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Preservation of Water Samples Containing Nitroaromatics and Nitramines

Thomas F. Jenkins, Philip G. Thorne, Erika F. McCormick
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Abstract

This study was conducted to develop a method for stabilizing water samples to be analyzed for nitroaromatic and nitramine explosives using SW846 Method 8330. Several options were tested using river water fortified with 15 nitroaromatic, nitramine, and aminonitroaromatic analytes. Acidification to pH 2 using sodium bisulfate was selected based on its ability to retard microbiological and chemical transformations, its ease of use under field conditions, and its usability with both the direct and preconcentration procedures in Method 8330. Holding-time studies were performed over a 64-day storage period using fortified river water and groundwaters with and without chemical stabilization. Nonacidified samples showed rapid loss of tetryl, TNB, and TNT and slower loss of the dinitroaromatics. These losses were accompanied by increasing concentrations of transformation products. Losses of these nitroaromatics were completely eliminated by acidification to pH 2. Nitramines were stable over the entire period whether samples were acidified or not. A small loss of the aminodinitroaromatics was observed for both acidified and unacidified samples. The rate of loss for acidified samples was initially greater than for nonacidified samples. Sample acidification caused no adverse effects on SW846 Method 8330, although samples to be preconcentrated using salting-out solvent extraction should be neutralized prior to extraction to prevent additional loss of aminodinitroaromatics.The use of sample acidification was tested in a production laboratory scenario using field-contaminated water samples. The loss of aminodinitrotoluenes due to acidification was again observed for some samples but not for others. A small interference near the retention time of TNT was observed and traced to the disposable syringes used for sample filtration prior to HPLC determination. Acidification completely eliminated losses of TNT and TNB over the 28-day study. As observed for fortified samples, HMX and RDX were stable whether samples were acidified or not.

For conversion of SI units to non-SI units of measurement consult ASTM Standard E380-93, *Standard Practice for Use of the International System of Units,* published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.

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Cold Regions Research & Engineering Laboratory

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PREFACE

This report was prepared by Dr. Thomas F. Jenkins, Research Chemist, and Philip G. Thorne, Physical Sciences Technician, Geological Sciences Division, Research and Engineering Directorate, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL), and Erika F. McCormick, Chemist, and Karen F. Myers, Biologist, Environmental Chemistry Branch, Environmental Engineering Division, Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi.

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Preservation of Water Samples Containing Nitroaromatics and Nitramines

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INTRODUCTION

There is an unavoidable delay between sample collection at hazardous waste sites and analysis at off-site environmental testing laboratories. Analytes must be sufficiently stable in the matrix under investigation using prescribed storage conditions or they must be stabilized, usually by the addition of chemical preservatives. Matrix-specific analytical methods typically include a section describing storage conditions and acceptable holding times between sample collection and analysis.

The most commonly used analytical method in the United States for nitroaromatics and nitramines in water is SW846 Method 8330 (EPA 1992). The preextraction holding time prescribed in this method is seven days, with samples maintained under refrigeration and no preservative specified. However, two recent holding-time studies have indicated that the nitroaromatics frequently associated with munitions wastes can be subject to significant losses while being held prior to analysis. Maskarinec et al. (1991) found rapid loss of 2,4-dinitrotoluene (2,4-DNT) in both surface water and groundwater even when stored at 4°C in the dark; they recommended a preextraction holding time of only four days for water samples to be analyzed for 2,4-DNT. Grant et al. (1993) found very rapid losses of 1,3,5-trinitrobenzene (TNB) and 2,4,6-trinitrotoluene (TNT) for surface water maintained at 2°C in the dark, but loss of 2,4-DNT was very small. Losses of 55% for TNB and 30% for TNT were observed after only seven days' storage, and the losses reached 77% for TNB and 45% for TNT after 14 days. The major loss mechanism appears to be microbiological transformation resulting in the reduction of one nitro group on the ring to amino (Won et al. 1974, McCormick et al. 1976). For TNB and TNT this results in a series of transformation products, with the most stable being 3,5-dinitroaniline (3,5-DNA) (from TNB) and the 2- and 4-amino-dinitrotoluenes (2ADNT and 4ADNT) (from TNT). Elsewhere, Goerlitz (1992) reported TNT losses as high as 75% after 20 days of storage for unpreserved groundwater samples from Hawthorne Army Ammunition Plant. Miller et al. (1983) reported that 2,4-DNT, TNB, and several other nitroaromatics fortified into groundwater from Sunflower Army Ammunition Plant demonstrated significant instability when stored at 4°C in the dark without preservation. Given the differences in results for different waters, it appears that variations in physical, chemical, and microbiological properties of specific waters can produce a wide range of effects, i.e., degradation mechanisms and rates are somewhat specific to a given water. Thus, preservation techniques appropriate for nitroaromatics in water are needed and should be used on a routine basis.

A protocol for preservation of 12 munitions-related chemicals in water was developed by Miller et al. (1983) at the Midwest Research Institute. Several parameters were evaluated relative to their effect on analyte stability with time, including pH, temperature, light, containers, and the presence of sediment, salt, and acetonitrile (used as an antibacterial agent). Several alternative preservation techniques were tested using munitions-fortified water. The resulting data did not demonstrate large differences among treatments, nevertheless, the following preservation technique was selected: water samples are preserved by adding acetonitrile to the sample to achieve a 10% solution, the pH is adjusted to 3.5 with glacial acetic acid, suspended particles are removed by centrifugation, and samples are stored at 4°C in amber glass bottles.

Tests using munitions-fortified tap water, monitoring well water, and sediment-deionized water were used to demonstrate the effectiveness of this procedure. The procedure, however, does not appear to be very practical on a large scale, particularly for routine use in the field.

Goerlitz and Franks (1989) tested chloroform and mercuric chloride as preservatives and found that addition of mercuric chloride at 60 mg/L was successful in significantly reducing the rate of alteration of TNT in groundwater samples from Hawthorne AAP. They attribute the alterations to aerobic microbiological transformation resulting in the generation of the 2- and 4-aminodinitrotoluene transformation products.

Maskarinec et al. (1986) investigated the use of solid-phase extraction, not only as a method of preconcentration of nitroorganics from water, but also as a means for storage/preservation prior to analysis. In their study, three groundwaters were fortified with a set of nitroaromatics, nitramines, and nitrate esters, extracted with Porapak R (a styrene-divinylbenzene copolymer with polyvinylpyrrolidone) and held for periods of up to 32 weeks prior to analysis. The authors report that the resin-adsorbed analytes were stable for at least 16 weeks. The analytical precision of the results for TNT in particular was quite poor, however, and drawing firm conclusions from these data seems risky. Nevertheless, the use of solid-phase extraction for this purpose was an innovative idea worthy of serious consideration, since preconcentration prior to determination is often necessary anyway to obtain the desired detection limits for these analytes in water.

Recently Maskarinec et al. (1990) reported on the use of acidification to pH 2 with sodium bisulfate as a method of preservation for volatile organics in water. This method proved very effective at extending the acceptable analytical holding times for aromatic hydrocarbons and ketones, both of which are subject to microbiological degradation. Acidification with sodium bisulfate appears to be a method that could be used conveniently in the field during sample collection.

OBJECTIVE

The objective of the studies described here was to evaluate several different approaches to preservation with regard to their effectiveness in maintaining the integrity of nitroaromatics in water samples without inducing instability in nitramines, often present in the same samples. In addition to preserving these analytes successfully for at least seven days after collection, acceptable techniques must be simple and inexpensive to implement, must not introduce chemicals that increase the cost of disposal, and must be compatible with SW846 Method 8330 (EPA 1992). Specifically, Method 8330 allows two analytical sequences depending on the detection limits required. If detection limits in the range of 10–20 µg/L are adequate, a direct injection, reversed-phase high-performance liquid chromatographic (RP-HPLC) method is used. If lower detection limits are required, water samples must first be preconcentrated prior to analysis by RP-HPLC. The method currently specified in Method 8330 is salting-out solvent extraction with acetonitrile, but the use of either cartridge or membrane solid-phase extraction (SPE) may be allowed as an alternative in the future. It was our hope to develop a stabilization method appropriate for samples analyzed by any of these procedures.

EVALUATION OF ALTERNATIVES

A number of potential options for preservation were considered, including the use of mercuric chloride, sodium azide, chloroform, sodium thiosulfate, acetonitrile, acetonitrile with acidification to pH 3.5 using acetic acid, and acidification to pH 2 using sodium bisulfate. The stabilization procedures that we decided to test were acidification to pH 2 using sodium bisulfate (Maskarinec et al. 1990) and the addition of acetonitrile combined with or without acidification to pH 3.5 with acetic acid (Miller et al. 1983). The use of acidification with sodium bisulfate was selected because it would only require addition of a preweighed amount of a solid material to each water sample and could be easily implemented in the field. It has also been tested for its effectiveness with other analytes such as volatile organics in water and was found to be effective at preventing biodegradation of aromatic hydrocarbons (Maskarinec et al. 1990). Acidification with sodium bisulfate should not result in disposal difficulties for residual samples since acidification is a common laboratory practice for sample pretreatment and is also often used for cleaning of containers and other glassware. Water samples at pH 2 may cause some difficulties in either the direct method of analysis or the two alternative preconcentration

schemes, but neutralization prior to analysis can be performed if necessary.

The second stabilization method selected for evaluation was addition of acetonitrile as a biocide by itself or in combination with pH adjustment to 3.5 with acetic acid. Miller et al. (1983) tested and recommended a variation of this method. Addition of acetonitrile is already a part of the salting-out solvent extraction method and hence disposal problems should not be increased. This method would be less easily implemented in the field, however, and the minimum concentration of acetonitrile required for effective preservation is not known.

EXPERIMENTAL MATERIALS

Chemicals

All standards and test solutions were prepared from Standard Analytical Reference Materials (SARMs) obtained from the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland. Methanol used in preparation of analytical standards and HPLC eluent was HPLC grade from Baker. HPLC eluent was prepared by combining equal volumes of methanol and water and vacuum filtering through a 0.45-µm nylon membrane to degas and remove particulate matter. Acetonitrile used in preparation of stock standards and as a chemical preservative was HPLC-grade from Baker. Glacial acetic acid, used to acidify water samples, was reagent-grade from Baker. Sodium bisulfate ($NaHSO_4$), used as a preservative, was obtained from Aldrich. Humic acid, sodium salt, was technical-grade from Aldrich.

Solid-phase extraction materials

Prepacked cartridges of Porapak RDX (Sep-Pak, 6 cc, 500 mg) were obtained from Waters Corporation; they were precleaned by eluting with 15 mL of acetonitrile followed by 30 mL of reagent-grade water. Empore SDB extraction membranes (47 mm) were obtained from 3M Corporation; they were precleaned in the same manner as the Porapak cartridges. Experimental Empore SDB-LS (extra clean) membranes were used for samples from the Naval Surface Warfare Center; they were cleaned as described above.

Water samples

Blank water samples were either reagent-grade water from a Milli-Q Type I water system, surface water from the Connecticut River in West Lebanon, New Hampshire, or groundwater samples from Vershire (PT), Hartland (TR), or Weathersfield (MW), Vermont. Groundwater samples were obtained in duplicate from the Naval Surface Warfare Center in Crane, Indiana. One sample of each duplicate was collected in a bottle containing sodium bisulfate such that the solution concentration was 1.5 g/L. A second sample of each duplicate was collected without acidification.

Analyte spiking

All analyte spiking solutions were prepared in water. SARMs for each analyte were placed in individual brown glass jugs, reagent-grade water was added, and the contents were stirred continuously at room temperature for a week. The solutions were then filtered through 0.45-µm nylon membranes into clean, brown glass jugs. No solvents other than water were used in the preparation of these solutions. The concentration of analyte in each aqueous spike solution was determined against standards prepared in methanol or acetonitrile and diluted 1:1 with reagent-grade water prior to analysis (EPA 1992).

ANALYSIS

All water samples were analyzed by RP-HPLC at either the U.S. Army Cold Regions Research and Engineering Laboratory (CRREL) or the U.S. Army Engineer Waterways Experiment Station (WES). At CRREL, analysis was conducted on a modular system composed of a Spectra-Physics Model SP8810 isocratic HPLC pump, a Spectra-Physics SP8490 UV variable-wavelength detector set at 254 nm (cell path 1 cm), a Spectra-Physics SP8875 autosampler equipped with a Rheodyne Model 7010 Sample Loop Injector (100 µL), a Hewlett Packard 3376 digital integrator, and a Linear strip-chart recorder. At WES, primary analysis was conducted using a Waters Model 600 system controller, Model 610 fluid unit, Model 717 plus Auto Injector set for a 50-µL injection, a 486 UV Variable Wavelength Detector set at 245 nm, and a Maxima Chromatography Work Station. Confirmation was conducted on a Waters LC Module 1 with a 486 UV Variable Wavelength Detector (245 nm), a 717 plus Auto Injector (50 µL), and a Maxima 820 Chromatography Work Station.

Depending on the specific test conducted, water samples were either analyzed using the direct method specified in SW846 Method 8330 (EPA

Table 1. Retention times for various separations used at CRREL.

Analyte	Retention time (min) LC-18*	LC-CN*
HMX	2.4	1.4
RDX	3.5	7.3
TNB	4.6	4.3
DNB	5.6	4.4
3,5-DNA	6.1	5.6
tetryl	6.2	9.2
NB	6.6	4.0
TNT	7.4	5.2
4ADNT	8.0	6.0
2ADNT	8.4	6.4
2,6-DNT	8.8	4.9
2,4-DNT	8.9	5.2
2NT	0.6	4.5
4NT	1.8	4.7
3NT	2.4	4.8
tetryl breakdown product	4.6	8.1
2-amino-4-nitrotoluene	5.6	4.2
3-nitroaniline	4.2	3.8
2,4-diamino-6-nitrotoluene	4.0	6.3
2,6-diamino-4-nitrotoluene	2.1	4.8
4-amino-2-nitrotoluene	8.1	4.3

*Separations were conducted at 1.5 mL/min with an eluent of 1:1 methanol/water.

Table 2. Retention times for various separations used at WES.

Analyte	Retention time (min) LC-18*	LC-CN*
HMX	0.3	13.0
RDX	0.1	8.3
TNB	0.7	5.2
DNB	0.3	5.2
3,5-DNA	9.2	6.6
NB	9.8	4.6
TNT	1.2	6.3
4ADNT	2.4	6.9
2ADNT	2.4	7.4
2,6-DNT	3.3	5.8
2,4-DNT	3.6	6.1

*Separations were conducted at 1.2 mL/min with an eluent of 1:1 methanol/water.

