Analysis of the key intermediates of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) in groundwater: occurrence, stability and preservation

Paquet, Louise; Monteil-Rivera, Fanny; Hatzinger, Paul B.; Fuller, Mark E.; Hawari, Jalal

Publisher's version / Version de l'éditeur:
https://doi.org/10.1039/C1EM10329F
Journal of Environmental Monitoring, 13, 8, pp. 2304-2311, 2011-07-06

Journal of Environmental Monitoring

Cite this: *J. Environ. Monit.*, 2011, **13**, 2304

www.rsc.org/jem

PAPER

Analysis of the key intermediates of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) in groundwater: occurrence, stability and preservation

Louise Paquet,[a] Fanny Monteil-Rivera,[a] Paul B. Hatzinger,[b] Mark E. Fuller[b] and Jalal Hawari*[a]

Received 15th April 2011, Accepted 7th June 2011
DOI: 10.1039/c1em10329f

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a widely used explosive that is present in soils at a number of military sites, including training and testing ranges. Because of its relatively weak adsorption to soil, RDX frequently migrates through the unsaturated zone and causes groundwater contamination. In the environment, RDX can transform to produce mono-, di-, and tri-nitroso derivatives (MNX, DNX, and TNX) and the ring cleavage products methylenedinitramine (MEDINA) and 4-nitro-2,4-diazabutanal (NDAB). The present study was undertaken to analyze RDX and its products in groundwater samples taken from various US military sites. The stability of some of the common transformation intermediates of RDX, including the nitroso derivatives, NDAB and MEDINA, under typical conditions in a groundwater aquifer is not well understood, and appropriate preservation methods for these compounds have not been established. Therefore, we studied the inherent stability of these compounds in deionized water and in groundwater, and evaluated various preservation techniques, including adjustment of pH, temperature, and salinity. NDAB and nitroso derivatives were stable under typical ambient environmental conditions, but MEDINA was highly unstable. The addition of sea salts (10% w/v) was found to stabilize MEDINA when the samples were stored at 4 °C. Using appropriate preservation techniques, we detected nitroso derivatives and NDAB, but no MEDINA, at some of the sites investigated. Stabilizing RDX intermediate products in field samples to allow detection is important because the presence of any of these chemicals can indicate past contamination by RDX and provide insight into the occurrence of *in situ* natural attenuation.

Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a powerful energetic chemical that is extensively used by the military.[1] RDX has been detected in soils, groundwater and marine environments at a large number of military training and testing facilities in the US and abroad. Extensive research has been conducted recently to understand the environmental fate of RDX, including its biotic and abiotic degradation pathways. In the last decade, we identified several key transformation products of RDX that contributed to an improved understanding of the abiotic and biotic degradation routes of the nitroamine under both aerobic and anaerobic conditions.[2–7] RDX was shown to transform by two distinctive pathways: (1) a reduction pathway initiated by

[a]*Biotechnology Research Institute, NRCC, 6100 Royalmount Ave, Montréal, Quebec, H4P 2R2, Canada. E-mail: Jalal.Hawari@cnrc-nrc.gc.ca*
[b]*Shaw Environmental & Infrastructure, 17 Princess Road, Lawrenceville, NJ, 08648, USA*

Environmental impact

RDX is an explosive that has been detected in soil and groundwater at numerous military facilities, including many testing and training ranges. The nitroamine can transform under both biotic and abiotic conditions giving characteristic and unique products including nitroso derivatives (MNX, DNX, and TNX), methylenedinitramine (MEDINA) and 4-nitro-2,4-diazabutanal (NDAB). Successful detection of RDX transformation products in field samples is important because it (1) demonstrates past or current RDX contamination, (2) proves the occurrence of *in situ* natural attenuation, and (3) will aid in the development of on site monitoring tools (*e.g.* chemical sensors). We evaluated the stability of the key RDX transformation products in groundwater under different conditions and developed a preservation method that allows the samples to be transported from the field to the laboratory without losses. Using this method, we detected RDX and several of its key transformation products in groundwater sampled from various US military sites.

NRCC 53366

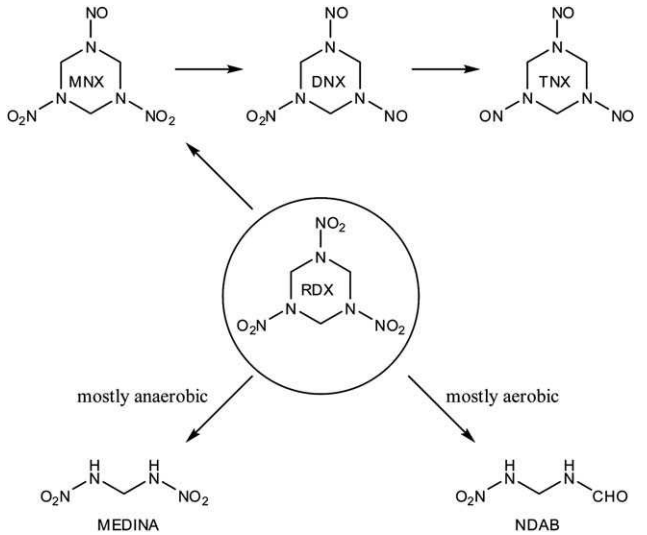

Fig. 1 Transformation routes of RDX; the nitroso route is mostly anaerobic whereas the denitration route is both aerobic (mostly NDAB) and anaerobic (mostly MEDINA).

