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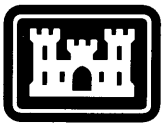

US Army Corps of Engineers

Cold Regions Research & Engineering Laboratory

Analytical method for determining tetrazene in soil

Marianne E. Walsh and Thomas F. Jenkins

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U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY
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For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380, Metric Practice Guide, *published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.*

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An ion-pairing RP-HPLC method was developed to determine tetrazene in soil. The method involves extracting a 2-g soil sample with 50 mL of a solvent containing 55/45 v/v methanol-water and 1-decanesulfonic acid, sodium salt at 0.01 M concentration. The soil and extracting solvent are vortexed for 15 s and shaken on a platform orbital shaker for a period up to 5 hr. The extract is filtered through a 0.5-µm Millex SR filter and analyzed. Determination was achieved using an LC-18 column, a mobile phase of 2/3 v/v methanol-water containing 1-decanesulfonic acid, sodium salt at 0.01 M concentration, and a UV detector set at 280 nm. The mobile phase pH was adjusted to 3 with glacial acetic acid, which was optimal for separation of tetrazene from potential interferences by other explosives. Retention time was 2.8 min. Kinetic studies show maximum tetrazene recoveries are achieved from undried soil within 5 hr of shaking at room temparature. Refrigeration is required for extracts that are not analyzed immediately.

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PREFACE

This report was prepared by Marianne E. Walsh, Physical Scientist, Applied Research Branch, Experimental Engineering Division, and Thomas F. Jenkins, Research Chemist, Geochemical Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory. Funding for this research was provided by the U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, Maryland (R-90 Multi-Analytical Services), Martin H. Stutz, Project Monitor.

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ILLUSTRATIONS

TABLES

ABBREVIATIONS

HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
IRPQAP	Installation Restoration Program Quality Assurance Program
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
RP-HPLC	reverse-phase, high-performance liquid chromatography
SARM	Standard Analytical Reference Material
TNT	2,4,6-trinitrotoluene
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
UV	ultraviolet

Analytical Method for Determining Tetrazene in Soil

MARIANNE E. WALSH AND THOMAS F. JENKINS

INTRODUCTION

Tetrazene is an organic amine used as a primary explosive by the U.S. Army (Fig. 1). Contamination of soil and water surrounding munitions manufacturing and storage facilities is an environmental concern. USATHAMA, under the Installation Restoration Program, directed the Cold Regions Research and Engineering Laboratory to develop methods for the determination of tetrazene in water and soil. An analytical method for determination of tetrazene in water is described elsewhere (Walsh and Jenkins 1987), and this report describes the development of a method for tetrazene in soil.

Most of the published analytical methods for tetrazene were developed for product quality control of primer mixes or caps. Primers must contain 2–8% tetrazene by weight to be activated by friction or impact. Quantitative methods for the determination of tetrazene in primer mixes are listed in Table 1 and include polarographic (Wild 1957, 1963; Traas and Ligtenberg 1962; Hetman 1964; Flack 1974; Semel 1980), spectrophotometric (Norwitz and Keliher 1979, Tummavuori and Surma-aho 1981), and thermoanalytical (Krien 1979) protocols. No analytical method for tetrazene in soil was found in the literature.

EXPERIMENTAL METHODS

Instrumentation

RP-HPLC determinations were conducted on a Perkin-Elmer Series 3/LC65T high-performance liquid chromatograph equipped with a variable-wavelength UV detector set at 280 nm. Previous work had established that this setting was optimal for tetrazene (Walsh and Jenkins 1987). A Rheodyne 7125 sample loop injector with a 100-μL sample loop was overfilled by injecting 500 μL of sample. Separations were achieved with an LC-18 (Supelco) analytical column. The mobile phase consisted of 2/3 v/v methanol–water modified with 0.1 M 1-decanesulfonic acid, sodium salt. The mobile phase pH was adjusted by adding 8 mL of glacial acetic acid to each liter of eluent pre-

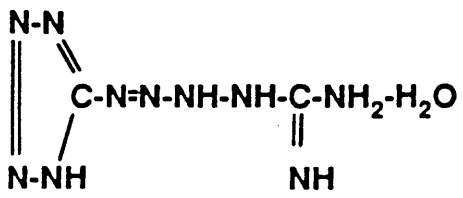

Figure 1. Structure of tetrazene.

Table 1. Existing analytical methods for the determination of tetrazene.

Matrix	*Method*	*Concentration range*	*Reference*
Percussion cap	Polarography	200 mg/g	Wild (1957, 1963)
Primer mix	Polarography	10–50 mg/g	Flack (1974)
Primer mix	Polarography	50 mg/g	Semel (1980)
Primer mix	Polarography	40 mg/g	Traas and Ligtenberg (1962)
Primer	Polarography	0.1–0.2 g	Hetman (1964)
Primer mix	Calorimetry	10–25 mg/g	Krien (1979)
Primer mix	Spectrophotometry	20–50 mg/g	Norwitz and Keliher (1979)
Primer mix	Spectrophotometry	130–660 mg/g	Tummavuori and Surma-aho (1981)
Water	Spectrophotometry	0.5–10 mg/L	USATHAMA (1981)
Surfaces	Color development	Qualitative	USATHAMA (1981)

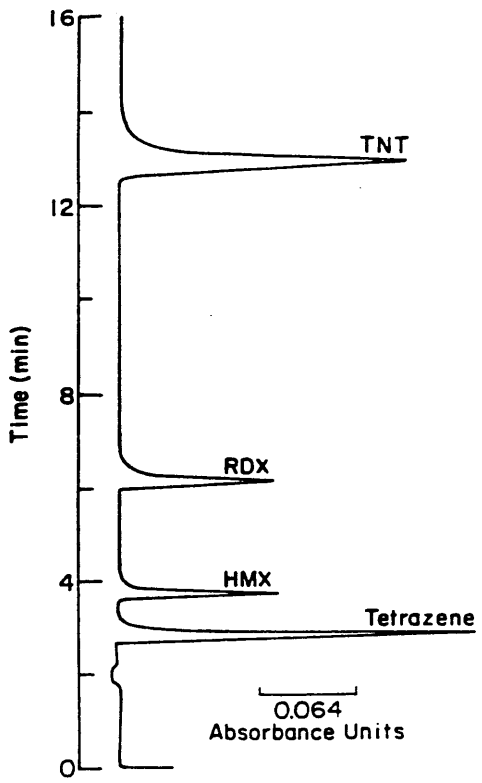

Figure 2. Typical chromatogram showing separation of tetrazene from other explosives.

pared. Flow was set at 1.5 mL/min. These parameters allow for elution of tetrazene without interference from other explosives such as HMX, RDX, and TNT (Fig. 2). Retention time for tetrazene is 2.8 min; based on an unretained peak for nitrate (1.6 min), the capacity factor for tetrazene is 0.75. The peak areas were measured using a Hewlett Packard HP3390A digital integrator.

Chemicals

Analytical standards for tetrazene were prepared from SARM obtained from USATHAMA, Aberdeen Proving Ground, Maryland. The SARM was dried to a constant weight in the dark over dry calcium chloride in a vacuum desiccator. The methanol used to prepare the tetrazene standards and the mobile phase for HPLC analysis was Baker HPLC grade. The ion-pairing agent for HPLC was 1-decanesulfonic acid, sodium salt (98%) obtained from Aldrich. The glacial acetic acid was Mallinckrodt (99.5%). Water used for the preparation of the mobile phase was purified by a MilliQ Type I Reagent Grade Water System (Millipore). The mobile phase was vacuum-filtered through a Whatman CF-F microfiber filter to remove particulates and degas the eluent. The blank soil used in the spike recovery study was USATHAMA standard soil. Other blank soils tested during the method development included Lebanon landfill soil, Fort Edwards clay, Manchester sand, and garden soil obtained locally. Properties of these soils are listed in Table 2. A Minneapolis soil spiked with tetrazene was prepared at the request of USA THAMA. This contaminated soil was used in kinetic studies.

Table 2. Soil properties.