1992) or were preconcentrated using either salting-out solvent extraction (SOE), cartridge solid-phase extraction (SPE-C), or membrane solid-phase extraction (SPE-M) (Jenkins et al. 1992).

Primary analysis was conducted on a 25-cm × 4.6-mm (5-µm) LC-18 column (Supelco) eluted with 1:1 methanol/water (v/v) at 1.5 mL/min at CRREL or 1.2 mL/min at WES (EPA 1992). At CRREL, samples were introduced by overfilling a 100-µL sampling loop. At WES, 50-µL samples were introduced using a 200-µL loop. Retention times of the analytes of interest are shown in Tables 1 and 2 for analyses conducted at CRREL and WES, respectively. Concentration estimates were obtained for most analytes from peak heights from the digital integrator. In some instances, particularly for samples preconcentrated using Porapak RDX SPE cartridges, better quantitative results were obtained using peak areas. At CRREL, the identities of transformation products were confirmed by analysis of some of the samples on LC-CN, using a 25-cm × 4.6-mm (5-µm) LC-CN column from Supelco eluted with 1:1 methanol/water (v/v) at 1.5 mL/min (EPA 1992). At WES, confirmation of analyte identities as well as quantitative results for 2ADNT and 4ADNT were obtained on an LC-CN column (Supelco) eluted with 1:1 methanol/water at 1.2 mL/min.

EXPERIMENTAL DESIGN AND RESULTS

Selection of initial test matrix

In a previous study (Grant et al. 1993), the preextraction holding times for nitroaromatics and nitramines in water were evaluated using three sample matrices: reagent-grade water, groundwater from Enfield, New Hampshire, and surface water from the Connecticut River in West Lebanon, New Hampshire. Of these, the most rapid rate of analyte transformation occurred in the Connecticut River water. After only seven days of refrigerator storage of Connecticut River water fortified with TNB and TNT, only 45% of the initially fortified TNB and 70% of the initially fortified TNT remained. The expected microbiological transformation products, 3,5-DNA from TNB and 2ADNT and 4ADNT from TNT (Won et al. 1974, McCormick et al. 1976), appeared as the concentrations of TNB and TNT decreased with time. Thus, fortified Connecticut River water appears to be a good choice as a test matrix for evaluation of alternative preservation techniques.

To ensure that Connecticut River water in the vicinity of West Lebanon, New Hampshire, could be relied upon to be degradative for nitroaromatics, a short holding-time study was conducted in a

manner similar to that described by Grant et al. (1993). Since TNB was the least stable analyte in the earlier study, it was selected as the test analyte and was fortified at 50 µg/L. Results indicated that after three days' storage at room temperature, the concentration of TNB was reduced by 80% and a buildup of 3,5-DNA had occurred. Thus, as observed previously, fortified Connecticut River water should be an excellent test matrix for the assessment of various stabilization techniques.

Preliminary evaluation of sodium bisulfate and percentages of acetonitrile necessary to stabilize samples

Connecticut River water was fortified with RDX, TNB, and TNT at 42, 26, and 41 µg/L, respectively. One subsample of the fortified water was stored under refrigeration without addition of any chemical preservative, and this sample served as the control sample for judging the effectiveness of the various chemical stabilization procedures examined. The water was analyzed at day 0, 4, 8, 20, and 34. A second subsample was acidified to pH 3.5 with acetic acid and stored over the same period under refrigeration. Acetonitrile (ACN) was added to 14 subsamples in the appropriate amounts to achieve acetonitrile concentrations in duplicate at 0.1, 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0% (v/v). One subsample at each acetonitrile concentration was acidified to pH 3.5 with acetic acid, and the pH of the remaining subsample at each ACN concentration was left unmodified. These 14 samples were stored and analyzed as described above. A final subsample of the fortified Connecticut River water was acidified to pH 2.0 with sodium bisulfate and held under refrigeration for 30 days. This sample was analyzed in the same manner as described above after 0, 4, 8, 16, and 30 days of storage.

Analytical results for the control sample agreed with those presented in Grant et al. (1993) for nitroaromatic and nitramine fortified Connecticut River water. The rate of loss of TNB was found to be very rapid (Fig. 1), the rate of loss of TNT was somewhat slower, and there was no evidence for loss of RDX. After only four days, the concentration of TNB in the unacidified control sample was reduced from 26.0 µg/L to 8.3 µg/L. The concentration of 3,5-DNA, the associated, relatively stable transformation product, increased as the TNB was lost, with a maximum concentration of 6.1 µg/L at 20 days (Fig. 2). The rate of loss of TNB was reduced by acidification to pH 3.5 with acetic acid (Fig. 1), and 3,5-DNA was not detected in this sample until day 20. By day 34, however, the TNB concentration had been reduced to 2.7 µg/L, which is about a 90% reduction in concentration relative to day 0, and the 3,5-DNA concentration was about 10 µg/L. This 3,5-DNA concentration was higher than that determined for the unpreserved sample

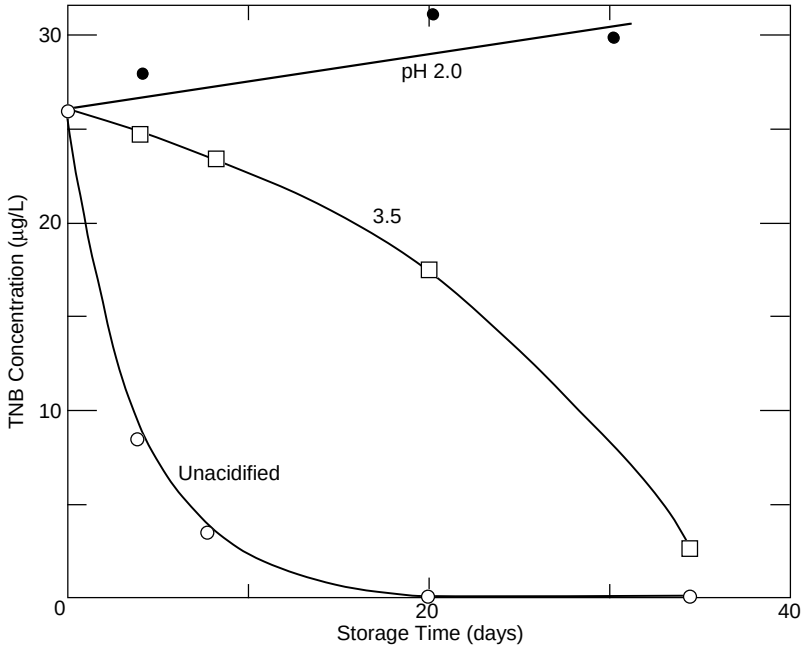

Figure 1. Stability of TNB in samples acidified to pH2, pH 3.5, and left unacidified.

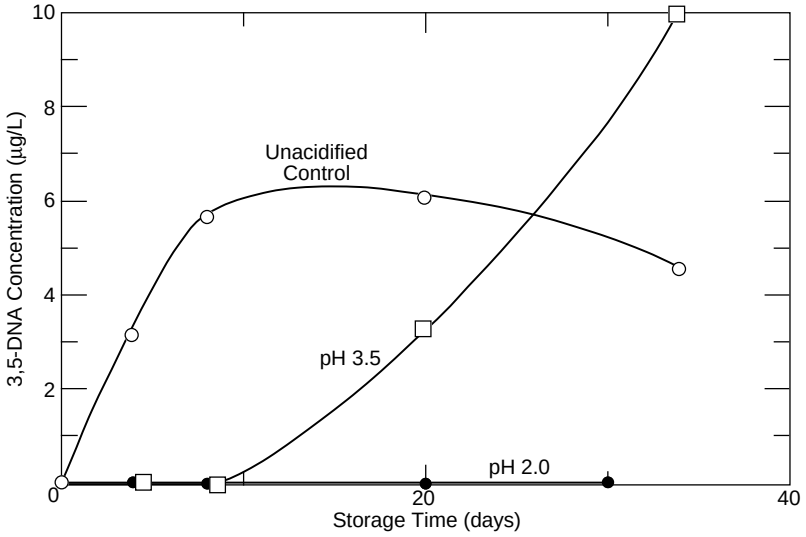

Figure 2. Concentration of 3,5-DNA for samples acidified to pH 2, pH 3.5, and left unacidified.

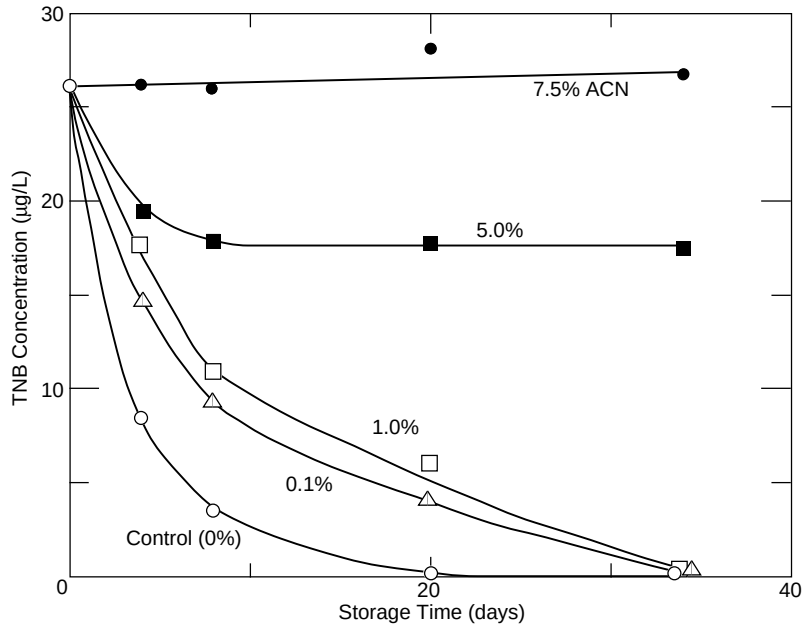

Figure 3. Stability of TNB as a function of the acetonitrile concentration for unacidified samples.

at day 20 and probably indicates that acidification to pH 3.5 is having a greater effect on the destructive mechanism for 3,5-DNA than on the mechanism of production. Acidification to pH 2 with sodium bisulfate, however, was very effective in preserving the TNB concentration over the entire 30-day period (Fig. 1), and no detectable concentration of 3,5-DNA was observed. The small increase in TNB concentration with time is attributed to day-to-day calibration error.

The effects of the addition of ACN for preservation depended on the ACN concentration used. For concentrations at or below 5.0%, increasing losses of TNB were found as a function of storage time (Fig. 3). For all of these samples, the loss of TNB was accompanied by an increase in the concentration of 3,5-DNA. For ACN concentrations below 5.0%, the rate of loss of TNB was inversely related to ACN concentration. The fastest rate of loss was observed for the control (no addition of ACN). No loss of TNB, or observable 3,5-DNA, were found for samples containing concentrations

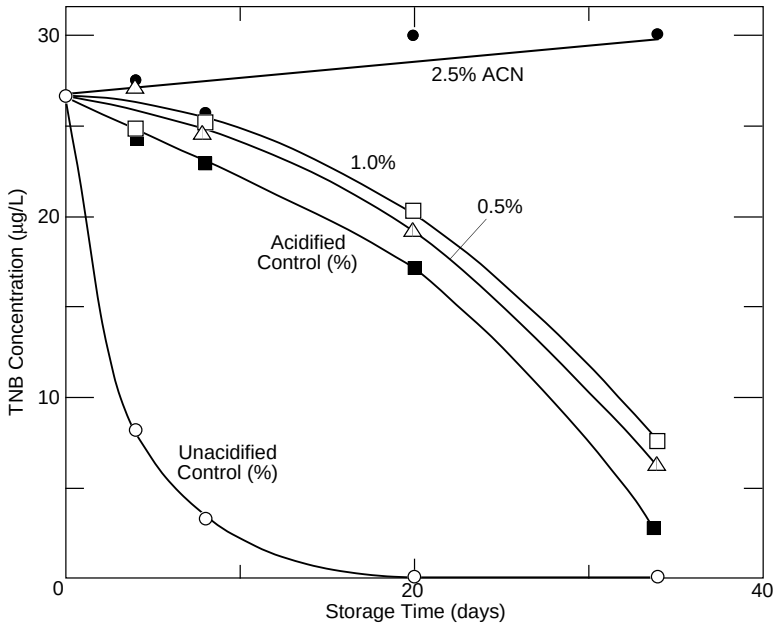

Figure 4. Stability of TNB as a function of the acetonitrile concentration for samples also acidified to pH 3.5.

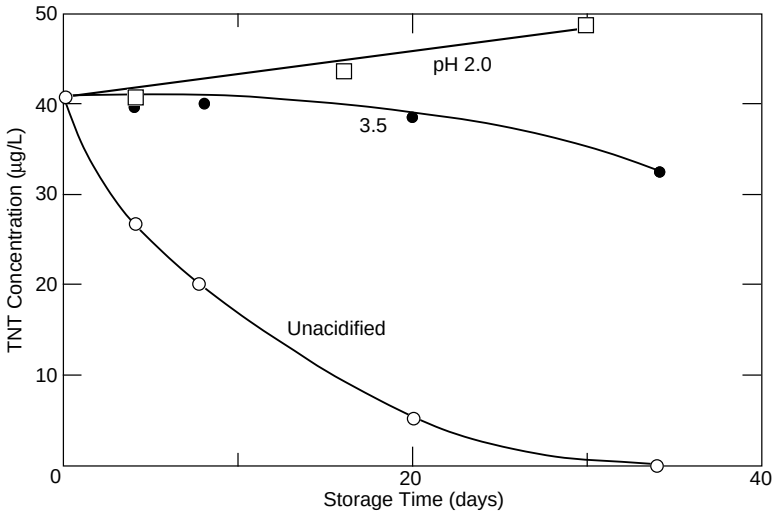

Figure 5. Stability of TNT in samples acidified to pH2, pH 3.5, and left unacidified.

of ACN of 7.5% (Fig. 3) or 10% (not shown), which appeared to be as effective as acidification to pH 2 for TNB stabilization in this matrix.

The concentration at which ACN was effective in retarding TNB loss was reduced when the samples were also acidified to pH 3.5 with acetic acid (Fig. 4). For this combination, no observable TNB loss or increase in 3,5-DNA concentration was found for samples containing ACN concentrations of 2.5% or higher. For samples containing 1.0%, 0.5%, and 0.1% ACN, TNB concentrations at 34 days were 7.6 µg/L, 6.3 µg/L, and $<d$, respectively. Acidification did reduce the rate of loss of TNB relative to samples containing the same percent ACN but without acidification (Fig. 3 and 4).

