SPECIAL REPORT 93-11

Experimental Assessment of Analytical Holding Times for Nitroaromatic and Nitramine Explosives in Soil

Clarence L. Grant, Thomas F. Jenkins and Susan M. Golden

June 1993

Abstract

A study was conducted to experimentally evaluate the maximum acceptable preextraction analytical holding times (MHTs) for three nitroaromatic compounds and two nitramines in soil. Three spiked soils and a field-contaminated soil were utilized in the study. Analytes investigated were HMX, RDX, TNB, TNT and 2,4–DNT, all at the low µg/g level. Subsamples of each soil were extracted with acetonitrile in an ultrasonic bath after being held for periods of 0, 3, 7, 14, 28 and 56 days at either room temperature (22°C), under refrigeration (2°C) or frozen (–15°C). Extracts were analyzed by RP-HPLC. The two nitramines, HMX and RDX, were stable over the entire period for all soils under all storage temperatures. For the three nitroaromatics (TNB, TNT and 2,4–DNT) the results were very different, in that all three analytes rapidly degraded in spiked soils at room temperature, more slowly degraded under refrigerator temperature and remained quite stable when frozen. Of the three, TNB degraded most rapidly, followed by TNT and 2,4-DNT. The degradation at room temperature and in the refrigerator was much faster for one soil than for the others. Even when frozen there was a small loss of 2,4–DNT in the soil showing the most rapid degradation. For the field-contaminated soil, the nitroaromatics were much more stable, even at room temperature, although some degradation occurred. Because of the large stability difference between fortified and field- contaminated soils, the efficacy of using fortified soils to estimate MHTs is discussed. The recommended MHT for soils containing only nitramines is eight weeks under refrigeration. When nitroaromatics are present, refrigeration is inadequate and soils should be frozen to preserve analyte integrity. When frozen, an MHT of eight weeks is recommended.

For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380-89a, *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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US Army Corps of Engineers
Cold Regions Research & Engineering Laboratory

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PREFACE

This report was prepared by Dr. Clarence L. Grant, Professor Emeritus, University of New Hampshire, and Dr. Thomas F. Jenkins, Research Chemist, Geological Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory, and Susan M. Golden, Chemist, Science and Technology Corporation. Funding was provided by the U.S. Army Environmental Center (formerly the U.S. Army Toxic and Hazardous Materials Agency), Aberdeen Proving Ground, Maryland, Martin H. Stutz, Project Monitor.

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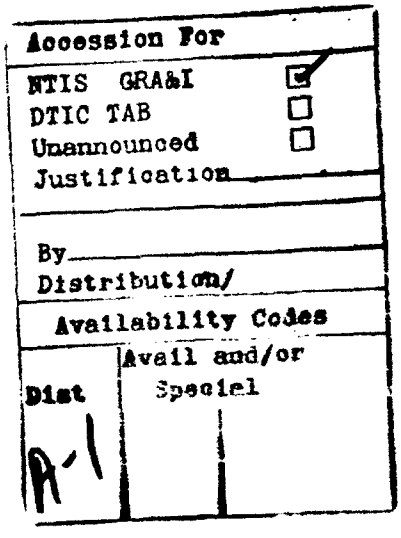

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Experimental Assessment of Analytical Holding Times for Nitroaromatic and Nitramine Explosives in Soil

CLARENCE L. GRANT, THOMAS F. JENKINS AND SUSAN M. GOLDEN

INTRODUCTION

Several years ago, CRREL developed a laboratory method for the determination of nitroaromatic and nitramine explosives in soil (Jenkins et al. 1989). This method was collaboratively tested (Bauer et al. 1990) and subsequently accepted by the American Society for Testing and Materials (ASTM 1991), the Association of Official Analytical Chemists (AOAC 1990) and the Environmental Protection Agency (EPA 1992) as a standard laboratory method for this determination.

One criterion that was not experimentally evaluated during this method development process was an acceptable preextraction sample holding time. Lacking available experimental data, the EPA method established a preextraction holding time of seven days for soil in SW846 Method 8330 (EPA 1992). This holding time was chosen to be consistent with those for other organics in a soil matrix and for contractual compliance.

Subsequently, the U.S. Army Environmental Center (USAEC) (formerly the Toxic and Hazardous Materials Agency), the U.S. EPA and the U.S. Navy jointly funded Oak Ridge National Laboratory to conduct an experimental study to recommend appropriate maximum preextraction holding times (MHTs) for soils contaminated with nitroaromatic and nitramine explosives (Maskarinec et al. 1991). In this study replicate 2-g aliquots of three different soils were placed in 40-mL glass vials and, three days before fortification, the soils were wetted with 0.5 mL of reagent grade water. This was done to allow bacterial activity to come to a steady state prior to fortification with the explosives. On the day the study began, each subsample was spiked with a 0.5-mL aliquot of each individual explosive stock solution. These stock solutions were in an acetonitrile matrix (Maskarinec et al. 1991) and since four different analytes were studied, a total of 2 mL of acetonitrile was added to each 2-g portion of soil. The spiked soils were then vortex mixed for 30 seconds and stored at room temperature (+20°C), refrigerator temperature (+4°C) or freezer temperature (–20°C) for eight time periods ranging up to 365 days. Quadruplicate subsamples for each combination of soil type and storage temperature were analyzed at each time period and the resulting concentrations of each analyte plotted as a function of holding time. While the effect of this large amount of acetonitrile on the soil biota is unknown, storage of soils under acetonitrile does not mimic the manner in which normal soil samples are stored prior to analysis for nitroaromatics and nitramines. In fact, acetonitrile is the extraction solvent of choice for analysis of soils for these analytes (Jenkins et al. 1989). In summary, while the Maskarinec et al. (1991) study seems to be carefully done and the statistical treatment of the data is extensive, we feel it suffers a flaw because of the use of acetonitrile for fortification and the resulting MHT estimates may not be appropriate for customary soil sample storage procedures.

We also have concerns with the data treatment. MHTs were estimated using two definitions: a modified version of an ASTM procedure (1986) and one reported by Prentice et al. (1986). In ASTM, MHT is defined as the "maximum period of time during which a properly preserved sample can be stored before such degradation of the constituent of interest occurs or change in sample matrix occurs that the systematic error exceeds the 99% confidence interval (not to exceed 15%) of the test about the mean concentration found at zero time." The zero time mean concentration and standard deviation are estimated from an appropriate number of samples (usually 10) analyzed immediately after collection. If an analyte concentration is less than one order of magnitude higher than the crite-

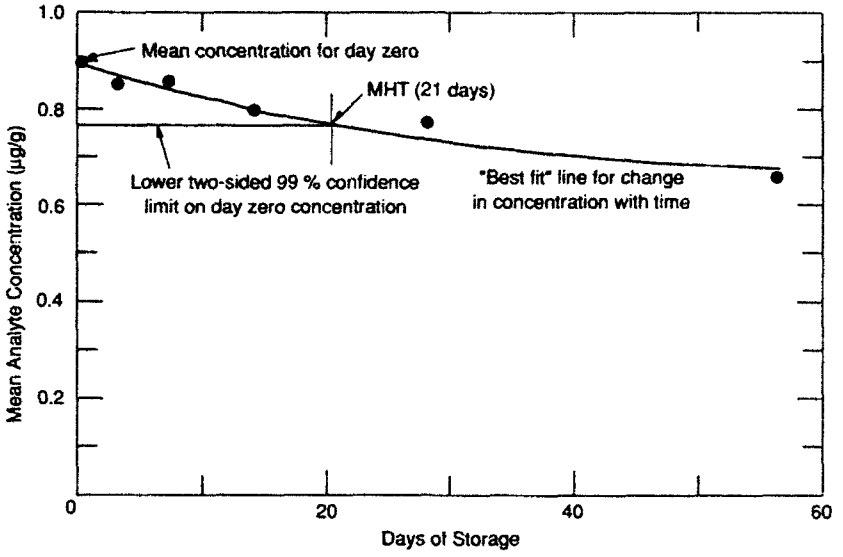

Figure 1. Illustration of ASTM method for estimating maximum holding time.

rion of detection, a bulk sample is fortified and the zero time mean and standard deviation are redetermined. Concentrations are then measured after various time intervals using a number of replicates calculated from the percent relative standard deviation (RSD) of the zero time results. The average concentration found at each analysis point is plotted vs. time on linear graph paper and the "best graphical fit" to the data points is drawn. A MHT is the point where the "best fit" line intersects the two-sided 99% confidence interval about the zero time mean. Figure 1 is an illustration of this definition using a hypothetical example. Note that the number of replicates used in the confidence interval calculation is the number used for each time interval measurement rather than the 10 replicates used to estimate the zero time mean.

According to Maskarinec et al. (1991), their "working definition differed slightly from the exact ASTM definition...." We believe it differs greatly. Their data are fitted via least squares to linear zero-order or first-order kinetic models or, in some

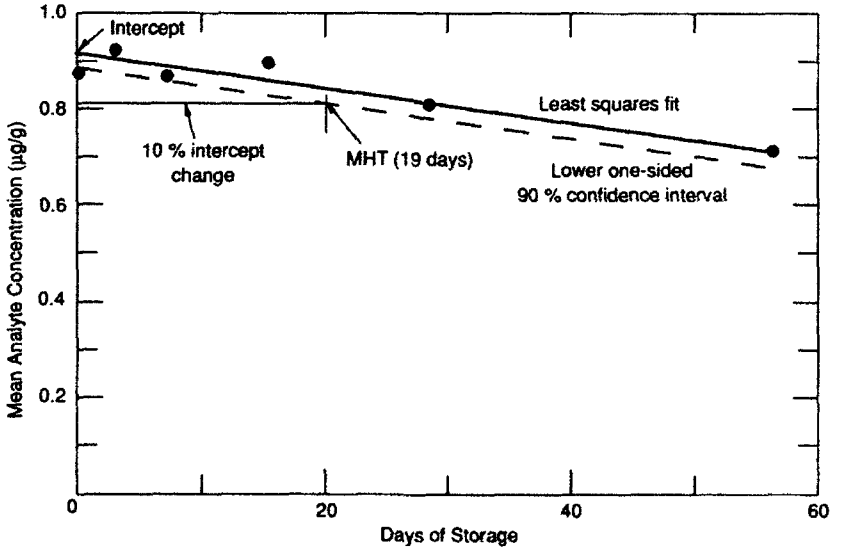

Figure 2. Illustration of ESE method for estimating maximum holding time.

cases, to other models such as a cubic spline. The zero time concentration is estimated as the intercept of the fitted model rather than the zero time mean, and the 99% confidence interval on the intercept is calculated using the standard deviation of the intercept. We are not passing judgment on the appropriateness of the Maskarinec et al. (1991) procedure, but it should be clearly understood that it differs markedly from the ASTM method. In some cases, the intercept differs substantially from the day zero mean and the standard deviation of the intercept differs greatly from the standard deviation of the day zero results.

