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## The Fate of the Cyclic Nitramine Explosive RDX in Natural Soil

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The sorption-desorption behavior and long-term fate of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was examined in sterilized and nonsterilized topsoil. Results of this study indicate that although RDX is not extensively sorbed by the topsoil ( $K_d$  of 0.83 L/kg), sorption is nearly irreversible. Furthermore, there was no difference in the sorption behavior for sterile and nonsterile topsoil. However, over the long-term, RDX completely disappeared within 5 weeks in nonsterile topsoil, and hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) metabolites formed in the aqueous phase. Over the same period, recovery of RDX from sterile topsoil was high (55–99%), and the nitroso metabolites were not detected. Only traces of RDX were mineralized to CO<sub>2</sub> and N<sub>2</sub>O by the indigenous microorganisms in nonsterile topsoil. Of the RDX that was mineralized to N<sub>2</sub>O, one N originated from the ring and the other from the nitro group substituent, as determined using N<sup>15</sup> ring-labeled RDX. However, N<sub>2</sub>O from RDX represented only 3% of the total N<sub>2</sub>O that formed from the process of nitrification/denitrification.

### Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, Figure 1) is an energetic compound that is commonly used as a military explosive. Various commercial and military activities that include manufacturing, waste discharge, testing and training, demilitarization, and open burning/open detonation (OB/OD) have resulted in extensive RDX contamination of soil and groundwater (1). The toxicity of RDX to humans and mammals is well established (2). Hence, remediation of contaminated soil and groundwater is necessary. Natural attenuation is an emerging remediation technology that is potentially less expensive, less intrusive, and offers a long-term solution (3). However, to gain acceptance by regulatory agencies, more data is required in order to accurately assess the fate of contaminants in the environment. Particularly, the fate of RDX depends on the processes of transformation, microbial degradation, and immobilization (3).

The metabolic pathways of polynitroaromatics, such as 2,4,6-trinitrotoluene, have been extensively characterized in

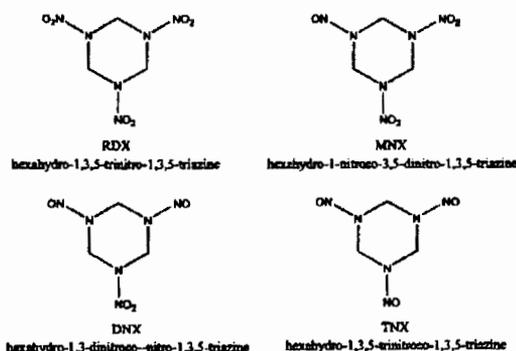


FIGURE 1. Molecular structures of RDX, MNX, DNX, and TNX.

liquid culture and natural soil systems (4–7). Two decades ago, McCormick et al. (8) identified nitroso metabolites of RDX. More recently, we identified several other metabolites and end products in the biodegradation of RDX with an anaerobic sludge (9). Particularly, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazazine (MNX, Figure 1) and hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazazine (DNX, Figure 1) were formed by the stepwise reduction of –NO<sub>2</sub> in RDX. Methylene-dinitramine (O<sub>2</sub>NNHCH<sub>2</sub>NHNO<sub>2</sub>) and bis(hydroxymethyl)-nitramine ((OHCH<sub>2</sub>)<sub>2</sub>NNO<sub>2</sub>) product formation was attributed to enzymatic hydrolytic ring cleavage of the inner C–N bonds of RDX. The four metabolites identified above disappeared to produce N-containing products (N<sub>2</sub>O, and traces of N<sub>2</sub>) as well as C-containing products (HCHO, CH<sub>3</sub>OH, HCOOH, and CO<sub>2</sub>). During the course of these experiments, 50–60% mineralization of RDX to CO<sub>2</sub> occurred. For aerobic biodegradation of RDX by *Phanerochaete chrysosporium*, we identified trace quantities of MNX and methanol as metabolites and N<sub>2</sub>O and CO<sub>2</sub> as major mineralization products (10).