The effects of the various chemical treatments were very similar for TNT, except the rates of change were reduced in all cases. For example, after four days, the TNT concentration in the control sample was reduced from 41.3 µg/L to 26.5 µg/L for a reduction of about 36% (Fig. 5). For TNB over the same period, the reduction was about

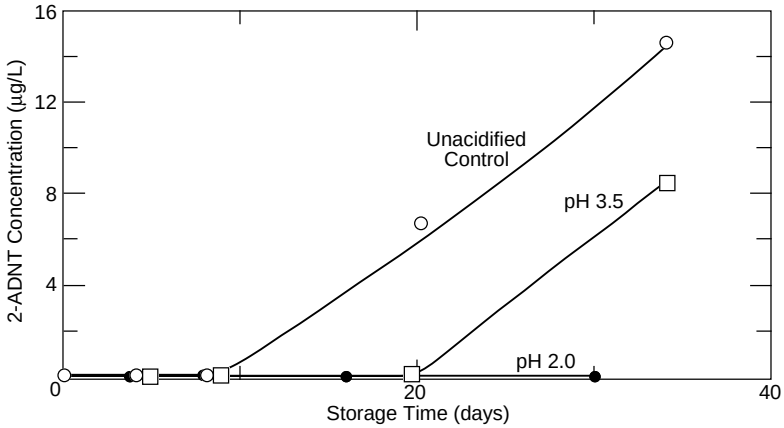

Figure 6. Production of 2ADNT in samples acidified to pH 2, pH 3.5, or left unacidified.

68%. Acidification to pH 3.5 with acetic acid resulted in increased stability of TNT relative to TNB with the concentration at day 34 of 32.6 µg/L (Fig. 5). No loss of TNT was observed for the sample acidified to pH 2 with sodium bisulfate over the entire 30-day storage period. As with TNB, the loss of TNT was accompanied by the production of the monoamino transformation products (Fig. 6). The effects of storage with various levels of ACN, with and without acidification to pH 3.5, were similar to that described above for TNB. Overall, the three storage conditions that were successful in preserving TNB were also successful in preserving TNT. As discussed above, RDX was stable in the control sample and was unaffected by any of the chemical preservatives tested.

Thus, of the various stabilization techniques investigated in this initial study, three appeared to be quite successful:

(1) acidification to pH 2 with sodium bisulfate,
(2) acidification to pH 3.5 with addition of ACN to a concentration of 2.5% or greater, and
(3) addition of ACN without acidification to achieve a concentration of 7.5% or greater.

In all cases examined, TNB, TNT, and RDX were stable for at least 30 days when samples were preserved using these three techniques. Without preservation, TNB and TNT were unstable in these matrices.

Further evaluation of successful methods of preservation

To further evaluate these options, the stability of several other SW846 8330 target analytes were evaluated over a 31-day period. The test was conducted using fortified Connecticut River water in a manner similar to that discussed above, except the analytes tested were tetryl, 2ADNT, 4ADNT, and 3,5-DNA, an analyte recommended for inclusion in Method 8330 (Walsh et al. 1993). Tetryl was chosen because it had been demonstrated to be unstable with regard to both reduction and hydrolysis when held in a soil matrix for very short periods (Jenkins 1994). The three amino compounds were selected because of the potential for protenation at the low pH used in two of the preservation techniques, which could affect their long-term stability.

The results for tetryl were similar to that reported earlier for TNB. The concentration of tetryl rapidly declined in the unpreserved control such that after seven days only about 50% remained (Fig. 7). The loss of tetryl was accompanied by the production of a transformation product that eluted about 1.6 min prior to tetryl (4.6 min vs. 6.2 min for tetryl). This transformation product was noted in the tetryl soil holding-time study discussed above, but the compound was not identified. Acidification to pH 2 and acidification to pH 3.5 with an acetonitrile concentration of 2.5% were very successful in stabilizing tetryl over the entire 31-day study. Stabilization using an acetonitrile concentration of 7.5% without acidification appeared to be a slightly less effective preservative, although no transformation products were observed even after 31 days of storage. The small differences in concentration for the three preservatives shown in Figure 7 could be a result of poor quantitation due to the development of a noisy baseline as the samples aged. This appears to be due to long-term storage of samples containing acetonitrile.

The results for 3,5-DNA were quite different from the results with tetryl. For the unpreserved control, the concentration only declined from 57 µg/L to 41 µg/L after 31 days. Concentrations of 3,5-DNA in the samples stabilized using the three different preservatives did not appear to be substantially different from one another. For the 2ADNT and 4ADNT, no apparent losses of these two compounds were observed with the unpreserved control sample held for 31 days. There appeared to be a slightly lower recovery of both compounds for the sample preserved at pH 2 using sodium bisulfate. This result was obtained using the direct-injection RP-HPLC method without neu-

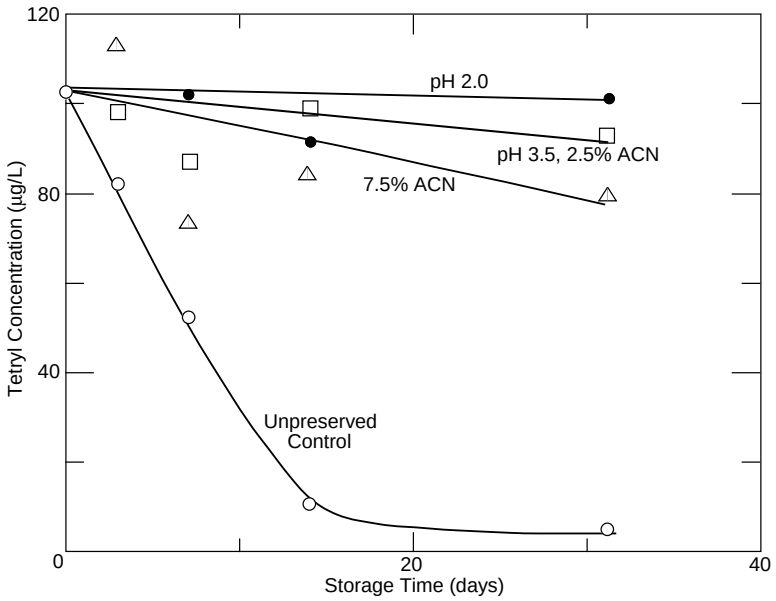

Figure 7. Stability of tetryl in samples preserved at pH 2, pH 3.5, with 2.5% acetonitrile, and with 7.5% acetonitrile.

tralization. Low recovery could be due to some protenation of the amines at pH 2, although pKa values for 2ADNT and 4ADNT have been reported as 0.59 and 1.23, respectively (Glover et al. 1977), indicating that most of both compounds should exist in the nonionized amino form at pH 2. Overall, the three stabilization techniques appear to be quite adequate in preserving these four analytes.

Evaluation of selected preservatives on EPA Method 8330 determination

Effects on preconcentration methods

The next phase of this study was to evaluate the effects these three potential stabilization techniques have on the most commonly used analytical method for nitroaromatics and nitramines, SW846 Method 8330. The following experiment was conducted to determine the effects of these preservatives on the extraction/preconcentration procedures used for low-level determination.

A solution was prepared containing HMX, RDX, TNB, DNB, 3,5-DNA, TNT, 2ADNT, and 2,4-DNT in reagent-grade water at concentrations ranging from 10.7 to 12.2 µg/L. The solution was divided into four portions; three were preserved as described above, and the fourth was left unpreserved to serve as a control for comparison of analytical results. Aliquots of each portion were immediately preconcentrated using salting-out solvent extraction with acetonitrile, cartridge solid-phase extraction (SPE) using Porapak RDX, and membrane SPE using Empore SDB membranes, according to the methods described in detail in Jenkins et al. (1994). The resulting extracts were analyzed using RP-HPLC as specified in Method 8330, and percent recoveries were calculated vs. initial spiked concentrations (Table 3). As you will note, the results for the membrane SPE method for the solution preserved with 7.5% ACN indicated that recoveries of the analytes ranged from only 3% for HMX and RDX to 45% for 2,4-DNT. Since these recoveries are unacceptably low, no additional experiments were conducted using this preservative.

Results for the SOE procedure, when samples were acidified to pH 2, indicated that low recovery was obtained for the two amino-containing analytes, 3,5-DNA (35%) and 2ADNT (54%). These results are consistent with some protenation of these compounds to form the corresponding ammonium cations, which would not be expected to partition favorably into the salted-out acetonitrile. A further test of the SOE procedure was conducted when the initially pH-2-preserved solution was neutralized with aqueous KOH to pH 6.6 before extraction. The recovery of 3,5-DNA and 2ADNT improves to 100 and 97%, respectively, after neutralization (Table 3). No problems were encountered with the pH-2-preserved solution with either the cartridge or membrane SPE methods, where recovery of the amino-containing compounds appears to be unaffected by the low pH. Recovery of HMX using the SDB membrane method, however, is low for both preserved solutions and the unpreserved solution, as was observed in our earlier study (Jenkins et al. 1992, 1994). An apparent high recovery of RDX was found for the cartridge SPE method for all three solutions. This again had been observed previously; part of the problem appears to be due to a narrowing of the peak width for RDX for cartridge SPE extracts compared with the unextracted standards used for establishing response factors. All three extraction methods appear to give good recovery for the portion of solution preserved with 2.5% ACN at pH 3.5.

These results indicate that acidification using sodium bisulfate to pH 2 or addition of ACN to

Table 3. Recovery of target analytes from fortified Milli-Q water preserved with either sodium bisulfate (pH 2), 2.5% acetonitrile at pH 3.5, or 7.5% acetonitrile without pH adjustment using SOE, SPE-C, and SPE-M.

	Analyte (% recovery)							
Treatment/preservation	*HMX*	*RDX*	*TNB*	*DNB*	*3,5-DNA*	*TNT*	*2ADNT*	*2,4-DNT*
SOE*								
control	100	112	102	102	106	107	104	100
pH 2	90	93	93	104	35	93	54	101
pH 2(neutralized)	95	103	96	96	100	95	97	93
2.5% ACN	5	103	96	95	98	96	102	98
SPE-C*								
control	06	132	107	114	112	106	110	104
pH 2	05	138	104	110	112	92	106	92
2.5% ACN	03	126	101	111	126	109	120	110
SPE-M*								
control	1	102	98	101	106	91	98	89
pH 2	2	106	103	107	112	96	104	94
2.5% ACN	9	76	103	108	114	96	105	96
7.5% ACN	3	3	11	14	19	40	40	45

* SOE – salting-out solvent extraction
SPE-C – cartridge solid-phase extraction (Porapak RDX)
SPE-M – membrane solid-phase extraction (Empore-SDVB)

achieve a 2.5% solution along with acidification to pH 3.5 do not cause major analytical problems for Method 8330. Neutralization will be required if SOE is used for preconcentration of pH-2-preserved samples, but this is not difficult to achieve and the pH need only be raised to 3.5 to enable complete recovery of the amino compounds.

Effects on the direct method

To assess the effects of acidification to pH 2 with sodium bisulfate on the direct analysis procedure of Method 8330, a sample of Connecticut River water was divided into four portions and fortified with the 15 target analytes in the four groups indicated in Table 4. Each of these four solutions was then divided into two aliquots; one aliquot was acidified to pH 2 with sodium bisulfate and the other was left unacidified. Six replicate portions of each solution were then processed as described in Method 8330 except that samples were diluted 1:1 with methanol rather than acetonitrile and analyzed by RP-HPLC (EPA 1992). The decision to use methanol rather than acetonitrile was made to matrix-match the sample and eluent to improve the quality of the chromatograms near the regions where HMX and RDX elute.

The mean value and standard deviation determined for each analyte in the preserved and unpreserved solutions are presented in Table 5. An *F*-test was conducted to compare the variances for acidified and unacidified portions for each analyte to assess whether acidification affected the analytical precision obtained for the acidified solution relative to the unacidified control. For all analytes, except TNT and 2ADNT, the variances obtained were not significantly different at the 95% confidence level. Thus, for these analytes, no detrimental effect on analytical precision was

Table 4. Concentrations of various analytes fortified into Connecticut River water for replicate study.

		Fortified Connecticut River concentration (µg/L)		
Group	*Analyte*	$\overline{X}$	±	S
1	DNB	39.9	±	0.52
	tetryl	41.2	±	0.58
	4ADNT	57.2	±	0.42
	3NT	59.9	±	1.95
2	HMX	64.6	±	1.33
	RDX	100.5	±	0.94
	TNB	52.7	±	0.85
	TNT	91.4	±	0.37
	2,4-DNT	69.7	±	0.38
	2NT	74.4	±	0.69
3	3,5-DNA	59.5	±	0.32
	2ADNT	79.5	±	1.03
	4NT	178.4	±	0.96
4	NB	43.6	±	0.48
	2,6-DNT	99.2	±	1.34

* $\overline{X}$ = mean; *S* = standard deviation

Table 5. Results of direct analysis of preserved (pH 2) and unpreserved Connecticut River water (six replicates each) using the direct analysis procedure of SW846 Method 8330.

Analyte	*Preserved (pH 2)*	$\overline{X}$	S	F*	t*
HMX	No	64.6	0.33	2.55	0.82
	Yes	66.2	0.83		
RDX	No	00.5	0.94	1.89	1.20
	Yes	02.8	1.30		
TNB	No	52.7	0.63	2.77	0.55
	Yes	53.4	0.97		
DNB	No	39.9	0.52	4.98	0.03
	Yes	39.9	0.23		
3,5-DNA	No	59.5	0.32	4.49	3.04
	Yes	58.2	0.15		
tetryl	No	41.2	0.58	2.28	0.61
	Yes	40.4	0.87		
NB	No	43.6	0.48	1.74	0.40
	Yes	43.2	0.63		
TNT	No	91.4	0.37	31.8	1.61†
	Yes	92.9	2.05		
4ADNT	No	57.2	0.42	4.14	0.21
	Yes	57.4	0.86		
2ADNT	No	79.5	0.02	5.68	3.43††
	Yes	77.8	0.43		
2,6-DNT	No	99.2	0.34	2.40	0.65
	Yes	97.2	2.08		
2,4-DNT	No	69.7	0.38	2.39	0.29
	Yes	69.9	0.59		
2NT	No	74.4	0.69	2.12	0.21
	Yes	74.1	1.01		
4NT	No	78.4	0.97	3.17	0.80
	Yes	76.5	1.73		
3NT	No	59.9	0.95	3.37	0.06
	Yes	60.1	1.06		

$\overline{X}$ = mean; *S* = standard deviation

* Critical values for *F* and *t* at the 95% confidence level are 5.05 and 2.23, respectively.

† Critical value for *t* is 2.78 (*d.f.* = 5), thus the *t* value for TNT is not significant at the 95% confidence level.