the sequential reduction of the nitro groups to give the corresponding nitroso derivatives 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)[8,9] (Fig. 1) and (2) a denitration pathway initiated by the cleavage of the N–NO_2 bond(s) leading to decomposition of the chemical to produce labile products such as nitrite (NO_2^-), ammonia (NH_3), nitrous oxide (N_2O), formaldehyde (HCHO) and formic acid (HCOOH).[3,10,11] RDX denitration leads to the formation of two characteristic intermediates, namely, 4-nitro-2,4-diazabutanal (NDAB; NO_2NHCH_2NHCHO) and methylenedinitramine (MEDINA; $NO_2NHCH_2NHNO_2$) (Fig. 1), the yields of which depend on the environmental conditions (*e.g.*, pH, presence of zero-valent iron or other reduced metals, specific microorganisms and enzymes, aerobic or anaerobic conditions) and the stoichiometry of the denitration step.[4–6,11]

None of the RDX nitroso-derivatives or its ring cleavage products MEDINA or NDAB are known to occur naturally. Since these chemicals are distinctive transformation products of RDX generated by either biotic or abiotic process, their detection in the environment is clear evidence for (1) current or past presence of RDX, and (2) the presence of microorganisms or chemical agents that can degrade RDX *via* biotic or abiotic mechanism, respectively. Therefore, methods to preserve and detect these intermediates in field samples are important for evaluating degradative processes taking place *in situ*.

The three nitroso derivatives of RDX (MNX, DNX and TNX) have been detected at a few RDX contaminated sites, including both the Iowa Army Ammunition Plant[12] and the Cornhusker Army Ammunition Plant,[13] but analysis for other RDX transformation products including NDAB and MEDINA has not been routinely conducted at explosive-contaminated sites. Thus, the extent to which RDX indicator products occur and persist in aquifers is largely unknown. We were able to detect the ring cleavage product, NDAB, in a soil sample collected from an ammunition plant in Valleyfield, Quebec, Canada.[14] However, the other ring cleavage product, MEDINA, which is unstable in water,[15] has never been detected in field samples.

Analytical methods to quantify RDX metabolites and its transformation products in synthetic solutions are now well established[2,3,13–15] but information concerning the stability and preservation of these intermediates in field samples is lacking. Our objective was therefore to determine appropriate sampling and preservation techniques for some of the more labile products of RDX transformation, such as NDAB and MEDINA. To meet this objective, we evaluated the stability of these intermediates as a function of various environmental factors, including pH, temperature, and salinity. The data provide both new insight into the lability of these products and potential methods to enhance their aqueous stability during sampling and shipping for laboratory analysis.

Experimental

Chemicals

1,3,5-Trinitro-1,3,5-triazine (RDX), methylenedinitramine (MEDINA), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) were provided by Defence Research and Development Canada (Valcartier, QC). 4-Nitro-2,4-diazabutanal (NDAB), hexahydro-3,5-dinitro-1-nitroso-1,3,5-triazine (MNX), and hexahydro-5-nitro-1,3-dinitroso-1,3,5-triazine (DNX) were purchased from SRI (Ron Spanggord, Menlo Park, Ca). Formaldehyde (HCHO) and sea salts were purchased from Sigma-Aldrich (Oakville, ON). Deionized water was obtained with a Milli-Q^{UV} plus (Millipore) system. Other chemicals were of reagent grade.

Groundwater sampling

Samples of contaminated groundwater were collected from November 2008 to January 2010 at various current and former US Army and US Navy sites. Sampling locations were selected based on geochemistry, available information concerning groundwater contamination with RDX, and ease of access. When possible, groundwater samples at a given site were collected from both RDX-contaminated wells and those outside of known contaminated areas so that the presence of potential RDX transformation products could be assessed against control wells (with no intermediates from RDX expected). Sites undergoing active *in situ* remediation *via* addition of carbon substrates to promote anaerobic degradation of RDX were also included. During sample collection, low-flow groundwater sampling procedures were used and field parameters (pH, oxidation-reduction potential, temperature, and conductivity) were allowed to stabilize before collection of samples from a given well. The groundwater was directed into a flow cell equipped with a calibrated multi-parameter meter that measured each of these parameters. The location of the sites and basic groundwater geochemistry are provided in Table 1. Samples collected in glass bottles were either kept without additives or mixed with preservative agents (*e.g.* sea salts), shipped on ice, and stored at 4 °C until further analyses. Each sample was analyzed for RDX, its NO-derivatives, NDAB, MEDINA, HCHO, N_2O, NO_2^-, and NO_3^- as described below.

Stability experiments

Solutions of MEDINA (30 mg L^{-1}), NDAB (20 mg L^{-1}) or nitroso derivatives (MNX: 0.4 mg L^{-1}, DNX: 1.0 mg L^{-1}, TNX: 0.4 mg L^{-1}) were prepared in deionized water or in groundwater (sample NJ-3 from the New Jersey site) and left static and away from light at the selected temperature (4 °C; 23 °C; 40 °C), pH (3–12), and sea salts concentration (0, 3, 5, 10% w/v). In the case of groundwater, samples were either used as sampled or sterilized by filtering the water through a 0.22 μm membrane before adding the chemical to be monitored, and all experimental manipulations were carried out under anaerobic conditions. At specific times, samples from either deionized water or groundwater solutions were collected and analyzed by high performance liquid chromatography (HPLC) as described below.

Chemical analyses

RDX and its nitroso products. Field samples without any additives were pre-concentrated using Porapak RDX cartridges (Waters, MA) according to USEPA method 3535 (ref. 17) and RDX, MNX, DNX, and TNX were analyzed by reverse phase HPLC as previously reported.[16] Briefly, RDX and its nitroso products were analyzed with a Waters HPLC system (Milford, MA, USA) using a Supelcosil LC-CN HPLC column (25 cm, 4.6 mm, 5 μm) (Oakville, ON) at 35 °C. The solvent system consisted of a methanol/water gradient at a flow rate of 1.5 mL min^{-1}. The initial solvent composition was 30% methanol and 70% water, which was held for 8 min. A linear gradient was run from 30% to 65% methanol over 12 min then changed to the initial conditions over 5 min and held there for an extra 5 min. Chromatograms were taken at a wavelength of 230 nm.

