Sorption and Degradation of Octahydro-1,3,5,7-tetranitro-1,3,5,7- tetrazocine in Soil

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · OCTOBER 2003

Impact Factor: 5.33 · DOI: 10.1021/es030407e · Source: PubMed

3 AUTHORS, INCLUDING:

Jalal Hawari
Université de Montréal-Ecole Polytechniqued

351 PUBLICATIONS **7,160** CITATIONS

SEE PROFILE

Environ. Sci. Technol. **2003**, *37,* 3878−3884

Sorption and Degradation of Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in Soil

FANNY MONTEIL-RIVERA, CARL GROOM, AND JALAL HAWARI*

Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montréal, Quebec, Canada H4P 2R2

The sorption/desorption and long-term fate of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) was examined using sterilized and nonsterilized soils. Two soils were used that differ mainly by the amount of total organic carbon (TOC): an agricultural topsoil (VT, 8.4% TOC) and a sandy soil (SSL, 0.33% TOC). The adsorption isotherms performed at room temperature were well-described by a linear model, which led to sorption distribution coefficients of 2.5 and 0.7 L kg^{-1} for VT and SSL soils, respectively. The organic content of soil did not significantly affect HMX sorption. Over a period of 20 weeks, HMX degraded (60% disappearance) in static anaerobic nonsterile VT soil preparations. In separate experiments using UL-[^{14}C]-HMX, 19% mineralization (liberated $^{14}CO_2$) was obtained in 30 weeks. In addition, four nitroso derivatives of HMX were detected. Knowing the sorption/desorption behavior and the long-term fate of HMX in soil will help assess the effectiveness of natural attenuation for HMX removal.

Introduction

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (Table 1) is a melt-cast explosive used in a wide range of commercial and military applications (*1*−*3*). Recently, soil samples were characterized from a NATO anti-tank firing range at Wainwright, AB (*4*), where past and present practices involve the extensive use of ammunitions containing the melt-cast explosive Octol. Although the latter is composed of HMX (70 wt %), TNT (30 wt %), and RDX (<1 wt %) (Table 1), only HMX was detected as the principal soil contaminant. Similar observations were obtained at anti-tank firing ranges at Valcartier, QC (*5*), and Fort Ord, CA (*6*). The pattern of HMX distribution was similar at all three sites as HMX concentrations decreased greatly with distance from the targets and were confined to depths of 15 cm or less from the soil surface. Pennington et al. (*7*) also observed that in field samples HMX persists in surface soil where oxygen is available but is not typically observed in deep aquifers where an anaerobic environment is expected to prevail.

Since HMX is toxic and mutagenic (*8*), understanding its fate in soil is of vital importance. This can be done by studying its abiotic (photodegradation, hydrolysis) and biotic transformations as well as its physical transport processes including volatilization, solubilization, and soil sorption. Abiotic transformation of HMX due to hydrolysis and photolysis is expected to be of limited extent. For instance, HMX hydrolyzes at pH 10 more slowly than RDX ($t_{1/2(HMX)}$ = 288 d; $t_{1/2(RDX)}$ = 4 d) (*9*) and is stable at neutral pH. Loss of HMX due to evaporation is negligible as a consequence of its low vapor pressure (3.3×10^{-14} mmHg at 25 °C) (*1*).

Although the water solubility of HMX is low (5 mg/L at 25 °C) as compared to that of TNT and RDX (145 and 60 mg/L, respectively) (*10*), HMX could migrate through subsurface soil and cause groundwater contamination. In this context, the extent and the reversibility of HMX sorption onto soil and its ability to undergo (bio)transformation will be major factors determining the fate of HMX in natural environments. HMX was shown to biodegrade only under anaerobic conditions in culture media (*11*), effluent waste sludge (*12*) and soil bioslurry (*13*, *14*).

Limited research has been conducted on HMX sorption in soil. Leggett (*15*) studied the sorption of RDX and HMX on bentonite drilling muds in batch experiments and found that the sorption of both explosives was linear. The samples used in the above study consisted mainly of montmorillonite, and no mention was made concerning the presence of organic matter. Myers et al. (*16*) studied the sorption of HMX in soil column experiments and observed some HMX disappearance in the column effluents that was tentatively attributed to reductive transformation. No further details on sorption mechanisms were given. Recently, Brannon et al. (*17*) studied the effects of changing the composition of simulated groundwater on adsorption of TNT, RDX, and HMX in low carbon aquifer soils and found that modifying the soil cation did not significantly affect the adsorption of HMX.

The fate of HMX in soil as determined by sorption and degradation is still inadequately characterized. The objective of the present study was therefore to determine the sorption/desorption behavior and the disappearance of HMX in sterile and nonsterile soils. Two soils were investigated: (i) an agricultural topsoil taken from Varennes, QC, which was used earlier to determine the fate of TNT (*18*) and RDX (*19*); (ii) a Sassafras sandy loam that contained less organic carbon than the Varennes topsoil. Knowing the sorption/desorption behavior and the long-term fate of HMX in soil will help assess the effectiveness of natural attenuation for HMX removal. It will also provide insight as to why only HMX persisted close to the soil surface (*5*) and accumulated in plant leaf tissues (*4*) at sites initially contaminated with TNT, RDX and HMX.

Experimental Section

Chemicals. HMX (>99% purity) was provided by Defence Research and Development Canada (DRDC) (Valcartier, QC, Canada). Uniformly labeled UL-[^{14}C]-HMX (chemical purity 98%, radiochemical purity 92%, specific activity 74.4 μCi/mmol) was also provided by DRDC. Radiochemical purity was determined by HPLC fractionation with scintillation counting of the collected fractions using a Tri-Carb 4530 liquid scintillation counter (model 2100 TR; Packard Instrument Company, Meriden, CT). All other chemicals used were of reagent grade.