†† Critical value for *t* is 2.37 (*d.f.* = 7), thus the *t* value for 2ADNT is significant at the 95% confidence level.

found due to acidification. For 2ADNT, the *F* ratio was only 5.68, compared with a critical value of 5.05, and this marginally significant value may be a result of the unusually good precision obtained for this experiment. For TNT, the cause of the significant *F* ratio was traced to a small and variable coeluting peak in the chromatogram obtained for the acidified Connecticut River water. This peak was not found in the unacidified water, nor was it found in the methanol or the sodium bisulfate. Subsequently this interference was found to be due to the use of a disposable syringe containing a rubber-tipped plunger for sample filtration prior to RP-HPLC analysis. This interference is only produced with acidified samples, and the degree of interference increases with time as samples are held prior to analysis. Since various samples are analyzed at different times after filtration, the size of this interference varies from analysis to analysis. We did not fully appreciate or understand the significance of this problem until after nearly all of the experimental work in this report was completed, including the evaluation using explosives-contaminated groundwater samples. This interference can be eliminated by the use of disposable plastic syringes that do not have a rubber-tipped plunger. This interference will be discussed again in a later section on the results of acidification on field-contaminated groundwaters from the Naval Surface Warfare Center.

Since variances, except for TNT and 2ADNT, were not significantly different, further analysis of these results was conducted by pooling the variances obtained for the acidified and unacidified samples for a given analyte and performing a *t* test to assess whether the mean values obtained for the acidified and unacidified solutions were statistically different. Since the variances were significantly different for TNT and 2ADNT, variances were not pooled and *t* values were calculated using the approach presented in Miller and Miller (1984). Except for 2ADNT and 3,5-DNA, means of acidified and unacidified samples were found to be "not significantly different" at the 95% confidence level. Thus, for these 13 analytes, there is no indication of a measurable effect on the accuracy of the direct analysis method under Method 8330 due to acidification of samples to pH 2. The significant differences in means for the 2ADNT and 3,5-DNA were not surprising in light of the problems discussed above for the amino compounds and others to be presented later. Nevertheless, these differences are too small to be of practical significance.

Holding-time study using pH 2 stabilization for fortified river water

Based on the results above, acidification to pH 2 with sodium bisulfate was selected as the option with the greatest potential for conveniently stabilizing waters containing nitroaromatics and nitramines. To evaluate this procedure more thoroughly, a replicated holding-time study was conducted using all 15 target analytes (14 current SW846 Method 8330 target analytes plus 3,5-DNA).

Connecticut River water was selected as the principal test matrix based on the results discussed above. The analytes were divided into four groups as described above (Table 4). The river water was fortified at concentrations ranging from 50 to 150 µg/L and divided into two portions. The first portion was divided into 11 subsamples and stored in 22-mL glass vials. These samples served as control samples to assess analyte losses without preservation. The second portion was acidified to pH 2.0 by addition of sodium bisulfate (1.5 g/L) and was also divided into 11 subsamples. On day 0 (about 4–16 hours after preparation), six replicates each of the control and preserved samples were analyzed to establish initial concentrations for later comparison. The remaining samples of control and preserved water were held under refrigeration in the dark for up to 64 days. One replicate sample of the control and acidified water was analyzed in triplicate on days 3, 7, 14, 28, and 64 (Table 6).

The behavior of the 15 analytes in this study varied significantly, falling into four different groups. In the first group, analyte concentrations appeared to be stable with or without acidification over the entire 64-day period. This group included HMX and RDX (Fig. 8), and their behavior is consistent with the results of our earlier study (Grant et al. 1993) and that of Maskarinec et al. (1991).

For the second group, the concentrations in the acidified samples remained stable for at least 28 days (and for all but tetryl for 64 days), but concentrations in the unacidified samples declined at variable rates over the entire period. This group includes tetryl, TNB, TNT, 1,3-dinitrobenzene (DNB), 2,6-DNT, and 2,4-DNT in decreasing order of the rate of concentration decline (Table 6, Fig. 9). The difference in stability of these analytes for acidified and unacidified samples is clearly shown in the chromatograms for fortification groups 1

Table 6. Results of holding-time study using fortified Connecticut River water.

		Concentration ($\overline{X}$ ±S) (µg/L)					
Analyte	*Preserved (pH 2.0)*	*Day 0*	*Day 3*	*Day 7*	*Day 14*	*Day 28*	*Day 64*
HMX	No	64.6 ±1.33	66.1 ±0.58	67.1 ±0.79	65.8 ±1.92	66.3 ±0.86	63.1 ±0.28
	Yes	66.2 ±0.83	64.3 ±2.85	62.2 ±0.94	64.2 ±0.86	63.3 ±0.34	59.5 ±0.48
RDX	No	100.5 ±0.9	97.1 ±1.0	97.0 ±0.6	98.9 ±2.9	100.5 ±0.7	97.0 ±0.38
	Yes	102.8 ±1.3	100.9 ±1.9	97.3 ±0.97	100.7 ±0.17	99.7 ±1.86	97.6 ±0.63
tetryl	No	41.2 ±0.58	10.3 ±0.44	11.0 ±0.33	*<d*	*<d*	*<d*
	Yes	40.4 ±0.87	41.8 ±0.76	42.4 ±0.37	41.7 ±0.90	41.2 ±0.58	32.5 ±5.10
TNB	No	52.7 ±1.63	29.8 ±0.48	17.7 ±0.08	7.5 ±0.59	0.7 ±0.0	*<d*
	Yes	53.4 ±0.97	52.0 ±1.06	51.3 ±0.22	54.1 ±0.63	51.8 ±0.23	45.9 ±2.72
TNT	No	91.4 ±0.37	75.4 ±1.24	65.9 ±0.36	50.8 ±3.42	32.7 ±0.31	13.4 ±0.45
	Yes	92.9 ±2.04	91.0 ±1.75	89.3 ±2.52	95.4 ±0.70	90.4 ±1.18	89.6 ±0.12
DNB	No	39.9 ±0.52	37.2 ±0.32	33.3 ±0.11	28.5 ±0.17	23.8 ±0.38	11.3 ±0.25
	Yes	39.9 ±0.58	39.8 ±0.29	39.0 ±0.76	39.1 ±0.20	39.0 ±0.18	38.5 ±0.37
2,6-DNT	No	99.2 ±1.34	96.2 ±1.38	90.7 ±0.60	84.4 ±1.38	84.9 ±0.82	73.5 ±0.88
	Yes	97.2 ±2.14	95.9 ±0.53	91.9 ±0.31	94.8 ±0.43	94.6 ±0.46	94.3 ±1.02
2,4-DNT	No	69.7 ±0.38	68.8 ±0.15	68.3 ±0.04	66.1 ±4.73	63.7 ±0.27	58.8 ±0.38
	Yes	69.9 ±0.59	68.6 ±0.51	67.2 ±0.48	71.5 ±1.07	67.3 ±0.44	66.1 ±0.05
4ADNT	No	57.2 ±0.42	57.5 ±0.09	55.3 ±0.23	54.0 ±2.39	54.0 ±0.80	50.4 ±0.30
	Yes	57.4 ±1.50	46.4 ±1.16	44.3 ±0.38	43.1 ±2.16	43.8 ±0.25	41.4 ±0.13
3,5-DNA	No	59.5 ±0.32	54.4 ±0.37	48.4 ±0.16	45.9 ±0.05	40.0 ±0.44	30.9 ±0.31
	Yes	58.2 ±0.15	53.3 ±2.29	52.1 ±0.49	52.7 ±0.84	51.8 ±0.21	51.3 ±0.11
2ADNT	No	79.5 ±1.03	77.0 ±0.68	73.1 ±0.89	73.4 ±0.49	69.0 ±0.53	62.9 ±0.30
	Yes	77.8 ±0.43	73.4 ±2.38	71.1 ±0.38	73.0 ±0.72	71.5 ±0.41	70.8 ±0.36
2NT	No	74.4 ±0.69	71.2 ±1.18	65.6 ±0.79	64.1 ±5.47	61.1 ±0.57	53.7 ±1.06
	Yes	74.1 ±1.01	68.4 ±0.55	63.5 ±0.98	68.7 ±0.76	60.9 ±1.13	49.5 ±4.35
3NT	No	59.9 ±1.95	54.4 ±0.44	51.3 ±0.24	49.5 ±0.41	46.4 ±1.10	40.9 ±0.61
	Yes	60.1 ±1.07	53.8 ±0.07	50.5 ±0.64	48.6 ±1.32	45.6 ±1.06	40.4 ±1.35
4NT	No	178.4 ±1.0	163.2 ±2.5	153.3 ±0.9	150.2 ±0.3	136.3 ±4.6	129.7 ±0.9
	Yes	176.5 ±1.7	161.6 ±4.8	150.4 ±0.78	149.0 ±0.50	138.6 ±0.69	126.8 ±2.61
NB	No	43.6 ±0.48	42.4 ±0.62	40.6 ±0.25	40.8 ±0.45	40.3 ±0.35	37.7 ±0.53
	Yes	43.2 ±1.46	41.2 ±0.58	40.2 ±0.86	40.6 ±0.27	39.6 ±0.90	37.9 ±0.64

* $\overline{X}$ = mean; S = standard deviation

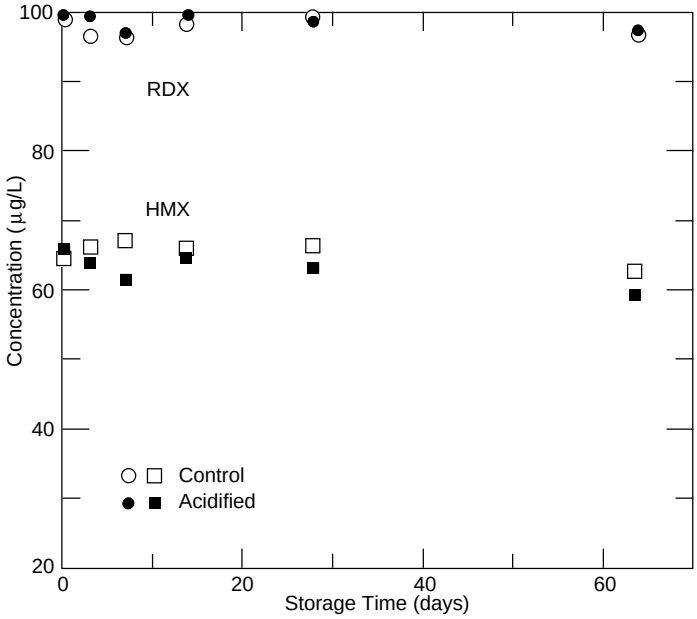

Figure 8. Stability of HMX and RDX in fortified Connecticut River water as a function of storage time for samples acidified to pH 2 or left unacidified.

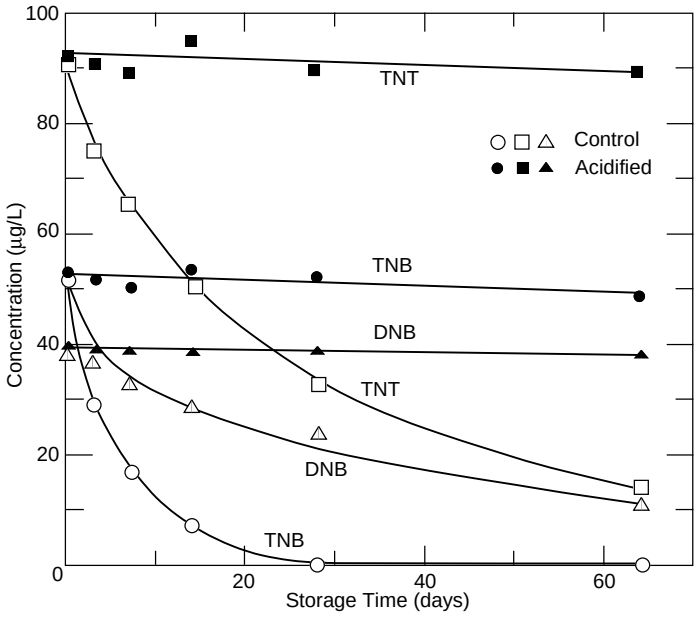

Figure 9. Stability of TNB, TNT, and DNB in fortified Connecticut River water as a function of storage time for samples acidified to pH 2 or left unacidified.

and 2 as a function of storage time (Fig. 10 and 11). In the worst case, tetryl declined when not acidified from about 41 µg/L to "not detected" in less than 14 days. For TNB, the concentration in the unacidified sample declined from about 53 µg/L to 0.7 µg/L in 28 days, and for TNT the concentration declined from about 91 µg/L to 13.4 µg/L over 64 days. Concentrations of tetryl, TNB, TNT, and DNB in the unacidified samples dropped by 73%, 66%, 28%, and 17%, respectively, during the currently accepted holding time of seven days. Transformation products from TNB, TNT, tetryl, DNB, and 2,6-DNT were observed in increasing concentrations as the concentrations of the fortified analytes declined. For TNB and TNT these transformation products were positively identified as the monoamino reduction products (McCormick et al. 1977). In the case of tetryl, however, a major transformation product was identified as picrate ion, and lesser amounts of a transformation product thought to be due to reduction of a nitro on the ring to amino was also observed (Fig. 10). For DNB and 2,6-DNT, the observed retention times of the transformation products were consistent with those expected for the monoamino transformation products, but we did not have the authentic compounds available to verify this assignment.

The third group of analytes included 4ADNT, 2ADNT, and 3,5-DNA. For solutions where these analytes were fortified, there appeared to be a rapid step-drop in concentration for acidified samples within the first several days, which differed substantially from their behavior in the unacidified samples (Fig. 12). For acidified samples, losses after seven days of storage were 9%, 10%, and 23% for 2ADNT, 3,5-DNA, and 4ADNT, respectively. After 64 days, losses were 9%, 12%, and 28% for the same three compounds, indicating that no significant additional loss had taken place. Unacidified controls were somewhat more stable initially and, after seven days of storage, losses were 8%, 19%, and 3% for 2ADNT, 3,5-DNA, and 4ADNT, respectively, but these losses increased to 21%, 48%, and 12% for these compounds by day 64. Losses in the unacidified samples are presumably due to microbial transformation. We will discuss the losses in acidified samples in more detail later.

