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Development of an analytical method for the determination of extractable nitroaromatics and nitramines in soils

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DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF EXTRACTABLE NITROAROMATICS AND NITRAMINES IN SOILS

by

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DISSERTATION

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DEDICATION

This dissertation is dedicated to three very special women. To Elizabeth, without whose encouragement the effort would never have begun, to Pamela, without whose patience and support it would never have come to fruition, and to my mother, whose unwavering confidence in me was a continual source of inspiration.

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ABBREVIATIONS

DNB	1,3-dinitrobenzene
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
NG	nitroglycerine
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SEX	octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine
TAX	hexahydro-1-(N)-acetyl-3,5-dinitro-1,3,5-triazine
TNB	1,3,5-trinitrobenzene
TNBA	2,4,6-trinitrobenzaldehyde
TNT	2,4,6-trinitrotoluene
Tetryl	Methyl-2,4,6-trinitrophenylnitramine
m-NT	Meta-Nitrotoluene
o-NT	Ortho-Nitrotoluene
p-NT	Para-Nitrotoluene
2-Am-DNT	2-amino-4,6-dinitrotoluene
4-Am-DNT	4-amino-2,6-dinitrotoluene
2,4-DAm-NT	2,4-diamino-6-nitrotoluene
2,6-DAm-NT	2,6-diamino-4-nitrotoluene
2,4-DNT	2,4-dinitrotoluene
2,6-DNT	2,6-dinitrotoluene
2,4,5-TNT	2,4,5-trinitrotoluene
AAP	Army Ammunition Plant
CRL	Certified Reporting Limit
CRREL	U.S. Army Cold Regions Research and Engineering Laboratory
GC	Gas Chromatography
HPLC	High-Performance Liquid Chromatography
MDL	Method Detection Limit
NP	Normal-Phase
RP	Reversed-Phase
RP-HPLC	Reversed-Phase, High-Performance Liquid Chromatography
RSD	Relative Standard Deviation
SARM	Standard Analytical Reference Material

USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
UV	Ultraviolet

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ABSTRACT

DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF EXTRACTABLE NITROAROMATICS AND NITRAMINES IN SOILS

BY

Thomas F. Jenkins
University of New Hampshire, December, 1989

An analytical method was developed to determine the concentrations of HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT in soil. The method relies on solvent extraction with analysis by reversed-phase liquid chromatography.

The extraction step was studied in terms of process kinetics and recovery. Two solvents (acetonitrile and methanol) and four extraction techniques (Soxhlet, ultrasonic bath, mechanical shaker and homogenizer-sonicator) were compared. Ultrasonic bath extraction with acetonitrile was selected based on extraction kinetics, overall analyte recovery, sample throughput, and instability of analytes at elevated temperature. The rate of extraction of analytes from field-contaminated soil was shown to be much slower than from spiked soils, indicating it is unwise to develop extraction procedures based solely on spiked materials.

A number of possible separations were examined. Adequate separation of the seven analytes was achieved on an LC-18 column eluted with 1:1 methanol/water with a run time under 15 minutes. Confirmation of analyte identities was recommended on LC-CN, also eluted with 1:1 methanol/water. Elution orders on the two columns were quite different due to different mechanisms of separation.

Additional tests were conducted to assess various sample processing alternatives. Removal of particulates from soil extracts was achieved by dilution of extracts 1:1 with aqueous $CaCl_2$. This resulted in flocculation of suspended particles, which were then easy to remove by settling and filtration. Stock standards were stable for at least a year, working standards at least 28 days, and soil extracts at least two months. Care needs to be taken to ensure that air drying of soil, prior to extraction, is not conducted in direct sunlight; otherwise losses of TNT and an increase in photochemical transformation products will result.

The overall method provides linear calibration curves over a wide range of analyte concentrations. Detection limits ranged from 0.03 to 1.27 $\mu g/g$ with no extract preconcentration. Recovery of spiked analyte was better than 80% for all analytes tested. The method was successfully tested in two collaborating laboratories.

CHAPTER 1

INTRODUCTION

1.1 Requirement for Method

Probably the most serious environmental problem facing the U.S. Army today is the presence of soil contaminated with munitions residues at military installations throughout the United States. Soils have become contaminated over the last fifty years by a) waste discharges from manufacturing of explosives and propellants, b) fabrication of finished munitions, c) destruction of out-of-specification material, and d) demilitarization of out-of-date bombs, rockets and ammunition.

Unlike many other organic chemical residues, many components of munitions are quite mobile in the soil. Thus contaminated soil can be a source of groundwater pollution. Recent field studies have shown this to be the case both on Army installations themselves and beyond installation boundaries (Spaulding and Fulton, 1988; Pugh, 1982).

Along with nitrocellulose 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are the explosives most widely used by the U.S. Army (Etnier, 1986). These explosives are used in pure form, in mixtures with other explosives or inert materials, or in concert in a wide range of explosives and propellants (Table 1.1). Because of their widespread use and their long-term stability in the environment, TNT and RDX are the two explosives most commonly observed in munitions-contaminated soils. Further, because of their mobility in the soil profile, they pose the greatest immediate problem for groundwater contamination.

Table 1.1. Composition of various military explosives. (Mikdiff and Washington, 1976; U.S. Army, 1984; Leggett et al., 1977).

Explosive	Composition*
Composition A	RDX (91%)†
Composition B	RDX (60%), TNT (40%)
Composition C	RDX (71%), TNT (4%), Tetryl (3%), DNT (10%), MNT (5%)
Composition C-4	RDX (91%)
Tetratol	TNT (20%), Tetryl (80%)
Military dynamite	RDX (75%), TNT (15%)
Octols**	TNT, HMX

* Remainder composed of binders and non-explosive additives.

† RDX - hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT - 2,4,6-trinitrotoluene
Tetryl - methyl-2,4,6-trinitrophenylnitramine
DNT - dinitrotoluenes
MNT - mononitrotoluenes
HMX - octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.

** Mil explosives TM 9-1300-214

Current methods of decontaminating soil rely on excavation followed by incineration. To minimize the cost of this very expensive process, the area of soil contamination must be carefully delineated. Consequently, a relatively quick, inexpensive analytical protocol is required. The method must be precise enough to allow quantification with a minimum number of replicate determinations. It must also be accurate over the range of concentrations measured to enable a judgement as to when soil levels have declined below regulatory criteria.

1.2 Choice of Analytes

In addition to TNT and RDX (Fig. 1.1), other nitroaromatics and nitramines are often used as components of military explosives (Table 1.1). Any method for TNT and RDX must therefore be able to distinguish between these analytes and impurities, and decomposition products with similar structure and functionality. For example, tetryl (methyl-2,4,6-trinitrophenylnitramine) was used in combination with TNT until 1979 (U.S. Army, 1984). Since portions of these residues were deposited over a 40-period prior to 1979, many residues may contain large amounts of tetryl. HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) has been used as an explosive in conjunction with TNT for some munitions, and it is also present as an impurity at the 5-10% level in RDX made by the Schiessler-Ross and Bachmann processes (Edward, 1987). A component of some smokeless powders and a common impurity in military grade TNT is 2,4-dinitrotoluene (2,4-DNT). Analyses of a wide variety of TNT-based munitions produced from 1945 to 1971 indicate that 2,4-DNT concentrations range from 0.02 to 0.11% (Leggett et al., 1977). While this is a relatively low level, 2,4-DNT has been identified as an EPA priority pollut-

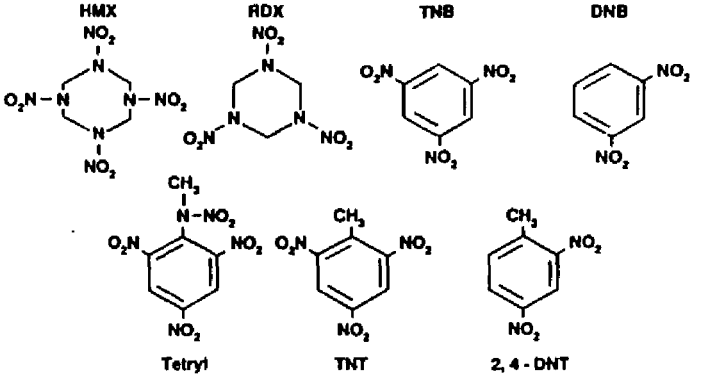


Fig. 1.1. Chemical structures of seven primary analytes

ant due to its potential for causing human injury, and hence it has been included as a primary analyte in the method development activity discussed here. Other DNT isomers are also present in military-grade TNT; however, their levels are much lower than 2,4-DNT. For example, 2,6-DNT is typically present at less than 5% of the 2,4-DNT concentration (Leggett et al., 1977).

Two other analytes chosen were 1,3,5-trinitrobenzene (TNB) and 1,3-dinitrobenzene (DNB). TNB, which is not present at significant levels in military-grade TNT, is thought to arise in environmental residues from photodegradation of TNT via oxidation of the methyl group followed by decarboxylation (Burlinson, 1980; Spanggord et al., 1980). Trace levels of DNB are also found where high levels of TNT are observed (Jenkins and Grant, 1987), presumably due to photodegradation of 2,4-DNT. Spanggord et al. (1982) have also identified DNB as a major impurity in effluent discharges from TNT manufacture.

Thus seven major analytes have been selected (Fig. 1.1). These fall into two groups. The first is nitroaromatics, which include TNT, TNB, DNB, and 2,4-DNT. The second is nitramines, which include HMX and RDX. Tetryl is both a nitroaromatic and a nitramine.

1.3 Objective

The objective of this research was to develop a method that could be used to determine the concentrations of extractable nitroaromatics and nitramines in soil. The method should be suitable for commercial use by contractors analyzing large numbers of soil samples from the 1391 current U.S. Army installations and the over 7000 former Army sites, any of which

could be contaminated with these compounds. Because of the numbers of samples involved, it was hoped that relatively uncomplicated, off-the-shelf equipment could be utilized to minimize costs, while still producing high-quality data.

CHAPTER 2

LITERATURE REVIEW

2.1 Physical and Chemical Properties of Selected Analytes

The chemical structures of the seven analytes of interest are shown in Figure 1.1. The nitroaromatic compounds are planar molecules, while RDX and HMX exist primarily in the chair and crown conformations, respectively (Freeman et al., 1976). These compounds are all polar, neutral organics which are chemically stable in the environment for extended periods. Water solubilities range from 5.0 mg/L for HMX to 460 mg/L for DNB (Table 2.1). In general they are all much more soluble in polar organic solvents than in nonpolar ones. For example, TNT's solubility in acetone is 109 g/100 g at 20°C, while its solubility in carbon tetrachloride is only 0.65 g/100 g at the same temperature (EPA, 1989). Most of the compounds are thermally unstable below their boiling points (Table 2.1).

2.2 Soil Composition and Mechanisms of Binding of Organic Solutes

To develop effective means of extracting these solutes from soil, a basic understanding of soil composition and soil/solute binding mechanisms is useful. Soil is a heterogeneous assembly of biotic and abiotic components. Soil composition varies widely, both horizontally from location to location and vertically with depth. On a volume basis the major components of soil are solid minerals, organic matter in various stages of decomposition, water and air, the percentages of each varying widely.

Table 2.1. Physical and chemical properties of nitroaromatics and nitramines.

Compound	Molecular Weight	Melting Pt. (°C)	Boiling Pt. (°C)	Water Solubility (mg/L)	Vapor Pressure (20°C) (torr)	Log K_{ow}*	Henry's Law Const. H_c (torr M^{-1})
TNT	227.13	80.1-81.6 (1)	240 (explodes) (2)	130 @ 20° (1)	1.1×10^{-6} (3)	1.86 (4)	0.18 (5)
RDX	222.26	204.1 (7)	(decomposes)	42 @ 20° (16)	4.1×10^{-9} (8)	0.86 (4)	2×10^{-5} (5)
HMX	296.16	286 (9)	(decomposes)	5.0 @ 25° (10)	3.3×10^{-14} (17)	0.061 (4)	
TNB	213.11	122.5 (11)	315 (11)	34 @ 20° (5)	2.2×10^{-4} (5)	1.18 (12)	1.3 (13)
DNB	168.11	89.6 (11)	300-303 (11)	460 @ 15° (11)	3.9×10^{-3} (8)	1.49 (12)	
Tetryl	287.14	129.5 (9)	(decomposes)	80 (14)	5.7×10^{-9} @ 25° (17)	1.65 (4)	
2,4-DNT	182.14	69.5-70.5 (15)	300 (decomposes)	270 @ 22° (15)	1.4×10^{-4} @ 25° (15)	2.01 (15)	3.4 (5)

* K_{ow} is the n-octanol/water partition coefficient.

(1) EPA (1989)
(2) Verscheuren (1983)
(3) Leggett (1977)
(4) This Thesis
(5) Spanggord et al. (1979)
(6) Spanggord et al. (1978)
(7) EPA (1988)
(8) Spanggord et al. (1980)
(9) Lindner (1980)
(10) Glover and Hoffsommer (1973)
(11) Wentzel et al. (1979)
(12) Hansch and Leo (1979)
(13) Maksimov (1968)
(14) Urbanski (1964)
(15) EPA (1980)
(16) Sikka et al. (1978)
(17) Burrows et al. (1989)

Soil minerals are composed of a variety of crystalline aluminosilicates whose specific composition is a function of the parent geological material from which it was derived and the weathering processes to which it has been subjected. Mineral surfaces are thought to be negatively charged due to isomorphic substitutions of aluminum for silicon and magnesium for aluminum within crystal lattices. Mineral surfaces are hydrated with a series of layers of adsorbed water in which various exchangable cations are present in the proper proportion to maintain an overall charge balance.

The size distribution of soil particles also varies widely from small cobbles to particles too small to be visible with standard optical microscopes. Soil scientists have subdivided these particles into the following size categories: gravel (2-70 mm), sand (0.05-2 mm), silt (0.002-0.05 mm) and clay (< 0.002 mm) (Klute, 1986). Surface areas of soil vary depending on both the types of minerals present and the distribution of particle sizes. Surface areas for soils with a large proportion of clay can be very high. Surface areas over 800 m^2/g have been observed for some expanding layer silicates such as montmorillonite (Klute, 1986). Thus a very large surface can be available for soil/solute binding.

A great deal of research has been conducted on the interaction between various chemicals and pure mineral surfaces. Recent evidence, however, indicates that mineral surfaces for most topsoils are coated with an amorphous layer of hydrous metal oxides and organic matter (Karickhoff, 1984). It is the latter which appears to control binding mechanisms between man-made organic pollutants and surface soils. Inter-

actions with mineral surfaces may be more important for deeper soils, which typically have a low organic matter content.

The chemical composition of soil organic matter has been the subject of a vast amount of research, but its complexity and inhomogeneity have thus far defied complete characterization. Soil organic matter is known to contain proteins, carbohydrates and other relatively degradable material from plant and animal debris. Soil chemists have concentrated, however, on the more fully degraded portion of soil organic matter, which has been subdivided operationally into several fractions based on solubility in various media. Two fractions that are thought to play an important role in binding mechanisms are humic and fulvic acids, which are often referred to together as humic material. Although many model structures for humics have been proposed, one developed by Stevenson (1982) is representative (Fig. 2.1).

There appears to be general agreement by most researchers that humic material is a mixture of complex polymers formed by reactions among partially decomposed plant and animal debris. These polymers contain a relatively large proportion of phenolic and carboxylic acids. Humic polymers contain both aliphatic and aromatic regions, the proportion of each varying as a function of their origin (Gauthier et al., 1987). Soil humic material, being terrestrial in nature, should be more aromatic in character than marine-derived humic matter (Jackson, 1975).

The mechanisms which bind humic materials to mineral surfaces are not completely understood, but one can speculate that they may include contributions from van der Waals interactions, hydrogen bonding, electrostatic attraction through exchangable cations and a degree of covalent bonding through silanol esters. The conformation of humic materials may

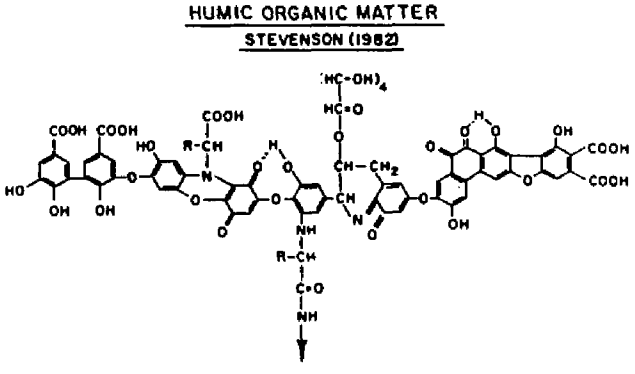


Fig. 2.1. Model structure of humic acid

be quite dynamic (Lochmüller and Saavedra, 1986) in response to changing solution ionic strength and pH, partially as a result of natural wetting and drying cycles. It is useful to consider humic materials bound to soil surfaces as analogous to the chemically bonded silica widely used in reversed-phase liquid chromatography.

The observation that binding of uncharged organic solutes to soil is largely controlled by organic matter is credited to Lambert and coworkers (Lambert, 1967, 1968; Lambert et al., 1965). Lambert reported that the distribution coefficients between water and a number of soils for a specific nonpolar pesticide were equivalent when the pesticide concentration in each soil was normalized to the percent organic matter present. This observation has since been confirmed by many other investigators, including Karickhoff et al. (1979), who observed a similar relationship for polynuclear aromatics and chlorinated pesticides in sediments. This type of interaction is often referred to as hydrophobic bonding.

Lambert is also credited with suggesting that the distribution coefficient for uncharged organics between soil organic matter and water was analogous to that between two immiscible liquids in solvent extraction. Briggs (1973) observed an excellent correlation between the degree of sorption for a series of herbicides to soil and their n-octanol/water partition coefficients. Karickhoff et al. (1979) correlated sorption to aquatic sediment with the chemical's octanol/partition coefficient and the sediment's percent organic carbon. A number of other investigators have confirmed the usefulness of this relationship for a variety of nonpolar solutes.

Chiou et al. (1979) extended this idea by proposing that a partitioning mechanism between a liquid-like layer of organic matter and water

could explain the linearity of sorption isotherms for hydrophobic bonding of nonpolar organics. Mingelgrin and Gerstl (1983) have challenged this interpretation and suggest that a range of interactions from two-dimensional adsorption at individual charge sites on the surface, to three-dimensional partitioning, may be operable for various non-ionic organics. They believe that the partitioning mechanism is most applicable to nonpolar organics. However, conclusive evidence has not been gathered to prove which, if either, of these interpretations is correct.

In natural soils, surfaces are largely covered by adsorbed water, with association dominated by hydrogen bonding interactions. Bond energies for hydrogen bonding range from about 0.5 to 15 kcal/mol (Morrill et al., 1982). Thermodynamic studies of the hydrophobic binding of nonpolar organics to soil indicate that bond energies are usually 1 to 2 kcal/mol, a range which is typical for van der Waals interactions (Voice and Weber, 1983). A nonpolar organic molecule must displace adsorbed water to sorb to a soil surface. Since bond energies at the surface are generally weaker for the nonpolar molecule, the driving force for this process appears to be entropic in nature. This apparently results from a decrease in the ordering of water molecules in solution when the solute is transferred to the surface. Nonpolar solutes are thought to be held in solution by an ice-like arrangement of water molecules surrounding the solute (Voice and Weber, 1983).

While interactions between nonpolar organics and soils have been studied rather extensively, few studies have concentrated on polar non-ionic organics. This is probably a result of the overriding interest among environmentalists with pollutants such as polynuclear aromatics, polychlorinated biphenyls and dioxin, which are nonpolar in character.

The interaction of nitroaromatics and nitramines with bentonite clays has been investigated by Leggett (1985). Nonlinear isotherms that could be resolved into two components were observed for TNT and 2,4-DNT. Leggett interpreted his results to indicate that both TNT and 2,4-DNT were sorbed by two different mechanisms. Class 1 sorption was attributed to hydrogen bonding mechanisms between negatively charged surfaces of the clays and the electron-deficient aromatic rings. Class 2 sorption for TNT and 2,4-DNT, and the linear sorption observed for RDX and HMX, were attributed to hydrophobic interactions. Since the organic carbon content of the two commercial bentonites was not determined, it is uncertain whether the hydrophobic interactions observed were with organic polymer additives or with clay mineral surfaces. Leggett (1985) also reevaluated the sorption data presented by Sikka et al. (1980) for TNT and RDX on aquatic sediments. He found the same two classes of sorptive behavior for TNT on sediments that he had observed for clays, while linear isotherms indicating only one type of sorption were observed for RDX. The difference in behavior for the nitroaromatics and nitramines was attributed to the lack of an aromatic ring structure for RDX and HMX and their non-planar conformation, which could affect their ability to approach high-energy binding sites closely.

Because of the inhomogeneity of soils and the variety of binding mechanisms that appear to be involved, an extracting solvent must perform several functions. First it must be capable of displacing analytes from high-energy binding sites. It should also maintain the soil organic matter in an open structure in which imbibed organics can diffuse. The extractant must provide the analytes with adequate solubility so that any equilibrium partitioning between the solvent and the soil organic matter

will be strongly in favor of the extracting solvent. For all of these functions, a relatively polar organic solvent is expected to be optimum for nitroaromatics and nitramines.

2.3 Previous Extraction Studies

Several methods for extraction and determination of nitroaromatic and nitramines in soil have been reported, although none has been thoroughly validated. Hoffsommer et al. (1972) extracted ocean floor sediment by stirring with benzene prior to gas chromatographic (GC) determination of TNT, RDX and tetryl. No evaluation of extraction efficiency was reported. Goerlitz and Law (1975) used acetone-hexane to extract TNT and RDX from soil prior to GC determination. Three successive extractions were used. Recovery studies using fortified soils indicated that only 55% of the spiked TNT and RDX were recovered in the first extraction. It was not clear whether this poor extraction efficiency was due to a poor distribution coefficient for the analytes between the extracting solvent and soil, poor solvent recovery or slow desorption kinetics. After three successive extractions, 85 ± 15% TNT and 93 ± 10% RDX were recovered. No tests were reported with field-contaminated soils.

Miller et al. (1983) evaluated acetone, acetonitrile, methylene chloride/methanol and hexane for extraction of eight munitions compounds from fortified sediment prior to HPLC determination. The compounds tested included TNT, RDX, TNB, DNB, tetryl and 2,4-DNT. A 1-hour extraction period was used on a wrist-action shaker with a 10/1 solvent-to-sediment (mL/g) ratio. The authors report that the best recovery was found for

methylene chloride/methanol, although recovery of tetryl was unsatisfactory. Recoveries ranged from less than 25% for tetryl to nearly complete recovery for TNB and RDX. No evaluation of the method using field-contaminated soils was reported.

Bongiovanni et al. (1984) report a method for extraction of TNT, RDX, HMX, tetryl, 2,4-DNT and 2,6-DNT and subsequent determination by reversed-phase HPLC. Fortified, 10-g soil samples were extracted with 20 mL of acetonitrile. Neither the actual method of extraction or the extraction times used are discussed, but the authors do report that extraction was "enhanced by sonication." Quantitative recovery was reported for all the analytes tested. The authors indicate that better extraction efficiency was obtained if soils retained 20-30% moisture when extracted.

Cragin et al. (1985) report an investigation in which various soil drying techniques were studied prior to determination of TNT, RDX and HMX. The authors tested acetone, methanol, acetonitrile and tetrahydrofuran with a field-contaminated sediment and soil. All solvents appeared to work equally well, and the authors selected methanol for further experiments "because of its lower toxicity." Of the drying methods tested, freeze drying was preferred by the authors, although air drying at room temperature was selected as a practical alternative. Oven drying at 105°C produced low recoveries for TNT and RDX. It was postulated that drying of sediments with large concentrations of organic matter could collapse the gel network, making it difficult to extract analytes trapped within. This could explain the results obtained by Bongiovanni et al. (1984), where better extraction efficiency was obtained if a residual water content of 20-30% was maintained.

Brueggemann (1985) reports a method for extraction of a series of nitroaromatics and nitramines from deactivation furnace ash and subsequent determination using RP-HPLC. A 5-g portion of ash was extracted with 10 mL of acetonitrile for 30 minutes on a wrist-action shaker. Recoveries from spiked ash samples ranged from about 90% for RDX to 98% for 2,4-DNT.

Folsom et al. (1988) describe a method of soil extraction in which 10 g of soil is extracted with 200 mL of benzene in an ultrasonic cell disrupter operated at full power for 5 minutes. Only fortified soils were tested, but recoveries of about 80% were reported for TNT. Analytical precision for this method was very poor, with relative standard deviations of 33 to 58%.

Pennington (1988) compared methanol, acetone, methylene chloride and benzene for extraction of TNT from soil. Methanol and acetone were found to be more efficient at removing radio-labeled TNT from soil (55-100%) than methylene chloride or benzene (29-50%). A 3-minute sonication procedure was used with a sonic probe.

While there are some inconsistencies in the extraction literature described above, polar solvents and binary solvents containing a polar constituent seem to be more efficient than nonpolar solvents at extracting these nitroaromatics and nitramines from soil. This is consistent with a general theory of solvent extraction proposed by Freeman and Cheung (1981). The optimum extraction solvent, according to their reasoning, should be a relatively polar solvent to (1) maximize swelling of humic acid gels in which the solute is imbibed, and (2) provide maximum solute solubility for polar organics. In this way both the highest equilibrium partition coefficient and the rate at which it is attained

are maximized. A polar extracting solvent should also be more effective at releasing TNT and other nitroaromatics from high-energy binding sites such as those found for bentonite clays by Leggett (1985).

Except for that of Cragin et al. (1985), the studies described above all used fortified soil for which the manner of incorporation and the equilibrium times for soil/analyte interaction have not been reported. None of the studies included kinetic measurements to determine whether equilibrium between the soil and solvent had been established. Georlitz and Law (1975) did indicate that poor extraction efficiency was obtained unless the soil was allowed to contact the extracting solvent for at least 12 hours. Most of the other methods use very short extraction periods: 3 minutes for Pennington (1988) to 1 hour for Miller et al. (1983). It appears that better analytical precision was obtained when longer extraction times were used, indicating that equilibrium was probably not obtained with short extraction times.

All of the methods described rely on favorable distribution coefficients between the extracting solvent and the soil. Contact between the soil and the solvent is maximized by some method of agitation, such as a mechanical shaker, or sonication using a sonic probe or ultrasonic bath. No comparison among these approaches or the classical Soxhlet continuous extraction method has been reported for these analytes. Comparisons for other analyte/solvent pairs have shown inconsistent results, the preferred solvent and method varying depending on the analyte and matrix studied.

Since the state of knowledge of the best solvent/method combination for a specific application is largely empirical at present, one of the objectives of the research described here was to compare the various

method/solvent options for extracting nitroaromatic and nitramine residues from soil. Since there is reason to believe that the extraction of analytes from fortified soil is kinetically different from field-contamined soil, the latter will be used for comparative purposes. Fortified soils will only be used to make those assessments requiring a knowledge of the total analyte content of the soil.

2.4 Chromatographic Separations

Most previous methods for determining nitroaromatics and nitramines in environmental matrices rely on chromatography. A separation is useful prior to the determination step because the analytes have similar chemical and spectral properties and are often found together (Tables 1.1 and 2.1). For soil, the potential for observing other organic pollutants is also large, and chromatography is effective at separating interferences from the analytes of interest.