Knowledge of the products and transformation pathways from the anaerobic (9, 11) and aerobic (10) biodegradation of RDX in liquid culture have provided us with information that is necessary in the design of effective remediation technologies. However, it is also necessary to assess the transformation, microbial degradation, and immobilization behavior of RDX in natural soil to assess the effectiveness of natural attenuation. Therefore, the objective of the present study was to determine the sorption-desorption behavior and the long-term fate of RDX in sterile and nonsterile topsoil.

### Experimental Section

**Chemicals.** RDX (>99% purity) was provided by Defense Research Establishment Valcartier (Valcartier, PQ, Canada). Uniformly labeled [UL-<sup>14</sup>C]RDX was synthesized and recrystallized to achieve chemical and radioactive purity of 99% and 97%, respectively (12). The specific activity of the radioactive compound was 28.7 μCi/mmol. The ring-labeled [<sup>15</sup>N]RDX (>98% purity) was synthesized similarly (13). The MNX and TNX were synthesized according to the methods of Brockman et al. (14). All other chemicals used were reagent grade.

**Soil.** An agricultural topsoil was obtained from Varennes, Quebec. Properties of the topsoil are summarized in Table 1. Qualitative mineralogical analysis was determined by X-ray diffraction (Geochemical Laboratories, McGill University, Montreal, Quebec).

**Soil Sterilization.** The topsoil was sterilized by gamma irradiation from a cobalt-60 source at the Canadian Irradia-

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**TABLE 1. Properties of the Topsoil Used in Experiments with RDX**

particle size distribution			% organic matter	pH	CEC,* mequiv/100 g
% clay (<2 μm)	% silt (2–53 μm)	% sand (>53 μm)			
4	12	83	8.4	5.6	14.6
major elements		minor elements		trace elements (<0.05%)	
quartz (SiO <sub>2</sub> )		albite-ordered (Na·Al·Si <sub>3</sub> O <sub>8</sub> )		crossite (Na <sub>2</sub> (Fe,Mg) <sub>5</sub> (Si,Al) <sub>8</sub> O <sub>22</sub> (OH) <sub>2</sub> )	

\* Cation exchange capacity.

tion Centre (Laval, Quebec) with minimum and maximum doses of 35.4 and 40.4 kGy, respectively. Gamma irradiation was examined for sterilization since it has minimal impact on the sorption of other contaminants by soil (15–17). Irradiated topsoil was combined with a solution that contained 0.1% (w/w) sodium pyrophosphate in water. Three dilutions were spread plated onto tryptone-yeast extract agar (18). The absence of colony forming units (CFUs) after 20 days incubation at 35 °C is indicative that gamma irradiation was effective.

**Sorption–Desorption.** Sorption was conducted in batch reactors at 25 °C. Aqueous RDX solutions were prepared from stock solutions in acetonitrile (10.27 mg/mL). A specific volume from the stock solution was added to deionized water to give the following initial RDX concentrations 5, 10, 15, 20, and 30 mg/L. In 16 mL borosilicate centrifuge tubes, fitted with Teflon coated screw caps, 15 mL of the aqueous RDX solutions was combined with 2 g of nonsterile topsoil.

Centrifuge tubes were wrapped in aluminum foil, sealed with Teflon coated caps without degassing, and agitated on a Wrist Action shaker (Burrell Corp., Pittsburgh, PA) for 22 h. The tubes were centrifuged for 30 min at 3500 rpm, and the supernatant was filtered using a Millex-HV 0.45 μm filter unit (Millipore Corp., Bedford, MA). Sorption of the RDX by the filter unit was negligible. The mass of RDX sorbed by topsoil was calculated by difference. All experiments were conducted in triplicate.

