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Detection of the cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX) and their degradation products in soil environments

Carl A. Groom, Sylvie Beaudet, Annamaria Halasz, Louise Paquet, Jalal Hawari*

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

Abstract

The cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX) were examined in field and microcosm soil samples to determine their patterns of degradation and environmental fates. A number of analytical techniques, including solid-phase microextraction with on-fiber derivatization, gas chromatography–mass spectrometry, gas chromatography with electron-capture detection, liquid chromatography–mass spectrometry, and micellar electrokinetic chromatography were required for the analyses. Two different classes of intermediates were detected, both of which lead ultimately to the formation of nitrous oxide (N_2O) and carbon dioxide (CO_2). The first class was identified as the nitroso derivatives formed by the sequential reduction of $-NO_2$ functional groups. The second class of intermediates, which was favored at higher humidities and in the presence of anaerobic sludge amendments, consisted of ring cleavage products including bis-(hydroxymethyl)-nitramine and methylenedinitramine. Rye-grass (*Lolium perenne*) present in field samples was found to extract and accumulate HMX from soil without further degradation. In all cases (excepting the plant samples), the indigenous microbes or amended domestic anaerobic sludge consortia degraded the cyclic nitramine explosives eventually to produce N_2O and CO_2.

Keywords: Soil; Environmental analysis; Hexahydro-1,3,5,7-trinitro-1,3,5,7-triazine; Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine; RDX; HMX; Explosives; Nitramines

1. Introduction

A recent survey of five Canadian anti-tank ranges [1] revealed low level soil contamination by the nitroaromatic explosive 2,4,6-trinitrotoluene (TNT), and the cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX). The weapon system employed on these ranges contains a nominal 70% HMX and 30% TNT formulation (%, w/w) with significant amounts of RDX present as an HMX production impurity. The remediation of these lands using cost effective in situ remediative methods including microbial degradation [2] and phytoremediation [3], is currently under consideration. A major prerequisite to the application of these methods is an understanding of the relevant degradative chemistry and the selection of appropriate

*Corresponding author. Tel.: +1-514-4966-267; fax: +1-514-4966-265.

E-mail address: jalal.hawari@nrc.ca (J. Hawari).

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analytical methods to determine the environmental fate of intermediates and associated population risks. The biotransformation of TNT and its potential for microbial degradation in soil are well documented and reported elsewhere [4,5]. In the case of RDX and HMX, the degradative chemistry in soil remains incomplete, and the detection of intermediates and products in soil samples requires a large number of analytical methods. In this work a selection of extractive and analytical methods are applied to determine RDX or HMX and their degradation products in contaminated field soil and plant samples. Methods including solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS), liquid chromatography–mass spectrometry (LC–MS), GC with electron-capture detection (GC–ECD), micellar electrokinetic chromatography with ultraviolet absorbance detection (MEKC–UV), and high-performance liquid chromatography with UV detection (HPLC–UV), along with ^{15}N- and ^{14}C-labeled RDX substrate and anaerobic sludge amendments are applied to identify some of the intermediates involved in the (bio)transformation of cyclic nitramine explosives in natural soil environments. The observed patterns of degradation are then discussed with reference to previously identified mechanisms for the degradation of cyclic nitramine explosives in liquid culture media [6,7].

2. Experimental

2.1. Materials

Commercial-grade RDX and HMX (with a purity >99%) were provided by Defense Research Establishment Valcartier, Quebec, Canada. Uniformly labeled [UL-^{14}C]RDX and HMX with respective specific activities of 28.7 μCi/mmol and 93.9 μCi/mmol were synthesized [8,9] and recrystallized to achieve respective chemical and radiochemical purities of 99% and 97% for RDX and 94% and 91% for HMX. Uniformly ring labeled [UL-^{15}N]RDX was synthesized as described by Ampleman et al. [9] (purity>98%). Mono- and trinitroso-RDX were synthesized according to the methods described by Brockman et al. [10]. All other chemicals were reagent grade. The sludge was obtained from a food factory located at the city of Cornwall, Canada, and was used as the exogenous source of microorganisms. A BBL dry anaerobic indicator (VWR, Canlab, Canada) was used to detect air leaks and to ensure anaerobic conditions. Contaminated soil samples were obtained from a firing range in western Canada and analyzed for explosives as described by the US Environmental Protection Agency (EPA) Method 8330 [11]. Topsoil was obtained from a local garden supplier and artificially contaminated with the energetic chemical.

