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Development of an analytical method for the determination of explosive residues in soil

Part III. Collaborative test results and final performance evaluation

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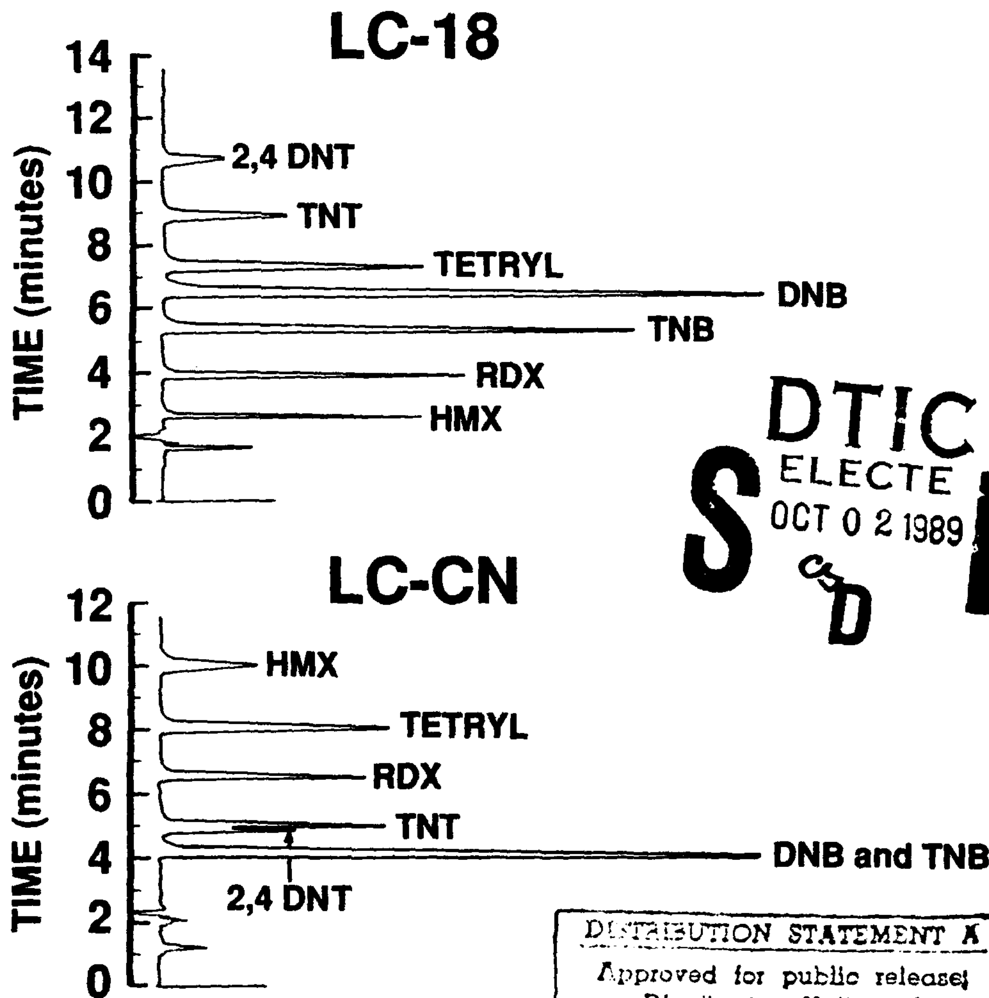

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Cover: Example of the separations achieved for a standard solution on the primary and confirmation columns using the same element (1:2 water–methanol). Note the differences in elution order, particularly for HMX, RDX and TNT.

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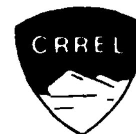

Development of an analytical method for the determination of explosive residues in soil

Part III. Collaborative test results and final performance evaluation

Christopher F. Bauer, Thomas F. Jenkins, Stephan M. Koza,
Patricia W. Schumacher, Paul H. Miyares, and Marianne E. Walsh

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A collaborative test of a method for the determination of nitroaromatic and nitramine explosives in soil was conducted at eight laboratories. The method involves extraction of a 2.00-g portion of soil with 10.0 mL of acetonitrile in a sonic bath, dilution of 5.00 mL of soil extract with 5.00 mL of aqueous $CaCl_2$, filtration and determination by RP-HPLC-UV at 254 nm. Certified reporting limits (CRLs) and method detection limits (MDLs) were obtained for HMX, RDX, TNT and ten other analytes. Values ranged from 0.07 to 2.15 µg/g for the CRLs and from 0.03 to 1.27 µg/g for the MDLs. The collaborative test was conducted under the auspices of the Association of Official Analytical Chemists. The analytes (HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT) were measured in eight field-contaminated soils and eight spiked standard matrix soils. Both sets of eight consisted of four individual samples in duplicate. Concentrations ranged from the limits of detection to nearly 1000 µg/g. The results were evaluated by means of analysis of variance and regression analysis with and without the inclusion of data identified as outliers. When outliers were excluded, method repeatability (the within-lab standard deviation) for all analytes except tetryl was excellent (less than 5% for spiked soils and less than 18% for field-contaminated soils). The relative standard deviation generally decreased as the analyte concentration increased. Reproducibility (the between-lab standard deviation), except for tetryl and DNT, was also good (less than 7% for spiked soils and 26% for field-contaminated soils). These results indicate that collaborators have nearly equivalent performance on spiked samples, and

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19. ABSTRACT (cont'd). that for field-contaminated soils the variability of extraction recoveries contributes to imprecision. The poor precision for tetryl was due to thermal decomposition during extraction. The relative precision for DNT in field-contaminated soils was poor because the concentrations were near the detection limit. A significant source of interlaboratory error seems to be inaccuracy in the preparation of the calibration standard. When outliers were not excluded, intralaboratory error increased by no more than a factor of two, in most instances, to about 5–20%. Outliers were often caused by electronic integrator miscalculations of chromatographic peak areas. Analyte recoveries were good, except for tetryl: 95–97% for HMX, RDX, TNT and DNT (similar to recoveries from aqueous samples), 92–93% for DNB and TNB, and 70% for tetryl. Precision and recoveries for tetryl would have been better if sonic bath temperatures had been maintained near ambient. The recoveries were insensitive to the elimination of outliers from the data set.

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PREFACE

This report was prepared by Dr. Christopher F. Bauer and Stephan M. Koza, Chemistry Department, University of New Hampshire; Thomas F. Jenkins, Patricia W. Schumacher and Paul H. Miyares, Geochemical Sciences Branch, and Marianne E. Walsh, Applied Research Branch, U.S. Army Cold Regions Research and Engineering Laboratory.

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The authors are also indebted to the Association of Official Analytical Chemists for their review and endorsement of the collaborative test of the method reported here.

The contents of this report are not to be used for advertising or promotional purposes. Citation of brand names does not constitute an official endorsement or approval of the use of such commercial products.

ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
SARM	Standard Analytical Reference Material obtained from USATHAMA, Aberdeen Proving Ground, Maryland
CRL	Certified Reporting Limit
MDL	Method Detection Limit
HMX	octahydro-1,3,5,6-tetranitro-1,3,5,7-tetrazocine
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
RP-HPLC	reversed-phase, high-performance liquid chromatography
TNT	2,4,6-trinitrotoluene
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
UV	ultraviolet
NG	nitroglycerine
Tetryl	methyl-2,4,6-trinitrophenylnitramine
TNB	1,3,5-trinitrobenzene
DNB	1,3-dinitrobenzene
2,4-DNT	2,4-dinitrotoluene
NB	nitrobenzene
AAP	army ammunition plant
o-NT	ortho-nitrotoluene
m-NT	meta-nitrotoluene
p-NT	para-nitrotoluene
2,6-DNT	2,6-dinitrotoluene
2-Am-DNT	2-amino-4,6-dinitrotoluene
4-Am-DNT	4-amino-2,6-dinitrotoluene
2,4,5-TNT	2,4,5-trinitrotoluene
2,4-DAm-NT	2,4-diamino-6-nitrotoluene
2,6-DAm-NT	2,6-diamino-4-nitrotoluene

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Development of an Analytical Method for the Determination of Explosive Residues in Soil
Part III. Collaborative Test Results and Final Performance Evaluation

CHRISTOPHER F. BAUER, THOMAS F. JENKINS, STEPHAN M. KOZA, PATRICIA W. SCHUMACHER, PAUL H. MIYARES AND MARIANNE E. WALSH

INTRODUCTION

Over the past few years it has become increasingly apparent that a large number of installations operated by the Defense Department are contaminated with residues from the manufacture, distribution, destruction or testing of munitions. The most frequently encountered residues contain TNT, RDX and their impurities and degradation products. Analytical methods are required to determine these materials in a variety of environmental matrices. In response to this need, CRREL developed a method for determining trace levels of TNT, RDX, HMX and 2,4-DNT in water (Jenkins et al. 1984, 1986). This method was accepted by the Association of Official Analytical Chemists (AOAC 1986) as the standard method for this analysis. A recently improved method for this analysis allows for the simultaneous analysis of TNB, DNB and tetryl as well as the original suite of analytes (Jenkins et al. 1988a).

A preliminary method was also developed for the determination of HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT in soil (Jenkins and Walsh 1987). This method involved extraction of soil with acetonitrile (Jenkins and Leggett 1985, Jenkins and Grant 1987) followed by dilution with water, filtration, and determination by reversed-phase high-performance liquid chromatography (RP-HPLC). Confirmation of analyte identity was recommended using a separation on a second RP-HPLC column, which gave a very different order of elution for the analytes of interest.

Further testing of this method revealed that the method performed well but that the filtration step was difficult to perform for soils containing a significant amount of clay-sized material (Jenkins et al. 1988b). It also appeared that the detection limits could be lowered by increasing the soil-to-solvent ratio. These possibilities were explored, along with the shelf life of the analytical standards and the stability of the soil extracts. To measure the sensitivity of the determinations to small procedural changes, a ruggedness test was also conducted in which several of the key analytical steps were varied systematically. The method was found to be relatively insensitive to small methodological changes (Jenkins et al. 1988b).

A major improvement was accomplished by diluting the acetonitrile extract 1:1 with an aqueous $CaCl_2$ solution rather than deionized water prior to filtration. The addition of $CaCl_2$ flocculated the clays in a very short period of time, and the floc was easily removed by settling and filtration. Careful testing indicated that this floc did not sorb or exclude the analytes of interest, nor did it modify the determined concentrations in any other way (Jenkins et al. 1988b). Finally, we sponsored an external test of the method at two cooperating laboratories, using field-contaminated soils. Both labs appeared to have good success using the method.

Based on the positive results discussed above, we submitted two final reports (Jenkins and Walsh 1987, Jenkins et al. 1988b), a draft of the step-by-step method in AOAC format, and a detailed protocol for a full-scale collaborative test (Appendix F) to the AOAC to solicit their sponsorship. The plan was approved and samples for the test were supplied to 12 collaborators in April 1988.

The objective of this report is to present the results of the final testing of the method. This includes the experiments conducted to establish certified reporting limits (USATHAMA 1987) and method detection limits (*Federal Register* 1984), as well as the results of the full-scale collaborative test.

STABILITY OF SPIKED SOILS

Objective

To evaluate the usability of this method by other laboratories, it was necessary to supply a set of homogeneous soil samples to each collaborator. Field-contaminated soil can be used to measure method precision, but it cannot be used to assess recovery since the "true" concentration of each analyte is unknown. Therefore, a set of spiked soils was prepared in which the actual analyte concentrations were accurately known. Because of the lag time between when the soils were spiked and the time each collaborator extracted the samples, it was necessary to first evaluate analyte stability in the soil matrix after spiking.

Experimental method

Individual stock standards of HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT were prepared by weighing dried Standard Analytical Reference Material for each analyte followed by dissolution in a known volume of acetonitrile.* A combined analyte spiking solution was prepared by volumetric addition of each stock to a volumetric flask and dilution to volume with acetonitrile. The spiking solution contained about 8 mg/L of each substance.

Fifteen replicate 2.0-g subsamples of USA THAMA standard soil were placed in individual 20-cm × 2.5-cm Teflon-capped glass test tubes. Three tubes were selected randomly and spiked with 1.00 mL of the spiking solution, which yielded a target concentration of about 4 µg/g for each analyte. The spiked tubes were allowed to stand uncapped for 48 hours in a dark fume hood to allow the solvent to evaporate. Once the solvent had visibly evaporated, the tubes were capped and stored in the refrigerator until the soil was extracted.

On each of four subsequent spiking days, three replicate tubes were chosen and spiked, and the solvent was allowed to evaporate as described above. The schedule for the spiking and analysis is given in *Table 1*. The first spiked tubes were stored for 62 days before extraction and analysis. The other four time periods examined were 32, 25, 1 and 0 days. A set of three tubes containing the same volume of solvent used for extraction, but containing no soil, was also spiked on the last day.

All samples were extracted and analyzed together to ensure that differences in day-to-day calibration, which would result if samples were

* Preparation of stock standards was similar to that described in detail in the *Collaborative Test* section.

Table 1. Schedule for preparation of spiked soils for stability test.

Treatment no.	*Date spiked*	*Date extracted*	*Storage days*
1	12 August	13 October	62
2	11 September	13 October	32
3	18 September	13 October	25
4	12 October	13 October	1
5	13 October	13 October	0
6*	13 October	13 October	0

* Treatment 6 refers to direct spiking of extraction solvent.

Table 2. Results of the spiked soil stability test.

Treatment no.	*Storage days*	*Mean concentration (µg/g)* HMX	RDX	TNB	DNB	Tetryl	TNT	2,4-DNT
1	62	3.8	4.1	4.4	4.1	4.9	4.1	4.2
2	32	3.9	4.0	4.1	4.0	4.6	4.0	3.9
3	25	4.1	4.3	4.3	4.0	4.8	4.3	4.2
4	1	4.0	4.2	4.5	4.1	4.9	4.2	4.2
5	0	4.2	4.2	4.3	4.2	4.9	4.2	4.2
6	Soln.	4.0	4.1	4.0	4.1	4.2	4.1	4.0
Slope*		4×10^{-3}	9×10^{-4}	4×10^{-4}	1×10^{-3}	2×10^{-3}	2×10^{-3}	1×10^{-3}
t†		2.04	0.48	0.22	1.19	0.49	1.01	0.57

* Slope of the regression line of concentration vs storage time (days).
† t statistic comparing the observed slope to a slope of zero. A significant slope for t at the 95% confidence level (df = 14) = 2.160.

analyzed on different days, did not confound differences due to the length of storage before extraction.

The extraction and analysis were conducted as described in detail in Appendix F.

Results

The results of this study are presented in Table 2. A regression analysis was conducted on individual measured concentrations as a function of storage time. The slopes obtained were tested to determine if they differed from a slope of zero at the 95% confidence level. In no case was the calculated slope statistically different from zero, indicating that there was no evidence of loss of any analyte over the 62-day storage period.

Thus, the spiked soils are sufficiently stable to serve as a test of analyte recovery if the collaborators conducted the analysis within two months after receiving the sample.

During this test, however, we did observe a peak in the chromatogram from the USATHAMA standard soil that interfered with the determination of TNB using the digital integrator. Since most analytical laboratories routinely use digital data collection devices, we decided to use Lebanon sandy silt for the spiked soils in the collaborative test because it was free from interferences. The spiked soil stability study reported here used data obtained from manual peak height measurement that were not affected by this problem.

COLLABORATIVE TEST

Rationale

The experimental design chosen for the collaborative study reflects the desire to obtain estimates of accuracy, intralaboratory precision (repeatability) and interlaboratory precision (reproducibility). Four field-contaminated soils and four spiked standard soils were analyzed, all in duplicate and blind to the collaborators. The four field-contaminated soils are from contaminated sites in four states and represent a range of analyte concentrations (determined using the proposed method) as shown in Table 3.

