to over 2 orders of magnitude higher than the overlying water in the Static Buried treatments, yet still well below the solubility limits for these compounds. The potential for heightened exposure to organisms residing in the sediment, however, appears to be limited to the immediate

vicinity to the fragment, as concentrations approached the relatively low overlying water concentrations just a few cm from the Composition B fragments (Figure 7.4). In addition, in the presence of Flow, porewater concentrations directly under the Comp B fragments were only minimally elevated relative to overlying water concentrations (Figure 7.4).

Toxicity of MC released from Composition B

No significant reductions in survival were observed for any test species relative to control tanks without Composition B fragments (Table 7.1). This was not unexpected, as water concentrations did not achieve previously reported lethal concentrations for amphipod, polychaete, minnow, or adult mussel (see Section 2). Somewhat reduced survival in some amphipod and polychaete treatments is likely related to issues associated with recovery from the field exposure chambers, as opposed to effects associated with contaminant exposure, as some reduced survival was also observed in the control treatments (Table 7.1).

Table 7.1. Test organism survival, and embryo-larval development success following exposures with Composition B fragments.

Species	Static Control	Flow Control	Static Exposed	Flow Exposed	Static Buried	Flow Buried
Survival after 10-d exposure [a]						
Sheepshead minnow *Cyprinodon variegates*	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100
Mussel (adult) *Mytilus galloprovincialis*	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100
Amphipod *E. estuarius*	100 (0)	90 (10)	90 (7.1)	90 (10)	90 (5)	95 (5)
Amphipod *L. plumulosus*	62 (2.9)	80 (7.1)	75 (21)	63 (13)	73 (20)	77 (32)
Polychaete *Neanthes arenaceodentata*	87 (11)	80 (0)	70 (14.1)	80 (20)	87 (12)	93 (12)
Development success after 2-d exposure [a]						
Mussel (embryo) *Mytilus galloprovincialis*	70 (16)	74 (16)	0	85 (13)	73 (22)	85 (13)

[a] Data is presented as mean (± 1 standard deviation)

The only test endpoint in which statistically significant effects were observed was for mussel embryo-larval development in which the SE treatment, the treatment representing the worst-case scenario, resulted in no normally developed larvae (Table 7.1). No other treatments resulted in significantly lower larval development success when compared to controls. The impacts to mussel larval development in the SE treatment are explained by relatively high overlying water TNT concentrations (0.4 mg L^{-1}) measured on the day that the mussel embryo exposures were initiated, which approached thresholds for TNT exposure in an experiment earlier in this study (median effective concentration = 0.75 mg L^{-1}; Rosen and Lotufo 2007a; see Section 4).

Bioaccumulation of MC released from Composition B

The bioaccumulation of TNT and RDX from Composition B and their transformation products in fish, mussel, polychaetes and amphipods were measured at the end of the 10-d exposures. Whole body concentrations (body burden) of RDX and SumTNT, for those tissues in which these compounds were detectable, are presented in Table 7.2. Neither TNT nor RDX were detectable in amphipod or polychaete tissues. Bioconcentration values for fish and mussel tissues were similar overall, and corresponded with overlying water concentrations, with SE (worst case) treatments having the highest body burdens.

Table 7.2. Body burdens from Composition B fragment exposures

Species	Treatment	Body Burden[a]		
		RDX (nmol g^{-1})	SumTNT (nmol g^{-1})	Am-DNTs as % of SumTNT
Sheepshead minnow *Cyprinodon variegatus*	Static Exposed	35.3 (29.9)	28.2 (12.6)	89 (6.6)
	Static Buried	0.4 (0.7)	BDL	BDL
	Flow Exposed	10.8 (4.4)	13.7 (4.4)	79 (4.4)
	Flow Buried	BDL	BDL	BDL
Mussel (adult) *Mytilus galloprovincialis*	Static Exposed	46.4 (9.1)	31.1 (8.2)	82 (8.4)
	Static Buried	BDL	BDL	BDL
	Flow Exposed	31.5 (7.5)	18.7 (8.5)	34 (5.1)
	Flow Buried	BDL	BDL	BDL
Amphipod *Eohaustorius estuarius*[b]	Static Exposed	108	74.4	87
	Static Buried	BDL	BDL	BDL
	Flow Exposed	BDL	BDL	BDL

[a] Data is presented as mean (± 1 standard deviation), [b] Replicates composited due to insufficient tissue mass. BDL - below detection limit.