2.2. Soil slurry microcosms

In a typical set up, a serum bottle (100 ml) was charged with 20 g of soil. A molasses mineral salt medium (10 ml) composed of 33 g/l molasses, 7.4 g/l $NaHCO_3$ and 8.8 g/l $KHCO_3$ was then added to serve as carbon source and RDX (final concentration 800 mg/l) served as the N-source for the degrading microorganisms. Anaerobic sludge (8 ml) and distilled water were then added to obtain a final volume of 40 ml. Some serum bottles (microcosms) were supplemented with a uniformly labeled [UL-^{14}C]RDX (100 000 dpm) and then fitted with a small test tube containing 1.0 ml of 0.5 *M* KOH to trap liberated carbon dioxide ($^{14}CO_2$). The headspace in each microcosm was flushed with nitrogen gas (N_2) to maintain anaerobic conditions and then sealed with butyl rubber septa and aluminum crimp seals to prevent the loss of CO_2 and other volatile metabolites. For the analysis of N_2 and nitrous oxide (N_2O) the head space was flushed with nitrogen-free argon gas. Uniformly ring labeled [UL-^{15}N]RDX was used to confirm the identity of RDX breakdown products such as N_2, N_2O, and other ring cleavage products. Each microcosm was wrapped with aluminum foil to protect the mixture against photolysis. Microcosms with [UL-^{14}C]RDX were routinely sampled (on hourly and/or daily basis) for the determination of $^{14}CO_2$ in the KOH trap using a Packard, Tri-Carb 4530 liquid scintillation counter (Model 2100 TR; Packard, Meriden, CT, USA). Microcosms that did not receive ^{14}C-labeled RDX were reserved for SPME–GC–MS, HPLC–UV, GC–ECD and LC–MS

analysis of intermediate degradation products (see below).

2.3. Extraction of HMX from plant tissue

The tissue extracts from rye-grass shoots (*Lolium perenne*) grown on contaminated firing range soil were prepared and analyzed using the methods outlined by Larson et al. [12]. Briefly, finely cut samples of the plant (approximately 4 g) were suspended in 10–20 ml of ice cold deionized water (18 MΩ cm resistivity) and homogenized using a Kinematica homogenizer (Kriens, Switzerland) fitted with a Brinkman Polytron PTA 20 S saw tooth generator (Brinkman Instruments, Mississauga, Canada) suitable for fibrous plant or animal tissues. The samples were then homogenized at full setting (20 000 rpm) until a frothy granular paste was obtained. Immediately after homogenization, the samples were transferred to tared 120-ml Labconco lyophilization flasks and immersed in a dry ice acetone bath for 20 min. The samples were lyophilized using a Virtis Freezemobile 24 lyophilizer (Virtis, Gardner, NY, USA) until no further change in flask mass was observed (average time 20 h). Approximately 0.2 g of freeze–dried material was then transferred to a 15-ml Kimax screw cap culture tube with 10 ml of acetonitrile. The capped tubes were then sonicated for 18 h in a Blackstone Ultrasonics Neptune Ultrasonic Generator (Blackstone Ultrasonics, Jamestown, NY, USA) cooled to 10°C using a Lauda RM6 refrigerated circulating bath (Brinkman Instruments). After sonication the Kimax tubes were centrifuged at 5000 *g* for 15 min using a Fisher Centrific benchtop centrifuge (Fisher Scientific, Montreal, Canada). The supernatant was then decanted and allowed to settle for 20 min. A 2-ml aliquot of the supernatant was then mixed with an equal volume of deionized water and filtered using Millex HV 0.45-μm filter cartridges.

2.4. CE–UV analysis of HMX and derivatives in plant extracts

Separations to detect HMX, RDX or their metabolites in plant extracts were performed using a Hewlett-Packard (HP) ^{3D}CE instrument interfaced with a HP Vectra personal computer running HP Chemstation software.The HP ^{3D}CE instrument was fitted with a HP G-1600-31232 fused-silica bubble capillary with a total length of 64.5 cm, and an effective length (inlet to detection window) of 56 cm. The voltage was set at 30 kV and the temperature at 25°C. Samples were injected by applying 50 mbar pressure to the capillary inlet for 5 s. The separation buffer was composed of 2.5 m*M* sodium tetraborate and 12.5 m*M* boric acid (pH 8.5) containing 50 m*M* sodium dodecyl sulfate. Absorbances were monitored at wavelengths of 214, 230 and 280 nm. Unless otherwise indicated the separation time was 8 min with post-conditioning flushes of the capillary after each run in the following sequence; methanol (0.5 min), 0.1 *M* NaOH (0.5 min) and running buffer (3 min). The total analysis time was therefore 12 min.