Each field-contaminated sample was extracted, flocculated, filtered and analyzed without splitting out duplicates at any point. Each extract was analyzed only once since the largest source of variation between samples is likely to be intersample heterogeneity. Despite the fact that the soils samples were dried, ground and homogenized, some heterogeneity may still exist. Thus, in a real situation one would be wise to analyze more samples rather than replicate analyses of extracts. In addition, by analyzing each extract only once, the total number of HPLC runs required for the entire test could be performed conveniently on a single day. This eliminates the confounding effect of a day-to-day calibration variation.

Since the real samples were duplicated, the variance between duplicates represents the within-lab method error. However, this error includes any heterogeneity of analyte composition in the original sample, which might be significant. This heterogeneity would inflate the apparent interlaboratory precision. To obtain a better estimate, spiked samples were also analyzed. Since there are no major compositional differences between replicate spikes, the resulting intralaboratory precision reflects the performance of the extraction and determination steps of the method without confounding from intersample differences. Comparison of intralaboratory precision for spiked samples vs field-contaminated samples will reveal the extent of residual heterogeneity in the field-contaminated samples after grinding and sieving. Interlaboratory and intralaboratory precision estimates for both field-contaminated and spiked samples are obtained by means of an analysis of variance.

The spiked samples are also used for evaluation of accuracy and for preliminary inspection of re-

Table 3. Composition of field-contaminated soils used in the collaborative test.

Site	Sample no.	Concentration (µg/g)*						
		HMX	RDX	TNB	DNB	Tetryl	TNT	2,4-DNT
Tennessee	2 & 4	30	135	5	1	—	5	—
Louisiana	3 & 13	255	960	2	—	5.7	14	—
Iowa	14 & 16	60	110	80	0.6	—	750	—
Nebraska	8 & 12	4	1	2	—	—	0.8	—

* Values determined at CRREL.
— = not detected

Table 4. True values for spiked soils used in the collaborative test.

Soil sample no.	Spike solution no.	Concentration (µg/g) HMX	RDX	TNB	DNB	Tetryl	TNT	2,4-DNT
11 and 15	A	10.7	10.04	10.03*	3.01	40.26	76.00	10.02
1 and 10	B	50.35*	50.2	12.54*	6.01*	25.17*	2.54	1.00
5 and 7	C	40.29*	100.35*	50.15	5.01*	20.13*	25.33*	4.01*
6 and 9	D	100.7	90.45*	2.01	1.00	5.04	30.40*	5.01*
Test sample†	E	50.35	50.20	50.15	50.05	50.35	50.65	50.05

* Data pairs used for Youden two-sample plots.
† Values for this sample were given to collaborators.

sults by means of Youden plots. Lebanon sandy silt, which does not contain any of the analytes, was weighed into extraction test tubes and spiked with standard solutions containing analytes. In this way the mass of analytes present in a given sample is known within pipeting accuracy. It was decided not to pursue the alternative of spiking contaminated soils and comparing "before" and "after" values to calculate recoveries. The "before" values can only be known by measurement using the method under evaluation; hence, the variance in recovery values would be much greater.

The samples were spiked at four concentration levels that span the linear range of the analytical method (Table 4). For each analyte, a pair of concentrations was selected within about 20% of each other for constructing Youden two-sample plots. To evaluate accuracy, a linear regression of found concentrations vs known values was performed. The slopes of the regression lines represent analyte recoveries.

Summary of protocol

The structure and style of the collaborative study protocol (Appendix F) were similar to those used in a previous study (Jenkins et al. 1984, Bauer et al. 1986). Strict adherence by the participating laboratories was essential if the statistical analysis of the results were to provide unbiased estimates of method performance. Known sources of random or systematic error were to be minimized, and any requested deviations from the protocol had to be approved prior to use.

A single analyst or group in each laboratory was responsible for all aspects of this study, from receipt of materials through data analysis. All activities were documented in duplicate in a project notebook.

The analytical work was performed in two steps. First, analysts became familiar with the procedures. During this period a calibration curve for each analyte was prepared and statistical tests were performed to verify linearity and applicability of a zero-intercept model. Previous experience had shown that in practice this behavior was expected (Jenkins and Walsh 1987). Consequently the laboratories participating in this study were expected to achieve the same results. When a linear model with a zero intercept is found to describe the calibration data accurately, daily calibration is simplified, resulting in significant time savings. These conditions were established by means of an unweighted least-squares linear regression with lack-of-fit testing, using models with and without a zero intercept. Unweighted least squares was used instead of a weighted approach, which may be considered more generally appropriate, because the former was easier for collaborators to carry out. In the lower concentration ranges, HPLC method variances are expected to be homogeneous (Jenkins and Walsh 1987). When variance is not a function of concentration, the weighted and unweighted approaches are equivalent.

A test sample was provided to the collaborators with "true values." If the results for this sample were acceptable, the analyses of the collaborative test samples could proceed.

The second portion of the work consisted of analyzing the 16 soil samples as described in Appendix E. All samples were extracted for 18 hours in an ultrasonic bath. An aliquot of supernatant was flocculated with $CaCl_2$ solution, filtered and stored. The analysis on a subsequent day proceeded as follows:

1. The most concentrated standard was analyzed, and its response was compared statistically with the previously established working curves.

Table 5. Weights of SARM used to prepare the spiking solution stocks for the collaborative test.

Substance	*Weight** *(g)*	*Stock conc. (mg/L)*
HMX	0.2518	1007
RDX	0.2509	1004
TNB	0.2507	1003
DNB	0.2503	1001
Tetryl	0.2516	1007
TNT	0.2533	1013
2,4-DNT	0.2504	1001

* Dissolved in acetonitrile and brought to volume in a 250-mL volumetric flask.

Table 6. Comparison of response factors from two sets of individually prepared stock standards.

Analyte	*Response factor (peak ht./conc.)* April 1988 *A*	*B*	*C*	$\bar{X}$	January 1988 *A*	*B*	*C*	$\bar{X}$	*Difference (%)*
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	47.1[illegible]	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-DNT	73.39	73.34	73.44	73.39	72.90	73.08	73.03	73.00	0.5

2. Barring unresolved discrepancies in the first step, each of the filtered extracts was analyzed along with five replicates of the highest standard. The analysis sequence was random.

3. The calibration curve for each analyte was based on the mean response of the five replicates of the highest standard, assuming a zero intercept.

Preparation of soils for the collaborative test

Standard Analytical Reference Materials (SARMs) for HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT were obtained from the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), Aberdeen Proving Ground, Maryland. The standards were dried to constant weight (0.1 mg) in a vacuum dessicator in the dark. Approximately 250 mg of each dried standard was weighed out to the nearest 0.1 mg, dissolved and diluted to volume with acetonitrile in individual 250-mL volumetric flasks. The actual weights for each analyte are given in Table 5.

To ensure that no major errors occurred in the preparation of the stock 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 a three-month-old set of single-analyte stock standards. The results indicated that the mean response factors for the seven analytes differ by an average of less than 2% (Table 6). 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.

Solutions A–E were prepared by combining the individual stock solutions as described in Table 7 and diluting to volume with acetonitrile in 100-mL volumetric flasks. In some cases a 1:10 dilution of the TNB, DNB and 2,4-DNT stock standards was prepared first by diluting 10.0 mL of each stock to volume with acetonitrile in individual 100-mL volumetric flasks.

One-hundred-thirty-five 2.00-g samples of Lebanon sandy silt soil were weighed into individual 20- × 2.5-cm Teflon-capped glass test tubes. These tubes were randomly divided into four sets of 30 tubes and one set of 15 tubes. Each set of 30 was spiked with 1.00 mL of one of the combined analyte spiking solutions described above (Solution A–D). The set of 15 tubes was spiked with 1.00 mL of solution E to give the soil concentrations shown in

Table 7. Volumes of stock spiking solutions used to prepare the combined analyte spiking solutions for the collaborative test.

Spike solution	*Volumes of stock standards (mL)/100 mL acetonitrile* *HMX*	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*2,4-DNT*
A	2	2	20*	6*	8	15	20*
B	10	10	25*	12*	5	0.5	2
C	8	20	10	10*	4	5	8*
D	20	18	4*	2	1	6	10*
E	10	10	10	10	10	10	10

* Volumes shown were for a 1:10 dilution of stock for that analyte.

Table 4. All spiking was done with 1.00-mL volumetric glass pipettes.

After spiking, the tubes were allowed to stand uncapped in a hood for two days to allow the solvent (acetonitrile) to evaporate. Following this drying period, no residual solvent was visible. Samples were than capped and refrigerated until shipment to the collaborators on the following day. Soil spiking was conducted as close to the required shipping date as possible to minimize the time lag between spiking and analysis.

The spiked soils described above represented half of the soil samples supplied to the collaborators. The other half of the samples were field-contaminated soils from four sites in Tennessee, Louisiana, Iowa and Nebraska (Table 3). These soils were prepared by air drying about 500 g of sample, grinding the dried soil to pass a #30 mesh sieve, and mixing thoroughly over an extended period by shaking and rotating the glass bottles in which the material was stored. A set of thirty 2.000-g (0.001-g) subsamples of each field-contaminated soil was weighed into Teflon-capped test tubes. Each set of 30 replicates was randomly subdivided into two sets of 15. Each set of 15 was labeled with a separate sample number. Thus two replicates of each soil were supplied blind to each collaborator.

Statistical analysis

Once we received the data from the participants, the sequence of tasks was as follows: inspection of raw data and construction of Youden two-sample plots to obtain a visual impression of the overall performance, rejection of extreme values (outliers), analysis of variance to extract estimates of precision, and regression analysis to evaluate overall accuracy. Most calculations were made using the computer program RS/1 (Bolt, Baranek and Newman Software Products Corporation, version 2.1), which is available on a VAX 8650 computer at the University of New Hampshire.

The eight laboratories that completed the study reported analyte concentration values in micrograms per gram of dry soil (Appendix A). This information was entered into a computer file twice, and the duplicate files subtracted to uncover transcription errors. The results reported as "not detected" or "less than" were entered as "0.0 µg/g." Individual laboratories were identified only by number to avoid potential bias. Laboratory 2 had unresolved instrumentation problems that led to serious deviations from the protocol. Hence, its results were excluded.

Initial results

An initial impression of analytical performance can be gleaned from the test sample determinations. Table 8 lists individual results, means, standard deviations and actual concentrations. The differences between the mean determined concentrations and the actual values are small: less than 15% for all analytes but tetryl.

Soil sample results were inspected for gross errors. Accuracy was checked by calculating the ratios of the reported concentrations to the known concentrations (the spiked value for standard soils and the CRREL determination for field-contaminated soils). Precision was checked by the difference in this ratio between duplicate soil samples. Ideally these marker values should be 1 and 0, respectively. Results that were far from these ideals were flagged, and the original chromatograms and notebooks were inspected. Seventeen miscalculated concentrations were discovered and corrected. Of another 46 flagged values, 11 were clear misintegrations (some of the collaborators noted these)

Table 8. Results of the test sample analysis.

	Concentration (µg/g)						
Laboratory	*HMX*	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*2,4-DNT*
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
true conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
mean	47.79	48.34	44.68	47.67	29.24	49.91	48.32
std dev	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% rsd	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% diff*	5.08	3.71	10.91	4.76	41.93	1.46	3.46

* Between true value and mean determined value.

and an additional 17 small but visible peaks were not integrated at all and thus reported as "0.0." Integrator errors appear to be a significant problem that does not seem to be well appreciated within the field of analytical chemistry.

Some field-contaminated samples contained negligible amounts of certain analytes. If the median of the reported results (including 0.0 values) was less than the method detection limit (described later in this report), that group of data was excluded from consideration. This applied to RDX, DNB and tetryl for Nebraska soil, and DNB for Louisiana soil.

Several significant variations from the protocol were noted upon review of the collaborator's notebooks. First, half of the labs neglected to reanalyze the dilutions of samples found to contain levels of HMX, RDX and TNT much higher than the highest calibration standard. The protocol required a 50-fold dilution to avoid absorbance values outside the linear range. A paired t-test of diluted vs undiluted extracts confirmed this concern: diluted samples had results 10% higher for TNT and 4% higher for RDX, but they were not different for HMX. These differences would contribute to the interlaboratory imprecision if diluted and undiluted results were considered together. However, because of the small number of labs, it was impractical to segregate labs according to whether the dilution was performed. Instead, undiluted results were used for all labs. Since dilutions were only required for field-contaminated soil extracts and recoveries from the real soils were not being evaluated, the slight inaccuracy introduced by this decision was inconsequential.

Second, injections immediately following an "over-range" sample were to be redone to check for a memory effect in the injector. Only one of the labs followed the protocol in this regard.

Third, a few labs performed analyses immediately after flocculating and filtering the extracts. The protocol specified a minimum overnight storage to control the TNB complexation problem observed in the Iowa soil sample (Jenkins et al. 1988b).

Fourth, a preliminary version of the protocol, circulated to collaborators for familiarization, was mistakenly used by two labs instead of the final version, which contained a few important revisions. The most significant change was extraction of the soils with 10 mL of solvent instead of 50 mL. The two labs that made this error therefore were unable to detect some analytes in some samples because of the extra five-fold dilution.

Last, collaborators had a number of difficulties with establishing statistical control of the method. These problems consisted of the lowest standards being below a lab's limit of detection, failure to verify calibration before analysis on the day extracts were analyzed, miscalculation of statistical tests, or in one case apparently ignoring the statistical tests entirely.

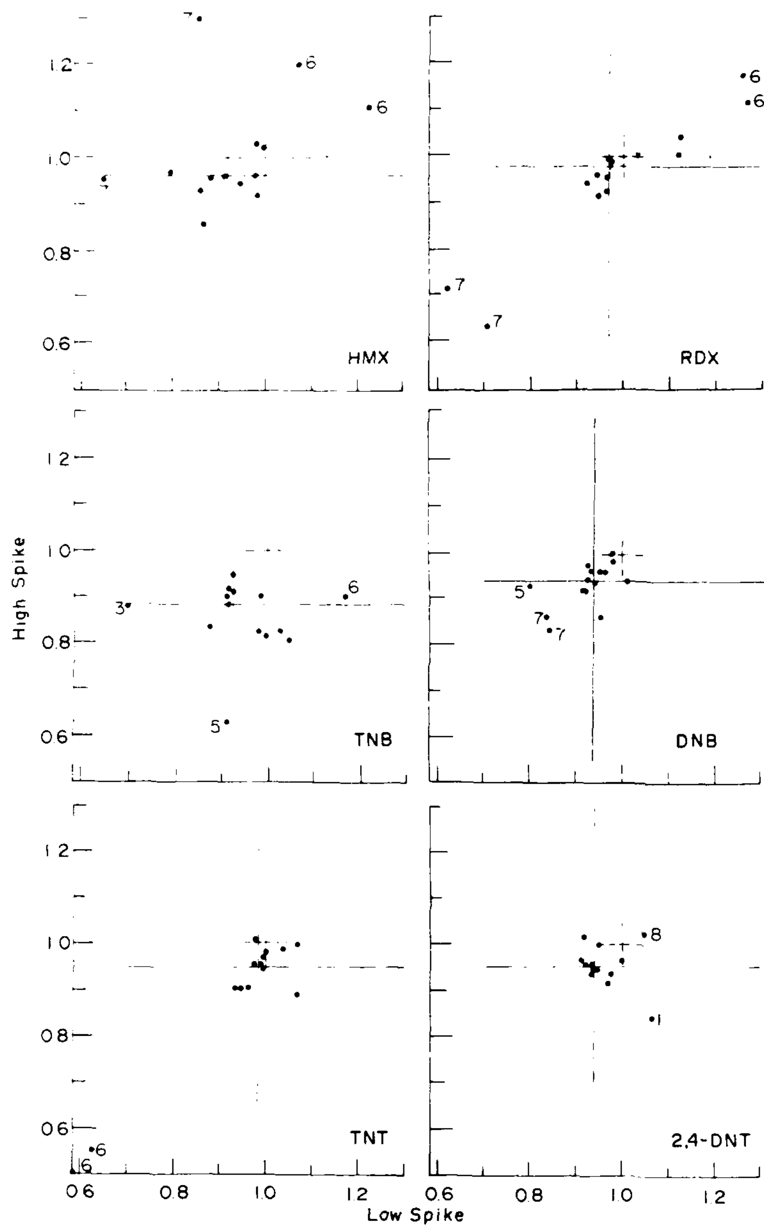
Because nearly every participant was at variance with some aspect of the protocol, nothing would be gained by eliminating labs from this already small group. The consequence is that the interlaboratory comparisons drawn herein certainly represent a realistic and rugged appraisal of the method.