2.4.1 Thin-Layer Chromatography

One of the earliest methods for separating nitroaromatics was thin-layer chromatography (TLC). Yasuda (1964) found that he could satisfactorily separate the isomers of dinitrotoluene and trinitrotoluene using two-dimensional TLC on silica gel.

Hoffsommer and McCullough (1968) found that TLC combined with visible spectrometry could be used to provide quantitative analysis of polynitroaromatics in complex mixtures. Neither of these methods was used for environmental analysis, and TLC has now been largely overshadowed for quantitative use by gas-liquid chromatography (GC) and high-performance liquid chromatography (HPLC). TLC may still have some utility,

however, as a rapid, semi-quantitative method for field detection of these analytes.

2.4.2 Gas Chromatography

The earliest use of GC for the separation of nitroaromatics dates to the early 1960s (Parsons et al., 1961). Gehring and Shirk (1967) demonstrated that the isomers of dinitro- and trinitrotoluenes could be separated and determined using GC. Rowe (1966) observed that RDX decomposed at the oven temperature he used to separate the nitroaromatics. He later found that at a column temperature of 180°C the decomposition of RDX was minimal and reproducible (Rowe 1967). However, HMX did not elute from the column under the thermal conditions needed to minimize RDX decomposition.

All of the GC methods described above were developed for determination of percent levels of compounds in solid explosives. They used relatively insensitive flame ionization and thermal conductivity detectors, which respond on a mass basis similarly to all organic molecules. The power of GC for environmental determinations of trace levels of nitroaromatics and nitramines is due to their selectivity and sensitivity of response on the electron capture detector (ECD). Murrmann et al. (1971) demonstrated this sensitivity by determining the composition of the vapor in equilibrium with production-grade TNT.

Hoffsommer and Rosen (1972) developed a method for determining TNT, RDX and tetryl in sea water using GC-ECD. The authors used benzene extraction and recovered of 70 ± 10%. Hoffsommer et al. (1972) extended the GC-ECD method to the determination of TNT, RDX and tetryl in ocean

floor sediment and fauna. By combining an initial TLC separation, interferences in GC-ECD were eliminated.

Goerlitz and Law (1975) reported a step-by-step method for determining TNT and RDX in soil. The soil is extracted with acetone-hexane and evaporated to a small volume on a Kuderna-Danish evaporator. The extract is added to the top of an alumina column and eluted with benzene. This step removes interferences which would otherwise be difficult to separate by GC. The column eluent is then analyzed by GC-ECD.

Routine determination of HMX by GC methods has proven troublesome. Thermal degradation on glass or metal columns appears to be the primary problem. Douse (1981) reports that the use of fused silica capillary columns permitted determination of HMX, RDX, TNT, tetryl and several organo-nitrates. The best results for HMX were found when the oven temperature was programmed from 140 to 240°C at 40°/min. The author observed that an "effective clean-up procedure must be developed" because of interference from other electron-capturing substances in sample extracts.

The combination of GC separations with the more selective pyrolysis-chemiluminescence detector (TEA) has been reported by Lafleur and Mills (1981), Douse (1983 and 1985) and Fine et al. (1984). This detector is very sensitive and selective for explosives. Nitric oxide (NO) is released by pyrolysis of nitroaromatics, nitramines or nitrate esters, and it then reacts with ozone to form NO_2 with the emission of light. Since this detector only responds to compounds that release NO upon pyrolysis, it eliminates the need for a clean-up step after extraction, prior to GC analysis. The major drawback to this detector is its high cost and limited applicability to other analytical problems.

Belkin et al. (1985) have reported a GC-ECD method for 2,6-DNT, 2,4-DNT, TNT and tetryl in water. The method employs a toluene extraction and fused silica capillary GC determinations. Poor results were reported for RDX due in part to poor extractability from water. No results for HMX were reported.

Richard and Junk (1986) reported a method for solid-phase extraction of water for munitions followed by GC-ECD analysis. The authors investigated TNT, TNB, DNB, NB, 2,4- and 2,6-DNT and RDX. Good recovery was found for all compounds except RDX. No results were given for HMX or tetryl.

Gas chromatography/mass spectroscopy (GC/MS) has also been investigated for environmental determination of nitroaromatics. Pereira et al. (1979) reported a method for determining 2,4-DNT, TNT and two isomeric aminodinitrotoluenes in groundwater. The method employed benzene extraction, evaporative concentration and clean-up on deactivated alumina prior to GC/MS analysis. No performance criteria or tests with HMX or RDX were reported. GC/MS has the advantage of unequivocal identification, but its disadvantages include poor precision, high detection limits and high cost of analysis.

Overall GC determination of these seven nitroaromatics and nitramines in soil extracts is a viable option. The TEA detector is the most selective detector for these types of compounds, with a sensitivity approaching that of the ECD. The TEA is an unattractive option from a cost standpoint, however, since the method being developed is to be used in commercial laboratories under contract to the Army. Currently GC instrumentation equipped with a TEA detector is only available in laboratories specializing in forensic analysis.

The ECD is the most sensitive detector for nitroaromatics and nitramines. The ECD is also very sensitive to a wide variety of other environmental contaminants, such as pesticides, PCBs and chlorinated solvents. Thus, routine use of ECD requires a clean-up step employing alumina or silica gel column chromatography. ECD also has a rather small dynamic range and poor reproducibility from run to run.

GC techniques have not been demonstrated that can simultaneously determine all seven of the major analytes of interest here. Temperature programming with ECD is much less desirable compared to other GC detectors due to baseline drift. However, it is necessary because room-temperature vapor pressures range from 3.3×10^{-14} torr for HMX to 3.9×10^{-3} torr for DNB (Table 2.1). No GC-ECD separation for these seven analytes at high sensitivity has been reported.

HMX, RDX, TNT and tetryl are quite thermally labile. While GC techniques on fused silica columns have been reported for these analytes, discussions with a number of analysts indicated that column performance rapidly degrades when used for real samples. This is attributed to the accumulation of other less volatile contaminants on the front of the column. These compounds appear to catalyze thermal degradation of RDX, HMX and TNT.

2.4.3 High-Performance Liquid Chromatography (HPLC)

The first reported use of HPLC for environmental analysis of nitroaromatic compounds was a reversed-phase (RP) application by Walsh et al. (1973). TNT and 2,4-DNT were separated on a bonded-phase octadecylsilane (C_{18}) column, eluted with 90:10 water-acetonitrile. Concentrations were determined with a UV detector (no wavelength specified) or a refractive

index detector. No information was provided on the behavior of other nitroaromatics or nitramines.

Doali and Juhasz (1974) reported several different normal-phase (NP) separations for TNT and tetryl; RDX and HMX; and TNT, tetryl and RDX using a silica gel column and several different eluents containing dioxane and cyclohexane. Concentrations were determined on either a refractive index detector or a UV detector at 254 nm.

Goerlitz (1979) provided a step-by-step method for RDX, 2,4-DNT and TNT determination in water using RP-HPLC. He used a C_{18} column eluted with 30:70 methanol-water and a UV detector at 254 nm.

The first use of HPLC-MS for explosives analysis was reported by Vouros et al. (1977). TNT, RDX and HMX were separated on an NP silica column eluted with 1,2-dichloroethane. This work and a later RP study by Yinon and Hwang (1983) were conducted with the MS in the chemical ionization mode. Because of the large amount of solvent entering the MS, true electron impact mass spectra have not as yet been demonstrated for these analytes. Voyksner and Yinon (1986) did, however, report the use of thermospray HPLC-MS, which yielded sufficiently diagnostic mass spectra to resolve several nitroaromatic components that co-eluted. At present, however, HPLC-MS systems are relatively rare, and routine environmental analysis on this equipment is very costly.

Stidham (1979) reported both NP and RP separations for TNT, RDX, HMX and several acetyl analogs of HMX and RDX (SEX and TAX). The NP separation was achieved on a LiChrosorb column eluted with 5:10:15:70 methanol-acetonitrile-chloroform-isooctane. The RP separation used a bonded-phase octylsilane (C_8) column with a ternary eluent containing water, methanol and acetonitrile under conditions of gradient elution. Determination was

achieved with a UV detector at 245 nm for the NP and 230 nm for the RP separations. In both cases, good separations were achieved in about 10 minutes. Lower detection limits were reported using the RP procedure, and overall chromatographic resolution appeared to be better than in the NP mode.

Application of the TEA to HPLC was reported initially by Lafleur and Morriseau (1980). Several gradient elution NP separations were reported, including separations of RDX and HMX. No suitable solvents were discovered to elute TNT or tetryl from the NP column. Still, the enormous selectivity of the TEA for explosives was demonstrated.

Other authors discussing the use of HPLC-TEA for explosives include Fine et al. (1984) and Selavka et al. (1987). Fine et al. demonstrated the excellent sensitivity of the TEA for forensic analysis of post-blast debris, handswabs and human blood. Because of the selectivity of the TEA, no cleanup steps were necessary. Selavka et al. incorporated a post-column UV irradiation step prior to the TEA, thereby permitting the use of reduced pyrolyzer temperatures to destroy the nitroaromatics. This modification drastically reduced background noise compared to the higher temperature required to pyrolyze the nitroaromatics without post-column irradiation. A major limitation of the TEA is that it can only be used with NP eluents because of the necessity for rapid evaporation of the eluent prior to the pyrolyzer unit.

An electrochemical detector can be used with HPLC for the determination of nitroaromatics, nitramines and nitrate esters, as first shown by Bratin et al. (1981). The detector was operated in the reductive mode because of the highly oxygenated state of these nitro-organics. A major limitation of this approach was the need to remove oxygen completely from

the mobile phase. Post-column photolysis to produce nitrite ion, followed by oxidative electrochemical detection, was reported by Krull et al. (1984). This method eliminated the need to remove oxygen from the mobile phase. The use of two detectors at different potentials was cited as giving useful qualitative information.

Maskarinec et al. (1984) combined electrochemical detection in the reductive mode with resin adsorption to reduce the detection limits for explosives in water. In attempting to refine this method, Bicking (1987) concluded that poor performance of the electrochemical detector limited applicability such that "routine implementation of the procedure may be difficult for contractor laboratories."

Krull et al. (1981) described an HPLC-electron capture detector (ECD) system for the analysis of post-blast explosive residues. The eluent from the HPLC was split, with one portion going to a 300°C oven where the eluent was evaporated before being directed into the ECD with oxygen-free nitrogen gas. Only NP operation was possible since polar RP eluents were not compatible with ECD operation. This limitation was recently addressed by Maris et al. (1988), who described a post-column extraction technique whereby analytes eluted under RP conditions could be continuously extracted into a nonpolar solvent which was compatible with the ECD. While the ECD is attractive due to its high sensitivity for nitroaromatics and nitramines, routine use with HPLC, particularly in the RP mode, has not been demonstrated.

The major reason for interest in TEA, ECD and electrochemical detectors has been the concern that UV detectors are not sufficiently selective to enable determination of nitroaromatics and nitramines without substantial interference from other environmental contaminants. In

addition, much of the referenced research was directed at forensic applications where any sensitivity advantage was worth the additional expense. For routine environmental analysis, however, HPLC-UV has remained popular. Bongiovanni et al. (1984) reported a method for trace analysis of HMX, RDX, tetryl, TNT, 2,4-DNT and 2,6-DNT in soil. Separation was achieved on a C_{18} column using 40:60 methanol-water and detection by UV at 254 nm. The method was successfully applied to a wide variety of field-contaminated soils. Brueggemann (1985) reported a similar method for the determination of SEX, HMX, TAX, RDX, tetryl, TNT, 2,6-DNT and 2,4-DNT in furnace ash from the thermal destruction of munitions. Separation was achieved on a C_{18} column operated in the gradient elution mode using methanol and water. A UV detector at 254 nm was used for determination. Detection limits quoted in both studies were about 1 μg/g.

Cragin et al. (1985) also reported a method for RP-HPLC determination of explosives in soil. The authors separated TNT, RDX and HMX on a C_8 column using water-methanol-acetonitrile (50:40:10) with UV determination at 230 nm. Analyte concentrations ranging from low μg/g to % levels were determined using this method.

Jenkins et al. (1984, 1986) also published a method for determining HMX, RDX, TNT and 2,4-DNT in munitions wastewater and groundwater. A C_8 column was used with a ternary eluent composed of 50:38:12 water-methanol-acetonitrile. After a full-scale collaborative test (Jenkins et al., 1984; Bauer et al., 1986) the method was accepted by the Association of Official Analytical Chemists as a standard method for this determination (AOAC, 1986).

2.4.4 Method Selection

Of the various options described, RP-HPLC with UV detection was selected for method development. The known thermal instability of most of these analytes makes HPLC a better choice for routine analysis than GC. In addition the vapor pressures for these substances vary over a range which includes many other commonly encountered organic environmental contaminants. Thus any GC-based method, with the possible exception of those using the TEA, would require a clean-up step. Many HPLC procedures potentially eliminate this feature, largely because separations are based on the polarity of these analytes, which differ from the most common suite of organic environmental pollutants.

The RP mode of HPLC was selected over NP due to the generally better resolution and lower detection limits reported and the ability to directly analyze polar solvents which are the most efficient extracting solvents for these analytes. A solvent exchange step would be necessary for NP-HPLC or for most GC methods, and this increases the complexity and cost of a method and usually reduces analytical precision. UV detection was selected based largely on the availability of such equipment and its demonstrated utility for environmental determination of these analytes. Because UV detection is potentially prone to interferences, the possible need for a clean-up step or confirmation procedure will be investigated.

CHAPTER 3

EXPERIMENTAL SECTION

3.1 Chemicals

All analytical standards for HMX, RDX, TNB, TNT, tetryl and 2,4-DNT were prepared from Standard Analytical Reference Materials (SARM) obtained from the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), Aberdeen Proving Ground, Maryland. All SARMs are greater than 98 mole % pure. Standards were dried to constant weight in a vacuum desiccator over dry calcium chloride in the dark.

The methanol and acetonitrile used to extract the soils and to prepare the mobile phase for HPLC determinations were either Mallinckrodt ChromAR HPLC or Baker HPLC grade. Water used to dilute extracts and to prepare the mobile phase was purified using a Milli-Q Type I Reagent-Grade Water System (Millipore Corporation). Methanol, acetonitrile and water were combined in the proper proportions and vacuum filtered through a Whatman CF-F microfiber filter to remove particulates and to degas the mobile phase.

3.2 Soils

Field-contaminated soils were obtained from the Iowa Army Ammunition Plant (Middletown, Iowa), the Louisiana Army Ammunition Plant (Shreveport, Louisiana), the Milan Army Ammunition Plant (Milan, Tennessee), the Nebraska Ordnance Works (Meade, Nebraska) and Weldon Springs Ordnance Works (Weldon Springs, Missouri). The soils were air dried to constant

weight at room temperature, ground with a mortar and pestle, passed through a No. 30 mesh sieve, and thoroughly homogenized in bottles placed on a roller mill or shaken thoroughly over a period of several days. Soil descriptions are given in Table 3.1 along with percent clay and percent organic carbon when a sufficient amount of soil was available to determine these parameters. Percent clay was determined by the standard hydrometer technique and percent organic carbon by standard C, H and N analysis, after explosive residues were extracted with methanol and the solvent removed by evaporation.

Standard soil obtained from USATHAMA was used for spike-recovery studies. Analysis indicated it was free of interferences for the analytes investigated in this study.

3.3 Instrumentation

All the RP-HPLC determinations were made on a modular system composed of the following:

1. A Perkin-Elmer series 3 or Spectra-Physics SP8810 pump.

2. A Dynatech Precision Sampling Model LC-241 autosampler containing a Rheodyne Model 7010A sample loop injector, or a manual Rheodyne 7125 loop injector equipped with a 100-μL sampling loop.

3. Either a Spectra-Physics Model SP8300 UV-254-nm fixed-wavelength detector, a Perkin-Elmer LC-65T variable-wavelength UV detector or a Spectra-Physics Model SP8490 variable-wavelength detector set at 254 nm.

4. A Hewlett Packard 3393A digital integrator equipped with a Hewlett Packard 9114B disk drive.

5. A Linear Model 500 strip chart recorder.

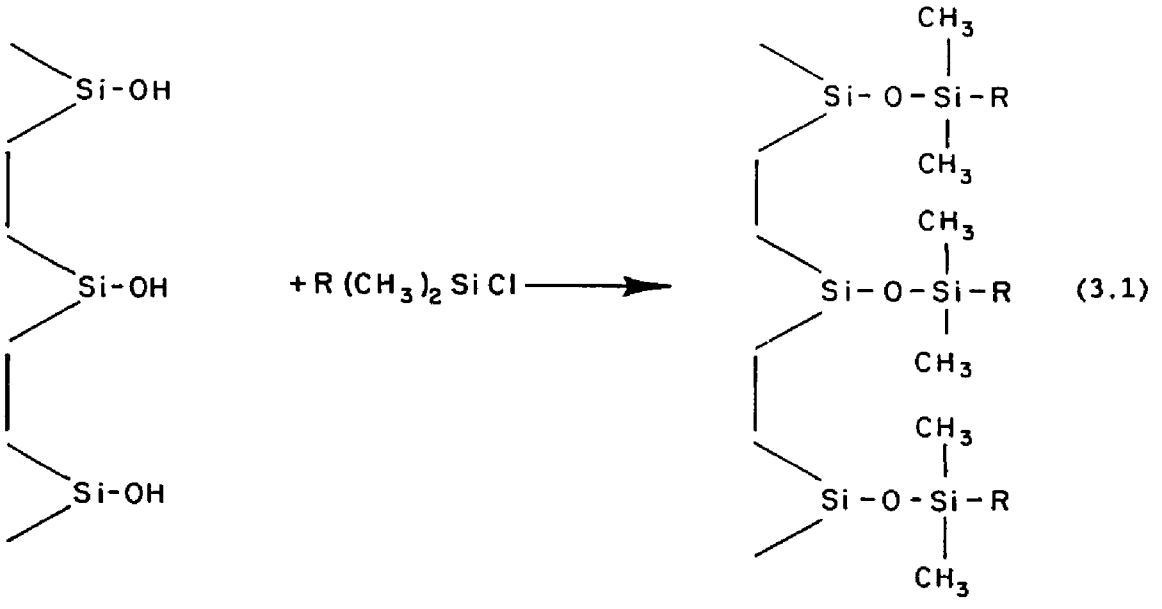
Table 3.1. Soils used in the method development

Soil No.	Description	Clay (%)	Organic Carbon (%)
Iowa AAP No. 1	Demolition area	67.9	<0.5
Iowa AAP No. 2	Surface of disposal lagoon	60.3	3.00
Iowa AAP No. 3	Surface of disposal lagoon	52.5	2.25
Iowa AAP No. 4	Soil near melt and pour buildings	65.3	1.25
Iowa AAP No. 5	Drainage ditch	56.6	1.37
Iowa AAP No. 6	Surface of ordnance-burning area	52.1	0.70
Iowa AAP No. 7	Control soil (uncontaminated)	48.6	2.62
Louisiana AAP 11	Sediment from disposal lagoon		
Louisiana AAP 12	Soil next to disposal lagoon		
Milan AAP 10	Subsurface soil near disposal lagoon		
Milan AAP 13	Surface of burning area		
Milan AAP 14	Subsurface (4-6 in.) below burning area		
Milan AAP 15	Soil near disposal lagoon		
Milan AAP 16	Subsurface (4-6 in.) below burning area		
Milan AAP 17	Soil near disposal lagoon		
Nebraska D-49	From Nebraska Ordnance Plant		
Nebraska D-16	From Nebraska Ordnance Plant		
USATHAMA standard soil	Control soil (uncontaminated)	53.6	1.45
New Hampshire soil	Windsor sandy loam (uncontaminated)	7.5	0.8
Indiana soil	Control soil (uncontaminated)		
Missouri soil	From Weldon Springs site		

3.4 Reversed-Phase HPLC Columns

Separations were obtained on several 25-cm by 4.6-mm (5 μm) RP-HPLC columns made by Supelco. These columns included LC-8, LC-18, LC-1, LC-CN, LC-DP and LC-diol. Quantitative results were obtained using one of the following column-eluent combinations: LC-18 using 1:1 (V/V) methanol-water, LC-CN using 1:1 methanol-water, or LC-8 using 50:38:12 water-methanol-acetonitrile. Samples were introduced by overfilling either a 20-μL or a 100-μL sampling loop.

Reversed-phase columns are prepared from microparticulate silica which has been reacted with a chloro-organo-silane as shown in equation 3.1



(3.1)

to form a modified surface in which the reactive silanol groups have been largely replaced by an organo-silane. For LC-18 the R group is an n-octadecyl group. The R groups for other RP columns are listed in Table 3.2.

Table 3.2 R groups for reversed-phase columns.

Column type	R group
LC-18	n-octadecyl
LC-8	n-octyl
LC-1	methyl
LC-CN	cyanopropyl
LC-DP	diphenyl
LC-diol	3-glycerylpropyl

3.5 Soil Extraction Devices

Solvent extraction of soil was accomplished with methanol or acetonitrile using one of the following:

(a) Burrell Model 75 wrist-action shaker.

(b) Cole-Parmer Model 8845-60 ultrasonic bath operated at 55,000 cycles/s at 200 W.

(c) Brinkman Model PT 10/35 soil-plant homogenizer with PTA 205 generator operated at an intermediate setting 4.

(d) Soxhlet extractor using Whatman cellulose extraction thimbles.

A Vanlab Model K-55-G vortex mixer was used to initially disperse the soil in the extracting solvent prior to use of the wrist-action shaker or bath sonifier.

CHAPTER 4

ANALYTICAL SEPARATIONS

4.1 RP-HPLC Column Selection

Initial work centered on finding an RP-HPLC column that would separate the principal analytes from each other and from potential interferences. The principal analytes identified in a number of contaminated soils were HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT. Potential interferences, known to be present in munitions wastewater or formed by decomposition, are octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX), hexahydro-1-(N)-acetyl-3,5-dinitro-1,3,5-triazine (TAX) and cyclohexanone (Stidham, 1979), other isomers of dinitrotoluene (Gehring and Shirk, 1967; Leggett et al., 1977), and the aminodinitrotoluenes and diaminonitrotoluenes (McCormick et al., 1976; Pereira et al., 1979; Spanggord et al., 1982). A secondary objective was to find a second RP-HPLC column that would give a very different elution order for the primary analytes, to serve as a confirmation column.

Tests were conducted with the following reversed-phase columns: LC-8, LC-18, LC-1, LC-DP, LC-diol and LC-CN. Eluents tested were various combinations of water-acetonitrile, water-methanol and ternary phases of water-methanol-acetonitrile. LC-8 using water-methanol or the ternary phase (Jenkins et al., 1986) gave good separations for HMX, RDX, TNB and TNT but failed to adequately separate TNT and tetryl (Appendix Table A1). A mobile phase of water-acetonitrile was unable to separate HMX and RDX.

LC-18 and LC-8 gave similar orders of elution, but TNT and tetryl were separated by over a minute with LC-18 (Fig. 4.1) using an eluent of 1:1 (V/V) water-methanol. The excellent separation for other major analytes using LC-8 was retained or improved using LC-18. However, several potential impurities do interfere. For example, SEX elutes only 0.04 minutes before HMX; 2,6-DAm-NT also elutes at about the same time as HMX, and 2,4,5-TNT elutes with TNT (Table 4.1).

The LC-1 and LC-DP columns were also tested with various combinations of methanol-water and the ternary mixture. Neither was successful in separating TNT and tetryl, and the overall performance was poorer than that of either LC-8 or LC-18 (Appendix Table A1, Table 4.1).

The LC-diol column was tested using eluents composed of methanol-water, acetonitrile-water and 100% water. Separations were very different from those on the LC-8, LC-18, LC-1 or LC-DP columns. In general, solvent strengths had to be reduced significantly to obtain any useful separations. The best separation was with an eluent of 95% water and 5% acetonitrile (V/V). TNT was separated from tetryl using this eluent, but for soil extracts in acetonitrile, it would be necessary to dilute the extract at least 10 to 1 with water so that the separation would not be degraded by the solvent strength of the injected sample. Consequently, LC-diol was rejected for this application.

The LC-CN column was tested with mobile phases consisting of various combinations of methanol-water, acetonitrile-water and the ternary mixture. Using 1:1 water-methanol (Fig. 4.1), separation of the primary analytes was good (with the exception of TNB and DNB, and TNT and 2,4-DNT), and the elution order was very different from the LC-18 column (Table 4.1). For example, HMX elutes first on the LC-8 and LC-18 columns

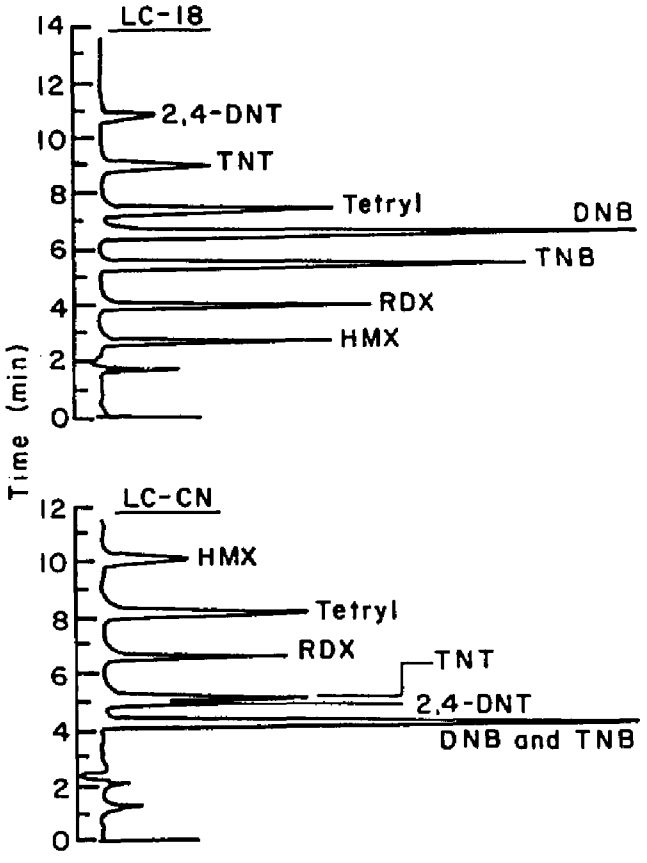


Fig. 4.1. Chromatograms of major analytes on LC-18 and LC-CN eluted with 1/1 (V/V) methanol-water at 1.5 mL/min

Table 4.1. Retention times and capacity factors for primary analytes and potential interferences on LC-18 and LC-CN columns using a flow rate of 1.5 mL/min 1:1 methanol/water.

