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Tracing the cycling and fate of the munition, Hexahydro-1,3,5-trinitro-1,3,5-triazine in a simulated sandy coastal marine habitat with a stable isotopic tracer, ^{15}N-[RDX]

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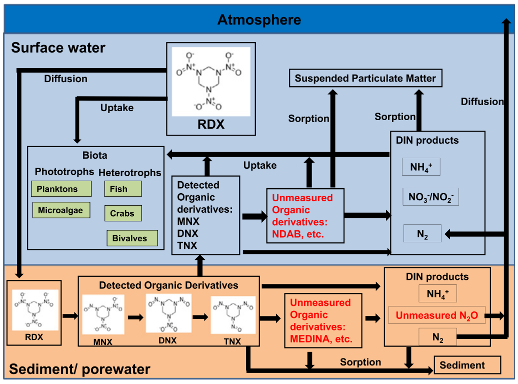
HIGHLIGHTS

- RDX transforms to inorganic nitrogen via series of organic derivatives.
- Coupled oxic and anoxic degradation help to transform RDX in marine environments.
- Major breakdown product of RDX is N_2 that majority escapes to the atmosphere.
- The low redox conditions in porewaters were likely important for RDX mineralization.
- RDX breakdown products can adsorb onto the sandy sediment in marine settings.

GRAPHICAL ABSTRACT



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ABSTRACT

Coastal marine habitats become contaminated with the munitions constituent, Hexahydro-1,3,5-trinitro-1,3,5-trazine (RDX), via military training, weapon testing and leakage of unexploded ordnance. This study used ^{15}N labeled RDX in simulated aquarium-scale coastal marine habitat containing seawater, sediment, and biota to track removal pathways from surface water including sorption onto particulates, degradation to nitroso-triazines and mineralization to dissolved inorganic nitrogen (DIN). The two aquaria received continuous RDX inputs to maintain a steady state concentration (0.4 mg L^{-1}) over 21 days. Time series RDX and nitroso-triazine concentrations in dissolved (surface and porewater) and sorbed phases (sediment and suspended particulates) were analyzed. Distributions of DIN species (ammonium, nitrate + nitrite and dissolved N_2) in sediments and overlying water were also measured along with geochemical variables in the aquaria. Partitioning of RDX and RDX-derived breakdown products onto surface sediment represented 13% of the total added ^{15}N as RDX (^{15}N-[RDX]) equivalents after 21 days. Measured nitroso-triazines in the aquaria accounted for 6–13% of total added ^{15}N-[RDX]. ^{15}N-labeled DIN was found both in the oxic surface water and hypoxic porewaters, showing that RDX mineralization accounted for 34% of the ^{15}N-[RDX] added to the aquaria over 21 days. Labeled ammonium ($^{15}NH_4^+$, found in

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sediment and overlying water) and nitrate + nitrite ($^{15}NO_X$, found in overlying water only) together represented 10% of the total added ^{15}N-[RDX]. The production of ^{15}N labeled N_2 ($^{15}N_2$), accounted for the largest individual sink during the transformation of the total added ^{15}N-[RDX] (25%). Hypoxic sediment was the most favorable zone for production of N_2, most of which diffused through porous sediments into the water column and escaped to the atmosphere.

1. Introduction

RDX has been extensively used in munitions compounds since World War II. Processes involving the production, handling, loading, disposal and detonation of explosives can release RDX into coastal marine habitats (Hawari et al., 2000). Although active dumping of unexploded ordnances in the United States was prohibited by Title I of the Marine Protection, Research and Sanctuaries Act in 1972, military training and weapon testing, as well as previously dumped underwater ordnances, continue to release RDX to some adjacent coastal settings (Smith et al., 2015a). Studies have reported that RDX has toxic effects on aquatic organisms in marine environments and concern has further increased due to the possible carcinogenicity of RDX and nitroso-derivatives in humans (Rosen & Lotufo, 2005; Sweeney et al., 2012).