Youden two-sample plots

To aid further with inspection of the results and to begin to consider the problem of outliers, Youden two-sample plots were constructed for each analyte (Fig. 1). A Youden plot (Youden and Steiner 1975) concisely summarizes the relative amount of systematic error between laboratories in comparison to the amount of random error in the method and also indicates the relative accuracy of the results. In these diagrams the reported concentrations for two of the spiked solutions normalized to the known values are plotted against each other. The two soils involved were the two that were purposely made similar to each other in concentration (Table 4). The higher-concentration spike was plotted vs the lower concentration. The origin of the solid axes locates the medians for the entire normalized data set for that analyte (Table 9). The shortened dashed axes locate the true values, which after normalization equal (1.0, 1.0). The medians have been used here instead of means because the former are unaffected by a few extreme values.

Table 9. Grand median values for each analyte in Youden plots normalized to known concentrations.

	Normalized grand median			
	High-concentration sample		*Low-concentration sample*	
Analyte	*Median*	*Range*	*Median*	*Range*
HMX	0.961	0.855–1.296	0.930	0.647–1.221
RDX	0.970	0.970–1.116	0.968	0.629–1.128
TNB	0.882	0.629–0.949	0.924	0.696–1.167
DNB	0.944	0.837–1.007	0.938	0.798–1.012
Tetryl	0.634	0.109–0.961	0.832	0.205–0.996
TNT	0.949	0.500–1.007	0.983	0.600–1.070
2,4-DNT	0.952	0.842–1.028	0.939	0.908–1.065



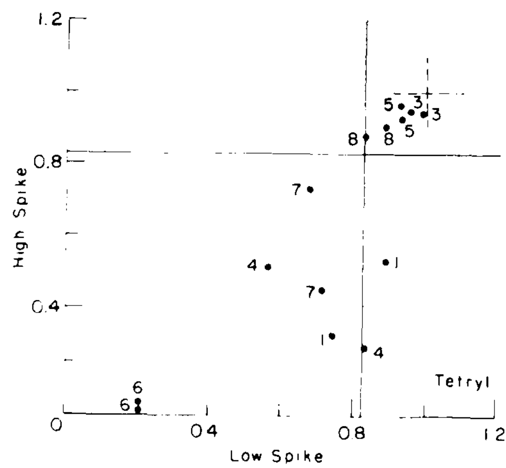


Figure 1. Youden plots for the spiked samples. The lab numbers for the extreme data are indicated. The large axes center on medians; the small axes center on known concentrations.

Both the tabulated values and the Youden plots show that the average accuracy is quite good, the disparity being 3–7% for all analytes except tetryl. The plot shapes for RDX, DNB and tetryl hug the 45° line. This indicates that the systematic error between laboratories is larger than the random error. For HMX, TNB, TNT and DNT the pattern is more circular, indicating that random errors are more dominant. The most likely source of the systematic error is the calibration procedure.

The results for lab 6 consistently stand apart for almost every analyte. Tetryl (Fig. 1) exhibits significant losses, and the loss is uniform for a given lab, some labs recovering better than 90% but others recovering much less. There is some indication that thermal decomposition during extraction in the sonic bath is responsible. Bath temperatures were 20, 25, 30, 34, 40, 42 and 53°C. The laboratory having the highest temperature shows the lowest recovery (lab 6), and there seems to be a correlation across the other labs. For lab 6, recoveries of TNT were also low, but those of other analytes were higher than for the other labs. We speculate that the high temperature not only promoted decomposition of labile compounds (tetryl and TNT) but also led to some solvent evaporation, which concentrated the more stable analytes.

Rejection of outliers

More sophisticated statistical methods had to be applied at this point to help us identify outliers and decide whether or not to reject them. The reasons for excluding outliers are 1) the extraction method is being tested here, not the individual laboratories, 2) there is no other way to identify errors that are not obvious by inspection, and 3) the analysis of variance assumes homogeneity of variance. It is inevitable that a data set this large will contain some outliers. By inspection, many suspect values were found that could not be corrected by reference to the laboratory notebooks. The collaborators should be commended for their honesty in reporting data that they could have censored when they observed apparently aberrant values. Rejecting outliers is more safely done with reference to the entire population of analytical results rather than with reference to the results within a single laboratory.

The statistical outlier tests applied are described in detail in Youden and Steiner (1975). The sequence of application was: a ranking test on laboratories, a Dixon's range test on individual data values, a range test for homogeneity of variance among laboratories, and a range test for homogeneity of variance between replicates. For comparison, accuracy and precision estimates were also obtained on the full, unedited data sets.

Ranking test

For each sample, laboratories were ranked according to their reported concentrations. These rankings were then summed across all samples.

Table 10. Summary of results of the laboratory ranking test.

Laboratory	*HMX*	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*2,4-DNT*
Results for spiked soils							
1							
3					0		
4						0	
5							
6	X	X			X		
7		X		0			
8							
Results for field-contaminated soils							
1							
3							X
4	0						
5							
6		X	X		X		0
7							
8			0		X		

X = Rank test indicates systematic error, and the results for this analyte were rejected for this laboratory.

0 = Rank test indicates systematic error, but the results were not rejected after inspection of the data.

Spiked samples were ranked separately from real samples, and zero values were included in the ranking. The distribution of total scores was compared to limiting scores expected for the case of completely random errors at the 95% probability level. Any laboratory having a score outside these limits indicates systematic error. Table 10 summarizes the results. The difficulties of lab 6 are very clear, so its data were eliminated where indicated. Three other cases could be eliminated unambiguously: RDX in spiked soils for lab 7, tetryl in field-contaminated soils for lab 8, and DNT in field-contaminated soils for lab 3. The last two data sets consisted entirely of zero values. In several other instances the ranking test indicated systematic error, but inspection of the data led us to decide not to reject the entire set because only a few of the concentration values were extreme.

These could be eliminated individually instead of eliminating the entire laboratory's data and throwing away many valid data points. Lab outliers are annotated in Appendix A.

Dixon's test

Dixon's test uncovers individual stray data. It is sensitive to values that lie outside the range expected for randomly distributed results. Data reported as zero or "not detected" were excluded from this and further consideration. Our rationale for elimination is that these data are arbitrarily "0.0" and thus not from the same population as the other data. Furthermore, duplicate zeros falsely lower the variance of the data set. Of 44 such values, 38 were from labs 3 and 8, which had used too large an extraction solvent volume (see above).

Table 11 lists the numbers of values rejected by applying Dixon's test at the 95% probability level. The relative number of outliers for each analyte is no more than 12% when excluded laboratories are not considered part of the total. This does not represent an excessive loss to the data set. It is similar to results in a previous collaborative study for munitions in aqueous samples (Jenkins et al. 1984, Bauer et al. 1986), and seems to be typical for collaborative studies in general (Horwitz 1982). Of the 11 outliers in spiked soils for HMX results from lab 7 and TNT results from lab 6, seven were verified misintegrations. Note that lab 6 accounted for 71% of all outliers. Dixon outliers are annotated in Appendix A.

Table 11. Number of data points rejected on the basis of Dixon's test and laboratory ranking test.

	Number rejected						
Laboratory	*HMX*	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*2,4-DNT*
Spiked soils							
1							
3			1	1			
4	1						
5			1				
6	8*	8*	2		8*	6	1
7	5	8*		1			
8							
Total rejected	14	16	4	2	8	6	1
Number before rejection†	56	56	54	56	54	56	55
Percent rejected	25	29	7	4	15	11	2
Percent rejected disregarding rejected labs	12	0	7	4	0	11	2
Field-contaminated soils							
1							
3						1	**
4							
5							
6		6*	8*		6*		
7	1			1			
8					**		
Total rejected	2	6	8	1	6	1	0
Number before rejection†	56	42	56	23	30	51	39
Percent rejected	4	14	14	4	20	2	0
Percent rejected disregarding rejected labs	4	0	0	4	0	2	0

* Entire lab rejected via laboratory ranking test.
† Less than 56 if data set contained zero values or samples with concentrations below the detection limit.
** Entire lab rejected via laboratory ranking test because all values were zero; these values are not included in the counts.

Analysis of variance

Normally a two-way analysis of variance (ANOVA) would be used to test for significant differences among the participating labs and for the existence of significant lab–sample interactions. However, for some analytes the data set was unbalanced because of the elimination of individual outliers and zero values. Under these circumstances, a two-way ANOVA yields ambiguous results (Freund 1980, Freund and Littel 1981). For this reason the data for each analyte were split into individual samples and a one-way ANOVA was performed for each sample. This option was preferable to either dropping good data to achieve balance or filling in missing values with estimates because the number of instances was considerable. An addi-

tional advantage is that the variance tends to increase with concentration. Thus, segregating samples offers a means for maintaining homogeneity of variance, an underlying assumption for ANOVA.

There are disadvantages to this decision. The significance test for differences among labs is less sensitive because fewer sets of duplicates exist for estimating the error variance. In addition, the ability to test explicitly for a lab– sample interaction is lost (recognizing that its interpretation would be complex for the unbalanced data). Where balanced data existed, a two-way ANOVA was also performed for comparison.

ANOVA assumes homogeneity of variance. For a two-way ANOVA, homogeneity must exist across all samples. A range test (Youden and Steiner 1975) found heterogeneity at the 95% probability level in several instances (TNB for both spiked and field-contaminated soils; HMX, RDX and TNT for field-contaminated soils). Segregating these samples into two concentration ranges remedied the problem (Appendix A). Data transformations were also investigated as a means of homogenizing the variance. No particular transformation was uniformly successful for all analytes, so we decided to abandon that option.

Another underlying assumption of ANOVA is homogeneity of the variance between duplicate samples. Again, a range test is used to verify this (Youden and Steiner 1975). For a given analyte the maximum range between a set of duplicates was compared to the sum of the ranges for all duplicates. This was performed for each sample (for a one-way ANOVA) or for each group of samples (for a two-way ANOVA). At the 95% probability level, heterogeneity was discovered in three cases, but the offending data were not so extreme as to warrant their elimination.

Appendices B and C contain one-way ANOVA results for the edited (outliers omitted) and unedited data sets, respectively. Spiked soils are listed above field-contaminated soils, and each group is sequenced from lowest to highest average found concentration. Table 12 summarizes these results. Laboratories were significantly different at the 95% probability level for only about half of the samples. These differences are somewhat more likely at higher concentrations, but the trend is not consistent for all analytes. Differences are more frequent in the unedited data but only for spiked samples. The heterogeneity of the field-contaminated soil samples apparently swamps out differences among

Table 12. Significant interlaboratory differences with respect to analyte concentration and soil type.

	Ranked† concentrations for spiked soils					*Ranked† concentrations for field-contaminated soils*				
Analyte	*1*	*2*	*3*	*4*	*Sum*	*1*	*2*	*3*	*4*	*Sum*
Unedited data										
HMX			*	*	2		*		*	2
RDX		*	*	*	3	—		*	*	2
TNB	*	*			2			*		1
DNB				*	1					0
Tetryl	*	*	*	*	4		*	*	—	2
TNT		*	*	*	3	*		*		2
2,4-DNT				*	1				*	1
Sum	2	4	4	6	16	1	2	4	3	10
Edited data										
HMX				*	1	*	*		*	3
RDX	*			*	2	—			*	1
TNB		*			1			*		1
DNB				*	1	*		—	—	1
Tetryl	*		*		2				—	0
TNT		*		*	2	*		*		2
2,4-DNT				*	1				*	1
Sum	2	2	1	5	10	3	1	2	3	9

* = F ratio for laboratory mean square significant at 95% probability level.
† = increasing integer means increasing analyte concentration.
— = no data for ANOVA.

Table 13. Repeatability and reproducibility estimates for edited data from the collaborative study.

	Spiked soils			*Field-contaminated soils*		
Analyte	*Mean conc. (µg/g)*	*Standard dev.*	*% rsd*	*Mean conc. (µg/g)*	*Standard dev*	*% rsd*
			Repeatability*			
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12.0	11.5
				877	29.6	3.4
TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
DNB	3.5	0.14	4.0	1.1	0.11	9.8
Tetryl	17	3.1	17.9	2.3	0.41	18.0
TNT	40	1.4	3.5	7	0.61	9.0
				669	55	8.2
2,4-DNT	5	0.17	3.4	1.0	0.44	42.3
			Reproducibility*			
HMX	46	2.6	5.7	14	3.7	26
				153	37.3	24
RDX	60	2.6	4.3	104	17.4	17
				877	67.3	7.7
TNB	8.6	0.61	7.1	2.8	0.23	8.2
	46	2.97	6.5	72	8.8	12.2
DNB	3.5	0.24	6.9	1.1	0.16	14.5
tetryl	17	5.22	30.7	2.3	0.49	21.3
TNT	40	1.88	4.7	7	1.27	18
				669	63.4	9.5
2,4-DNT	5	0.22	4.4	1.0	0.74	74

* After pooling

the laboratories. Least-significant-difference tests were conducted to identify which laboratories' results were different from one another. Generally where the ANOVA indicated that a difference existed, a single laboratory's results were found to be different from the remaining laboratories. The specific laboratory that differed varied from analyte to analyte and sample to sample.

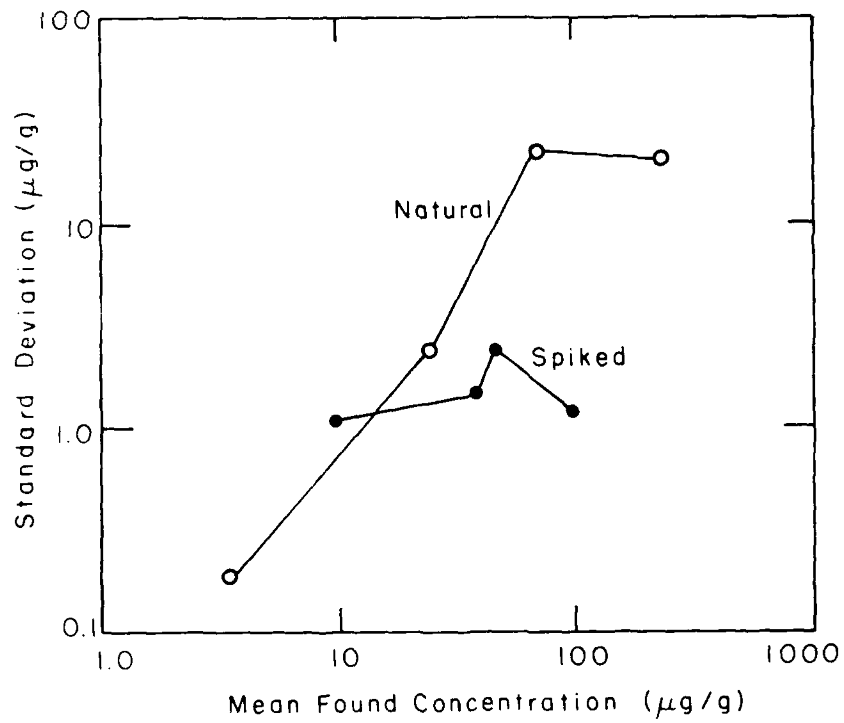
Appendices B and C also list repeatability (the intralaboratory precision calculated from the square root of the error mean square in the ANOVA) and reproducibility (the interlaboratory error that is obtained as in Youden and Steiner (1975) from the mean square error and mean square across labs). These data are summarized in Table 13 by pooling them into homogeneous groups as discussed above.