2.5. HPLC–UV and LC–MS analysis

The concentrations of RDX and HMX were monitored by observing peak absorbances at 230 and 254 nm and accompanying diode array spectra (HPLC–UV) as described earlier [7]. The nitroso derivatives and ring cleavage products were analyzed by HPLC–UV and by LC–MS with a Micromass Plattform 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 culture medium were injected into a Supelcosil LC-CN column (25 cm× 4.6 mm; 5 μm particle size) thermostated at 35°C. The solvent system consisted of a methanol–water gradient at a flow-rate of 1 ml/min. A first linear gradient was run from 10% to 20% methanol over 15 min followed by a second linear gradient from 20% to 60% over 5 min which was held for 3 min. This solvent ratio was returned to the initial conditions over 2 min and held for an extra 10 min. Analyte ionization was done in a negative electrospray ionization (ESI) mode producing mainly $[M-H]^-$ mass ions. The electrospray probe tip potential was set at 3.5 kV with a skimmer voltage of 30 V at an ion source temperature of 150°C. The mass range was scanned from 40 to 400 u with a cycle time of 1.6 s and the resolution was set to 1 u (width at half-height).

2.6. SPME–GC–MS

Formaldehyde was analyzed with an SPME–GC method as described by Martos and Pawlyszin [13]. A fiber coated with poly(dimethylsiloxane)–divinylbenzene (Supelco) was placed inside the head space of vial containing the derivatizing agent *O*-(2,3,4,5,6 - pentafluorobenzyl)hydroxylamine·hydrochloride salt (PFBHA). The SPME fiber, coated with the derivatizing agent, was then placed inside the headspace of a fixed volume (1 ml) of sludge treating RDX at 55°C with stirring for 20 min. The SPME assembly was then allowed to undergo desorption (250°C) inside the injector port of a GC (HP 6890)–mass selective detection (HP5973) system. A Supelco MDN-5S column (30 m×250 μ I.D., 0.25 μm film thickness) with He as a carrier gas was used with an oven temperature set at 45°C (hold 1 min) that was increased at a rate of 10°C/min to 200°C. The MS range was set from 35 to 400 u. A HCHO standard solution (37%, w/w, water), Fisher Scientific (Nepean, Canada) was derivatized in a similar way to confirm the identity of RDX metabolite. An adsorption time of 20 min followed by 20 min desorption in the GC system were found to be ideal conditions for quantifying HCHO. Detection limit<100 μg/l.

2.7. Measurement of gaseous end products (CH_4, N_2, N_2O)

A SRI 8610 GC (INSUS Systems) connected to a Supelco Porapack Q column (2 m) and coupled with either an ECD system (330°C) for the detection of nitrous oxide (N_2O), or a GC–RAM radio activity detector (750°C) for the detection of radiolabeled methane ($^{14}CH_4$) was used. The gaseous products from the head space of the culture medium were sampled using a gas-tight syringe for subsequent injection inside the GC system using helium as a carrier gas (21 ml/min) at 60°C. Gas identification was confirmed by comparison with reference materials. The detection limits for RAM and ECD were 150 dpm and 12 ppm, respectively. The presence of N_2 and N_2O as products was confirmed using GC–MS analysis and monitoring the masses at m/z 29 ($^{15}N^{14}N$), and 45 u ($^{15}N^{14}NO$), respectively.

3. Results and discussion

3.1. Primary degradation products of RDX and HMX in soil: the effect of indigenous soil microorganisms and higher plants

Fig. 1 is a HPLC chromatogram (230 nm) for an acetonitrile extract of firing range soil. The predominance of HMX (0.10 m*M*) is noted, along with the presence of its reduction product octahydro-1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazine (MN-HMX). Fig. 2 illustrates the transport of HMX and MN-HMX to the blade tissue of rye-grass previously grown on a soil contaminated with the explosive. No degradation occurred although the transport of the explosive to the plant tissue might constitute a potential future in situ remediation technology (phytoextraction). The translocation of RDX without degradation has also been observed for tree species [14].