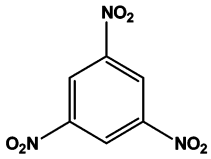
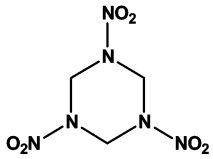
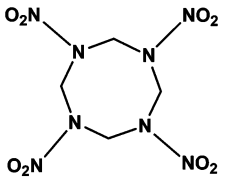
Soils. Two well-characterized soils were used in this study: an agricultural topsoil (VT) originating from Varennes, QC, Canada, and a Sassafras sandy loam (SSL) sampled in an uncontaminated open grassland at the Aberdeen Proving Ground (Edgewood, MD). Properties of both soils are summarized in Table 2. Soil sterilization was accomplished by γ-irradiation from a cobalt-60 source at the Canadian Irradiation Center (Laval, QC) as described in ref *19*.

General Sorption/Desorption Procedure. Batch sorption experiments were conducted at room temperature. A volume of HMX solution (15 mL) was added to 2 g of dried soil in 16-mL borosilicate centrifuge tubes fitted with Teflon-coated

* Corresponding author phone: (514)496-6267; fax: (514)496-6265; e-mail: jalal.hawari@cnrc-nrc.gc.ca.

10.1021/es030407e CCC: $25.00 Published 2003 by the Am. Chem. Soc.
Published on Web 08/05/2003

TABLE 1. Physicochemical Properties[a] of TNT, RDX, and HMX

Name	TNT	RDX	HMX
Formula			
Log K_{ow}	1.6, 2.2, 2.7	0.87 (0.90)	0.13 (0.16)
Water Solubility at 20° C (mg L^{-1})	130	42.3 (42.6)	6.6 (3.3)
Vapor Pressure at 25 °C (mm Hg)	1.99×10^{-4}	4.03×10^{-9}	3.33×10^{-14}

[a] data from ref *1* and references therein. Values in parentheses are from the present study.

TABLE 2. Properties of Soils Used for Sorption of HMX

	particle size distribution					
soil	% clay (<2 μm)	% silt (2−53 μm)	% sand (> 53 μm)	total org C (%)	pH	CEC[a] (mequiv/100 g)
VT	4	12	83	8.4	5.6	14.6
SSL	11	18	71	0.33	5.1	4.3

[a] CEC, cationic exchange capacity.

screw caps without degassing. The tubes were wrapped in aluminum foil and agitated on a Wrist Action shaker for the desired contact time. After centrifugation (30 min; 1170*g*), the supernatant was filtered through a 0.45-μm Millipore filter and analyzed by HPLC after discarding the first 3 mL.

Desorption experiments were conducted by adding 15 mL of distilled water to the pellet remaining in the tube after removing the supernatant and agitating the suspensions for the required contact times. Samples were then centrifuged, filtered, and analyzed as described for sorption experiments.

To estimate the amount of HMX loss during the sorption and desorption experiments, sorbed HMX was extracted with CH_3CN from the solid recovered after sorption or desorption as described in the EPA SW-846 Method 8330 (*20*). The solution volume that remained with the soil at the end of sorption and desorption was estimated gravimetrically, and corrections were made to take into account the amount of HMX present in this volume. A percent recovery was then calculated.

Sorption Kinetics. Sorption kinetics experiments were carried out for both soils according to the above procedure. An aqueous stock solution of HMX (3 mg/L) was prepared by weighing the appropriate amount of HMX and dissolving it in water. The solution was then contacted with soil over a period ranging from 1 h to 7 d. Resazurin was added under a CO_2 blanket to test for the presence of oxygen after 1 week of contact time. Triplicate experiments were performed for each contact time.

Desorption Kinetics. The soil was first contacted with a 3 mg/L HMX solution for 60 h. After centrifugation, the supernatant was removed, and the pellet was contacted with 15 mL of freshwater during periods varying from 1 h to 7 d. Controls ($n = 3$) containing an aqueous solution of HMX (3 mg L^{-1}) and no soil were allowed to shake from the beginning of sorption to the end of the longer desorption time (10 d in total). Recoveries of 100% were obtained, demonstrating the absence of HMX degradation or adsorption to the container material (glass or Teflon) during sorption/desorption cycles.

Sorption/Desorption Isotherms. Aqueous HMX solutions were prepared from a filtered saturated aqueous solution to give initial HMX concentrations ranging from 0.5 to 4 mg/L. Sorption experiments were conducted over a period of 60 h, with 6 replicates for each concentration. Three of the six samples were extracted with acetonitrile while the three remaining samples were subjected to desorption in water (40 h). After desorption, samples were extracted with acetonitrile.

Long-Term Fate. The long-term fate of HMX was monitored in sterile and nonsterile VT soil. A sterile aqueous solution (15 mL) of HMX (1.39 mg/L) was combined with 2 g of either sterile or nonsterile VT soil in 16-mL vials keeping an empty headspace volume of 0.5 mL. The vials were kept static, away from light at room temperature for 16 weeks. A sufficient number of vials were prepared to allow for sacrificial sampling. Soil suspensions were centrifuged, and the resulting soil pellets were subjected to acetonitrile extraction. The supernatant aqueous phases and acetonitrile soil extracts were then analyzed for HMX, as described below. At termination of the study, sterile and nonsterile tubes were augmented with resazurin to test for the presence of oxygen.