Excluding tetryl, the relative repeatability for spiked soils was less than 5% and the relative reproducibility 7% or less. For spiked samples, therefore, the collaborators were able to perform at a level nearly equivalent to the method's random error. The small differences are probably due to calibration differences.

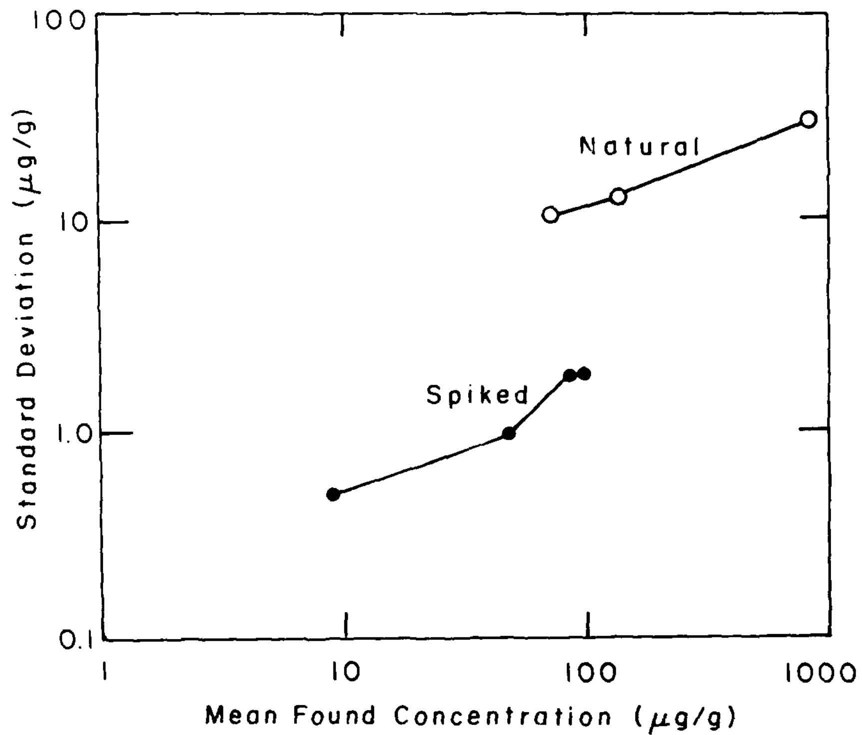
For field-contaminated soils, repeatability and reproducibility were always larger than for spiked soils—up to 18% and 26%, respectively, except for DNT. The poorer repeatability for field-contaminated vs spiked soils represents real differences in sub-sample composition, despite precautions taken by CRREL to homogenize the soils before distribution. These differences thus increase the repeatability estimates by 1–10% (from Table 13, excluding tetryl and DNT). Furthermore, we should expect the variation *between* laboratories for field-contaminated soils to exceed that for spiked soils by 1–10% as well; i.e. the reproducibility for field-contaminated soils should be about 5–16% (the reproducibility for spiked soils plus 1–10%). In fact, it is somewhat greater: 8–26%. This additional imprecision is likely due to variable extraction recoveries between laboratories.

The behaviors of absolute and relative repeatability as a function of concentration are interesting (Fig. 2 and 3, respectively). Repeatability estimates almost always increase with analyte concentration, except for DNT (in the 0.5- to 10-µg/g range) and spiked HMX and TNT (in the 10- to 100-µg/g range). Relative repeatability almost always decreases or at least stays fairly constant. This behavior is indicative of mass-constant errors at low concentration and mass-proportional errors at higher concentration. No consistent trend of repeatability across analytes is observed when field-contaminated soils are compared with spiked soils. However, in specific cases the percent error for field-contaminated soils was greater (RDX, DNT and HMX at 100 µg/g). Sample heterogeneity is suspected in these cases.

To evaluate the effect of outlier rejections, Appendix D shows the ratios of the means and standard deviations of the unedited data to those of the edited data (Appendix B vs Appendix C). Table 14 summarizes these data by pooling precisions into the same groups as in Table 13. Percent relative standard deviations for the unedited data are within a factor of two larger than the edited data in most cases. The differences for reproducibilities tend to be greater where all or most of a lab's data were eliminated—HMX, RDX, tetryl and TNT for spiked soils, and RDX, TNB and tetryl for field-contaminated soils (Table 11). Note that in some cases eliminating only one datum reduces the standard deviation tremendously. This reinforces our view that data screening is necessary to obtain representative performance evaluation. Even when outliers are included, interlaboratory precision is not unreasonable for homogeneous spiked soils—5 to 20% excluding tetryl.

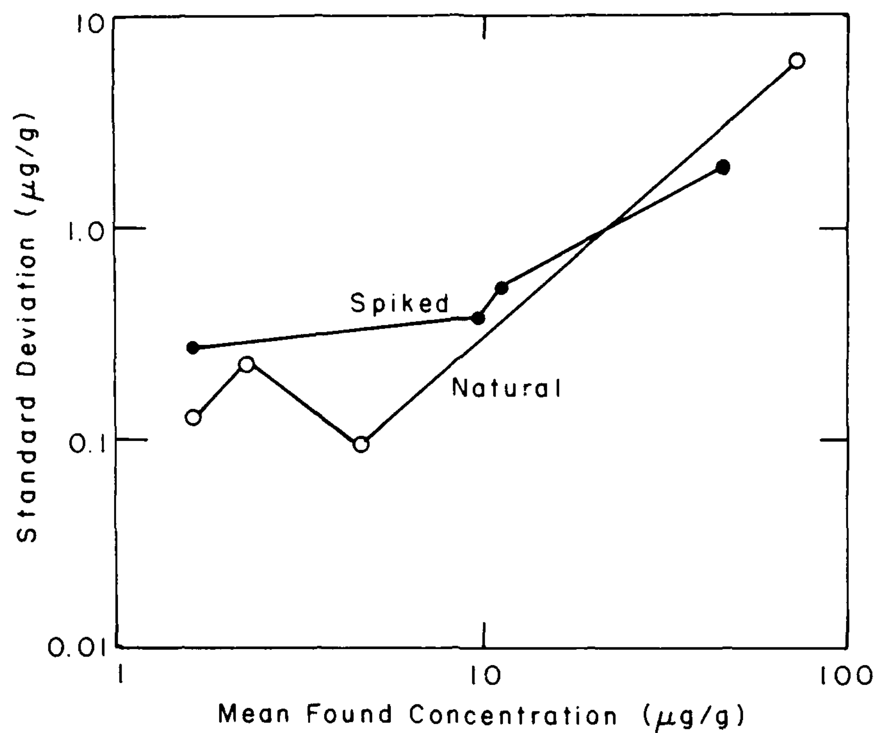


a. HMX.

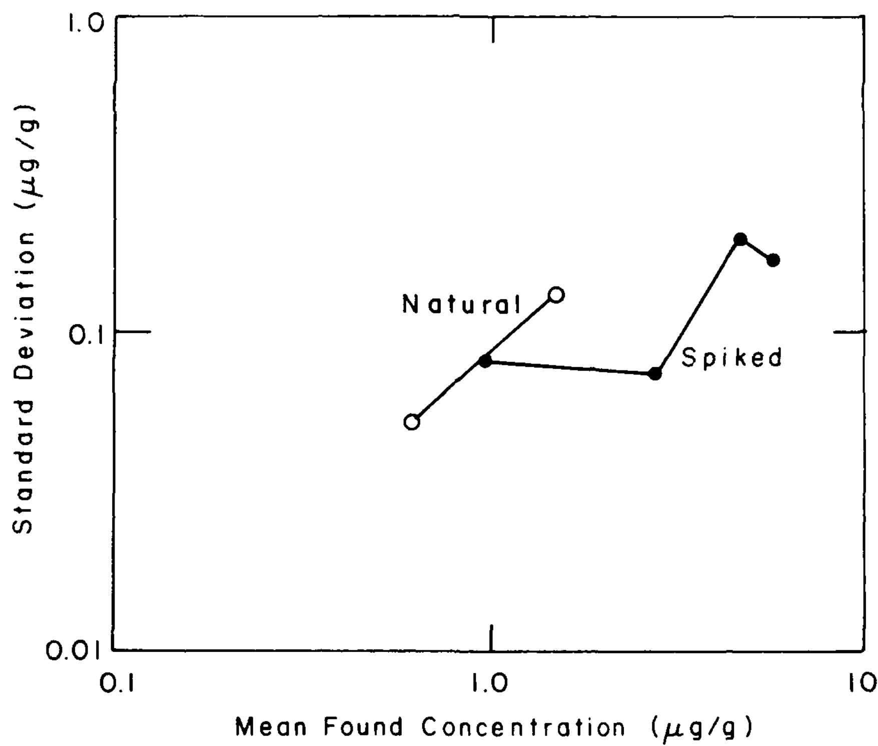


b. RDX.

Figure 2. Dependence of intralaboratory absolute precision on analyte concentration.

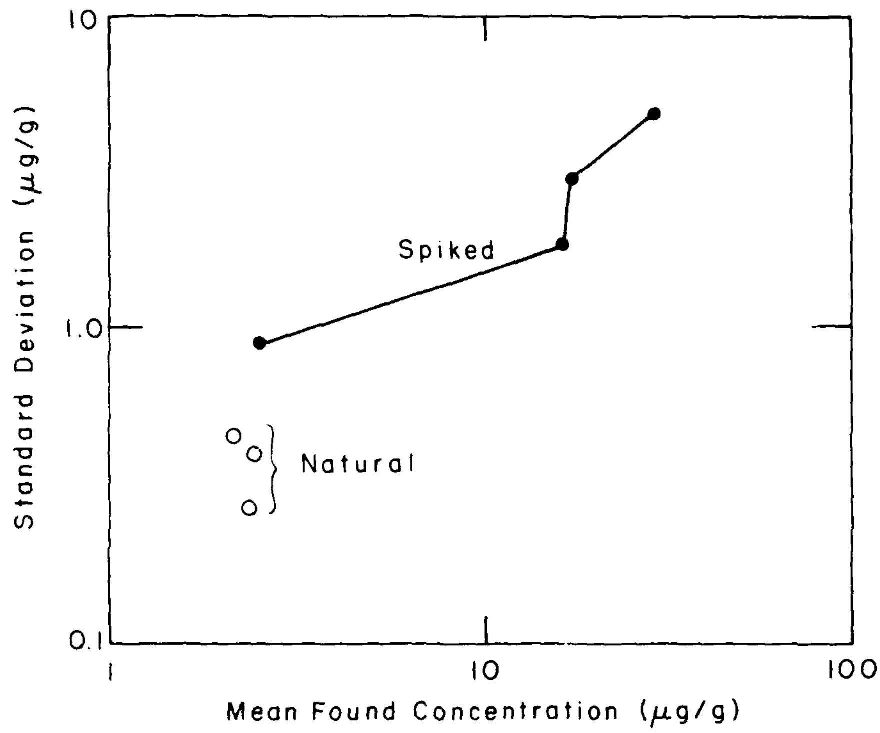


c. TNB.

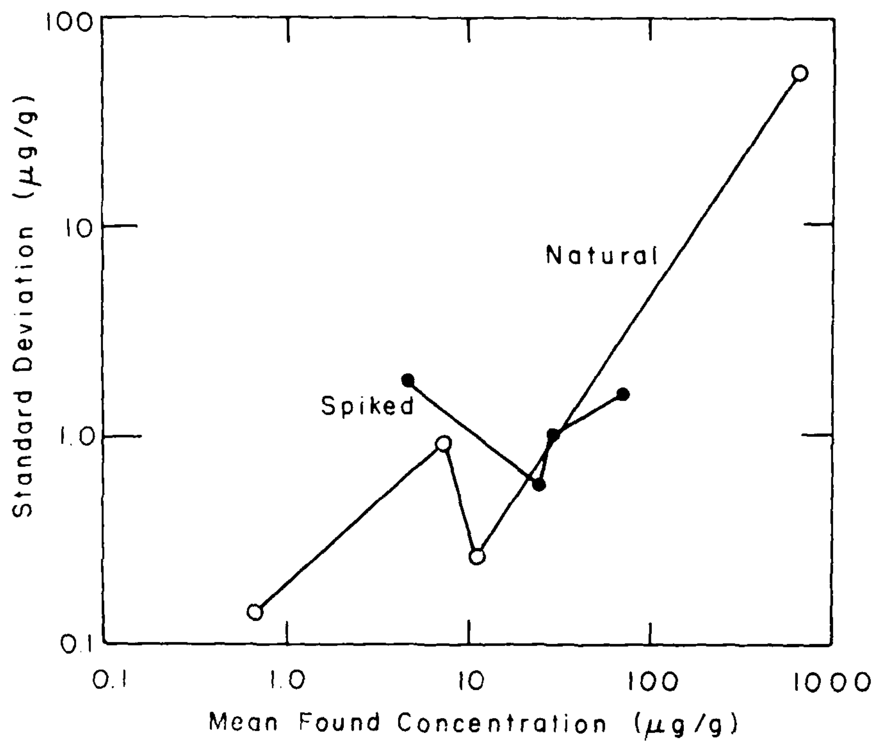


d. DNB.

Figure 2 (cont'd). Dependence of intralaboratory ab. lute precision on analyte concentration.

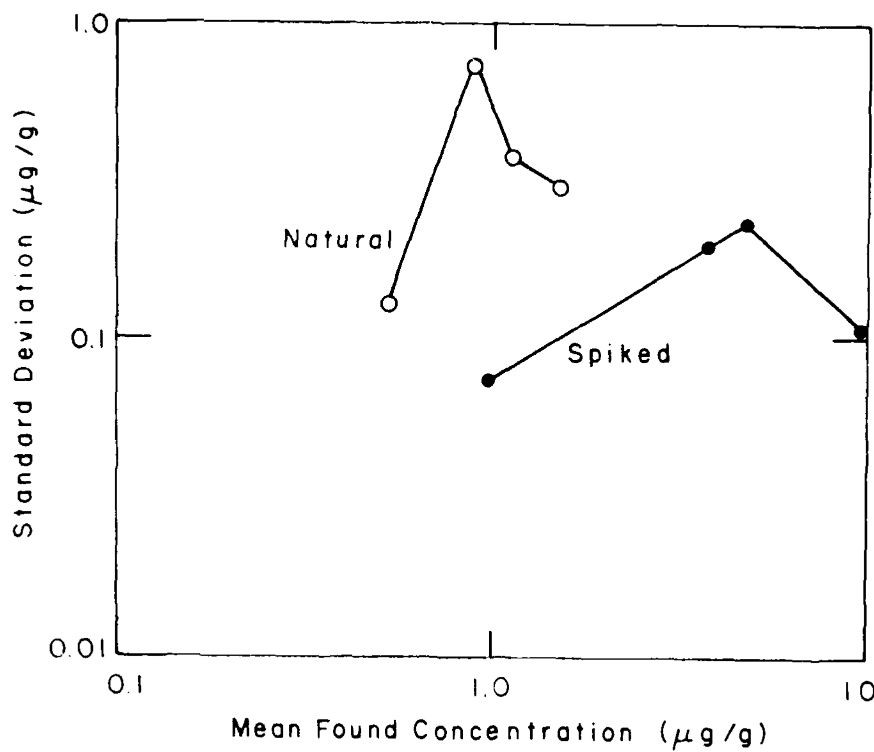


e. Tetryl.