Substance	Retention time (min)		Capacity factor (k')*	
	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
TNB	5.11	4.05	2.12	0.71
DNB	6.16	4.18	2.76	0.76
TNT	8.42	5.00	4.13	1.11
2,4-DNT	10.05	4.87	5.13	1.05
tetryl	6.93	7.36	3.23	2.11
NG	7.74	6.00	3.72	1.53
NB	7.23	3.81	3.41	0.61
m-NT	14.23	4.45	7.68	0.88
p-NT	13.26	4.41	7.09	0.86
o-NT	12.26	4.37	6.48	0.84
2-Am-DNT	9.12	5.65	4.56	1.38
4-Am-DNT	8.88	5.10	4.41	1.15
SEX	2.40	5.07	0.46	1.14
TAX	2.78	3.70	0.70	0.56
2,4,5-TNT	8.44	5.89	4.15	1.49
2,4-DAm-NT	3.16	4.20	0.93	0.77
2,6-DAm-NT	2.39	3.70	0.46	0.56
2,6-DNT	9.82	4.61	4.99	0.95
benzene	11.22	3.48	5.84	0.47
toluene	23.0	3.93	13.02	0.66

* Capacity factors are based on an unretained peak for nitrate at 1.64 min on LC-18 and 2.37 min on LC-CN.

and elutes last among the primary analytes on the LC-CN column. RDX, which elutes ahead of TNT on the other columns, elutes after TNT on LC-CN. TNT and tetryl are very well separated on LC-CN. The LC-CN also resolves TNT and 2,4,5-TNT very efficiently; the LC-18 column was unable to effect this separation. LC-CN also separates HMX from TAX and the diaminonitrotoluenes, which interferred with HMX on LC-18 (Table 4.1). However LC-CN is unsuitable as the primary analytical column because a number of major analytes and interferences coelute. For example, TNT is not well separated from either of the two tested isomers of dinitrotoluene; TAX and TNB are not well separated nor are RDX and 2,4,5-TNT (Table 4.1).

The conclusion from these tests was to use an LC-18 column as the primary analytical column for quantitative results and the LC-CN to confirm peak identities. The eluent for both columns should be 1:1 methanol-water. Elution times for all the analytes of interest on the LC-18 column using 1:1 methanol-water are approximately 75% shorter than for the 40:60 methanol-water used by Bongiovanni et al. (1984), yet separations are adequate. Where two channels of HPLC equipment are available, the primary determination and confirmation can be conducted simultaneously using a common eluent.

The utility of a second column for analyte confirmation requires that the mechanism of separation on the two columns be different. Otherwise analytes and interferences would elute together on both columns, and the second column would provide no additional information.

In gas chromatography, second-column confirmations have long been used for analyte identifications because different separations are easily

accomplished through the use of columns differing in polarity. In contrast, RP-HPLC separations are based on solvophobic behavior, where retention correlates strongly with octanol-water partition coefficients (K_{ow}). McDuffie (1981) demonstrated that this relationship was so predictable that K_{ow} values could be estimated by correlation with RP-HPLC capacity factors (corrected retention times, K'). An example of this type is shown in Figure 4.2, where log K' values for nine nitroaromatic compounds on LC-18 are plotted versus their K_{ow} values (Table 4.2). An excellent correlation with a positive slope was found ($R^2 = 0.963$), indicating that retention times increase systematically with increasing values of K_{ow}. Thus, normal solvophobic behavior is observed for these nitroaromatic compounds on LC-18 where retention time increases with decreasing polarity. This makes sense for LC-18 because the silica surface is covered by bonded long-chain alkyl groups which are very nonpolar. Table 4.2 contains the experimental K' values and literature K_{ow} values for the nine nitroaromatics plotted in Figure 4C. K_{ow} values for seven other nitroaromatics and nitramines, whose values were not available in published K_{ow} tabulations, are estimated based on the correlation equation presented in Figure 4.2.

A correlation of the log K' for nitroaromatics and nitramines on LC-CN versus log K_{ow} is shown in Figure 4.3. This correlation is much poorer, and the behavior is nearly opposite to that found for LC-18. The slope of the correlation equation for LC-CN is negative, indicating that retention increases with increasing solute polarity for this series of compounds. A similar relationship for LC-CN is presented for chlorinated organics, benzene and toluene in Figure 4.4. For these nonpolar com-

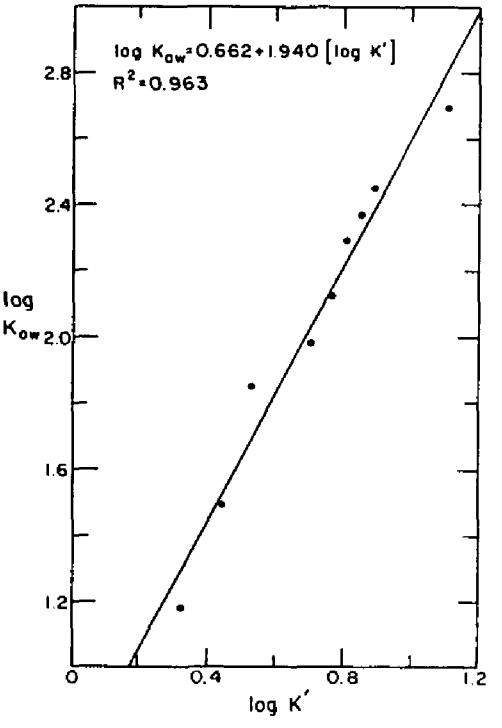


Fig. 4.2. Correlation between log octanol-water partition coefficient (K_{ow}) and log capacity factor (K') for nitroaromatics on LC-18

Table 4.2. Relationship of capacity factors (K') on LC-18 column with octanol-water partition coefficients (K_{ow}).

Substance	K'	log K'	log K_{ow}	K_{ow}
1,3,5-trinitrotoluene	2.1	0.326	1.18*	15.1
1,3-dinitrotoluene	2.76	0.441	1.49*	30.9
nitrobenzene	3.41	0.533	1.85*	70.8
2,4-dinitrotoluene	5.13	0.710	1.98*	95.5
benzene	5.84	0.766	2.13*	135
o-nitrotoluene	6.48	0.812	2.30*	200
p-nitrotoluene	7.09	0.851	2.37*	234
m-nitrotoluene	7.68	0.885	2.45*	282
toluene	13.0	1.11	2.69*	490
HMX	0.49	-0.31	0.061**	1.15
RDX	1.27	0.104	0.86**	7.31
2,4,6-trinitrobenzaldehyde			1.16**	14.4
tetryl	3.23	0.509	1.65**	44.6
nitroglycerine	3.72	0.571	1.77**	58.7
2,4,6-trinitrotoluene	4.13	0.616	1.86**	72.0
4-amino-2,6-dinitrotoluene	4.41	0.644	1.91**	81.5
2-amino-4,6-dinitrotoluene	4.56	0.659	1.94**	87.2
2,6-dinitrotoluene	4.99	0.698	2.02**	104

* From Hansch and Leo (1979).

** Estimates obtained from correlation shown in Figure 4.3.

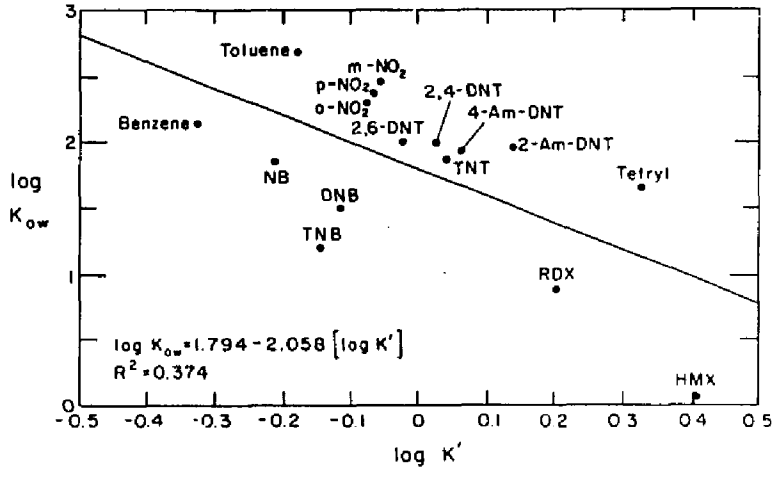


Fig. 4.3. Correlation between log octanol-water partition coefficient (K_{ow}) and log capacity factor (K') for aromatics, nitroaromatics and nitramines on LC-CN column

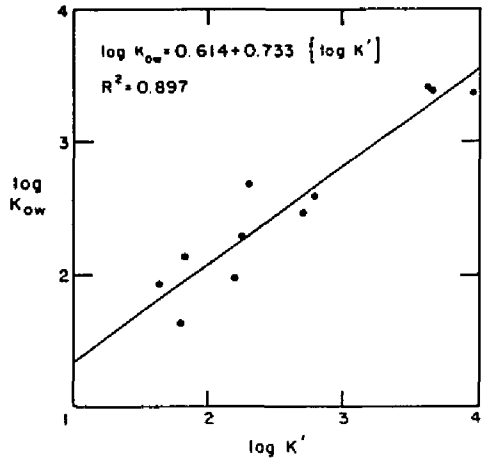


Fig. 4.4. Correlation between log octanol-water partition coefficient (K_{ow}) and log capacity factor (K') for aromatics and chlorocarbons on LC-CN column

pounds, the correlation is much better than for the nitroaromatics and nitramines, and retention on LC-CN parallels that found on LC-18.

Recalling that the silica surface of the particles used in the LC-CN column are covered with bonded cyanopropyl groups, the retention behavior seems to indicate that two types of interactions occur. For nonpolar compounds such as the chlorinated organics, benzene and toluene, solvophobic interaction is occurring through the alkyl portion of the cyanopropyl group. Thus retention times for these compounds increase with decreasing solute polarity. The correlation shown in Figure 4.4 is excellent, indicating that retention is dominated by this single type of interaction.

For the more polar nitroaromatics and nitramines, solute interaction is probably strongest at the polar -CN portion of the cyanopropyl group. Thus, retention times tend to increase with increasing solute polarity, although the correlation observed with log K_{ow} is not nearly as good (Fig. 4.3) as with the chlorinated compounds (Fig. 4.4). This indicates that some additional interaction probably occurs with the nonpolar alkyl portion of the cyanopropyl group but to a varying extent depending on the specific solute. Thus a two-column identification/confirmation sequence has been successfully demonstrated where the mechanisms of separation on the two columns are substantially different.

4.2 UV Wavelength Selection

As discussed in Chapter 2, the UV detector is the best compromise choice for determination of nitroaromatic and nitramine analytes by RP-HPLC. Proper wavelength selection is important to maximize sensitivity while minimizing interference from other potential co-elutants. The λ_{max}

values for these seven analytes in methanol are shown in Table 4.3. Based on λ_{max} alone, a wavelength near 230 nm would be a good choice.

Another criterion for wavelength selection is the availability of instrumentation. In addition to variable-wavelength detectors, a variety of fixed-wavelength 254-nm detectors have been commercially available for many years. The popularity of this fixed-wavelength-detector is due to the simplicity of building a monochromatic detector based on mercury emission at 253.6 nm. This detector is often used for determining aromatic compounds since many other nonaromatic compounds are transparent or only absorb weakly at 254 nm.

A ratio of the absorptivity at 230 and 254 nm for the seven major analytes in the 1:1 (V/V) methanol-water mobile phase is included in Table 4.3. In all cases the absorptivity is greater at 230 nm, with ratios ranging from 1.16 for DNB to 2.85 for HMX. While methanol-water is reasonably transparent in the UV at 230 and 254 nm, its absorptivity increases substantially as wavelengths are reduced below 230 nm. Thus the use of wavelengths below 230 nm is subject to increased noise levels from flow rate fluctuations.

A wide variety of organic pollutants may be encountered in soil analysis. Consequently, a major concern in method development is the potential for interference. This is why a second column analyte confirmation was recommended. As discussed in Section 4.1, the retention characteristics for specific compounds on LC-18 can be predicted on the basis of their log K_{ow} values. Compounds that could interfere with the determination of these seven analytes on LC-18 should have log K_{ow} values ranging between 0 and 2 (Table 4.2). Hansch and Leo (1979) have tabulated log K_{ow} values for a wide variety of organic compounds. Among the

Table 4.3. Spectral characteristics for seven primary analytes.

Compound	UV λ_{max} (nm) in methanol	Response Ratio (1/1 V/V MeOH/water) 230 nm/254 nm
TNT	226.0 (1) (ϵ=18700)	1.45
RDX	235 (2)	1.66
HMX	< 220 (2)	2.85
TNB	222.8 (1) (ϵ=27200)	2.01
DNB	233.8 (1) (ϵ=17700)	1.16
Tetryl	225 (3) (in ethanol)	1.79
2,4-DNT	240.0 (1) (ϵ=14300)	1.76

(1) Spanggord et al. (1978)
(2) Dalton (1981)
(3) Yinon and Zitrin (1981)

compounds whose log K_{ow} values fall in this range, the most important classes of potential interferences are low-molecular-weight halogenated alkanes, medium-molecular-weight aliphatic amines, alcohols and ketones, and phenol and aniline and their mono-nitro substituted analogs. Except for the aromatic anilines and phenols, the other groups do not significantly absorb 254 nm. Many individuals in these groups, however, absorb weakly at 230 nm, increasing the potential for interference if 230 nm is chosen. Some components of natural organic matter may also absorb at these wavelengths, however, they should be well separated from the analytes of interest on both columns.

Since the low-molecular-weight halogenated alkanes are fairly common environmental pollutants and the potential for interference is an overriding concern, 254 nm was selected as the best compromise choice for analyte determination. When an increased sensitivity of a factor of 2-3 is advantageous and samples involve a relatively clean matrix, 230 nm may be acceptable. It should be pointed out that the response ratios presented in Table 4.3 can be used as an additional criterion for confirmation of analyte identity. This may be particularly useful for situations not resolvable using the two column approach discussed in Section 4.1.

CHAPTER 5

INSTRUMENT CALIBRATION

5.1 Preparation of Standards

Analytical stock standards of TNT, RDX, HMX, TNB, DNB, tetryl and 2,4- DNT were prepared by carefully weighing out approximately 100 mg of each dried SARM to the nearest 0.01 mg, transferring it to individual 100-mL volumetric flasks and diluting to volume with acetonitrile. Flask closures were wrapped with Parafilm to retard evaporation, and the flasks were stored at 4°C in the dark.

Combined analyte stock standards were prepared by pipetting 10.0 mL of the TNT, TNB and DNB stock standards, 1.00 mL of the 2,4-DNT stock standard and 20 mL of the HMX, RDX and tetryl stock standards into a 100-mL volumetric flask. This solution contained about 100 μg/mL of TNT, TNB and DNB, about 200 μg/mL of HMX, RDX and tetryl, and about 10 μg/mL of 2,4-DNT. The solution was stored at 4°C in the dark.

For testing the linearity of calibration curves, a series of standards was prepared by pipetting the volumes given in Table 5.1 into individual volumetric flasks.

For each working standard, 10.0 mL of standard and 10.0 mL of water was added to a glass scintillation vial using glass pipets. The vials were capped, shaken and allowed to stand 15 minutes prior to injection. These injection standards were actually half the concentrations given in Table 5.1; however, this extra dilution can be ignored since the samples and standards are processed identically.

Table 5.1 Calibration standards.

Std.	Aliquot of combined Analyte Stock (mL)	Size of Volumetric Flask (mL)	Approximate Solution Conc (µg/L) TNT,TNB DNB	HMX,RDX tetryl	2,4-DNT
A	10	25	40,000	80,000	4,000
B	10	100	10,000	20,000	1,000
	Aliquot of Std. B				
C	10	25	4,000	8,000	400
D	10	50	2,000	4,000	200
E	10	100	1,000	2,000	100
F	10	250	400	800	40
G	5	250	200	400	20
H	5	500	100	200	10
I	1	250	40	80	4
J	1	500	20	40	2
K	0.5	500	10	20	1

5.2 Calibration

The calibration injection standards made from solutions C through K (Table 5.1) and a blank were randomly injected in duplicate. Peak areas were obtained from a digital integrator (Appendix Tables A2-A8). Only those concentrations that produced measurable peak areas are reported (Table 5.2). Solution blanks yielded zero response for all seven analytes.

These data were subjected to least-squares regression analysis using both a linear model with an intercept ($y = a + bx$) and a zero-intercept linear model ($y = bx$), where y = peak area and x = concentration. Both regression equations were tested for lack of fit to determine if the linear models adequately described the data. For all the analytes except TNB, the F ratio for lack of fit relative to random error was less than the critical value for 95% confidence, yielding the conclusion that linear models did adequately represent these data over the concentration ranges given in Table 5.2. A model calculation using the HMX results is shown in Table 5.3.

A test was then conducted to determine if the intercepts obtained using the model with an intercept were significantly different from zero at the 95% confidence level. This was done by comparing the difference in the residual sum of squares for the model through the origin and the residual sum of squares of the model with an intercept to the residual mean square for the model with an intercept. A model calculation for HMX is shown in Table 5.3. The F ratios for all cases except TNB were below the critical values at the 95% confidence levels (Appendix Table A9). Therefore, we conclude that linear models through the origin adequately

Table 5.2. Concentration ranges tested and linear ranges obtained using integrator peak areas.*

Analyte	Lowest Standard Tested (µg/L)	Lowest Standard Measureable (µg/L)	Highest Standard Tested (µg/L)	Linear Range** (µg/L)
HMX	20.2	202	8096	202-4048
RDX	21.2	21.2	8480	21.2-8480
TNB	9.2	19.4	3888	19.4-3888†
DNB	10.4	10.4	4176	10.4-4176
Tetryl	21.1	211	8448	211-4224
TNT	10.2	20.4	4076	20.4-2038
2,4-DNT	1.56	15.6	624	15.6-624

* 254-nm UV detector, LC-18 column using 1:1 water-methanol at 1.5 mL/min, 100-µL injection volume.
** Determined using lack-of-fit statistics at the 95% confidence level.
† Lack of fit was significant for zero-intercept model.

Table 5.3. Example of lack-of-fit and zero-intercept tests for HMX.

Model With Intercept: $y = 2231.15 + 340.174\ x$

Source of Variation	SS	df	MS	F
Residual	96468990	8	12058620	
Total error	38256310	5	7651263	
Lack of fit	58212680	3	19404230	2.54

Critical $F_{0.95}$ (3,5) = 5.41

Model Without Intercept: $y = 340.957\ x$

Source of Variation	SS	df	MS	F
Residual	120586200	9	13398470	
Total error	38256310	5	7651263	
Lack of fit	82329930	4	20582480	2.69

Critical $F_{0.95}$ (4,5) = 5.19

Zero-Intercept Test

$$F = \frac{\text{Difference in residual SS for two models}}{\text{Difference in df in residual for two models}} = 1.8$$
Residual MS for model with intercept

Critical $F_{0.95}$ (1,8) = 5.32

describe the calibration data for six of the seven analytes over the ranges listed in Table 5.2.

For TNB the lack of fit was not significant for the model with an intercept, but it was significant at the 95% confidence level for the zero- intercept model. This was due to excellent replication, particularly at the high end of the concentration range. A plot of the data appears linear (Fig. 5.1), even with the zero-intercept hypothesis. Because the zero intercept linear model was accepted for the other six analytes and no major departure from linearity was observed by inspection of the TNB plot, this model was accepted for TNB as well. Thus a replicated high-range standard can be analyzed and the mean response used to obtain a response factor for each of the seven analytes. This offers major advantages for daily calibration and quality control during routine use.

While the calibration results using peak area measurements demonstrated a wide range of linearity, low analyte concentrations could not be reproducibly measured. With HMX and tetryl, for example, the lowest standards giving reproducible measurements were 202 and 211 μg/L, respectively (Table 5.2). The problem appeared to be due to the inability of the electronic integrator in locating the end of a peak. During discussions with representatives of the integrator manufacturer, the suggestion was made to try using integrator peak height measurements instead of peak areas. Initial tests gave promising results, so a repeat calibration experiment was conducted except that detector responses were obtained in the peak height mode.

The range of standards tested and the lowest concentration standard resulting in measurable peak heights are presented in Table 5.4. For all

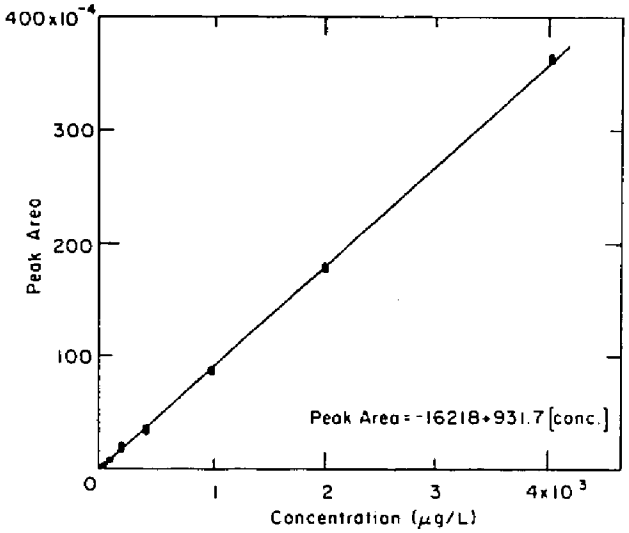


Fig. 5.1. Calibration curve for TNB

Table 5.4. Summary of results of linearity testing using integrator peak height measurements.*

Analyte	Lowest Standard Tested (µg/L)	Lowest Standard Measureable (µg/L)	Highest Standard Tested (µg/L)	Linear Range** (µg/L)
HMX	5.1	20.3	20144	20.3-10072
RDX	5.0	10.0	20074	10.0-10037
TNB	2.5	5.0	20052	5.0-20052
DNB	2.6	2.6	20026	2.6-10013
Tetryl	2.3	4.6	20130	4.6-20130
TNT	2.3	2.3	20264	2.3-10132
2,4-DNT	2.5	2.5	20028	2.5-5007

* 254-nm UV detector, LC-18 column using 1:1 water-methanol at 1.5 mL/min, 100-µL injection volume.
** Determined using lack-of-fit statistics at the 95% confidence level.

analytes it was possible to obtain measurable responses at lower concentrations in the peak height mode. Statistical linearity tests were conducted as described earlier for peak area data, and a linear equation with zero intercept adequately described the data for all analytes except HMX at the 95% confidence level. For HMX, visual inspection of the plot of peak height versus concentration did not reveal detectable departure from linearity up to 10,000 μg/L. Thus it appears that either peak height or peak area measurements are acceptable, but better reproducibility at very low concentrations are obtained in the peak height mode.

These calibration results were also used to estimate detector sensitivity values for the seven analytes at 254 nm. Sensitivities were calculated from peak height measurements of the highest standard considered to be in the linear range. These values, calculated in absorbance units/μg/L, are presented in Table 5.5. Sensitivities at 254 nm varied over a factor of 2.3 ranging from 6.82×10^{-6} to 1.60×10^{-5} absorbance units/μg/L for RDX and DNB, respectively. Sensitivities at 230 nm can be obtained by multiplying the sensitivities at 254 nm (Table 5.5) by the response ratios (Table 4.3).

5.3 Stability of Stock Standards

A major question in all analytical procedures is how often stock standards must be replaced. To address this question I took advantage of the availability of stock standards of these explosives prepared over a period of 19 months. In all cases these stock standards were prepared by weighing out SARM-grade material, transferring it to volumetric flasks, and diluting it to volume with either methanol or acetonitrile. The

Table 5.5. Sensitivities for primary analytes using a 254-nm UV detector, a 100-μL sample loop injector and an LC-18 column.

Analyte	Sensitivity* (Absorbance units/μg/L)
HMX	8.25×10^{-6}
RDX	6.82×10^{-6}
TNB	1.30×10^{-5}
DNB	1.60×10^{-5}
Tetryl	7.13×10^{-6}
TNT	9.52×10^{-6}
2,4-DNT	1.36×10^{-5}

* LC-18 column eluted with 1:1 methanol-water at 1.5 mL/min.

stock standards were stored in a refrigerator at 4°C in the dark, and the stoppers were wrapped with Parafilm to retard solvent evaporation.

Three sets of individual stock standards were tested. The first set was prepared in methanol in August 1985. For the 1985 HMX and RDX stocks, the solution contained 40% acetonitrile to assist in initial dissolution, since these two substances dissolve very slowly in methanol. The second and third sets of standards were prepared in June 1986 and March 1987, and they were diluted to volume with acetonitrile.

In July 1987 the three sets of stock standards were compared as follows. Three replicate composite standards were prepared for each set of stock standards by adding 4.00 mL of each individual stock (3.00 mL for RDX) in a 50-mL volumetric flask (100-mL volumetric flask for the 1986 replicates) and diluting to volume with acetonitrile. Diluted working standards of each combined solution were prepared by diluting 10.00 mL to volume with acetonitrile in a 100-mL volumetric flask.

The diluted working standards were analyzed as usual using the mean integrator response of the working standard to obtain response factors for each analyte. Quantitative results for all diluted working standards were obtained using these response factors. While 2,4-DNT was not intentionally added to the 1986 standard, a small peak eluted at the proper retention time for DNT. We discovered that this impurity originated from the 1986 TNB stock standard. This impurity was also observed in the 1985 TNB stock standard at the same level relative to the response of TNB as in the 1986 stock. Both of these stock solutions were prepared from the same bottle of SARM, so it was probably due to an impurity in the solid. Since the level was the same in both 1985 and 1986 standards, it was not due to decomposition of TNB in solution. In contrast the 1987 TNB stock

was prepared from a different bottle of SARM, and no 2,4-DNT was observed in this stock standard.

The results of the analysis of the various diluted combined standards are presented in Appendix Table A10. The estimates normalized to their expected concentrations are shown in Table 5.6. Except for TNB in the 1986 standard and TNT in the 1985 standard, all recoveries were within 100 ± 5%. The 7% low recovery for the 1986 TNB standard is understandable since it contained a known impurity that amounted to about 4% on a peak area basis, whereas the 1987 standard, on which the response factor was based, did not contain this contaminant. The 6% high recovery of TNT for the 1985 standard appears to be due to replicate a, which also showed a high value for tetryl.

None of the analytes exhibited a consistent trend toward decreasing concentrations as a function of storage time. When an analysis of variance was conducted on the data in Table 5.6, there were significant differences among the years for all analytes. This indicates that our ability to replicate the combination and dilution while preparing working standards from individual stock standards is better than our ability to prepare the stock standards themselves. Replicating the preparation of stock standards involves the reproducibility of the SARM from bottle to bottle as well as long-term stability of the analytical balance used to weigh out the solid.

Overall, the variation in standards prepared and stored over 23 months is minimal. We conclude that stock standards of these explosives stored in glass at 4°C in the dark, with precautions taken to minimize solvent evaporation, can be safely used for up to a year. A replacement schedule of 1 year is recommended.

Table 5.6. Determined concentrations of diluted combined standards normalized to expected values.*

		Normalized concentration						
Standard	Replicate	HMX	RDX	TNB	DNB	tetryl	TNT	DNT
1987	a	1.01	1.01	1.01	1.01	1.00	1.00	1.01
	b	1.00	1.00	1.00	1.00	1.00	1.01	1.00
	c	0.99	0.99	0.99	0.99	0.99	0.98	0.99
	mean	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1986	a	0.95	0.97	0.93	0.97	1.01	0.96	--
	b	0.93	0.93	0.91	0.95	1.01	0.94	--
	c	0.98	0.99	0.96	0.99	1.07	1.00	--
	mean	0.95	0.96	0.93	0.97	1.03	0.97	--
1985	a	1.02	**	0.99	--	1.08	1.09	**
	b	0.99	**	0.96	--	1.03	1.05	**
	c	0.97	**	0.95	--	1.04	1.03	**
	mean	0.99	**	0.97	--	1.05	1.06	**

* Actual determined concentrations presented in Appendix Table A10.
** Volumes of these standards too small to allow confident use of stock.