HMX Degradation Products. Experiments to identify the degradation products of HMX in topsoil (VT) were performed with 1.28 μmol of HMX/sample. Stock acetone solutions of HMX were added in 20-mL headspace vials, and solvent was evaporated under aseptic conditions. The vials were then filled with 1 g of sterile or nonsterile VT soil and 7.5 mL of sterile deionized water before crimping with Teflon-coated rubber septa. The vials were then sparged with argon for 20 min and incubated away from light at 37 °C. After 35 weeks, the samples were sacrificed. The liquid and solid fractions were analyzed for HMX and possible metabolites.

Mineralization experiments were performed at 35 °C in 120-mL serum bottles containing 3 g of VT soil and 15 mL of aqueous solutions of HMX (0.13 mg) spiked with UL-[^{14}C]-HMX (0.033 μCi). Liberated $^{14}CO_2$ was collected in KOH traps and measured periodically as described in ref *19*. At the end of the mineralization experiments (30 weeks), the samples were acidified by the aseptic addition of 0.5 mL of 0.05 M H_3PO_4 and agitated (16 h) to collect remaining CO_2 trapped as carbonate in solution. After decanting the 15.5-mL aqueous phase, the soils were then filtered (Whatman No 1 filter paper) and rinsed with three 15-mL volumes of distilled water. The remaining washed soil fraction (with filter) was then sonicated as in the case of sorption/desorption studies. The volumes of the resultant CO_2 trap, aqueous phase (including soil washes), and soil extracts were analyzed by liquid scintillation

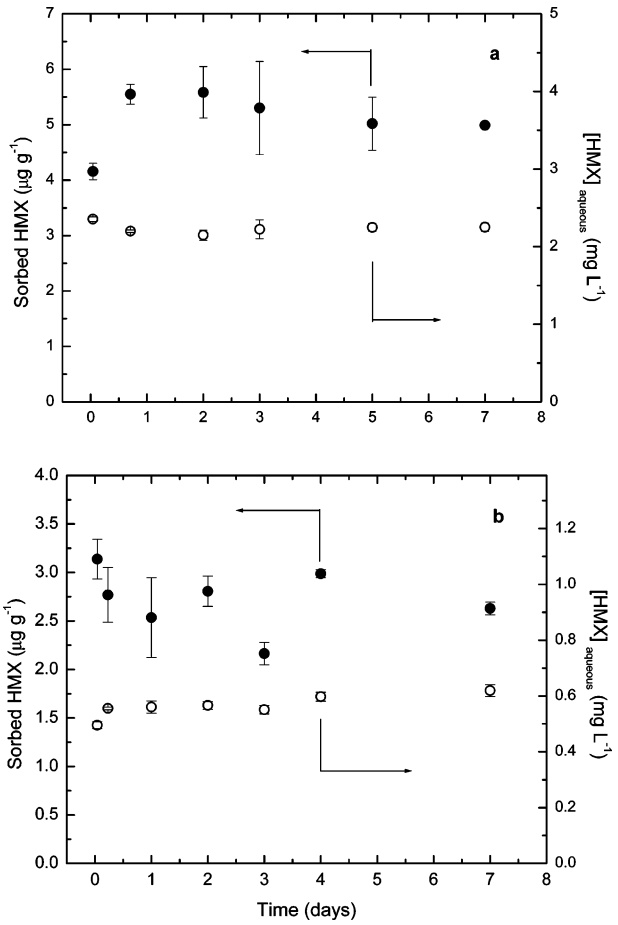


FIGURE 1. Kinetics results for sorption (a) and desorption (b) of HMX on VT soil: (●) sorbed HMX determined by extraction from the solid; (○) aqueous concentration of HMX; error bars represent standard deviations of three replicates.

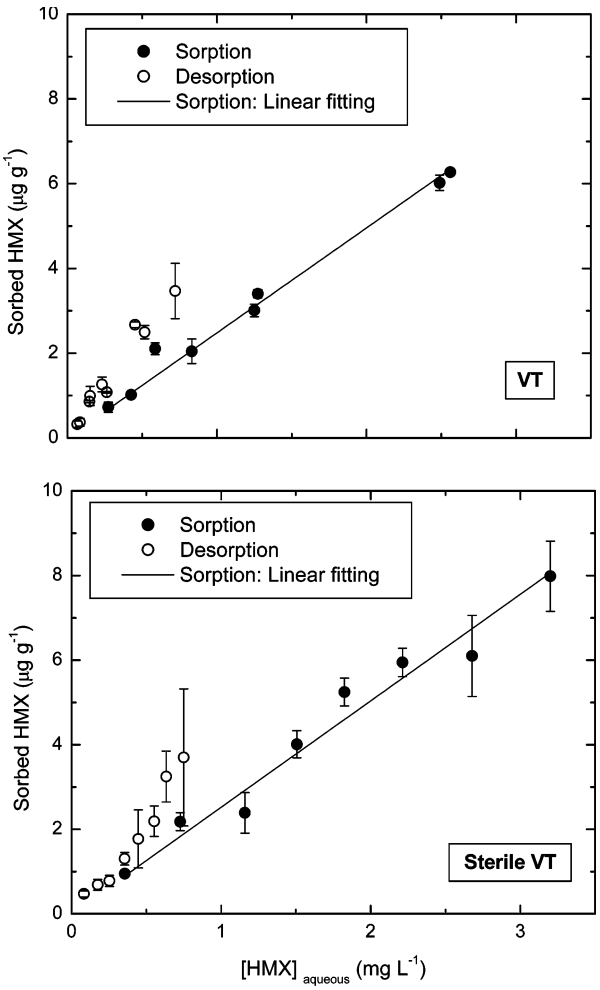


FIGURE 2. Sorption and desorption isotherms of HMX on sterile and nonsterile VT soil. Error bars represent the standard deviation of three replicates. Fitting parameters are given in Table 3.

counting as described in ref *19*.