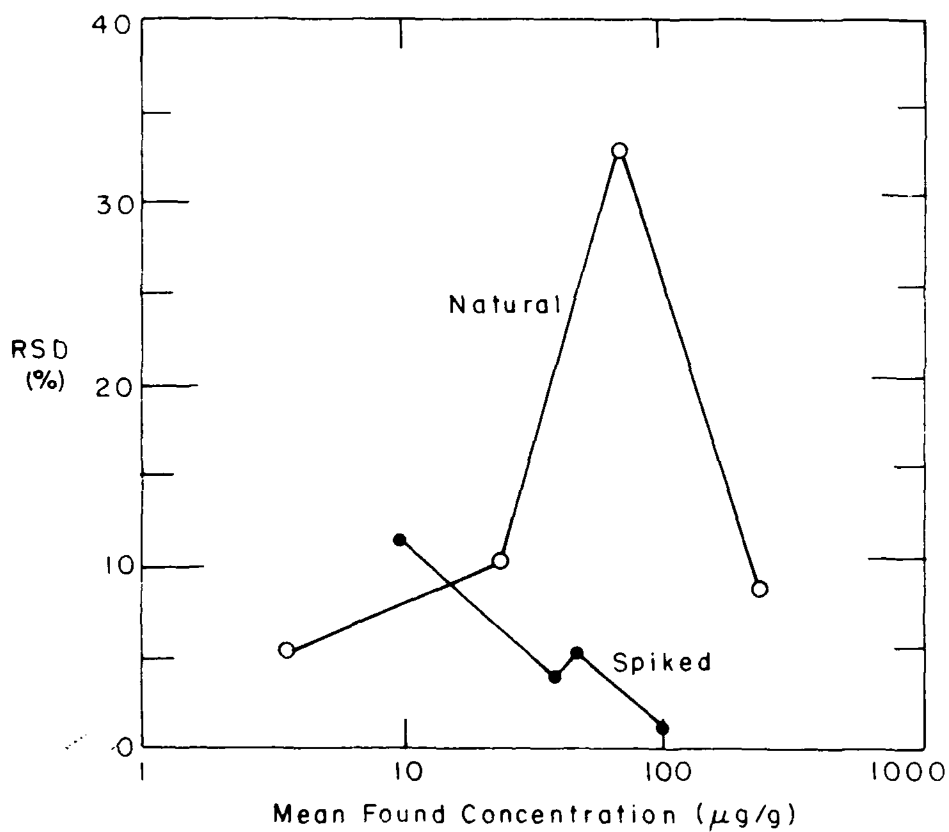
f. TNT.

Figure 2 (cont'd).



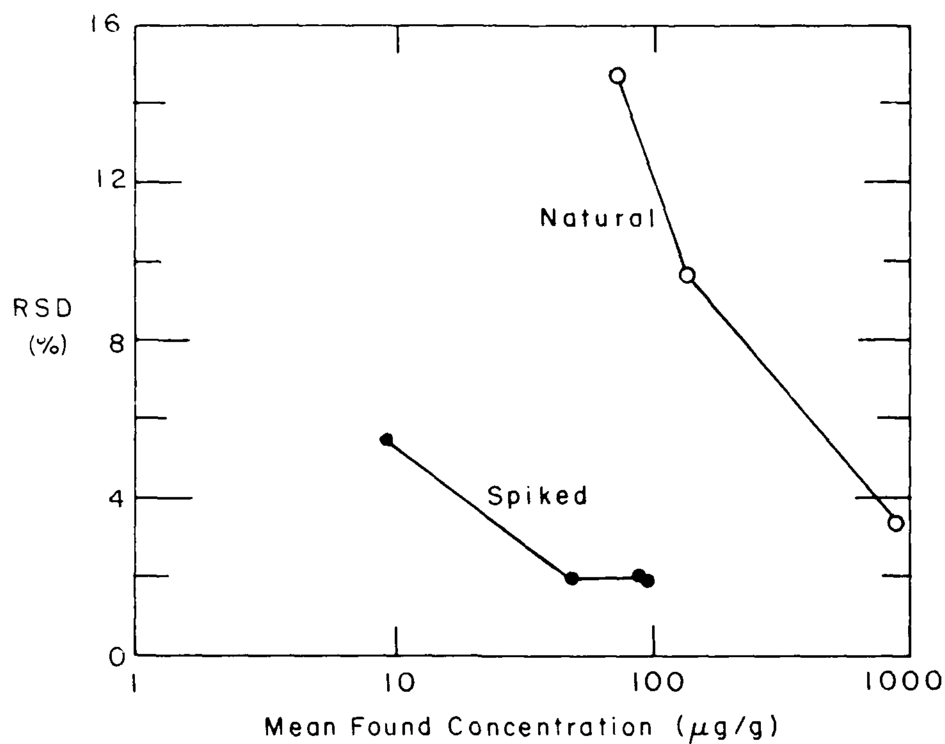
g. DNT.

Figure 2 (cont'd). Dependence of intralaboratory absolute precision on analyte concentration.

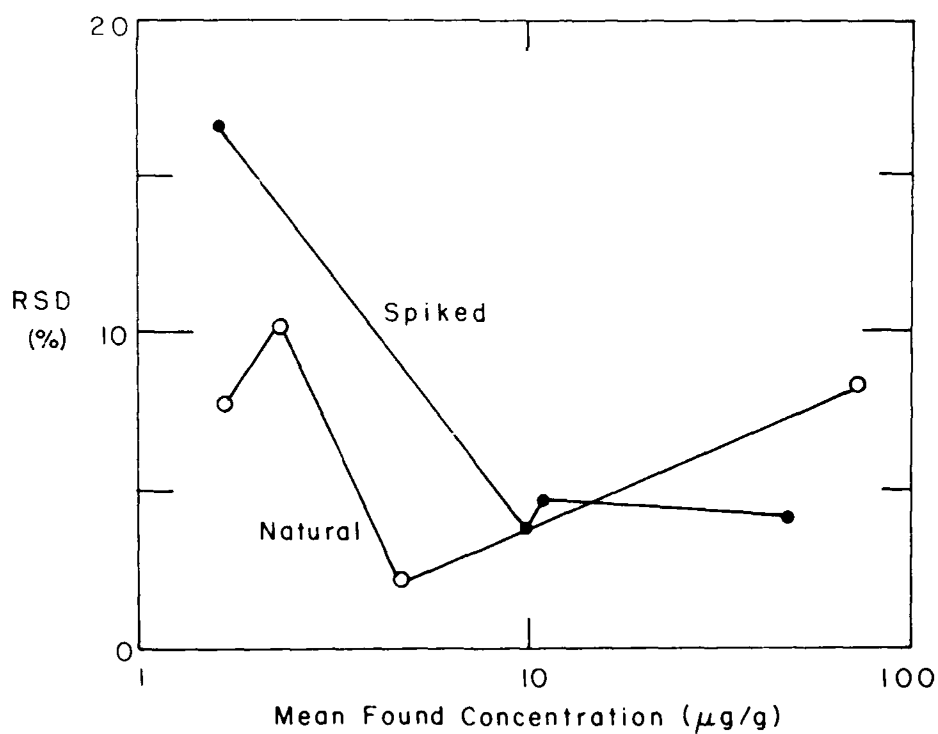


a. HMX.

Figure 3. Dependence of intralaboratory relative precision on analyte concentration.

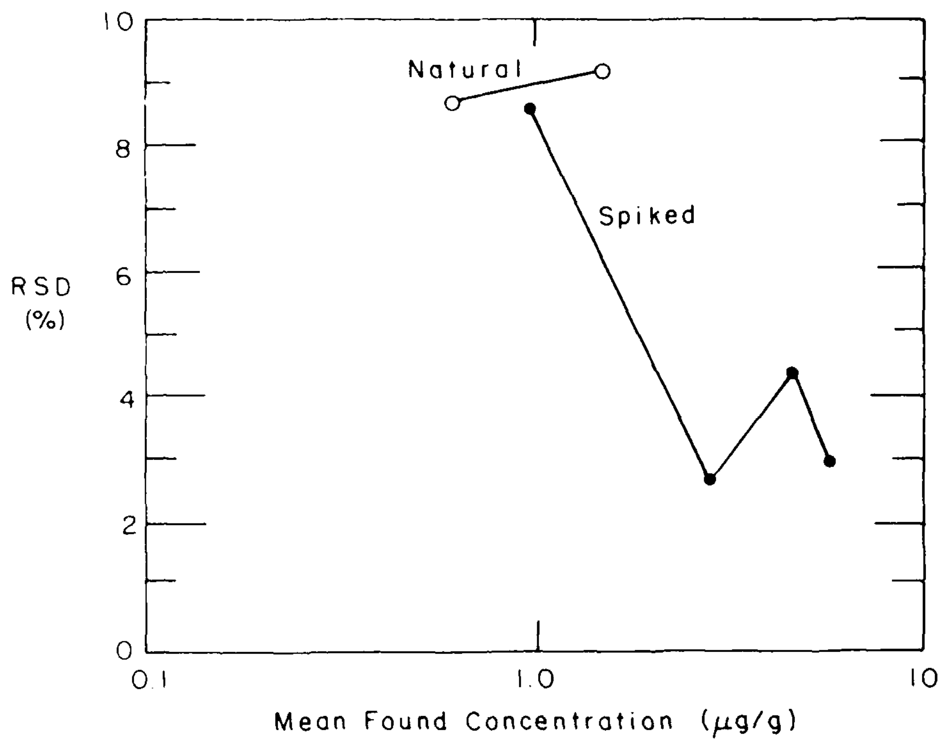


b. RDX.

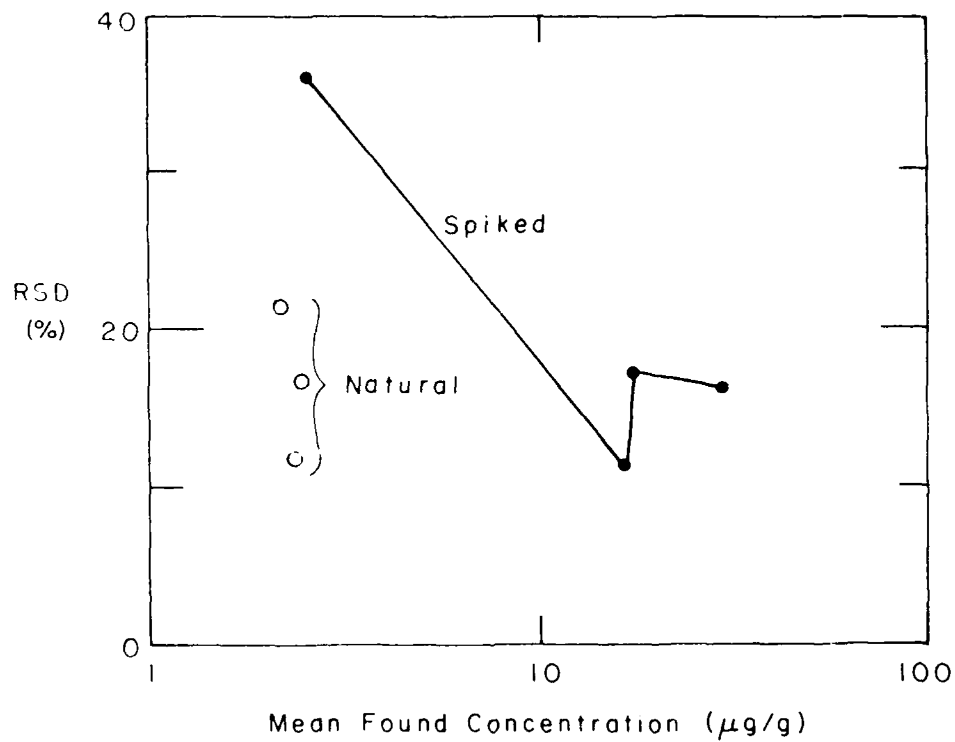


c. TNB.

Figure 3 (cont'd).

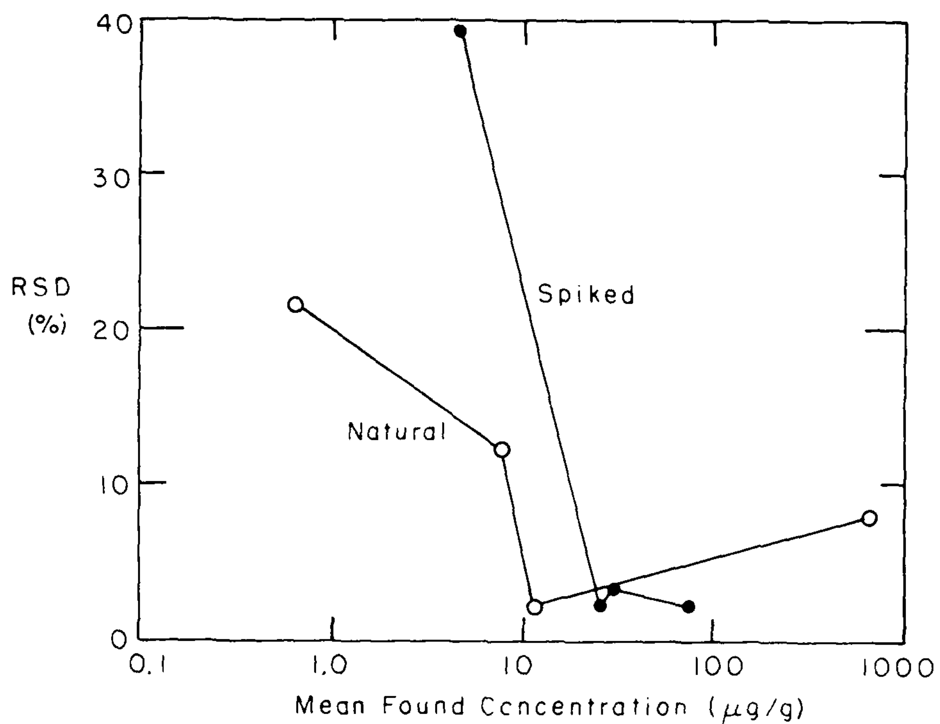


d. DNB.

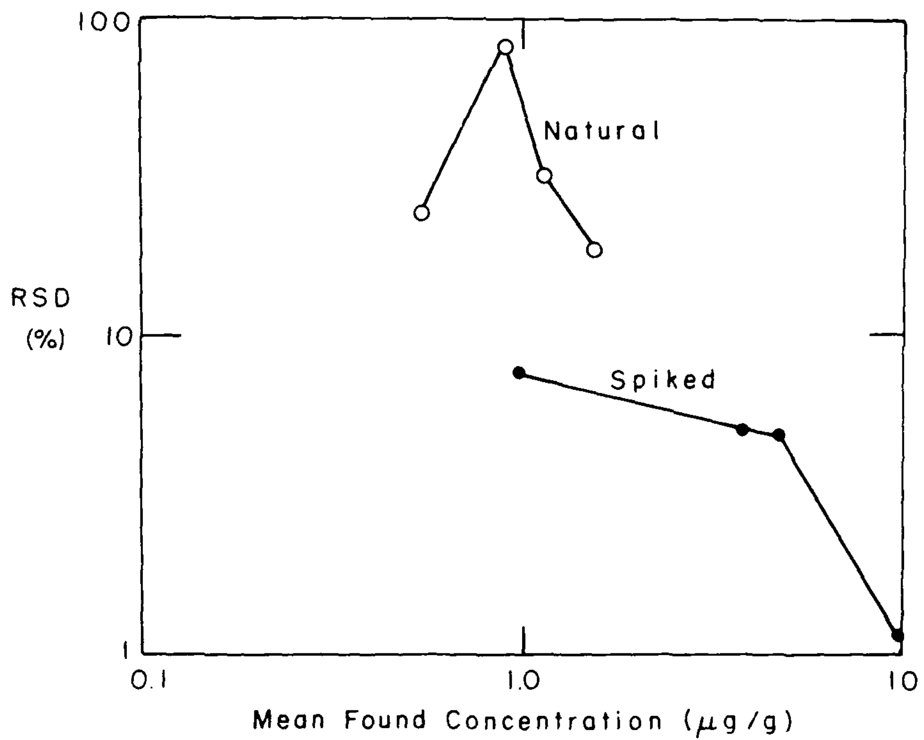


e. Tetryl.