The fourth group of analytes includes the three isomers of nitrotoluene and nitrobenzene (2NT, 3NT, 4NT, NB). Concentrations for a given analyte declined slowly in acidified and unacidified solutions at identical rates over the entire 56-day pe-

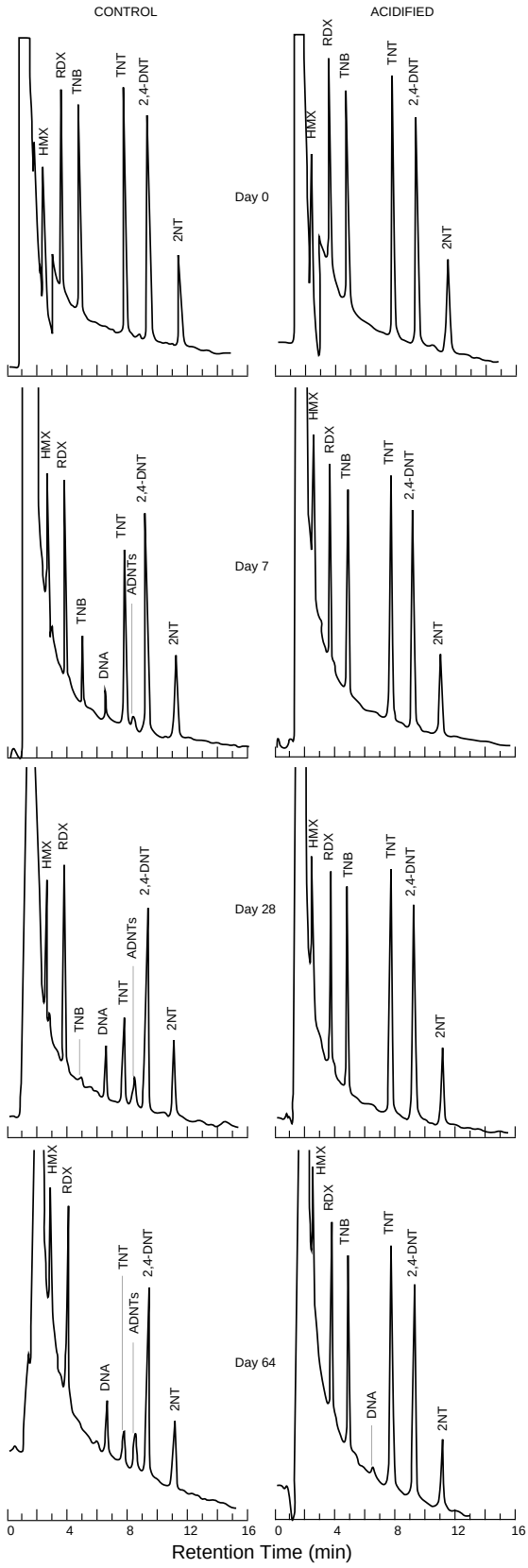

Figure 10. RP-HPLC chromatograms for Connecticut River water samples fortified with DNB, tetryl, 4ADNT, and 3NT.

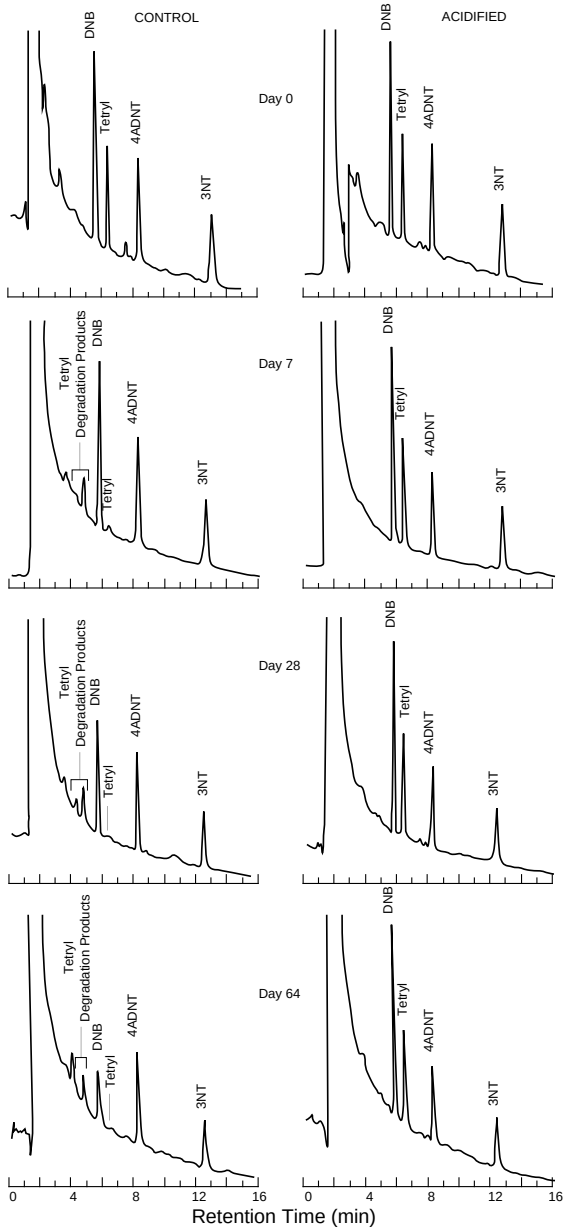

Figure 11. RP-HPLC chromatograms for Connecticut River water samples fortified with HMX, RDX, TNB, TNT, 2,4-DNT, and 2NT.

riod (Fig. 13). A mechanism that could explain this loss is partitioning into the polyethylene cap liner. This behavior has been observed elsewhere for Teflon in contact with hydrophobic organics, including these three nitrotoluenes (Parker et al. 1990, Leggett and Parker 1994).

To test the hypothesis that losses of the mononitrotoluenes was due to partitioning into the polyethylene cap liners, caps taken from vials stored with and without acidification for 28 days were rinsed thoroughly with reagent-grade water

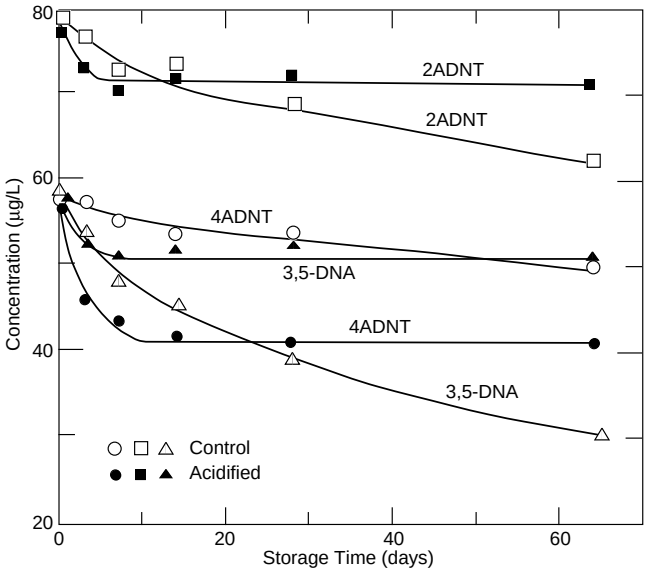

Figure 12. Stability of 2ADNT, 4ADNT, and 3,5-DNA in fortified Connecticut River water as a function of storage time for samples acidified to pH 2 or left unacidified.

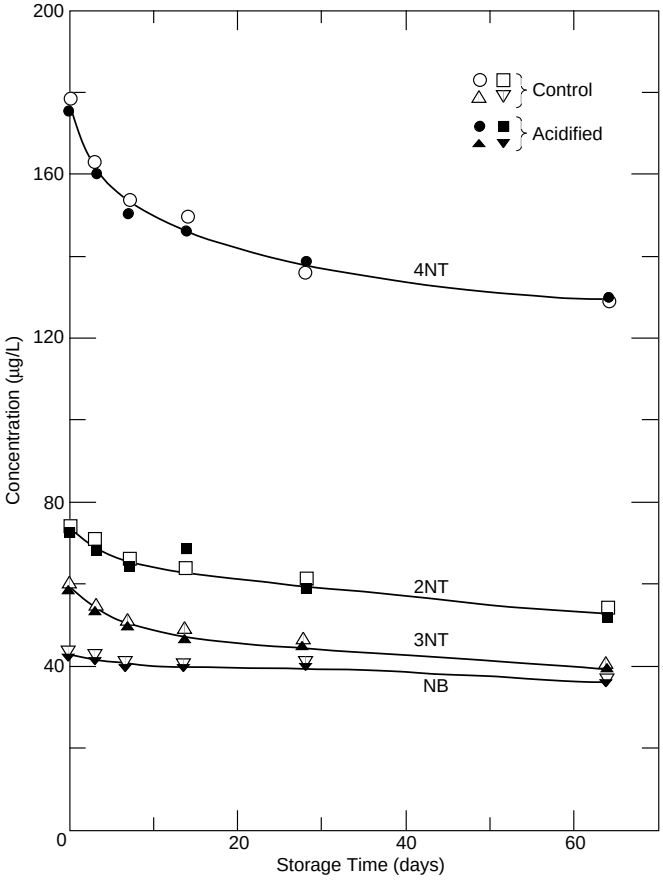

Figure 13. Stability of 2NT, 3NT, 4NT, and NB in fortified Connecticut River water as a function of storage time for samples acidified to pH 2 or left unacidified

and placed in new vials filled to capacity with blank reagent-grade water. The vials were inverted to ensure that solution contacted the cap liner, and they were held under refrigeration for 36 days. At 7, 19, and 36 days, a small portion of the water was withdrawn and analyzed. Detectable concentrations of the three isomers of nitrotoluene were found, and the concentrations increased as exposure times increased (Table 7). Cap liners from the 64-day acidified samples were rinsed with reagent-grade water and placed in new vials containing 2 mL of acetonitrile and inverted to extract sorbed analytes. These samples were stored overnight in the dark at room temperature. Detectable concentrations of the three isomers of mononitrotoluenes, NB, and the two isomers of the dinitrotoluenes were found (Table 7). The percentage recovery ranged from 21 to 70% of the mass lost from the water for the various compounds. Thus, sorption by the cap liners appears to be responsible for the slow losses of the nitrotoluenes and nitrobenzene observed and accounts for the loss of the dinitrotoluenes from acidified samples as well.

Table 7. Recoveries of analytes from polyethylene cap liners (0.44 cm^3).

Mass of analytes recovered when caps (day 28) were equilibrated with reagent-grade water for various lengths of time.

	Mass (µg) recovered		
	*Day 7 C/A**	*Day 19 C/A*	*Day 36 C/A*
2NT	0.18/0.15	0.18/0.19	0.19/0.21
3NT	0.15/0.11	0.17/0.15	0.21/0.19
4NT	0.31/0.37	0.55/0.57	0.65/0.64

* C/A—Control/Acidified

Mass of analytes recovered when caps (day 64) were extracted for 18 hours with ACN.

	Mass of analyte lost from solution (µg)	*Mass of analyte recovered from cap liners (µg)*
2NT	0.56	0.20
3NT	0.48	0.10
4NT	1.17	0.32
NB	0.14	0.06
2,4-DNT	0.10	0.06
2,6-DNT	0.10	0.07

Table 8. Results of holding-time study using three fortified ground waters.

Analyte/matrix*		Preserved (pH 2.0)	Concentration ($\overline{X} \pm S$)** (µg/L) Day 0	Day 3	Day 7	Day 14	Day 28	Day 64
TNB	1	No	53.1 ±1.39	51.4 ±0.30	51.3 ±0.41	35.2 ±0.20	18.4 ±0.22	7.9 ±0.04
		Yes	51.8 ±0.69	51.2 ±0.07	56.7 ±1.96	53.9 ±0.30	53.5 ±0.47	51.8 ±0.56
	2	No	52.4 ±0.58	52.3 ±0.49	54.7 ±0.18	45.2 ±0.51	33.8 ±0.93	16.5 ±1.20
		Yes	52.8 ±1.56	52.0 ±0.10	54.9 ±0.13	54.0 ±0.35	54.6 ±1.42	53.4 ±0.62
	3	No	53.6 ±0.62	52.5 ±0.09	53.4 ±0.54	42.2 ±0.85	20.7 ±0.68	4.2 ±0.60
		Yes	52.7 ±0.81	52.9 ±0.04	55.6 ±0.15	54.8 ±0.46	56.6 ±0.26	54.7 ±0.16
tetryl	1	No	42.5 ±1.17	39.2 ±0.02	29.0 ±0.28	13.9 ±0.58	7.2 ±0.01	3.0 ±0.13
		Yes	41.5 ±0.67	41.6 ±0.53	45.8 ±0.39	43.1 ±0.13	42.7 ±0.41	39.5 ±0.44
	2	No	41.4 ±0.48	39.7 ±0.48	38.6 ±0.48	25.8 ±0.27	14.4 ±0.02	3.7 ±0.80
		Yes	41.5 ±1.54	41.6 ±0.40	44.4 ±0.53	42.6 ±0.72	42.4 ±0.11	40.4 ±0.42
	3	No	170.3 ±0.1	168.5 ±0.0	147.4 ±2.5	83.1 ±0.48	70.2 ±0.01	26.3 ±0.53
		Yes	170.8 ±0.6	173.7 ±2.0	179.9 ±3.5	174.7 ±2.2	176.3 ±0.1	158.4 ±0.63
TNT	1	No	89.4 ±2.27	89.1 ±0.24	93.3 ±0.45	80.9 ±0.43	74.6 ±0.89	61.8 ±1.05
		Yes	88.3 ±0.71	91.0 ±0.75	96.1 ±2.96	92.6 ±0.98	90.7 ±1.15	91.6 ±0.60
	2	No	89.9 ±1.18	91.8 ±1.45	94.0 ±0.40	85.4 ±1.05	74.1 ±2.25	63.4 ±0.37
		Yes	89.1 ±2.82	92.1 ±0.96	94.6 ±0.76	92.5 ±0.02	92.3 ±1.73	91.5 ±0.29
	3	No	90.5 ±1.51	91.3 ±0.26	94.7 ±0.96	88.1 ±0.84	80.2 ±0.18	62.5 ±0.51
		Yes	88.5 ±0.07	93.5 ±1.82	95.0 ±0.15	89.9 ±0.48	94.3 ±0.11	92.7 ±0.27
4-ADNT	1	No	56.0 ±1.67	55.7 ±0.32	57.5 ±0.16	56.9 ±0.01	56.5 ±0.04	56.4 ±0.87
		Yes	55.3 ±1.29	45.2 ±0.47	39.6 ±0.22	34.8 ±0.16	35.0 ±0.31	31.1 ±0.10
	2	No	55.5 ±0.94	55.7 ±0.23	57.0 ±0.46	56.5 ±0.17	54.6 ±1.13	54.5 ±0.42
		Yes	56.2 ±1.69	45.9 ±0.32	41.0 ±0.71	36.1 ±1.56	32.7 ±1.00	29.8 ±0.14
	3	No	54.3 ±2.07	51.1 ±2.79	55.8 ±1.80	54.9 ±0.28	53.4 ±0.38	54.5 ±0.25
		Yes	54.6 ±0.46	47.8 ±1.85	45.2 ±0.52	41.4 ±0.76	40.9 ±1.02	38.7 ±0.15

* Matrix 1 = PT, matrix 2 = MW, matrix 3 = TR

** $\overline{X}$ = mean; S = standard deviation

To determine if these losses could be eliminated using Teflon cap liners, a study was conducted as follows. Reagent-grade water was fortified with TNT, NB, the three nitrotoluenes, and the two DNTs. Samples were stored in an inverted position, under refrigeration, and analyzed after 7, 14, 28, and 64 days. No measurable losses were observed. Thus, the losses for this group of analytes would have been eliminated if vials using Teflon cap liners had been used in the holding-time study.