Whole body residues were below levels associated with biological effects determined in previous studies (Section 3). The highest mean body residue in mussels (Table 7.2) was lower than the no-observed-effect residues (NOER) reported for that species (45.3 and 86.2 nmol g^{-1}, for TNT and RDX, respectively) (Rosen and Lotufo 2007a). The highest mean body residues in the sheepshead minnow were 28.2 nmol g^{-1} sumTNT (the sum of 25.1 nmol g^{-1} Am-DNTs and 3.1 nmol g^{-1} TNT) and 35.3 nmol g^{-1} RDX. Those residues are lower by approximately 6-fold relative to the 5-d median lethal residue (LR$_{50}$) determined for TNT (20.4 nmol g^{-1}), 2-Am-DNT (275 nmol g^{-1}), and approximately 4-fold relative to the RDX 10-d NOER (135 nmol g^{-1}) (Lotufo et al. 2010a, 2010b). Because of the large difference in the toxicity of TNT and Am-DNTs, measurement of the concentration of the TNT and its major transformation products in the exposure water is critical for the appropriate interpretation of body residue data.

Conclusions

This laboratory experiment simulated real world exposure of marine receptors to Composition B following a low order detonation, providing insight into more realistic assessment of fate and effects of MC in underwater environments. A conservative flow rate resulted in substantially reduced TNT and RDX overlying and porewater concentrations (2-3 fold) and lower body residues (about a factor of 2) when compared to static treatments. Burial of Composition B fragments resulted in very limited movement of MC compounds to overlying water and no detectable tissue concentrations to organisms. Therefore, it is expected that buried munitions in the field would pose a lower risk to organisms inhabiting the water column relative to surface exposed munitions. Porewater TNT and RDX concentrations were highest immediately under the Composition B fragments, yet were dramatically lower a few centimeters from the fragment, suggesting only very localized elevated exposure potential to infaunal organisms residing directly under Composition B fragments. TNT and RDX approached steady concentrations in overlying water and porewater that were much lower than maximum attainable concentrations based on solubility limits of these compounds. Adverse effects to amphipods, adult mussels, fish, and polychaetes under these conservative exposure scenarios (e.g. low or no flow, sandy sediment) were not observed. Mussel embryo-larval development was the only endpoint for which effects were observed, and only under static conditions with exposed fragment. As this experiment was designed to represent a worst case scenario, and resulted in little bioaccumulation and toxicity, it is expected that ecological risk from Composition B, and likely other MC, in marine systems is negligible.

8   Summary of aquatic toxicology data for select MC

This section provides an abbreviated summary of the available data on fate toxicity and bioaccumulation of explosive MC in aquatic environments. More detailed information and citations are provided in Sections 2-7 of this report.

TNT

The molecular formula for 2,4,6-trinitrotoluene (TNT) is $C_7H_5N_3O_6$, with a corresponding molecular weight of 227.1 g mol^{-1}. At room temperature, TNT is a yellow solid that is slightly soluble in water. The water solubility of TNT is 130 mg L^{-1} at 25°C. Dissolved TNT will diffuse and disperse with the water in which it is dissolved.

Photochemical transformation (i.e., photolysis or photooxidation) of TNT in aqueous solutions has been investigated. When associated with organic matter in the water column, soil or sediment, TNT typically transforms, sometimes extensively, to reduced aminated compounds, first to produce aminodinitrotoluene (Am-DNT) and preferentially 4-Am-DNT, with further reduction producing diaminonitrotoluene (DANT) and preferentially 2,4-DANT. Strong sorption to sediment organic matter not attributed to hydrophobic partitioning has been reported for both freshwater and marine sediment.

In water, lethal toxicity values, reported for ten marine species, varied widely, from 0.98 mg L^{-1} (LC$_{50}$ for a mysid shrimp) to 19.5 mg L^{-1} (LC$_{50}$ for the adult stage of a mussel). Lethal toxicity to freshwater species has been reported within a similar range. Sublethal toxicity has been reported as impairment of embryo development at 0.75 mg L^{-1} (LOEC) for a mussel and 12 mg L^{-1} (reported effect concentration) for a sea urchin, as well as decrease growth of an alga germling at 0.61 mg L^{-1} (EC$_{50}$). Experiments on the fate and effects of TNT on fish showed no significant impacts on body indices, hematological variables, hepatic detoxification, and antioxidant enzyme activities.

In sediment, lethal toxicity values, reported for three marine invertebrate species, varied from 38 mg kg^{-1} (LOEC for an amphipod) to 508 mg kg^{-1} (LOEC for a polychaete). Lethal toxicity values for freshwater invertebrates, as LOEC, varied widely from 0.1 mg kg^{-1} to 69 mg kg^{-1}.

Bioconcentration factor values were 0.31 ml g^{-1} (for a mussel) and 9.71 ml g^{-1} (for sheepshead minnow), indicating that tissue residues are expected to reach only up to few folds the exposure concentration. For freshwater species, bioconcentration factor values ranged from 0.8 to 5.0 ml g^{-1}. TNT is efficiently transformed to 2-Am-DNT and 4-Am-DNT in the tissue. Parent and transformation products are quickly eliminated. Elimination half lives were 0.33 (mussel) and 0.91 h (sheepshead minnow), indicating that release from exposure would result in fast depuration and likely recovery from toxic effects.