Soil	*Clay (%)*	*Total organic carbon (%)*	*pH*
USATHAMA standard soil	53.6	1.45	6.4
Iowa AAP No. 7	48.6	2.62	7.7
Fort Edwards clay	100	0.52	8.4
Lebanon landfill	11.3	0.3	6.2
Manchester sand	0	0.3	5.5
Garden soil (local)	—	—	7.0

Calibration standards

An analytical stock standard of 102 mg/L tetrazene was prepared by accurately weighing 20.4 mg of dried SARM into a 200-mL volumetric flask. The flask was filled to volume with methanol, a stir bar was added, and the flask was placed in an ice bath on top of a stirring plate for 60 min. After stirring, the flask was manually inverted for 10 min, whereupon crystals of tetrazene were no longer visible.

To test the linearity of the detector response a series of intermediate standards was prepared. First, the stock solution was allowed to warm to room temperature and standard A was prepared by pipetting a 4-mL aliquot of stock into a 100-mL volumetric flask and bringing it to volume with methanol. Additional intermediate standards were prepared by pipetting the volumes shown in Table 3 into individual volumetric flasks and filling the flasks to volume with methanol. All standards were prepared in duplicate.

Prior to determination, 4.0 mL of each intermediate standard were mixed with 6.0 mL of chilled water in glass scintillation vials. Each vial was capped and shaken, then placed in an ice bath. Portions of these chilled, diluted standards were analyzed as described above.

Table 3. Calibration standards.

Std.	Aliquot of sol. A (mL)	Capacity of volumetric flask (mL)	Solution concentration (μg/L)	Injection solution concentration (μg/L)*	Equivalent concentration in soil (μg/g)†
A**	—	100	4080	1632	40.8
B	25	100	2040	816	20.4
C	20	100	816	326	8.16
D	10	100	408	163	4.08
E	5	100	204	81.6	2.04
F	2	100	81.6	32.6	0.816
G	1	100	40.8	16.3	0.408
H	0.5	100	20.4	8.16	0.204
I	0	100	0	0	0.8

* Concentrations correspond to dilution of 4 mL standard with 6 mL of water.

† Concentrations correspond to 100% extraction from 2 g of soil with 50 mL of solvent.

** Sol. A was prepared by diluting the 102 mg/L stock solution 1/25 v/v with methanol.

Soil extraction

All soils were extracted by adding 50.0 mL of extracting solvent to 2-g soil subsamples in 125-mL Erlenmeyer flasks equipped with ground glass stoppers, vortexing for 15 s, and shaking at 200 rpm on a platform shaker. Shaking time was varied for the kinetic studies and was 1.5 hr for the spike–recovery study. The extracting solvent was 100% methanol for the initial experiments and was changed to a mixture of 55/45 v/v methanol–water, modified with 0.01 M 1-decanesulfonic acid, sodium salt. The latter solvent produced higher recoveries from spiked soil samples. All extracts were filtered through a 0.5-μm membrane and chilled in an ice bath before analysis.

Spike–recovery study

Reporting limits were obtained using the Hubaux and Vos (1970) method outlined in the USA THAMA Installation Restoration Program Quality Assurance Program for Class 1 certification. Samples of USATHAMA standard soil were spiked, extracted, and analyzed on four separate days. The spiking solution stock for the recovery study was prepared by dissolving tetrazene in methanol in a manner similar to that described for the calibration stock, except that 20.5 mg of SARM material were used. The stock solution was diluted to volume with methanol. A series of spiking solutions were prepared by the dilutions shown in Table 4.

Table 4. Spiking solutions.

Std.	Aliquot of sol. A (mL)	Capacity of volumetric flask (mL)	Solution concentration (mg/L)	Equivalent concentration in soil (μg/g)*
A†	—	200	51	25.6
B	25	50	25.5	12.8
C	20	100	10.2	5.12
D	10	100	5.1	2.56
E	5	100	2.55	1.28
F	0	100	0	0

* Assuming 1.0 mL spiking solution added to 2 g soil.

† Spike solution A was prepared by diluting the stock 1/1 v/v with methanol.

Several 2-g subsamples of USATHAMA standard soil were weighed out to the nearest 0.1 g in 125-mL Erlenmeyer flasks equipped with ground glass stoppers. Each soil subsample was spiked with 1.0 mL of one of the spiking solutions and allowed to stand for 1 hr uncapped. Then 50.0 mL of 55/45 v/v methanol-water, 0.01 M 1-decanesulfonic acid, sodium salt were added. Each flask was vortexed for 15 s and shaken for 1.5 hr. Samples were allowed to settle for 5 min, then the extracts were filtered and analyzed. Filtered extracts were stored in an ice bath before analysis.

RESULTS AND DISCUSSION

Instrument calibration

To determine if the detector response for tetrazene was a linear function of analyte concentration, the calibration data was subjected to a regression analysis for a non-zero-intercept model ($y = a + bx$) and a zero-intercept model ($y = bx$). The regression coefficients a and b were estimated using the method of least squares (Fig. 3).

The fitted equations for both models were subjected to the lack-of-fit (LOF) test. A linear model

PRECERTIFICATION ANALYSIS

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Method Name: SOIL
Compound: TETRAE
Units of Measure: UGG

Laboratory: CR
Analysis Date: 09/14/87
Matrix: SO

ANALYSIS OF RESIDUAL VARIATIONS

	--- Model with Intercept ---			- Model through the Origin -		
	Y = (4912.543750) + (58863.66370)X			Y = (59037.84640)X		
	(SS)	(df)	(MS)	(SS)	(df)	(MS)
Residual:	2294673080	14	163905220.0	2549185210	15	169945680.7
Total Error:	2263284860	8	282910607.5	2263284860	8	282910607.5
Lack of Fit:	31388220.00	6	5231370	285900350.0	7	40842907.14
	LOF F-Ratio(F): 0.018491247			LOF F-Ratio(F): 0.144366828		
	Critical 95% F: 3.58			Critical 95% F: 3.50		

ZERO INTERCEPT HYPOTHESIS

Zero Intercept Accepted Calculated F: 1.552800637 Critical 95% F: 4.60

****

TABLE OF DATA POINTS Targets: 8 Measures per Target: 2

	Target Value	Instrument Values	
1:	0.2040000	15231	15874
2:	0.4080000	26223	33273
3:	0.8160000	55696	47487
4:	2.0400000	128270	127640
5:	4.0800000	242210	245170
6:	8.1600000	499020	472780
7:	20.400000	1206100	1204500
8:	40.800000	2437100	2376200

Figure 3. Lack-of-fit and zero-intercept test for calibration standards.

was found to be acceptable at the 95% confidence level. The intercept was then tested to determine if it was significantly different from zero. The *F*-ratio was calculated by dividing the differences between the residual sum of squares for the non-zero- and zero-intercept models by the residual mean square for the model with non-zero intercept. Since the calculated *F*-ratio was less than the critical value at the 95% confidence level, the zero-intercept linear model was accepted. Thus, daily calibration may be obtained using a zero-intercept model.

Soil extraction

The first step in the determination of organics in soil involves some type of extraction procedure. Jenkins and Walsh (1987) outline several extraction techniques for organic chemical residues in soil. The techniques include Soxhlet extractors, shakers (manual and wrist action), ultrasonic baths, and sonic probes. Since tetrazene is heat labile and shock sensitive, Soxhlet and sonic extraction procedures were ruled out. Mechanical shakers include various wrist-action and rotary configurations. A wrist-action shaker was tested, but it was observed to be ineffective in dispersing the soil in the solvent. Maximum contact between soil and solvent was achieved with a variable-speed rotary platform shaker set so that the soil and solvent are gently swirled in an Erlenmeyer flask.

Kinetic studies with methanol

Initially, kinetic studies were conducted to determine the length of contact time required for maximum recovery of analyte. Methanol was used as the extracting solvent. Since previous experience (Walsh and Jenkins 1987) has indicated that tetrazene is unstable in solution at room temperature, all samples were kept cold throughout the extraction procedure. Two-gram subsamples of the spiked Minneapolis soil obtained from USA THAMA were extracted with methanol by shaking at 0°C for 75.5 hr. Samples of the extract were removed and analyzed at 5 min, 1, 4, 6, 24, 30, 48 and 75.5 hr. The results are presented in Figure 4. Maximum concentration was achieved rapidly, between 0 and 4 hr, followed by a decrease in concentration.