Analytical Methods. HMX concentrations were determined by reversed-phase high-pressure liquid chromatography (HPLC) equipped with a photodiode array (PDA) detector as described in ref *12*.

Values of the octan-1-ol/water partition coefficient (K_{ow}) were measured for HMX and RDX using the flask-shaking method as described in OECD Guideline 107 (*21*). Solubility in water for both compounds was determined by stirring an excess amount of solid explosive in water at 20 °C according to the procedure described in ref *22*.

HMX and its nitroso derivatives were analyzed with a liquid chromatography/mass spectrometer (LC/MS) using a Micromass Plattform Benchtop single quadrupole mass detector fronted by a Hewlett-Packard 1100 series HPLC system connected to a Supelcosil LC-CN column (25 cm × 4.6 mm; 5 μm particle size). A methanol/water gradient (10−70% methanol over a period of 20 min, 70% methanol for 3 min, 70−10% methanol over a period of 2 min, 10% methanol for 10 min) was used at a flow rate of 1 mL/min. Analyte ionization was done in a negative electrospray ES(−) ionization mode producing mainly $[M - H]^-$. The instrumental operating conditions used to analyze the ring-cleavage product, methylene dinitramine, are reported elsewhere (*23*).

Results and Discussion

Sorption/Desorption Kinetics. The minimum time required to reach sorption and desorption equilibria for VT soil is shown in Figure 1a,b, respectively. The results indicated that HMX retention was rapid and appeared to reach equilibrium within approximately 1 d. Likewise, the desorption of HMX in water was rapid and showed little time dependence after 1 d of contact time. Analogous results were obtained with SSL soil (results not shown). To our knowledge, no data have been reported on the sorption kinetics for HMX, but for RDX, a rapid sorption was reported to occur in less than 24 h for different soils (*16*, *19*, *24*, *25*). Analysis of the acetonitrile extracts of both soils led to recoveries greater than 96%, indicating that no significant HMX transformation occurred during the time frame of the present experiments. The addition of resazurin after 1 week of contact time indicated that oxygen was still present in the samples.

Sorption/Desorption Mechanisms. The isotherms of the sorption and desorption of HMX in soils are presented in Figures 2 and 3 for VT and SSL soils, respectively. Sorption was measured after 60 h of contact time, while desorption was measured after contacting the sorbed solid with freshwater for 40 h. It is common to estimate the amount of sorbed contaminant by difference from the aqueous concentration, but where contaminants are partially degraded this can lead to an overestimation of the adsorbed fraction. We thus analyzed HMX in both the aqueous phase and the sorbed soil and used the resulting data to construct the sorption isotherms presented in Figures 2 and 3.

Linear, Freundlich, or Langmuir equations are frequently used to describe the distribution of solute at equilibrium. On the basis of visual observation of the experimental data obtained for HMX, the isotherms appear to follow a linear trend. Legget (*15*) also reported linear sorption of RDX and

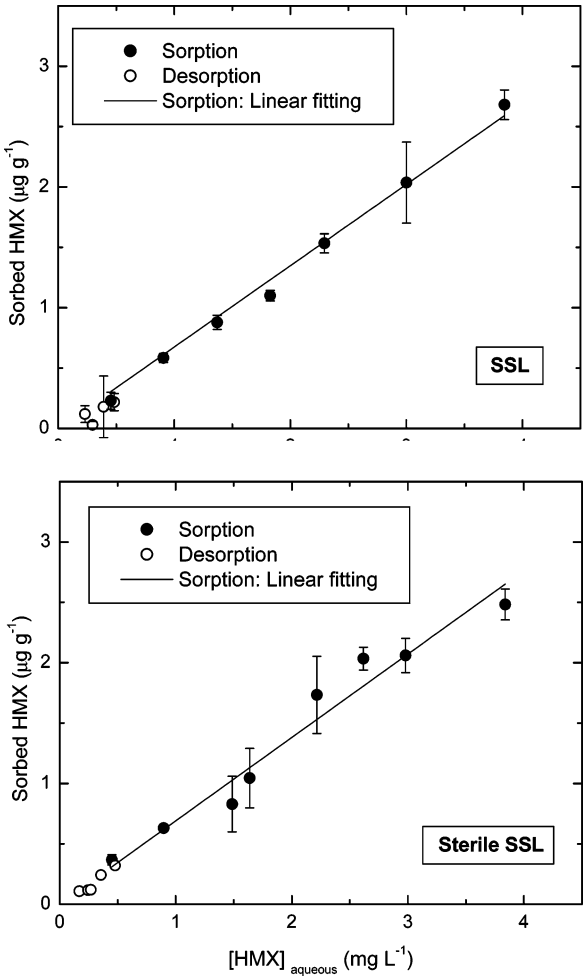


FIGURE 3. Sorption and desorption isotherms of HMX on sterile and nonsterile SSL soil. Error bars represent the standard deviation of three replicates. Fitting parameters are given in Table 3.

HMX onto drilling muds, and Townsend et al. (*26*) found that RDX and HMX column elution curves could be adequately described using a linear equilibrium model. The linear eq 1 was thus used to describe the equilibrium sorption and desorption of HMX in soil:

$$\frac{x}{m} = K_d C \qquad (1)$$

where x/m is the mass of solute sorbed per unit mass of dried soil at equilibrium (μg g^{-1}), K_d is the distribution coefficient (L kg^{-1}), and C is the aqueous equilibrium phase solute concentration (mg L^{-1}). The distribution coefficient for sorption will be denoted as K_d^S, while for desorption it will be denoted as K_d^D. The K_d^S and K_d^D values for HMX are reported for both soils in Table 3 along with the corresponding K_{oc} ($= K_d^S/f_{oc}$, where f_{oc} corresponds to the weight fraction of organic carbon).