Desorption was conducted by adding 15 mL of distilled water to the soil pellets for all five initial concentrations following sorption. The initial desorption step was 24 h, as this time frame was determined to be sufficient to achieve equilibrium (results not shown). The interstitial solution volume that remained with the soil pellet was estimated gravimetrically. Using the same methods just described, a second desorption step that lasted 88 h was also employed.

**Sorption Kinetics (Sterile and Nonsterile Soils).** To verify that the results for 22 h sorption were not coupled with biotic losses of RDX, sorption was studied with nonsterile and sterile topsoil. These results were also used to verify the time required to achieve equilibrium with respect to sorption. The initial RDX concentration was 10 mg/L, and samples were analyzed in triplicate following 1, 19, 24, 44, and 72 h of sorption.

**Long-Term Fate.** The long-term fate of RDX in sterile and nonsterile topsoil was monitored over 7 weeks. Particularly, 15 mL of 10 mg/L RDX was combined with 2 g of topsoil, as was done for the sorption–desorption experiments. Sufficient vials were prepared to allow for sacrificial sampling. The flasks were sealed with aluminum coated capes with degassing. Each week, the aqueous phase and the remaining soil pellet were subjected to acetonitrile extraction (see next section), in triplicate. The aqueous phases and acetonitrile extracts were analyzed for RDX (described subsequently). These experiments were static and were conducted at 25 °C.

**Acetonitrile Extraction.** RDX was extracted from sterile and nonsterile topsoil using the EPA SW-846 Method 8330 acetonitrile extraction procedure (19). Briefly, the soil was combined with 10.0 mL of acetonitrile, vortexed, and placed in a sonicator bath (20 kHz) cooled to 22 °C (Blackstone Ultrasonics, Jamestown, NY) for 18 h. After sedimentation, 5.0 mL of the supernatant was combined with 5.0 mL of a 5 g/L CaCl<sub>2</sub> solution. The solutions were agitated and settled for 15 min prior to sample preparation for HPLC/LC-MS analysis. Based on a material balance, the percent recovery of RDX was calculated as follows

$$\% \text{ recovery} = ((RDX_{\text{solid}} + RDX_{\text{aqueous}}) / RDX_{\text{total}}) \times 100\% \quad (1)$$

where  $RDX_{\text{solid}}$  is the RDX recovered by acetonitrile extraction of soil,  $RDX_{\text{aqueous}}$  is the RDX present in the aqueous phase, and  $RDX_{\text{total}}$  is the total amount of RDX present. All terms in eq 1 are expressed in units of moles.

**Mineralization.** Mineralization experiments were prepared in 120 mL serum bottles with 3 g of topsoil and 15 mL of distilled water. For RDX mineralization to CO<sub>2</sub>, 0.051 μCi of [UL-<sup>14</sup>C]RDX was combined with unlabeled RDX for a total RDX amount of 1.27 mg in the serum bottle. Samples were prepared in triplicate with sterile and nonsterile topsoil and incubated statically at 35 °C. The headspace was not augmented with air or oxygen. It was determined that the RDX was completely soluble at this temperature. Each serum bottle was fitted with a small test tube containing 1 mL of 0.5 M KOH to trap liberated <sup>14</sup>CO<sub>2</sub>. Microcosms with [UL-<sup>14</sup>C]RDX were routinely sampled (every 2–3 days) for determination of <sup>14</sup>CO<sub>2</sub> in the KOH trap using a Tri-Carb 4530 liquid scintillation counter (model 2100 TR; Packard Instrument Company, Meriden, CT). At the end of the experiments, the unfiltered and filtered aqueous phase (Millex-HV 0.45 μm filter unit) was also analyzed by liquid scintillation counting to determine the residual radioactivity remaining in the respective phases.

For RDX mineralization to N<sub>2</sub>O, microcosms were prepared as just described for CO<sub>2</sub> mineralization, except that only unlabeled RDX (1.27 mg) was used (the headspace was sampled every 2–3 days and analyzed by GC, as described subsequently). Microcosms were similarly prepared with ring-labeled [<sup>15</sup>N]RDX, and the headspace was analyzed by gas chromatography–mass spectroscopy (GC-MS), as described subsequently.