The second method (ESE method) of estimating MHTs (after Prentice et al. 1986) is defined as the time when a one-sided 90% confidence interval on the concentration predicted by the least squares model selected to represent the concentration vs. time data falls below a 10% change in the intercept of the model. This definition is illustrated in Figure 2, again using a hypothetical example. When a linear model is used, the slope is tested to see if it differs significantly from zero. If it doesn't, the MHT is the longest time tested. Based on statistical considerations, Maskarinec et al. (1991) found that there was not much consistency in the pattern of models chosen. Often three different models were chosen for the same soil when tested at different storage temperatures but a given temperature pattern did not hold for other soils or even for the same soil at a different analyte concentration. For high concentrations of explosives in soils the Prentice et al. method gave MHT estimates that were always longer (up to 2.5 times longer) than corresponding estimates using the modified ASTM definition. However, for low explosives concentrations the modified ASTM definition sometimes gave longer estimates by as much as a factor of 6, although the trend was not consistent.

Because of these very large inconsistencies, Maskarinec et al. (1991) had to interpret their results very conservatively. Briefly, they recommended storage of RDX, HMX, and 2,4–DNT contaminated soils at 4°C (refrigerator) with a MHT of six weeks and TNT contaminated soils at –20°C (freezer) also with a MHT of six weeks.

In the following study, we re-examine the issue of MHTs for explosives-contaminated soils with emphasis on 1) avoidance of organic solvent addition during soil fortification, 2) alternative approaches to data analysis/interpretation, and 3) comparison of stability of fortified soils to a field-contaminated soil.

EXPERIMENTAL

Chemicals

All standards and test solutions were prepared from Standard Analytical Reference Materials (SARMs) obtained from the USAEC. Aqueous standards and test solutions were prepared in reagent grade water obtained from a Milli-Q Type I Reagent Grade Water System (Millipore Corp.). Methanol used in the preparation of HPLC eluent and acetonitrile used for soil extraction were HPLC grade from Alltech and Baker, respectively. Eluent was prepared by combining equal volumes of methanol and water and vacuum filtering through a nylon membrane (0.45 µm) to degas and remove particulate matter.

Analyte spiking solutions

All analyte spiking solutions were prepared in water. SARMs for 2,4,6–trinitrotoluene (TNT), 2,4–dinitrotoluene (2,4–DNT), 1,3,5–trinitrobenzene (TNB), 1,3,5–hexahydro–1,3,5–trinitrotriazine (RDX) and 1,3,5,7–octahydro–1,3,5,7–tetranitrotetrazocine (HMX) were placed in individual brown glass jugs, reagent grade water was added, and the contents were stirred 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 diluted 1:1 with reagent grade water prior to analysis (Jenkins et al. 1986, EPA 1992). A multianalyte spiking solution was prepared by combining appropriate volumes of these individual analyte solutions and filtering through a 0.2-µm nylon membrane. The combined analyte spike solution was stored in a brown glass bottle in the refrigerator until used.

Soils

Blank test soils were obtained locally from Vermont (Windsor), New Hampshire (Charlton) and New York (Ft. Edwards). These soils were air dried, ground with a mortar and pestle and passed through a 30-mesh sieve (590 µm). Some physical and chemical properties of these soils are presented in Table 1. Replicate 5.0 ± 0.1-g subsamples of each blank soil were placed in individual 20-mL glass scintillation vials.

A field-contaminated soil was obtained from the Rockeye site at the Naval Surface Warfare

Table 1. Physical and chemical properties of test soils.

Property	Soil: Ft. Edwards clay	Windsor sandy loam	Charlton silty loam
pH	8.4	6.2	6.0
TOC (%)*	0.5	1.1	1.8
Clay (%)	70	30	20
CEC (meq/100 g)**	>150	3.5	7.3

* Total organic carbon
** Cation exchange capacity

Center, Crane, Indiana, courtesy of Karen Myers from the U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi. This soil contained measurable concentrations of HMX, RDX, TNT, TNB, two isomeric microbiological transformation products of TNT (McCormick et al. 1976, Walsh 1990), 2–amino–4,6–dinitrotoluene (2–Am-DNT) and 4–amino–2,6–dinitrotoluene (4–Am-DNT) and 3,5–dinitroaniline (3,5–DNA), which is a microbiological transformation product of TNB. This bulk soil was air dried, ground with a mortar and pestle and sieved. Subsamples of this soil were weighed into separate glass scintillation vials in an identical manner as described above except that since less of this soil was available, only 2.00 ± 0.01 g subsamples were used.

Soil wetting and analyte spiking

Prior to the onset of the experiment, previously air-dried test soils were rewetted. Because the texture and water holding capacity of the various soils differed, the volume of water added to each soil was varied such that after spike additions were made, there was no evidence of free-standing water. For the three initially blank soils, 0.20 mL of reagent grade water was added to the Windsor sandy loam and 1.00 mL was added to the Ft. Edwards Clay and Charlton silty loam. For the field-contaminated soil from Crane, 0.50 mL of reagent grade water was added. After water addition, all soils were allowed to stand at room temperature in the dark for three days to allow microbiological activity to be reestablished (Maskarinec et al. 1991).

Fortification of the three initially blank soils was made by carefully adding 1.00 mL of a combined aqueous spiking solution with known concentrations of HMX, RDX, TNB, TNT and 2,4-DNT (Table 2) to each test vial. Except for the soils designated as "day zero exposure" and those to be stored frozen, the spiked soils were immediately placed in the appropriate storage temperature in the dark. The day zero samples and the samples to be frozen were permitted to stand for two hours after fortification to allow time for the analytes to interact with the soils prior to either extraction or freezing. The vials containing the field-contaminated soil were treated and stored in an identical manner as described above except that no fortification was made. An estimate of the initial analyte concentrations in the field-contaminated soil is also presented in Table 2.

Soil respiration

To ensure that the rewetted, fortified and field-contaminated soils had regained microbial activity, three vials of each soil were placed in separate 250-mL Erlenmeyer flasks enclosed with a two-hole rubber stopper. Air was slowly drawn through two aqueous NaOH scrubbers, through an Erlenmeyer containing a given soil and into a CO_2 collection tube containing standard aqueous NaOH. The CO_2 evolved from the soils was collected as carbonate over a period of two weeks and the carbonate level determined by back titration with 0.5 N HCl. The levels of CO_2 evolved are shown in Table 3.

Soil holding time test parameters

A summary of the test parameters used for the soil holding time study is presented in Table 4. For both the fortified and field-contaminated soils, three storage conditions were examined, room temperature (22 ± 2°C), refrigerator storage (2 ± 2°C) and

Table 2. Concentration of combined analyte spiking solution and initial analyte concentrations in test soils.

Analyte	Soil spiking Solution (mg/L)	Concentration Spiked Windsor, Ft. Edwards and Charlton (µg/g)*	Field contaminated Crane soil (µg/g)
HMX	1.48	0.30	2.60
RDX	6.68	1.33	0.44
TNB	4.92	0.98	0.83
TNT	5.06	1.01	2.32
2,4-DNT	4.13	0.83	—
4-Am-DNT	—	—	1.85
2-Am-DNT	—	—	1.18
3,5-DNA	—	—	0.67

* Calculated based on measured composition of spiking solution.

Table 3. Soil respiration measurements, 0–14 days at room temperature.

Soil	CO_2 evolved (mg evolved as C/g of soil)
Windsor sandy loam	0.93
Charlton silty loam	0.33
Ft. Edwards clay	0.48
Crane	0.31

Table 4. Experimental factors for soil holding time study.

Factors	No. of levels	Levels
		Fortified soils
Analytes	5	HMX,RDX,TNB,TNT,2,4-DNT
Soils	3	Ft. Edwards, Charlton, Windsor
Storage temp. (°C)	3	-15°, 2°, 22°
Storage time (days)	6	0,3,7,14,28,56
Replicates	3	a,b,c
		Field-contaminated soils
Analytes	7	HMX,RDX,TNB,TNT,2-Am-DNT,4-Am-DNT,3,5-DNA
Soils	1	Crane
Storage temp. (°C)	3	-15°, 2°, 22°
Storage time (days)	6	0,3,7,14,28,56
Replicates	3	a,b,c

freezer storage (–15 ± 2°C). Portions stored under these conditions were extracted after 0, 3, 7, 14, 28 and 56 days of storage and the analyte concentrations determined. Because of expected variability among subsamples, triplicate portions were analyzed for each storage temperature for each storage time.

Soil extraction

For soil extraction, the vials containing the soil were warmed to room temperature and 9.00 mL of acetonitrile added. The vials were vortex mixed for 1 minute and placed in a sonic bath for 18 hours. The temperature of the bath was maintained at less than 25°C with cooling water. The vials were then removed from the bath and allowed to stand undisturbed for 30 minutes. A 10.00-mL aliquot of aqueous $CaCl_2$ (5 g/L) was then added and the soil particles were allowed to flocculate for 30 minutes before a 5-mL aliquot of the supernatant was filtered through a 0.5 µm Millex SR filter.

This extraction procedure was based on the method developed by Jenkins et al. (1989) (SW846 Method 8330) with two differences. First the soils were not air dried prior to extraction, because it was judged that the time required to dry the soil in the vials at room temperature could result in analyte loss and confound the effect of the holding time temperatures. Second, a 5-g portion of soil was used for the fortified samples instead of the usual sample size of 2 g This was necessary because the solubility of HMX and RDX in the aqueous spiking solution is limited (4 mg/L and 42 mg/L, respectively) as was the moisture-holding capacity of the test soils. Thus to obtain sufficiently high extract concentrations of these analytes without exceeding the moisture-holding capacity of the soils, larger soil samples were required.