A second kinetic study was performed over a 2-hr time period with aliquots removed at 5, 15, 30, 60, and 120 min. In addition to the samples shaken at 0°C, a duplicate set was shaken at room temperature. Results are presented in Figure 5. Equilibrium was reached faster at room temperature, and degradation was not observed within the 2-hr time period as compared to extracts from the 0°C subsamples.

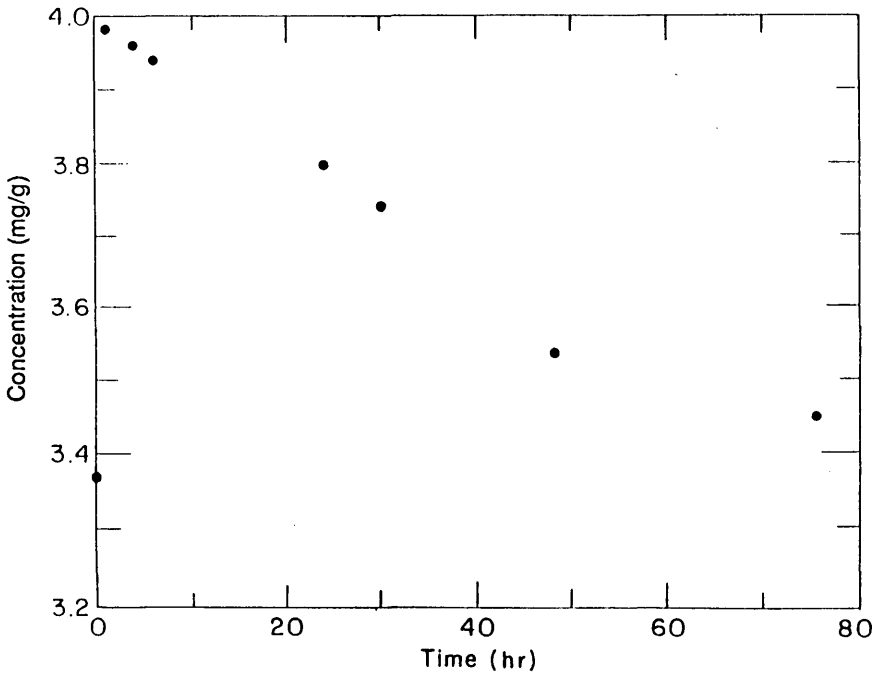

Figure 4. Kinetics study: Extraction of Minneapolis soil with methanol.

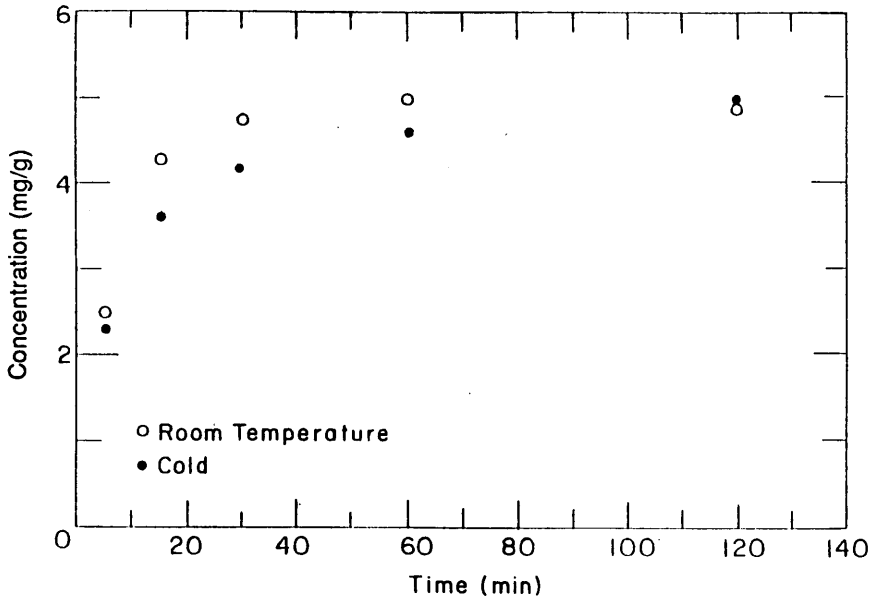

Figure 5. Kinetics study: Extraction of Minneapolis soil with methanol at room temperature and at 0°C.

Extraction of undried and dried soils

To see if a drying step is required prior to extraction, both wet and dried soils were analyzed. Twelve 2-gram subsamples of the spiked Minneapolis soil obtained from USATHAMA were randomly divided into two groups. Six of the subsamples were allowed to air-dry overnight, while the other six were kept in a sealed container to prevent evaporation. Both sets of samples were extracted with methanol and analyzed. The results are presented in Table 5. Although the mean concentration for the undried samples was 5% lower than the mean concentration for the dried samples (probably due to dilution with water present in the soil), the difference was not statistically significant at the 0.05 significance level [calculated t value = 1.49 vs a table value of 2.23 for $t_{0.95}(df = 10)$]. Thus a drying step is not necessary, but it will improve homogenization prior to subsampling.

Table 5. Comparison of found concentrations of tetrazene in dried and undried soils.

	Conc. in soil (mg/g)	
Rep.	*Dried*	*Undried**
1	4.78	4.16
2	4.44	4.22
3	4.53	4.33
4	4.32	4.66
5	4.59	4.35
6	4.53	4.55
Mean	4.53	4.38
Std. dev.	0.155	0.192
Variance	0.0240	0.0367

Calculated t = 1.49

$t_{0.95}(df = 10)$ = 2.23

* Concentration based on dry soil weight.

Screening experiment to isolate possible sources of analyte loss

Since tetrazene is prone to degradation, a factorial experiment was performed to test if analyte loss occurs 1) while the spiked soil is aging, 2) during vortexing, or 3) during exposure to ambient temperatures. The 2^3 factorial design matrix is shown in Table 6. Samples were prepared in duplicate by spiking the blank USATHAMA standard soil. Half of the samples contained soil spiked with a tetrazene–methanol solution to yield a target concentration of 51 μg/g. For the other half, sample 50-mL aliquots of methanol were spiked with the same amount of spike solution that was added to the soil samples. Half of the samples were vortexed and the other half were not. Half of the samples were prepared at ambient temperature and the other half were prepared at 0°C. All extracts were diluted 2/3 v/v with water before filtration and analysis.

Table 6. Design matrix and found concentrations of tetrazene for screening experiment to isolate sources of analyte loss.

Design matrix							Found conc.*
X1	*X2*	*X3*	*X1X2*	*X1X3*	*X2X3*	*X1X2X3*	*(μg/g)*
1	1	1	1	1	1	1	25.8
1	1	1	1	1	1	1	25.3
-1	1	1	-1	-1	1	-1	49.6
-1	1	1	-1	-1	1	-1	46.3
1	-1	1	-1	1	-1	-1	24.7
1	-1	1	-1	1	-1	-1	24.5
-1	-1	1	1	-1	-1	1	48.2
-1	-1	1	1	-1	-1	1	48.7
1	1	-1	1	-1	-1	-1	31.8
1	1	-1	1	-1	-1	-1	30.7
-1	1	-1	-1	1	-1	1	52.2
-1	1	-1	-1	1	-1	1	51.3
1	-1	-1	-1	-1	1	1	30.8
1	-1	-1	-1	-1	1	1	30.6
-1	-1	-1	1	1	1	-1	49.7
-1	-1	-1	1	1	1	-1	50.1

X1—spiked matrix (1 = soil, -1 = methanol).
X2—vortexing (1 = yes, -1 = no).
X3—temperature (1 = 23 °C, -1 = 0 °C).

* Target concentration = 51 μg/g.