Figure 3 (cont'd). Dependence of intralaboratory relative precision on analyte concentration.



f. TNT.



g. DNT.

Figure 3 (cont'd).

The results of the two-way ANOVA, where completely balanced data sets existed, are listed in Table 15. Laboratories were always significantly different from each other despite the fact that differences did not exist in every concentration range (Table 12). Significant lab–sample interactions were sometimes found. In the unedited data set, these interactions seem to be due to those values that were classified as outliers. Figure 4 shows plots of the residuals for the regression of found concentrations vs known concentrations for HMX, RDX, DNB and TNT spiked soils, respectively, showing how each lab deviates from the regression line. An interaction arises because of the different behaviors of each lab's residuals vs concentration.

Significant trends all tend to show increasing deviation from the zero-residual line as concentration increases. This reflects the calibration method, which uses a single high standard and assumes a zero intercept. The appearance of linear upward or downward trends is probably due to systematic differences in the concentration of that high standard between labs. Since trends are not the same for every analyte, the errors must arise in the preparation of the single-analyte stock solution (SARM assay variability, mass measurement error, volumetric measurement error) or of the combined-analyte stock solution (aliquot transfer or dilution error). Labs 6 and 7 are most seriously affected. Outlier tests eliminated the extreme values of HMX (Fig. 4a), RDX (Fig. 4b) and TNT (Fig. 4d).

Table 14. Effect of rejecting outlying data on method repeatability and reproducibility.

		Repeatability		*Reproducibility*		
Analyte	*Mean conc. (µg/g)*	*% rsd edited*	*% rsd unedited*	*Outlier percent*	*% rsd edited*	*% rsd unedited*
Spiked soils						
HMX	46	3.7	8.0	25	5.7	21.9**
RDX	60	2.3	4.2	29	4.3	16.3**
TNB*	8.6	4.6	8.5	10	7.1	10.7
	46	4.1	4.1	0	6.5	6.5
DNB	3.5	4.0	5.1	4	6.9	7.8
Tetryl	17	17.9	16.7	15	30.7	42.3**
TNT	40	3.5	3.5	11	4.7	13.2
2,4-DNT	5	3.4	3.8	2	4.4	4.5
Field-contaminated soils						
HMX*	14	12.8	23.7	4	26	36.1
	153	14.1	17.0	4†	24	26.2
RDX*	104	11.5	10.7	14	17	18.4**
	877	3.4	5.8	14	7.7	11.8**
TNB*	2.8	7.1	15.5	14	8.2	22.5**
	72	8.3	11.4	14	12.2	16.5**
DNB	1.1	9.8	43.7	4†	14.5	44.4
Tetryl	2.3	18.0	15.4	20	21.3	39.0**
TNT*	7	9.0	9.0	3	18	24.7
	669	8.2	8.2	0	9.5	9.5
DNT	1.0	42.3	42.3	0	74	74

* Segregated into ranges of homogeneous variance.
† A single outlier was eliminated.
** Includes rejected labs.

Table 15. Two-way ANOVA results.

Analyte	F *across labs*	F *for lab sample interaction*
Unedited data		
HMX, spiked soil	24.1*	11.4*
RDX, spiked soil	77.7*	12.0*
DNB, spiked soil	8.3*	1.7
TNT, spiked soil	68.4*	15.5*
HMX, field-contaminated soil		
two lower conc.	2.5	5.3*
two higher conc.	4.2*	3.6*
RDX, real soil	6.8*	2.4
TNB, real soil	8.6*	1.8
Edited data		
RDX, field-contaminated soil	4.5*	1.8
TNB, field-contaminated soil	5.4*	1.8

* Significant at the 95% probability level.

Regression analysis

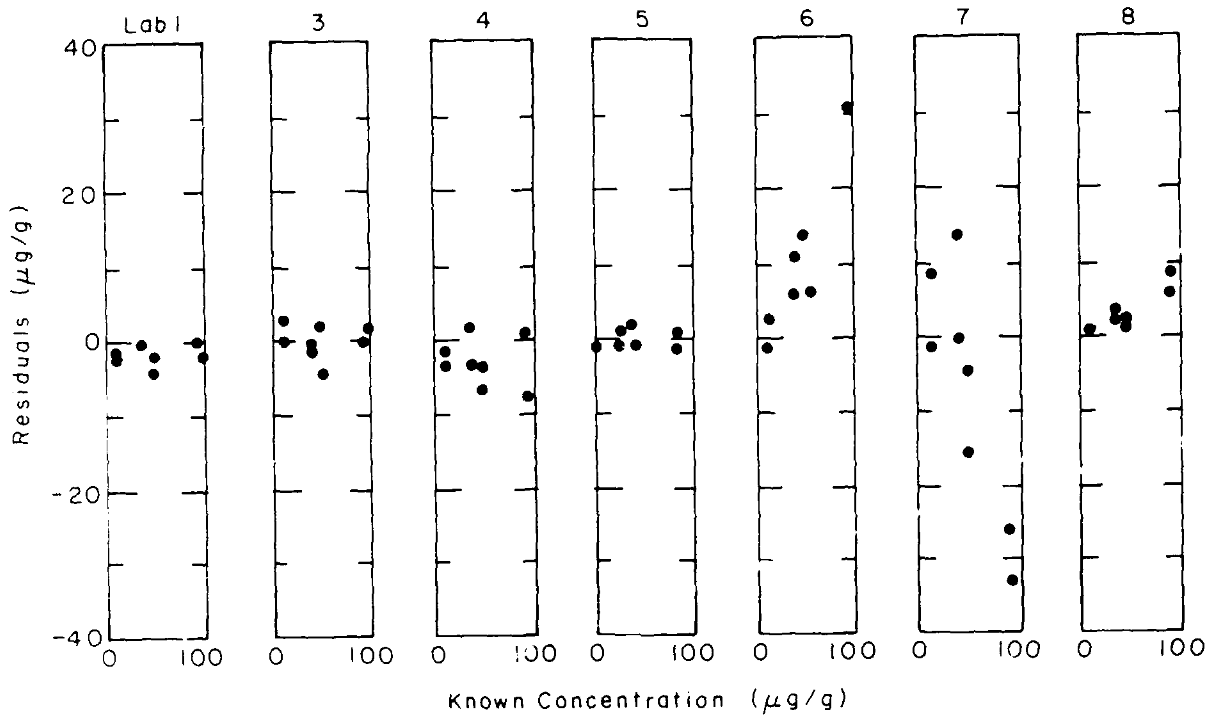
Accuracy, or recovery, was evaluated by linear least-squares regression analysis of "found" concentrations (y) plotted vs "known" concentrations (x). A perfectly accurate method should have an intercept of 0 and a slope of 1.00.

Table 16 lists the regression equations for each analyte in the edited data set for a linear model with and without a zero-intercept constraint. Also listed is the zero-intercept model for the unedited data

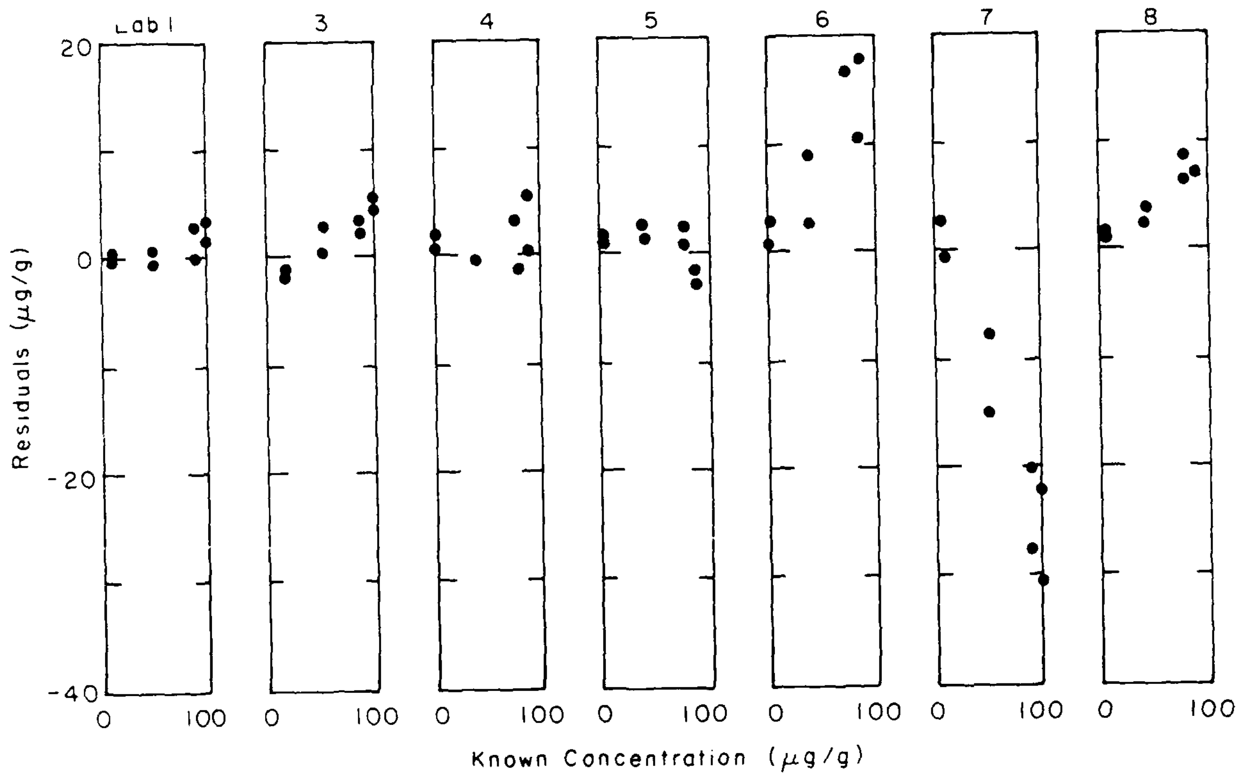
Table 16. Linear least-squares regression equations* for each analyte.

	Edited data		*Unedited data*
Analyte	*Model with intercept*	*Model through origin*	*Model through origin*
HMX	$-1.543 + 0.977x$	$0.954x$	$0.959x$
RDX	$-0.928 + 0.979x$	$0.968x$	$0.944x$
TNB	$-0.142 + 0.924x$	$0.920x$	$0.919x$
DNB	$-0.001 + 0.930x$	$0.930x$	$0.925x$
Tetryl	$-0.643 + 0.768x$	$0.747x$	$0.670x$
TNT	$1.473 + 0.941x$†	$0.968x$	$0.925x$
2,4-DNT	$-0.001 + 0.960x$	$0.960x$	$0.960x$

* Equations:
[Found Value] = intercept + [true value] recovery
[Found Value] = [true value] recovery
† Lack-of-fit test and residuals plot indicate significant nonlinearity.

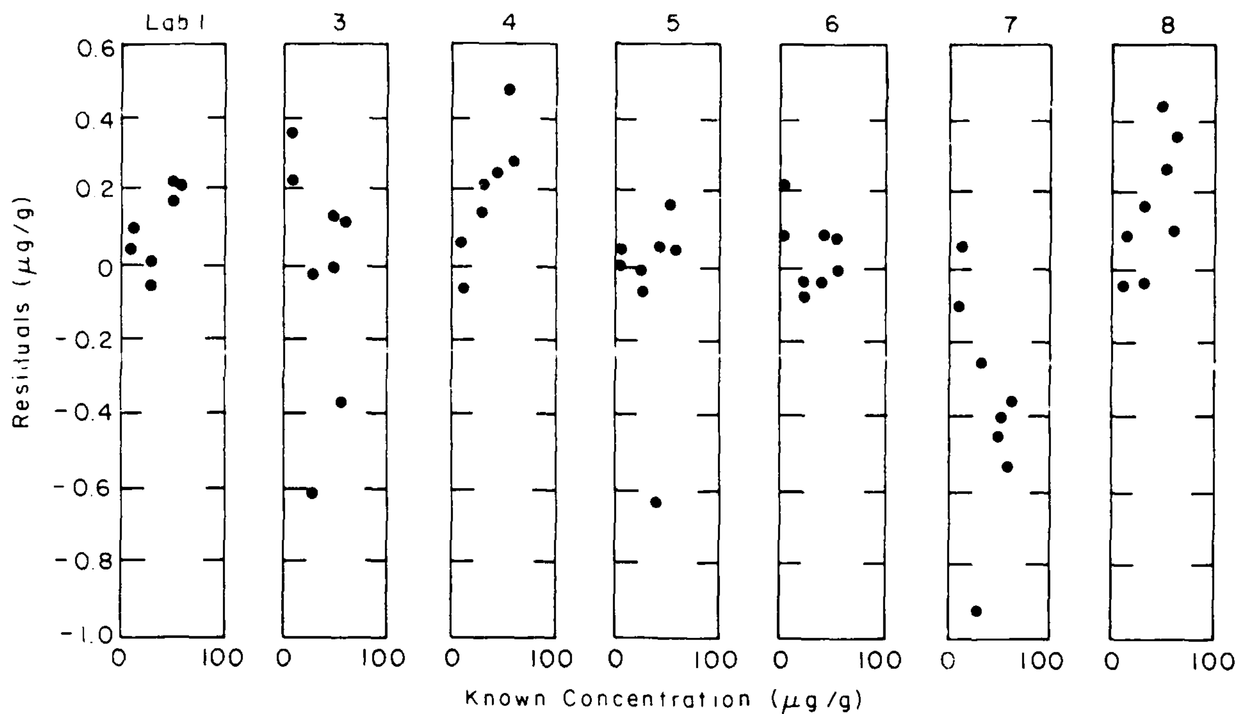


a. HMX.

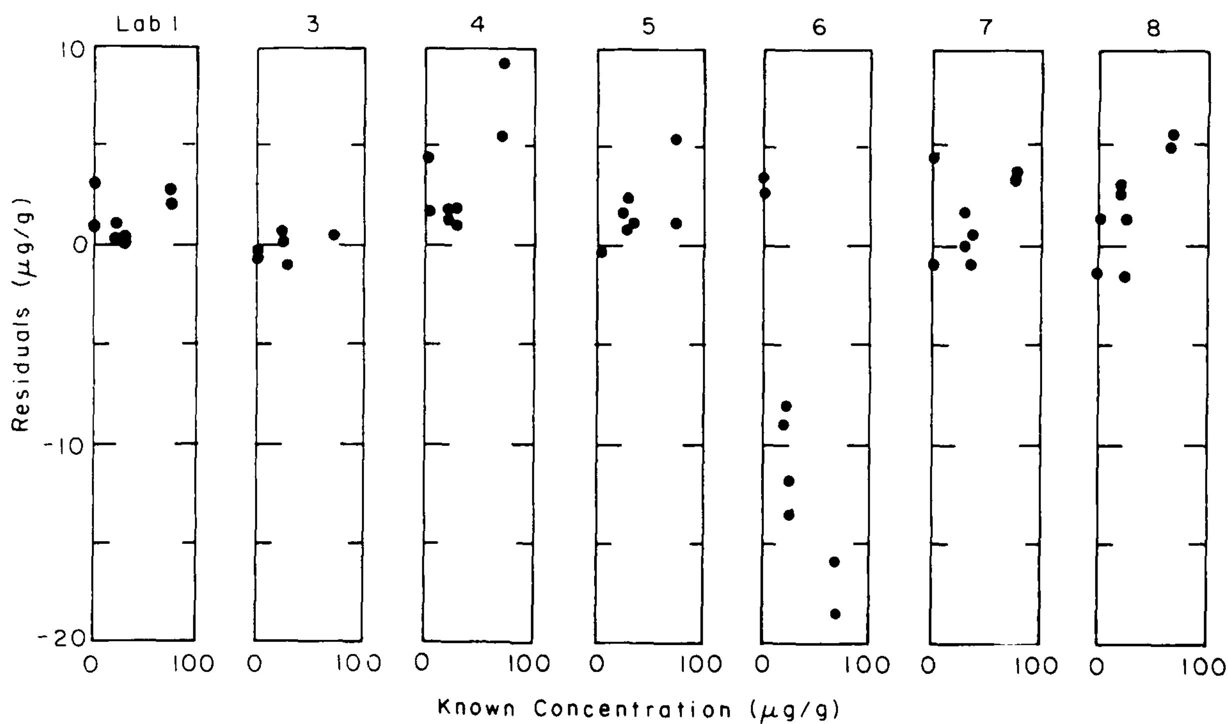


b. RDX.