RP-HPLC analysis

All soil extracts were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC). Analysis was conducted on a modular system composed of a Spectra-Physics Model SP8800 ternary HPLC pump, a Spectra-Physics Spectra 100 UV variable wavelength detector set at 254 nm (cell path 1 cm), a Dynatech Model LC 241 auto sampler equipped with a Rheodyne Model 7125 sample loop injector, a Hewlett-Packard 3393A digital integrator and a Linear strip chart recorder.

All extracts were analyzed 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 (Jenkins et al. 1989). Samples were introduced by overfilling a 100-µL sampling loop. Retention times of the analytes of interest are shown in Table 5. Confirmation of identities of analytes and transformation products were obtained on a 25-cm × 4.6-mm (5-

Table 5. Retention times of test analytes and transformation products for two reversed-phase columns.

Compound	Retention time (min) LC–18	LC–CN
HMX	2.6	9.1
RDX	3.8	6.1
TNB	4.9	4.0
4-amino-2-nitrotoluene	5.1	3.7
2-amino-4-nitrotoluene	5.5	3.8
1,3-dinitrobenzene	6.0	3.9
3,5-dinitroaniline	6.8	5.0
TNT	7.8	4.9
4-amino-2,6-dinitrotoluene	8.7	5.3
2-amino-4,6-dinitrotoluene	9.0	5.6
2,4-DNT	9.4	4.7

μm) LC-CN column (Supelco) under the same operational conditions (Table 5).

Data analysis

The mean and standard deviation for each of 416 sets of triplicate measurements were calculated. Suspect individual measurements were flagged on the basis of extreme values of the % RSD (> 50%) and inconsistencies in the overall pattern for that compound. Each suspect value was checked for possible computation or transcription errors. Twelve individual extreme values (four for HMX, three for RDX, three for TNT and two for 2,4-DNT) with no assignable cause were arbitrarily excluded because they produced large distortions of both means and standard deviations. In no case was more than one datum excluded from a triplicate set. These exclusions amounted to less than 1% of the values.

A modified version of the ASTM procedure was used to estimate MHTs where appropriate. Due to time constraints and the small amount of field-contaminated soil available, triplicate measurements were used throughout. To gain degrees of freedom and to fairly represent precision for the entire experiment, pooled standard deviations were calculated for the six sets of triplicates for each soil/storage condition where rapid degradation was absent. This produced more degrees of freedom for the standard deviation than the nine that would have been obtained if we had been able to run ten replicates on day zero as suggested by ASTM. Where a 99% confidence interval exceeded ±15% of the day zero mean, the limits were set at ±15% as specified in the ASTM procedure. This procedure worked well for the fortified soils where standard deviations were small and the results should be very comparable to the standard ASTM procedure. For the field-contaminated soil, however, more replicates would have improved the results. The major weakness of this approach is the larger than desirable uncertainty in the day zero mean due to the small number of replicates.

Using the day zero values as true values, percent recoveries were calculated for each time period. Where substantial degradation was absent an estimate of the overall recovery was obtained by averaging across the five periods.

RESULTS AND DISCUSSION

Initial analyte concentrations

It is instructive to compare the day zero extractable analyte concentrations in the three fortified soils (Table 6) with the expected concentrations calculated from the multianalyte spiking solution (Table 2). Both HMX and RDX gave slightly higher extractable concentration estimates than expected in Windsor and Charlton soils, while the RDX value for Ft. Edwards was the expected one. The HMX concentration in Ft. Edwards soil could not be reliably estimated because of a large peak eluting very early that tails badly and causes serious quantitation problems for both HMX and RDX. We feel this peak results from a large number of colloidal particles in the extract of this high clay content soil. All three soils showed very similar 2,4-DNT concentrations, which were in good agreement with the expected value. The precision of these

Table 6. Initial concentration of nitroaromatics and nitramines in fortified and field-contaminated soils estimated by RP-HPLC. The fortified soils were extracted two hours after spiking solution was added.

	Mean soil concentration and relative standard deviation							
	Windsor		*Charlton*		*Ft. Edwards*		*Crane*	
Compound	$\bar{X}$ *(μg/g)*	*RSD (%)*	$\bar{X}$ *(μg/g)*	*RSD (%)*	$\bar{X}$ *(μg/g)*	*RSD (%)*	$\bar{X}$ *(μg/g)*	*RSD (%)*
HMX	0.37	0.7	0.39	2.6	a	a	2.60	37.4
RDX	1.50	0.5	1.62	2.4	1.33	6.4	0.44	12.4
TNB	0.91	0.4	0.82	1.8	0.57	26.1	0.83	26.9
TNT	0.97	0.5	0.98	2.1	0.60	15.0	2.32	15.1
4-Am-DNT	—	—	—	—	—	—	1.85	8.3
2-Am-DNT	—	—	—	—	—	—	1.18	8.7
2,4-DNT	0.85	0.3	0.86	2.4	0.88	1.5	—	—
3,5-DNA	—	—	—	—	—	—	0.67	12.3

a – Interference from colloidal particles from high clay content soil.

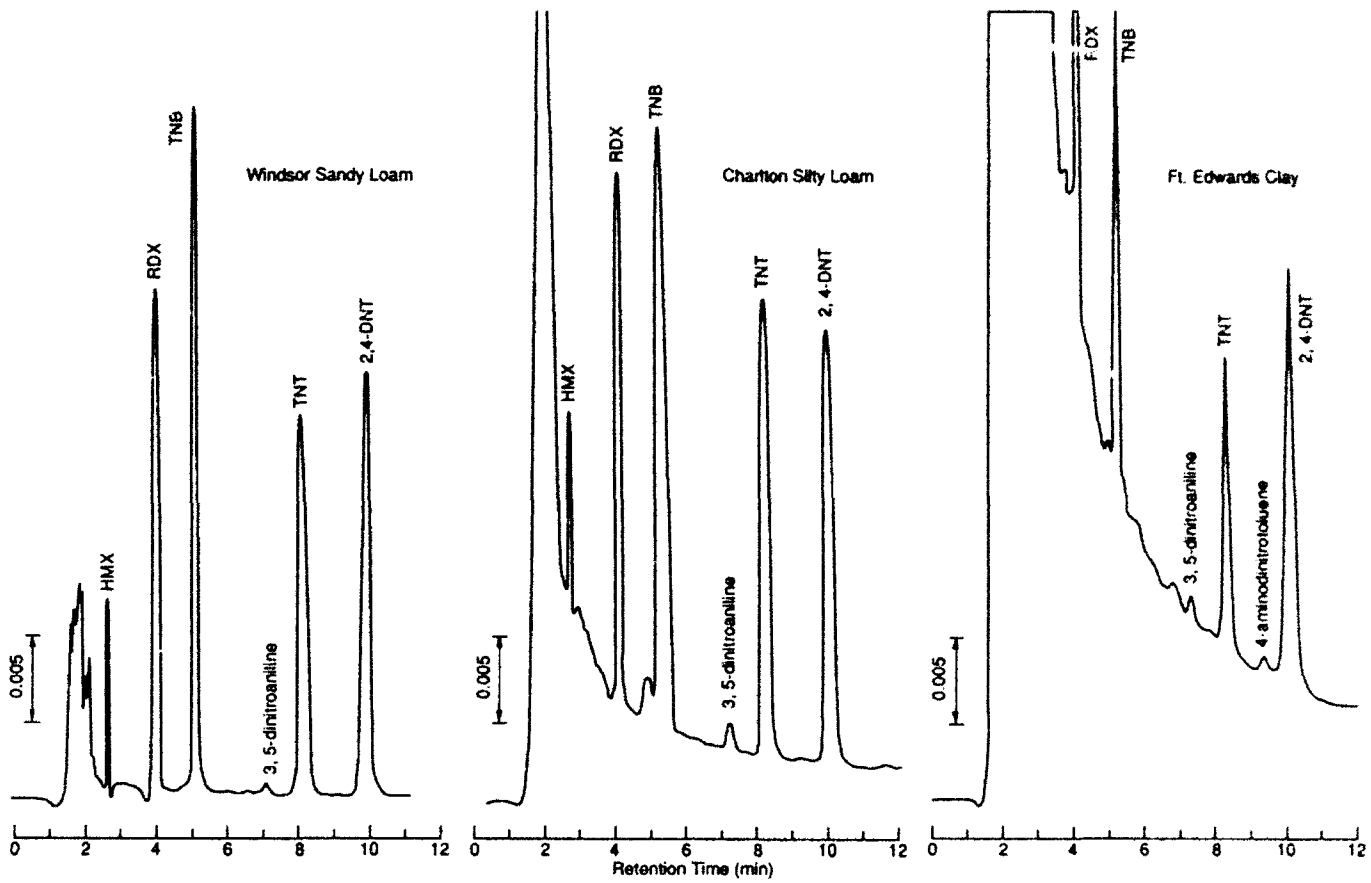

Figure 3. Chromatograms for soil extracts, day zero.

determinations was excellent; only RDX in Ft. Edwards soil had an RSD greater than 2.6%.

For TNB and TNT in Windsor and Charlton soils, the extractable concentration estimates were only moderately lower than the expected values and the RSDs were again very low. The Ft. Edwards soil, however, gave low recoveries of both TNB and TNT and the RSDs were much higher than for the other two soils. Two hypotheses that would explain low recoveries from Ft. Edwards soil are 1) TNB and TNT were bound in non-extractable forms during the two hours between spiking and extraction, and 2) TNB and TNT were partially degraded during that brief period. Experimental evidence indicates that the second hypothesis is the correct one. For the Windsor and Charlton soils (Fig. 3), no chromatographic peaks other than those for the five added analytes were observed in the day zero extracts except for a small peak corresponding to the retention time of 3,5–DNA and a background peak eluting just before TNB. This latter peak has often been observed in acetonitrile extracts of blank soils (Walsh et al. 1993). However, peaks corresponding to 4-Am-DNT and 2-Am-DNT are also present in day zero extracts from Ft. Edwards clay (Fig. 3) and the peak for 3,5-DNA is larger than observed for the Windsor and Charlton extracts. Since 3,5-DNA is a microbiological transformation product of TNB, and 4-Am-DNT and 2-Am-DNT are similarly derived from TNT, the most plausible explanation for their presence in the day zero extracts is as a consequence of TNB and TNT degradation during the two hours between spiking and extraction.