Holding-time study using pH 2 stabilization for fortified groundwaters

Since preservation by acidification to pH 2 was successful for most of these compounds in Connecticut River water, an additional study was conducted using three local groundwaters. These groundwaters were fortified with TNB, TNT, tetryl, and 4ADNT at the same initial concentrations as used above. The concentration of tetryl was inadvertently fortified at a higher concentration for one groundwater matrix. We studied TNB, tetryl, and TNT because they were the least stable of those tested in the river water matrix. 4ADNT was of interest because of its behavior in acidified samples compared with unacidified. Preserved and unpreserved samples were prepared and treated as described for the previous study, except that only duplicates were analyzed for each storage time. The results of this study are shown in Table 8.

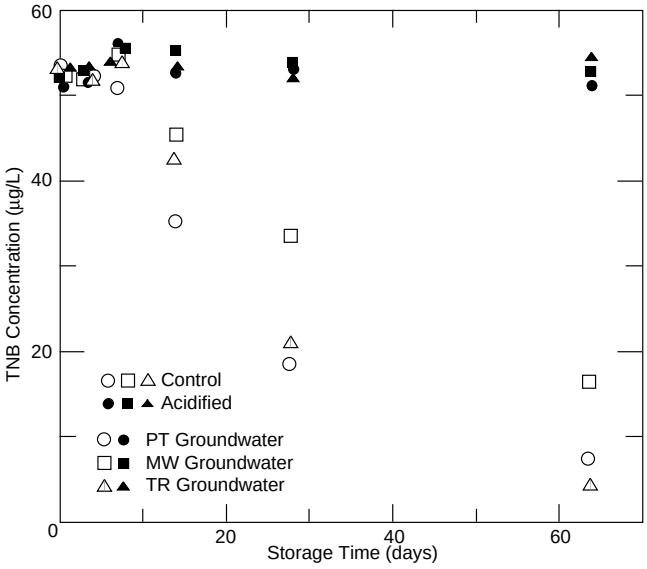

Figure 14. Losses of TNB in acidified and unacidified groundwater samples as a function of storage time.

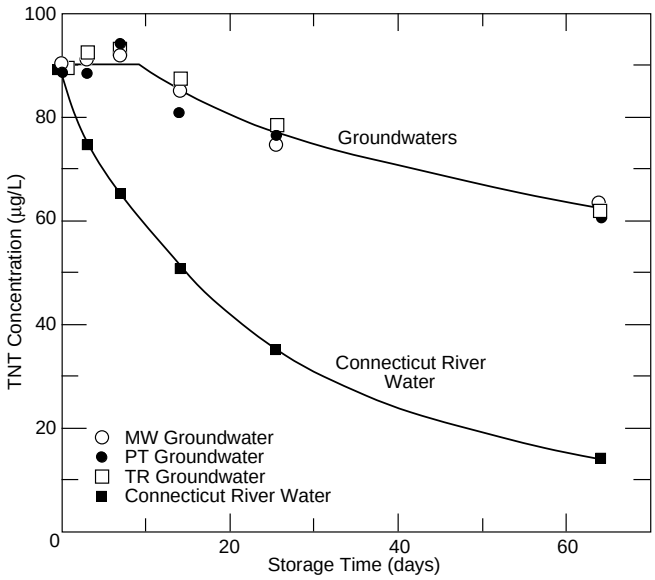

Figure 15. Losses of TNT in unacidified groundwaters and Connecticut River water samples as a function of storage time.

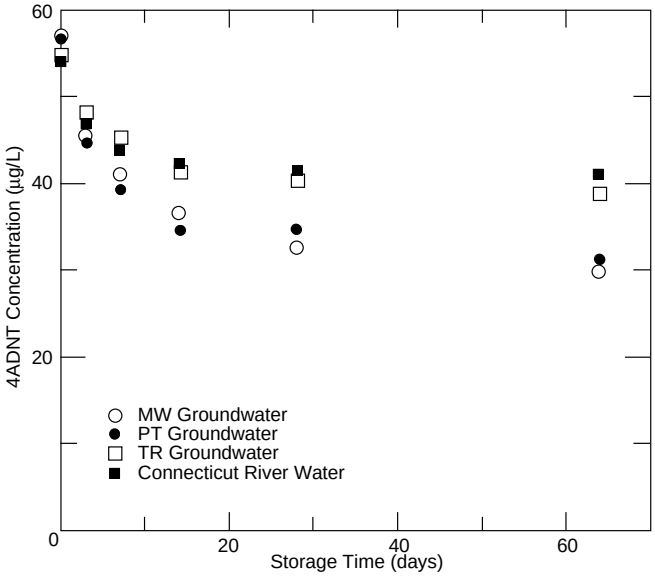

Figure 16. Losses of 4ADNT in acidified groundwaters and Connecticut River water samples as a function of storage time.

Acidification to pH 2 eliminated losses of TNB and TNT over the entire 64-day study for all three fortified groundwaters. Acidification also eliminated losses of tetryl for two of the three groundwaters over the 64-day period, but some loss of tetryl was observed after day 28 for tetryl in matrix no. 3 (Table 8). Substantial losses of tetryl, TNB, and TNT were observed in all of the unacidified samples, however. The pattern of TNB and TNT loss in the three unacidified groundwaters was similar, with the rate of change being greatest for TNB. Both analytes were stable for the first seven days, but substantial losses were observed by day 14 (Fig. 14). This behavior is somewhat different from that found in the fortified Connecticut River water, where major losses of these two analytes were observed by day 3 (Fig. 15). The behavior in both matrices is consistent with microbiological transformation being the major loss mechanism for these compounds. For Connecticut River water, a large population of aerobic microorganisms is undoubtedly present initially that requires little or no acclimation time before being capable of transforming these compounds. In the groundwater, the initial population of aerobic microorganisms is probably many orders of magnitude lower, and it appears that several days are required for the populations to increase sufficiently to result in significant analyte losses.

The behavior of tetryl in the unacidified groundwaters is somewhat different from TNB and TNT. For tetryl, a small loss in all three groundwaters was observed by day 3, with substantial loss for two of the three groundwaters by day 7 (Table 8). This behavior is consistent with earlier research showing that losses of tetryl can occur by hydrolysis as well as microbiological transformation (Kayser et al. 1984, Jenkins 1994).

The behavior of 4ADNT in these fortified groundwaters is similar to that observed in fortified Connecticut River water for acidified samples, but somewhat different for unacidified controls. For unacidified samples, no losses of 4ADNT were observed over the 64-day study. For the acidified samples, substantial losses were observed for all three groundwaters that amounted to 29%, 44%, and 47% for the TR, PT, and MW groundwaters, respectively, after 64 days (Fig. 16). Percent loss of 4ADNT in the TR groundwater was similar to that found in the Connecticut River water, and losses in the other two matrices were greater.

Evaluation of acidification using field-contaminated groundwater

Since chemical preservation using acidification to pH 2 with sodium bisulfate looked promising, this technique was further evaluated with 36 groundwater samples from monitoring wells at the Naval Surface Warfare Center (NSWC) in Crane, Indiana. At each well, two water samples were collected in 125-mL glass bottles. A 0.2-g

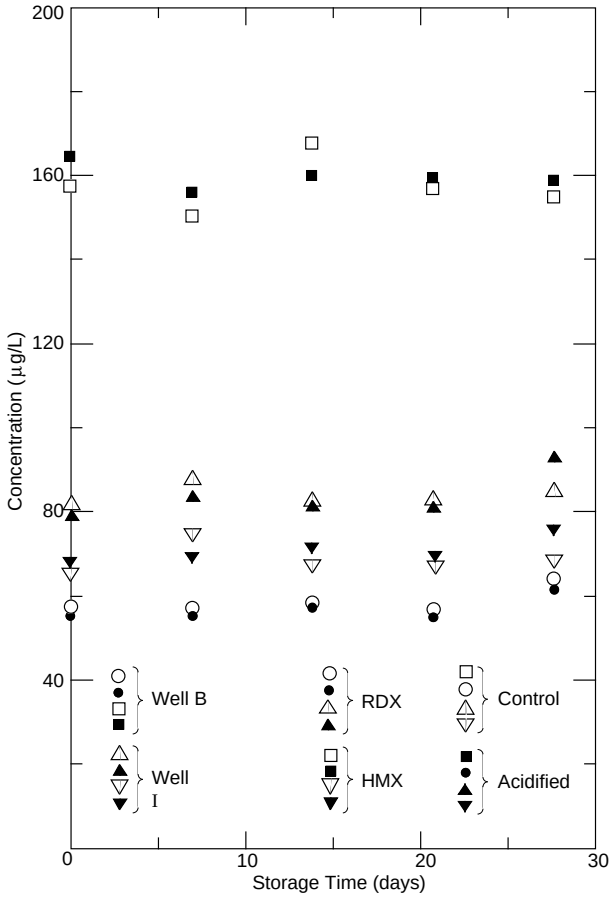

Figure 17. Stability of HMX and RDX in groundwater samples from the Naval Surface Warfare Center.

portion of sodium bisulfate had been added to one bottle in each pair so that an acidified and an unacidified subsample from each well were returned to the laboratory. Samples were shipped cold by overnight carrier.

Upon receipt in the laboratory the day after sample collection, the pH of all samples was measured and all 36 unacidified samples were screened using several commercial enzyme immuno-assay kits to estimate the TNT concentration (Thorne and Myers, in press). Based upon the TNT concentration obtained, nine samples were selected to conduct holding-time studies, and 40-mL aliquots of both the acidified and unacidified portions of these samples were fortified with additional TNT and TNB. Fortification was accomplished by addition of TNT and TNB in aqueous solution prepared without use of organic solvents. The pH for each sample and the fortification level for TNT and TNB is given in Appendix A. A second set of 40-mL aliquots of each pair of acidified and unacidified samples was also retained without fortification. All of these samples were stored in 40-mL glass vials with Teflon-lined caps and analyzed on day 0 (the day they arrived in the laboratory) and at 7, 14, 21, and 28 days after holding at 4°C in the dark. Samples were diluted 1:1 with methanol and filtered prior to analysis. Analytical results are presented in Appendix A.

Of the nine unfortified groundwater samples from the NSWC, seven contained detectable concentrations of HMX and RDX, four had detectable TNT, six had detectable 2ADNT/4ADNT, two had a very low but detectable TNB concentration, and one acidified sample had a very low concentration of 2,4-DNT. As observed for the fortified waters discussed earlier, HMX and RDX were stable in all seven samples over the entire 28-day holding time, whether samples were acidified to pH 2 or not (Fig. 17). The behavior of 2ADNT and 4ADNT was sample-dependent. For three samples, the concentrations of these compounds in the acidified subsamples were substantially lower than for the unacidified subsamples (Fig. 18). In three others, some at nearly identical initial concentrations and measured pH, no loss of 2ADNT or 4ADNT was found due to acidification (Fig. 19). As observed previously, when loss occurred, the major portion occurred rapidly over the first few days.

For TNT, acidification to pH 2 proved to be effective in preserving TNT whether samples initially had TNT present or were fortified with TNT. For their unacidified counterparts, the results were mixed: TNT was stable over the 28-day holding time in two unfortified samples but declined in two others. The worst case was for well F (Table A6), where the acidified sample had a mean concentration of about 22 µg/L over the study, but the unacidified sample showed a consistent decline from 14 µg/L to less than detectable (detection limit estimated at 2 µg/L). The concentration at 7 days, the currently accepted holding time, was 9 µg/L, indicating that nearly two-thirds of the TNT had been lost over this period. The concentration of TNT in the unacidified portion, even in the day 0 sample, was reduced relative to the acidified portion, apparently due to loss occurring during the one-day shipping time from the field to the laboratory. TNT stability in the fortified samples was similarly unpredictable; TNT concentrations in some remained stable but declined significantly in others (Fig. 20) over the 28-day holding time.

The behavior of TNB in fortified samples paralleled that of TNT, but the rate of loss appeared to be faster in samples that showed losses (Fig. 21).

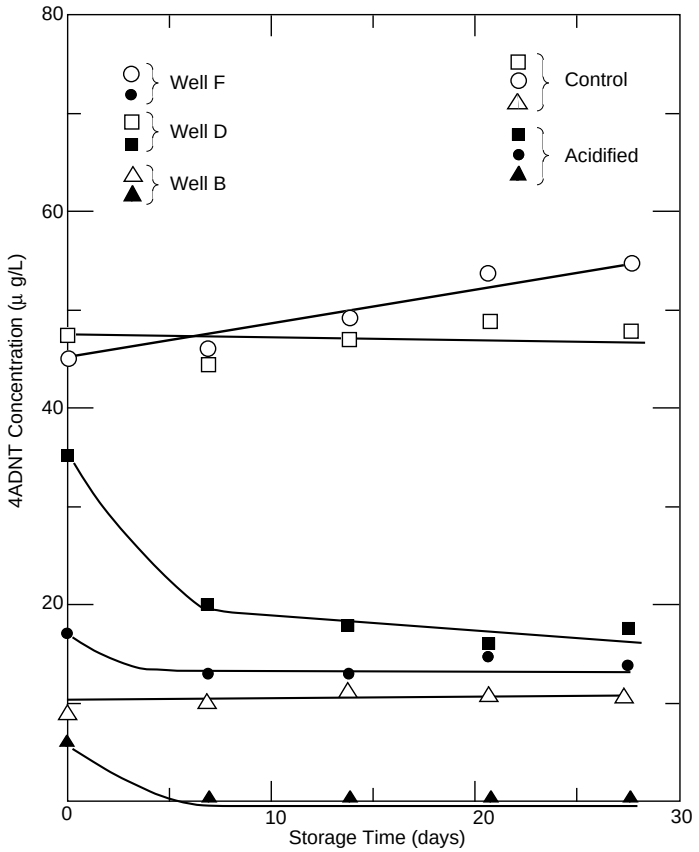

Figure 18. Stability of 4ADNT in three groundwater samples from the Naval Surface Warfare Center.