NDAB and MEDINA. RDX ring-cleavage products, NDAB and MEDINA, were analyzed as previously reported using a Waters HPLC system with an AnionSep Ice-Ion-310 Fast organic acids column (St Louis, MO, USA) maintained at 35 °C.[18] The mobile phase was acidified water (pH 2.0) at a flow rate of 0.6 mL min^{-1}. Chromatograms were taken at a wavelength of 225 nm.

N_2O. N_2O was analyzed in the headspace of collected samples using an Agilent 6890 gas chromatograph equipped with an electron capture detector (ECD). Headspace gas samples were manually injected with a gastight syringe on a 3.65 m × 3 mm Chromosorb 102 column (60–80 mesh) maintained at 50 °C for 5 min. Helium at 30 mL min^{-1} was the carrier gas and the injector and the detector were maintained at 125 °C and 350 °C, respectively.

NO_2^- and NO_3^-. NO_2^- and NO_3^- were analyzed by ion chromatography according to EPA Method 300.0 (ref. 19) using a Dionex DX 120 ion chromatograph (Sunnyvale, CA, USA) equipped with a 4 mm × 50 mm AG18 guard column and 4 mm × 250 mm AS18 analytical column. NO_2^- and NO_3^- were eluted from the column with 30.4 mM KOH and quantified by suppressed conductivity detection.

Results and discussion

Effect of temperature, pH, and salts on stability of MEDINA

The MEDINA route for RDX degradation (Fig. 1), which is most common under anaerobic conditions, appears to be an effective way to remediate RDX judging from the high mineralization (60–70%) obtained during biodegradation *via* this pathway.[20,21] However, as noted above, the general instability of MEDINA in water makes its detection in field samples unlikely without preservation during sample collection. The present study was thus conducted to evaluate preservation methods for

Table 1 Physicochemical[a] characterization of groundwater samples collected from various military sites in the US

Site location	Well ID	*T*/°C	pH (S.U.)	DO/mg L^{-1}	ORP/mV	Sp. cond./μS cm^{-1}	TOC/mg L^{-1}
Virginia	VA-1	17.36	5.20	1.47	288.9	0.079	5.0
	VA-2	17.54	5.45	3.70	236.3	0.073	3.4
	VA-3	16.89	4.76	4.12	425.3	0.052	3.6
	VA-4	15.51	5.02	1.88	293.2	0.039	2.8
Maryland	MD-1	17.81	6.70	0.33	−102.3	0.735	8.0
	MD-2	19.22	6.32	0.41	−36.8	1.285	12.6
New Jersey	NJ-1	12.74	4.87	2.13	265.8	0.149	3.5
	NJ-2	11.36	5.76	1.13	144.5	0.185	3.4
	NJ-3	11.09	6.31	0.19	−120.4	0.358	4.2
	NJ-4	11.35	5.57	3.56	143.9	0.115	2.5
Massachusetts	MA-1	9.77	5.90	12.25	182.3	63.000	N.D.
	MA-2	9.75	5.54	12.19	213.4	71.000	N.D.
Colorado	CO-1	14.84	7.39	6.62	91.9	0.716	4.8
	CO-2	15.50	7.89	8.12	156.0	0.611	5.1
	CO-3	14.34	7.15	1.33	88.0	0.818	3.4
	CO-4	14.62	7.52	4.62	58.0	0.759	1.4
Texas	TX-1	15.88	8.08	6.31	74.0	0.666	2.3
	TX-2	18.40	7.64	7.03	99.0	0.465	2.6
	TX-3	16.39	7.61	6.18	98.0	0.551	2.2
	TX-4	18.37	7.90	5.99	107.0	0.475	1.9
Oregon	OR-1	7.27	7.82	10.00	160.2	494.000	2.7
	OR-2	14.27	7.91	9.95	157.7	485.000	2.2

[a] DO: dissolved oxygen; ORP: oxidation-reduction potential; Sp. Cond.: specific conductivity; TOC: total organic carbon.

MEDINA. The stability of MEDINA was first studied in deionized water under various pH conditions (pH 3: H_3PO_4; pH 5.5: H_2O alone; pH 12: NaOH) (Fig. 2). Complete disappearance of MEDINA was observed after 1 d in pure deionized water. Although low pH stabilized the dinitramine slightly (30% loss after 1 d), alkaline conditions had a better effect, with only 5% loss after 1 d at pH 12. These results are in agreement with the observation by Urbanski[22] that MEDINA readily decomposes when the pH ranges between 3 and 8 but is more stable at pH 1 and 10.

Acidification is commonly used to stabilize field samples containing organic compounds including explosives, due to its ability to limit microbial activity. In the present case, however, acid was not sufficiently effective as a preservative for MEDINA in aqueous samples. Similarly, although adjusting samples to high pH led to better stability of MEDINA, alkaline conditions are not desirable to preserve explosive compounds in field samples. Indeed some of these compounds, including RDX, are prone to degradation *via* alkaline hydrolysis, resulting in the formation of transformation intermediates after sample collection.