Agricultural soil artificially contaminated with RDX (0.34 m*M*) and aged for 49 days without any other additions under a blanket of argon showed LC peaks representing mono- and dinitroso-RDX derivatives in the acetonitrile extract of the soil pellet (Fig. 3). A sterilized soil did not produce the above intermediates, indicating the biological transformation of the explosive by soil indigenous microorganisms.

The appearance of degradation products from

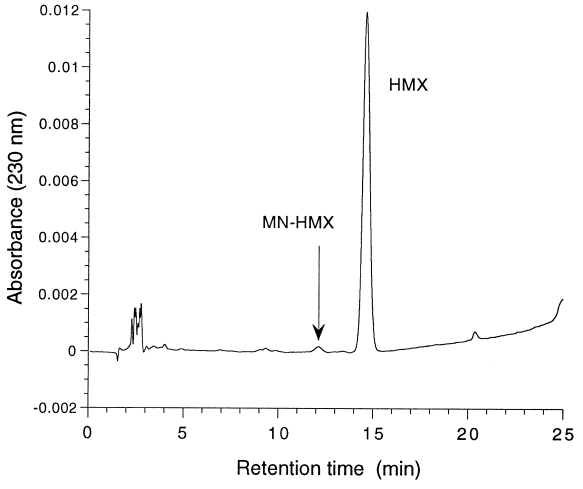

Fig. 1. HPLC chromatogram of an acetonitrile extract of firing range soil showing HMX and MN-HMX.

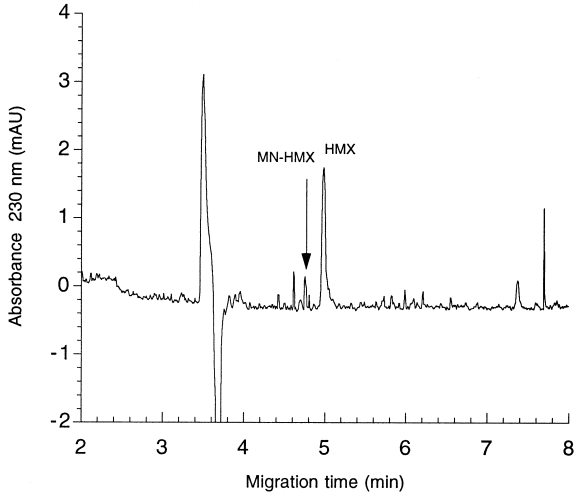

Fig. 2. Sodium dodecyl sulfate-MEKC electropherogram of a rye-grass (*Lolium perenne*) shoot acetonitrile extract containing HMX and MN-HMX.

either RDX or HMX in soil can be accelerated using anaerobic sludge. Fig. 4 is an LC–ESI-MS chromatogram obtained during biodegradation of RDX (3.59 m*M*) in a soil slurry reactor using domestic anaerobic sludge. After 7 days of incubation, several LC–MS peaks were detected as shown in Fig. 4. The LC–MS profile showed two different groups of peaks as identified by their mass data using their

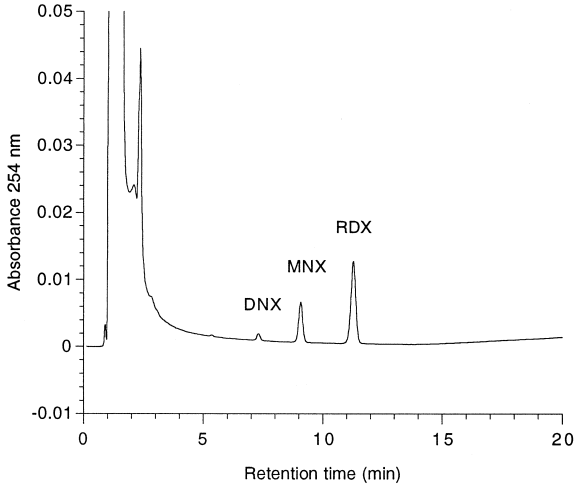

Fig. 3. HPLC chromatogram (254 nm) of non-sterile agricultural soil spiked with 0.39 m*M* RDX. RDX, MNX and DNX were identified using photodiode array detection spectra.