The linearity of the adsorption isotherm implies that HMX binding to soil is not strongly concentration-dependent, suggesting that HMX is adsorbed onto soil mainly by nonspecific interactions. However, only a narrow concentration range could be used due to the low solubility of HMX in water (3.3 mg/L at 20 °C). At low surface coverage, linear isotherms have also been observed for nitroaromatic compounds whose adsorption isotherms are usually curved and converge to a saturation level (*27*). Therefore, specific interactions between HMX and soils are not excluded. Desorption experiments were thus performed using both VT and SSL soils to provide more insight into the strength of HMX binding onto the soil matrix. Data obtained using VT soil ($K_d^D = 4.5-5.0$ L kg^{-1} > $K_d^S = 2.5$ L kg^{-1}) (Figure 2) clearly indicate a sorption/desorption hysteresis, which implies the occurrence of specific interactions between HMX and VT soil. As for SSL soil (Figure 3), the low extent of sorption led to desorption data points that are gathered at the bottom left of the isotherm, making it difficult to determine the reversibility of HMX sorption. The hysteresis observed with VT soil is not due to the formation of covalent bonds between HMX and the organic matter present in the soil since the nitramine was fully recovered when extracting the sorbed matrix with acetonitrile.

TABLE 3. Sorption and Desorption Distribution Coefficients for HMX and Sterile and Nonsterile VT and SSL Soils

parameters	VT	sterile VT	SSL	sterile SSL
K_d^S (L/kg)	2.47	2.52	0.67	0.69
r^2	0.983	0.964	0.992	0.960
K_d^D (L/kg)	5.04	4.50	0.615	nd[a]
r^2	0.966	0.936	0.876	nd
log K_{oc}[b]	1.47	1.48	2.31	2.32

[a] nd, not determined. [b] Based on measured K_d^S values and $f_{oc} = 0.084$ and 0.0033 for VT and SSL, respectively.

TABLE 4. K_d^S Values Obtained When Sorbing TNT, RDX, and HMX onto Different Matrixes

soil	sand (%)	silt (%)	clay (%)	TOC[a] (%)	K_d^S TNT	K_d^S RDX	K_d^S HMX
LAAP A[b]	89	5	6	0.31	26		1
LAAP C[b]	77	11	12	0.08	64	0.3	
LAAP D[b]	27	41	32	0.20	167	0.7	2
montmorillonite[b]	na[c]	na	na	nd[d]	416	3.2	22.5
Aqua-Gel[e]	>5		> 87	nd	131	6.6	8.0
VT	83	12	4	8.4	4.2[f]	1.9[g]	2.5[h]
SSL	71	18	11	0.33			0.7[h]

[a] Total organic carbon in wt %. [b] Data from ref *17*. LAAP, Louisiana Army Ammunition Plant. K_d^S values obtained with montmorillonite saturated with K^+. [c] na, not applicable. [d] nd, not determined. [e] Data from ref *15*. [f] Data from ref *18*. [g] Data from ref *19*. [h] Data from the present study.

Table 4 gives a comparison of sorption parameters for TNT, RDX, and HMX obtained by our group and by others (*15*, *17*−*19*). Several trends appear from this table that can also be seen in Figure 4: (i) the sorption distribution coefficients K_d^S for HMX are slightly higher than those for RDX but significantly lower than those of TNT for the same soil; (ii) the percentage of organic matter does not govern the sorption of HMX, and (iii) HMX sorption grows exponentially with the amount of clay.

The higher K_d^S values obtained for TNT are consistent with the nonlinear shape observed for its sorption isotherms (*15*, *27*, *28*). TNT is a nitroaromatic compound that can interact with soil through the formation of electron donor−acceptor complexes between the oxygen atoms present at the mineral surface (e^- donor) and the π-system of the aromatic ring in TNT (e-acceptor) (*27*). Also, TNT can transform quite readily under both abiotic and biotic conditions, leading to the formation of amines, which can irreversibly bind onto soil through amide linkages (*29*) or be intercalated between negative clay plates by cationic exchange when protonated. This last assumption is consistent with the good correlation observed between TNT K_d^S values and cation exchange capacities (CEC) (*17*) or pH (*18*).

Such interactions cannot exist with RDX and HMX because cyclic nitramines are nonaromatic and do not form stable

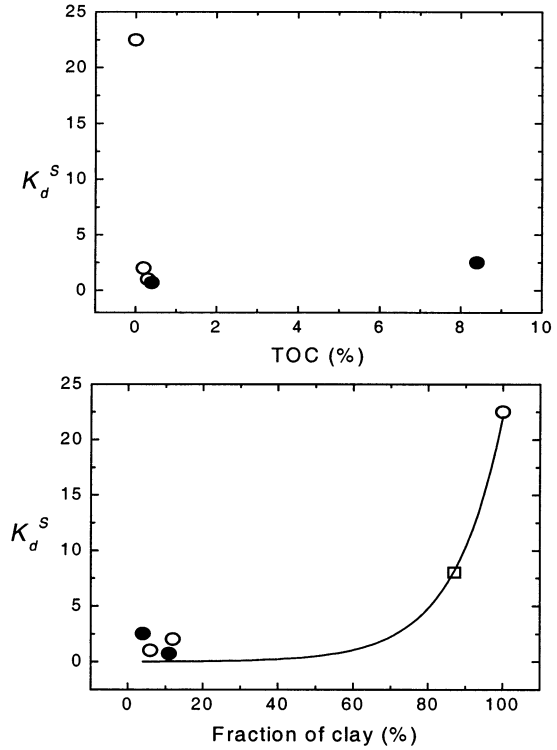


FIGURE 4. Effect of total organic content (TOC) and clay content on sorption of HMX onto soils: (□) data from ref *15*; (○) data from ref *17*; (●) data from the present study.