The concentrations of analytes in the Crane soil differ slightly from the fortified soils; HMX is a factor of seven higher, TNT is a factor of 2.5 higher, RDX is a factor of 3.5 lower, TNB is about the same, and concentrations of 3,5-DNA, 4-Am-DNT and 2-Am-DNT range from 0.64 to 1.85 µg/g. The concentration of 2,4-DNT in the Crane soil was too low to accurately quantify. Relative standard deviations for the analytes in the Crane soil range from 8.3% for 4-Am-DNT to 37.4% for HMX, indicating that attempts to homogenize the soil prior to subsampling were not completely successful. This

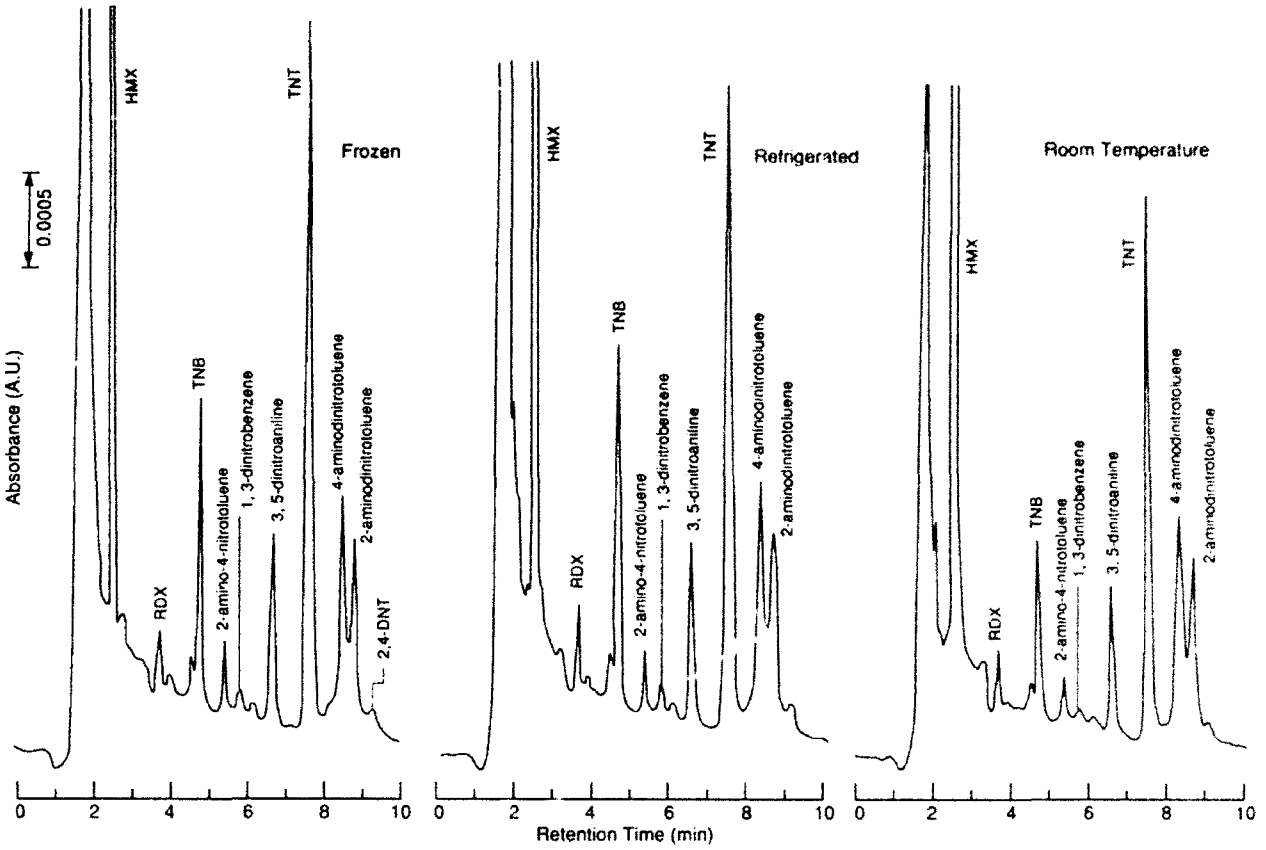

Figure 4. Chromatograms for extracts of Crane soil after seven days of soil storage at different temperatures.

condition is not unusual for field-contaminated soils. Further, the limited amount of soil available made it necessary to use 2.0-g test samples and this small size undoubtedly contributed to the poor precision by increasing the sampling error.

Chromatograms for the extracts from the Crane soil also reveal small peaks corresponding to the presence of 1,3-dinitrobenzene (1,3-DNB) and 2-amino-4-nitrotoluene (Fig. 4). The identification of this variety of nitroaromatics and nitramines in the field-contaminated soil from Crane is consistent with what has been reported elsewhere (Walsh and Jenkins 1992, Walsh et al. 1993) although the identification of 2-amino-4-nitrotoluene has not been previously reported. The reason for the presence of these compounds in soils initially contaminated with production grade TNT and RDX wastes is discussed elsewhere (Walsh 1990, Walsh and Jenkins 1992, Walsh et al. 1993).

Since the stability of these chemicals in the fortified soils and the field-contaminated soils was found to be quite different, the two cases are discussed separately.

Behavior of analytes in fortified soil as a function of holding time

The mean concentrations of the five fortified analytes and three transformation products are presented in Tables 7–9 as a function of holding time and storage condition for the Windsor, Charlton and Ft. Edwards soils, respectively. Of the five fortified analytes, TNB shows the most rapid rate of degradation. For all three soils TNB concentration rapidly decreases at room temperature with only an average of 6.5% remaining in these soils after three days. This result reinforces our conclusion that the low initial value found for TNB in the Ft. Edwards clay was due to degradation in the first two hours of exposure. For refrigerator storage, the rate of disappearance of TNB is slower than at room temperature, but even so, only an average of 15.3% remains after 7 days. Further reduction of TNB occurs by 14 days, and by 28 days the concentration of TNB is below its detection limit. This disappearance is accompanied by the appearance of an increased level of 3,5-DNA, the expected initial microbiological transformation (re-

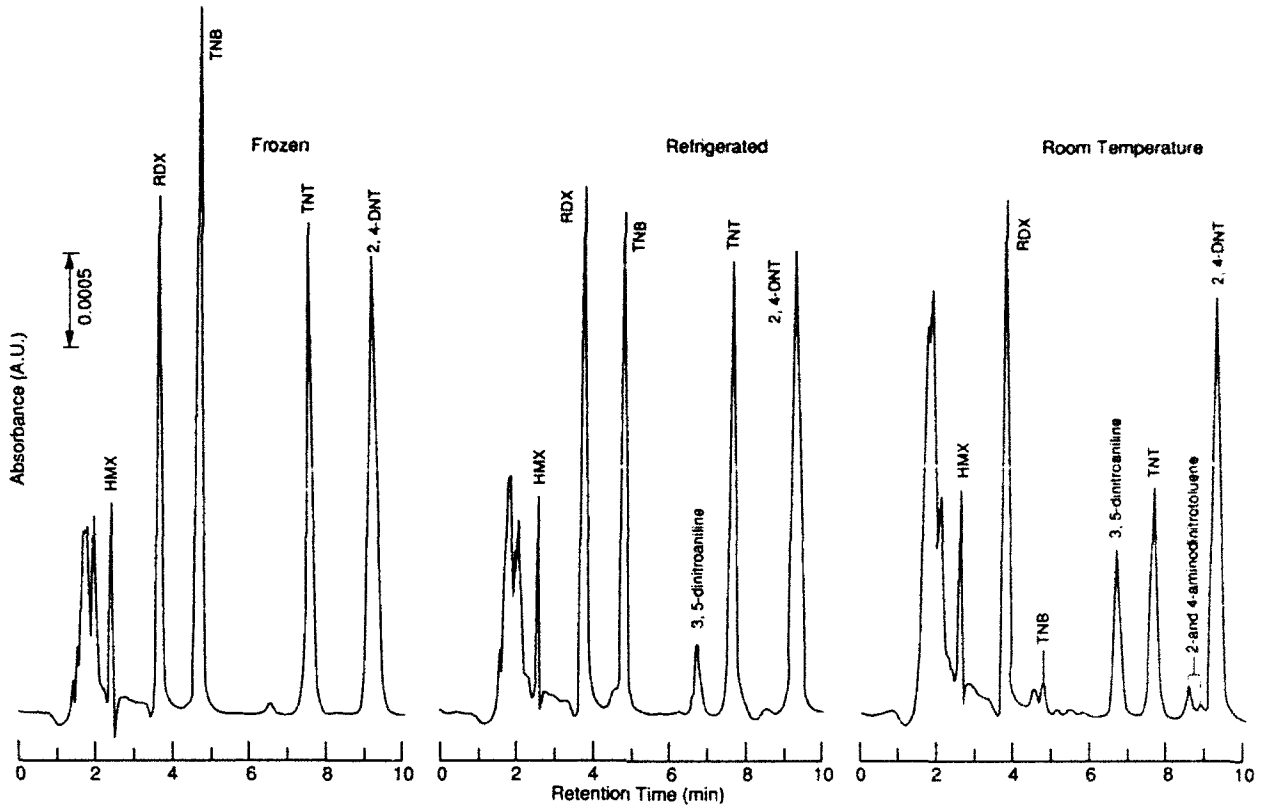

Figure 5. Chromatograms for extracts of Windsor soil after three days of soil storage at different temperatures.

duction) product (McCormick et al. 1976). These changes can be seen in four chromatograms for the Windsor soil (Fig. 5–8) and they are summarized for all three soils at refrigerator storage in Figure 9. On a molar basis, a maximum of 36%, 47% and 15% of the TNB lost could be accounted for as 3,5-DNA for the Windsor, Charlton and Ft. Edwards soils, respectively. It is also interesting to note the slow decrease in 3,5-DNA concentration in all three soils once the TNB precursor is gone. Clearly this is a very dynamic system even under refrigeration.