5.4 Stability of Dilute Working Standards

A question remains as to how often diluted working standards need to be prepared. To test the stability of the dilute working standards, duplicate combined stock standards and duplicate dilute working standards were prepared about every five days over a 28-day period. These dilute working standards were stored at 4°C in the dark over this period. The stoppered joints were wrapped with Parafilm to retard evaporation. Another set of duplicates was prepared at the same time as those for day 28, but they were warmed to room temperature and a small portion was removed every five days to simulate a working standard that was being used over this 28-day period. The 16 individual working standards were analyzed as a group in random order on the day following the last preparation. Response factors were obtained from the mean responses of the most recent working standard. The results are presented in Table 5.7. Each concentration represents a mean of two determinations.

An analysis of variance was done for each of the seven analytes. For all the analytes except tetryl, differences were not statistically significant at the 95% confidence level, in spite of excellent agreement between duplicates. Relative standard deviations ranged from 0.52 to 1.15%. For tetryl a statistically significant difference was observed ($F = 4.7$ compared to a table value $F_{0.95}(7,8) = 3.5$). A least-significant-difference computation indicated that only the standard stored for 24 days was significantly different from the most recent standard, while those stored 28 days were not significantly different. Thus the results for tetryl are inconsistent and suggest that the 24-day result was

Table 5.7. Results of working standard stability study.

Days after preparation	Concentration (μg/L)						
	HMX	RDX	TNB	DNB	tetryl	TNT	2,4-DNT
1	3108	3522	3189	3232	3368	3315	3225
	3132	3518	3199	3244	3294	3309	3239
6	3097	3478	3178	3206	3086	3269	3210
	3120	3501	3184	3235	3314	3346	3251
10	3091	3462	3174	3214	3055	3274	3213
	3115	3493	3192	3224	3075	3257	3204
15	3108	3448	3180	3233	3054	3273	3205
	3102	3467	3190	3102	2966	3265	3210
20	3101	3493	3161	3203	3214	3242	3203
	3120	3473	3189	3211	3355	3300	3233
24	3077	3452	3190	3202	2899*	3233	3190
	3117	3456	3196	3235	3002*	3265	3208
28†	3098	3490	3185	3222	3356	3280	3233
	3107	3478	3189	3227	3205	3283	3231
28	3061	3412	3159	3196	3186	3260	3193
	3115	3475	3217	3246	3069	3278	3228

* Significantly different from freshest standard at the 95% confidence level using a least-significant-difference test.

† Aliquot withdrawn at periods corresponding to 24, 20, 15, 10, 6 and 1 day to simulate a working standard being used over the period.

anomalous. We conclude that working standards can be prepared and used over a 28-day period if they are refrigerated and kept in the dark when not in use.

CHAPTER 6

SOIL EXTRACTION

6.1 Comparison of Extraction Techniques and Solvents

All experiments to develop extraction methodology used field-contaminated soils from a variety of Army installations (Table 3.1). All soils were air dried, ground, sieved and homogenized as thoroughly as possible to reduce the error associated with replicate subsampling.

The first two sets of experiments compared extraction methodologies using either methanol or acetonitrile where soils were dispersed with a wrist-action shaker, an ultrasonic bath or a soil-plant homogenizer and compared with classical Soxhlet extraction. These experiments utilized two field-contaminated soils from the Iowa Army Ammunition Plant (AAP soils #2 and #6, Table 3.1). The first experiment was designed to give information on the kinetics of the extraction processes. In the second study, referred to as the replication study, the extraction efficiency of the four techniques and two solvents were compared using extensive replication and analysis of variance (ANOVA) of the results. Samples were processed for the four techniques as described below.

6.2 Wrist-Action Shaker

For the kinetic study, two 2.00-g samples of soil were placed in 2.5-cm x 20-cm screw-cap glass test tubes followed by 50.0 mL of either methanol or acetonitrile containing 545 μg/L 2,4-DNT as an internal standard. The soil was dispersed using a vortex mixer for 1 minute and

then shaken at maximum speed on a wrist-action shaker for periods ranging from 10 minutes to 24 hours for soil 6 and from 30 minutes to 48 hours for soil 2. Periodically the tubes were centrifuged at 3500 rpm for 5 minutes, and 5-mL portions of the supernatant were removed, mixed with 5 mL of water and filtered through a 0.45-μm Gelman Acrodisc CR disposable filter assembly. Soils were redispersed using the vortex mixer and returned to the shaker. For the reproducibility studies, six replicates of each soil were shaken for 24 hours, after which samples were collected and processed as described above.

6.3 Bath Sonifier

For this procedure 2.00-g subsamples were prepared as described in the previous paragraph and placed in a sonic bath for periods ranging from 1 minute to 4 hours for soil 6 and 15 minutes to 7 hours for soil 2. The bath sonifier operated at 55,000 cycles/sec at 200 W. Samples were sonified by the indirect method in which four to six tubes were placed in 1-L beakers which were suspended in a perforated sample basket. Soil particles were constantly in motion throughout sonication. For the replicate studies, a 4-hour equilibration time was used for soil 6 and a 24-hour period for soil 2. Samples were removed and processed as described for the wrist-action shaker.

6.4 Soil-Plant Homogenizer

Two 1.00-g subsamples were placed in 45-mL Pyrex centrifuge tubes, and 25 mL of extracting solvent was added. Each sample was ground in a soil-plant homogenizer for periods ranging from 1 to 16 minutes for soil

6 and 5 to 60 minutes for soil 2. Although significant solvent evaporation was observed, the internal standard corrected for this. For the replicate studies, total grinding times of 10 minutes and 30 minutes were used for soils 6 and 2, respectively.

6.5 Soxhlet Extractor

Two 16.0-g subsamples of soil were placed in Soxhlet extraction thimbles (Whatman, cellulose) and extracted with 400 mL of solvent. The cycle time for the extractors was about 15 minutes. For the kinetic study, samples were withdrawn at periods ranging from 1 to 37 hours for soil 6 and from 1 to 48 hours for soil 2. For the replicate study, a 24-hour extraction period was used for both soils.

6.6 Extraction Kinetics

Kinetic studies were undertaken to determine the time required to approach equilibrium using the three batch extraction techniques and the time required to obtain maximum extraction using the Soxhlet method. The results are presented in Appendix Tables A11 and A12. No critical comparison of final concentrations with respect to solvent or method is possible since different subsamples were used without replication, and, therefore, sampling error may be substantial. Such a comparison will be made later based on replicated trials. To ensure that emphasis is on the shapes of the curves, concentrations have been individually normalized to the highest concentration for that trial. The results for RDX and TNT in soil 6 are presented in Figures 6.1 and 6.2. Maximum times were based on practical constraints. For example, the soil-plant homogenizer was studied only up to 16 minutes since this is a one-at-a-time method and

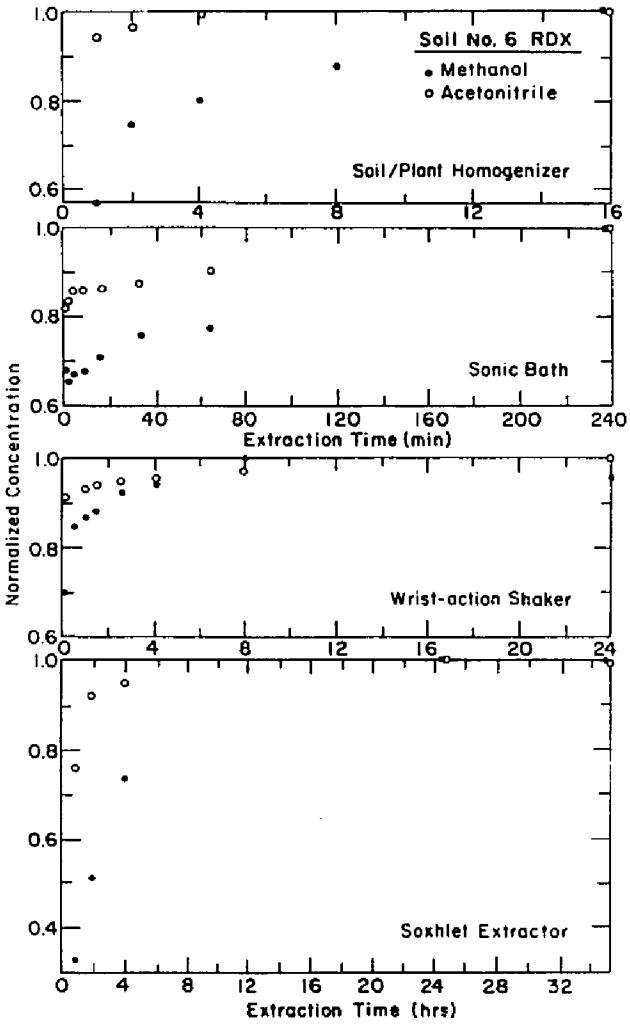


Fig. 6.1. Concentration of extractable RDX as a function of time for various extraction techniques, soil 6

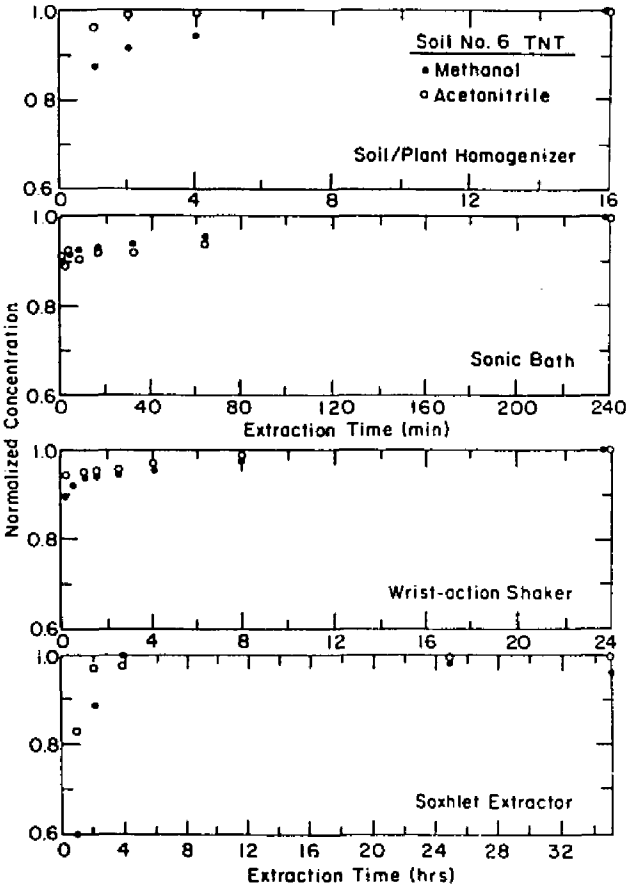


Fig. 6.2. Concentration of extractable TNT as a function of time for various extraction techniques, soil 6

much longer periods seemed impractical. Furthermore, substantial solvent evaporation occurs during grinding.

Because of the very low concentrations in soil 2, equilibration times were increased from those used for soil 6. In particular, the longest time increments for the Soxhlet and wrist-action shaker were extended to 48 hours. The time for the soil-plant homogenizer was extended to 60 minutes despite the conviction that this would be impractical in routine use. The results of this kinetic study are presented in Figure 6.3 for TNT. Only trace levels of TNB, RDX and HMX were observed; since integrated areas exhibited large uncertainty, no attempt was made to plot these data.

With the wrist-action shaker, fairly constant values were obtained for soil 6 after about 4 hours for all four components (see RDX and TNT in Fig. 6.1 and 6.2), the amount of increase from the 4-hour to the 24-hour samples being 5% or less. The results for the bath sonifier indicated a somewhat greater increase in going from a 1-hour to a 4-hour extraction time, particularly for RDX in methanol. With the soil-plant homogenizer, equilibrium appeared to be reached much more quickly for all four analytes using acetonitrile than with methanol, which had generally not leveled off by 16 minutes. For the Soxhlet, values very close to the maxima were generally reached within 4 hours for both solvents. One exception was HMX in methanol, where values increased by a factor of 2.5 in going from 4 to 24 hours (Appendix Table A11).

TNT levels in soil 2 were about a factor of 100 lower than those found for soil 6, and the kinetics of desorption were slower for all four extraction methods. One possible explanation for this behavior is that the low level of TNT present is preferentially adsorbed to high-energy

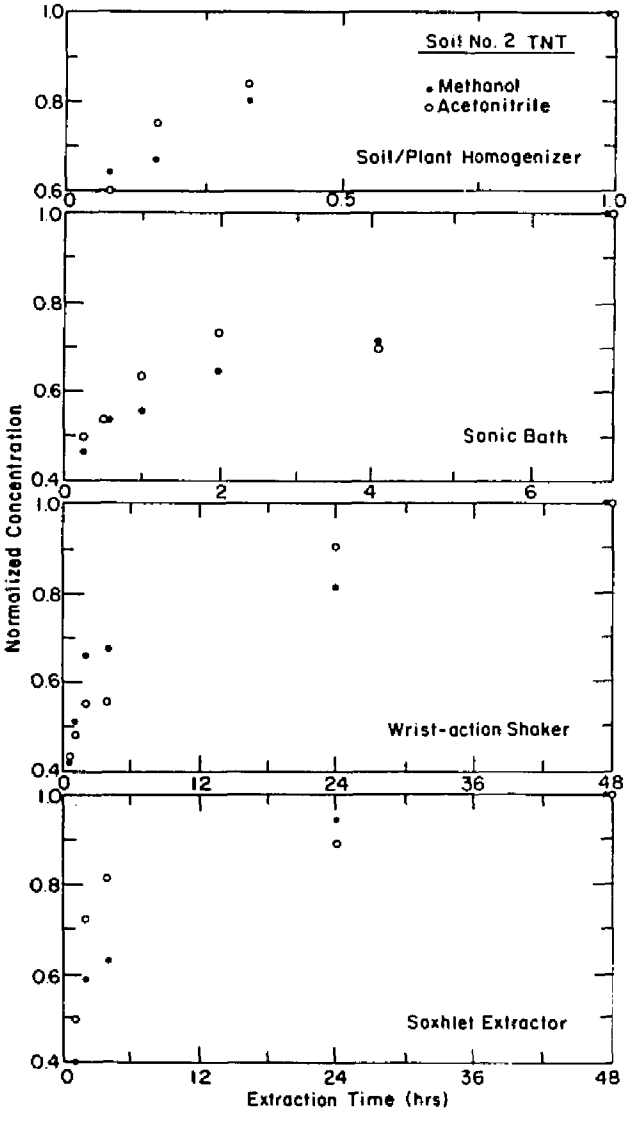


Fig. 6.3. Concentration of extractable TNT as a function of time for various extraction techniques, soil 2

binding sites. Since the abundance of these types of sites is limited, the large amount of TNT associated with soil 6 exceeds what can be adsorbed in this fashion, and the bulk of the TNT may, therefore, be loosely bound. This explanation is consistent with the results obtained by Leggett (1985), where two types of binding sites for TNT were observed on Bentonite clays. The energy barrier for desorption of less tightly bound material should be lower, resulting in faster desorption kinetics for the majority of the TNT sorbed by soil 6.

For soil 2 (Fig. 6.3), it appears that equilibrium was not established during the experiment with either solvent. The Soxhlet and wrist-action shaker come closer to a terminal value for the times studied than do the soil-plant homogenizer or the sonic bath. Little difference was observed between solvents. Clearly the use of the soil-plant homogenizer for extended periods is impractical since it is a one-sample-at-a-time technique; in addition, it results in a large degree of solvent evaporation because the tubes are required to be open to the atmosphere during grinding. The use of the sonic bath for longer periods is possible, however. The wrist-action shaker and Soxhlet appear to have come fairly close to final values after 24 hours, and from a practical point of view, this is about the maximum time acceptable for soil equilibration-extraction.

6.7 Replicate Study

To compare the extraction efficiencies of the four extraction methods and solvents, six subsamples of soil 6 were processed by each method using both methanol and acetonitrile (Appendix Table A13). Equilibration periods were 10 minutes and 4 hours for the soil-plant

homogenizer and sonic bath, respectively, and 24 hours for the wrist-action shaker and Soxhlet. A similar comparison was conducted with soil 2 except that the extraction times for the soil-plant homogenizer and the sonic bath were increased from 10 to 30 minutes and from 4 to 24 hours, respectively (Appendix Table A14). Means and standard deviations for each method and solvent are summarized in Tables 6.1 and 6.2. The statistical significance ($p = 0.05$) of differences among means was established using ANOVA.

Based on the relative standard deviations (RSD) for both acetonitrile and methanol extraction of soil 6, the analytes fall into two distinct groups. The RSD for TNT and TNB is generally in the range of 1-3%, indicating very good analytical precision, as well as good analyte homogenization prior to subsampling. The RSD for RDX and HMX, on the other hand, is generally at or above 20%, and for the homogenizer sometimes over 50%. The mean concentrations for HMX and RDX are about the same as that for TNB, and hence this difference in RSD is apparently not an analytical problem related to concentration. Since replicate injections of solutions do not reflect this pattern of variation, the most likely explanation is that RDX and HMX are distributed less homogeneously than TNT and TNB in this soil. Because of the much larger sample size for the Soxhlet procedure, RSDs are lower compared to the other methods for HMX and RDX in both solvents. In contrast, RSD values for the homogenizer, where 1-g subsamples were used, were generally higher than for the shaker and sonic bath, where 2-g subsamples were used. This behavior is typical for heterogeneously distributed analytes (Grant and Pelton, 1973). However, I am unable to explain why these two analytes

Table 6.1. Comparison of extraction results for soil 6.

Analyte	Method	Extractable* concentration (µg/g)	Standard** deviation (µg/g)	(%) RSD†
		Acetonitrile Extract		
TNT	Shaker	881 a	13	1.4
	Sonic bath	883 a	10	1.1
	Homogenizer	849 b	13	1.5
	Soxhlet	881 a	8	0.9
TNB	Shaker	55 b	1.0	1.9
	Sonic bath	56 b	0.6	1.0
	Homogenizer	51 c	0.8	1.5
	Soxhlet	62 a	1.1	1.8
RDX	Shaker	54 a	10	18
	Sonic bath	55 a	13	24
	Homogenizer	64 a	56	87
	Soxhlet	65 a	11	16
HMX	Shaker	82 a	32	39
	Sonic bath	56 a	14	26
	Homogenizer	65 a	27	41
	Soxhlet	84 a	12	14
		Methanol Extract		
TNT	Shaker	895 a	14	1.6
	Sonic bath	840 b	25	3.0
	Homogenizer	870 a	39	4.4
	Soxhlet	891 a	5	0.6
TNB	Shaker	56 b	0.8	1.4
	Sonic bath	53 c	1.5	2.9
	Homogenizer	53 c	1.1	2.0
	Soxhlet	58 a	0.6	1.1
RDX	Shaker	37 a	12	32
	Sonic bath	40 a	8	20
	Homogenizer	31 a	17	54
	Soxhlet	48 a	5	11
HMX	Shaker	22 a	5	24
	Sonic bath	33 a	11	32
	Homogenizer	28 a	18	64
	Soxhlet	59 a	6	10

* Values for given analyte and solvent flagged with the same letter are not significantly different at the 95% confidence level.

** Standard deviations determined from six replicates used to produce each mean.

† RSD is relative standard deviation.

Table 6.2. Comparison of extraction results for soil 2.

Analyte	Method	Mean* extractable concentration (µg/g)	Standard** deviation (µg/g)	RSD (%)
		Acetonitrile Extract		
TNT	Shaker	2.46 c	0.12	5.0
	Sonic bath	3.54 b	0.22	6.3
	Homogenizer	2.10 d	0.11	5.1
	Soxhlet	4.35 a	0.33	7.5
TNB	Shaker	0.37 a	0.05	13
	Sonic bath	0.45 a	0.11	23
	Homogenizer	0.35 a	0.13	37
	Soxhlet	0.33 a	0.05	15
		Methanol Extract		
TNT	Shaker	2.76 b	0.25	9.1
	Sonic bath	3.91 a	0.19	5.0
	Homogenizer	2.23 c	0.08	3.6
	Soxhlet	3.70 a	0.11	2.9
TNB	Shaker	0.31 a,b	0.09	28
	Sonic bath	0.44 a	0.12	27
	Homogenizer	0.27 b	0.08	29
	Soxhlet	0.27 b	0.06	22

* Values for given analyte and solvent indicated with the same letter are not statistically different at the 95% confidenc level.

** Standard deviations determined from six replicates used to produce each mean.

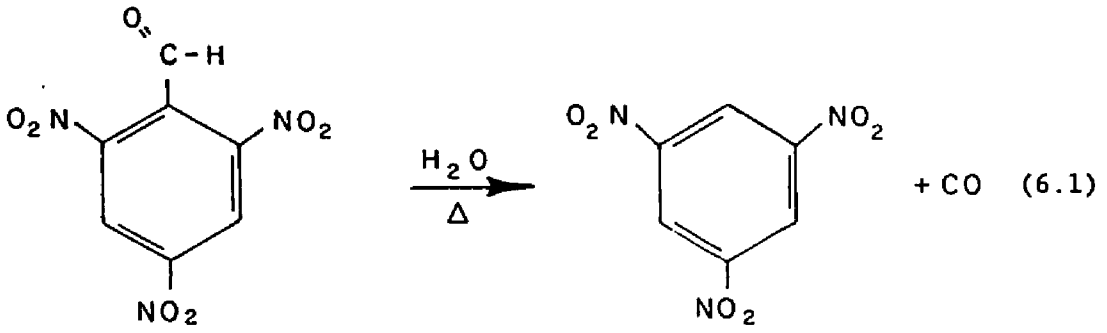
were heterogeneous while TNT and TNB seemed relatively homogeneous in distribution.

Because of the very good precision obtained for TNT, ANOVA indicates a significant difference for the methods at the 95% confidence level. The Soxhlet and wrist-action shaker give very similar results with both solvents, but the soil-plant homogenizer gives significantly lower values with acetonitrile, while the sonic bath is low for methanol. This causes a significant interaction between method and solvent at the 95% confidence level, even though the difference between solvents is not significant overall.

For TNB, excellent precision again enables sensitive comparison, and method, solvent and interaction are all significant at the 95% confidence level (Table 6.1). With both solvents the Soxhlet procedure is distinctly superior to the other three methods with respect to the amount extracted, the largest differences being observed with acetonitrile. The reason for this superiority of the Soxhlet for TNB but not for TNT was not immediately obvious, particularly in view of the similarity in structure of the two substances. However, later research indicated that soil 6 contained 2,4,6-trinitrobenzaldehyde (TNBA) in addition to TNB. TNBA is converted to TNB by decarbonylation (Burlinson, 1980) in the heated reservoir of the Soxhlet but not in the three bath extraction methods (equation 6.1). Thus the higher result obtained by the Soxhlet method for TNB is due to the inclusion of TNBA in the TNB concentration, and therefore the results are inaccurate.

The large uncertainties observed for RDX and HMX make it impossible to assign significance to any small systematic differences observed in

the various methods. Nonetheless, solvent was found to be a significant



(6.1)

effect. For RDX and HMX, acetonitrile consistently yielded a higher extraction efficiency. The solubilities of RDX and HMX are over 20 times higher in acetonitrile than in methanol*; therefore, partitioning should be more favorable into acetonitrile (Freeman and Cheung, 1981). Overall, acetonitrile is superior to methanol for RDX and HMX extraction and is as good as methanol for TNT and TNB.

Mean values and standard deviations for TNT and TNB in soil 2 are presented in Table 6.2. Experimental precision for TNT averaged about 6% (RSD), indicating very good analyte homogenization prior to subsampling. This excellent precision for such a low concentration allowed powerful comparisons using ANOVA. Significant differences were found among methods ($\ell = 0.05$), but as with soil 6, there was no consistent TNT concentration difference between solvents. For acetonitrile the Soxhlet was significantly better than the other three procedures, and the sonic bath was second best. These differences were much greater on a percentage basis than found for the higher concentration in soil 6. For methanol the sonic bath and the Soxhlet were not different, but they extracted

* D.C. Leggett, personal communication.

significantly higher concentrations than the shaker or homogenizer. Again, there was a significant interaction between method and solvent, indicating that some methods worked better in one solvent while others worked better in the other.

For TNB the relative standard deviation was considerably larger than for TNT, averaging over 20%. This is a consequence of the very low levels of TNB present in this soil (about 0.4 $\mu g/g$), which approached the detection limit, estimated to be 0.1 $\mu g/g$. RSD values typically increase as analyte concentrations approach the detection limit (Horwitz, 1982).

ANOVA for the TNB results revealed a significant difference among methods but not for solvent type or method-solvent interaction. This significant difference in methods was only apparent for methanol, where the sonic bath produced significantly higher results than the Soxhlet or homogenizer. The shaker was not significantly different from any of the other methods at the 95% confidence level.

Overall, the bath sonifier using acetonitrile seems to be the best compromise. This is based not only on performance with both soils and the four analytes tested, but from practical considerations such as apparatus and solvent cost, convenience and sample size requirements. In contrast to the Soxhlet, the bath sonifier is also capable of extracting TNBA without conversion to TNB, and as we will see later, using acetonitrile as the extraction solvent, it is capable of extracting tetryl without significant degradation of this thermally labile analyte.

6.8 Optimization of Sonic Bath Extraction

Since acetonitrile and the sonic bath extraction method were chosen as the best overall compromise, the next series of kinetic experiments

was conducted to optimize this procedure. Six Iowa AAP field-contaminated soils with varying properties were studied to better define the length of time required to achieve equilibrium for the ultrasonic bath extraction procedure. The concentrations of explosives residues ranged from detection limits to 15,000 μg/g in these soils.

To conduct these experiments, a 2-g subsample of each soil was weighed out to the nearest 0.01 g and transferred to a 2.5-cm by 20-cm glass screw-cap test tube equipped with a Teflon liner. Aliquots of 50 mL of acetonitrile were added to each test tube, and the soil was extracted as described in the previous section. Five-milliliter aliquots were removed for analysis after 5 minutes, 1, 4, 8, 24 and 48 hours in the sonic bath.

The concentrations of TNT, HMX, TNB, RDX, tetryl and DNB observed in these extracts, expressed on a μg/g-dry soil basis, are shown in Table 6.3. The concentrations of TNT from soils 2, 3 and 4 reached a maximum within 24 hours. The concentration of TNT from soil 6 continued to rise through 48 hours (Fig. 6.4) but the increase was only 3% between 24 and 48 hours. The concentrations of TNT in soils 1 and 5 were too low to provide much information, but it appeared that 24 hours was an adequate extraction time.

The values for HMX showed a similar pattern, with the highest concentrations at 24 hours for soils 3, 4 and 5 and at 48 hours for soil 6. RDX levels reached a maximum by 24 hours for soil 3 and 48 hours for soil 6. A statistical analysis of the data for soil 6 indicated that the mean concentrations for HMX and RDX at 48 hours are not significantly different from the mean values at 8 or 24 hours at the 95% confidence level. Soil 6, obtained from the surface of the ordnance-burning area, may,

Table 6.3. Summary of kinetic study results for TNT, HMX, TNB, RDX, tetryl and DNB in Iowa AAP soils, using the sonic bath extraction method with acetonitrile.