2-Am-DNT

The molecular formula for 2-amino-4,6-dinitrotoluene (2-Am-DNT) is $C_7H_7N_3O_4$ with a corresponding molecular weight of 197.2 g mol^{-1} (USEPA 2012). The water solubility of 2-Am-DNT is 42 mg L^{-1} at 25°C. The presence of 2-Am-DNT in the environment results primarily from the microbial reduction of the 2-nitro group of TNT.

In water, the lethal toxicity values for 2-Am-DNT (8.6 mg L^{-1}, LC$_{50}$ for sheepshead minnow and a marine copepod (no lethal effects at 50 mg L^{-1}) were higher than those for TNT. The toxicity of 2-Am-DNT was lower than that of TNT for freshwater fish and most invertebrate species investigated with the exception being cladocerans. Marine toxicity data for 2-Am-DNT in sediment have not been found. For a freshwater midge, 2-Am-DNT was less toxic (59 mg kg^{-1}, LOEC) than TNT.

A single bioconcentration factor value (13.1 ml g^{-1} for sheepshead minnow) has been reported. For freshwater species, bioconcentration factor values were 10.2 ml g^{-1} for 2-Am-DNT and 12.4 for 4-Am-DNT. Elimination half-life was 0.72 h (sheepshead minnow), indicating that release from exposure would result in fast depuration and likely recovery from toxic effects.

4-Am-DNT

The molecular formula for 4-amino-2,6-dinitrotoluene (4-Am-DNT) is $C_7H_7N_3O_4$ with a corresponding molecular weight of 197.2 g mol^{-1}. The

water solubility of 4-Am-DNT is 42 mg L^{-1} at 25°C. No data on the fate, biotransformation or marine toxicity was found for 4-Am-DNT. The presence of 4-Am-DNT in the environment results primarily from the bacterial reduction of the 4-nitro group of TNT.

Lethal toxicity values for freshwater aquatic vertebrates and invertebrates varied widely, from 5.1 mg L^{-1} (LC$_{50}$ for a cladoceran) to 22.7 mg L^{-1} (LC$_{50}$ for the larval stage of a frog). Sublethal reproductive effects (EC$_{50}$) on a freshwater cladoceran were noted at 5.1 mg L^{-1}. Freshwater sediment toxicity data for 4-Am-DNT have not been found.

Although marine bioaccumulation data for 4-Am-DNT have not been found, based on the low log K$_{ow}$ the potential for bioconcentration in aquatic organisms is considered low. A single freshwater bioconcentration factor (12.4 ml g^{-1} for an oligochaete) has been reported.

2,4-DANT

The molecular formula for 2,4-diamino-6-nitrotoluene (2,4-DANT) is $C_7H_9N_3O_2$ with a corresponding molecular weight of 167.2 g mol^{-1}. The presence of 2,4-DANT in the environment results primarily from the microbial reduction of the 2 and 4-nitro group of TNT.

In saline water, exposure to 2,4-DANT at 50 mg L^{-1} failed to promote lethal effects on sheepshead minnows or copepods, suggesting that near complete nitro group reduction may substantially decrease the toxicity of TNT. Conversely, the toxicity of 2,4-DANT was substantially higher (0.05 mg L^{-1}, LOEC) than that of TNT for the reproduction of a cladoceran. In sediment, lethal toxicity values reported for two invertebrate species, were 3.0 mg kg^{-1} (LOEC for an amphipod) and 574 mg kg^{-1} (LOEC for a polychaete). For freshwater invertebrates, sediment-associated 2,4-DANT (0.3 and 59 mg kg^{-1} LOEC for an amphipod and midge, respectively) and TNT were toxic at similar concentrations. Marine or freshwater toxicity data for 2,4-DANT in sandy sediment have not been found.

A single bioconcentration factor value (0.51 ml g^{-1} for sheepshead minnow) has been reported. A bioconcentration factor of 2.8 ml g^{-1} has been reported for a freshwater oligochaete. The elimination half life for 2,4-DANT for sheepshead minnow was 0.25 h, indicating that release from exposure would result in fast depuration and likely recovery from toxic effects.

2,6-DANT

The molecular formula for 2,6-diamino-4-nitrotoluene (2,6-DANT) is $C_7H_9N_3O_2$ with a corresponding molecular weight of 167.2 g mol^{-1}. The presence of 2,6-DANT in the environment results primarily from the microbial reduction of the 2 and 6-nitro group of TNT. No data on the fate, biotransformation or marine toxicity was found for 2,6-DANT.

In water, exposure to 2,6-DANT at 50 mg L^{-1} failed to promote lethal effects on marine copepods (Won et al. 1976). The toxicity of 2,6-DANT was substantially lower (0.33 mg L^{-1}, LOEC) than that of TNT for the reproduction of a cladoceran. No sediment toxicity data for 2,6-DANT were found.