Because temperature is another factor that can be used to limit the loss of organic compounds in samples, the stability of aqueous solutions of MEDINA (30 mg L^{-1}) was investigated at various temperatures (Fig. 3). Decreasing temperature from 40 to 4 °C increased significantly the stability of MEDINA in water (pH 5.5) but did not completely prevent degradation during incubation. Considering three days as a general minimum time necessary between sampling and analysis, one would expect a loss of at least 15% MEDINA to take place before analysis if samples are stored at 4 °C without additional preservation. Although keeping samples at 4 °C will significantly increase the chances of detecting MEDINA, another preservation agent is necessary for long-term preservation in aqueous solution.

Previously, while studying the decomposition of RDX and HMX by zerovalent iron in marine media (unpublished data), we observed significant amounts (0.5 equiv.) of MEDINA in marine water but not in deionized water. Based on this earlier observation, we decided to test the effect of sea salts on the stability of MEDINA. Adding sea salts to deionized water was found to increase the stability of MEDINA irrespective of storage

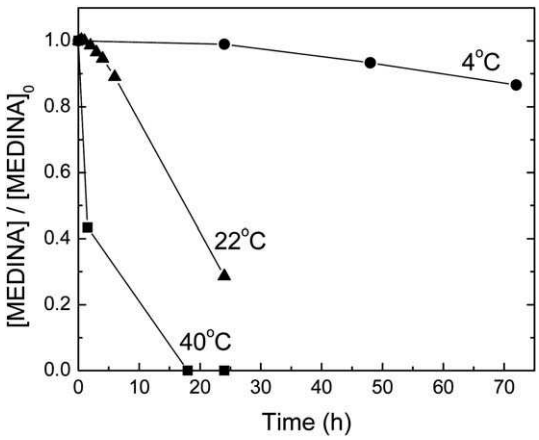

Fig. 3 Effect of temperature on MEDINA decomposition in deionized water.

temperature (Fig. 4). For instance, after 6 d at 4 °C, a loss of only 5.7, 2.4 and 0.5% of the MEDINA originally added to deionized water was observed for 3, 5 and 10% (w/v) sea salts added compared to 34% loss without added salts. When sodium chloride (3% w/v) was added to water instead of sea salts, the MEDINA concentration declined as quickly as in distilled water, thus suggesting that salinity alone was not responsible for the stabilization. The improved stability of MEDINA in sea water may thus have been caused by the slight alkalinity of marine water (pH 7.9) and/or the presence of other components (metals, sulfates, carbonates) that may form coordination complexes with MEDINA. The presence of sulfate ions was tested individually and found to have no effect on MEDINA stability. A more refined pH study was thus conducted using carbonate buffer solutions (at 9 mM, the concentration present in sea salts) at various pH values (Fig. 5). In this experiment, MEDINA was unstable at pH 5 to 6 (>80% loss in 24 h, 23 °C) but much more stable at pH 7.9 (90% recovery after 24 h, 23 °C). The agreement of this last value with the recovery measured in water containing sea salts at 23 °C (see Fig. 4) suggests that the stability of MEDINA in marine water is mainly due to its mild alkalinity.

Given the positive results obtained using sea salts in deionized water, we investigated their potential use to stabilize MEDINA

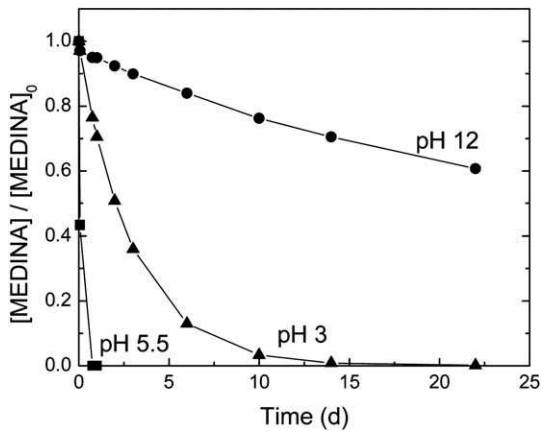

Fig. 2 Effect of pH on MEDINA decomposition in water (40 °C).

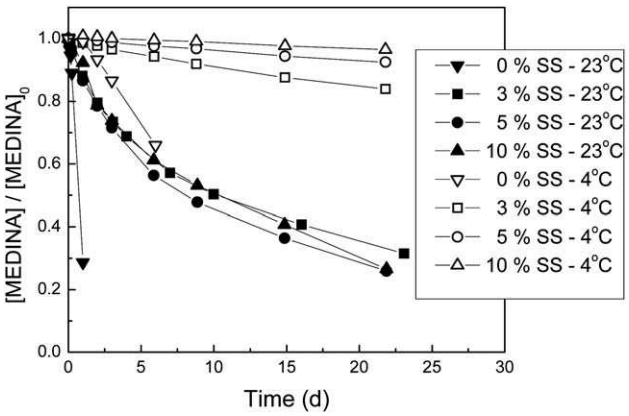

Fig. 4 Effect of sea salts (SS) addition on MEDINA stability in deionized water.