Results were compared using analysis of variance (ANOVA) (Table 7). Two factors were found to be significant: matrix and temperature. The interaction between them was also significant although of much smaller magnitude than the main effects. While the samples prepared at 0°C yielded higher recoveries than those prepared at 23°C, the most significant differences were between the spiked soil and spiked methanol samples. Recoveries averaged 43% lower for spiked soils than spiked methanol. Clearly, there is some sort of interaction between the tetrazene and the soil; either sorption or degradation is occurring, and the process is slower at 0°C.

Table 7. ANOVA table for screening experiment.

	Effect	*SS*	*df*	*MS*	*F*
Total	—	25,990	16	—	—
Correction factor	—	24,048	1	—	—
X1 (matrix)	-21.5	1,847	1	1,847	2,284*
X2 (vortexing)	0.712	2.03	1	2.03	1.51
X3 (temperature)	-4.26	72.7	1	72.7	89.9*
X1X2	0.038	0.006	1	0.006	0.007
X1X3	-1.64	10.7	1	10.7	13.2*
X2X3	-0.488	0.95	1	0.95	1.17
X1X2X3	0.688	1.89	1	1.89	2.33
Error	—	6.47	8	0.809	—

* Significant at the 95% confidence level.
$F_{0.95}(1, 8) = 5.32$.

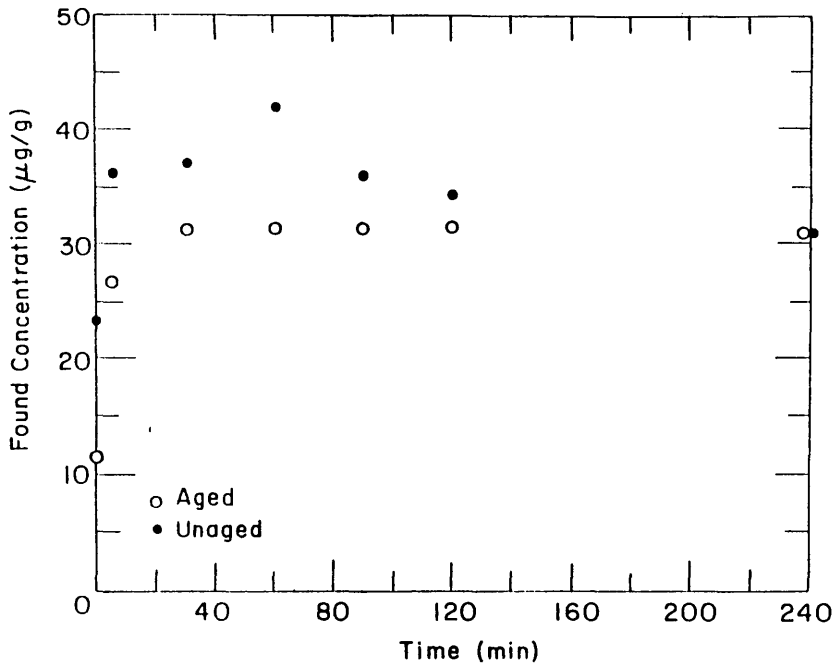

Figure 6. Kinetics study: Extraction of aged and unaged spiked soils.

Recovery from aged and unaged spiked soils

To determine if the soil-tetrazene interaction is occurring while the spiked soils age, an experiment was performed to compare recoveries from aged and unaged spiked samples. Two 2-g subsamples of USATHAMA standard soil were spiked with a tetrazene-methanol solution to yield a target concentration of 51 μg/g. One soil sample was extracted immediately with 50 mL of methanol while the other sample was allowed to age 1 hr prior to extraction with methanol. Both samples were vortexed and shaken. Immediately and after 5, 30, 60, 90, 120, and 240 min of shaking, aliquots were removed for analysis. The results are presented in Figure 6. Recovery from the unaged spiked sample was initially higher than from the aged spiked sample, but at 240 min, recoveries were equal.

This result indicates that interactions between tetrazene and the soil occur very rapidly or occur in the presence of methanol during extraction. Thus, the aging process is not responsible for incomplete analyte recovery. The inability to recover additional analyte when sequential extraction was attempted suggests that the lost analyte is either degraded or chemi-sorbed in a manner that cannot be released by simple solvent extractions.

Effect of soil type and extracting solvent on recovery of analyte

To see if recovery is a function of soil type and/or extracting solvent, several different soils were spiked with a tetrazene-methanol solution, aged for 1 hr, and then extracted with various combinations of methanol and water. The target concentration was 51 μg/g.

Initially five soils from different locations were spiked. For two of the soils, both wet and dry samples were used. The results for methanol extractions are presented in Table 8. Recoveries ranged from 23.2% for the Fort Edwards clay sample to 86.5% for the Lebanon landfill sample. Recoveries appear to be higher for sandy soils (Table 2).

Several methanol-water ratios were tested on spiked samples of Fort Edwards clay, USATHAMA standard soil, and Manchester sand (Tables 9–11). Some of the extracting solvents contained small amounts of acetic acid and/or 1-decanesulfonic acid, sodium salt. By adding water to the methanol, the soils were much better dispersed during shaking and higher recoveries were noted. At the same time, however, the addition of water to the methanol made the soil extacts more difficult to

Table 8. Recovery from various soils using methanol.

Soil	Found conc. (μg/g)	Recovery* (%)
Lebanon landfill	44.1	86.5
Fort Edwards clay	11.8	23.2
Iowa #7	27.5	54.0
Garden soil (undried)	40.3	78.9
Garden soil (dried)	37.7	73.9
USATHAMA std. soil (dry)	27.9	54.7
USATHAMA std. soil (wetted)	24.1	47.2
Solvent spike	52.5	103

* Target concentration = 51 μg/g.

Table 9. Recovery from Fort Edwards clay with various extracting solvents.

Methanol conc. (%)	Water conc. (%)	Acetic acid conc. (%)	1-decanesulfonic acid, sodium salt molar conc. (%)	Recovery (%)
100	0	0	0	23.2
				32.7
90	10	0.8	0.005	64.5
90	10	0.4	0.005	65.8
80	20	0.6	0.005	70.3
70	30	0.8	0.005	73.6
70	30	0.4	0.005	73.4
				73.2
55	45	0	0	72.6
				69.9
55	45	0	0.01	73
50	50	0	0	71.6
45	55	0	0	73.4
40	60	0	0.005	67.2
40	60	0	0.01	67.7
40	60	0.4	0	65.7
40	60	1.6	0	62.4
40	60	0	0	67.8
				70.6
40	60	0.4	0.005	65.8
40	60	0.4	0.01	67.4
40	60	1.6	0.005	62.5
40	60	1.6	0.01	65.4
40	60	0.8	0.01	73.8

Table 10. Recovery from USATHAMA standard soil with various extracting solvents.

Methanol conc. (%)	Water conc. (%)	Acetic acid conc. (%)	1-decanesulfonic acid, sodium salt molar conc. (%)	Recovery (%)
100	0	0	0	58.1
				54.7
				49
90	10	0.8	0.005	61.1
90	10	0.4	0.005	65
80	20	0.6	0.005	62.6
40	60	0.8	0.01	55.2
70	30	0.8	0.005	67.9
70	30	0.4	0.005	66.3
				65.1
55	45	0	0	71.4
				70.1
55	45	0	0.01	73.5
50	50	0	0	67.7
				70
50	50	0.4	0	58.4
50	50	0	0.005	71.1
50	50	0.4	0.005	60
50	50	1.6	0.005	53.2
45	55	0	0	68.6
40	60	0	0.005	64.3
40	60	0	0.01	65.5
40	60	0	0	65.4
40	60	0	0	68.9
45	55	0	0	68.6

Table 11. Recovery from Manchester sand with various extracting solvents.

Methanol conc. (%)	Water conc. (%)	Acetic acid conc. (%)	1-decanesulfonic acid, sodium salt molar conc. (%)	Recovery (%)
55	45	0	0	90.5
		0		87.7
55	45	0	0.01	87.7
50	50	0	0	88
45	55	0	0	88.3
40	60	0	0	87.6

filter than when methanol alone was used. The highest recoveries were obtained with a mixture of 55/45 v/v methanol–water. Addition of 1-decanesulfonic acid, sodium salt made filtration easier and did not adversely affect recovery. Therefore, a combination of 55/45 v/v methanol–water modified with 0.01 M 1-decanesulfonic acid, sodium salt was deemed optimal for recovery of tetrazene-spiked soil.