In sediment, lethal toxicity values, reported for two invertebrate species, were 3.0 mg kg^{-1} (LOEC for an amphipod) and 574 mg kg^{-1} (LOEC for a polychaete). For freshwater invertebrates, 2,4-DANT (0.3 and 59 mg kg^{-1} LOEC for an amphipodand midge, respectively) and TNT were toxic at similar concentrations. Marine or freshwater toxicity data for 2,4-DANT in sandy sediment have not been found.

A single bioconcentration factor value (0.51 ml g^{-1} for sheepshead minnow) has been reported. A bioconcentration factor of 2.8 ml g^{-1} has been reported for a freshwater oligochaete. The elimination half life for 2,4-DANT for sheepshead minnow was 0.25 h, indicating that release from exposure would result in fast depuration and likely recovery from toxic effects.

1,3,5-TNB

The molecular formula for 1,3,5-trinitrobenzene (1,3,5-TNB) is $C_6H_3N_3O_6$, with a corresponding molecular weight of 213.1 g mol^{-1} (USEPA 2012). At room temperature, 1,3,5-TNB is a clear to light yellow solid. The water solubility of 1,3,5-TNB is 385 mg L^{-1} at 25°C. 1,3,5-TNB is also formed as a by-product of TNT manufacture and as a product of TNT environmental transformation, including its photolysis. Extensive transformation of 1,3,5-TNB has been shown to occur in freshwater and marine sediment. Reductive transformation of 1,3,5-TNB, leading to the formation of dinitroaniles (or amino-dinitrobenzenes) has been reported. In marine and freshwater fine-grained sediment, extensive transformation of parent compound to aminated products has been reported.

In water, lethal toxicity values, reported for four marine species, varied narrowly from 0.74 mg L^{-1} (LC$_{50}$ for a mysid shrimp) to 1.6 mg L^{-1} (LC$_{50}$ for a polychaete). Lethal toxicity to freshwater species has been reported within a similar range (1.0 – 2.7 mg L^{-1}). Sublethal toxicity to marine invertebrates has been reported as impairment of sea urchin embryo development at 1.3 mg L^{-1} (LOEC) and impaired reproduction for a polychaete (0.4 mg L^{-1}, EC$_{50}$).

In sediment, lethal toxicity values, reported for two marine invertebrate species, were 13 mg kg^{-1} (LOEC for an amphipod) and 107 mg kg^{-1} (highest concentration tested, no significant effect for a polychaete). The lowest lethal toxicity value for a freshwater invertebrate was 1 mg kg^{-1} (LOEC for an amphipod). Although no data for the bioaccumulation of 1,3,5-TNB in marine or freshwater fish and invertebrates were found, based on the low log K$_{ow,}$ the potential for bioconcentration in aquatic organisms is considered low.

2,4-DNT

The molecular formula for 2,4-dinitrotoluene (2,4-DNT) is C$_7$H$_6$N$_2$O$_4$ with a corresponding molecular weight of 182.2 g mol^{-1}. At room temperature, 2,4-DNT is a yellow crystalline solid. The water solubility of 2,4-DNT is 270 mg L^{-1} at 25°C. Dissolved 2,4-DNT will diffuse and disperse with the water it is dissolved in.

DNTs are a primary product in propellants and are also formed as a byproduct during the TNT manufacturing process. There are six isomers of DNT, the most common one is 2,4-DTN. Technical grade DNT is composed of approximately 75% 2,4-DNT, 20% 2,6-DNT, and 5% other isomers. Like TNT, DNTs can be reduced to the corresponding mono or diamino derivatives. Strong sorption of 2,4-DNT to soil organic matter has been reported. There are six isomers of DNT, the most common one is 2,4-DNT.

In water, lethal toxicity values, reported for four marine species, varied widely, from 4.4 mg L^{-1} (LC$_{50}$ for a mysid shrimp) to 48 mg L^{-1} (LC$_{50}$ for redfish). Sublethal toxicity to marine invertebrates has been reported as impaired sea urchin embryo development at 10 mg L^{-1} (LOEC) and impaired polychaete reproduction (5.2 mg L^{-1}, EC$_{50}$). No toxicity data for 2,4-DNT in marine or freshwater sediment were found.

Although no data for the bioaccumulation of 2,4-DNT in marine fish and invertebrates were found, based on the low log K_{ow} the potential for bioconcentration in aquatic organisms is considered low. For freshwater species, reported bioconcentration factor values were 4.5 ml g^{-1} for gold fish and 9.2 ml g^{-1} for carp.