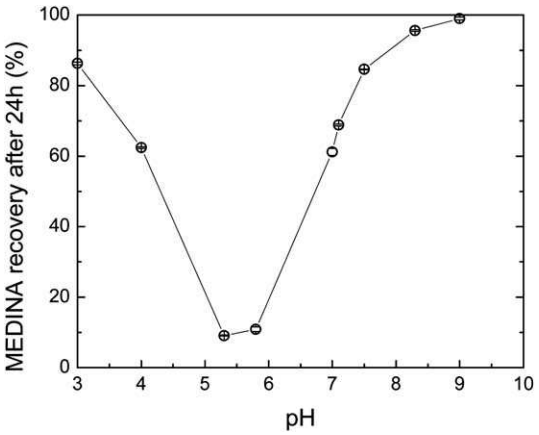

Fig. 5 Recovery of MEDINA after 24 h in 0.009 M carbonate buffers of various pHs (temperature = 23 °C; error bars represent the error of duplicate experiments).

in groundwater samples. A sample (NJ-3, Table 1) collected within the active zone of an *in situ* biostimulation project in a military facility in New Jersey was sterilized by filtration through a 0.22 μm filter or left unfiltered, and both treatments were amended with sea salts (10%, pH 7.9) and MEDINA (0.147 mM). Only 2.5 and 4% losses of MEDINA were measured after 3 days at 4 °C in sterile and non-sterile samples containing sea salts as opposed to 36% in the absence of sea salts (Fig. 6). The similarity of disappearance curves in filtered and unfiltered samples suggests that biological activity did not cause any decline in MEDINA in the groundwater samples. However, the addition of salts to the groundwater may have inhibited the groundwater microbial community, which is not adapted to high salinity.

In summary, the present study suggests that the addition of 10% sea salts can be used to effectively preserve MEDINA in groundwater samples. If the samples are also stored at 4 °C, recoveries above 99% are expected after 1 week of preservation. Since MEDINA is the hardest metabolite to preserve, the above method (10% sea salts at 4 °C) was selected and subsequently evaluated with other RDX metabolites.

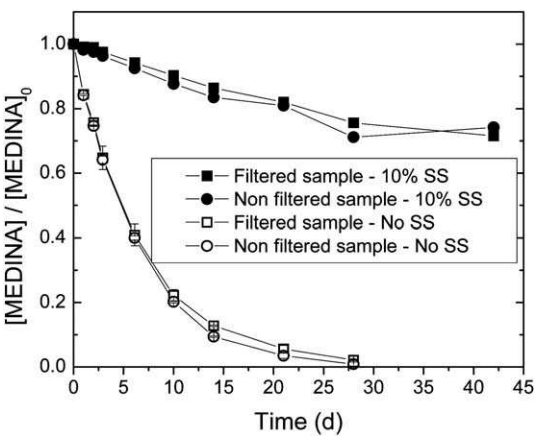

Fig. 6 MEDINA stability in groundwater (NJ-3) with or without sea salts (SS) at 4 °C (error bars represent the error of duplicate experiments).

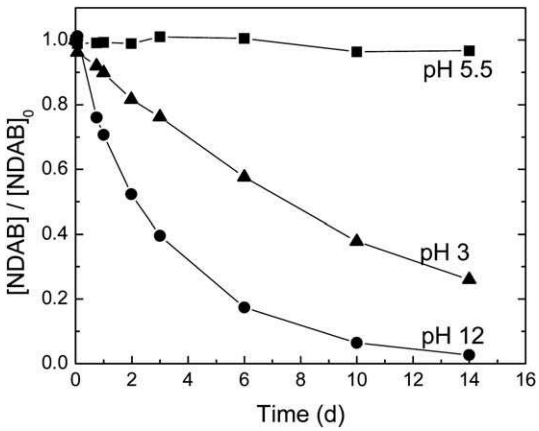

Fig. 7 Effect of pH on NDAB decomposition in water (40 °C).

Stability of NDAB under conditions optimized for MEDINA preservation

NDAB is an intermediate product of RDX that can be observed biotically under aerobic conditions or abiotically during hydrolysis[4] and photolysis[23] (Fig. 1). We previously detected it in soil samples collected from an ammunition plant in Valleyfield, Quebec, Canada,[14] and it has also been detected in plant tissues during phytophotolysis of RDX in leaves of reed canary grass.[24] The stability of NDAB was investigated under various pH conditions. Unlike MEDINA, NDAB was found to be stable in neutral deionized water but degraded under extreme alkaline (pH 12) or acid (pH 3) conditions (Fig. 7). When tested in the groundwater sample used to evaluate stability of MEDINA (NJ-3 from the NJ military site) at 4 °C, NDAB remained stable for one month with or without sea salts (Fig. 8). Filtration did not have any effect on the persistence of NDAB, suggesting a lack of degradation by microorganisms in the site samples. Thus, NDAB was apparently stable in the groundwater under *in situ* conditions, and we verified that adding sea salts to the samples did not reduce its natural stability (Fig. 8).

Although NDAB is formed as a dead end metabolite during aerobic RDX metabolism by *Rhodococcus* strains,[25] the chemical

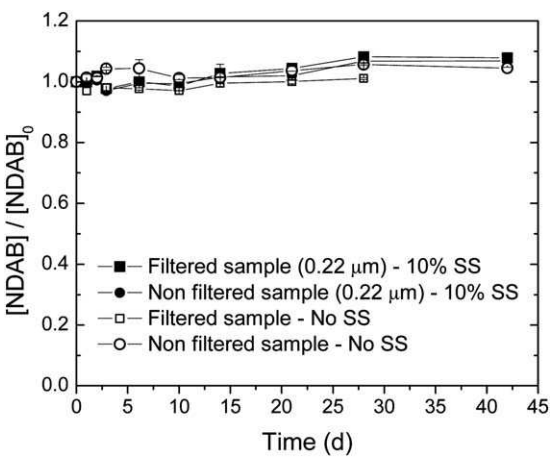

Fig. 8 Stability of NDAB in groundwater (NJ-3) with or without sea salts (SS) at 4 °C (error bars represent the error of duplicate experiments).