In contrast to the rapid degradation found at room temperature and under refrigeration, TNB is quite stable in the frozen soils (Tables 7–9). According to our modified ASTM test, TNB is stable for the entire 56-day test period in Windsor soil. In Charlton soil, TNB concentration does rise slightly above the upper 99% confidence interval after 14 days of storage. This finding is due to a very small pooled standard deviation for this data set, and it is of no practical importance because the concentration change is still only 7.6% after 56 days. The greatest change occurred in Ft. Edwards soil. After 28 days the concentration decreased to the lower 99% confidence interval representing a 15% decrease, but it was no lower after 56 days. When estimates from the five storage times were averaged and compared to day zero estimates, the mean percent recovery of TNB for freezer storage of the three fortified soils was 99.3% and the average for the 56-day test was 98.6%. Given the considerable variability in texture among these soils, and the unavoidable daily calibration error, the overall mean recovery is surprisingly close to 100%.

The behavior of TNT in these fortified soils parallels that of TNB except that the rate of disappearance is reduced. The expected transformation products, 2- and 4-Am-DNT (McCormick et al. 1976), are observed to increase as TNT concentrations decline. The rate of loss of TNT varies from soil to soil in the following order: Ft. Edwards > Charlton > Windsor, the same order that was found for TNB. However, the difference between Charlton and Windsor was very small. The rapid loss of TNT for the room temperature storage condition parallels that observed by Maskarinec et al. (1991), in

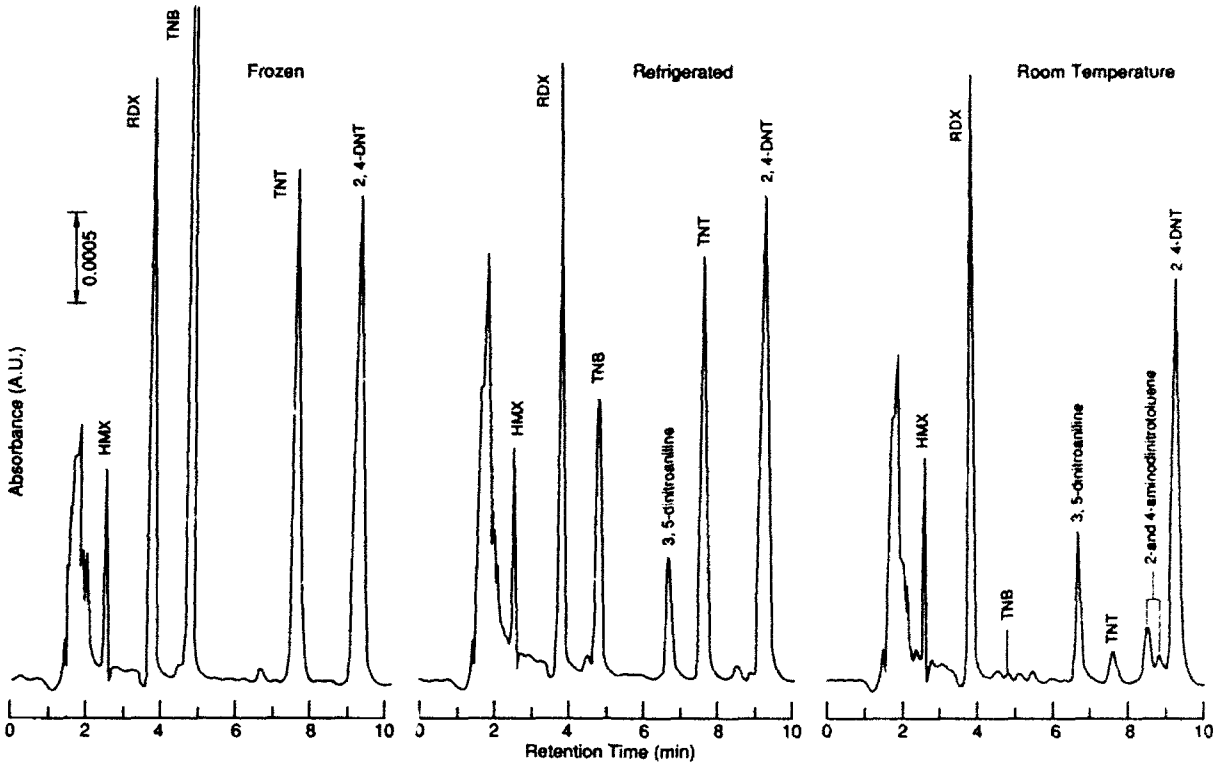

Figure 6. Chromatograms for extracts of Windsor soil after seven days of soil storage at different temperatures.

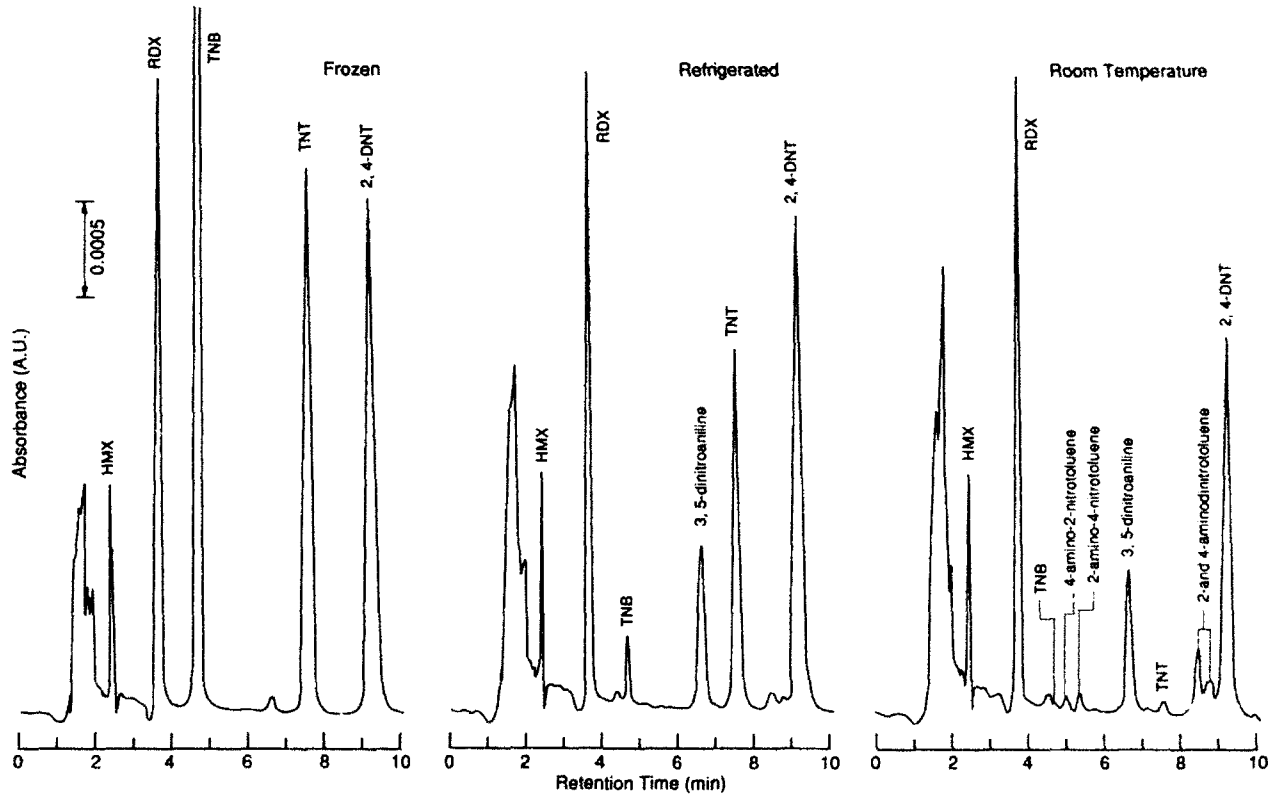

Figure 7. Chromatograms for extracts of Windsor soil after 14 days of soil storage at different temperatures.

Table 7. Concentrations of analytes and transformation products as a function of holding time and storage condition, Windsor sandy loam.

		Mean concentration (μg/g) ± standard deviation (μg/g) *Holding time*											
		00 Days		*03 Days*		*07 Days*		*14 Days*		*28 Days*		*56 Days*	
Compound	*Storage*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*
HMX	Room. temp.	0.373	0.003	0.353	0.007	0.385	0.012	0.377	0.001	0.392	0.009	0.349	0.008
	Refrigerator	0.373	0.003	0.360	0.004	0.375	0.004	0.381	0.009	0.379	0.004	0.350	0.009
	Freezer	0.373	0.003	0.354	0.003	0.377	0.011	0.377	0.005	0.399	0.018	0.354	0.008
RDX	Room. temp.	1.500	0.007	1.355	0.019	1.608	0.005	1.568	0.003	1.622	0.015	1.572	0.016
	Refrigerator	1.500	0.007	1.374	0.006	1.612	0.009	1.590	0.015	1.597	0.006	1.570	0.046
	Freezer	1.500	0.007	1.368	0.008	1.600	0.023	1.575	0.004	1.633	0.025	1.586	0.010
TNB	Room. temp.	0.914	0.004	0.013	0.023								
	Refrigerator	0.914	0.004	0.598	0.020	0.300	0.030	0.090	0.027			0.013	0.001
	Freezer	0.914	0.004	0.885	0.008	0.946	0.037	0.952	0.001	0.937	0.054	0.949	0.010
3,5-DNA	Room. temp.			0.277	0.007	0.274	0.016	0.238	0.007	0.191	0.008	0.127	0.009
	Refrigerator			0.116	0.005	0.220	0.013	0.283	0.003	0.277	0.007	0.255	0.022
	Freezer									0.014	0.024	0.028	0.001
TNT	Room. temp.	0.969	0.005	0.465	0.030	0.067	0.010						
	Refrigerator	0.969	0.005	0.861	0.005	0.777	0.013	0.637	0.043	0.309	0.026	0.086	0.017
	Freezer	0.969	0.005	0.926	0.006	0.975	0.026	0.978	0.003	0.980	0.024	0.954	0.010
4-Am-DNT	Room. temp.			0.109	0.005	0.202	0.006	0.215	0.005	0.211	0.010	0.169	0.004
	Refrigerator			0.025	0.002	0.041	0.006	0.067	0.010	0.124	0.002	0.169	0.003
	Freezer			0.004	0.003								
2-Am-DNT	Room. temp.			0.037	0.002	0.074	0.004	0.088	0.002	0.092	0.000	0.079	0.003
	Refrigerator			0.010	0.004	0.016	0.004	0.031	0.002	0.051	0.002	0.065	0.018
	Freezer											0.004	0.007
2,4-DNT	Room. temp.	0.850	0.002	0.741	0.016	0.716	0.021	0.626	0.006	0.573	0.004	0.419	0.020
	Refrigerator	0.850	0.002	0.802	0.004	0.837	0.007	0.828	0.013	0.772	0.005	0.675	0.015
	Freezer	0.850	0.002	0.799	0.006	0.863	0.020	0.856	0.005	0.857	0.017	0.808	0.007