Time	Mean Concentrations (μg/g) Soil #1	Soil #2	Soil #3	Soil #4	Soil #5	Soil #6
			TNT			
5 min	1.16(a)*	2.40(b)	14570	1285	<d(h)	849
1 hr	1.03(a)	2.77(b)	15110(e)	1410(f)	0.67(i)	872
4 hr	1.17(a)	3.96(c,d)	15140(e)	1450(g)	0.32(i)	883
8 hr	0.87(a)	3.52(b,c)	15130(e)	1405(f)	0.17(h,i)	891
24 hr	1.08(a)	4.90(d)	15380(e)	1485(g)	0.63(i)	902
48 hr	1.25(a)	4.67(c,d)	15220(e)	1470(g)	0.39(i)	928
			HMX			
5 min	<d	<d	1963	5330	<d(e)	53.0(f)
1 hr	<d	<d	2042(a,b)*	5580(c)	0.74(e)	55.5(f)
4 hr	<d	<d	2025(a,b)	5595(c)	1.13(e)	54.2(f)
8 hr	<d	<d	2016(a,b)	5580(c)	0.43(e)	56.1(f)
24 hr	<d	<d	2048(b)	5700(d)	2.45	55.0(f)
48 hr	<d	<d	2004(a)	5645(c,d)	<d(e)	59.1(f)
			TNB			
5 min	<d	<d	470	107(c)	<d	52.2(e)
1 hr	<d	<d	514(a)*	122(c,d)	<d	54.9(e)
4 hr	<d	<d	524(a,b)	126(d)	<d	52.8(e)
8 hr	<d	<d	526(b)	118(c,d)	<d	56.4(e)
24 hr	<d	<d	549	119(c,d)	<d	53.2(e)
48 hr	<d	<d	567	116(c,d)	<d	53.5(e)
			RDX			
5 min	<d	<d	13400(a)*	<d	<d	91.5(d)
1 hr	<d	<d	13790(b,c)	<d	<d	94.7(d,e)
4 hr	<d	<d	13740(b,c)	<d	<d	94.2(d,e)
8 hr	<d	<d	13710(b,c)	<d	<d	95.3(e,f)
24 hr	<d	<d	13890(c)	<d	<d	97.4(e,f)
48 hr	<d	<d	13570(a,b)	<d	<d	98.5(f)
			tetryl			
5 min	<d	<d	279	<d	<d	<d
1 hr	<d	<d	329(a)*	<d	<d	<d
4 hr	<d	<d	324(a)	<d	<d	<d
8 hr	<d	<d	325(a)	<d	<d	<d
24 hr	<d	<d	346(a)	<d	<d	<d
48 hr	<d	<d	336(a)	<d	<d	<d

Table 6.3 (Con't.)

Time	Mean Concentrations (μg/g) Soil #1	Soil #2	Soil #3	Soil #4	Soil #5	Soil #6
			DNB			
5 min	<d	<d	37.1	<d	<d	<d
1 hr	<d	<d	42.6(a)*	<d	<d	<d
4 hr	<d	<d	41.6(a)	<d	<d	<d
8 hr	<d	<d	43.5(a)	<d	<d	<d
24 hr	<d	<d	45.2(a)	<d	<d	<d
48 hr	<d	<d	44.5(a)	<d	<d	<d

*Values with the same letter are not significantly different at the 95% confidence level. Values which are not designated with a letter are different from all the others.

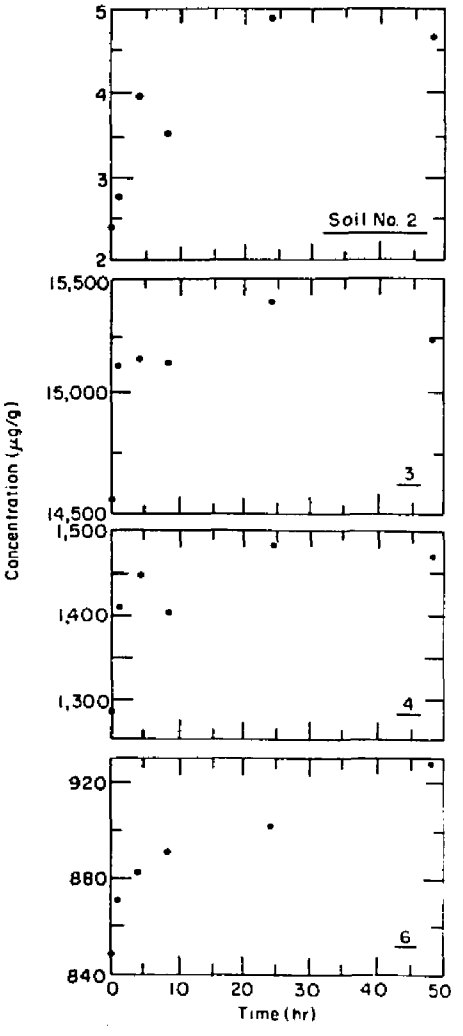


Fig. 6.4. TNT concentration as a function of time for the kinetic study of Iowa AAP soils 2,3,4 and 6

however, have a different mode of adsorption/interaction, which may account for the different trends observed.

DNB and tetryl were found only in soil 3, and both analytes reached maximum levels within 24 hours. For both analytes the mean concentration values for 5 minutes through 48 hours were not significantly different at the 95% confidence level.

The results for TNB were different for each soil where it was identified. TNB values peaked rapidly in soils 4 and 6 at 4 hours and 8 hours, respectively. In soil 6 the mean TNB concentration values for 5 minutes through 48 hours were not significantly different at the 95% confidence level, nor were the values from soil 4 for 1 hour through 48 hours. In contrast, TNB concentration failed to reach equilibrium by 48 hours for soil 3, although the difference between concentrations at 24 and 48 hours was only 3%.

Overall, equilibrium is approximated within 24 hours for the majority of the soils and analytes studied. Longer extraction times may produce slight increases for some analytes but may also result in analyte loss, as noted for HMX and RDX. Harrold and Young (1982) also observed analyte loss during extraction periods greater than 24 hours. For practical reasons an extraction time of 18 hours was chosen. Samples prepared in the afternoon could be sonically extracted overnight, with extracts available for analysis the following morning. Because an extended exposure to the noise from the ultrasonic bath is very uncomfortable to some individuals, use of the baths during periods when the laboratory is unoccupied is quite convenient.

6.9 Soil-to-Solvent Ratio

Initially the method employed 2 g of soil and 50 mL of acetonitrile. To determine if this ratio could be increased without affecting method performance, we selected two field-contaminated soils from Iowa and Louisiana which had very different concentrations of analytes. A set of 18 replicate 2.00-g subsamples of each soil was randomly divided into three groups of six. One group of six subsamples for each soil type was extracted with 50 mL of solvent, as usual. The other two groups were extracted with 25 mL and 10 mL of solvent, respectively (Appendix Tables A15-A20). The results are summarized in Table 6.4. An analysis of variance of analyte concentrations showed significant differences among the three treatments in four of the ten cases where comparisons were possible: RDX for both soils, HMX for the Louisiana soil and TNT for the Iowa soil. In the Iowa soil the concentrations of TNT and RDX exceeded 1% of the dry weight of soil, and poorer recovery was found when the 2-g subsample was extracted with only 10 mL of acetonitrile. These differences, however, amounted to only 7.1% for RDX and only 3.0% for TNT on a $\mu g/g$ basis.

For the Louisiana soil a similar result was observed for RDX, where the result was 6.0% lower for the 10-mL extracts than for the 50-mL extracts. For HMX, analyte concentrations were 17.7% higher in the 10-mL extracts than in the 50-mL extracts on a $\mu g/g$ basis. This anomalous result for HMX may be due to some interference from unretained salts and polar compounds which elute just before HMX. In the 10-mL extracts the concentration of these compounds can be as much as five times higher than in the 50-mL extracts and may overload the column, thereby causing greater interference with the early-eluting HMX.

Table 6.4. Summary of results for soil-to-solvent ratio test.

Analyte	Mean concentration (µg/g) 2 g/50 mL		2 g/25 mL		2 g/10 mL		% difference Highest-Lowest / lowest x 100	
				Iowa Soil				
HMX	1,990	(a)†	2,000	(a)	1,970	(a)	1.6	NS*
RDX	13,600	(b)	13,300	(b)	12,700	(c)	7.1	**
TNB	484	(d)	479	(d)	474	(d)	2.1	NS
DNB	38.4	(e)	38.3	(e)	39.6	(e)	3.4	NS
Tetryl	390	(f)	420	(f)	398	(f)	7.7	NS
TNT	14,900	(g)	14,800	(g,h)	14,500	(h)	3.0	**
				Louisiana Soil				
HMX	224	(i)	228	(i)	264	(j)	17.8	**
RDX	878	(k)	871	(k,l)	828	(l)	6.0	**
TNB	1.8	(m)	1.7	(m)	1.7	(m)	5.9	NS
DNB	<d		<d		0.15		--	
TNT	12.2	(n)	12.0	(n)	11.6	(n)	5.2	NS

* NS indicates that difference between three treatments was not significant at the 95% confidence level using ANOVA.

† Numbers identified with the same letter are not significantly different at the 95% confidence level by ANOVA and least-significant-difference test.

** Differences were significant at the 95% confidence level using ANOVA.

The higher solution concentrations achieved for the extracts with 2 g in 10 mL did permit quantitation of DNB for the Louisiana soil which was impossible for the 25-mL and 50-mL extracts. This was expected since the concentration in the extract was approaching the lower limit of detection.

Overall the method is quite rugged with respect to soil-to-solvent ratio. A 2-g to 10-mL ratio was selected since it gave the best low-level detection capability. Higher soil-to-solvent ratios were not tested because a sufficient volume of processed extract was needed to allow both primary analysis on LC-18 and secondary analyte confirmation on LC-CN.

6.10 Stability of Soil Extracts

Another question which required investigation is the long-term stability of soil extracts. To investigate this question a series of five field-contaminated soils from four locations were extracted and processed. The extracts were allowed to stand at room temperature for 24 hours and were then analyzed immediately. The extracts were also analyzed after being stored at 4°C in the dark for 3, 6, 18, 27 and 71 days. The results are presented in Table 6.5.

HMX, RDX, DNB and TNT were stable over the entire 71-day period in these extracts. Insufficient data were obtained for 2,4-DNT to be certain of its stability, although I have no reason to suspect it to be less stable than the other analytes. Tetryl was not present in these samples, so it was not possible to generalize about its behavior.

It appears that the concentration of TNB in the extracts from Iowa 6 and Nebraska D-16 slowly increased over the time the extracts were

Table 6.5. Stability of soil extracts.

Storage Time (days)	Concentration (μg/g) HMX	RDX	TNB	DNB	tetryl	TNT	2,4 DNT
			Milan 16 soil				
0	23.1	101	4.7	1.6	<d*	8.3	<d
3	22.5	101	4.5	1.5	<d	8.1	<d
6	25.7	104	5.1	1.7	<d	8.7	<d
18	22.6	103	5.1	1.5	<d	8.8	<d
27	24.8	104	5.3	1.4	<d	8.1	<d
71	22.1	103	5.2	1.6	<d	8.4	<d
			Louisiana 11 soil				
0	226	676	2.1	<d	<d	13.1	<d
3	219	663	1.6	<d	<d	11.8	<d
6	239	709	2.2	<d	<d	12.7	<d
18	240	701	2.1	<d	<d	12.1	<d
27	238	706	2.2	<d	<d	11.7	<d
71	232	704	2.3	<d	<d	11.6	<d
			Iowa 6 soil				
0	55.8	67.1	78.6	0.5	<d	698	<d
3	57.0	67.7	80.9	0.4	<d	715	<d
6	56.5	66.8	84.3	0.3	<d	711	<d
18	55.1	66.5	86.5	0.4	<d	707	<d
27	55.0	68.4	86.8	0.3	<d	702	<d
71	54.6	67.0	92.6	0.5	<d	683	<d
			Nebraska D-49				
0	3.3	<d	2.1	<d	<d	<d	<d
3	2.0	<d	1.4	<d	<d	<d	<d
6	3.2	<d	2.4	<d	<d	<d	<d
18	4.6	<d	2.3	<d	<d	1.5	<d
27	4.7	<d	2.7	<d	<d	<d	<d
71	5.3	<d	2.7	<d	<d	1.3	<d
			Nebraska D-16 soil (diluted 1:10)				
0	8	<d	360	2	<d	7589	<d
3	18	<d	378	1	<d	7785	<d
6	16	<d	410	4	<d	7798	<d
18	12	<d	438	3	<d	7454	9
27	18	<d	444	5	<d	7763	9
71	<d	<d	475	5	<d	7629	11

* Concentrations were less than certified reporting limits given in Table 8.4.

stored. The increase amounted to about 18% for Iowa 6 and 32% for Nebraska D-16. The increase in TNB was not accompanied by a measurable loss in the concentration of other analytes, but the small peak attributed to TNBA, discussed earlier, declined over storage. Thus the increase in TNB concentration was probably a result of TNBA decarbonylating and releasing TNB during the extended storage period.

Thus, with the possible exception of TNB, it appears that extracts can be held for extended periods at 4°C without adverse effect. Holding times of up to two months have been demonstrated with extracts from five field-contaminated soil samples from four states.

6.11 Comparison of Extraction Kinetics for Spiked Versus Field-Contaminated Soils.

All of the extraction experiments described thus far have utilized field-contaminated soils. These are soils in which the analytes of interest have had years to associate with the soil during wet and dry periods and
wide variations in ambient temperature. Often, however, methods are developed for environmental analysis using soils in which the analytes of interest are artificially incorporated in one manner or another and are allowed to equilibrate over relatively short periods of time. It has been my observation that this practice is quite common, particularly in commercial laboratories charged with rapidly developing a method for environmental analysis.

The experiment described below was conducted to investigate whether the kinetics of analyte extraction were similar when field-contaminated

soil and uncontaminated soils spiked with the same analyte were extracted using acetonitrile and the sonic bath method.

For this comparison, two field-contaminated soils (Iowa AAP soil 2 and Nebraska soil D-16) and two uncontaminated soils from Indiana and New Hampshire were chosen. The field-contaminated soils contained TNT and were air dried to constant weight as usual, ground with a mortar and pestle and homogenized. The uncontaminated soil was treated in an identical manner, and 2-g subsamples were spiked with a TNT spiking solution in acetonitrile. The solvent was allowed to evaporate slowly over 48 hours.

The four soils were extracted for 50 hour using 50 mL of acetonitrile. At various time increments the samples were removed from the sonic bath and 5 mL aliquots were removed. After removal the tubes were replaced in the sonic bath, and extraction continued. The TNT concentration normalized to the highest concentration found for that soil over the 50-hour period is plotted versus extraction time for the spiked (Fig. 6.5) and field- contaminated soils (Fig. 6.6). Very different behavior was observed. For the two spiked soils, nearly 95% TNT was recovered in only 1 hour. For the field-contaminated soils, an average of about 62% was recovered in 1 hour, and it took 18 hours to get greater than 90% recovery. Analyte spiked onto a soil and allowed to interact for only a short period of time does not associate with the soil in the same manner as analyte allowed to "age" on the soil for an extended period of time under environmental conditions. Long interaction periods with alternating wetting and drying cycles allow solutes to slowly redistribute to the highest-energy binding sites. Clearly these results

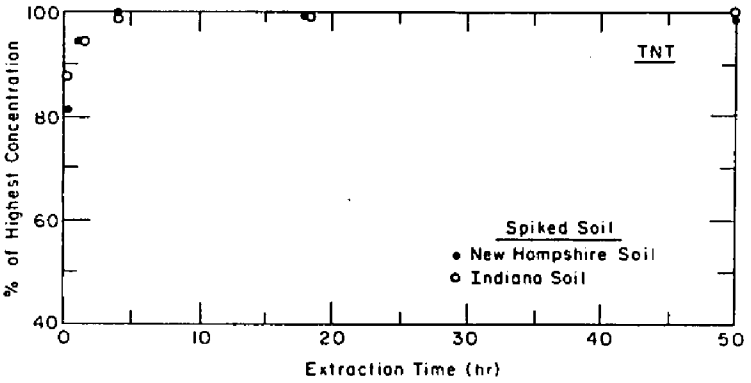


Fig. 6.5. Kinetics of extraction of TNT from two spiked soils

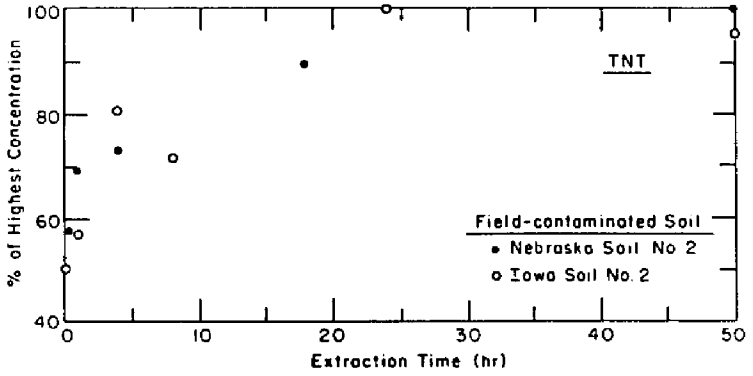


Fig. 6.6. Kinetics of extraction of TNT from two field-contaminated soils

indicate that it is unrealistic to develop extraction methods for soils based solely on spiked material.

6.12 Power Dissipation in Sonic Bath

A question arose* whether the sonic bath extraction efficiency depended on the number of samples being processed in the bath simultaneously. The concern was that processing a large number of tubes at a time could lessen the efficiency of sonic dispersion.

To investigate this, eight replicate 2-g subsamples of Iowa AAP soil 6 were placed into test tubes. Four tubes were randomly selected and extracted for 18 hours as usual with no other tubes in the bath. The remaining four tubes were processed in an identical manner except that 32 additional tubes were processed simultaneously.

After extraction both sets of replicates were processed and analyzed as usual (Table 6.6). No significant differences were found between the two treatments for any of the analytes at the 95% confidence level. For TNB and TNT the RSD averaged 2.1%, so the ability to observe a difference between treatments, if one was present, was powerful. For HMX and RDX, analytical precision was poorer, so the ability to observe a difference was also poor. Nevertheless, it does not appear that there is a meaningful difference in analyte concentrations whether sonic bath extraction is conducted with a full rack of 36 tubes or as few as 4.

* Dr. Bruce Tomkins (Oak Ridge National Laboratory), personal communication.

Table 6.6. Results of sonic power study (Iowa 6 soil).

	Concentration (μg/g)									
	HMX		RDX		TNB		DNB		TNT	
Replicate	four in rack	full rack	four in rack	full rack	four in rack	full rack	four in rack	full rack	four in rack	full rack
1	77.2	99.7	66.8	94.0	61.7	59.4	0.53	0.48	735	750
2	48.8	85.1	100.6	99.1	60.7	60.5	0.60	0.63	754	751
3	62.8	150.4	74.8	77.3	59.7	60.2	0.65	0.57	748	769
4	49.8	64.6	85.6	58.0	62.9	59.8	0.58	0.40	806	753
$\bar{X}$	59.7	100.0	82.0	82.1	61.3	60.0	0.59	0.52	761	756
s	13.3	36.6	14.6	18.6	1.4	0.48	0.05	0.10	31	8.9
	t = 2.07		t = 0.01		t = 1.76		t = 1.24		t = 0.31	

Critical value for $t_{0.95}$ (df = 6) = 2.45.

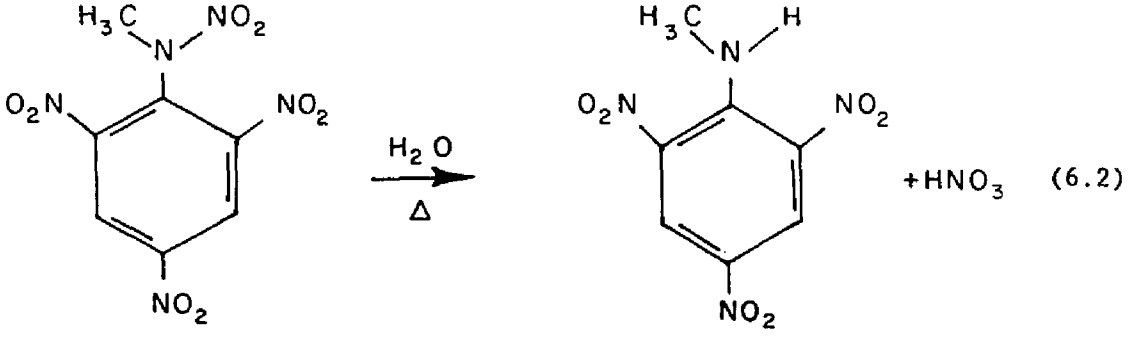
6.13 Loss of Tetryl at High Sonic Bath Temperatures

When the sonic bath is used to extract soils over an extended period of time, the water in the bath is heated well above room temperature. For an 18-hour extraction period, temperatures up to 45°C have been observed. Initial tests indicated that analytes were stable at this temperature, as evidenced by the successful use of the Soxhlet extraction method where extracts are maintained at the boiling point of acetonitrile (81.6°C) for many hours. These tests, however, used field-contaminated soils which did not contain significant amounts of tetryl.

When the soil method described here was subsequently subjected to a collaborative test, all the analytes of interest except tetryl were recovered nearly quantitatively (Bauer et al., 1989; Bauer et al., in press). Tetryl recovery was variable from collaborator to collaborator, and it was suggested that recovery was related to the sonic bath temperature attained. Since various models of sonic baths were used, final temperatures varied from room temperature to 45°C.

To investigate this possibility an experiment was conducted to determine if tetryl loss was indeed increased by high sonic bath temperatures during solvent extraction. Two 2.0-g subsamples of the New Hampshire soil used for spiked samples in the collaborative test were placed in glass vials and spiked with a dilute solution of tetryl in acetonitrile. The acetonitrile was allowed to evaporate for two days. A 10.0-mL aliquot of acetonitrile was added to each vial, and both subsamples were extracted in sonic baths for 18 hours. One bath was allowed to warm to 45°C, while the temperature in the other was kept constant at 11°C. Chromatograms of the extracts for each of these

subsamples are presented in Figure 6.7. Clearly the level of tetryl in the extract obtained at 45°C is much reduced from that held at 11°C, and the peak corresponding to the tetryl degradation product is much larger. Because this peak elutes near TNT, it could influence TNT quantitation. This peak is thought to be n-methylpicramide (equation 6.2), a known hydrolysis product of tetryl (Tamiri and Zitrin, 1986). While the rate of hydrolysis of tetryl may differ from soil to soil, it appears necessary to maintain sonic bath temperatures near ambient levels to get good analyte recovery.



(6.2)

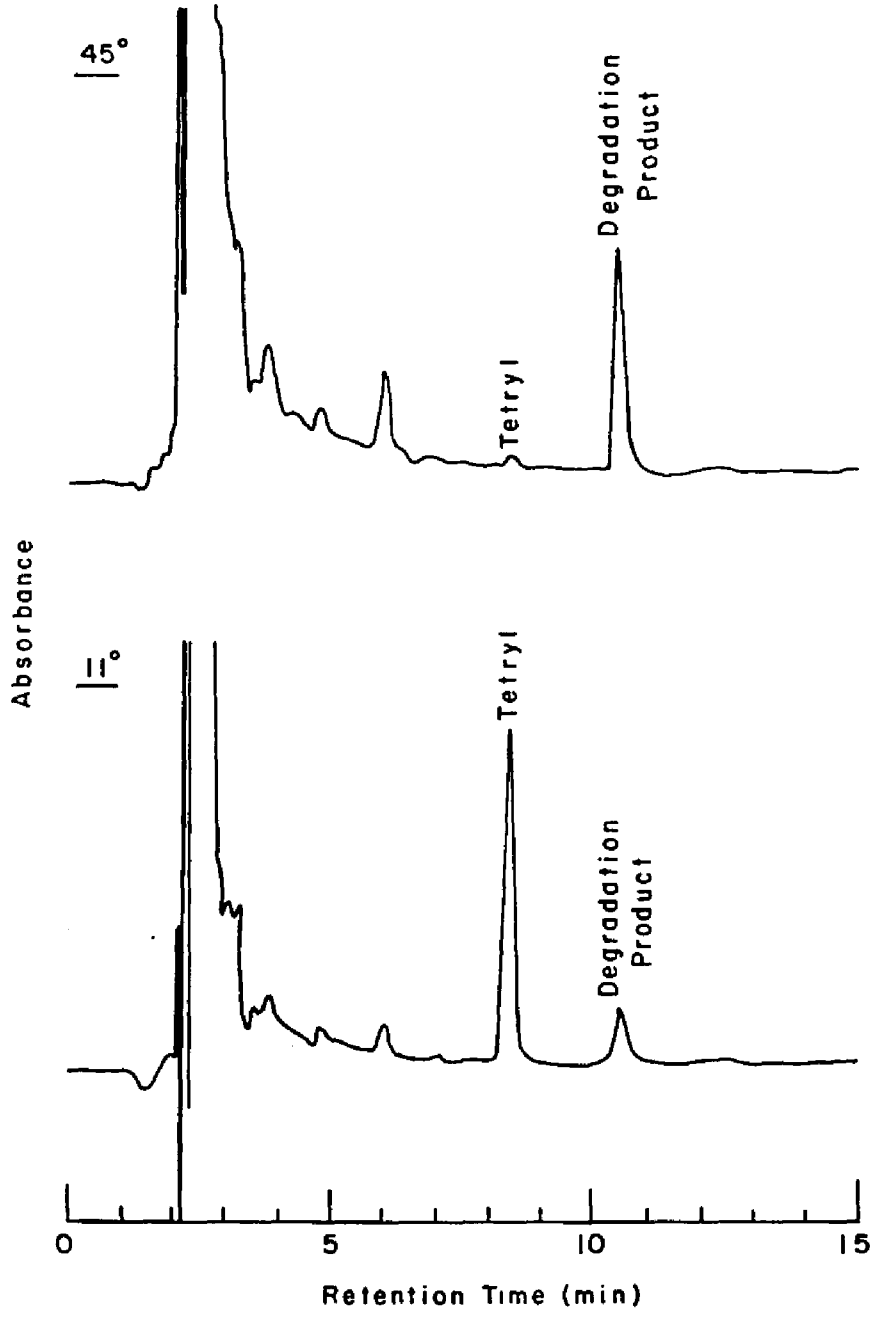


Fig. 6.7. Loss of tetryl at high sonic bath temperature

CHAPTER 7

SAMPLE PROCESSING

7.1 Photodegradation Study

It is well known that TNT photodegrades in solution (Burlinson, 1980). However, the susceptibility of TNT and other munitions to photodegradation when associated with soil is unknown. In general, soils to be analyzed for explosives are air dried for periods of at least 24 hours prior to extraction. It is important to know how sensitive these components are to light exposure during the drying period to assess whether special precautions are necessary.