**Analytical Methods.** RDX concentrations were determined by reversed-phase high-pressure liquid chromatography (HPLC) with a photodiode array (PDA) detector. The Waters (Waters Associates, Milford, MA) HPLC system consisted of a model 600 pump, 717 Plus autosampler, and a 996 PDA detector (λ = 254 nm). A Supelcosil LC-CN column (25 cm × 4.6 mm, particle size 5 μm) was coupled with a Temperature Control Module held at 35 °C. The methanol/water gradient was at a flow rate of 1.5 mL/min. The initial solvent composition was 30% methanol and 70% water that was held for 8 min. Following this, a linear gradient was run from 30 to 65% methanol over 12 min. The solvent ratio was changed to the initial conditions over 5 min and held for another 5 min. The system was outfitted with Millennium data acquisition software.

Liquid chromatography/mass spectrometry (LC/MS) was used to verify the presence of RDX, MNX, DNX, and TNX. Analyte ionization was achieved in a negative electrospray ionization ES (–) mode. This system consisted of a Micromass Platform II benchtop single quadrupole mass detector fronted by a Hewlett-Packard 1100 series HPLC system equipped with a photodiode array detector. Samples (50 μL) from the microcosms were injected into a Supelcosil LC-CN column (25 cm × 4.6 mm; 5 μm particle size) at 35 °C. The instrument

**TABLE 2. Freundlich Sorption and Desorption Parameters for RDX and Nonsterile Topsoil\***

operation	$K_d$	$n$	$r^2$
sorption ( $K^s_d$ )	0.83	1.1	0.96
desorption # 1 ( $K^{d1}_d$ )	4.13	1.1	0.96
desorption # 2 ( $K^{d2}_d$ )	16.05	1.0	0.88

\* 95% confidence intervals are shown for values of  $K_d$  and  $n$  determined from 18 data points.

conditions used are reported elsewhere (9). Confirmation of the identity of targeted metabolites (MNX and TNX) was accomplished by comparison with reference compounds simulated according to the methods of Brockman et al. (14).

A Perkin-Elmer Sigma 2000 GC connected to a Chromsorb 102 (60–80 mesh, 12' x 1/8") stainless steel column (Supelco, ON) coupled with an electron capture detector (ECD) (350 °C) was used for  $N_2O$  detection. Gas samples from the headspace of the serum bottles were sampled using a gastight syringe for subsequent injection to the GC using helium as a carrier gas (30 mL/min) at 50 °C. Identification was confirmed by comparison with a reference compound. The presence of  $N_2O$  as a product of RDX was confirmed by analyzing the headspace of microcosms containing ring-labeled [ $^{15}N$ ]RDX by GC-MS to monitor the masses at 45 Da ( $^{15}N^{14}NO$ ). A Hewlett-Packard 6890 GC (Mississauga ON), coupled with a 5973 quadrupole mass spectrometer, was used for this analysis. A GS-Gas Pro (30 m x 0.32 mm) capillary column (J & W Scientific, Folsom CA) was used under splitless condition. The column was maintained at 150 °C and 280 °C, respectively. The injection volume was 50  $\mu$ L.

## Results and Discussion

**Sorption-Desorption.** The following Freundlich isotherm has been found to be adequate to describe equilibrium sorption and desorption of explosives by and from soil (5, 20–22)