their holding time study, and also by Pennington and Patrick (1990) and Cragin et al. (1985) (Fig.10) for their low concentration spiked soils. Our results are quite different from those found by Maskarinec for refrigerated storage, however. We found that for seven days of storage, the concentrations of TNT remaining were only 80%, 72% and 0%, respectively, for Windsor, Charlton and Ft. Edwards while Maskarinec et al. (1991) found no significant TNT loss until after day 7 for the three soils studied. The accumulation of TNT biodegradation products is shown in Figure 11, where the sums of 2- and 4-Am-DNT are plotted along with TNT concentrations. In contrast to 3,5-DNA, the Am-DNT concentrations continue to increase throughout the storage period, albeit at a slow rate after 28 days.

When soils were frozen our modified ASTM criterion showed no significant change for TNT in Windsor or Charlton soils during the 56-day test period. With Ft. Edwards soil the TNT concentration reached the lower 99% confidence interval (15% change) after about 20 days. However, the total decrease was still only 16.1% after 56 days. When averaged across the five storage times and three soils, the mean percent recovery of TNT for freezer storage was 95.6% of the day zero concentration and the average for the 56 day test was 94.4%.

The stability of 2,4-DNT in these fortified soils is much greater than that of either TNB or TNT. At room temperature an average of 68.4% remained after three days. This increased stability of 2,4-DNT relative to TNT at room temperature agrees with the results of Maskarinec et al. (1991). Under refrigeration an average of 86% remained after seven days of storage. A slow rate of loss continued throughout the study and, by 14 days, peaks corresponding to the expected reduction products,

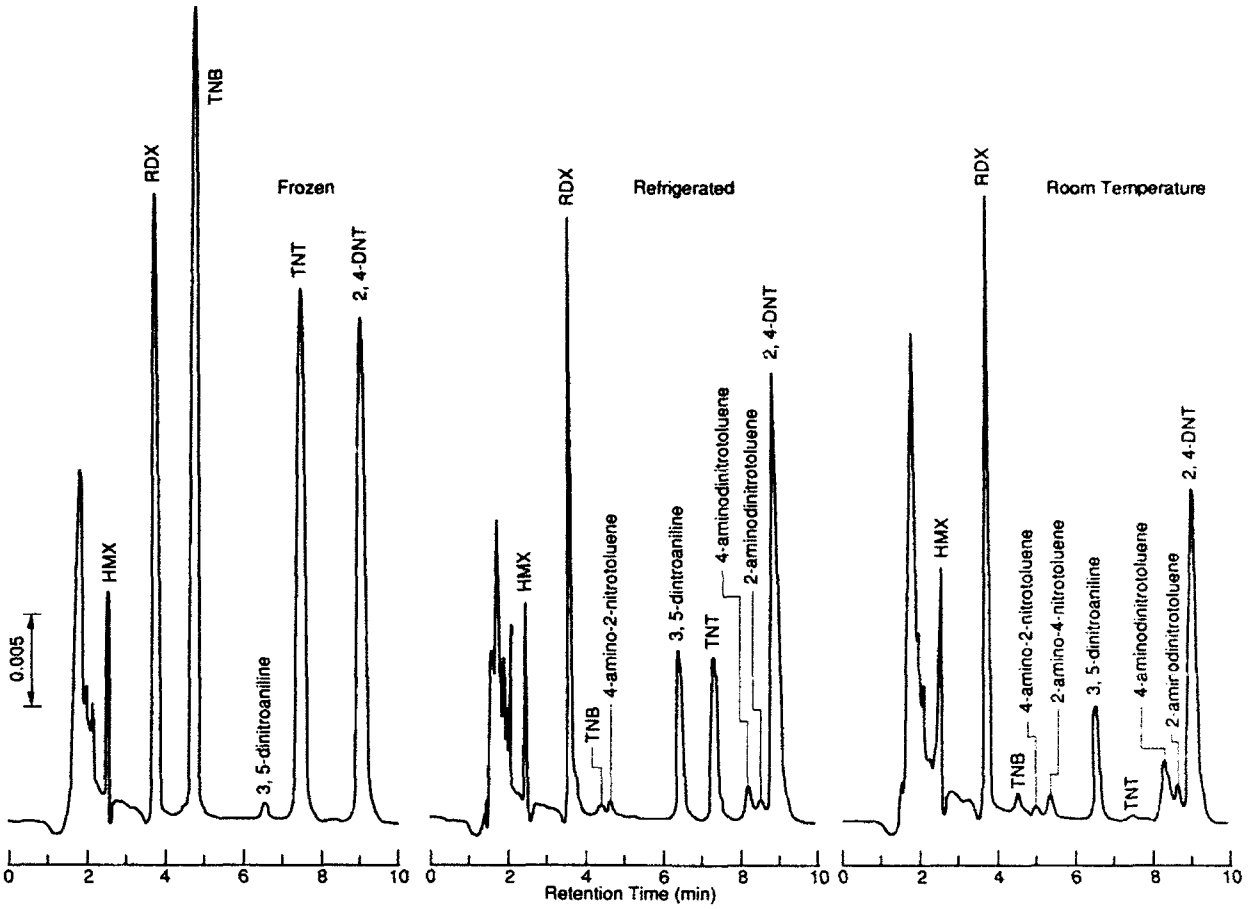

Figure 8. Chromatograms for extracts of Windsor soil after 28 days of soil storage at different temperatures.

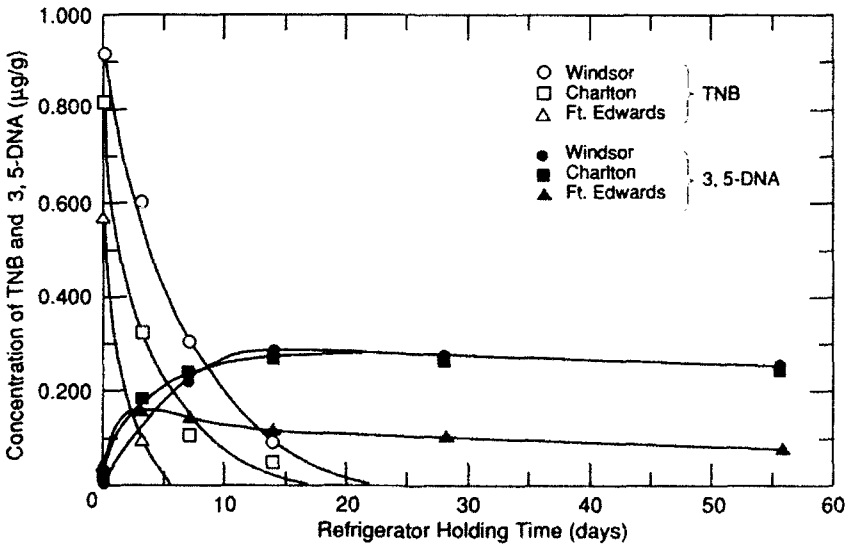

Figure 9. Refrigerator storage effects on TNB for three fortified soils.

Table 8. Concentrations of analytes and transformation products as a function of holding time and storage condition, Charlton silty loam.

		Mean concentration (μg/g) ± standard deviation (μg/g) Holding time											
		00 Days		*03 Days*		*07 Days*		*14 Days*		*28 Days*		*56 Days*	
Compound	*Storage*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*
HMX	Room. temp.	0.387	0.010	0.414	0.032	0.389	0.026	0.399	0.027	0.378	0.009	0.358	0.008
	Refrigerator	0.387	0.010	0.400	0.006	0.369	0.013	0.403	0.005	0.387	0.017	0.363	0.009
	Freezer	0.387	0.010	0.389	0.010	0.391	0.006	0.409	0.016	0.390	0.003	0.371	0.013
RDX	Room. temp.	1.618	0.038	1.448	0.025	1.604	0.043	1.570	0.004	1.500	0.058	1.349	0.051
	Refrigerator	1.618	0.038	1.475	0.013	1.588	0.069	1.672	0.026	1.654	0.017	1.542	0.128
	Freezer	1.618	0.038	1.439	0.064	1.666	0.010	1.682	0.021	1.633	0.041	1.668	0.028
TNB	Room. temp.	0.817	0.014	0.119	0.036	0.059	0.004						
	Refrigerator	0.817	0.014	0.320	0.030	0.108	0.001	0.054	0.003			0.013	0.012
	Freezer	0.817	0.014	0.820	0.034	0.854	0.012	0.884	0.012	0.833	0.020	0.879	0.029
3,5-DNA	Room. temp.	0.016	0.028	0.282	0.005	0.278	0.008	0.258	0.016	0.218	0.005	0.166	0.006
	Refrigerator	0.016	0.028	0.178	0.006	0.224	0.011	0.270	0.008	0.274	0.002	0.252	0.017
	Freezer	0.016	0.028			0.014	0.024					0.044	0.004
TNT	Room. temp.	0.977	0.021	0.437	0.028	0.190	0.006	0.072	0.008			0.008	0.007
	Refrigerator	0.977	0.021	0.876	0.018	0.702	0.029	0.601	0.014	0.372	0.021	0.225	0.005
	Freezer	0.977	0.021	0.940	0.041	0.963	0.014	0.993	0.018	0.944	0.037	0.984	0.026
4-Am-DNT	Room. temp.			0.130	0.007	0.175	0.010	0.190	0.009	0.179	0.009	0.132	0.010
	Refrigerator			0.021	0.005	0.041	0.004	0.077	0.009	0.111	0.004	0.135	0.010
	Freezer												
2-Am-DNT	Room. temp.			0.061	0.003	0.081	0.004	0.096	0.007	0.097	0.004	0.087	0.007
	Refrigerator			0.011	0.004	0.018	0.003	0.037	0.009	0.051	0.002	0.071	0.003
	Freezer												
2,4-DNT	Room. temp.	0.860	0.021	0.793	0.009	0.751	0.024	0.667	0.040	0.574	0.011	0.426	0.027
	Refrigerator	0.860	0.021	0.828	0.013	0.783	0.037	0.843	0.021	0.803	0.005	0.726	0.057
	Freezer	0.860	0.021	0.813	0.037	0.850	0.014	0.869	0.016	0.825	0.032	0.839	0.018