amino derivatives. However, as seen in Figure 4, the presence of clay in the soil plays a significant role in the sorption of HMX. Hydrogen bonds between surface hydroxyl groups (−OH) in soil and nitro groups ($-NO_2$) of nitramines have been reported as a possible sorption mechanism (*15*). Boyd and co-workers (*30*) also suggested that nitroaromatic compounds could be sorbed by complexation between metals in soil, such as K^+, and the various $-NO_2$ groups. The higher K_d^S values obtained for HMX, which contains one extra $-NO_2$ group, as compared to that obtained with RDX, and the positive effect of clay both support the possible interaction of $-NO_2$ groups with surface hydroxyl groups of clay or metals present in the soil.

The absence of correlation between TOC and HMX K_d^S values (Figure 4) shows that organic carbon does not govern HMX sorption, as substantiated also by the different K_{oc} values calculated from sorption of HMX on VT and SSL soils (Table 3). Moreover, the relative K_{ow} values for RDX and HMX (Table 1) fail to explain the difference in sorption between both compounds (K_d^S (HMX) > K_d^S (RDX)), thus reinforcing the limited role of hydrophobicity on HMX sorption onto soils. Pennington and Brannon (*31*) reported that in contrast to TNT, only small amounts of RDX were actually associated with soil organic matter. Similar behavior is observed here with HMX.

Finally, data shown in Figures 2 and 3 and the corresponding K_d values reported in Table 3 indicate that there is no difference in the sorption/desorption behavior of HMX using sterile or nonsterile soils. Also, recoveries of greater than 96% were measured for HMX, indicating the absence of biotic or abiotic losses of the chemical within the time course of sorption/desorption cycles (1 week in total).

Long-Term Fate. The fate of HMX in sterile and nonsterile topsoil systems was subsequently studied in static experiments over a period of 16 weeks. Following the first 3 weeks, almost all HMX was recovered from both sterile and nonsterile systems (Figure 5a). After 16 weeks, the percentage of HMX

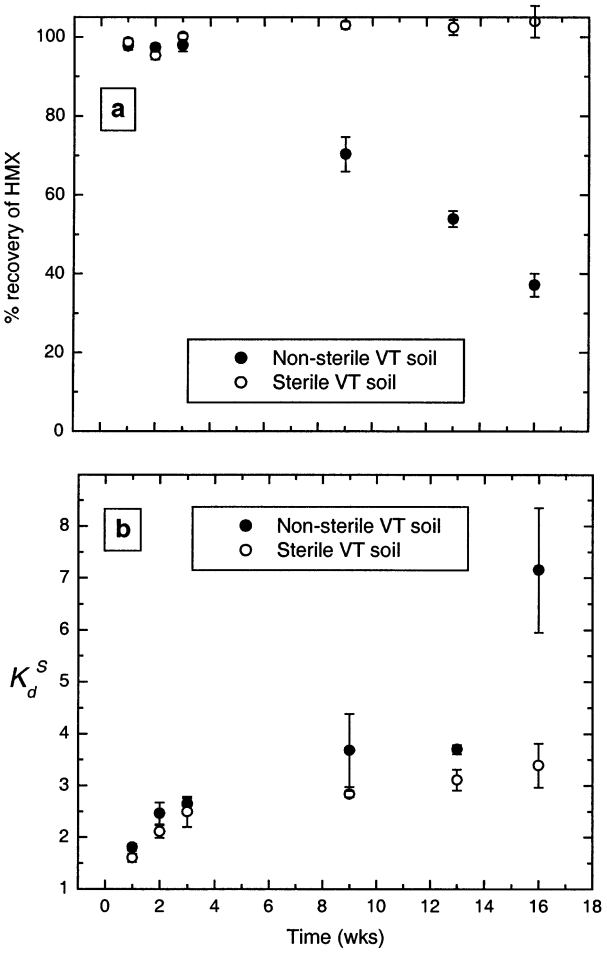


FIGURE 5. Long-term static sorption experiments on sterile (open circles) and nonsterile (solid circles) VT soil. Error bars represent the standard deviation of three replicates: (a) total HMX recovered from the aqueous and soil-sorbed phases; (b) K_d^S values.

recovery for sterile and nonsterile soils was 95% and 40%, respectively, indicating the occurrence of biotransformation in the latter case. The addition of resazurin at the end of the experiment confirmed the presence of an anaerobic environment in the soil samples. RDX was also found to degrade in nonsterile VT soil but at a much faster rate since total disappearance was observed after 4 weeks under similar conditions (*19*).

The K_d^S values were determined for each time interval of the fate study (Figure 5b). K_d^S values measured with sterile and nonsterile soils were in agreement with each other during the first 3 weeks, but past this time, K_d^S values became higher with nonsterile soil. HMX sorption/desorption on VT soil is faster than its biotransformation; therefore, if sorption was fully reversible, K_d^S values would remain unchanged whatever the total amount of HMX in the medium. The increase in K_d^S when using nonsterile VT soil supports the occurrence of hysteresis with this soil and suggests that biotransformation takes place in the aqueous phase.