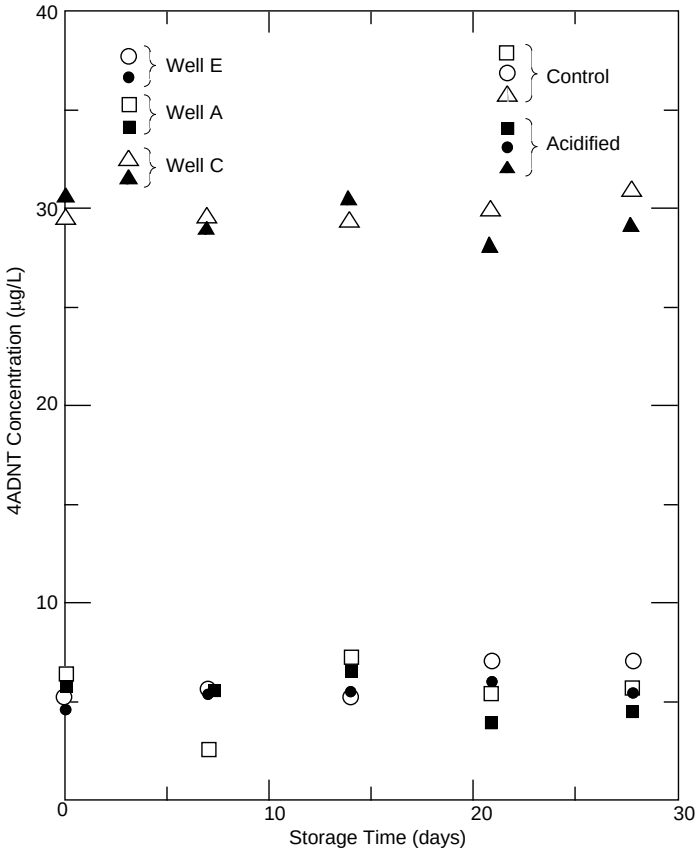

Figure 19. Instability of 4ADNT in three other groundwater samples from the Naval Surface Warfare Center.

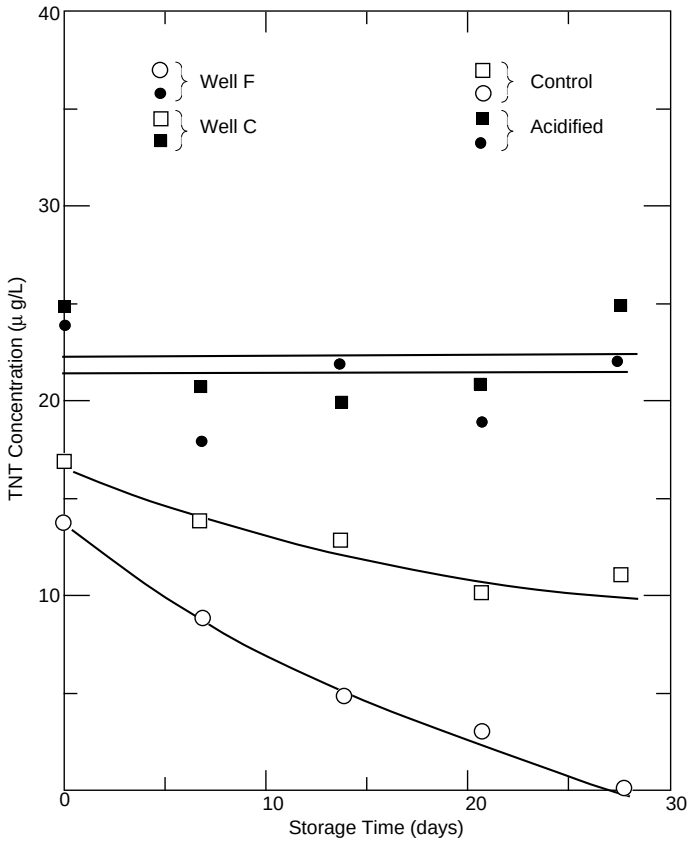

Figure 20. Behavior of TNT in several groundwater samples from the Naval Surface Warfare Center.

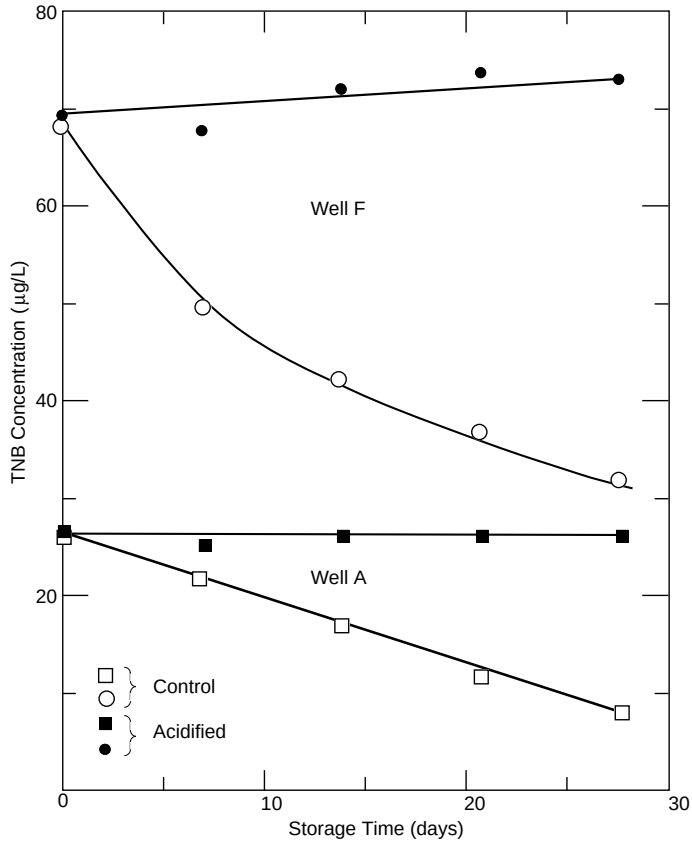

Figure 21. Behavior of TNB in several groundwater samples from the Naval Surface Warfare Center.

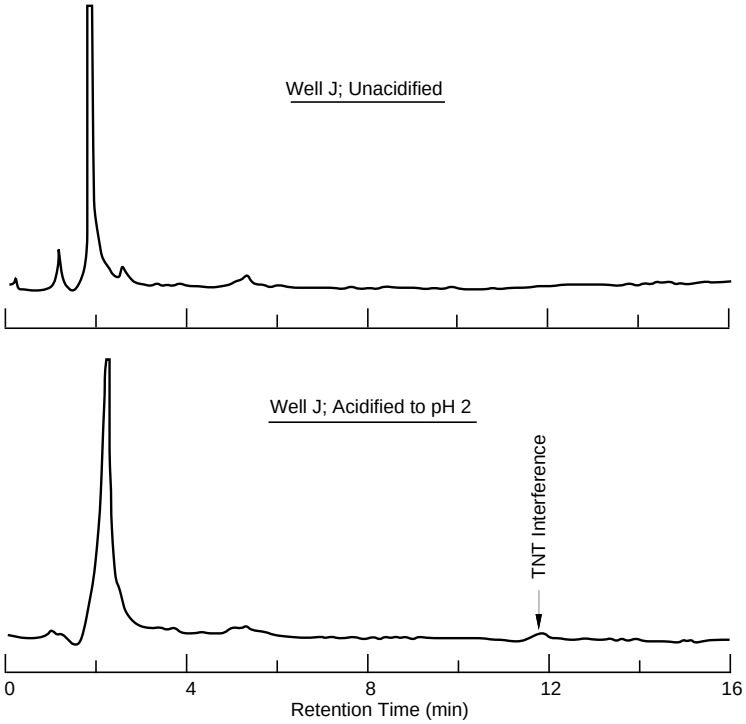

Figure 22. Chromatograms for the acidified groundwater samples from the Naval Surface Warfare Center.

The loss of TNB in several samples was accompanied by the appearance of 3,5-DNA, the expected transformation product. When TNT was either already present, or fortified, at concentrations above about 150 µg/L, no losses of TNT or TNB were observed. This may be due to a toxicity effect on the microorganisms present. Well F (Table A6) had a detectable concentration of 2,4-DNT, but only in the acidified portion. Apparently this analyte was unstable in this sample if not acidified. This result is consistent with observations of Maskarinec et al. (1991). These results confirm that the stability of TNT and TNB in unacidified groundwater samples is very sample-specific, but that acidification to pH 2 is a very effective stabilization technique for nitroaromatics.

Chromatograms obtained for the acidified groundwater samples on the LC-18 column revealed the presence of a small but detectable interference with a similar retention time to TNT (Fig. 22). This interference had been observed earlier with acidified groundwater samples that had been filtered using a syringe containing a rubber-tipped plunger. Analysis of these samples on the LC-CN confirmation column indicated that this peak was definitely not TNT. When these samples were preconcentrated by a factor of 100 prior to analysis, using either salting-out solvent extraction with acetonitrile or solid-phase extraction where the retained compounds were eluted with acetonitrile, this peak was not observed, which was also consistent with the hypothesis that it was due to the rubber-tipped plunger.

Problems with acidification for the amino compounds

As discussed above, some decrease in concentration was found for the amino-containing compounds in acidified samples in the fortified Connecticut River water, the three fortified groundwaters, and some, but not all, of the field-contaminated groundwaters from the NSWC. We initially suspected that this behavior was due to protenation of a significant portion of the amino functional groups to form the corresponding ammonium ions ($HADNT^+$) at pH 2 (eq 1). This could result in low recovery of the parent amine when conducting direct analysis using RP-HPLC.

$$HADNT^+ \rightleftharpoons ADNT + H^+ \qquad (1)$$

$$Ka = [ADNT]\,[H^+] \,/\, [HADNT^+] \qquad (2)$$

The pKa values (eq 2) for the ammonium compounds corresponding to 4ADNT and 2ADNT are

reported to be 1.23 ±0.02 and 0.59 ±0.03, respectively (Glover and Hoffsommer 1977). If we assume an initial concentration of 50 µg/L for 4ADNT, the equilibrium concentrations of 4ADNT and 4HADNT$^+$ for a [H$^+$] of 0.01M would be 42.7 µg/L and 7.3 µg/L, respectively. Similarly, for an initial concentration of 50 µg/L of 2ADNT, the equilibrium concentrations would be 48.1 µg/L and 1.9 µg/L, respectively. If the protenated species were separated from the unprotenated amino compounds during RP-HPLC analysis, recoveries of 85.4% and 97.2% for 4ADNT and 2ADNT, respectively, would result. When solutions of the amino compounds at about 50 µg/L in reagent-grade water were prepared and analyzed quickly after acidification, we observed no losses of the amino compounds relative to unacidified samples. Since acid–base reactions are kinetically very fast, the pH of the sample, once injected into the HPLC, must shift sufficiently that essentially all of the ADNT reverts to the amino form regardless of initial sample pH. Thus, the protenation of the 4ADNT is not directly responsible for the losses we observed.

Figures 16, 18, and 19 indicate that the amount of 4ADNT lost for various water matrices varies substantially, but for matrices where loss is found, the pattern is quite similar. In these cases, loss is fairly rapid over the first several days and then the concentrations become fairly stable at a reduced level. Thus, the loss mechanism appears to have a finite capacity, but the capacity differs from matrix to matrix. Losses were not found to correlate with the actual pH achieved upon acidification for the well waters from NSWC. However, problems with sorption of dinitroaniline herbicides from water on surfaces such as glass, stainless steel, Nalgene, and Teflon have been reported by Strachan and Hess (1982). While there was no association of this sorption with acidification, these results demonstrate how sorptive these types of compounds can be even in the absence of particulate matter. Variations in the colloidal/suspended matter content for specific water samples may be responsible for the apparently inconsistent behavior observed. It is possible that acidification to pH 2 activates surfaces on the colloidal/suspended matter thereby making it more sorptive for these amino compounds.

CONCLUSIONS

The most important conclusion from this study is that holding times for water samples containing nitroaromatics such as TNT, TNB, and tetryl can be extended to at least 28 days by acidification to pH 2 using sodium bisulfate. Acidification does not affect the stability of nitramines that were stable over this period with or without preservation. Unfortunately, in some samples there can be a loss of aminodinitroaromatics such as 4ADNT, 2ADNT, and 3,5-DNA due to acidification to pH 2, and the lost analytes cannot be recovered by neutralization. The maximum loss due to this process after 28 days was 71% for 4ADNT in well F from NSWC (Table A6). These amino-containing compounds can also be lost in unacidified samples, probably as a result of microbiological transformation, but at a slower rate than that due to acidification during the first few days. If samples are to be preconcentrated using salting-out solvent extraction, they must be neutralized prior to extraction, or incomplete recovery of the amino compounds will be found. If preconcentration is to be accomplished using solid-phase extraction, neutralization prior to extraction does not appear to be necessary.

A slow loss of the mononitrotoluenes and nitrobenzene was also found during sample storage whether samples were acidified or not, and the loss was found to be due to sorption into the polyethylene cap liners used for sample storage. When samples were stored in vials with caps containing Teflon liners, this loss was drastically reduced.

If acidified samples are to be analyzed using the direct-analysis protocol in SW846 Method 8330, it is important that filtration be conducted using disposable syringes that do not have a rubber-tipped plunger. If a rubber-tipped plunger is used, a small but detectable interference will be found near the retention time of TNT, and the size of this interference will depend on the time between filtration and analysis.

Because acidification can affect the stability of the amino transformation products in solution, we do not recommend acidification to pH 2 for R&D projects studying the fate of explosives under various treatment protocols. In these cases, samples can be stabilized if necessary by addition of acetonitrile to achieve 2.5% and acidification to pH 3.5 using acetic acid.

LITERATURE CITED

EPA (1992) Nitroaromatics and nitramines by HPLC. Second Update SW846 Method 8330.

Glover, D.J., J.C. Hoffsommer and D.A. Kubose (1977) Analysis of mixtures of 2-amino-4,6-

dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene. *Analytica Chimica Acta*, **88**: 381–384.

Goerlitz, D.F. (1992) A review of studies of contaminated groundwater conducted by the U.S. Geological Survey Organics Project, Menlo Park, California, 1961–1990. In *Groundwater Contamination and Analysis at Hazardous Wastes Sites* (Suzanne Lesage and Richard E. Jackson, Eds.), p. 295–355. New York: Marcel Dekker, Inc.

Goerlitz, D.F. and B.J. Franks (1989) Use of on-site high performance liquid chromatography to evaluate the magnitude and extent of organic contaminants in aquifers. *Ground Water Monitoring Review*, **9**: 122–129.

Grant, C.L., T.F. Jenkins and S.M. Golden (1993) Evaluation of pre-extraction analytical holding times for nitroaromatic and nitramine explosives in water. USA Cold Regions Research and Engineering Laboratory, CRREL Special Report 93-24.