Sequential extraction

Since not all of the tetrazene in the spiked USA THAMA soil samples was recovered, a sequential extraction was performed to see if recovery could be improved by more than one extraction. Spiked subsamples of USATHAMA soil were extracted with 55/45 v/v methanol–water by shaking for 1.5 hr. An aliquot of the extract was removed for analysis, and the rest of the sample was vacuum-filtered. The soil was then re-extracted with fresh solvent. Analysis revealed that, for the first extraction, found concentrations averaged 36 μg/g for a target concentration of 51 μg/g; for the second extraction, found concentrations averaged only 1.1 μg/g, probably due to residual solvent. Clearly, the missing tetrazene was not recoverable with a second extraction.

Kinetic study with methanol and water

A kinetic study was performed with 55/45 v/v methanol–water, 0.01 M 1-decanesulfonic acid, sodium salt. Two soils were used. Duplicate 2-g subsamples of the spiked Minneapolis soil obtained from USATHAMA were extracted. This soil had been spiked approximately 6 months before this experiment. The target concentration was 2.8 mg/g. In addition, duplicate 2-g subsamples of the blank USATHAMA standard soil were spiked with a tetrazene–methanol solution to yield a target concentration of 25.6 μg/g. Soils were aged 1 hr before extraction. Each of the soil subsamples was extracted with 50 mL of solvent. Aliquots were removed from the shaking samples at 15 min, 1, 1.5, 2, 4, 6, and 23 hr. The results are presented in Figures 7 and 8.

Maximum recovery was achieved from the Minneapolis soil after 5 hr of shaking (Fig. 7), with no degradation apparent after 23 hr. Such a pattern suggests that the extracting solvent became saturated. If field samples contain tetrazene at levels exceeding 0.5 mg/g, either sequential extractions must be performed or a lower soil-to-solvent ratio should be used.

For the USATHAMA standard soil spiked with a tetrazene–methanol solution (target concentration = 25.6 μg/g) and aged 1 hr, extraction kinetics were quite different (Fig. 8). Using the mixed extracting solvent, maximum recovery of 70% was achieved after only 15 min of shaking. Shaking beyond 2 hr resulted in tetrazene degradation. After 23 hr, recovery was only 20%.

Based on these data, a set shaking time cannot be recommended. Additional work with low-level field-contaminated soils is indicated since soils

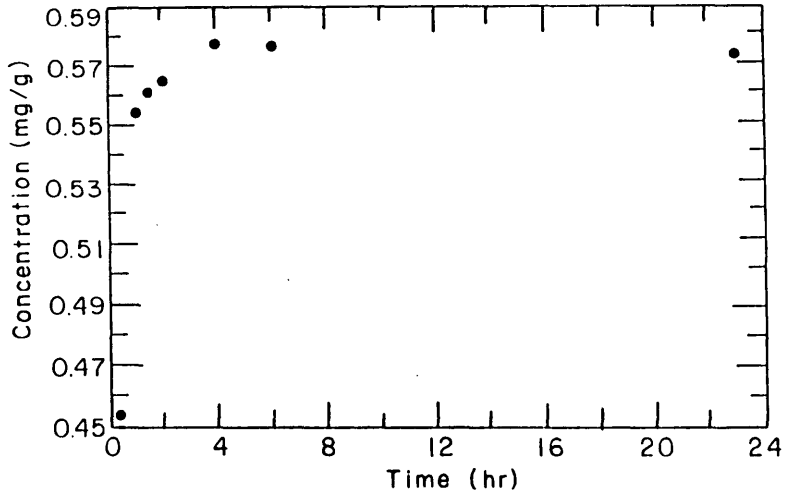

Figure 7. Kinetics study: Extraction of Minneapolis soil with 55/45 v/v methanol–water, 0.01 M 1-decanesulfonic acid, sodium salt.

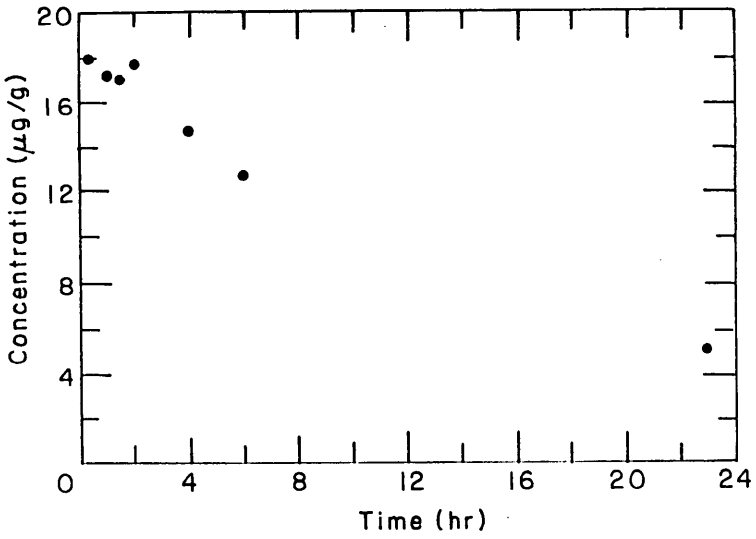

Figure 8. Kinetics study: Extraction of the spiked USATHAMA soil with 55/45 v/v methanol-water, 0.01 M 1-decanesulfonic acid, sodium salt.

contaminated at the low μg/g level are hard to simulate in the laboratory. For the spike-recovery study, a shaking time of 1.5 hr was chosen as a tradeoff between maximum extraction and minimum degradation.

Effect of soil type on precision and accuracy

To assess the effect of soil type on the method accuracy, four different soils, chosen because their clay contents ranged from 0 to 100% (Table 2), were spiked to yield a target concentration of 25.6 μg/g. To measure the precision of this method, six replicates of each soil type were spiked. Spiked soils were aged 1 hr and shaken for 1.5 hr. The results are presented in Table 12. The accuracy of the method was 68% for the USATHAMA standard soil and the Fort Edwards clay and 88% for the Lebanon landfill and Manchester sand. The accuracy was higher for the two sandy soils; however, other factors such as total organic carbon content, cation exchange capacity, pH, and mineralogy probably affect recovery as well. The precision of the method ranged from 0.15 to 0.35 μg/g, and the relative standard deviations averaged 1.28% for the four soils. Figure 9 contains chromatograms obtained when these different types of soils were spiked and extracted. No interfering peaks were observed.

Table 12. Recovery from various spiked soils using 55/45 v/v methanol-water, 0.01 M 1-decanesulfonic acid, sodium salt.

	USATHAMA std. soil		*Lebanon landfill*		*Fort Edwards clay*		*Manchester sand*	
Rep.	*Found conc. (μg/g)*	*Recovery (%)*	*Found conc. (μg/g)*	*Recovery (%)*	*Found conc. (μg/g)*	*Recovery (%)*	*Found conc. (μg/g)*	*Recovery (%)*
1	17.83	69.66	22.42	87.59	17.72	69.21	22.46	87.74
2	17.03	66.53	22.42	87.56	17.39	67.93	22.29	87.06
3	17.73	69.24	22.50	87.88	17.52	68.45	22.37	87.40
4	17.38	67.87	22.38	87.40	17.44	68.12	22.02	86.01
5	17.02	66.48	22.74	88.84	17.69	69.09	22.47	87.76
6	17.69	69.11	22.66	88.52	17.39	67.93	23.07	90.12
Mean	17.45	68.15	22.52	87.97	17.53	68.46	22.45	87.68
Std. dev.	0.3516	1.406	0.1470	0.5839	0.1476	0.5721	0.3469	1.358
Variance	0.1293	1.976	0.0216	0.3410	0.0218	0.3273	0.1203	1.843
% RSD	2.061	2.063	0.6526	0.6638	0.8423	0.8358	1.545	1.548