2,6-DNT

The molecular formula for 2,6-dinitrotoluene (2,6-DNT) is $C_7H_6N_2O_4$ with a corresponding molecular weight of 182.2 g mol^{-1}. At room temperature, 2,6-DNT is a tan crystalline solid. The water solubility of 2,6-DNT is 206 mg L^{-1} at 25°C. Dissolved 2,6-DNT will diffuse and disperse with the water it is dissolved in.

In saline water, lethal toxicity values, reported for three marine species, varied widely, from 5.0 mg L^{-1} (LC$_{50}$ for a mysid shrimp) to 28 mg L^{-1} (LC$_{50}$ for redfish). Lowest sublethal toxicity values to marine invertebrates were reported as impaired sea urchin embryo development at 6.7 mg L^{-1} (LOEC) and impaired reproduction (2.1 mg L^{-1}, EC$_{50}$ for a polychaete). In marine sediment, no significant lethal effects to an amphipod were observed at the highest 2,6-DNT measured tested concentration (5 mg kg^{-1}).

Although no data for the bioaccumulation of 2,6-DNT in marine fish and invertebrates has been found, the potential for bioconcentration in aquatic organisms is considered low based on its low log K_{ow}. For freshwater species, the sole reported bioconcentration factor value was 1.7 ml g^{-1} for carp.

Tetryl

The molecular formula for tetryl (methyl-2,4,6-trinitrophenylnitramine) is $C_7H_5N_5O_8$ with a corresponding molecular weight of 287.2 g mol^{-1}. At room temperature, tetryl is a yellow, crystalline solid. The water solubility of tetryl is 75 mg L^{-1} at 25°C. Dissolved tetryl will diffuse and disperse with the water it is dissolved in.

Photolysis and hydrolysis are major environmental transformation processes for tetryl in aqueous media. In natural, sunlit waters, tetryl is transformed via photolysis and hydrolysis to several degradation products, including picric acid, which is the primary hydrolysis product of tetryl. Information on the fate of tetryl in sediments is limited, and it is reported

that the formation of unknown breakdown products occurs in sandy and fine-grained marine sediments spiked with tetryl. Picric acid, a primary hydrolysis product of tetryl, was detected in sandy sediment only.

In saline water, lethal toxicity values, reported for three marine species, varied widely, from 0.03 mg L^{-1} (LC$_{50}$ for a polychaete) to 1.1 mg L^{-1} (LC$_{50}$ for redfish). Sublethal toxicity to marine invertebrates has been reported as impaired polychaete reproduction (0.01 mg L^{-1}, EC$_{50}$), impaired sea urchin embryo development (0.05 mg L^{-1}, EC$_{50}$), and decrease growth of an alga germling at 0.20 mg L^{-1} (EC$_{50}$). In sediment, the sole lethal toxicity value reported was 4.0 mg kg^{-1} (LOEC for sandy sediment) for an amphipod species (Table 5.3). Although no data for the bioaccumulation of tetryl in marine or freshwater fish and invertebrates were found, based on the low log K$_{ow,}$ the potential for bioconcentration in aquatic organisms is considered low.

Explosive D (Ammonium Picrate)/Picric Acid

The molecular formula for ammonium picrate (2,4,6-trinitrophenol ammonium salt), which is also known as Explosive D, is $C_6H_3N_3O_7 \cdot H_3N$ with a corresponding molecular weight of 246.1 g mol^{-1}. Ammonium picrate is a salt formed from picric acid and ammonium cation. The molecular formula for picric acid is $C_6H_3N_3O_7$ with a corresponding molecular weight of 229.1 g mol^{-1}. At room temperature, ammonium picrate and picric acid are bright yellow, crystalline solids. The water solubility of ammonium picrate is 10,200 mg L^{-1} and for picric acid is 12,800 mg L^{-1}, both at 25°C. Ammonium picrate is an ammonium salt of picric acid and under normal aqueous conditions will dissociate to ammonium and picrate ions and will reside in the water column as picric acid.

In sediment slurry tests, picric acid was found to remain in the aqueous phase with little partitioning to the sediments. Transformation to picramic acid was limited. It is likely that the ions transform further depending on the oxidation-reduction potential.

Similar to TNT, picric acid is also degraded through reduction, and the microbial biodegradation of picric acid results in the formation of 2A-dinitrophenol. Under aerobic conditions, the mono- or dinitrophenol can then be oxygenated with subsequent ring cleavage. Investigation of the fate of picric acid in marine sediments revealed that transformation rates were highest in incubated, fine-grained sediment and major transforma-

tion products included 4-dinitrophenol, aminodinitrophenols (including picramic acid), 3,4-diaminophenol, amino nitrophenol, and nitrodiaminophenol. Picric acid has been reported to bind to sediment and resist hydrolysis, biodegradation and photolysis.