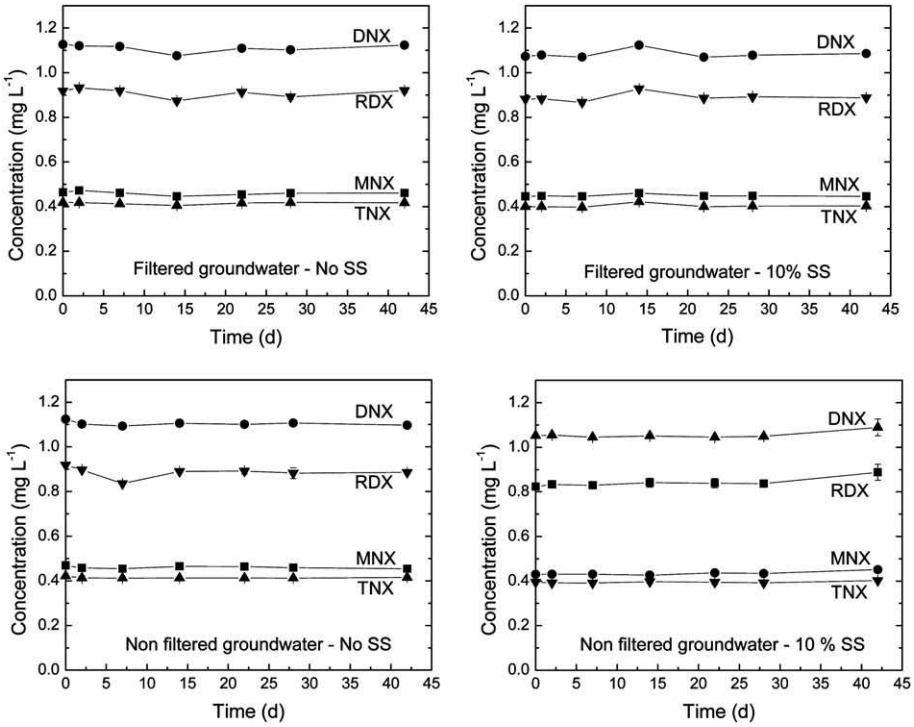

Fig. 9 Stability of RDX and its nitroso derivatives in groundwater (NJ-3) with or without sea salts (SS) at 4 °C (error bars represent the error of duplicate experiments).

can be degraded by *Methylobacterium*[26] and by *P. chrysosporium* species.[14] Similarly, although RDX can be hydrolyzed under alkaline conditions (pH 10) to give NDAB as a stable product,[4] in a recent study, NDAB (20 mg L^{-1}) was found to decompose at pH 12.3 to N_2O, HCHO, NH_3, and HCOOH with neither NO_2^- nor NO_3^- formed.[27]

Stability of nitroso compounds under conditions optimized for MEDINA preservation

Nitroso-derivatives of RDX are important intermediate transformation products that may occur under anoxic field conditions. The stability of the three nitroso derivatives MNX, DNX, and TNX as well as RDX was thus studied in actual groundwater (Well NJ-3) at 4 °C and in the presence or in the absence of sea salts (Fig. 9). At the time of sampling, groundwater from Well NJ-3 was anoxic and had a negative ORP (Table 2) due to the previous addition of cheese whey to promote biodegradation of RDX and other explosives in groundwater at the site. The parent RDX and its three nitroso derivatives were all found to be stable in the studied groundwater, whether salts were added or not and whether the sample had been filtered or not. Thus, the conditions that have been selected to limit MEDINA degradation during sampling and analysis (*i.e.*, 10% sea salts with storage at 4 °C) should also be compatible with the preservation of RDX and its NO-compounds in groundwater.

Analyses of RDX and its transformation products in groundwater

Groundwater samples collected from various military sites were analyzed for RDX and its nitroso-products after preconcentration, while samples preserved with sea salts and shipped at 4 °C were used for the analysis of NDAB and MEDINA (Table 2). RDX was found to be present in groundwater of most of the sites investigated, in agreement with its high predisposition to migrate downward through the vadose zone. Levels of contamination by RDX varied from tens of μg L^{-1} (most sites) to hundreds of μg L^{-1} at the Texas site, which is a former US Army loading and packing facility. RDX nitroso-derivatives were detected in two sites, in New Jersey and Texas, respectively. As previously noted, groundwater at the former site was previously amended with cheese whey to promote explosives biodegradation, a process that resulted in the formation of the nitroso-derivatives. The occurrence of these products at the second location in Texas may be the result of *in situ* natural attenuation, although *in situ* enhanced bioremediation *via* carbon source addition had also been conducted at this site over the years in areas different from the ones where the groundwater was collected.

MEDINA was not detected in any of the samples (Table 2). The natural pH values (pH 4–6) measured at the first four sites presented in Tables 1 and 2 (Virginia, Maryland, New Jersey, Massachusetts) would have caused MEDINA to rapidly degrade *in situ*. However MEDINA also was not detected in the latter three sites even though they were slightly alkaline (pH 7–8). Decomposition of MEDINA in water is known to produce formaldehyde (HCHO) and nitrous oxide (N_2O).[15] Formaldehyde was detected in most samples, but it is not a specific indicator of RDX degradation (*i.e.*, it has numerous sources), and its presence was not correlated with the presence or concentration of RDX (results not shown). In contrast, the concentration of N_2O appeared to be related to the presence of RDX at several sites,