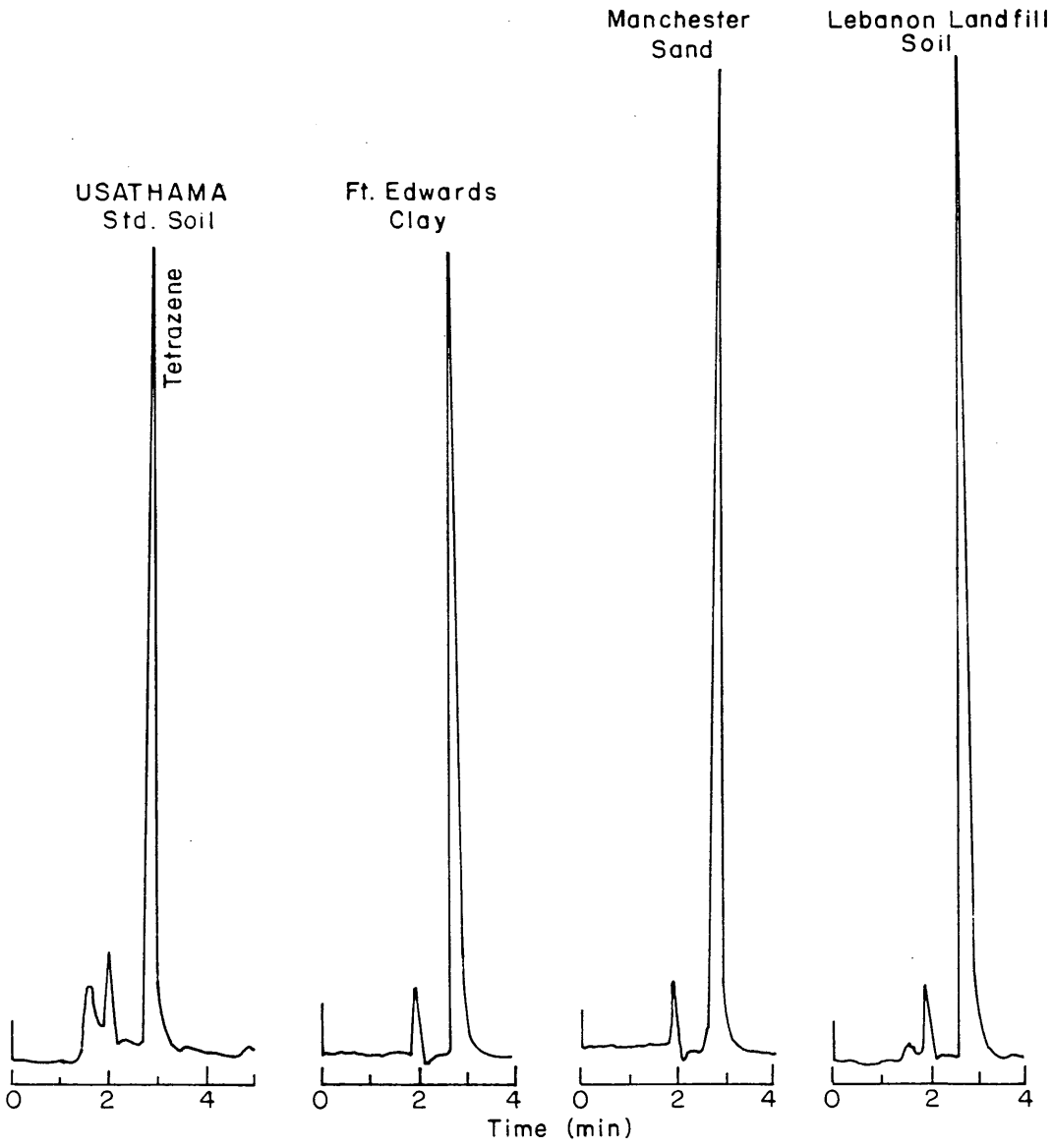

Figure 9. Chromatograms of four different soil types.

Spike-recovery study

A spike-recovery study was conducted to allow estimation of the method reporting limit. Spike solutions were prepared in such a way that the spiked soil samples would have analyte concentrations ranging from 0.5 to 10 times a target reporting limit of 2.56 μg/g. The results are presented in Table 13.

The certified reporting limit was calculated using the method of Hubaux and Vos (1970). First the mean and variance at each target level were calculated, and the variances were compared using Bartlett's test (Table 14). The range of homogeneous variance included the entire data set.

The data were statistically analyzed using the IRPQAP software provided by USATHAMA (Appendix A contains the output from this program). The data from each of the four days were pooled and tested for lack of fit. The linear range was limited to 0.5 to 5 times the target reporting limit, therefore the data set entered into the computer for the calculation of the certified reporting limit included the found concentrations for the target concentrations 0–12.8 μg/g. The method reporting limit was obtained from the X value corresponding to the point on the lower confidence limit curve where the Y value matches the value of Y on the upper confidence limit curve at $X = 0$ (Fig.

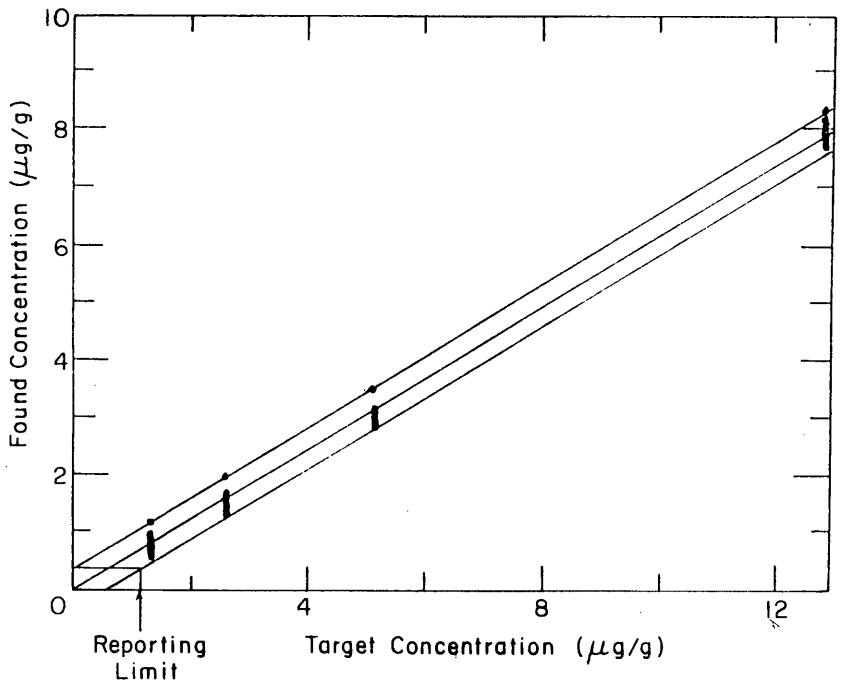

Figure 10. Reporting limit determination of tetrazene.

10). A method reporting limit of 1.10 μg/g was calculated. As dictated by the USATHAMA protocol, the certified reporting limit must be equal to or greater than the lowest spike level. Otherwise the lowest tested concentration is the minimum value that can be reported as the certified reporting limit. In this case the lowest tested concentration was 1.28 μg/g. Therefore, the certified reporting limit is reported as 1.28 μg/g.

Table 13. Recovery of tetrazene during 4-day spike-recovery study.

Spike level	*Target conc. (μg/g)*	*Found conc. (μg/g) Day 1*	*Day 2*	*Day 3*	*Day 4*
0.5 X	1.28	0.633	0.614	1.180	0.810
		0.928	0.951	0.711	0.712
1 X	2.56	1.44	1.55	1.50	1.44
		1.48	1.56	2.02	1.39
2 X	5.12	2.91	2.85	2.98	3.51
		2.98	2.88	2.99	3.09
5 X	12.8	7.75	7.77	7.96	7.70
		7.59	8.04	8.38	8.15
10 X	25.6	17.2	16.7	17.4	17.3
		16.7	16.8	17.6	17.2

Table 14. Means and variances of found concentrations at each target level for reporting limit test.