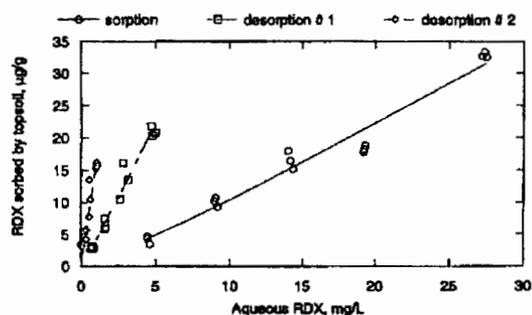
$$\frac{x}{m} = K_d C^n \quad (2)$$

where  $x/m$  is the mass of solute sorbed per unit mass of soil at equilibrium ( $\mu$ g/g),  $K_d$  is the capacity constant (L/kg),  $C$  is the aqueous equilibrium phase solute concentration (mg/L), and  $n$  is a constant. The capacity constant for sorption is denoted as  $K^s_d$ , and for desorption it is denoted as  $K^{d1}_d$ . When  $K^s_d < K^{d1}_d$ , sorption-desorption hysteresis exists.

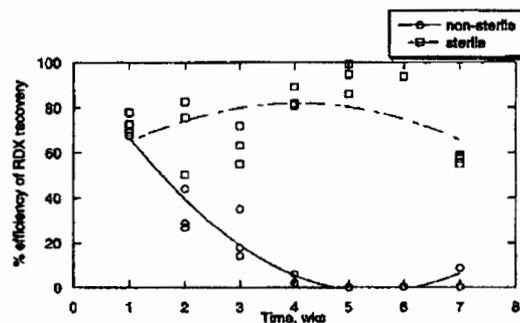
The sorption capacity constant for RDX is considerably less than ( $K^s_d$  of 0.83 L/kg, Table 2) those of 2,4,6-trinitrotoluene and its two amino metabolites for the same topsoil (6.38–11.96 L/kg (5)). For other surface soils, values of  $K^s_d$  that range from 0.58 to 11 and 0.21–0.33 L/kg have been reported for TNT and RDX, respectively (23). However, since the desorption isotherms lie above the sorption isotherm (Figure 2), and the capacity constants for desorption are 10 to 100 times greater than for sorption (Table 2), there is considerable sorption-desorption hysteresis.

**Long-Term Fate.** Results of kinetic experiments indicate that equilibrium was achieved within 1 h and that there were no differences between the sterile and nonsterile systems over 72 h (data not shown). Therefore, the fate of RDX in sterile and nonsterile topsoil systems was studied over 7 weeks. The results indicate that following the first week, similar quantities of RDX were recovered from both sterile and nonsterile systems (Figure 3). Therefore, the sorption-desorption hysteresis described in the previous section (Figure 2) is attributable to abiotic processes such as sorption.

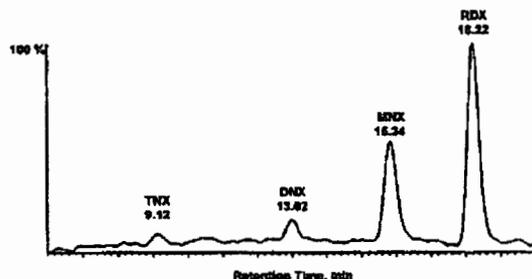
Between the second and seventh weeks, the recovery of RDX (eq 1) from sterile systems remained between 55 and



**FIGURE 2. Sorption-desorption isotherms for RDX and topsoil.**



**FIGURE 3. Efficiency of total RDX recovered from the aqueous and solid-sorbed phases of sterile and nonsterile topsoil.**



**FIGURE 4. LC-MS chromatogram for aqueous phase following 4 weeks of contacting RDX (10 mg/L) with nonsterile topsoil.**

99%. In contrast, the recovery of RDX from nonsterile topsoil fell from 43 to less than 1% during the same period. Furthermore, the three nitroso metabolites were evident (mostly in the aqueous phase) for the nonsterile topsoil between two and seven weeks. The nitroso metabolites were absent in sterile topsoil over the entire experiment. The presence of these three metabolites in the aqueous phase in nonsterile topsoil (4 weeks contact) was confirmed by LC-MS, and a representative chromatogram is shown in Figure 4. Anaerobic conditions were evidenced by the stench when vials were sampled, and since sampling was sacrificial, air was not introduced to the vials during the course of the experiment.