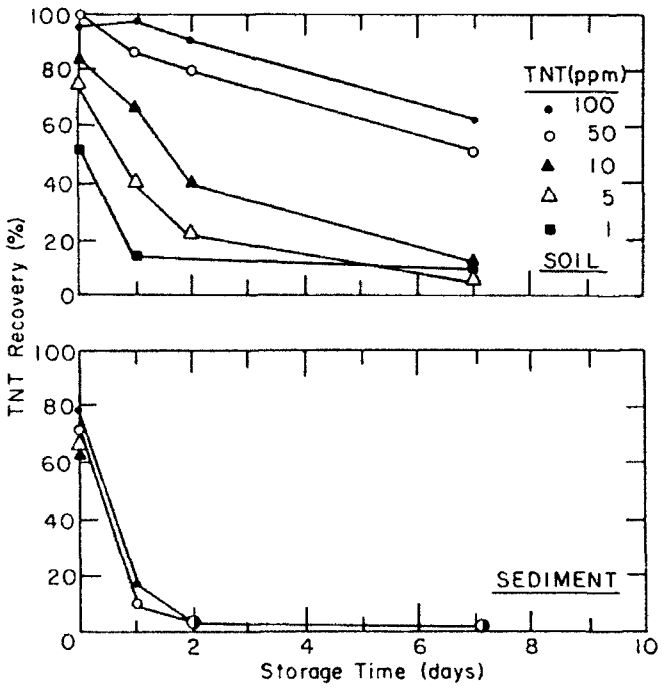

Figure 10. Time variation of recoveries of TNT from spiked Charlton soil and Kewanee sediment samples (from Cragin et al. 1985).

Table 9. Concentrations of analytes and transformation products as a function of holding time and storage condition, Ft. Edwards clay.

		Mean concentration (µg/g) ± standard deviation (µg/g)											
		Holding time											
		00 Days		*03 Days*		*07 Days*		*14 Days*		*28 Days*		*56 Days*	
Compound	*Storage*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*
HMX	Room. temp.												
	Refrigerator												
	Freezer												
RDX	Room. temp.	1.335	0.085	1.241	0.005	1.365	0.125	1.240	0.123	1.214	0.071	1.343	0.011
	Refrigerator	1.335	0.085	1.206	0.054	1.324	0.037	1.220	0.089	1.372	0.081	1.375	0.017
	Freezer	1.335	0.085	1.180	0.004	1.259	0.097	1.220	0.086	1.279	0.046	1.296	0.039
TNB	Room. temp.	0.566	0.148	0.020	0.034								
	Refrigerator	0.566	0.148	0.102	0.018								
	Freezer	0.566	0.148	0.609	0.310	0.477	0.221	0.538	0.108	0.480	0.041	0.477	0.081
3,5-DNA	Room. temp.	0.027	0.046	0.082	0.010	0.042	0.037					0.028	0.001
	Refrigerator	0.027	0.046	0.166	0.008	0.144	0.013	0.112	0.009	0.099	0.009	0.080	0.005
	Freezer	0.027	0.046	0.131	0.055	0.062	0.009	0.065	0.018	0.054	0.047	0.070	0.017
TNT	Room. temp.	0.596	0.089										
	Refrigerator	0.596	0.089	0.130	0.025								
	Freezer	0.596	0.089	0.530	0.015	0.479	0.215	0.553	0.077	0.504	0.007	0.500	0.060
4-Am-DNT	Room. temp.	0.110	0.035	0.205	0.034	0.143	0.018	0.109	0.006	0.086	0.024	0.056	0.021
	Refrigerator	0.110	0.035	0.226	0.031	0.208	0.008	0.174	0.019	0.223	0.008	0.187	0.023
	Freezer	0.110	0.035	0.210	0.054	0.087	0.013	0.106	0.027	0.106	0.030	0.135	0.036
2-Am-DNT	Room. temp.					0.049	0.017	0.027	0.010	0.029	0.006	0.032	0.004
	Refrigerator					0.044	0.005	0.028	0.012	0.052	0.006	0.117	0.005
	Freezer					0.011	0.010	0.004	0.006				
2,4-DNT	Room. temp.	0.875	0.013	0.226	0.063	0.194	0.024	0.113	0.025	0.060	0.052	0.047	0.005
	Refrigerator	0.875	0.013	0.768	0.024	0.586	0.057	0.426	0.050	0.391	0.036	0.315	0.017
	Freezer	0.875	0.013	0.840	0.006	0.719	0.152	0.783	0.090	0.749	0.062	0.697	0.010

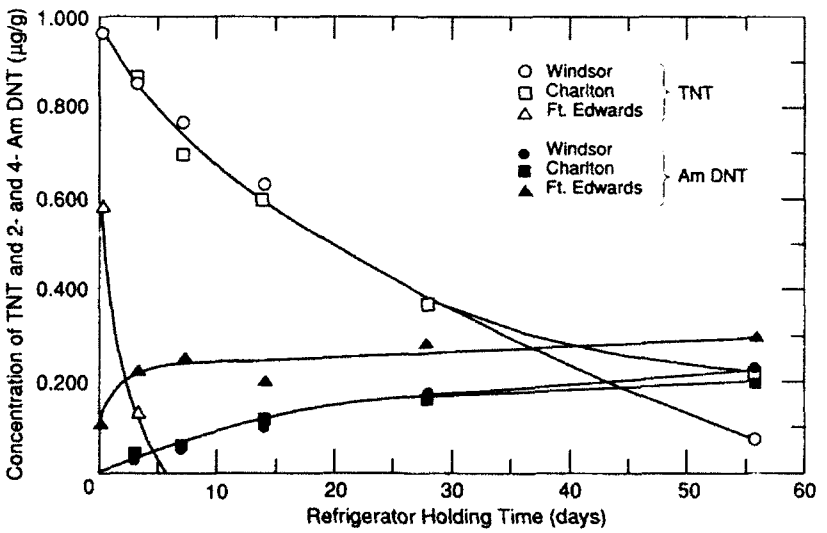

Figure 11. Refrigerator storage effects on TNT for three fortified soils.

2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene (McCormick et al. 1976), were observed (Fig. 7).

With freezer storage, 2,4-DNT was quite stable. For the Windsor soil, our modified ASTM criterion was exceeded on the low side after 42 days. Once again, this occurred because of a very small pooled standard deviation and, at 56 days, the concentration decrease was only 4.9%. Charlton soil showed no significant change during the 56-day test period. However, the Ft. Edwards soil produced a significant 2,4-DNT decrease after 30 days and the loss after 56 days was 20.3%. Still, the overall mean recovery relative to day zero for the three soils was 94.2% and the mean for the 56-day time was 90.8%.

The stability of HMX and RDX in these three fortified soils is much greater than that of TNB, TNT or 2,4-DNT. This agrees with the results obtained by Maskarinec et al. (1991) and Harvey et al. (1991) and is consistent with results from Hoffsommer et al. (1978) and Spanggord et al. (1980) who showed that RDX does not biodegrade under aerobic conditions. Regardless of storage conditions, no loss of HMX or RDX was observed over the entire 56-day study. The overall mean recoveries for HMX and RDX were, respectively, 99.8% and 97.1% for room temperature storage, 99.1% and 99.5% for refrigerator storage, and 100.3% and 99.0% for freezer storage. When HMX and RDX are the only analytes of interest, these data indicate that all three storage conditions are acceptable for at least 56 days.

Holding time behavior of analytes in a field-contaminated soil

The mean concentrations of four explosives (2,4-DNT concentration was too low to quantitate) and three transformation products in Crane-Rockeye soil are presented in Table 10 as a function of hold time and storage condition. Several differences from the fortified soils are evident from these data. First, it is apparent that triplicate analysis of 2.0-g samples of this field-contaminated soil failed to yield satisfactory precision despite efforts at homogenization. RSDs often exceeded ±25%, with the poorest results found for HMX and TNT. Secondly, and most important, the rapid loss of

Table 10. Concentrations of analytes and transformation products as a function of holding time and storage condition, Crane-Rockeye soil.