Two soils, Louisiana AAP soil 12 and Iowa AAP soil 6, were selected for study based on their previously determined concentrations of TNT. Under low light conditions, a bulk sample of each was air dried, ground, sieved, homogenized, and divided into two portions. One portion of each soil was spread in a thin layer in aluminum pans and exposed to room light and sunlight for 10 days. The pans were kept on the sill of a south-facing window,
ensuring maximum exposure to whatever sunlight was available over the period. Two days were sunny and the other eight days were mostly overcast. Fluorescent lights in the room were left on continuously during the ten days. The pans were shaken several times per day to refresh the soil surface exposed to light.

A second portion of each soil was similarly spread in aluminum pans which were kept in the dark in the same room as the exposed samples. The

residual moisture contents of the soils maintained in the dark and those exposed to room light were found to be equivalent as determined by the standard gravimetric method.

After the ten-day exposure, six 2-g subsamples of each soil treatment were extracted and analyzed as usual (Table 7.1). Statistically significant (95% confidence level) differences in analyte concentrations for the two treatments were observed for RDX and TNT in Louisiana 12 and for TNB and TNT in Iowa 6. A loss of 8.6% and 10.8% for TNT was observed for the light-exposed subsamples of Louisiana 12 and Iowa 6, respectively. A 5.0% increase in RDX concentration was observed in the light-exposed subsamples for Louisiana 12, and a 6.9% increase in TNB concentration was observed in Iowa 6.

The loss of TNT on exposure to light is consistent with its known susceptibility to photodegradation. The coincident increase in TNB concentration in Iowa 6, where the largest change in TNT concentration was observed, supports the notion that the presence of TNB in these soils is a result of photodegradation of TNT. The increase in RDX in the Louisiana 12 soil exposed to light was unexpected. RDX cannot be a degradation product of TNT and is unlikely to come from other potential contaminants, but it might be released from soil organic matter or mineral complexes.

While the loss of TNT due to photodegradation was clearly demonstrated for both soils, the loss averaged only about 10% for conditions in which light exposure was maximized. When air-drying soils, it is therefore recommended that the soils be isolated from direct sunlight and that exposure to room light be minimized. Grinding and sieving will

Table 7.1. Results of photodegradation experiment.

Replicate	Concentration (μg/g)									
	HMX		RDX		TNB		DNB		TNT	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
	Louisiana 12									
1	51.4	51.0	162	174	2.5	2.3	<d	<d	11.9	10.8
2	53.4	54.2	162	165	2.4	2.5	<d	<d	11.1	10.7
3	51.6	50.0	158	164	2.5	2.3	<d	<d	11.6	10.7
4	61.5	58.2	161	175	2.2	2.8	<d	<d	10.8	10.7
5	55.2	60.3	164	165	2.2	2.3	<d	<d	11.6	10.1
6	55.9	--	160	--	2.5	--	<d	<d	12.5	--
$\bar{X}$	54.8	54.7	161	169	2.4	2.4			11.6	10.6
S	3.7	7.4	2.1	5.4	0.14	0.21			0.59	0.2
	t = 0.04		t = 3.23*		t = 0.48				t = 3.36*	
	Iowa 6									
1	61.1	76.6	71.6	80.5	63.9	73.3	0.76	0.42	712	648
2	46.0	47.3	82.1	125.6	65.3	71.9	0.21	0.72	718	649
3	71.5	69.9	60.5	90.3	67.9	71.8	0.62	0.40	745	666
4	67.2	96.2	80.9	61.7	66.7	65.3	0.62	0.59	740	661
5	52.1	53.7	66.5	81.1	60.0	69.7	0.69	0.62	756	656
6	109.0	52.6	80.2	84.4	67.7	66.3	0.71	0.61	734	649
$\bar{X}$	67.8	66.1	73.6	87.3	65.2	69.7	0.60	0.56	734	655
S	22.3	18.5	8.9	21.1	3.1	3.3	0.20	0.12	16.6	7.5
	t = 0.15		t = 0.77		t = 2.46*		t = 0.43		t = 10.7*	

* Exceeds critical value for $t_{0.95}$ (9 df) = 2.26, $t_{0.95}$ (10 df) = 2.23.

generally take place only after the soil is dry, so the surface area actually exposed to light during drying will be much less than in this experiment.

7.2 Particulate Removal from Extracts

The procedure used in previous experiments to remove particles from extracts prior to RP-HPLC analysis was to dilute the extract 1:1 with water and filter through a 0.5-μm syringe filter. Filtration is essential to prevent particles from accumulating and destroying expensive RP-HPLC columns. Extracts from the ultrasonic extraction process are very difficult to filter, even after extensive centrifugation. Soil aggregates are dispersed into very fine particles with long settling times during this 18-hour period of sonication. With small syringe filters the pressure required to force extracts through these membranes often caused the membranes to rupture, resulting in sample loss.

Another option for extract processing is to filter prior to dilution with water. Water dilution is still necessary before sample introduction into the HPLC to reduce the solvent strength of the injected sample to match that of the HPLC eluent. Otherwise, chromatographic resolution is degraded. This option was rejected because analyte solubilities are much reduced in acetonitrile-water compared with pure acetonitrile. Thus if very high concentrations of analyte are present in an extract, small crystals of analyte could precipitate when the extract is diluted with water. When this dilution occurs after filtration, these crystals would be introduced into the sample loop of the HPLC, resulting in severe carryover between samples. Since very high analyte concentrations (% levels) are occasionally observed in field samples, extracts with high

analyte concentrations are sometimes encountered and protection against such carryover is a real concern.

A second alternative is to dilute with water as described and centrifuge at high speeds for long periods of time. This requires unbreakable, solvent-resistant centrifuge tubes that also seal sufficiently to inhibit evaporative loss of solvent. Centrifugation is also time consuming, especially when analytical lots of twenty or more samples are processed.

A third alternative was suggested by observing that addition of $CaCl_2$ to the acetonitrile-water extracts caused particles to settle rapidly.* The Ca^{++} ions flocculate clay particles into large aggregates that settle rapidly. A question remained whether this flocculation would affect analyte concentrations due to selective adsorption or rejection by the floc.

An experiment was conducted to identify the $CaCl_2$ concentration range over which flocculation occurred. These tests utilized an acetonitrile extract of Louisiana AAP soil 11 which was very difficult to filter using the normal procedure. A series of 5.00 mL aliquots of this extract was placed in individual test tubes and diluted with 5.00 mL aliquots of aqueous $CaCl_2$ solutions with concentrations ranging from 0.01 to 80 g/L. All solutions were shaken and allowed to stand undisturbed for 30 minutes at room temperature.

For the two highest $CaCl_2$ concentrations (60 and 80 g/L), two layers formed due to salting out of acetonitrile. Flocculation was not effective for the 0.01-g/L solution. With solutions ranging from 0.1 to

* Patricia W. Schumacher (U.S. Army Cold Regions Research and Engineering Laboratory), personal communication.

40 g/L, only one liquid layer was visible at room temperature, and complete settling of the floc occurred within 15 minutes. The rate of flocculation and settling appeared to be a function of $CaCl_2$ concentration, with higher concentration solutions settling most rapidly. Additionally, when acetonitrile solutions were mixed with aqueous $CaCl_2$ solutions with concentrations in excess of 20 g/L and cooled in the refrigerator overnight, two layers formed. This low-temperature salting-out was not observed when the $CaCl_2$ concentration was 10 g/L or less. From these results, $CaCl_2$ concentration in the range of 4 to 10 g/L is recommended for achieving efficient flocculation without causing the acetonitrile to salt out. To be safe I also recommend that refrigerated filtered samples be mixed prior to analysis because some soils contain substantial levels of native salts.

To test whether this flocculation technique affected analyte concentrations in extracts, an experiment was conducted on a series of eight field- contaminated soils. The explosives were extracted as usual using 50-mL portions of acetonitrile. Two 10-mL aliquots of each extract were placed in separate scintillation vials. A 10-mL portion of water was added to one subsample, and the solution was centrifuged at 2000 rpm for 15 minutes and filtered through a 0.5-μm Millex SR filter. To the second subsample, a 10-mL portion of 40-g/L $CaCl_2$* solution was added, the mixture was allowed to stand for 30 minutes, and the supernatant was filtered through a 0.5-μm Millex SR filter. Each subsample to which $CaCl_2$ was added formed a visible floc that settled rapidly. The result-

* This experiment was conducted prior to the observation that 40 g/L $CaCl_2$ would result in salting-out of acetonitrile at refrigerator temperatures.

ing supernatants were remarkably clear, while the subsamples that were centrifuged were turbid even after extensive centrifugation. When filtration was conducted, the samples flocculated with $CaCl_2$ filtered very easily, while subsamples that were mixed with water and centrifuged were extremely difficult to filter (several membranes ruptured).

The filtered solutions of all subsamples were analyzed as usual for explosives. The experimental data are presented in Appendix Table A21 for HMX, RDX, TNB and TNT. A summary of the mean ratios of the analyte conentrations in centrifuged subsamples over the analyte concentrations in flocculated subsamples is presented in Table 7.2. These mean values were very close to 1.0 for all four analytes, showing that the analytical results were nearly equivalent for these two sample preparation methods.

While the results of this initial experiment were encouraging, no analytical replication was used, so it was impossible to determine whether the small differences between centrifuged and flocculated treatments for individual soils were statistically significant relative to analytical variability. To further pursue this question, three of these soils were selected for an additional study (Iowa AAP soil 6 and Milan AAP soils 13 and 16). Two of these soils were among those with the largest difference between the two types of processing in the initial study. A 2-g subsample of each was extracted as usual with 50 mL of acetonitrile, and 10-mL aliquots of each extract were processed by each of the two procedures. Centrifugation was conducted at 5000 rpm for 20 minutes. A 40-g/L aqueous $CaCl_2$ solution was used for flocculation. The resulting solutions from the two treatments for each soil were analyzed in quadruplicate by the usual procedure (Table 7.3).

Table 7.2. Mean and standard deviation for ratio of concentrations for centrifuged to $CaCl_2$-flocculated subsamples of extract from eight field-contaminated soils.

Analyte	Concentration Ratio (centrifuged/flocculated)* Mean	Standard deviation†
HMX	1.00	0.13
RDX	0.98	0.03
TNB	1.00	0.14
TNT	1.00	0.23

* Experimental data in Appendix Table A21.
† Standard deviation of individual ratios from single determinations for eight soils.

Table 7.3. Comparison of centrifugation (C) and flocculation (F) procedures with determinations conducted in quadruplicate.

Replicate	Concentration (μg/g)											
	HMX		RDX		TNB		DNB		tetryl		TNT	
	F	C	F	C	F	C	F	C	F	C	F	C
Milan 13												
1	72.3	70.5	437	437	1.6	2.1	0.81	0.88	34.5	34.0	27.4	27.7
2	70.0	71.7	434	436	2.3	1.9	0.58	0.58	33.4	34.1	27.3	27.8
3	71.5	71.6	448	437	2.0	2.0	1.12	0.73	35.6	33.9	28.0	27.3
4	70.8	70.4	436	435	1.7	2.5	0.93	0.90	35.2	34.7	29.4	28.7
$\bar{X}$	71.2	71.1	439	436	1.9	2.1	0.86	0.77	34.7	34.2	28.0	27.9
S	0.98	0.70	6.3	0.96	0.32	0.26	0.23	0.15	0.96	0.36	0.97	0.59
	t = 0.17		t = 0.79		t = 1.09		t = 0.65		t = 0.97		t = 0.26	
Milan 16												
1	23.8	22.7	172	173	5.3	3.4	1.7	1.4	<d	<d	10.2	9.8
2	23.3	23.7	170	172	4.8	5.0	1.3	1.0	<d	<d	10.5	10.2
3	21.8	23.4	170	172	4.0	4.9	1.6	1.2	<d	<d	10.5	11.0
4	28.0	22.7	171	173	4.8	5.5	1.5	1.3	<d	<d	9.9	11.3
$\bar{X}$	24.2	23.1	171	173	4.7	4.7	1.5	1.2	--	--	10.3	10.6
S	2.7	0.51	0.96	0.58	0.54	0.91	0.17	0.17	--	--	0.29	0.69
	t = 0.81		t = 3.13*		t = 0.05		t = 2.48*				t = 0.80	
Iowa 6												
1	115	118	83.3	78.3	65.5	80.8	<d	<d	<d	<d	757	756
2	117	117	79.1	80.6	65.9	82.3	<d	<d	<d	<d	756	758
3	117	118	79.1	79.6	67.5	83.1	<d	<d	<d	<d	755	756
4	116	120	81.0	80.1	68.0	84.7	<d	<d	<d	<d	748	756
$\bar{X}$	116	118	80.6	79.7	66.7	82.7	--	--	--	--	754	757
S	0.96	1.26	2.0	0.99	1.21	1.63	--	--	--	--	4.1	1.0
	t = 2.53*		t = 0.88		t = 15.8*						t = 1.19	

* Exceeds critical value for $t_{0.95}$ (df = 6) = 2.447.

In 4 of the 15 analyte-method comparisons that could be made, mean values for the two treatments were found to be significantly different at the 95% confidence level. For two of these cases (RDX in Milan 16 and HMX in Iowa 6), the percentage difference was 1.2% and 1.7%, respectively. From a practical point of view these differences are unimportant compared to the known variability of analytes in soils. These small differences are statistically significant because of the excellent analytical precision (RSD < 1%). The concentrations of DNB in the two treatments for Milan 16 were also significantly different at the 95% confidence level but just barely ($t = 2.48$ compared to a critical value of 2.447). Concentrations of DNB for this soil were very low (1.5 and 1.2 $\mu g/g$), and the significance is again because the analytical precision was excellent ($s = 0.17$ $\mu g/g$), particularly for such low concentrations.

The fourth statistically significant difference was TNB in Iowa 6. The mean values were 66.7 and 82.7 $\mu g/g$ for flocculated and centrifuged aliquots, respectively, a difference of 24%. Analytical replication was excellent in both cases, so the difference appears both real and important. Chromatograms for these extracts are presented in Figure 7.1. Clearly the TNB peak is lower in the flocculated subsample than in the centrifuged one. However, a small, broad peak eluted just ahead of the TNB peak in the flocculated subsample. This peak is probably due to 2,4,6-trinitrobenzaldehyde (TNBA) as discussed earlier. When we collected the eluent fraction corresponding to the TNBA peak and reinjected into the HPLC, it eluted at the same retention time as TNB (Fig. 7.1). Thus TNBA is apparently decarbonylating to TNB during RP-HPLC analysis. This explains the unusual breadth of the TNBA peak and the fact that it has

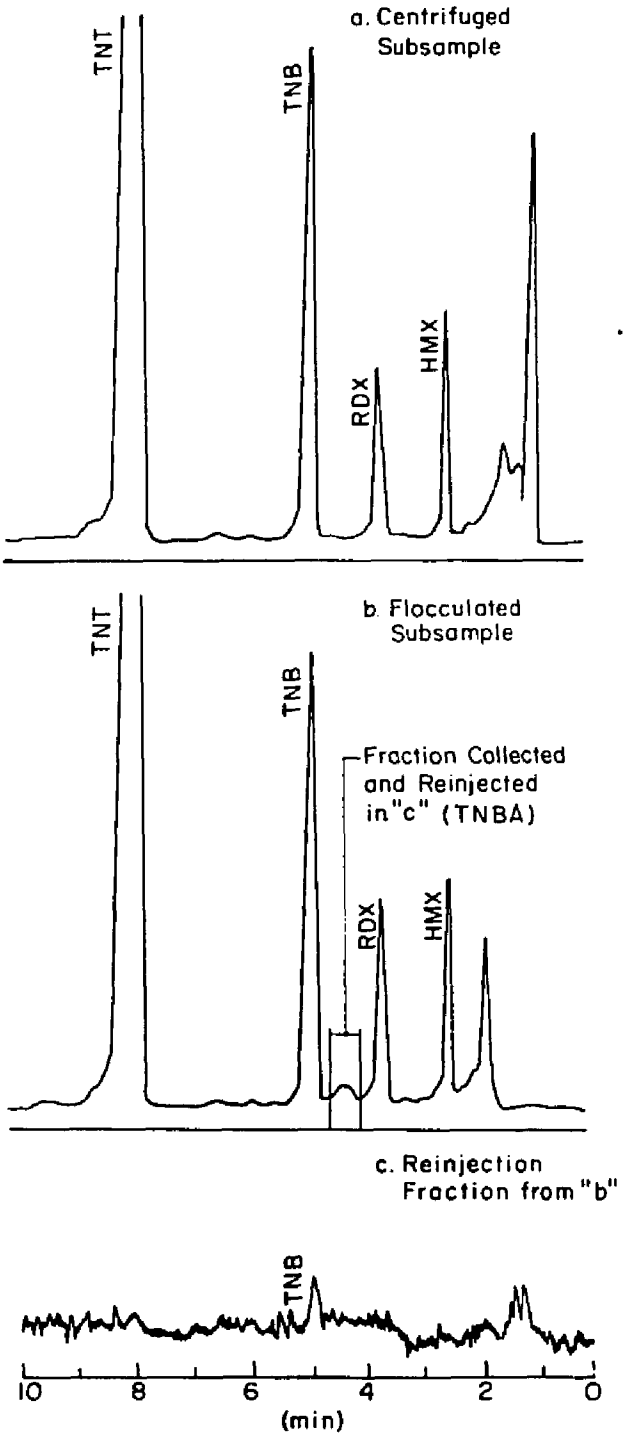


Fig. 7.1. Chromatograms showing extracts of Iowa 6 soil processed by centrifugation and flocculation techniques and reinjection results of the broad peak eluting just ahead of TNB in the flocculated subsample

been converted to TNB when the fraction is reinjected. Subsequent observation* indicated that decarbonylation proceeds more rapidly in methanol solution than in acetonitrile. The eluent in the HPLC analysis is 1:1 water-methanol, which accounts for the rapid decarbonylation that occurs during separation and subsequent reinjection compared to the rate of reaction in the acetonitrile extract.

The reasons for the stability of TNBA in the flocculated samples compared to centrifuged samples are uncertain. The high $CaCl_2$ concentration could be stabilizing TNBA by complexation. Whatever the reason, TNB results using the flocculation procedure are less affected by TNBA decomposition and are therefore more indicative of the actual TNB concentration in the soil.

Overall the flocculation method of extract processing is a major improvement over other methods tested. Dilution of extracts 1:1 with aqueous $CaCl_2$ followed by a standing time of 15 minutes yields a supernatant that is easy to filter. This procedure does not cause sorption of the analytes of interest, and for TNB it actually produces a more accurate result than other processing options.

7.3 Filtration Tests

Early work on filtration of totally aqueous solutions of these explosives indicated that statistically significant losses of analyte occurred on some types of filters (Walsh et al., 1988). To determine if analyte loss during filtration is also a problem for solutions of 50:38:12 water-methanol-acetonitrile, analyte concentrations in two un-

* Marianne E. Walsh (U.S. Army Cold Regions Research and Engineering Laboratory), personal communication.

filtered samples were compared with aliquots filtered through 11 different commercially available filters. The filter pore sizes were between 0.4 and 0.5 μm. Four replicates were analyzed in random order for each type of filter for each solution (Appendix Tables A22-A25). Mean concentrations and standard deviations are presented in Table 7.4. An analysis of variance indicated that there were no significant losses of analyte for any of the four test compounds at either of the two tested concentrations.

The 50:38:12 water-methanol-acetonitrile was tested rather than 1:1 water-acetonitrile because the filtration experiment was conducted before the final extractant and eluent were selected. Tests for solubility, however, indicated that HMX and RDX are 20 times more soluble in acetonitrile than in methanol.* Thus, the absence of losses for 50:38:12 solutions suggests that 1:1 water-acetonitrile solutions of these analytes should pose no problem.

* Daniel C. Leggett (U.S. Army cold Regions Research and Engineering Laboratory), personal communication.

Table 7.4. Summary of filtration results for HMX, RDX, TNT and 2,4-DNT in 50:38:12 water-methanol-acetonitrile.

	HMX Concentration (μg/L)			
	Low		High	
Filter type	Mean	Standard deviation	Mean	Standard deviation
Unfiltered	237	11.2	474	6.3
Millex-HV	227	13.6	503	22.8
Nalgene (green)	240	12.2	475	14.5
Millex-SR	240	11.2	487	13.0
Spartan-T	234	7.7	469	30.2
Bio Rad Prep Disc	230	10.0	475	7.9
Spartan 3	243	9.8	477	7.5
Spartan 25	236	12.9	492	32.8
Nalgene (yellow)	239	8.8	474	5.8
Spectra/Por	249	18.2	492	19.6
Gelman Acro LC25	239	9.1	482	17.6
Nuclepore	232	5.0	505	25.0
F Ratio*	1.09		1.53	

	RDX Concentration (μg/L)			
	Low		High	
Filter type	Mean	Standard deviation	Mean	Standard deviation
Unfiltered	205	4.6	410	11.3
Millex-HV	207	6.3	408	4.8
Nalgene (green)	212	3.3	406	7.9
Millex-SR	204	2.2	400	14.2
Spartan-T	203	4.0	392	8.8
Bio Rad Prep Disc	210	4.4	394	4.0
Spartan 3	212	6.6	403	12.6
Spartan 25	203	4.0	398	8.7
Nalgene (yellow)	206	10.2	397	10.6
Spectra/Por	207	8.1	408	13.3
Gelman Acro LC25	209	7.2	396	13.2
Nuclepore	205	3.9	392	6.2
F Ratio*	1.18		1.62	

* Critical value for $F_{0.95} = 2.074$

Table 7.4. (cont.)

	TNT Concentration (μg/L)			
	Low		High	
Filter type	Mean	Standard deviation	Mean	Standard deviation
Unfiltered	107	6.3	208	9.0
Millex-HV	107	3.7	201	2.6
Nalgene (green)	107	11.1	209	2.2
Millex-SR	105	5.0	211	11.7
Spartan-T	113	6.1	196	3.6
Bio Rad Prep Disc	114	2.3	208	7.6
Spartan 3	109	4.2	204	6.1
Spartan 25	102	6.0	206	4.4
Nalgene (yellow)	107	6.6	199	5.4
Spectra/Por	107	4.9	204	9.0
Gelman Acro LC25	106	5.2	205	5.4
Nuclepore	106	4.6	208	5.9
F Ratio*	1.23		1.71	

	2,4-DNT Concentration (μg/L)			
	Low		High	
Filter type	Mean	Standard deviation	Mean	Standard deviation
Unfiltered	78	3.5	159	8.8
Millex-HV	79	4.6	157	3.5
Nalgene (green)	80	4.8	159	5.6
Millex-SR	79	0.7	157	7.0
Spartan-T	82	7.3	158	8.7
Bio Rad Prep Disc	79	3.2	158	3.9
Spartan 3	81	5.6	158	2.9
Spartan 25	75	5.4	156	5.9
Nalgene (yellow)	75	3.7	160	6.2
Spectra/Por	77	1.2	161	3.9
Gelman Acro LC25	76	3.2	162	6.7
Nuclepore	81	4.3	154	5.0
F Ratio*	1.31		0.57	

* Critical value for $F_{0.95} = 2.074$

CHAPTER 8

DESCRIPTION OF METHOD AND PERFORMANCE EVALUATION

8.1 Method Description

The results of the various tests described in Chapters 3-7 led to the establishment of the step-by-step method described below.

8.1.1 Soil Drying and Grinding

Soils are spread uniformly in a 9-in. aluminum pie pan out of direct light in a fume hood and air dried 1-2 days to constant weight. Dried soil is inspected to ensure that solid pieces of explosive are absent before grinding with a mortar and pestle to a fine powder. Originally the ground soil was sieved to remove small stones and other debris but it was found that cross contamination between samples was encountered if sieves were not carefully washed and solvent-rinsed between samples. Since this is inconvenient when processing large numbers of samples and might easily be abused in commercial practice, sieving is no longer recommended. Instead manual removal of plant debris and stones during grinding is specified.

8.1.2 Extraction

Bottled ground soil is homogenized by shaking and rolling the bottle extensively. A representative 2.00-g subsample is weighed into a 6-dram glass vial, 10.0 mL of acetonitrile is added, and the vial is closed with a Teflon-lined cap.

The vial is vortex-mixed for 1 minute and placed in an ultrasonic bath for 18 hours. The bath temperature is maintained below 25°C by

passing cooling water through coils immersed in the bath. Cooling is necessary to minimize loss of tetryl by thermal degradation, and it also minimizes the potential for evaporative loss of extraction solvent.

8.1.3 Removal of Particles

Vials are removed from the sonic bath and allowed to stand undisturbed for at least 30 minutes. A 5.00-mL aliquot is then removed and combined with 5.00 mL of 5-g/L aqueous $CaCl_2$, and the vial is shaken and allowed to stand for at least 15 minutes. Generally the addition of $CaCl_2$ results in flocculation of the particles and rapid settling. A 5-mL portion is then filtered through a 0.5-μm Millex SR disposable filter into a clean scintillation vial. Vials are refrigerated at 4°C in the dark until analyzed.

8.1.4 RP-HPLC Analysis

On the day of analysis, vials are warmed to room temperature and shaken vigorously to ensure that the mixed aqueous-acetonitrile solution is homogeneous. Experience has shown that some soils have native salt concentrations sufficient to salt-out the acetonitrile at refrigerator temperatures. The extracts are then analyzed on an LC-18 column, eluted with 1.5 mL/min of 1:1 (V/V) methanol-water. Samples are introduced by overfilling a 100-μL sample loop, and determined on a 254-nm UV detector.

When potential analytes are observed at the proper retention times (Table 4.1), a confirmation analysis is conducted using identical analytical conditions on an LC-CN column. While the separation on LC-18 parallels the order of octanol-water partition coefficients of the analytes (Table 4.2), the separation on LC-CN is very different, with the order of elution very analyte specific (Table 4.1). Combination of the two chromatograms gives a powerful means of distinguishing among analytes

and possible interferences. Examples of the chromatograms obtained for extracts of four field-contaminated soils from different locations are shown in Figure 8.1. Experience with well over 100 soil samples from 13 states has confirmed the general utility of this method.

8.2 Rationale for Performance Evaluation

In order to decide whether an analytical method is suitable for a specific application, knowledge of the performance characteristics of the method is essential. Important performance criteria include method accuracy and precision, the concentration range covered, its sensitivity, and its ruggedness in the hands of other analysts.

Two protocols have been widely used in the field of environmental analysis to estimate the low-concentration measurement capability for an analytical method. These are the method detection limit (MDL) used by the Environmental Protection Agency (Federal Register, 1984) and the Certified Reporting Limit (CRL) used by the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA, 1985) and based on an approach suggested by Hubaux and Vos (1970).