In water, lethal toxicity values for picric acid, reported for seven marine species, varied widely, from 13 mg L^{-1} (LC_{50} for a mysid shrimp) to 265 mg L^{-1} (LC_{50} for a polychaete). Sublethal toxicity as impaired reproduction has been reported as 60 mg L^{-1} for a copepod and 155 mg L^{-1} for a polychaete (EC_{50} values), 28 mg L^{-1} for impairment of oyster shell deposition (EC_{50}), 281 mg L^{-1} for impairment of sea urchin embryo development (EC_{50}), and decrease growth of an alga germling at 94 mg L^{-1} (EC_{50}). The breakdown products picramic acid and 2,4-DNP have been reported as more toxic than the parent compound for marine species, typically by about an order of magnitude. In freshwater species, there was about an order of magnitude difference in toxicity for trout and a cladoceran but three orders of magnitude greater toxicity for 2,4-DNP to the bluegill.

In marine sediment, the sole lethal toxicity value was 162 mg kg^{-1} (LOEC for sandy sediment) for an amphipod species. In fine-grained sediment, amphipod mortality was highest at an intermediate concentration in which picric acid was below the detection limit, with the toxicity attributed to unknown transformation products. Although no data for the bioaccumulation of picric acid in marine or freshwater fish and invertebrates were found, based on the low log K_{ow} the potential for bioconcentration in aquatic organisms is considered low.

RDX

Royal Demolition Explosive, or RDX (cyclo-1,3,5-trimethylene-2,4,6-trinitramine), has the molecular formula $C_3N_6O_6H_6$ and has a molecular weight of 222.26 g/mol. At room temperature, RDX is a colorless crystal. The water solubility of RDX is 56.3 mg L^{-1} at 25°C. Military grade RDX generally contain up to 10% HMX as an impurity of the manufacturing process.

The log K_{oc} is 2.00 and indicates that moderate partitioning into organic carbon will occur. However, the log K_{ow} value is very low such that almost no partitioning into a non-polar substance will occur. With all physical factors of RDX taken into account, it is a compound that will reside on the

water body floor as a solid with dissolution into the water column over time.

The biodegradation of RDX occurs under both aerobic and anaerobic conditions but the anaerobic process is significantly faster. Unlike nitroaromatic explosives, RDX undergoes ring cleavage and mineralization under both aerobic and anaerobic conditions. Aerobically, it has been found that microbial cleavage of one of the $N-NO_2$ bonds produces unstable intermediates. Subsequent degradation reactions are largely governed by the interactions of the newly formed intermediates with water and eventually produce NO^-_2, N_2O, NH_3, HCHO, and HCOOH. Anaerobically, sequential reduction of the nitro groups to produce mono-, di- and trinitroso derivatives (MNX, DNX and TNX, respectively) is most frequently observed. The nitroso derivatives may be further transformed to produce the unstable hydroxylamine derivatives, leading to ring cleavage.

A study of microbial degradation of RDX in tropical marine sediments collected in Hawaii indicated that cyclic nitramine contaminants are likely to be degraded upon release from munitions into tropical marine sediment, suggesting natural attenuation *in situ*. Extensive transformation of RDX occurred following spiking to fine-grained sediment, but the transformation products were not identified. Different from the fate of RDX in organically-rich sediment, transformation was minimal in sandy sediment.

In water, lethal toxicity was evaluated for sixteen marine species. For most species, no significant lethality has occurred at concentrations reported as at or near the solubility limit. Effects concentration ranged from 2.4 mg L^{-1} (LC_{50} for speckled sand dab) to 26 mg L^{-1} (EC_{50} for reproduction impairment of a polychaete). Lethal toxicity to freshwater species has been reported within similar range.

In sediment, no significant lethality has been reported for marine or freshwater species in sandy and fine-grained sediments, with no effects concentrations ranging from 102 to 2229 mg kg^{-1}.

Bioconcentration factor values were 0.7 ml g^{-1} (for a mussel) and 1.7 ml g^{-1} (for sheepshead minnow), indicating that tissue residues are expected to only slightly exceed the exposure concentration. For freshwater species, bioconcentration factor values ranged from 1.0 to 2.1 ml g^{-1}. No reports of RDX biotransformation in fish or invertebrates were found. Elimination

half lives were 0.27 (mussel) and 7.7 h (sheepshead minnow), indicating that release from exposure would result in fast depuration and likely recovery from toxic effects.

HMX

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, also known as high-melting explosive (HMX), is a colorless polycrystalline solid with a molecular formula of $C_4H_8N_8O_8$ and a molecular weight of 296.20 g/mol. The water solubility of HMX is 4.5 mg L^{-1} at 25°C. Military grade RDX generally contains up to 10% HMX as an impurity of the manufacturing process (Talmage et al. 1999).

HMX exists in four polymorphic forms: α, β, γ, and δ. The β form of HMX is the least sensitive to impact, and the most preferred for use as a high explosive. The log K_{ow} value for HMX is very low such that almost no partitioning into a non-polar substance will occur. With all physical factors of HMX taken into account, it is a compound that will reside on the water body floor as a solid with dissolution into the water column over time.