Figure 4. Residuals for the regressions of found concentrations vs known concentrations for the four spiked soils that had balanced data sets.

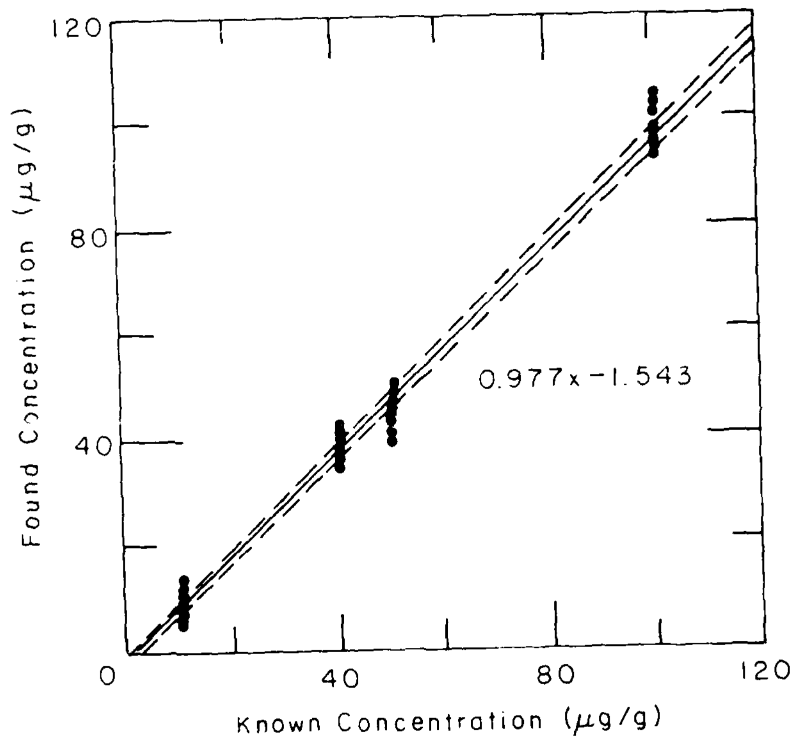


c. DNB.

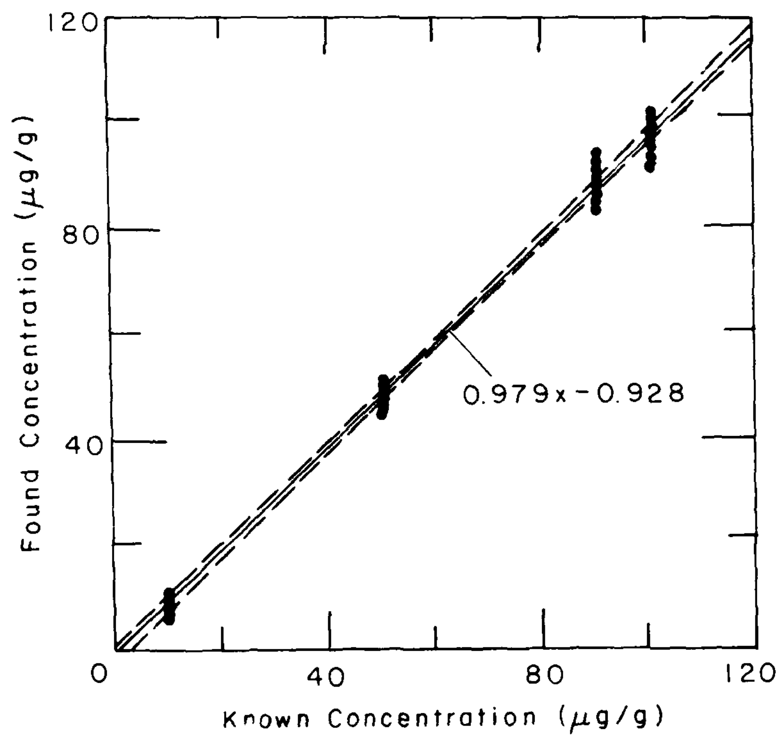


d. TNT.

Figure 4 (cont'd). Residuals for the regressions of found concentrations vs known concentrations for the four spiked soils that had balanced data sets.

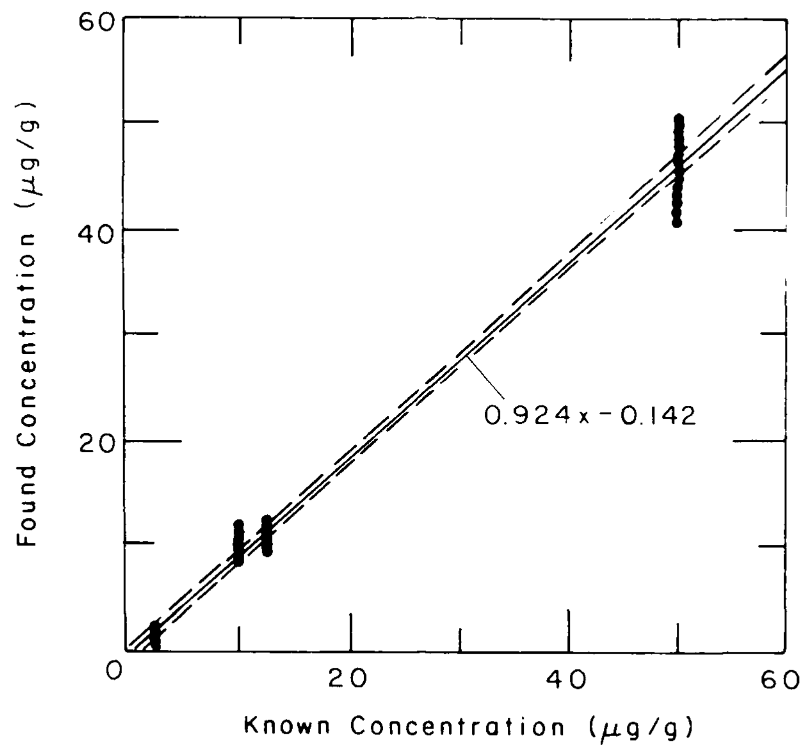


a. HMX.

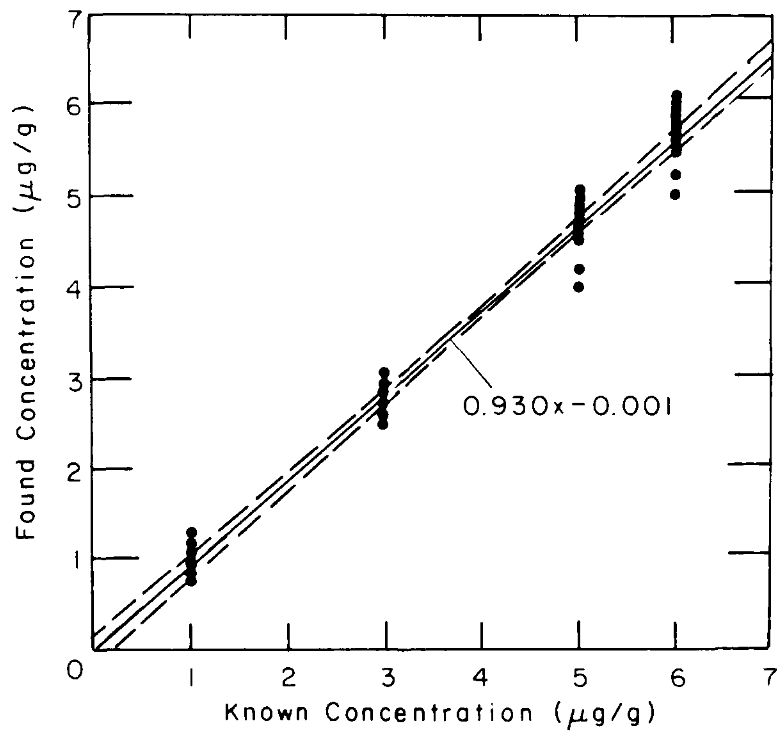


b. RDX.

Figure 5. Recovery from the spiked soils (edited data).

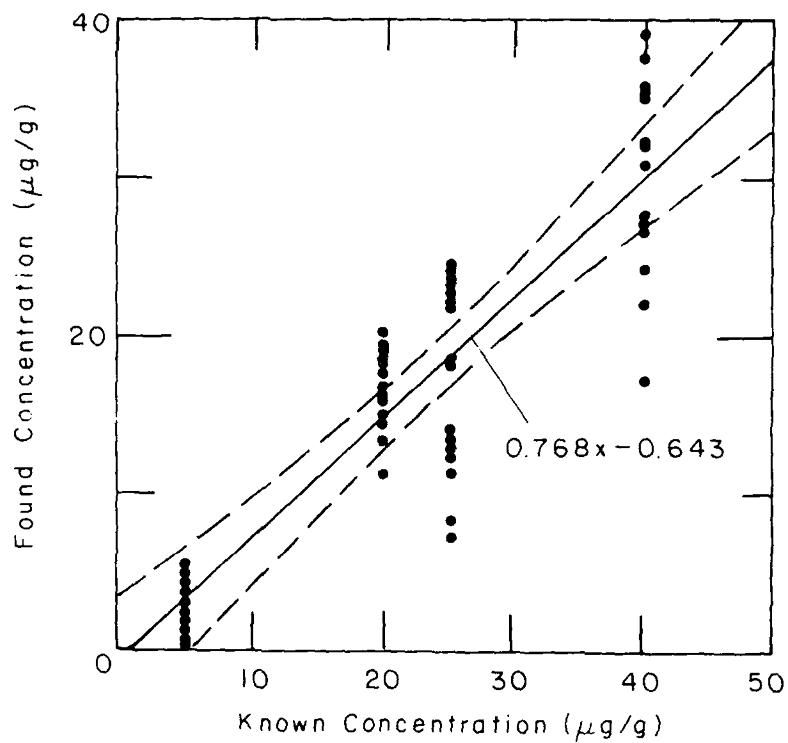


c. TNB.

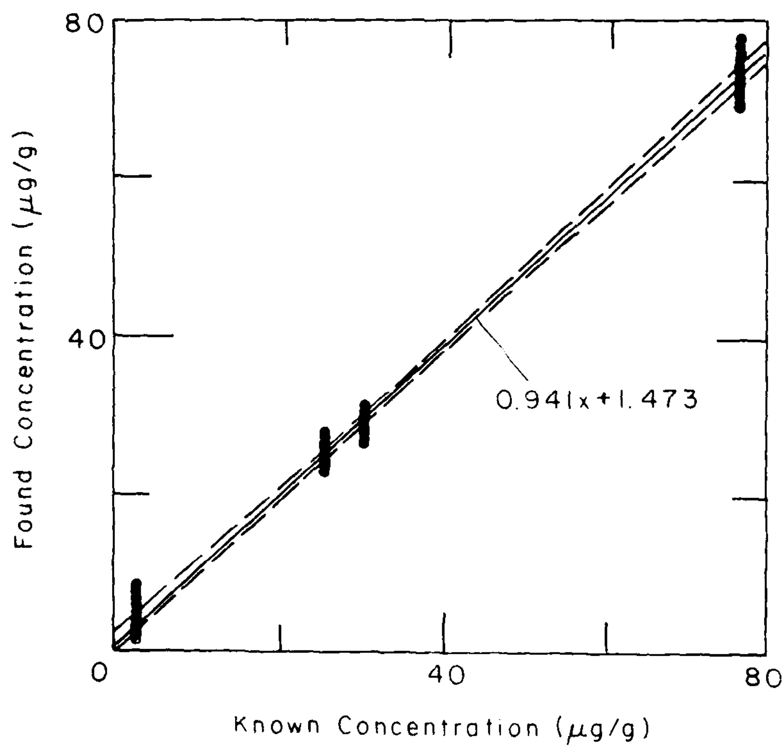


d. DNB.

Figure 5 (cont'd). Recovery from the spiked soils (edited data).

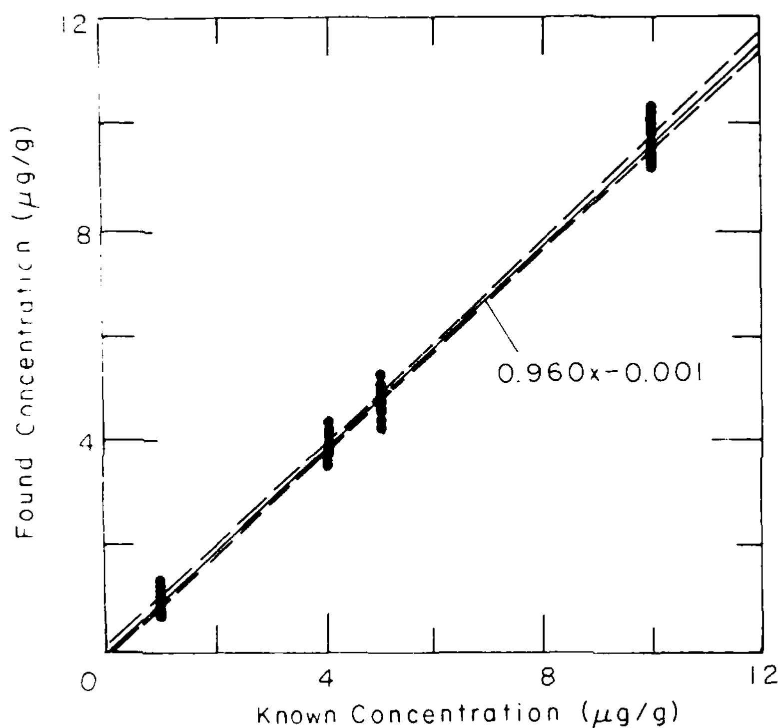


e. Tetryl.



f. TNT.

Figure 5 (cont'd).



g. DNT.

Figure 5 (cont'd). Recovery from the spiked soils (edited data).

set. Figure 5 shows the regression plots with 95% confidence bands for the edited data using the model with an intercept.

Formal testing for lack of fit confirmed that the TNT relationship was not linear. Inspection of the TNT plot indicated that there was curvature at the lowest concentration. This is discussed below.

It is clear from the plots that confidence bands incorporate the origins. A formal test indicated that the intercept is not significantly different from zero in all cases. This demonstrates that no constant bias exists in the recoveries.

Table 16 also indicates that rejection of outliers had no major effect on recoveries. The small improvements for tetryl and RDX were, in both cases, the result of eliminating the data from a particular lab whose results were consistently very low.

Regression residuals tended to increase with concentration, a fact noted in variance homogeneity tests and elsewhere (Bauer et al. 1986). Rejection of outliers reduced heterogeneity for some analytes. In cases of nonuniform variance, a weighted regression is more rigorously correct; however, this approach, when performed on the unedited data set, had no significant effect on regression slopes.

The TNT nonlinearity was suspected to be due to a coeluting substance, probably a tetryl decomposition product. The original chromatograms for the samples containing the lowest TNT concentration were inspected. By chance these samples also were spiked with high levels of tetryl. For three of the collaborators, the TNT peak was accompanied by a shoulder, or closely eluting peak. In a few cases the shoulder was incorrectly integrated with the TNT peak. Figure 6 is a plot of the apparent gain in TNT vs the loss of tetryl relative to their known spiking levels for samples 1 and 10. Clearly a positive correlation exists, tending to confirm our suspicions.