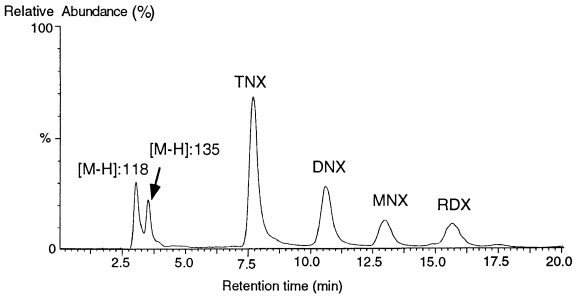

Fig. 4. A typical LC–ESI-MS of RDX (3.59 m*M*) degradation in soil slurry (40%, w/w) microcosms with anaerobic sludge (0.46%). Indicated peaks: RDX, mononitroso-RDX (MNX), dinitroso-RDX (DNX), and trinitroso-RDX (TNX), [M−H] 135 methylenedinitramine, [M−H] 118 unidentified ring cleavage product.

deprotonated molecular mass ions and retention times ([M−H], u, t_R, min). Further confirmation was also obtained by comparison with reference materials when available. The group of peaks appearing after 7 min were identified as 1,3,5-trinitroso-1,3,5-triazine, TNX (173, 8.1 min), 1,3-dinitroso-5-nitro-1,3,5-triazine, DNX (189, 10.5 min), 1-nitroso-3,5-dinitro-1,3,5-triazine, MNX (205, 13.0 min) and RDX (221, 16.5 min), respectively. The formation of nitroso-RDX derivatives has been observed during earlier studies involving biodegradation of RDX under anaerobic conditions using sludge in a liquid culture medium [6,15] and horse manure [16,17] as sources of microorganisms.

The earlier eluting group of peaks in Fig. 4 have their [M−H] and t_R at 118 u, 3.0 min and 135 u, 4.7 min, respectively. The peak at 4.7 min with [M−H] at 135 u (M_r: 136), matched a molecular mass formula of $CH_4N_4O_4$. In liquid culture the above metabolite was tentatively identified as the ring cleavage product methylenedinitramine, $O_2NNHCH_2NHNO_2$ using [UL-^{15}N]RDX, with the [M−H] at 137 u [7]. The peak at 3.3 min which had its [M−H] at 118 u (M_r: 119) matched a molecular formula of $C_2H_5N_3O_3$. This product is also suspected of being a ring cleavage product but requires further investigation.

3.2. Secondary intermediates

All the LC–MS peaks observed in Fig. 4 did not

persist and transformed further to produce several other products including HCHO, N_2O, N_2 and CO_2. In earlier RDX studies [7] methylenedinitramine formed in a liquid culture medium, did not accumulate, and its disappearance was accompanied by the formation of the above gaseous products. After 60 days of incubation close to 60% mineralization (liberated $^{14}CO_2$) was obtained. Formaldehyde was detected as its pentafluorobenzyl oxime derivative (Fig. 5) in the headspace of RDX-treated microcosms using the SPME–GC–MS method described earlier by Martos and Pawliszyn [13]. The formation of carbon dioxide (CO_2) and nitrous oxide (N_2O) is an indication of the continued biological and/or abiotic decomposition of the primary degradation products of cyclic nitramines including the ring cleavage products. RDX and HMX are reportedly degraded by anaerobic sludge in liquid culture via a sequential reduction to 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) (Fig. 6, path a) [6]. The nitroso compounds were suggested to undergo further transformation to unstable hydroxylamine-RDX derivatives, HOHN-RDX, that undergo ring-cleavage to eventually yield formaldehyde (HCHO), methanol (CH_3OH), hydrazine (NH_2NH_2) and 1,1-dimethyhydrazine [$(H_3C)_2NNH_2$] [6]. No data to support the formation of hydroxylamine derivatives or any direct ring cleavage intermediates was presented. Recently Hawari et al. [7] reported the formation of CO_2 (mineralization), and N_2O as major products from the degradation of RDX with a domestic anaerobic sludge in liquid culture. Two-ring cleavage intermediate products were identified,

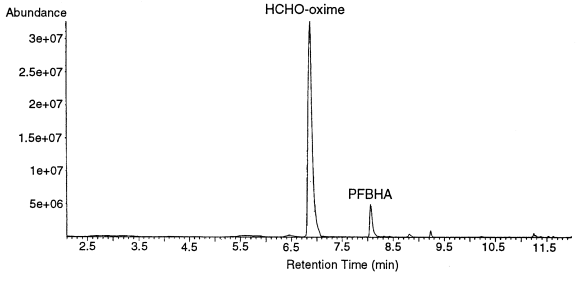

Fig. 5. Total ion current GC–MS chromatogram of *O*-(2,3,4,5,6-pentafluorobenzyl)-oxime derivative of formaldehyde obtained during degradation of RDX with domestic anerobic sludge.