In water, lethal toxicity was evaluated for only two marine species. Concentrations reported as at or near the solubility limit have not caused significant lethality or sublethal effects. In freshwater, toxicity was evaluated for eight species; only fathead minnows at 7 d post hatch were lethally impacted at the target concentration of 15 mg L^{-1}, which is higher than the reported solubility limit.

In sediment, no significant lethality was reported for marine or freshwater species in fine-grained sediments, with no effects concentrations ranging from 115 to 353 mg kg^{-1}.

Bioconcentration factor values were 0.31 ml g^{-1} (for a mussel) and 0.51 ml g^{-1} (for sheepshead minnow), indicating that tissue residues are expected to be lower than the environmental concentration. No reports of HMX biotransformation in fish or invertebrates were found. Elimination half-lives were 1.5 (mussel) and 5.8 h (sheepshead minnow), indicating that release from exposure would result in fast depuration and likely recovery from toxic effects.

Nitroguanidine

Nitoguanidine is also known by its military abrievation: NQ. The molecular formula for nitroguanidine is $CH_4N_4O_2$ with a corresponding molecular weight of 104.1 g mol^{-1}. At room temperature, nitroguanidine is a colorless, crystalline solid. The water solubility of nitroguanidine is 4,400 mg L^{-1} at 25°C. Degradation of nitroguanidine was shown to be negligible in activated sludge under aerobic conditions and in sterile sludge under reducing conditions.

In water, no lethal effect to a marine copepod was reported at the highest concentration tested (683 mg L^{-1}). Similarly, exposure of freshwater fish and aquatic invertebrates to exceedingly high concentrations (1,024 to 2,714 mg L^{-1}) of nitroguanidine also failed to result in toxicity. No sediment toxicity data for nitroguanidine were found. Although no data for the bioaccumulation of nitroguanidine in marine or freshwater fish and invertebrates were found, based on the low log K_{ow}, the potential for bioconcentration in aquatic organisms is considered low.

Nitroglycerin

Nitroglycerin or glycerol trinitrate (propane-1,2,3-triyl trinitrate) is also known by its military abrievation: NG. Nitroglycerin has the molecular formula $C_3H_5N_3O_9$ with a corresponding molecular weight of 227.1 g mol^{-1}. The water solubility of nitroglycerin is 1,800 mg L^{-1} at 25°C. At room temperature, nitroglycerin is a colorless liquid. The predominant products of nitroglycerine via hydrolysis are calcium nitrate and calcium nitrite. Nitroglycerine has a low log K_{ow} value, suggesting hydrophilic behavior and a low log K_{oc} value, indicating limited sorption. Nitroglycerine has been documented to transform via microbial mediation in both aerobic and anaerobic conditions, ultimately forming glycerol.

No marine toxicity data for nitroglycerin were found. Lethal toxicity values for freshwater aquatic vertebrates and invertebrates varied widely, from 0.7 mg L^{-1} (LC_{50} for rainbow trout) to 16 mg L^{-1} (LOEC for a cladoceran). No freshwater sediment toxicity data for nitroglycerin were found. Although no data for the bioaccumulation of nitroglycerin in marine or freshwater fish and invertebrates were found, based on the low log K_{ow} the potential for bioconcentration in aquatic organisms is considered low.

Nitrocellulose

Nitrocellulose is also known by its military abrievation: NC. Nitrocellulose is a nitrate ester produced with varying levels of nitration. Thus, the molecular formula is variable and the molecular weight ranges from 10^5 to 10^6 g mol^{-1}. Nitrocellulose is insoluble in water. At room temperature, nitrocellulose is a filamentous yellowish-white particulate. Nitrocellulose is very recalcitrant to microbial degradation. No fate and biotransformation data for nitrocelullose were found.

No marine toxicity data for nitrocelullose were found. Studies with nitrocellulose indicated no toxicity at concentrations up to 1000 mg L^{-1} when tested with several species of freshwater fish and invertebrates. The overall lack of toxicity of nitrocellulose is likely a result of its insolubility in water. No freshwater sediment toxicity data for nitrocelullose were found. No data for the bioaccumulation of nitrocelullose in marine or freshwater fish and invertebrates were found.

PETN

PETN, or (pentaerythritol tetranitrate) is used in some booster charges, demolition explosives, and initiating explosives. PETN has the molecular formula $C_5H_8N_4O_{12}$ and has a molecular weight of 316.2 g/mol. At room temperature, PETN is a white, crystalline solid. The water solubility of PETN is uncertain with a reported range of 2.1 to 43 mg L^{-1} at 25°C. PETN is not prone to hydrolyze at ambient temperature and is recalcitrant to microbial degradation.