The MDL protocol requires that 7-10 replicate samples be spiked with a single known concentration of analyte near the "detection limit" and the spiked samples be carried through the entire analytical procedure. The standard deviation of the measured concentrations (S) is calculated and multiplied by the "t" statistic for the appropriate number of degrees of freedom at the 99% confidence level. Two major assumptions of the protocol are that the distribution of results at the measured concentration is normal (Gaussian) and that the standard deviation at the measured

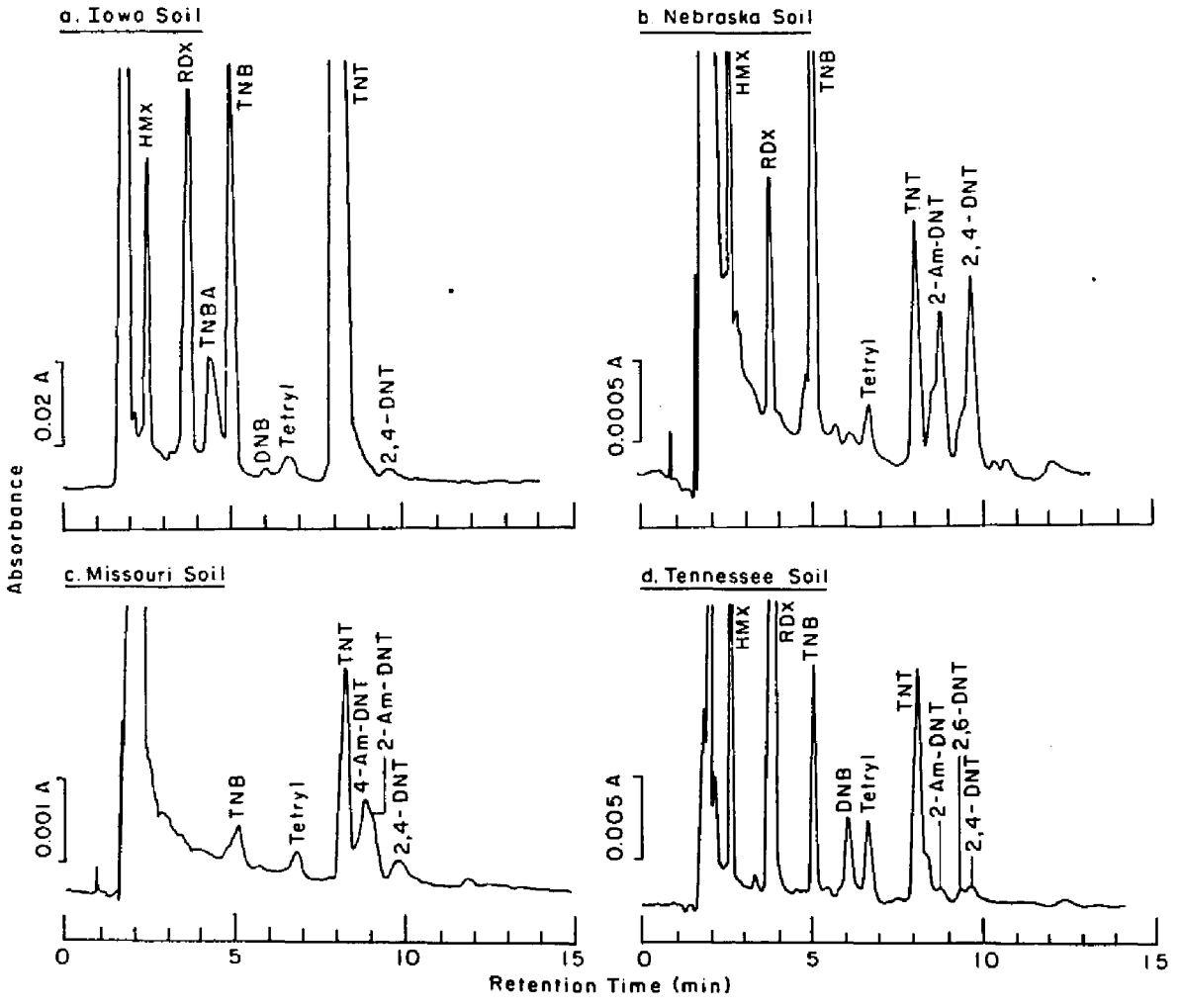


Fig. 8.1. LC-18 chromatograms obtained for extracts of four field-contaminated soils

concentration is equivalent to the standard deviation at zero concentration.

The normal distribution about the mean found concentration, $\bar{X}$, is defined by S (shown as distribution a in Figure 8.2). Because of the properties of the normal distribution, 99% of measured concentrations should be less than $\bar{X} + (S \cdot t_{0.99})$, where $t_{0.99}$ is the one-tailed t statistic at the 99% confidence level. The distribution is then shifted to the left such that $\bar{X}$ is centered on zero (distribution b in Figure 8.2). Assuming the same standard deviation is applicable, 99% of the measured concentrations for a true value of zero should fall to the left of the point defined by $S \cdot t_{0.99}$. In other words, we are 99% confident that a result greater than $S \cdot t_{0.99}$ does not come from a sample with a true concentration equal to zero.

The method detection limit protocol is conducted on a single day, and thus the estimates obtained do not include day-to-day calibration variability (Grant et al., in press). Since MDLs are established from measurements at a single concentration, the information obtained can only be used to estimate precision and accuracy at that one concentration.

The certified reporting limit (CRL) protocol is also designed to provide an estimate of low-concentration measurement capability, but it also provides data which can be used to assess precision and accuracy over a wide concentration range. The CRL protocol requires that a target reporting limit (TRL) be estimated and a minimum of one spiked sample at 0, 0.5, 1, 2, 5 and 10 times the TRL be carried through the complete method on each of four days.

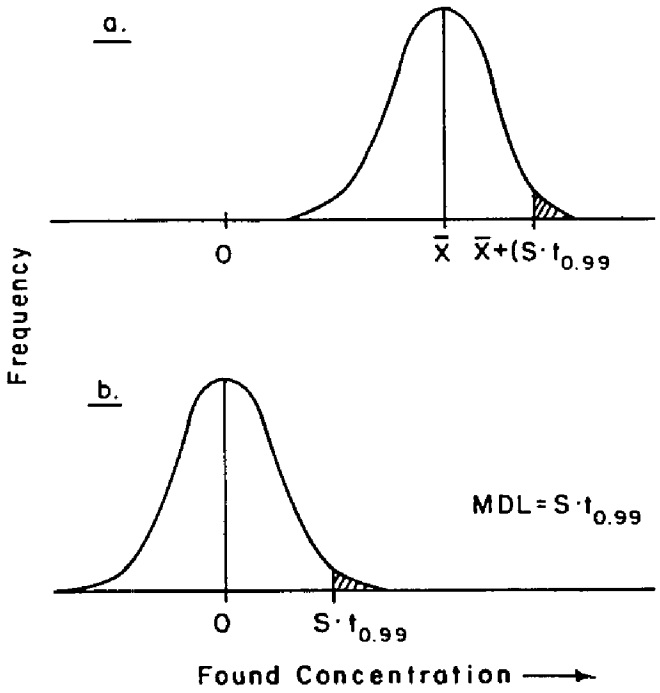


Fig. 8.2. Pictorial representation for establishment of the method detection limit (MDL).

The CRL is computed from the results from the 0.5 to 10 TRL spiked samples as shown in Figure 8.3. A least-squares linear regression equation is fitted to the plot of found concentrations (F_i) versus spiked (taken) concentrations (T_i). Confidence bands about the regression line are established at the 90% confidence level ($\alpha = \beta = 5\%$). A horizontal line is drawn from the intersection of the upper confidence band and the y axis until it intersects the lower confidence band. A vertical line is dropped to the xaxis and the intersection point is defined as the CRL. Using this approach, we are 95% confident that any found concentration greater than or equal to the CRL corresponds to a true concentration greater than zero.

Assumptions in the CRL approach are that a) the distribution of measurements at each spiked level is normal, b) the variance is homogeneous over the concentration range used (0.5 to 10 TRL), and c) this variance is equivalent to the variance at zero concentration. In other words the standard deviation is not a function of concentration over this range of concentration. If this assumption is violated, the estimated confidence bands will be inflated about zero and the CRL calculated will be too high. Elimination of one or more high-concentration standards may overcome this problem. Since the data used to establish the CRL are obtained over a four-day period, day-to-day calibration variability is included in the estimate.

8.3 Preparation of Spiked Soils for Performance Tests

A separate set of analyte spiking standard solutions were prepared from those described for calibration in Chapter 5. For HMX, RDX, TNB, DNB, NB, tetryl, TNT, 2,4-DNT and 2,6-DNT these standards were prepared

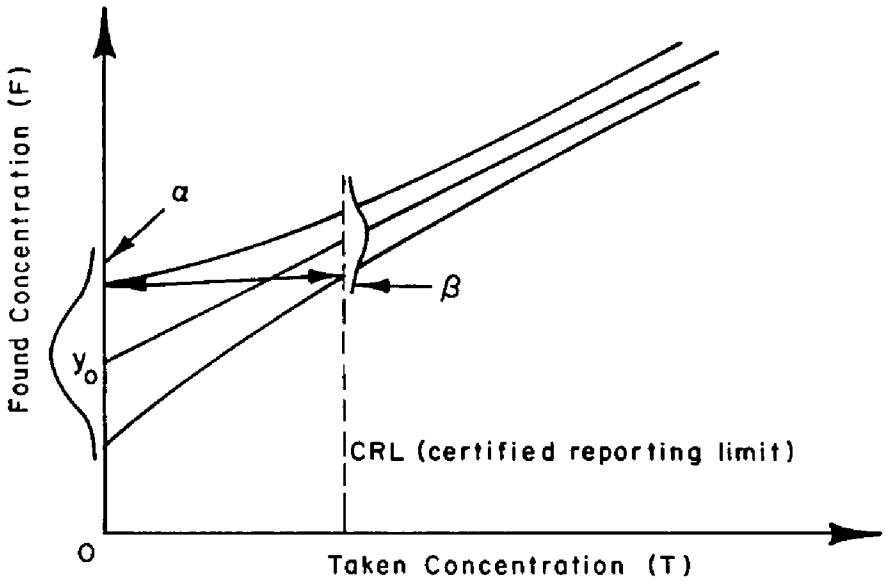


Fig. 8.3. Pictorial representation for establishment of the method detection limit (CRL)

from SARM obtained from the U.S. Army Toxic and Hazardous Materials Agency. Standard material for 2-Am-DNT was obtained from D. Kaplin, U.S. Army Natick Laboratories. Standard material for o-NT, m-NT and p-NT were commercial reagent- grade material from Baker. Approximately 250 mg of each dried standard was weighed out to the nearest 0.1 mg, transferred to individual 250-mL volumetric flasks and diluted to volume with acetonitrile.

To ensure that no major errors occurred in the preparation of the spiking standards, three replicate combined analyte solutions were prepared. In each case 2.00 mL of each stock standard was combined in a 100-mL volumetric flask and brought to volume with acetonitrile. These solutions were compared with three identical solutions prepared from calibration stock standards. The results indicate that mean response factors for the seven analytes differ by an average of less than 2% (Table 8.1). The largest difference was found for DNB, where the mean response factors differed by 4.4%. Thus, no large errors were associated with the preparation of the stock spiking standards.

In order to conduct spike/recovery tests, two separate sets of combined analyte spiking standards were prepared because retention times of NB and tetryl differ by only 0.30 minutes and retention times of 2,6-DNT and 2,4-DNT differ by only 0.23 minutes (Table 4.1), making it difficult to quantitate accurately each pair in the same sample at equivalent concentrations. Combined analyte spiking stock standard X was prepared by adding 2.00-mL aliquots of HMX, RDX, TNB, DNB, NB, TNT, 2,6-DNT, o-NT, p-NT and m-NT stock standards and bringing to volume with acetonitrile in a 200-mL volumetric flask. Combined analyte spiking standard Y was prepared in an identical manner using stock standards of tetryl, 2-Am-DNT

Table 8.1. Comparison of response factors from two sets of individually prepared stock standards.

Analyte	Response Factor (peak ht./conc.) April 1988				January 1988				Difference (%)
	A	B	C	$\bar{X}$	A	B	C	$\bar{X}$	
HMX	56.39	56.44	56.50	56.44	55.91	56.16	55.96	56.01	0.8
RDX	47.34	47.38	47.44	47.39	47.22	47.04	47.04	47.11	0.6
TNB	89.32	90.16	88.78	89.42	87.67	88.75	87.33	87.92	1.7
DNB	110.75	110.67	110.84	110.75	105.87	105.95	105.78	105.87	4.4
Tetryl	56.14				54.91				2.2
TNT	64.21	64.32	64.09	64.21	63.30	63.60	63.30	63.40	1.3
2,4-TNT	73.39	73.34	73.44	73.39	72.90	73.08	73.03	73.00	0.5

and 2,4-DNT. Analyte concentrations in both solutions were about 10 μg/mL.

In the protocol required to establish certified reporting limits (CRLs), a target reporting limit (TRL) must be estimated for each analyte to choose the concentrations to be tested. Because a water method developed earlier (Jenkins et al., 1988b) is procedurally similar to the soil method being tested, TRLs were estimated from CRL values for the water method performance tests using the following equation:

$$TRL_{soil}\ (\mu g/g) = CRL_{water}\ (\mu g/L) \cdot \left[\frac{\text{extract vol. (L)}}{\text{sample wt. (g)}}\right]$$

Since the soil method uses 10 mL of acetonitrile (0.01 L) and a 2-g subsample of soil, TRL values for soil in μg/g are 0.005 times the CRL values (μg/L) obtained for the water method (Table 8.2). The mean soil TRL for the 13 analytes was about 0.05 μg/g. Since interferences in soil analysis are generally much greater than in water analysis and the USATHAMA standard soil was known to have at least one large peak that elutes near TNB, I chose a higher TRL of 0.5 μg/g for performance tests. The dilutions required to prepare spiking solutions covering the range 0.5 TRL to 10 TRL are shown in Table 8.3.

To conduct the CRL test, duplicate 2.0-g subsamples of USATHAMA standard soil (Table 3.1) were spiked at all six concentration levels (Table 8.3) on each of four days for both groups of analytes. To obtain MDL estimates, 10 replicate 2.0-g subsamples were spiked at the TRL level on a single day for each group of analytes. All samples were processed as described in Section 8.1 except that the ultrasonic bath was not

Table 8.2. Estimates of TRL values from experimentally determined CRL values for water method.*

Analyte	CRL_w^* (µg/L)	TRL_s^{**} (µg/g)
HMX	15.3	0.08
RDX	13.9	0.07
TNB	7.3	0.04
DNB	4.0	0.02
Tetryl	43.6	0.22
TNT	6.9	0.03
2,4-DNT	5.7	0.03
NB	6.4	0.03
2,6-DNT	9.4	0.03
o-NT	11.7	0.06
m-NT	7.9	0.04
p-NT	8.5	0.04
2-Am-DNT	--	0.03 (est)

* CRL values for water method from Jenkins et al. (1988b).

** TRL estimates for the soil method based on 2-g soil sample and 10-mL extraction volume.

Table 8.3. Preparation of spiking solutions from combined stock standards X and Y for performance tests.

Designation	Aliquot of Solution X or Y (mL)	Size of Volumetric Flask (mL)	Approximate Conc. (µg/mL)	(µg/g)*
10 TRL	Straight		10	5.0
5 TRL	50	100	5	2.5
2 TRL	20	100	2	1.0
1 TRL	10	100	1	0.5
0.5 TRL	5	100	0.5	0.25
Blank	0	100	0.00	0.000

* Assuming a 1.00-mL volume is spiked onto a 2.00-g soil sample.

cooled during extraction. Thus the results for tetryl are poorer than would have resulted if the bath had been kept below 25°C.

8.4 Method Detection Limit Estimation

To determine method detection limits (MDLs), the standard deviation for the set of 10 replicates for each analyte at the 0.5-μg/g level was obtained and multiplied by the t statistic appropriate for 10 replicates at the 99% confidence level (Federal Register, 1984). Since I did not consistently get measurable responses for HMX at the 0.5 μg/g level, the MDL reported for HMX was obtained from the standard deviation of eight replicates obtained over four days at the 2.5-μg/g level in the CRL tests described below.

Except for HMX, MDL values for the other analytes were all less than 1 μg/g. Values ranged from 0.03 μg/g for 2,4-DNT and 2-Am-DNT to 1.27 μg/g for HMX (Table 8.4).

8.5 Certified Reporting Limits

Estimates of Certified Reporting Limits (CRLs) were obtained according to the protocol in USATHAMA (1985). To do so, the mean and variance were obtained for each target concentration (Table 8.5). Bartlett's test was used to determine over what concentration range the variances were homogeneous. For all analytes where a range of at least three successive target levels were found to be homogeneous, a regression of found versus target concentrations was performed. The best-fit linear regression equations were obtained, and confidence intervals about the regression lines established at the 90% confidence level (5% α risk and 5% β risk). Certified reporting limit estimates were obtained as described earlier.

Table 8.4. Detection capability estimates.

Analyte	CRL* (µg/g)	MDL** (µg/g)
HMX	2.15	1.27***
RDX	1.03	0.74
TNB	0.24	0.29
DNB	0.12	0.11
NB	0.11†	0.08
TNT	0.24†	0.08
2,6-DNT	0.16†	0.07
o-NT	0.24	0.07
p-NT	0.22†	0.07
m-NT	0.25†	0.07
Tetryl	0.65†	0.12
2-Am-DNT	0.11†	0.03
2,4-DNT	0.07†	0.03

* Certified reporting limit calculated over the widest range of homogeneous variance according to the USATHAMA (1985) protocol.

** Method detection limit obtained from 10 replicate measurements at the 0.5-µg/g level on a single day according to EPA protocol (Federal Register, 1984).

*** Estimate obtained from eight measurements over four days spiked at the 2.5-µg/g level.

† Variances were not found to be homogeneous at 95% confidence level.

Table 8.5. Variance analysis of measured concentrations for certified reporting limit tests.

Analyte	Target Concentration (µg/g)	Found Concentration Mean (µg/g)	Found Concentration Variance (µg/g)	Bartlett's Test (X^2)*
HMX	0.504	0.543	4.50×10^{-1}	
	1.01	1.51	3.34×10^{-1}	
	2.52	2.32	1.79×10^{-1}	1.41
	5.04	4.18	2.97×10^{-2}	10.47*
RDX	0.251	0.423	4.23×10^{-2}	
	0.502	0.609	3.24×10^{-2}	
	1.00	1.15	3.41×10^{-2}	0.14
	2.51	2.35	1.64×10^{-3}	13.57*
	5.02	4.60	2.01×10^{-2}	13.62*
TNB	0.251	0.269	4.87×10^{-3}	
	0.501	0.510	3.03×10^{-3}	
	1.00	1.17	1.60×10^{-2}	5.16
	2.51	2.94	4.09×10^{-2}	13.18*
	5.01	5.91	1.78×10^{-1}	33.54*
DNB	0.250	0.289	2.81×10^{-3}	
	0.501	0.534	4.55×10^{-4}	
	1.00	1.06	1.11×10^{-3}	
	2.50	2.64	3.00×10^{-3}	6.69
	5.01	5.25	1.49×10^{-2}	21.95*
NB	0.264	0.294	2.14×10^{-3}	
	0.528	0.543	3.70×10^{-4}	
	1.06	1.07	7.98×10^{-4}	5.11
	2.64	2.67	3.06×10^{-3}	8.04*
	5.28	5.28	1.69×10^{-2}	27.84*
TNT	0.253	0.323	1.26×10^{-2}	
	0.507	0.503	1.59×10^{-3}	6.32*
	1.01	1.04	2.28×10^{-3}	8.72*
	2.53	2.62	1.35×10^{-2}	10.90*
	5.07	5.18	6.42×10^{-2}	27.09
26DNT	0.256	0.316	6.08×10^{-3}	
	0.511	0.568	7.93×10^{-4}	6.16*
	1.02	1.11	1.59×10^{-3}	7.29*
	2.56	2.79	1.01×10^{-2}	11.99*
	5.11	5.51	4.18×10^{-2}	28.94*
2NT	0.254	0.279	5.76×10^{-3}	
	0.508	0.475	2.51×10^{-3}	
	1.02	0.941	7.30×10^{-3}	5.37
	2.54	2.38	4.99×10^{-2}	15.34*
	5.08	4.66	1.77×10^{-1}	41.81*

Table 8.5 (Con't.)

Analyte	Target Concentration (μg/g)	Found Concentration Mean (μg/g)	Found Concentration Variance (μg/g)	Bartlett's Test (X^2)*
4NT	0.249	0.315	9.23×10^{-3}	
	0.498	0.531	1.38×10^{-3}	
	0.997	1.03	5.14×10^{-3}	5.37
	2.49	2.62	3.24×10^{-2}	15.34*
	4.98	5.15	1.84×10^{-1}	41.80*
3NT	0.253	0.274	1.85×10^{-2}	
	0.505	0.526	5.67×10^{-4}	14.8*
	1.01	1.02	1.64×10^{-3}	20.0*
	2.53	2.59	1.34×10^{-2}	21.0*
	5.05	5.08	7.27×10^{-2}	36.9*
Tetryl	0.252	0.05	2.84×10^{-4}	
	0.503	0.20	1.76×10^{-3}	
	1.01	0.69	1.54×10^{-2}	21.99*
	2.52	1.85	7.39×10^{-2}	41.33*
	5.03	4.10	2.32×10^{-1}	59.9*
2AmDNT	0.250	0.226	1.70×10^{-4}	
	0.500	0.449	1.07×10^{-3}	
	1.00	0.928	2.19×10^{-3}	8.99*
	2.50	2.34	7.96×10^{-3}	20.32*
	5.00	4.54	6.04×10^{-2}	53.43*
2,4-DNT	0.256	0.268	1.64×10^{-4}	
	0.511	0.514	1.13×10^{-4}	0.244
	1.02	1.03	1.31×10^{-3}	12.36*
	2.56	2.61	1.68×10^{-2}	46.91*
	5.11	5.10	3.08×10^{-2}	58.97*

* Critical values of $X^2_{0.95}$ are 5.99 (2 df), 7.81 (3 df) and 9.949 (4 df).

For TNT, m-NT, 2,6-DNT, 2-Am-DNT and 2,4-DNT no homogeneous range of three values was found (Table 8.5). In these cases, the CRLs were established using data from the lowest three target concentrations to minimize the widening of confidence bands due to larger random error variances at higher target levels. A similar situation existed for the tetryl data, but target values over the four lowest levels were used in this case, since the slope of the regression line using only the three lowest concentrations differed by more than 10% compared to the full range (USATHAMA, 1985).

A detailed comparison of MDLs and CRLs has been reported elsewhere (Grant et al., in press), but I observed an interesting comparison here (Table 8.4). For analytes where CRLs were established over a range of homogeneous variance which included the level where the MDL was obtained, CRLs averaged about 1.8 times the MDL. For analytes where the random-error variances were not homogeneous over the concentration range used, CRLs averaged 3.6 times higher than MDLs. Thus it is clear why MDLs and CRLs for some methods correspond rather closely while differing substantially for others.

8.6 Method Accuracy and Precision

An estimate of the method accuracy (% recovery) was obtained from the slope of the least-squares regression line of found versus taken concentration in the CRL tests described in Section 8.5. Percent recovery estimates averaged 98% and ranged from 80% for HMX to 117% for TNB (Table 8.6).

Within-day analytical precision was estimated from the results for the 10 replicate samples in the MDL tests. Standard deviations ranged

Table 8.6. Method accuracy and precision estimates.

Analyte	Accuracy* (% recovery)	Precision: Within Day† (s, μg/g)	Precision: Total†† (s, μg/g)
HMX	80	--	0.56
RDX	84	0.27	0.19
TNB	119	0.11	0.09
DNB	105	0.04	0.04
Tetryl	83	0.04	0.15**
TNT	102	0.03	0.09**
2,4-DNT	102	0.01	0.07**
NB	100	0.03	0.03
2,6-DNT	107	0.03	0.05**
o-NT	92	0.03	0.07
m-NT	101	0.03	0.09**
p-NT	103	0.03	0.07
2-Am-DNT	91	0.01	0.03

* Obtained from the slope of the least-squares regression line of found versus taken results from the CRL test (model without an intercept).

† Within-day estimates are standard deviations of 10 replicate samples spiked at the 0.5-μg/g level for the MDL test.

†† Total estimates are pooled standard deviations over the ranges of homogeneous variance in CRL test and include both within- and between-day variations.

** No homogeneous range, estimates based on lowest three or four concentrations.

from 0.01 μg/g for 2-Am-DNT to 0.27 μg/g for RDX with a median value of 0.03 μg/g (Table 8.6). Total analytical precision (within plus between day) was estimated from the pooled standard deviation over the ranges of homogeneous variance shown in Table 8.5. These estimates ranged from 0.03 μg/g to 0.56 μg/g with a median value of 0.07 μg/g (Table 8.6).

8.7 Ruggedness Testing

Analytical chemists often find that methods described in the literature are difficult to reproduce in their laboratory. This is sometimes due to lack of inclusion of all the important parameters by the authors. Practices which may be common practice in the authors' laboratory may be uncommon elsewhere. A procedure which is used to reduce the impact of this occurrence is to subject the method to a ruggedness test (Youden and Steiner, 1975) before it is considered ready for external use. In a ruggedness test the method is carefully scrutinized, and factors which could potentially affect performance are identified. These factors are then systematically varied in a factorial experiment to assess the sensitivity to each variable or their interaction. In this way those factors which must be carefully controlled can be identified and carefully specified.

Two full 2^4 factorial experiments were conducted in duplicate, and the results reported elsewhere (Jenkins et al. 1988a). One test used Iowa AAP soil 2 and the other the Nebraska D-49 soil (Table 3.2). The four factors tested were (a) the degree of grinding prior to extraction, (b) the use of either vortex mixing or manual shaking prior to extraction in the ultrasonic bath, (c) the concentration of $CaCl_2$ used to flocculate the clay particles prior to filtration and (d) the settling time required

prior to filtration. Analysis of variance was applied to the results for each analyte to discover whether any of these factors had a significant effect on analyte recovery.

Overall the method was found to be very rugged, with few effects significant at the 99% probability level. No single factor was found to be dominant for all soil types or analytes. Based on this test the method was considered sufficiently rugged for external testing.

8.8 Initial Method Testing in Other Laboratories

All results discussed thus far were obtained in the laboratory at CRREL. To obtain an initial assessment of the utility of the method for more general application, the method and two different previously characterized field-contaminated soils were supplied to two laboratories that had no previous experience with the determination of explosive residues in soil but were acquainted with the use of HPLC.

The results of these analyses are presented in Table 8.7 along with values obtained at CRREL for the same soils (known values). For both laboratories the results compared favorably with those obtained at CRREL, particularly considering that the laboratories analyzed different subsamples of field-contaminated soils that had some inherent inhomogeneity.

8.9 Collaborative Test Results

Subsequently the method has been subjected to a full-scale collaborative test under the auspices of the Association of Official Analytical Chemists (AOAC). The test involved blind analysis of 16 soil samples, 4 field-contaminated and 4 fortified soils in duplicate, at eight laboratories using the method described here. The results of this test are

Table 8.7. Results of method testing in two collaborating laboratories using field-contaminated soils.