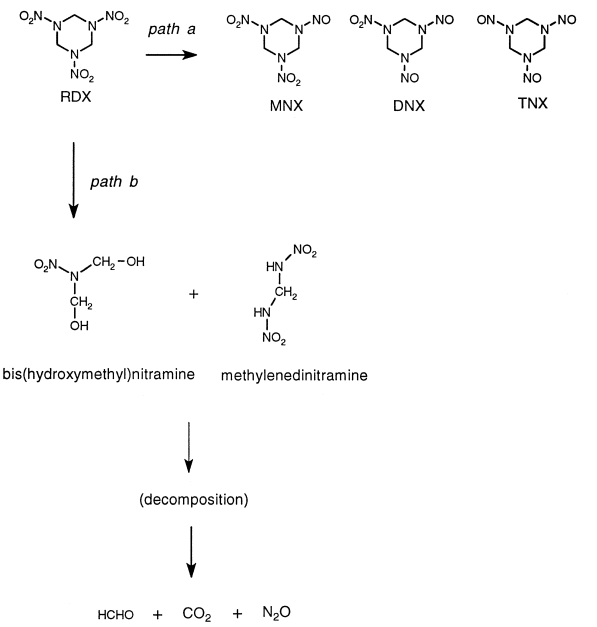

Fig. 6. A constructed pathway for the biodegradation of RDX (0.89 m*M*) with domestic anaerobic sludge (pH 7.0). Path a represents degradation of RDX via reduction to the nitroso compounds. Path b represents degradation via ring cleavage.

namely methylenedinitramine ($O_2NHNCH_2NHNO_2$) and bis(hydroxymethyl)nitramine ($HOCH_2NNO_2$-CH_2OH) which transformed to give N_2O and CO_2 (Fig. 6, path b). It is presumed that the degradation pathway for cyclic nitramine explosives in soil would proceed with the same pathways described earlier in liquid medium (Fig. 6, paths a and b) [7]. Equivalent degradative pathways in both anaerobic liquid cultures and soil have been demonstrated for many soil contaminants including atrazine [18,19] and trinitrotoluene [5].

3.3. Implications to field measurement and the assessment of environmental fate

In the case of contaminated soil, one cannot expect such an active or varied population of biocatalysts to exist as in the case of anaerobic sludge and for this reason only the initial nitroso-intermediates, and traces of gaseous end products (N_2O, CO_2) can be observed in detectable quantities from soil samples. The detection of N_2O and CO_2 in closed systems is promising for the in situ biomedia-

tion of RDX and HMX in soil. Where detectable, the rate of N_2O evolution may be of use to indicate the remediative capacity of a given soil environment.

Further method development is required to observe the accumulation of cyclic nitramines and their degradation products in plants. The effect of this process on environmental fate is best illustrated by a recent greenhouse study [3] in which sufficiently irrigated wheat and rye-grass cultivars accumulated HMX in their scenescent leaf tissue to over 500 mg/kg (plant dry mass basis) from soil with an average HMX concentration of 30 mg/kg (dry mass basis). RDX and HMX are of limited solubility in water (0.18 m*M* and 0.091 m*M*, respectively), but tend not to complex with stationary soil or plant tissue components and are readily concentrated in leaf tissue where bulk transpiration occurs. In addition, indigenous plant samples analyzed for HMX exhibited a high content of fatty acids which were readily extracted by acetonitrile, (the solvent normally applied in EPA Method 8330) and interfered with HPLC–UV quantifications (data not shown). While the use of MEKC permitted the resolution of HMX and its mononitroso-derivative (Fig. 2) in agricultural plant extracts, the excessively large fatty acid peaks observed in other samples had deleterious effects on reproducibility and quantification. The use of extraction procedures of greater selectivity, perhaps with cyclodextrin solutions or supercritical fluids, and other sample clean-up procedures prior to analysis are two directions for further experimentation.