LC/MS analysis of separate experiments containing a higher concentration of HMX (54.5 mg L^{-1}) with nonsterile VT soil showed after 35 weeks the disappearance of HMX and the formation of the four nitroso derivatives: octahydro-1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazocine (**2**), octahydro-1,5-dinitroso-3,7-dinitro-1,3,5,7-tetrazocine or octahydro-1,3-dinitroso-5,7-dinitro-1,3,5,7-tetrazocine (**3**), octahydro-1,3,5-trinitroso-7-nitro-1,3,5,7-tetrazocine (**4**), and octahydro-1,3,5,7-tetranitroso-1,3,5,7-tetrazocine (**5**) (Figure 6). These metabolites could not be quantified due to the lack of

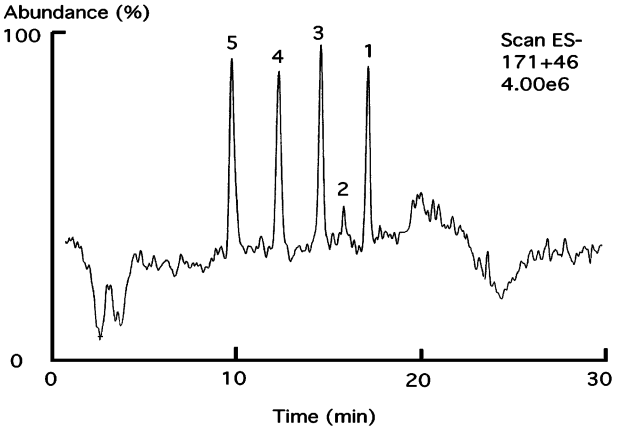


FIGURE 6. LC−MS chromatogram (negative electrospray ionization; extracted ions *m/z* 171 and 46) for supernatant of nonsterile VT soil microcosm after 35 weeks at 37 °C. The peaks correspond to the following: 1, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; 2, octahydro-1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazocine; 3, octahydro-1,5-dinitroso-3,7-dinitro-1,3,5,7-tetrazocine or octahydro-1,3-dinitroso-5,7-dinitro-1,3,5,7-tetrazocine; 4, octahydro-1,3,5-trinitroso-7-nitro-1,3,5,7-tetrazocine; 5, octahydro-1,3,5,7-tetranitroso-1,3,5,7-tetrazocine.

TABLE 5. Mineralization of ^{14}C-Labeled HMX Using Sterile and Nonsterile VT Soil[a]

soil	KOH trap	KOH trap after acidification	aqueous phase	soil extract	total detected
sterile VT	2.0 (0.1)	0.1 (0.1)	69 (1.9)	27.0 (3.5)	98.1
nonsterile VT	14.3 (2.4)	5.2 (2.4)	64.1 (6.9)	15.4 (4.9)	93.8

[a] Values are reported in % of total ^{14}C. Standard deviations of duplicate samples are in parentheses.

commercial products. The transformation of HMX to its corresponding nitroso derivatives was first suggested by McCormick and co-workers when studying the anaerobic degradation of HMX in culture media (*11*) and later reported at contaminated sites (*32*). We were unable to detect methylenedinitramine, that has been previously observed during incubation of HMX in sludge (*12*). However, when methylenedinitramine was added to a VT soil slurry, it disappeared rapidly to form N_2O and HCHO, demonstrating that the ring-cleavage product, methylenedinitramine, cannot be used as a marker to follow the fate of nitramines in soil. Mineralization experiments in the presence of sterile and nonsterile VT soil led to a slow release of $^{14}CO_2$ in the latter case. At the end of the 30-week experiment, a C mass balance of 94% was obtained (Table 5). Most of the radioactivity was present in the aqueous phase showing that degradation of HMX to products that would be strongly bound to the soil is not a relevant process, in contrast to TNT (*29*).

The results of the present study suggest that HMX will persist at active anti-tank firing ranges, as its low volatility (*1*), low aqueous solubility (*10*), and slow (bio)chemical reactivity in aerobic environments (*1*) do not allow for natural attenuation to effectively compete with constant soil loading. However, at abandoned sites, the compound's poor soil affinity (K_d^S, 0.7−2.5 L kg^{-1}, Table 3) will permit its slow migration to subsurface soil where anaerobic conditions prevail, thus allowing anaerobic degradation to take place. HMX degradation should proceed through the formation of nitroso derivatives and be accompanied by partial mineralization. These experimental findings are promising for the natural attenuation of HMX at contaminated sites.

Acknowledgments

We thank Annamaria Halasz and Louise Paquet for their technical assistance for LC/MS and HPLC analyses. We also thank Guy Ampleman and Sonia Thiboutot (DRDC, Valcartier, Canada) for providing HMX and UL-[^{14}C]-HMX. Funding was provided by the U.S. DoD/DoE/EPA Strategic Environmental Research and Development Program (SERDP 1213).