Table 2 Analysis of RDX and its transformation products in groundwater samples

Site location	Well ID	RDX/ mg L^{-1}	MNX/ μg L^{-1}	DNX/ μg L^{-1}	TNX/ μg L^{-1}	MEDINA/ μg L^{-1}	NDAB/ μg L^{-1}	N_2O/ μg L^{-1}	NO_2^-/ μg L^{-1}	NO_3^-/ mg L^{-1}
Virginia	VA-1	18.8	<0.1	<0.1	<0.1	<10	<10	30.7	<0.1	1.2
	VA-2	31.6	<0.1	<0.1	<0.1	<10	<10	14.5	<0.1	0.9
	VA-3	98.6	<0.1	<0.1	<0.1	<10	<10	20.9	<0.1	<0.1
	VA-4[a]	<0.2	<0.1	<0.1	<0.1	<10	<10	4	<0.1	0.3
Maryland	MD-1	<0.2	<0.1	<0.1	<0.1	<10	<10	0	<0.1	<0.1
	MD-2	14.4	<0.1	<0.1	<0.1	<10	<10	16.8	<0.1	1.6
New Jersey	NJ-1	35.3	*0.5*	<0.1	<0.1	<10	<10	27.59	<0.1	0.3
	NJ-2	86.0	*3.9*	<0.1	<0.1	<10	<10	7.20	<0.1	0.1
	NJ-3	<0.2	<0.1	<0.1	<0.1	<10	<10	3.88	<0.1	<0.1
	NJ-4	28.0	*1.4*	<0.1	<0.1	<10	<10	4.5	<0.1	0.1
Massachusetts	MA-1	<0.2	<0.1	<0.1	<0.1	<10	<10	0.93	<0.1	<0.1
	MA-2	10.5	0.1	<0.1	<0.1	<10	<10	8.30	<0.1	0.2
Colorado	CO-1	13.3	<0.1	<0.1	<0.1	<10	<10	5.1	<0.1	8.8
	CO-2[a]	<0.2	<0.1	<0.1	<0.1	<10	<10	4.4	<0.1	2.9
	CO-3	21.8	<0.1	<0.1	<0.1	<10	<10	133.8	0.2	20.9
	CO-4	0.3	<0.1	<0.1	<0.1	<10	<10	4.8	<0.1	0.5
Texas	TX-1	708.0	*3.4*	*6.1*	*45.4*	<10	*59.0*	38.8	<0.1	0.9
	TX-2	684.0	<0.1	<0.1	*48.1*	<10	*15.0*	27.1	<0.1	2.2
	TX-3	177.5	<0.1	*2.1*	*10.6*	<10	<10	27.7	<0.1	1.2
	TX-4[a]	<0.2	<0.1	<0.1	<0.1	<10	<10	4.3	<0.1	1.2
Oregon	OR-1[a]	<0.2	<0.1	<0.1	<0.1	<10	<10	8.2	<0.1	7.3
	OR-2	54.9	<0.1	<0.1	<0.1	<10	<10	15.3	<0.1	7.9

[a] Well without history of RDX contamination.

with most RDX contaminated groundwater exhibiting higher levels of N_2O than in control wells from each site (Table 2). For example, at the Texas and Virginia sites, N_2O concentrations were ~4 μg L^{-1} in the pristine groundwater, and exceeded 25 μg L^{-1} in the RDX-contaminated wells at the Texas site and 14 μg L^{-1} at the Virginia site. At these sites, the detection of N_2O might thus be attributed to the degradation of RDX *via* the MEDINA route. Stable isotope ratios of O and N in N_2O derived from nitrification and denitrification differ,[28] and it should thus be possible to use these ratios as a forensic tool to discriminate N_2O derived from RDX from that produced by other sources. Stable isotope analysis has previously been applied to assess whether explosives are sources of NO_3^- in groundwater,[29] but to our knowledge, this technique has not yet been applied for N_2O in field samples.

NDAB was also detected in the two wells at the Texas site with the highest current RDX concentrations, suggesting that multiple degradation pathways may be occurring simultaneously at this site. The contamination of RDX at the Texas site may extend back to the 1940s, when this facility was used as a packing plant during World War II or may have originated from testing activities in more recent decades. According to the kinetics we measured previously for RDX hydrolysis,[30] at a pH of 8.08 and a temperature of 16 °C (289 K), as measured in well TX-1, one can predict a half-life of 15 years for the hydrolysis of RDX. The measured content of NDAB in well TX-1 could thus be explained by the alkaline hydrolysis of RDX. In addition, the lower content of NDAB in well TX-2, despite the same level of RDX contamination agrees well with the hydrolysis kinetics expected at the slightly lower pH (7.64) of this well (assuming that RDX contamination occurred at the same time).

The sporadic and variable detection of NO_2^- and NO_3^- in groundwater could not be readily correlated with the presence of RDX because of the numerous sources and sinks for these compounds. However, as previously discussed for N_2O (where a correlation with RDX was apparent at some sites), natural abundance stable isotope techniques may be a valuable tool for discriminating explosive *versus* natural sources of these anions.[29]

Conclusion

Detection of any of the unique transformation products of RDX (*i.e.*, MNX, DNX, TNX, MEDINA, and NDAB) in the environment indicates past RDX contamination and the occurrence of *in situ* attenuation processes for this explosive. Preserving RDX intermediates in field samples is thus important because their detection can provide insight into both the occurrence and rate of natural attenuation of RDX and its degradation route(s). Our studies indicate that NDAB is stable in aqueous media including groundwater at ambient environmental conditions, thus allowing accurate detection after sampling and transport from the field to the laboratory. However since NDAB degrades under acidic conditions (pH < 3), EPA Method 8330B (ref. 31) which requires acid preservation may not be suitable for the detection of NDAB. In contrast, MEDINA is unstable in aqueous solutions and decomposes under ambient conditions. Although MEDINA was found to be preserved by the addition of 10% w/v sea salts and storage at 4 °C, it is likely that this intermediate will rapidly degrade in most groundwater aquifers, and be detected only if it is currently being formed in reasonable quantities. Other related RDX products, including the nitroso derivatives MNX, DNX and TNX, were found to be stable under ambient conditions and should be easily detected in groundwater if they are present. Our data also revealed a possible correlation between N_2O and RDX in some groundwaters, a relationship that should be further examined using N and O stable isotope analysis as a forensic tool to discriminate RDX-derived N_2O from that formed *via* other processes.