4. Conclusions

A wide range of analytical techniques, including SPME–GC–MS, LC–MS, GC–ECD, MEKC–UV and HPLC–UV, along with ^{15}N- and ^{14}C-labeled RDX substrate and anaerobic sludge amendments, were required to compare the degradation of the explosives RDX and HMX in field soil and plant samples. The biodegradation of the cyclic nitramine explosives in soil was observed to produce the same degradation patterns observed earlier in liquid culture microcosms. Two pathways were possible; one involved the sequential reduction of the $-NO_2$ groups to produce MNX, DNX and TNX whose fate requires further investigation. The second route involved the enzymatic cleavage of C–N bonds to produce nitramine ring cleavage products that autodecomposed to eventually produce CO_2 and N_2O. A rye-grass plant species (*Lolium perenne*) was observed to extract and accumulate HMX from contaminated soils without further degradation and this is an important consideration in the assessment of environmental fates.

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References

[1] S. Thiboutot, G. Ampleman, A. Gagnon, A. Marois, T.F. Jenkins, M.E. Walsh, P.G. Thorne, T.A. Ranney, DREV Report-R-9809, Defense Research Establishment Valcartier, Val Bélair, 1998.
[2] D.G. Karamanev, J. Sci. Ind. Res. 58 (1999) 764.
[3] Report 21590.4, Biotechnology Research Institute, National Research Council Canada–Beak International Incorporated (BEAK), Brampton, 2000.
[4] J. Hawari, A. Halasz, L. Paquet, E. Zhou, B. Spencer, G. Ampleman, S. Thiboutot, Appl. Environ. Microbiol. 64 (1998) 2200.
[5] A. Preuß, P.-G. Rieger, in: J.C. Spain (Ed.), Biodegradation of Nitroaromatic Compounds, Plenum Press, New York, 1995, p. 69, Chapter 5.
[6] N.G. McCormick, J.H. Cornell, A.M. Kaplan, Appl. Environ. Microbiol. 42 (1981) 817.
[7] J. Hawari, A. Halasz, T. Sheremata, S. Beaudet, C. Groom, L. Paquet, C. Rhofir, G. Ampleman, S. Thiboutot, Appl. Environ. Microbiol. 66 (2000) 2652.
[8] G. Ampleman, S. Thiboutot, J. Lavigne, A. Marois, J. Hawari, A.M. Jones, D. Rho, J. Label. Compd. Radiopharm. 36 (1995) 559.
[9] G. Ampleman, A. Marois, S. Thiboutot, J. Hawari, C.W. Greer, J. Godbout, G.I. Sunahara, C.F. Shen, S. Guiot, Report DREV-TR-1999-99, Defense Research Establishment Valcartier, Val Bélair, 1999.

[10] F.J. Brockman, D.C. Downing, G.F. Wright, Can. J. Res. 27B (1949) 469.
[11] US EPA Method 8330 SW-846 Update III part 4(B), EPA Office of Solid Waste, Washington, DC, 1997.
[12] S.L. Larson, A.B. Strong, S.L. Yost, B.L. Escalon, D. Parker, Tech. Report IRRP-98-5, US Army Corps of Engineers, Washington, DC, 1998.
[13] P.A. Martos, J. Pawliszyn, Anal. Chem. 70 (1998) 2311.
[14] P.L. Thompson, L.A. Ramer, J.L. Schnoor, Environ. Toxicol. Chem. 18 (1999) 279.
[15] J. Hawari, in: J.C. Spain, J.B. Hughes, H.-J. Knackmuss (Eds.), Biodegradation of Nitroaromatic Compounds and Explosives, CRC Press, Boca Raton, FL, 2000, p. 277, Chapter 11.
[16] C.L. Kitts, D.P. Cunningham, P.J. Unkefer, Appl. Environ. Microbiol. 60 (1994) 4608.
[17] D.M. Young, P.J. Unkefer, K.L. Ogden, Biotech. Bioeng. 53 (1997) 515.
[18] D.D. Kaufman, P.C. Kearney, Resid. Rev. 32 (1970) 235.
[19] E. Topp, L. Tessier, E.G. Gregorich, Can. J. Soil Sci. 76 (1996) 403.