Spike level	*Target conc. (μg/g)*	*Found conc. (μg/g) Mean*	*Variance*	*Bartlett's test (X^2)**
0.5 X	1.28	0.817	3.68E-02	—
1 X	2.56	1.55	3.92E-02	—
2 X	5.12	3.02	4.48E-02	—
5 X	12.8	7.92	7.11E-02	—
10 X	25.6	17.1	1.31E-02	4.11

* Critical X^2 value (α = 0.05, df = 4) = 9.49.

SUMMARY AND CONCLUSIONS

A method was developed for determining tetrazene in soil. The method involves extracting 2-g subsamples of soil with a solvent consisting of 55/45 v/v methanol-water and 1-decanesulfonic acid, sodium salt at a 0.01 M concentration using a platform shaker. The extract is filtered through a 0.5-μm Millex SR filter and chilled to 0°C. A portion of each extract is analyzed using an ion-pairing RP-HPLC technique. An LC-18 column is eluted with a methanol-water 2/3 v/v eluent modified with 1-decanesulfonic acid, sodium salt and gla-

cial acetic acid. Tetrazene was detected with a variable-wavelength UV detector set at 280 nm. The tetrazene retention time using this method was 2.8 min. The capacity factor was 0.78. No interferences were found.

Kinetic studies showed that tetrazene is extracted within 5 hr of shaking and is prone to degrade during extended periods of shaking. Soils contaminated in excess of 500 μg/g will result in solvent saturation. The accuracy of the method varied with soil type from 68% to 88%. The highest recoveries were from sandy soils. The method precision ranged from 0.15 to 0.36 μg/g or a 1.28% relative standard deviation at the level tested.

A four-day spike-recovery test was conducted to allow estimation of a method reporting limit. Using data over the linear range, a method reporting limit of 1.1 μg/g was calculated. Since this value is less than the lowest concentration tested, 1.28 μg/g is the certified reporting limit.

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APPENDIX A: COMPUTER OUTPUT FROM THAMA IRPQAP SOFTWARE INCLUDING CALCULATED REPORTING LIMIT

CERTIFICATION ANALYSIS — Report Date: 09/17/87

Method Name: SOIL — Laboratory: CR
Compound: TET — Analysis Date: 09/17/87
Units of Measure: UGG — Matrix: SO

ANALYSIS OF RESIDUAL VARIATIONS

	--- Model with Intercept --- Y = (-0.04143506) + (0.619257019)X			- Model through the Origin - Y = (0.614709008)X		
	(SS)	(df)	(MS)	(SS)	(df)	(MS)
Residual:	1.474817490	30	0.049160583	1.496952270	31	0.048288783
Total Error:	1.343003320	28	0.047964404	1.343003320	28	0.047964404
Lack of Fit:	0.131814170	2	0.065907085	0.153948950	3	0.051316317
	LOF F-Ratio(F): 1.374083260			LOF F-Ratio(F): 1.069883332		
	Critical 95% F: 3.34			Critical 95% F: 2.95		

ZERO INTERCEPT HYPOTHESIS

Zero Intercept Accepted — Calculated F: 0.450254628 Critical 95% F: 4.17

****

TABLE OF DATA POINTS — Targets: 4 — Measures per Target: 8

	Target Value	Found Concentration				
1:	1.2800000	0.6334070	0.9284720	0.6141980	0.9512800	1.1772040
		0.7108340	0.8096030	0.7124870		
2:	2.5600000	1.4381960	1.4814050	1.5487200	1.5632310	1.5031960
		2.0168050	1.4417330	1.3910160		
3:	5.1200000	2.9096580	2.9814770	2.8474940	2.8775110	2.9829990
		2.9957060	3.5135710	3.0886790		
4:	12.800000	7.7457090	7.5878440	7.7711340	8.0435160	7.9620930
		8.3854010	7.7054400	8.1543210		

*** END OF CERTIFICATION LACK OF FIT DATA TABLE ***

CERTIFICATION ANALYSIS

Report Date: 09/17/87

Method Name: SOIL
Compound: TET
Units of Measure: UGG

Laboratory: CR
Analysis Date: 09/17/87
Matrix: SO

TABLE OF RESULTS FOR ENTIRE DATA SET

Target Concentration	Standard Deviation	Percent Inaccuracy	Percent Imprecision
1.2800000	0.1917169	-36.15737	23.460636
2.5600000	0.1980067	-39.52978	12.790816
5.1200000	0.2117401	-40.92506	7.0005145
12.800000	0.2665740	-38.12944	3.3660747

TABLE OF DATA POINTS

Target Concentration	Found Concentration
0	0
	0
	0
	0
	0
	0
	0
	0
1.2800000	0.6334070
	0.9284720
	0.6141980
	0.9512800
	1.1772040
	0.7108340
	0.8096030
	0.7124870
2.5600000	1.4381960
	1.4814050
	1.5487200
	1.5632310
	1.5031960
	2.0168050
	1.4417330
	1.3910160
5.1200000	2.9096580
	2.9814770
	2.8474940
	2.8775110
	2.9829990
	2.9957060
	3.5135710
	3.0886790

CERTIFICATION ANALYSIS

Report Date: 09/17/87

Method Name: SOIL
Compound: TET
Units of Measure: UGG

Laboratory: CR
Analysis Date: 09/17/87
Matrix: SO

TABLE OF DATA POINTS
(continued)

Target Concentration	Found Concentration
12.800000	7.7457090
	7.5878440
	7.7711340
	8.0435160
	7.9620930
	8.3854010
	7.7054400
	8.1543210

-- REGRESSION EQUATION --
Y = 0.6175155X + -0.025569

-- CERTIFIED REPORTING LIMIT --
1.1043353

-- UPPER REPORTING LIMIT --
12.800000

-- ACCURACY --
0.6175155

-- SLOPE --
0.6175155

APPENDIX B: METHOD IN USATHAMA FORMAT

REVERSE-PHASE HPLC METHOD FOR THE DETERMINATION OF TETRAZENE IN SOIL

I. SUMMARY

A. ANALYTES. The compound tetrazene can be determined using this method.

B. MATRIX. This method is suitable for determination of tetrazene in soil.

C. GENERAL METHOD. The method involves extraction of soil samples with a mixed solvent containing water, methanol, and 1-decanesulfonic acid, sodium salt by shaking for 5 hr on a platform shaker followed by filtration and determination using ion-pairing reverse-phase HPLC—UV 280 nm.

II. APPLICATION

A. TESTED CONCENTRATION RANGE. Linearity tests were conducted using peak area measurements. For a 100-μL injection volume, this method was found to be linear over the concentration range of 0.204–40.8 μg/g.

B. SENSITIVITY. The response of the UV detector at 280 nm for tetrazene was estimated at 7.53×10^{-4} absorbance units/μg/g using the conditions described below and a 100-μL injection volume.

C. REPORTING LIMIT. The reporting limit as determined over four days using the method of Hubaux and Vos was 1.28 μg/g using a 100-μL injection volume.

D. INTERFERENCES. No interferences were found. However, tetrazene elutes early, and if a computing integrator is used for peak quantitation, the baseline setting may have to be customized to exclude baseline aberrations. While these abberations are insignificant when high concentrations of tetrazene are determined, they can cause large errors when low concentrations are determined. A blank run will help determine where the true baseline should be set.

E. ANALYSIS RATE. Approximately 40 samples can be analyzed in a day, provided the samples are not contaminated with late-eluting compounds such as TNT.

F. SAFETY INFORMATION. Tetrazene is extremely explosive in the dry state. Only small portions of the SARM material should be dried to prepare analytical standards. Methanol is a flammable organic solvent, and established safety precautions should be used.