The fact that the three nitroso metabolites were mainly observed in the aqueous phase, and not in the solid sorbed phase (i.e. in the acetonitrile soil-extracts), is not surprising since the sorption capacity constant of the topsoil for RDX is quite low ( $K^s_d$  of 0.83 L/kg, Table 1). Hence, the nitroso metabolites form in the aqueous phase, and they are not subsequently sorbed by topsoil. However, since we did not have access to pure forms of the nitroso metabolites (they are not commercially available), it was not possible to further characterize their behavior in soil.

**Mineralization of RDX.** In our previous work, we identified CO<sub>2</sub> and N<sub>2</sub>O as products from the mineralization of RDX by *Phanerochaete chrysosporium* (10) and an anaerobic consortium from food processing sludge (9, 11). By using ring labeled [<sup>15</sup>N]RDX, both studies confirmed that one nitrogen in N<sub>2</sub>O originates from RDX. Previous to these studies, it was not known that N<sub>2</sub>O could form from RDX by ring cleavage. In the present study, we sought to determine whether N<sub>2</sub>O formed from RDX by microbial activity of the indigenous microorganisms in the topsoil. Results of this study indicate that only traces of the N<sub>2</sub>O that formed originated from RDX. Particularly, one N originated from the ring, and the other from the nitro substituent. This was concluded by analyzing the N<sub>2</sub>O formed in experiments using ring labeled [<sup>15</sup>N]RDX. In particular, the headspace was analyzed by GC-MS, and the mass spectra showed a mass of 45 Da (indicative of N<sup>15</sup>N<sup>14</sup>O (9)). The mass of 45 Da represented a maximum of 3% of the total N<sub>2</sub>O that formed. However, N<sub>2</sub>O formation by indigenous microorganisms, presumably by nitrification and denitrification, was far greater than what was formed by ring cleavage of RDX. In particular, nonsterile controls (i.e. topsoil with no RDX) showed N<sub>2</sub>O evolution with time. Coupled with the fact that there was little conversion of RDX to CO<sub>2</sub>, it can be concluded that RDX was not extensively mineralized in the topsoil.

Attempts to identify other ring cleavage products or metabolites by LC-MS were not successful. McCormick et al. (8) postulated that the biodegradation of RDX proceeds by successive reduction of the nitro groups to a point where destabilization and fragmentation of the ring occurs in liquid culture. In two later studies, we showed that N<sub>2</sub>O and CO<sub>2</sub> were the final end products in both anaerobic and aerobic liquid cultures (9, 10). The absence of extensive mineralization in the present system may be due to the adsorption of ring cleavage products by soil or binding with dissolved organic matter. The topsoil contained 8.4% organic carbon (Table 1), and the presence of dissolved organic matter was visible. Binding of organic contaminants by such solid soil particles and possibly by dissolved organic matter are known to reduce the availability of organic contaminants to microorganisms for biodegradation in soil (24–27). To further investigate this possibility, the radioactivity in the aqueous phase was measured at the end of the [<sup>14</sup>C]RDX mineralization experiments. Results indicate that although RDX had disappeared, and the nitroso metabolites were no longer present, 1.93% (± 0.59) of radioactivity was recovered as <sup>14</sup>CO<sub>2</sub>, and 53.06% (± 2.47) of the radioactivity was recovered from the aqueous phase. Therefore, by difference, approximately 45% of the radioactivity partitioned with the solid-sorbed phase. Such partitioning may have rendered the sorbed phase as unavailable to the indigenous microorganisms for mineralization. However, at this point, the chemical structures of the breakdown products in the aqueous and solid-sorbed phases remain unknown. Based on the results of the present study, in contrast to what we observed in liquid culture (9, 10), N<sub>2</sub>O formation in topsoil is slow and the yield is small; hence, measurement of N<sub>2</sub>O for monitored natural attenuation does not appear to be a useful option.

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