Jenkins, T.F. (1994) Evaluation of pre-extraction analytical holding times for tetryl in soil. USA Cold Regions Research and Engineering Laboratory, Special Report 94-5.

Jenkins, T.F., P.H. Miyares, K.F. Myers, E.F. McCormick and A.B. Strong (1992) Comparison of cartridge and membrane solid phase extraction with salting-out solvent extraction for preconcentration of nitroaromatic and nitramine explosives from water. USA Cold Regions Research and Engineering Laboratory, Special Report 92-25.

Jenkins, T.F., P.H. Miyares, K.F. Myers, E.F. McCormick and A.B. Strong (1994) Comparison of solid phase extraction with salting-out solvent extraction for preconcentration of nitroaromatic and nitramine explosives from water. *Analytica Chimica Acta*, **289**: 69–78.

Kayser, E.G., N.E. Burlinson and D.H. Rosenblatt (1984) Kinetics of hydrolysis and products of hydrolysis and photolysis of tetryl. Naval Surface Weapons Center Report NSWC TR 94-68, Dahlgren, Virginia.

Leggett, D.C. and L.V. Parker (1994) Modeling the equilibrium partitioning of organic contaminants between PTFE, PVC, and groundwater. *Environmental Science and Technology*, **28**: 1229–1233.

Maskarinec, M.P., C.K. Bayne, L.H. Johnson, S.K. Holladay, R.A. Jenkins and B.A. Tomkins (1991) Stability of explosives in environmental water and soil samples. Oak Ridge National Laboratory Report ORNL/TM–11770, Oak Ridge, Tennessee.

Maskarinec, M.P., D.L. Manning and R.W. Harvey (1986) Application of solid sorbent collection techniques and high performance liquid chromatography with electrochemical detection to the analysis of explosives in water samples. Oak Ridge National Laboratory Report ORNL/TM–10190, Oak Ridge, Tennessee.

Maskarinec, M.P., L.H. Johnson, S.K. Holladay, R.L. Moody, C.K. Bayne and R.A. Jenkins (1990) Stability of volatile organic compounds in environmental water samples during transport and storage. *Environmental Science and Technology*, **24**: 1665–1670.

McCormick, N.G., F.E. Feeherry and H.S. Levinson (1976) Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. *Applied and Environmental Microbiology*, **31**(6): 949–958.

Miller, H.H., M.V. Crook and J.L. Spigarelli (1983) Development of sampling and preservation techniques to retard chemical and biological changes in water samples. U.S. Army Toxic and Hazardous Materials Agency Report DRXTH-TE-CR-82182, Aberdeen Proving Ground, Maryland.

Miller, J.C. and J.N. Miller (1984) *Statistics for Analytical Chemistry*, p. 55. Chichester, England: Ellis Horwood Ltd.

Parker, L.V., A.D. Hewitt and T.F. Jenkins (1990) Influence of casing materials on trace chemicals in well water. *Ground Water Monitoring Review*, **10**: 146–156 (Spring).

Thorne, P.G. and K.F. Myers (in prep.) Evaluation of commercial enzyme immunoassays for the field screening of TNT and RDX in water. USA Cold Regions Research and Engineering Laboratory, Special Report.

Strachan, S.D. and F.D. Hess (1982) Dinitroaniline herbicides adsorb to glass. *Journal of Agricultural Food Chemistry*, **30**: 389–391.

Walsh, M.E., T.F. Jenkins, P.S. Schnitker, J.W. Elwell and M.H. Stutz (1993). Evaluation of analytical requirements associated with sites potentially contaminated with residues of high explosives. USA Cold Regions Research and Engineering Laboratory, Special Report 93-5.

Won, W.D., R.J. Heckly, D.G. Glover and J.C. Hoffsommer (1974) Metabolic disposition of 2,4,6-trinitrotoluene. *Applied Microbiology*, **27**: 513–516.

APPENDIX A: HOLDING-TIME STUDY RESULTS

Table A1. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well A, samples 48622 and 48635.

		Conc. fortified(µg/L)		Determined concentration (µg/L)						
Acidified?		TNB	TNT	HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
				Day 0						
No	pH = 7.1	0	0	134	365				12	5
Yes	pH = 2.2	0	0	135	359				11	5
No		30.0	24.5	133	375	26		24		
Yes		30.0	24.5	135	373	27		24		
				Day 7						
No		0	0	144	378				5	4
Yes		0	0	126	370				11	3
No				138	376	22		19	14	4
Yes				128	370	25		25	11	6
				Day 14						
No		0	0	135	372				14	4
Yes		0	0	135	385			4	13	3
No				137	373	17	1	21	14	3
Yes				132	367	26		26	10	4
				Day 21						
No		0	0	133	372			1	11	3
Yes		0	0	134	366			3	8	5
No				130	370	12	4	19	11	5
Yes				135	368	26		29	9	4
				Day 28						
No		0	0	133	377			2	11	2
Yes		0	0	133	373		5	9	5	
No				136	380	8	4	17	11	6
Yes				130	371	26		27	8	4

Table A2. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well B, samples 48725 and 48726.

		Conc. fortified(µg/L)		Determined concentration (µg/L)						
Acidified?		TNB	TNT	HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
				Day 0						
No	pH = 6.0	0	0	165	58				9	7
Yes	pH = 2.5	0	0	158	55				6	5
No		75.0	36.7	166	51	70		35	11	7
Yes		75.0	36.7	155	50	69		40	4	3
				Day 7						
No		0	0	156	57				10	7
Yes		0	0	150	56					
No				152	60	57	4	32	7	6
Yes				149	56	69		36		
				Day 14						
No		0	0	160	58				11	6
Yes		0	0	168	58			5		
No				159	55	53	5	34	11	5
Yes				163	55	72		39		
				Day 21						
No		0	0	157	56				11	9
Yes		0	0	160	55				6	
No				156	55	47	4	31	10	8
Yes				158	56	70		34		
				Day 28						
No		0	0	155	64				11	11
Yes		0	0	159	61					
No				156	62	44	7	30	11	10
Yes				177	70	78		40		

Table A3. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well C, samples 48727 and 48728.

Acidified?		Conc. fortified(µg/L) TNB	TNT	Determined concentration (µg/L) HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
			Day 0							
No	pH = 6.40	0	0	173	76		2	17	59	54
Yes	pH = 2.47	0	0	173	66		2	25	61	52
No		75.0	61.2	168	69	71	3	77	58	50
Yes		75.0	61.2	170	66	69	1	81	57	51
			Day 7							
No		0	0	164	72		4	14	59	53
Yes		0	0	170	71		3	21	58	53
No				170	85	67	4	75	58	56
Yes				166	69	68	3	79	57	51
			Day 14							
No		0	0	167	70		3	13	59	56
Yes		0	0	175	71		3	20	61	54
No				164	75	63	4	75	58	53
Yes				174	71	70	2	81	60	53
			Day 21							
No		0	0	172	69		4	10	60	53
Yes		0	0	163	65		2	21	56	52
No				169	70	62	4	73	63	54
Yes				176	68	72	2	81	59	53
			Day 28							
No		0	0	168	82	2	3	11	62	56
Yes		0	0	175	74		3	25	58	52
No				165	83	57	3	71	59	56
Yes				177	76	73	2	84	59	52

Table A4. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well D, samples 48731 and 48732

Acidified?		Conc. fortified(µg/L) TNB	TNT	Determined concentration (µg/L) HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
			Day 0							
No	pH = 6.3	0	0	252	157	5	7	110	47	65
Yes	pH = 2.5	0	0	247	155	4	6	115	35	54
No		75.0	49.0	243	148	74	8	563	45	61
Yes		75.0	49.0	245	144	73	3	565	36	51
			Day 7							
No		0	0	249	150	4	7	102	44	63
Yes		0	0	247	156	3	4	109	20	36
No				244	152	72	6	557	44	62
Yes				240	144	72	3	557	16	35
			Day 14							
No		0	0	258	161	5	7	109	47	64
Yes		0	0	255	158	3	2	115	18	33
No				252	155	75	9	573	50	65
Yes				252	157	76	3	581	19	30
			Day 21							
No		0	0	268	154	4	7	109	49	66
Yes		0	0	251	153	5	4	118	16	31
No				263	151	74	8	575	41	67
Yes				259	155	78	4	596	22	32
			Day 28							
No		0	0	255	161	4	7	109	48	65
Yes		0	0	283	175	5	2	123	18	33
No				255	161	74	9	568	46	66
Yes				252	154	74	3	576	16	30

Table A5. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well E, samples 48743 and 48744.

Acidified?		Conc. fortified(µg/L) TNB	Conc. fortified(µg/L) TNT	Determined concentration (µg/L) HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
			Day 0							
No	pH = 4.49	0	0	112	608	8		180	10	8
Yes	pH = 2.22	0	0	101	555	3		170	9	8
No		75.0	24.5	107	601	80		405	10	6
Yes		75.0	24.5	99	550	73		399	11	8
			Day 7							
No		0	0	103	598	5		179	11	7
Yes		0	0	99	552	3		167	11	8
No				105	595	72		401	10	9
Yes				98	551	72		394	9	9
			Day 14							
No		0	0	112	624	5		183	10	8
Yes		0	0	105	587	3		175	11	9
No				111	612	73		411	9	7
Yes				102	575	74		409	11	7
			Day 21							
No		0	0	116	618	6	3	183	14	9
Yes		0	0	106	590	5	2	184	12	8
No				112	610	76	3	418	14	9
Yes				103	566	75	3	411	13	9
			Day 28							
No		0	0	114	627	4	3	178	14	10
Yes		0	0	108	590	3	2	178	11	8
No				114	619	73	2	416	14	12
Yes				107	586	76	1	414	12	7

Table A6. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well F, samples 48745 and 48746.

Acidified?		Conc. fortified (µg/L) TNB	Conc. fortified (µg/L) TNT	Determined concentration(µg/L) HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT	2,4-DNT
			Day 0								
No	pH = 5.78	0	0	325	102		7	14	51	40	
Yes	pH = 2.30	0	0	316	102		2	24	17	21	4
No		75.0	122.3	373	100	69	5	128	45	39	
Yes		75.0	122.3	314	97	70	1	135	17	20	3
			Day 7								
No		0	0	318	102		4	9	49	40	
Yes		0	0	306	103		7	18	13	18	4
No				316	101	50	7	122	46	40	
Yes				305	100	68	3	129	11	17	5
			Day 14								
No		0	0	328	104		5	5	47	39	
Yes		0	0	317	106		2	22	13	15	6
No				321	104	42	10	114	49	39	
Yes				323	109	72	4	140	11	17	4
			Day 21								
No		0	0	336	100		4	3	52	42	
Yes		0	0	333	108		4	19	15	21	4
No				325	102	37	11	115	54	45	
Yes				336	114	74	4	142	17	21	4
			Day 28								
No		0	0	331	108			5	53	9	
Yes		0	0	334	114		1	22	14	21	4
No				333	110	32	13	114	55	43	
Yes				322	111	73	3	145	15	21	4

Table A7. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well G, samples 48741 and 48742.

		Conc. fortified(µg/L)		Determined concentration (µg/L)						
Acidified?		TNB	TNT	HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
				Day 0						
No	pH = 3.69	0	0							
Yes	pH = 2.30	0	0							
No		75.0	36.7			67		33		
Yes		75.0	36.7			70		40		
				Day 7						
No		0	0							
Yes		0	0							
No						66		33		
Yes						70		38		
				Day 14						
No		0	0							
Yes		0	0							
No						66		36		
Yes						72		40		
				Day 21						
No		0	0							
Yes		0	0							
No						68		35		
Yes						74		41		
				Day 28						
No		0	0							
Yes		0	0							
No						65		35		
Yes						73		39		

Table A8. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well H, samples 48719 and 48720.

		Conc. fortified(µg/L)		Determined concentration (µg/L)						
Acidified?		TNB	TNT	HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
				Day 0						
No	pH = 4.50	0	0							
Yes	pH = 2.27	0	0							
No		75.0	36.7			68		32		
Yes		75.0	36.7			68		39		
				Day 7						
No		0	0							
Yes		0	0							
No						71		36		
Yes						69		33		
				Day 14						
No		0	0							
Yes		0	0							
No						68		32		
Yes						71		36		
				Day 21						
No		0	0							
Yes		0	0							
No						63		30		
Yes						69		35		
				Day 28						
No		0	0							
Yes		0	0							
No						64		34		
Yes						71		38		

Table A9. Results of holding-time study for groundwater samples from the Naval Surface Warfare Center, Crane, Indiana, well I, samples 48616 and 48629.

Acidified?		Conc. fortified(µg/L) TNB	Conc. fortified(µg/L) TNT	Determined concentration (µg/L) HMX	RDX	TNB	3,5-DNA	TNT	4ADNT	2ADNT
			Day 0							
No	pH = 7.10	0	0							
Yes	pH = 2.18	0	0							
No		30.0	24.5	65	82	28		23	4	
Yes		30.0	24.5	68	79	28		24		
			Day 7							
No		0	0							
Yes		0	0							
No				74	88	20		20		
Yes				69	84	25		23	3	
			Day 14							
No		0	0							
Yes		0	0							
No				67	83	14	3	19	3	
Yes				71	83	26		24	4	3
			Day 21							
No		0	0							
Yes		0	0							
No				67	83	6	3	14	3	2
Yes				69	82	27		24	2	3
			Day 28							
No		0	0							
Yes		0	0							
No				68	85	3	5	15	5	2
Yes				75	93	29		27	2	2

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13. ABSTRACT (*Maximum 200 words*)

This study was conducted to develop a method for stabilizing water samples to be analyzed for nitroaromatic and nitramine explosives using SW846 Method 8330. Several options were tested using river water fortified with 15 nitroaromatic, nitramine, and aminonitroaromatic analytes. Acidification to pH 2 using sodium bisulfate was selected based on its ability to retard microbiological and chemical transformations, its ease of use under field conditions, and its usability with both the direct and preconcentration procedures in Method 8330. Holding-time studies were performed over a 64-day storage period using fortified river water and groundwaters with and without chemical stabilization. Nonacidified samples showed rapid loss of tetryl, TNB, and TNT and slower loss of the dinitroaromatics. These losses were accompanied by increasing concentrations of transformation products. Losses of these nitroaromatics were completely eliminated by acidification to pH 2. Nitramines were stable over the entire period whether samples were acidified or not. A small loss of the aminodinitroaromatics was observed for both acidified and unacidified samples. The rate of loss for acidified samples was initially greater than for nonacidified samples. Sample acidification caused no adverse effects on SW846 Method 8330, although samples to be preconcentrated using salting-out solvent extraction should be neutralized prior to extraction to prevent additional loss of aminodinitroaromatics.

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