		Mean concentration (µg/g) ± standard deviation (µg/g)											
		Holding time											
		00 Days		*03 Days*		*07 Days*		*14 Days*		*28 Days*		*56 Days*	
Compound	*Storage*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*	*X*	*S*
HMX	Room. temp.	2.478	0.927	1.882	0.302	1.936	0.030	2.534	1.351	2.475	0.853	2.199	0.780
	Refrigerator	2.478	0.927	2.188	0.711	1.850	0.067	1.986	0.475	1.848	0.410	2.101	0.894
	Freezer	2.478	0.927	2.668	0.850	1.938	0.516	3.068	1.888	1.946	0.432	1.915	0.784
RDX	Room. temp.	0.421	0.052	0.398	0.033	0.432	0.070	0.335	0.027	0.380	0.015	0.399	0.034
	Refrigerator	0.421	0.052	0.421	0.035	0.447	0.100	0.355	0.037	0.467	0.042	0.351	0.058
	Freezer	0.421	0.052	0.390	0.039	0.383	0.044	0.403	0.090	0.404	0.029	0.375	0.071
TNB	Room. temp.	0.794	0.213	0.912	0.133	0.671	0.320	0.406	0.103	0.701	0.084	0.563	0.084
	Refrigerator	0.794	0.213	0.842	0.122	1.010	0.012	0.559	0.009	0.927	0.310	0.636	0.122
	Freezer	0.794	0.213	1.035	0.238	0.672	0.170	0.689	0.027	0.825	0.133	0.644	0.071
3,5-DNA	Room. temp.	0.643	0.079	0.770	0.038	0.692	0.162	0.493	0.051	0.512	0.074	0.416	0.041
	Refrigerator	0.643	0.079	0.705	0.110	0.786	0.050	0.625	0.010	0.775	0.133	0.633	0.041
	Freezer	0.643	0.079	0.762	0.085	0.650	0.100	0.639	0.017	0.707	0.052	0.604	0.047
TNT	Room. temp.	2.209	0.334	2.346	0.313	2.085	0.510	1.520	0.135	2.400	0.469	1.692	0.371
	Refrigerator	2.209	0.334	2.085	0.032	2.348	0.383	2.369	0.584	2.137	0.171	1.718	0.321
	Freezer	2.209	0.334	2.631	0.271	2.642	0.449	2.117	0.035	2.044	0.183	2.421	0.241
4-Am-DNT	Room. temp.	1.765	0.147	2.034	0.134	1.909	0.308	1.587	0.061	1.667	0.087	1.501	0.097
	Refrigerator	1.765	0.147	1.848	0.099	2.068	0.091	1.699	0.068	1.939	0.155	1.818	0.142
	Freezer	1.765	0.147	1.955	0.158	1.754	0.130	1.700	0.053	1.802	0.129	1.695	0.090
2-Am-DNT	Room. temp.	1.130	0.099	1.285	0.129	1.205	0.187	1.077	0.055	1.149	0.056	1.087	0.105
	Refrigerator	1.130	0.099	1.167	0.090	1.262	0.086	1.059	0.044	1.204	0.086	1.137	0.081
	Freezer	1.130	0.099	1.236	0.074	1.083	0.062	1.081	0.032	1.155	0.082	1.085	0.061

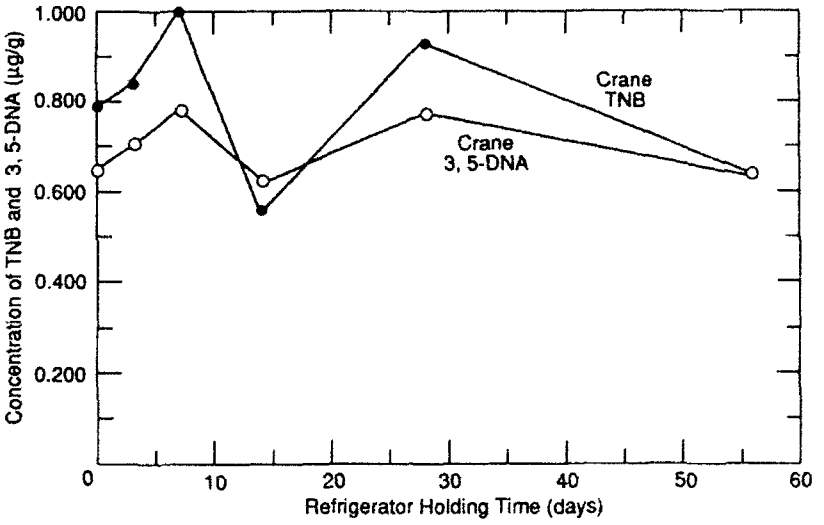

Figure 12. Refrigerator storage effects on TNB and 3,5-DNA for field-contaminated soil.

nitroaromatics observed with the fortified soils is not evident in the field contaminated soil. Also, there is no significant increase with time for the concentrations of degradation products. However, these compounds are initially present at much higher concentrations than ever found in the fortified soils. These points are illustrated in Figure 12 for TNB and 3,5-DNA. The striking similarity in the patterns of variation with time for these two analytes offers convincing evidence that random sampling errors are a dominant factor controlling results.

In view of the poor precision, our modified ASTM procedure to estimate MHTs was not applied. Only the isomers of Am-DNT yielded 99% confidence intervals that were less ±15%. However, overall mean recoveries relative to day zero were calculated. For freezer storage, mean recoveries were 93.1%, 92.9%, 97.4% and 107.3% for HMX, RDX, TNB and TNT, respectively. Comparable values for refrigerator storage given in the same order were 80.5%, 97.0%, 100.8% and 96.5%. If we consider the large uncertainties in the day zero means, these recovery estimates do not suggest rapid degradation of these analytes in the Crane soil.

ADDITIONAL COMMENTS

Least squares models were not fitted to our data. Maskarinec et al. (1991) required five different models to fit all their data and there was no consistent pattern of the "best fit" models as a function of soil or storage condition. We doubt these empirical fits imply any fundamental relationships. The problem is further exacerbated by 1) nonrandom calibration errors (day-to-day) that cannot be separated from real changes in analyte concentrations, and 2) by distortion of experimental errors caused by transformations of data (Motulsky and Ransnas 1987). We believe that our modified ASTM procedure using a pooled standard deviation is an acceptable way to estimate MHTs. However, future work should employ preliminary studies to estimate the required number of replicates for acceptable precision of means, and more replicates should be used for day zero data. For organic analytes in field-contaminated soils, consideration should be given to relaxing the limits for 99% confidence from ±15% to ±20 or 25% to accommodate insurmountable sample heterogeneity problems.

Results from fortified soils appear most applicable to freshly contaminated soils such as one might find near the front of a moving groundwater plume. If we assume that future studies will confirm the difference observed here between fortified and field-contaminated soils, MHTs for sites with long-standing contamination could be based on soils from similar sites because this offers the potential to extend MHTs. Where this is impractical, MHTs should be based on worst case results, which appear to result from fortified soils. Based on results for fortified soils, when HMX and RDX are the only analytes of interest, either refrigeration or freezing are acceptable storage conditions for at least eight weeks. When nitroaromatics are to be

determined, soil differences become important, as noted from the much more rapid degradation found in Ft. Edwards clay compared to Windsor and Charlton loams. Samples should be immediately frozen. At –15°C, TNB and TNT remain acceptably stable for eight weeks and 2,4-DNT is stable for four weeks. From practical considerations, 2,4-DNT will still give acceptable results after being frozen for eight weeks.

There remains a further unresolved issue relative to the effects of air drying. In an earlier study, Bauer et al. (1989) spiked air-dried soils with a series of nitroaromatics and nitramines in ACN, evaporated the ACN and studied the stability of these analytes over a 62-day period under refrigeration. Their results indicate that these analytes are stable once the soil is dry. In our study, however, we find substantial degradation in only two hours for TNB and TNT added to wet soils maintained at room temperature. This raises the issue of when to dry soils for homogenization and subsampling. Regardless of whether soils are air dried after sampling and before freezing, or not until they are thawed, significant loss of TNB and TNT is possible. Furthermore, there is some evidence to suggest more microbiological activity on thawing than before freezing (Skagland et al. 1988). Is it possible that freezing would be unnecessary if soils are first dried? Is there any way to speed the drying process to minimize microbiological degradation? Although freeze drying could possibly minimize the problem (Cragin et al. 1985), a question remains as to the practicality of this alternative for general usage. Will drying have a different effect on fortified samples compared to field-contaminated samples? Must we avoid drying and, therefore, homogenization, in order to prevent large losses of analytes such as TNB and TNT? Separate samples could be air dried for moisture correction. All of these issues should be addressed in future work.

The large difference in the behavior of nitroaromatics behavior between fortified and field-contaminated soils is extremely important. Even with avoidance of the addition of unnatural solvents during fortification, the behavior of nitroaromatics appears not to accurately mimic soils contaminated in the field over extended periods of time. To better define these differences, there is need for more extensive studies of field-contaminated soils under conditions where sample size and replicate numbers are not arbitrarily limited. If analytes in field-contaminated soils are consistently found to be much more stable than in fortified soils, continued use of fortified soils to estimate MHTs will lead to unnecessarily restrictive sample handling procedures and storage times. At some time these comparisons should be extended to other biodegradable organic compounds with different binding strengths to soils.

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13. ABSTRACT (*Maximum 200 words*)

A study was conducted to experimentally evaluate the maximum acceptable preextraction analytical holding times (MHTs) for three nitroaromatic compounds and two nitramines in soil. Three spiked soils and a field-contaminated soil were utilized in the study. Analytes investigated were HMX, RDX, TNB, TNT and 2,4–DNT, all at the low µg/g level. Subsamples of each soil were extracted with acetonitrile in an ultrasonic bath after being held for periods of 0, 3, 7, 14, 28 and 56 days at either room temperature (22°C), under refrigeration (2°C) or frozen (–15°C). Extracts were analyzed by RP-HPLC. The two nitramines, HMX and RDX, were stable over the entire period for all soils under all storage temperatures. For the three nitroaromatics (TNB, TNT and 2,4–DNT) the results were very different, in that all three analytes rapidly degraded in spiked soils at room temperature, more slowly degraded under refrigerator temperature and remained quite stable when frozen. Of the three, TNB degraded most rapidly, followed by TNT and 2,4-DNT. The degradation at room temperature and in the refrigerator was much faster for one soil than for the others. Even when frozen there was a small loss of 2,4–DNT in the soil showing the most rapid degradation. For the field-contaminated soil, the nitroaromatics were much more stable, even at room temperature, although some degradation occurred. Because of the large stability difference between fortified and field-contaminated soils, the efficacy of using fortified soils to estimate MHTs is discussed. The recommended MHT for soils containing only nitramines is eight weeks under refrigeration. When nitroaromatics are present, refrigeration is inadequate and soils should be frozen to preserve analyte integrity. When frozen, an MHT of eight weeks is recommended.

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