Literature Cited

(1) Hawari, J.; Halasz, A. In *The Encyclopedia of Environmental Microbiology*; Bitton, G., Ed.; John Wiley & Sons Ltd: New York, 2002; pp 1979−1993.
(2) Myler, C. A.; Sisk, W. In *Environmental Biotechnology for Waste Treatment*; Sayler, G., Fox, S. R., Blackburn, J. W., Eds.; Plenum Press: New York, 1991; pp 137−146.
(3) Urbanski, T. *Chemistry and Technology of Explosives*; Pergamon Press: Oxford, UK, 1983; Vol. 3, pp 17−47.
(4) Groom, C. A.; Halasz, A.; Paquet, L.; Morris, N.; Olivier, L.; Dubois, C.; Hawari, J. *Environ Sci. Technol.* **2002**, *36*, 112.
(5) Dubé, P.; Ampleman, G.; Thiboutot, S.; Gagnon, A.; Marois, A. *Report DREV-TR-1999-13*; Defence Research Establishment Valcartier, Department of National Defence Canada: 1999.
(6) Jenkins, T. F.; Walsh, M. E.; Thorne, P. T.; Miyares, P. H.; Ranney, T. A.; Grant, C. L.; Esparza, J. R. *CRREL Special Report 98-9*; Cold Regions Research and Engineering Laboratory, U.S. Army Corps of Engineers, Office of the Chief of Engineers: 1998.
(7) Pennington, J.; Brannon, J. M.; Gunnison, D.; Herrelson, D. W.; Zakikhani, M.; Miyares, P.; Jenkins, T. F.; Clarke, J.; Hayes, C.; Ringleberg, D.; Perkins, E.; Fredrickson, H. *Soil Sediment Contam*. **2001**, *10*, 45.
(8) Robidoux, P. Y.; Hawari, J.; Thiboutot, S.; Ampleman, G.; Sunahara, G. I. *Environ. Pollut.* **2001**, *111*, 283.
(9) Balakrishnan, V. K.; Halasz, A.; Hawari, J. *Environ. Sci. Technol.* **2003**, *37*, 1838.
(10) Talmage, S. S.; Opresko, D. M.; Maxwell, C. J.; Welsh, C. J. E.; Cretella, F. M.; Reno, P. H.; Daniel, F. B. *Rev. Environ. Contam. Toxicol.* **1999**, *161*, 1.
(11) McCormick, N. G.; Cornell, J. H.; Kaplan, A. M. *The Anaerobic Biotransformation of RDX, HMX and Their Acetylated Derivatives*; Technical Report A149464 TR-85/007; United States Army Natick Research & Development Center: Natick, MA, 1984.
(12) Hawari, J.; Halasz, A.; Beaudet, S.; Paquet, L.; Ampleman, G.; Thiboutot, S. *Environ. Sci. Technol.* **2001**, *35*, 70.
(13) Shen, C. F.; Hawari, J.; Ampleman, G.; Thiboutot, S.; Guiot, S. R. *Biorem. J.* **2000**, *4*, 27.
(14) Boopathy, R. *Soil Sediment Contam*. **2001**, *10*, 269.
(15) Leggett, D. C. *CRREL Report 85-18*; U.S. Army Cold Regions Research and Engineering Laboratory: Hanover, NH, 1985.
(16) Myers, T. E.; Brannon, J. M.; Pennington, W. M.; Myers, K. F.; Townsend, D. M.; Ochman, M. K.; Hayes, C. A. *Technical Report IRRP-96-1*; U.S. Army Corps of Engineers, Waterways Experiment Station: Vicksburg, MS, 1996.
(17) Brannon, J. M.; Price, C. B.; Hayes, C.; Yost, S. L. *Soil Sediment Contam*. **2002**, *11*, 327.
(18) Sheremata, T. W.; Thiboutot, S.; Ampleman, G.; Paquet, L.; Halasz, A.; Hawari, J. *Environ. Sci. Technol.* **1999**, *33*, 4002.
(19) Sheremata, T. W.; Halasz, A.; Paquet, L.; Thiboutot, S.; Ampleman, G.; Hawari, J. *Environ. Sci Technol.* **2001**, *35*, 1037.
(20) U.S. Environmental Protection Agency. *Method 8330 SW-846 Update III Part 4*: *1* (*B*), *Nitroaromatics and Nitramines by High Performance Liquid Chromatography* (*HPLC*); Office of Solid Waste: Washington, DC, 1997.
(21) OECD Guideline for Testing of Chemicals 107: Partition Coefficient (*n*-Octanol/Water) (Flask-Shaking Method). Adopted on May 12, 1981.
(22) Lynch, J. C.; Myers, K. F.; Brannon, J. M.; Delfino J. J. *J. Chem. Eng. Data* **2001**, *46*, 1549.
(23) Halasz, A.; Spain, J.; Paquet, L.; Beaulieu, C.; Hawari, J. *Environ. Sci. Technol.* **2002**, *36*, 633.
(24) Xue, S. K.; Iskandar, I. K.; Selim, H. M. *Soil Sci.* **1995**, *160*, 317.
(25) Singh, J.; Comfort, S. D.; Hundal, L. S.; Shea, P. J. *J. Environ. Qual.* **1998**, *27*, 572.
(26) Townsend, D. M.; Myers, T. E.; Adrian, D. D. *Technical Report IRRP-96-8*; U.S. Army Engineer Waterways Experiment Station: Vicksburg, MS, 1996.

(27) Haderlein, S. B.; Weissmahr, K. W.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1996**, *30*, 612.
(28) Townsend, D. M.; Myers, T. E. *Technical Report IRRP-96-1*; U.S. Army Engineer Waterways Experiment Station: Vicksburg, MS, 1996.
(29) Bruns-Nagel, D.; Steinbach, K.; Gemsa, D.; von Löw, E. In *Biodegradation of Nitroaromatic Compounds and Explosives*; Spain, J. C., Hughes, J. B., Knackmuss, H.-J., Eds.; CRC Press: Boca Raton, FL, 2000; p 357.
(30) Boyd, S. A.; Sheng, G.; Teppen, B. J.; Johnston, C. T. *Environ. Sci. Technol.* **2001**, *35*, 4227.
(31) Pennington, J. C.; Brannon, J. M. *Thermochim. Acta* **2002**, *384*, 163.
(32) Spanggord, R. J.; Mabey, W. R.; Chou, T. W. In *Chemical Industry Institute of Toxicology Series. Toxicity of Nitroaromatic Compounds*; Rickert, D. E., Ed.; Hemisphere Publishing Corp.: Washington, DC, 1985; p 15.

Received for review March 24, 2003. Revised manuscript received June 9, 2003. Accepted June 19, 2003.

ES030407E