In water, no lethal effect to a marine copepod (the only marine organism tested) occurred at the highest concentration tested (32 mg L^{-1}). Similarly, exposure of a freshwater invertebrate to 49 mg L^{-1} (target concentration) also failed to produce a lethal effect, but immobilization was reported after 48 h. No sediment toxicity data for PETN were found. Although no data for the bioaccumulation of PETN in marine or freshwater fish and invertebrates were found, based on the low log K_{ow} the potential for bioconcentration in aquatic organisms is considered low.

DEGDN

Diethylene glycol dinitrate (DEGDN) is a colorless, odorless, viscous, oily liquid. DEGDN has the molecular formula $C_4H_8N_2O_7$ and has a molecular

weight of 196.1 g/mol. The water solubility of DEGDN is 4,000 mg L^{-1} at 25°C. Environmental fate studies indicate that DEGDN is a stable compound once dissolved in water.

No marine toxicity data for DEGDN were found. Lethal toxicity values for freshwater aquatic vertebrates and invertebrates varied from 90.1 mg L^{-1} (LC$_{50}$ for a cladoceran) to 491 mg L^{-1} (LC$_{50}$ for fathead minnow). No freshwater sediment toxicity data for DEGDN were found. Although no data for the bioaccumulation of DEGDN in marine or freshwater fish and invertebrates were found, based on the low log K$_{ow}$, the potential for bioconcentration in aquatic organisms is considered low.

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Appendix A: Acronyms and Abreviations

Am-DNT	Aminodinitrotoluene
AP	Ammonium perchlorate (Explosive D)
BCF	Bioconcentration Factor
BIP	Blow-in-Place
BRAC	Base Realignment and Closure
CBR	Critical Body Residue
DMM	Discarded Military Munitions
DNB	Dintrobenzene
DoD	Department of Defense
DANT	Diaminonitrotoluene
DEGDN	Diethylene glycol dinitrate
DNT	Dinitrotoluene
EGDN	Ethyleneglycoldinitrate
FUDS	Formally Used Defense Sites
HMX	High Melting Explosive (or octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
LD50	Lethal dose 50
log K_{OC}	Organic carbon soil partition coefficient
log K_{OW}	Octanol/water partition coefficient

MC	Munitions Constituents
MEC	Munitions and explosives of concern
NC	Nitrocellulose
NG	Nitroglycerine
NQ	Nitroguanidine
PEP	propellants, explosives, or pyrotechnics
PBX	Plastic or Polymer Bonded Exposives
PETN	Pentaerythrite tetranitrate
RDX	Royal Demolition Explosive (Cyclo-1,3,5-trimethylene-2,4,6-trinitramine, hexahydro-1,3,5-trinitro-1,3,5-triazine or cyclonite)
1,3,5-TNB	1,3,5-trinitrobenzenes
TNT	2,4,6-Trinitrotoluene
USACE	US Army Corps of Engineers
USEPA	US Environmental Protection Agency
UXO	Unexploded Ordnance

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14. ABSTRACT
Military munitions are present in waters around the world, including those waters located at current and former Department of Defense sites. This report provides a review of the aquatic ecotoxicology of munitions constituents (MC), including nitroaromatics (2,4,6-trinitrotoluene (TNT), dinitrotoluenes (DNTs), 1,3,5-trinitrobenzene (TNB), 2,4,6-trinitrophenylmethylnitramine (tetryl) and 2,4,6-trinitrophenol (picric acid)); nitrate esters (nitrocellulose (NC), pentaerythritoltetranitrate (PETN), nitroglycerine (NG)); and nitramines (hexahydro-1,3,5- trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)). The major focus of this report is on the fate and effects of MC in the marine environment. Most MC observed in this study rapidly degraded in aqueous exposure systems and nitroaromatics showed a significant binding affinity with organic matter. To support the assessment of risk from MC in aquatic environments, laboratory-based toxicity data have been derived for a variety of aquatic species for both lethal and sublethal exposure endpoints using spiked water or sediment. Frequently, unrealistically high concentrations were used to derive toxicity benchmarks. In general, nitramines were less toxic than nitroaromatics, with a wide range of sensitivity among species. MC are weakly hydrophobic and bioaccumulative potential was low, as expected. High elimination rates for MC resulted in a virtually complete loss of body residue within hours to days following transfer to clean water. Uptake of TNT resulted in the substantial formation of bound residues. For fish, aqueous exposure was the dominant route of exposure to explosive compounds, with dietary uptake providing only minimal contribution. More realistic exposures using Composition B and multiple species found the presence of munitions in aquatic environments unlikely to result in biological effects. Verification of this conclusion should be pursued by determining site-specific exposure risk.

15. SUBJECT TERMS Bioaccumulation Composition B	Explosives Munitions constituents Sediment	Toxicity Unexploded ordnance

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