	Laboratory 1*				Laboratory 2†			
	Soil 1 conc. (μg/g)		Soil 2 conc. (μg/g)		Soil 3 conc. (μg/g)		Soil 4 conc. (μg/g)	
Analyte	known**	determined	known**	determined	known**	determined	known**	determined
HMX	4.2	2.1	124	117	79	88	30	25
RDX	<d*	<d	1162	1120	68	93	135	149
TNB	2.0	2.6	159	170	75	62	5	5
DNB	<d	<d	<d	0.5	<d	1.3	<d	1.6
Tetryl	<d	<d	<d	<d	<d	<d	<d	<d
TNT	<d	1.0	380	375	740	718	5	8
2,4-DNT	<d	<d	4.2	3.3	<d	<d	<d	<d

* Chemistry Department, University of New Hampshire (Ms. Dee Cardin).

† Wilson Laboratory, Salina, Kansas (Dr. Clifford Baker).

** Values determined at CRREL.

reported elsewhere (Bauer et al., 1989; Bauer et al., in press). A summary of the percent recoveries (method accuracy) for fortified samples and the intra- and inter-laboratory precision (repeatability and reproducibility) for both fortified and field-contaminated soils is presented in Table 8.8.

Overall, results of the collaborative test were excellent, particularly considering the levels of analytes present and the use of field-contaminated soils that had some inherent inhomogeneity. The results for tetryl are poorer than for the other six analytes. This is a result of the absence of temperature control on the sonic baths used in the test. Improved tetryl performance into the range found for the other analytes is expected with ultrasonic bath temperature maintained under 25°C as now recommended.

The method developed here, together with the results of the collaborative test described above, have been submitted to the Association of Official analytical Chemists (AOAC), which has granted the method "Interim First Action" status. This is the first step in adoption as the AOAC Standard Method for this determination.

The method has also served as the basis of a procedure for nitroaromatics and nitramines in soil for the American Society of Testing Materials (ASTM), where it is has passed subcommittee balloting in the Committee of Soils and Rock (Tracking number D18.06.87.02). The results have also been submitted to the U.S. EPA Office of Solid Waste, where it is being considered for adoption within their Standard Methods for Solid Waste Analysis (SW846).

Table 8.8. Results of full-scale collaborative test.

	Accuracy		Precision		
Analyte	Mean Concentration* (µg/g)	% Recovery	Mean Concentration** (µg/g)	Repeatability† (% RSD)	Reproducibility†† (% RSD)
HMX	46	95.9	153	14.1	24.0
RDX	60	94.4	877	3.4	7.7
TNB	46	91.9	72	8.3	12.2
DNB	3.5	92.5	1.1	9.8	14.5
tetryl	17	67.0	2.3	18.0	21.3
TNT	40	92.5	669	8.2	9.5
2,4-DNT	5	96.0	1.0	42.3	74

* Fortified samples.
** Field-contaminated samples.
† Within-laboratory precision.
†† Total precision resulting from combination of within-laboratory. error, between-laboratory error and error associated laboratory-sample interaction.

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APPENDIX A

Appendix Data Tables

Table A1. Retention times and capacity factors for primary analytes and potential interferences on LC-8 eluted with 50:38:12 water-methanol acetonitrile, and LC-DP and LC-1 eluted with 60:40 water-methanol, all at 1.5 mL/min.

	Retention time (min)			Capacity factor*		
Substance	LC-8	LC-DP	LC-1	LC-8	LC-DP	LC-1
HMX	3.20	4.05	3.20	0.808	1.34	0.78
RDX	4.17	4.70	4.05	1.36	1.72	1.25
TNB	4.93	5.87	4.26	1.79	2.39	1.37
DNB	5.70	6.78	4.96	2.22	2.92	1.76
Tetryl	7.23	10.88	5.87	3.08	5.29	2.26
TNT	7.56	9.44	5.85	3.27	4.46	2.25
2,4-DNT	8.36	10.03	6.93	3.72	4.80	2.85
Benzene	--	7.04	5.13	--	3.07	1.85
SEX	2.62	3.45	2.91	0.480	0.99	0.62
TAX	2.92	3.91	2.91	0.650	1.26	0.62
2-Am-DNT	8.06	8.72	7.06	3.55	4.04	2.92
4-Am-DNT	8.23	10.88	7.06	3.65	5.29	2.92
2,4-DAm-NT	2.91	4.77	3.96	0.644	1.76	1.20
2,6-DAm-NT	2.69	3.81	3.47	0.520	1.20	0.93
2,6-DNT	8.77	12.40	6.75	3.95	6.17	2.75
2,4,5-TNT	8.43	12.70	7.06	3.76	6.34	2.92
Nitrobenzene	6.27	7.27	5.57	2.54	3.20	2.09
Cyclohexanone	3.76	4.86	6.10	1.12	1.81	2.39

* Capacity factors based on an unretained peak for nitrate at 1.77 min for LC-8, 1.73 min for LC-DP, and 1.80 for LC-1.

Table A2. Instrument calibration results for HMX.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
202.4	5.06	68,408	74,373
404.8	10.12	135,740	139,010
809.6	20.24	280,100	274,720
2,024	50.60	694,980	695,270
4,048	101.2	1,377,900	1,376,800
8,096	202.4	2,747,100	2,722,900

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A3. Instrument calibration results for RDX.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
21.2	0.53	0	10,884
42.4	1.06	17,786	12,699
84.8	2.12	54,238	43,156
212	5.30	107,830	101,010
424	10.60	188,250	191,910
848	21.20	391,600	363,520
2,120	53.00	965,320	950,090
4,240	106.0	1,894,500	1,896,700
8,480	212.0	3,788,300	3,774,200

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A4. Instrument calibration results for TNB.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
19.4	0.48	5,055	15,238
38.9	0.97	28,322	23,080
97.2	2.43	77,372	71,908
194.4	4.86	178,900	152,630
388.8	9.72	350,280	334,870
972	24.3	872,490	861,550
1,944	48.6	1,776,900	1,767,800
3,888	97.2	3,646,100	3,600,500

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A5. Instrument calibration results for DNB.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
10.4	0.26	16,241	18,802
20.9	0.52	24,368	30,398
41.8	1.04	66,488	54,108
104.4	2.61	136,160	144,070
208.8	5.22	290,620	270,490
417.6	10.44	562,890	583,330
1,044	26.10	1,430,000	1,431,900
2,088	52.20	2,855,000	2,864,700
4,176	104.4	5,757,300	5,692,900

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A6. Instrument calibration results for tetryl.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
211.2	5.28	130,590	111,640
422.4	10.56	267,410	265,800
844.8	21.12	504,900	530,590
2,112	52.80	1,321,100	1,265,300
4,224	105.60	2,758,500	2,677,500

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A7. Instrument calibration results for TNT.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
20.4	0.51	15,912	15,938
40.8	1.02	51,943	52,094
101.9	2.55	98,478	116,680
203.8	5.10	202,850	233,580
407.6	10.20	462,230	433,740
1,019	25.47	1,089,200	1,071,200
2,038	50.95	2,083,700	2,116,100

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A8. Instrument calibration results for 2,4-DNT.

Concentration		Peak Area	
Solution (µg/L)	Soil* (µg/g)	Replicate 1	Replicate 2
0	0	0	0
15.6	0.39	18,755	22,328
31.2	0.78	61,461	45,119
62.4	1.56	97,645	110,030
156	3.90	269,500	270,800
312	7.80	512,060	497,591
624	15.60	1,015,500	1,010,300

* Calculated relative to a 2-g soil sample extracted with 50 mL of acetonitrile.

Table A9. Summary of lack-of-fit (LOF) statistics for evaluation of linearity of integrator peak area responses.

	Model with intercept ($y = b_0 + b_1x$)			Model without intercept ($y = b_1 x$)		Zero-intercept hypothesis
Analyte	Regression Coefficient b_0	Coefficient b_1	LOF F Ratio	Regression Coefficient b_1	LOF F Ratio	F Ratio
HMX	2,231.2	340.2	2.54	341.0	2.69	2.00
RDX	3,617.7	445.8	1.06	446.4	1.16	1.80
TNB	-19,360.4	932.9	2.16	925.6	4.48*	--
DNB	-594.8	1,370.9	0.07	1,370.7	0.06	0.00
Tetryl	-23,509.5	644.2	1.71	636.3	1.89	1.93
TNT	9,902.8	1,031.6	1.80	1,038.6	2.07	2.54
2,4-DNT	3,232.5	1,620.2	2.52	1,627.7	2.25	0.74

* F ratio is significant at the 95% confidence level.

Table A10. Results of tests on long-term stability of stock standards.

Standard		Concentration (µg/L) HMX	RDX	TNB	DNB	Tetryl	TNT	2,4-DNT
1987	a	3142	2659	3216	3266	3333	3324	3258
	b	3108	2638	3196	3231	3347	3330	3222
	c	3093	2604	3147	3193	3303	3261	3196
known value		3120	2640	3194	3238	3331	3312	3232
1986	a	3841	3096	3634	4069	4280	3932	--
	b	3757	2972	3540	3971	4281	3841	--
	c	3974	3152	3728	4154	4507	4058	--
known value		4048	3180	3888	4176	4224	4076	--
1985	a	3881	*	3557	--	3940	3631	*
	b	3754	*	3448	--	3786	3514	*
	c	3732	*	3436	--	3670	3477	*
known value		3792	2458	3597	--	3661	3341	1248

* Volume of remaining stock solution too small to allow confident use of this standard.

Table A11. Kinetic study of Iowa AAP soil no. 6.

Time (min)	Extracted concentrations (μg/g) HMX	RDX	TNB	TNT	Extract
		Soil-Plant Homogenizer			
1	36.8	48.2	52.2	857	Acetonitrile
2	39.9	49.4	52.3	883	
4	37.4	51.0	54.3	887	
16	37.7	51.3	46.3	891	
1	14.1	42.3	49.4	910	Methanol
2	21.1	55.3	51.7	953	
4	25.1	59.8	54.1	981	
8	29.4	65.2	56.9	1002	
16	34.0	74.1	58.6	1041	
		Sonic Bath			
1	114	40.1	55.8	860	Acetonitrile
2	111	40.8	56.8	854	
4	112	41.9	56.8	871	
8	111	41.7	55.9	861	
16	112	42.2	57.9	882	
32	111	42.7	57.4	876	
64	112	44.1	58.2	896	
240	115	48.9	67.4	952	
1	13.9	35.6	50.3	885	Methanol
2	16.1	34.2	52.8	901	
4	18.0	35.0	53.6	912	
8	17.6	35.3	54.3	919	
16	23.2	36.9	54.9	912	
32	21.9	39.5	56.4	934	
64	25.7	40.4	57.6	943	
240	38.9	52.2	66.5	992	
		Wrist-Action Shaker			
10	62.0	54.3	52.5	926	Acetonitrile
60	61.7	55.6	56.6	931	
90	62.5	56.0	56.7	931	
150	62.0	56.7	57.8	935	
240	62.3	57.0	56.0	952	
480	62.5	58.0	57.3	963	
1440	63.7	59.8	60.1	979	

Table A11 (cont'd.)

Time (min)	Extracted concentrations (µg/g) HMX	RDX	TNB	TNT	Extract
10	10.9	24.3	50.8	854	Methanol
30	17.1	29.6	53.0	878	
60	18.5	30.1	54.9	894	
90	18.8	30.7	55.1	894	
150	20.6	32.3	55.5	906	
240	19.8	32.8	56.0	910	
480	21.2	34.9	58.7	933	
1440	22.2	33.4	62.4	955	
			Soxhlet		
1	69.5	45.5	50.9	746	Acetonitrile
2	83.5	55.4	61.2	877	
4	85.7	57.1	62.0	882	
25	88.2	60.1	62.5	902	
37	90.0	59.2	59.1	903	
1	10.3	13.3	29.5	537	Methanol
2	16.1	20.9	47.3	799	
4	20.3	30.0	56.4	899	
25	47.0	40.8	56.4	890	
37	51.1	40.4	55.1	860	

Table A12. Kinetic study of TNT extraction, Iowa AAP soil no. 2.

Time (min)	Extracted concentrations (μg/g) Acetonitrile	Methanol
	Soil-Plant Homogenizer	
5	2.05	2.03
10	2.57	2.12
20	2.87	2.53
60	3.42	3.16
	Sonic Bath	
15	2.25	2.51
30	2.43	2.91
60	2.87	2.99
120	3.30	3.50
240	3.15	3.89
420	4.54	5.43
	Wrist-Action Shaker	
30	1.72	1.87
60	1.94	2.28
120	2.22	2.92
240	2.25	2.98
1440	3.62	3.60
2880	4.01	4.42
	Soxhlet	
1	2.39	1.82
2	3.49	2.68
4	3.93	2.86
24	4.31	4.31
48	4.85	4.55

Table A13. Replicate study of Iowa AAP soil no. 6 (μg/g).

Extracted concentrations (μg/g)				
Wrist-action shaker	Sonic bath	Soil-plant homogenizer	Soxhlet extractor	Extract
	TNT			
900.7	882.2	868.5	890.7	Acetonitrile
884.1	880.8	846.4	874.4	
867.6	896.7	859.5	874.9	
872.0	891.6	832.6	876.8	
871.5	872.2	844.8	890.5	
888.4	873.9	841.3	879.6	
895.5	811.9	857.1	892.2	Methanol
882.7	828.8	863.0	893.7	
895.2	854.2	878.5	894.5	
913.9	815.1	849.4	896.9	
876.8	867.9	942.4	887.9	
908.5	864.7	831.0	882.9	
	TNB			
55.7	56.1	50.7	63.6	Acetonitrile
55.4	55.8	51.5	61.8	
53.8	55.3	52.1	61.6	
54.2	55.9	50.3	63.1	
54.7	55.7	51.2	62.0	
56.6	54.5	52.3	60.4	
56.5	50.5	53.0	59.3	Methanol
55.4	52.4	52.0	58.6	
56.3	52.7	53.6	58.3	
57.0	51.1	53.9	57.8	
55.0	54.4	55.1	57.9	
55.5	53.9	52.9	57.6	

Table A13 (Cont'd.)

Extracted concentrations (μg/g)				
Wrist-action shaker	Sonic bath	Soil-plant homogenizer	Soxhlet extractor	Extract
		RDX		
47.4	47.4	45.1	66.8	Acetonitrile
49.0	69.1	44.3	68.8	
58.6	48.4	37.5	83.0	
46.0	42.3	33.4	62.7	
52.5	46.1	177.8	53.1	
71.7	73.4	48.4	56.4	
40.7	31.8	33.3	48.1	Methanol
30.1	38.6	31.3	57.2	
30.6	37.7	56.9	44.7	
33.5	54.9	34.7	48.2	
27.5	39.4	30.9	41.5	
59.1	36.8	28.4	48.3	
		HMX		
71.9	74.8	92.7	85.5	Acetonitrile
131.0	55.3	104.9	68.3	
88.8	73.2	50.4	72.5	
52.1	41.9	53.2	87.1	
101.6	46.5	44.6	96.2	
46.4	46.4	43.8	94.8	
19.7	39.4	25.9	54.0	Methanol
21.4	39.4	19.3	67.6	
14.4	48.3	19.3	64.3	
22.8	28.0	27.1	57.2	
24.8	24.3	63.9	54.3	
30.6	21.1	14.6	55.3	

Table A14. Replicate study of Iowa AAP soil no. 2

Extracted concentrations (μg/g)				
Wrist-action shaker	Sonic bath	Soil-plant homogenizer	Soxhlet extractor	Extract
		TNT		
2.50	3.75	2.07	3.76	Acetonitrile
2.44	3.29	2.07	4.36	
2.36	3.32	2.02	4.30	
2.47	3.69	2.03	4.39	
2.68	3.41	2.31	4.72	
2.33	3.77	2.11	4.55	
2.86	3.80	2.15	3.66	Methanol
2.92	3.76	2.29	3.63	
2.44	3.78	2.21	3.69	
2.67	4.28	2.26	3.64	
2.54	3.92	2.33	3.88	
3.11	3.91	2.13	3.57	
		TNB		
0.417	0.331	0.321	0.380	Acetonitrile
0.285	0.556	0.294	0.263	
0.369	0.432	0.271	0.345	
0.401	0.596	0.343	0.322	
0.380	0.378	0.598	0.290	
0.342	0.403	0.260	0.392	
0.231	0.330	0.238	0.270	Methanol
0.325	0.350	0.226	0.301	
0.244	0.379	0.410	0.337	
0.250	0.541	0.201	0.246	
0.456	0.628	0.294	0.312	
0.360	0.436	0.221	0.172	

Table A15. Soil-to-solvent ratio test for HMX.

Soil/ solvent ratio	Extract Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
Replicate			
1	2011	1986	1897
2	1981	2052	1987
3	1991	2047	2019
4	2031	1964	1921
5	1962	1998	2013
6	1961	1952	1972
$\bar{X}$	1990	2000	1968
S	27.7	41.7	49.5
	Louisiana 11		
1	219	224	302
2	234	224	302
3	219	218	281
4	242	226	214
5	222	225	276
6	210	250	210
$\bar{X}$	224	228	264
S	11.6	11.2	41.8

Table A16. Soil-to-solvent ratio test for RDX.

Soil/ solvent ratio	Extract Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
Replicate			
1	13585	13480	12474
2	13570	13732	12910
3	13525	13388	12644
4	14113	13383	12526
5	13332	13093	13071
6	13354	12644	12442
$\bar{X}$	13580	13287	12678
S	283	376	257
	Louisiana 11		
1	860	862	879
2	890	856	863
3	873	873	832
4	917	867	808
5	902	846	810
6	825	923	777
$\bar{X}$	878	871	828
S	32.9	27.0	37.9

Table A17. Soil-to-solvent ratio test for TNB.

Soil/ solvent ratio	Extract Concentration (μg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
Replicate			
1	479	471	477
2	480	480	469
3	497	491	504
4	477	466	440
5	485	479	495
6	487	488	457
$\bar{X}$	484	479	474
S	7.3	9.6	23.7
	Louisiana 11		
1	1.9	1.7	1.7
2	1.8	1.7	1.7
3	2.2	1.6	1.6
4	6.6*	1.7	1.6
5	1.3	1.9	1.6
6	1.8	1.7	1.7
$\bar{X}$	1.8	1.7	1.7
S	0.3	0.1	0.1

* An outlier using Dixon's Test and not used in statistical analysis.

Table A18. Soil-to-solvent ratio test for DNB.

Soil/ solvent ratio	Extract Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
		Iowa 3	
Replicate			
1	--	38.6	38.7
2	38.9	39.4	40.4
3	40.4	39.4	41.3
4	37.1	41.3	38.3
5	37.5	37.8	38.7
6	38.0	33.4	40.1
$\bar{X}$	38.4	38.3	39.6
S	1.3	2.7	1.2
		Louisiana 11	
1	< d	< d	0.25
2	< d	< d	0.16
3	< d	< d	0.12
4	< d	< d	0.10
5	< d	< d	0.15
6	< d	< d	0.13
$\bar{X}$	--	--	0.15
S	--	--	0.05

Table A19. Soil-to-solvent ratio test for tetryl.

Soil/ solvent ratio	Extract Concentration (μg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
Replicate			
1	364	455	457
2	409	419	331
3	379	368	419
4	378	451	342
5	367	366	637*
6	442	462	443
$\bar{X}$	390	420	398
S	30.1	43.8	58.2
	Louisiana 11		
1	4.3	3.4	3.7
2	6.0	4.3	3.4
3	7.3	3.3	3.0
4	3.4	3.9	3.1
5	4.1	3.3	2.6
6	3.7	3.2	3.0
$\bar{X}$	4.8	3.1	3.1
S	2.2	1.4	0.4

* An outlier using Dixon's Test and not used in statistical analysis.

Table A20. Soil-to-solvent ratio test for TNT.

Soil/ solvent ratio	Extract Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
		Iowa 3	
Replicate			
1	15888	15044	13960
2	14731	14762	14084
3	14612	15326	14474
4	15019	14449	13519
5	14827	14699	14495
6	14326	14306	14406
$\bar{X}$	14901	14764	14460
S	536	376	481
		Louisiana 11	
1	11.9	12.6	12.5
2	19.6*	11.8	11.5
3	12.8	10.9	12.3
4	11.4	12.0	11.2
5	14.3	12.5	11.3
6	10.7	25.5*	11.0
$\bar{X}$	12.2	12.0	11.6
S	1.4	0.7	0.6

* An outlier using Dixon's Test and not used in statistical analysis.

Table A21. Comparison of analytical results for HMX, RDX, TNB and TNT samples flocculated with $CaCl_2$ vs those centrifuged prior to filtration.

	HMX (µg/g)		Concentration Ratio
Sample	Centrifuged	Flocculated	(centrifuged/floc.)
Iowa 3	1786	1926	0.93
Iowa 6	60	70	0.86
Louisiana 11	254	258	0.98
Louisiana 12	64	68	0.94
Milan 13	84	86	0.98
Milan 14	75	79	0.95
Milan 16	30	27	1.11
Milan 17	4.7	3.7	1.27
			mean = 1.00
			S.D. = 0.13

	RDX (µg/g)		Concentration Ratio
Sample	Centrifuged	Flocculated	(centrifuged/floc.)
Iowa 3	11918	12807	0.94
Iowa 6	108	115	0.94
Louisiana 11	952	972	0.98
Louisiana 12	186	185	1.01
Milan 13	470	465	1.01
Milan 14	592	616	0.96
Milan 16	137	139	0.99
Milan 17	< d	< d	--
			mean = 0.98
			S.D. = 0.03

	TNB (µg/g)		Concentration Ratio
Sample	Centrifuged	Flocculated	(centrifuged/floc.)
Iowa 3	487	468	1.04
Iowa 6	80	80	1.00
Louisiana 11	2.1	2.1	1.00
Louisiana 12	3.9	3.8	1.03
Milan 13	3.0	2.5	1.20
Milan 14	< d	< d	--
Milan 16	4.6	6.1	0.75
Milan 17	< d	< d	--
			mean = 1.00
			S.D. = 0.14

	TNT (µg/g)		Ratio
Sample	Centrifuged	Flocculated	(centrifuged/floc.)
Iowa 3	9249	9237	1.00
Iowa 6	686	784	0.88
Louisiana 11	13.2	14.8	0.89
Louisiana 12	15.1	12.4	1.22
Milan 13	33	35	0.94
Milan 14	1.1	1.3	0.85
Milan 16	4.1	5.5	0.75
Milan 17	1.6	1.1	1.45
			mean = 1.00
			S.D. = 0.23

Table A22. Results of filtration experiment for HMX in 50:38:12 water-methanol-acetonitrile.

Filter type	Concentrations (µg/L) in Replicates 1	2	3	4
		Solution A		
Unfiltered	476	466	473	481
Millex-HV	492	509	478	531
Nalgene (green)	460	478	494	469
Millex-SR	485	481	505	475
Spartan-T	489	500	436	452
Bio Rad Prep Disc	470	467	479	484
Spartan 3	467	476	479	485
Spartan 25	529	461	510	468
Nalgene (yellow)	482	468	474	473
Spectra/Por	474	480	518	494
Gelman Acro LC25	500	459	478	490
Nuclepore	498	526	473	523
		Solution B		
Unfiltered	253	227	233	235
Millex-HV	223	232	243	211
Nalgene (green)	252	230	249	229
Millex-SR	237	241	227	254
Spartan-T	232	230	228	245
Bio Rad Prep Disc	241	235	219	224
Spartan 3	242	243	232	256
Spartan 25	249	231	220	243
Nalgene (yellow)	228	243	235	248
Spectra/Por	232	236	271	256
Gelman Acro LC25	252	234	237	232
Nuclepore	227	236	237	229

Table A23. Results of filtration equipment for RDX in 50:38:12 water-methanol-acetonitrile.

Filter type	Concentrations (µg/L) in Replicates 1	2	3	4
		Solution A		
Unfiltered	407	395	420	417
Millex-HV	401	410	408	412
Nalgene (green)	403	416	406	397
Millex-SR	392	393	421	393
Spartan-T	393	404	384	387
Bio Rad Prep Disc	394	397	396	388
Spartan 3	396	393	401	421
Spartan 25	394	392	396	411
Nalgene (yellow)	393	412	393	388
Spectra/Por	404	396	405	427
Gelman Acro LC25	413	385	386	400
Nuclepore	385	390	391	400
		Solution B		
Unfiltered	206	204	199	210
Millex-HV	198	216	206	213
Nalgene (green)	212	208	213	216
Millex-SR	207	202	203	204
Spartan-T	198	207	205	201
Bio Rad Prep Disc	208	215	205	212
Spartan 3	209	205	194	199
Spartan 25	209	201	201	201
Nalgene (yellow)	200	199	221	204
Spectra/Por	201	218	201	209
Gelman Acro LC25	199	211	211	216
Nuclepore	203	209	206	200

Table A24. Results of filtration experiment for TNT in 50:38:12 water-methanol-acetonitrile.

Filter type	Concentrations (µg/L) in Replicates 1	2	3	4
		Solution A		
Unfiltered	219	197	208	209
Millex-HV	199	205	201	200
Nalgene (green)	207	209	212	208
Millex-SR	228	206	205	203
Spartan-T	201	197	194	193
Bio Rad Prep Disc	202	201	216	213
Spartan 3	201	198	212	206
Spartan 25	202	204	212	204
Nalgene (yellow)	204	191	199	200
Spectra/Por	191	212	204	207
Gelman Acro LC25	213	201	203	203
Nuclepore	206	207	202	216
		Solution B		
Unfiltered	106.7	100.5	104.1	115.2
Millex-HV	110.0	108.7	101.8	108.6
Nalgene (green)	110.6	120.2	100.0	95.5
Millex-SR	101.2	102.8	103.8	112.3
Spartan-T	113.3	106.3	110.4	120.7
Bio Rad Prep Disc	114.7	111.1	116.0	116.1
Spartan 3	110.9	114.2	104.4	107.9
Spartan 25	100.9	106.3	108.0	94.8
Nalgene (yellow)	109.3	97.7	107.9	113.2
Spectra/Por	104.1	112.7	101.7	108.8
Gelman Acro LC25	101.7	108.0	102.2	112.7
Nuclepore	101.0	112.1	106.0	105.8

Table A25. Results of filtration experiment for 2,4-DNT in 50:38:12 water-methanol-acetonitrile.

Filter type	Concentrations (μg/L) in Replicates 1	2	3	4
		Solution A		
Unfiltered	169	148	162	158
Millex-HV	159	153	156	161
Nalgene (green)	152	161	157	165
Millex-SR	166	151	152	159
Spartan-T	170	156	150	154
Bio Rad Prep Disc	163	154	158	156
Spartan 3	155	160	156	161
Spartan 25	151	157	164	152
Nalgene (yellow)	166	153	164	156
Spectra/Por	157	164	165	159
Gelman Acro LC25	171	158	162	156
Nuclepore	147	155	153	159
		Solution B		
Unfiltered	80.2	73.2	81.0	77.7
Millex-HV	77.6	83.1	72.8	81.3
Nalgene (green)	73.6	82.4	84.3	77.8
Millex-SR	79.7	78.3	80.0	79.2
Spartan-T	91.2	81.5	73.5	81.2
Bio Rad Prep Disc	81.1	77.6	75.9	82.8
Spartan 3	84.7	79.6	74.2	86.7
Spartan 25	73.6	80.8	68.0	77.0
Nalgene (yellow)	70.0	73.2	78.0	77.3
Spectra/Por	75.1	77.2	78.0	76.9
Gelman Acro LC25	77.0	76.4	71.7	79.4
Nuclepore	80.0	77.1	87.2	81.4