There is considerable interest in exploring natural remediation techniques for the removal of RDX from contaminated marine coastal habitats (Public works technical bulletin 200-1-95., 2011). The fate of RDX in coastal marine habitats is determined by the physico-chemical properties of the compound (solubility, octanol-water partition constant, vapor pressure, etc.) (Kalderis et al., 2011) and the environmental conditions (including sediment properties, ionic strength, pH, redox conditions and biological factors). RDX has a relatively low sorption affinity to sediments (Pichtel, 2012; Ariyarathna et al., 2015) and a low bioconcentration potential in marine aquatic organisms (Ballentine et al., 2016). Abiotic degradation of RDX via hydrolysis and reduction by Fe (magnetite, ferrous iron, zero valent iron) was also previously reported (Balakrishnan et al., 2003; Monteil-Rivera et al., 2008; Gregory et al., 2004; Larese-Casanova & Scherer, 2008; Naja et al., 2008), but the rates were significantly lower compared to the degradation by microorganisms (Pichtel, 2012). The heterocyclic ring in RDX makes it unstable after the reduction of only one nitro group (Anke et al., 2003) and several benchtop studies have proven the potential of microbial degradation of RDX under both aerobic and, more favorably, anaerobic conditions (Hawari et al., 2000; Sheremata et al., 2001; Best et al., 1999; Kwon et al., 2011). A diverse range of active microorganisms responsible for RDX degradation in reducing environments including *Proteobacteria*, *Clostridia*, *Spirochaetes*, and *Bacteroidia* have been identified using carbon and nitrogen isotope probing techniques (Cho et al., 2015; Cho et al., 2013; Cho et al., 2016) which differ from stable isotope tracer methods used in this study. These organisms assimilate carbon and/or nitrogen from RDX and its metabolites differently in different electron accepting conditions, and are more complex than in isolated pure cultures (Cho et al., 2015; Cho et al., 2016). In contrast, several studies have shown the persistence of RDX in well aerated aquatic systems (Smith et al., 2013; Felt et al., 2009) suggesting the dependence of RDX biodegradation on the prevailing redox conditions of the system. Therefore, it is vital to examine the extent of biodegradation and its specific pathways in different micro-environments with a range of redox conditions including surface water, porewater and sediment (Smith et al., 2015a). Furthermore, studying the ultimate fate of RDX in coastal marine habitats by assessing the role of different pathways (partitioning, biodegradation and mineralization) in these different environments is critical for assessment of the natural remediation capacity of RDX in coastal environments. Complete mineralization of RDX is the optimal scenario of removal of RDX from marine environments (Smith et al., 2015a; Smith et al., 2013).

Studies examining RDX transformations have mainly focused on groundwater and fresh water systems (Hawari et al., 2000; Sheremata et al., 2001; Best et al., 1999; Kwon et al., 2011), as opposed to marine environments (Smith et al., 2015a; Montgomery et al., 2013). Existing records are also limited primarily to laboratory flask-type studies leaving a considerable data gap for biodegradation and mineralization of RDX at larger scales containing natural microbial assemblages and multiple complimentary or competing pathways of RDX transformations. Using isotopically labeled [^{15}N]-RDX helps to trace the pathways of RDX processing in such a setting at the aquarium scale. This project is a multidisciplinary study that focuses on the mineralization of RDX derived nitrogen products in systems of varying complexity. RDX was first traced in Smith et al. (2015a) in a similar system using a single pulse input and no biota other than sediment microbial assemblages. This study reports the differences in a similar system with a continuous addition of RDX and includes biota. The biotic portion of this work has been reported by Ballentine et al., 2016 (Ballentine et al., 2016) while this paper focuses on the RDX biogeochemistry and ^{15}N balance of the entire system.

Here, we evaluate the major pathways controlling the fate of RDX in contaminated shallow marine systems (Fig. 1) by introducing isotopically labeled ^{15}N-[RDX] into aquarium-scale, laboratory-simulated coastal marine habitats over 21 days. RDX and its transformation products, including nitroso-triazines in dissolved (surface water and porewater) and solid (suspended particulate matter, sediment and biota) phases, and DIN and N gas mineralization product, N_2 were measured (Chemical formulas and structures are shown in Table S1). ^{15}N enrichments of bulk sediment ($^{15}N_{Sed}$), suspended particulate matter ($^{15}N_{SPM}$) and biota ($^{15}N_{biota}$) were also measured in order to acquire a RDX-derived tracer ^{15}N mass balance of the aquaria. Incorporation of ^{15}N derived from RDX onto coastal marine biota in these aquaria has been comprehensively analyzed in Ballentine et al. (2016).

2. Methods

2.1. Experimental design

The experimental tanks consisted of two 70 L glass aquaria linked to each other via a glass reservoir to obtain common circulation and aeration conditions as described in (Ballentine et al., 2016; Smith et al., 2013). Experimental aquaria were loaded with a 8 cm deep layer of sandy, low (0.2%) organic carbon (OC) sediment collected from a subtidal habitat in Long Island Sound (LIS; 41° 19′ 13″ N, 72° 2′ 59″ W) and both aquaria were used for composite samples. The aquaria were maintained under flow-through conditions with sea water from LIS (30 PSU) for two weeks to achieve stabilized redox conditions in sediment and then, switched to a closed loop recirculation at 24 h before the start of the experiment. The two experimental aquaria were loaded with macroalgae (*Fucus vesiculosus* and *Ulva lactuca*), epifaunal (*Littorina littorea* and *Carcinus maenas*), bivalve (*Crassostrea virginica*, *Mytilus edulis*, and *Mercenaria mercenaria*), and fish species (*Pseudopleuronectes americanus* and *Fundulus heteroclitus*) (Ballentine et al., 2016) and water recirculation was continued over the course of the experiment (21 days). One day after the biota was introduced and the plumbing switched to recirculation mode, RDX labeled with ^{15}N in all three nitro positions (50 atom %) (Smith et al., 2015a) was introduced to the