Acknowledgements

We thank Ms Annamaria Halasz for valuable comments on the manuscript. This project was supported by the US Strategic Environmental Research and Development Program (Project ER-1607). Views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of Defense position or decision unless so designated by other official documentation.

References

1 T. F. Jenkins, A. D. Hewitt, C. L. Grant, S. Thiboutot, G. Ampleman, M. E. Walsh, T. A. Ranney, C. A. Ramsey, A. J. Palazzo and J. P. Pennington, *Chemosphere*, 2006, **63**, 1280.
2 J. Hawari, A. Halasz, T. Sheremata, S. Beaudet, C. Groom, L. Paquet, C. Rhofir, G. Ampleman and S. Thiboutot, *Appl. Environ. Microbiol.*, 2000, **66**, 2652.
3 D. Fournier, A. Halasz, J. Spain, P. Fiurasek and J. Hawari, *Appl. Environ. Microbiol.*, 2002, **68**, 166.
4 V. Balakrishnan, A. Halasz and J. Hawari, *Environ. Sci. Technol.*, 2003, **37**, 1838.
5 G. Naja, A. Halasz, S. Thiboutot, G. Ampleman and J. Hawari, *Environ. Sci. Technol.*, 2008, **42**, 4364.
6 J.-S. Zhao, Y. Deng, D. Manno and J. Hawari, *PLoS ONE*, 2010, **5** (2), 1–22, e9109.
7 J.-S. Zhao, D. Manno and J. Hawari, *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 491.
8 N. G. McCormick, J. H. Cornell and A. M. Kaplan, *Appl. Environ. Microbiol.*, 1981, **42**, 817.
9 J.-S. Zhao, L. Paquet, A. Halasz and J. Hawari, *Appl. Microbiol. Biotechnol.*, 2003, **63**, 187.
10 B. Bhushan, A. Halasz, J. C. Spain, S. Thiboutot, G. Ampleman and J. Hawari, *Environ. Sci. Technol.*, 2002, **36**, 3104.
11 R. G. Jackson, E. Raylot, D. Fournier, J. Hawari and N. C. Bruce, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 16822.
12 H. R. Beller and K. Tiemeier, *Environ. Sci. Technol.*, 2002, **36**, 2060.
13 D. A. Cassada, S. J. Monson, D. D. Snow and R. F. Spalding, *J. Chromatogr., A*, 1999, **844**, 87.
14 D. Fournier, A. Halasz, J. Spain, R. J. Spanggord, J. C. Bottaro and J. Hawari, *Appl. Environ. Microbiol.*, 2004, **70**, 1123.
15 A. Halasz, J. Spain, L. Paquet, C. Beaulieu and J. Hawari, *Environ. Sci. Technol.*, 2002, **36**, 633.
16 C. A. Groom, A. Halasz, N. Morris, L. Olivier, C. Dubois and J. Hawari, *Environ. Sci. Technol.*, 2002, **36**, 112.
17 U.S. Environmental Protection Agency, *Method 3535A SW-846 Revision 1, Solid-Phase Extraction (SPE)*, Office of Solid Waste, Washington, DC, 2007.
18 B. Bhushan, L. Paquet, A. Halasz, J. C. Spain and J. Hawari, *Biochem. Biophys. Res. Commun.*, 2003, **306**, 509.
19 U.S. Environmental Protection Agency, *Method 300.0 Revision 2.1, Determination of Inorganic Ions by Ion Chromatography*, Environmental Monitoring Systems Laboratory—Office of Research and Development, Cincinnati, OH, 1993.
20 J.-S. Zhao, A. Halasz, L. Paquet, C. Beaulieu and J. Hawari, *Appl. Environ. Microbiol.*, 2002, **68**, 5336.
21 C. F. Shen, J. Hawari, G. Ampleman, S. Thiboutot and S. R. Guiot, *Biorem. J.*, 2000, **4**, 27.
22 T. Urbanski, *Chemistry and Technology of Explosives*, Pergamon Press Ltd, Oxford, UK, 1967, vol. 3, pp. 17–18.
23 J. Hawari, A. Halasz, C. Groom, S. Deschamps, L. Paquet, C. Beaulieu and A. Corriveau, *Environ. Sci. Technol.*, 2002, **36**, 5117.
24 C. L. Just and J. L. Schnoor, *Environ. Sci. Technol.*, 2004, **38**, 290.
25 M. E. Fuller, J. Hawari and N. Perreault, *Lett. Appl. Microbiol.*, 2010, **51**, 313.
26 D. Fournier, S. Trott, J. Hawari and J. Spain, *Appl. Environ. Microbiol.*, 2005, **71**, 4199.
27 A. Halasz, D. Manno, S. E. Strand, N. C. Bruce and J. Hawari, *Environ. Sci. Technol.*, 2010, **44**, 9330.
28 K. R. Kim and H. Craig, *Science*, 1993, **262**, 1855.
29 G. Bordeleau, M. M. Savard, R. Martel, G. Ampleman and S. Thiboutot, *J. Contam. Hydrol.*, 2008, **98**, 97.
30 F. Monteil-Rivera, L. Paquet, R. Giroux and J. Hawari, *J. Environ. Qual.*, 2008, **37**, 858.
31 U.S. Environmental Protection Agency, *Method 8330B Revision 2, Nitroaromatic, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)*, Washington, DC, 2006.