III. APPARATUS AND CHEMICALS

A. GLASSWARE/HARDWARE

1. Injection syringe—Hamilton, liquid syringe, 500-μL
2. Filters—0.5-μm Millex-SR, disposable
3. Pipettes—50-mL volumetric, glass
4. Scintillation vials—20-mL, glass
5. Disposable syringes—Plastipak, 10-mL
6. Analytical balance— ± 0.1 mg
7. Erlenmeyer flasks, ground glass stoppers—125-mL, glass

B. INSTRUMENTATION

1. HPLC—Perkin-Elmer Series 3 (or equivalent) equipped with a 100-μL loop injector and a 280-nm UV detector.
2. Strip chart recorder.
3. Digital integrator—HP3390A (or equivalent)
4. LC-18 (Supelco) RP-HPLC column, 25 cm $\times$ 4.6 mm (5 μm)
5. Platform orbital shaker

C. ANALYTE

Tetrazene
Boiling point—NA
Melting point—140-160°C
Solubility in water at 22°C is 4.5 mg/L
CAS REG No. 31330-63-9

D. REAGENTS AND SARMS

1. Tetrazene—SARM quality
2. Methanol—HPLC grade
3. Water—Reagent grade
4. 1-decanesulfonic acid, sodium salt—HPLC grade
5. Glacial acetic acid—Reagent grade

IV. CALIBRATION

A. INITIAL CALIBRATION

1. Preparation of Standards. SARM is dried to constant weight in a vacuum desiccator in the dark. About 20 mg are weighed into a 200-mL volumetric flask and diluted to volume with methanol. The flask is inverted several times while the tetrazene slowly dissolves. (Because the tetrazene dissolves very slowly, the flask containing the methanol and undissolved tetrazene may be placed in the freezer for several hours. The flask is then removed from the freezer and continuously inverted until the solution is warmed to ambient temperature and the tetrazene is dissolved.) The stock solution is stored in the freezer at -10°C in the dark. The stock solution concentration is about 100 mg/L and is usable for one week from date of preparation.

A series of intermediate standards are prepared by first diluting the stock 1/25 v/v with methanol to make a 4000-μg/L standard. Intermediate calibration standards containing 0, 20, 40, 80, 200, 400, and 800 μg/L are prepared by placing 0, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 mL of the 4000-μg/L standard in a series of 100-mL volumetric flasks and filling to volume with methanol. An intermediate standard containing 2000 μg/L is prepared by placing 25.0 mL in a 50-mL volumetric flask and filling to volume with methanol.

Injection standards are prepared by diluting 4.0 mL of each of the intermediate standards with 6.0 mL of water. The resulting concentrations will be 0, 8.0, 16.0, 32.0, 80.0, 160, 300, 800 and 1600 μg/L, which correspond to 0.20, 0.40, 0.80, 2.0, 4.0, 8.0, 20, and 40 μg/g assuming 100% extraction from 2 g of soil with 50 mL solvent.

All solutions should be either refrigerated or kept in an ice bath following dilution.

2. Instrument Calibration. Duplicate injections of each standard over the concentration range of interest are sequentially analyzed in random order. Peak areas or peak heights are obtained. The retention time is 2.8 min.

3. Analysis of Calibration Data. The acceptability of a linear model with zero intercept is assessed using the protocol specified in USATHAMA QA (2nd Edition, March 1987). Experience indicates a linear model with zero intercept is proven to be appropriate; thus the slope of the best-fit regression line is then equivalent to a response factor. This response factor can be compared with values obtained from replicate analyses of a single calibration standard each day.

B. DAILY CALIBRATION. The highest standard can be used for daily calibration. A 4.0-mL aliquot of this standard is added to 6.0 mL of water in a scintillation vial. This standard is analyzed in triplicate at the beginning of the day, singly after five samples, and singly after the last sample of the day. The standard is maintained at 4°C throughout the analysis. A response factor is obtained from the mean peak area or peak height obtained

over the course of the day and compared with the response factor obtained for the initial calibration. These values must agree within ±10%, or a new initial calibration must be obtained.

V. CERTIFICATION TESTING

A. PREPARATION OF SPIKING SOLUTIONS. An analyte spiking solution is prepared in a manner identical to that described for the calibration stock. The stock is diluted 1/1 v/v with methanol to make a 50-mg/L spike solution. A series of spiking standards (0, 2.5, 5.0, and 10 mg/L) are prepared by placing 0, 5.0, 10.0, and 20.0 mL of the 50-mg/L spike solution in a series of 100-mL volumetric flasks and diluting to volume with methanol. The spike solution containing 25 mg/L is prepared by placing 25.0 mL into 50-mL volumetric flasks and diluting to volume with methanol.

B. PREPARATION OF CONTROL SPIKES. Spiked soil samples containing 0, 1.25, 2.5, 5.0, 12.5, and 25.0 μg/g are prepared by adding 1.0 mL of each spiking standard to 2.0 g of soil. Duplicate spiked soil samples are prepared and aged 1 hour.

C. EXTRACTION AND ANALYSIS OF SOIL SPIKES. Soil spikes are processed and analyzed as described below for real samples.

VI. SAMPLE HANDLING AND STORAGE

A. SAMPLING PROCEDURE. Representative subsamples are taken for analysis.

B. CONTAINERS. All containers used to store soil samples should be cleaned according to procedures specified in the USATHAMA QA Manual and rinsed with methanol.

C. STORAGE. All soil samples must be stored at 4°C before and throughout the analysis.

D. HOLDING TIME LIMITS. Samples should be processed as soon as possible after receipt, preferably within a day.

VII. PROCEDURE

A. SOIL EXTRACTION. Two-gram soil subsamples are weighed into 125-mL Erlenmeyer flasks equipped with ground glass stoppers. Then 50 mL of solvent are added to each flask, the flasks are vortexed for 15 s and shaken for 5 hr at 200 rpm on a platform shaker. The extracting solvent is a mixture of 55/45 v/v methanol–water modified with 0.01 M 1-decanesulfonic acid, sodium salt.

B. FILTRATION. A 10-mL portion of each soil extract sample is placed in a Plastipak syringe and filtered through a 0.5-μm Millex-SR filter unit. The first 5 mL of filtrate are discarded, and the remainder is retained for analysis.

C. DETERMINATION. Determination of analyte concentration in the filtered soil extracts is obtained by ion-pairing RP-HPLC on a 280-nm UV detector. The eluent is prepared by adding to a solution of 2/3 v/v methanol–water enough 1-decanesulfonic acid, sodium salt to obtain a 0.01 molar concentration level and adjusting the pH to about 3 with glacial acetic acid. For 1 L of eluent, 2.44 g of 1-decanesulfonic acid, sodium salt are dissolved in 400/600 v/v methanol–water and 8.0 mL of glacial acetic acid is added to the mixture. A 100-μL loop is overfilled by injecting 500 μL of sample through the loop and onto an LC-18 column eluted at 1.5 mL/min. The retention time for tetrazene is 2.8 min, and a capacity factor based on an unretained peak for nitrate is 0.795. A chromatogram obtained for tetrazene and potential interferences is shown in Figure 2.

VIII. CALCULATIONS

A. RESPONSE FACTOR. Since a linear calibration curve with zero intercept is to be expected, calculation of results on a daily basis is obtained using a response factor. The mean

response ($\bar{R}$) for tetrazene is obtained in either peak area or peak height units. The response factor is obtained by dividing the mean response by the known equivalent soil concentration (C) in units of μg/g:

$$RF = \frac{\bar{R}}{C}$$

B. ANALYTE CONCENTRATIONS. Soil concentrations (μg/g) in the soil samples (C_a) are obtained by dividing the response obtained for each sample (R_a) by the response factor:

$$C_a = \frac{R_a}{RF}$$

IX. DAILY QUALITY CONTROL

A. CONTROL SPIKES. Spiked soil samples are prepared as described for Class 1 methods in the USATHAMA QA Manual (2nd Edition, March 1987). For each analytical lot, a method blank, a single spike at two times the certified reporting limit, and duplicate spikes at ten times the certified reporting limit are analyzed for each analytical lot. Control spikes are prepared using the appropriate spiking solution in a manner identical to that described in Section V.

B. CONTROL CHARTS. The control charts required are described for Class 1 methods in the USATHAMA QA Manual (2nd Edition, March 1987). Standard Shewhart $\overline{X}$ and R charts for the duplicate high spikes and moving average $\overline{X}$ and R charts for the single low spikes are required. Details on the charting procedures are specified in the USATHAMA QA Manual (2nd Edition, March 1987).

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