Method recoveries are all quite good: 95–97% for HMX, RDX, TNT and DNT, 92–93% for DNB and TNB, and about 70% for tetryl. The 96.8% recovery for TNT is based on the assumption that the model with a zero intercept is appropriate. Since there is evidence that the lowest concentration results are inflated, the zero-intercept model would tend to override this effect by forcing the line through zero.

It is worthwhile to compare these recoveries from soils with those from aqueous matrices in Table 17 (Jenkins et al. 1984). The fact that recoveries from soils are comparable to those from water indicates the success with which the method is able to extract analyte from soil substrates.

We can speculate regarding the somewhat lower recoveries for DNB and TNB. All the DNB concentrations were very low (< 6 µg/g). It is possible that extraction for samples with very low analyte concentrations is less efficient because stronger binding sites are involved. We have no evidence of

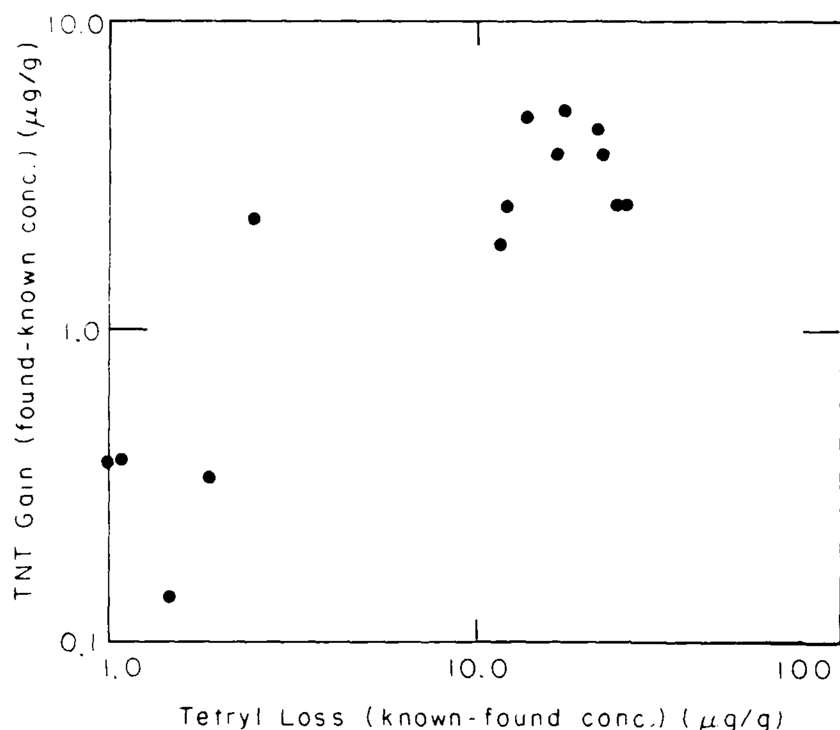


Figure 6. Correlation of TNT and tetryl concentration changes for samples 1 and 10.

behavior at higher concentrations to test this supposition.

For TNB, a problem has been noted with the formation of some sort of complex in some soil extracts (Jenkins et al. 1988b), which decreases the concentration of free TNB. Proper storage conditions apparently eliminate this problem. Chromatograms were inspected but no sign of the TNB-complex peak was observed.

For tetryl, average recoveries were low, but recall that the Youden plot (Fig. 1e) shows that a few labs had no particular problem recovering nearly 100%. As mentioned above, the extraction temperature may have promoted tetryl decomposition. Decomposition during storage both before and after extraction was also considered. Lab 6, which had the greatest tetryl loss, also had the longest delay before samples were extracted (4 weeks). All other labs had delays of about 1–2 weeks, but no clear correlation was found with tetryl loss.

An experiment was subsequently conducted to determine if tetryl loss was indeed caused by high sonic bath temperatures during solvent extraction. Two 2.0-g subsamples of the same 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 2 days in the same manner described for the collaborative test samples. 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 other was kept constant at 11°C. Chromatograms of the extracts for each of these subsamples are presented in Figure 7. Clearly the level of tetryl in the extract obtained at 45°C is much reduced from that held at 11°C. The peak corresponding to the tetryl degradation product is also much larger. This peak elutes near TNT and could have influenced the results for TNT in the spiked soils as discussed earlier. While the degradation of tetryl probably differs from soil to soil, it appears necessary to maintain sonic bath temperatures near ambient levels to get good analyte recovery.

Table 17. Comparison of method accuracy for soil and aqueous samples.

	Recovery (%)	
Analyte	*Soil method*	*Aqueous method**
2,4-DNT	96.0	98.6
TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

* Taken from Jenkins et al. (1984).

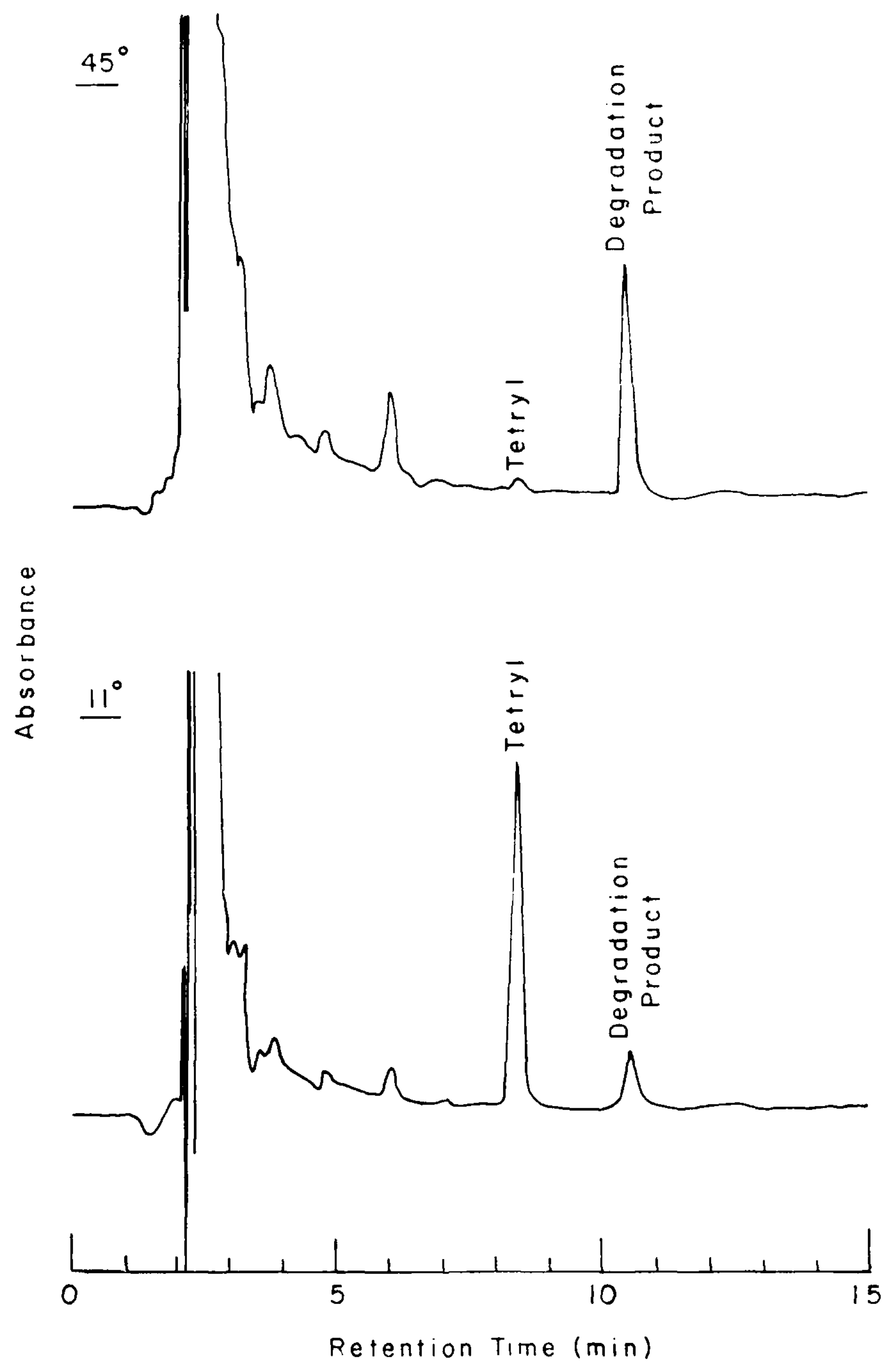


Figure 7. Loss of tetryl at high sonic bath temperatures.

FINAL PERFORMANCE TESTS

Rationale

We waited to conduct the final performance tests for the method until after the collaborative test results were inspected. This was to ensure that if major procedural modifications were warranted they could be incorporated in the final tests used to estimate certified reporting limits (CRLs), method detection limits (MDLs), percent recovery and within-lab precision. Since the collaborative test results for all the analytes except tetryl indicate that the various participants could obtain satisfactory results, no methodological changes were made. The identification of high sonic bath temperature as the cause of low recovery of tetryl was not discovered until these tests were completed. Thus the CRL and MDL for tetryl would be reduced if the bath temperature was maintained at less than 25°C. Except for the lack of sonic bath temperature control, the procedure used in the final testing phase of the work is that described in detail in Appendix E.

Preparation of spiked soils for CRL and MDL tests

The individual stock spiking standards described for the collaborative test (Table 5) were used for HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT. Stock solutions of 2-Am-DNT, o-NT, m-NT, p-NT and NB were prepared in a like manner. Two combined analyte spiking standards were prepared from these individual stock solutions. The first (solution X) was prepared by combining 2.00 mL of the HMX, RDX, TNB, DNB, NB, TNT, 2,6-DNT, o-NT, m-NT and p-NT in a 200-mL volumetric flask and diluting to volume with acetonitrile. Analyte concentrations in this solution were about 10 mg/L.

Similarly solution Y was prepared by combining 2.00 mL of the tetryl, 2,4-DNT and 2-Am-DNT stock standards in a 200-mL volumetric flask and diluting to volume with actonitrile. Analyte concentrations in solution Y were also about 10 mg/L.

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 the water method we developed (Jenkins et al. 1988a) is procedurally similar to the soil method discussed here, we obtained preliminary estimates of TRLs from CRL values from the water method certification study 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 obtained for the water method in µg/L (Table 18). The mean TRL for the 13 analytes reported for the water method was about 0.05 µg/L. Since interferences in soil analysis are generally much greater than those found in water analysis and the USATHAMA standard soil was known to have at least one large peak that elutes near TNB, we selected a higher TRL (0.5 µg/g) for this test. The dilutions required to prepare spiking solutions covering the range of 0.5–10 TRL are shown in Table 19.

A separate set of spiking standards was prepared from solution X and solution Y. This was done because 2,6-DNT and 2,4-DNT elute only 0.23

Table 18. Estimates of TRL values from CRL values determined using the 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

* CRL values for water method from Jenkins et al. (1988a).
† TRL estimates for the soil method based on a 2-g soil sample and 10-mL extraction volume.

Table 19. Preparation of spiking solutions from combined stock standard X or Y for the reporting limit tests.

Sample	*Aliquot of standard (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		0.00	0.000

minutes apart, and it is difficult to quantitate one accurately in the presence of an equal amount of the other.

To conduct the CRL test, duplicate 2-g subsamples of USATHAMA standard soil were spiked at all six concentration levels (Table 19) on each of four days for both groups of analytes. To obtain MDLs, 10 replicate 2-g subsamples were spiked at the TRL level on a single day for each group of analytes. In all cases, samples were processed and analyzed as described in Appendix E.

Results of final performance tests

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 we did not consistently get measureable responses for HMX at the 0.5-µg/g level, the MDL reported for HMX was obtained in a like manner from the standard deviation of eight replicates obtained over four days at the 2.5-µg/g level. Except for HMX, MDL values for the 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 20).

Table 20. Detection capability estimates.

Analyte	*CRL** (µg/g)		*MDL†* (µg/g)
HMX	2.15		1.27**
RDX	1.03		0.74
TNB	0.24	(0.25)††	0.29
DNB	0.12	(0.25)††	0.11
NB	0.11	(0.26)††	0.08
TNT	0.24***	(0.25)††	0.08
2,6-DNT	0.16***	(0.26)††	0.07
o-NT	0.24	(0.25)††	0.07
p-NT	0.22	(0.25)††	0.07
m-NT	0.25***		0.07
Tetryl	0.65***		0.12
2-Am-DNT	0.11***	(0.25)††	0.03
2,4-DNT	0.07***	(0.25)††	0.03

* Certified reporting limit calculated over the widest range of homogeneous variance according to USATHAMA (1987) protocol.
† Method detection limit obtained from 10 replicate measurements at the 0.6-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.
†† Accepted CRL value reverts to the lowest tested level according to USATHAMA (1987) protocol.
*** Variances not found to be homogeneous at the 95% confidence level.

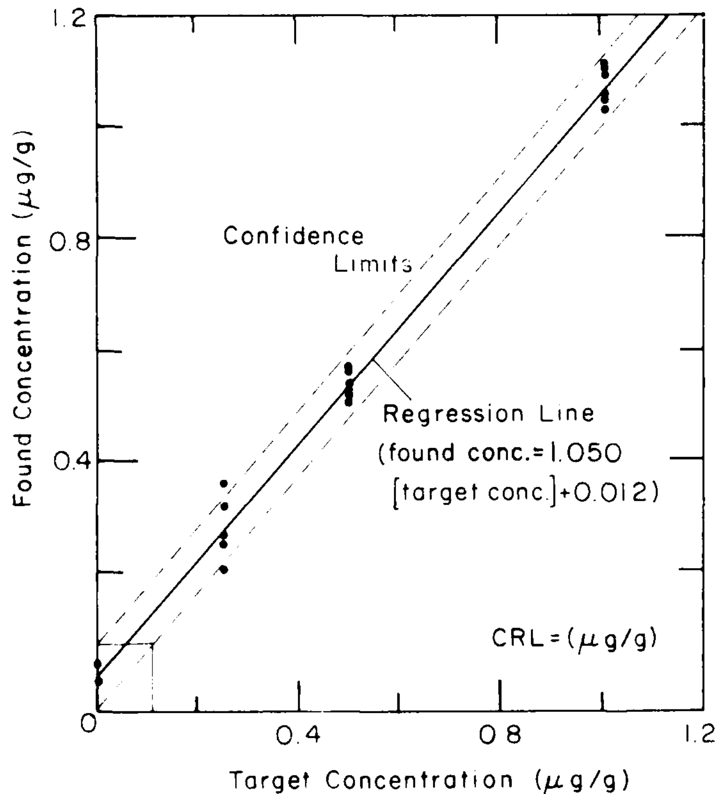


Figure 8. CRL estimation.

Table 21. Variance analysis of measured concentrations for reporting limit tests.

Analyte	*Target concentration (µg/g)*	*Found concentration (mg/g)*		*Bartlett's test* (X^2)*
		Mean	*Variance*	
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
2,6-DNT	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*
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*
2-Am-DNT	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*

* X^2 values for the 95% confidence level are 5.99 (2 df), 7.81 (3 df), and 9.49 (4 df).

Estimates of certified reporting limits (CRLs) were obtained according to the protocol in USATHAMA QA Program (1987), which was adapted from the method of Hubaux and Vos (1970). To do so, the mean and variance were obtained for each target concentration (Table 21). 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 vs target concentrations in this range was performed. The best-fit linear regression equations were obtained, and confidence intervals about the regression lines established at the 95% confidence level (5% α risk and 5% β risk). The certified reporting limit was defined as the point on the "target concentration" axis that corresponds to the point on the lower confidence band where the value in the upper confidence intersects the "found concentration" axis (Fig. 8). For TNT, m-NT, 2,6-DNT, 2-Am-DNT and 2,4-DNT, no homogeneous range of three values was found (Table 21). In these cases the CRLs were established using data from the lowest three target concentrations to minimize the widening of confidence bonds 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 ranges differed by more than 10% compared with the full range (USATHAMA 1987).

A detailed comparison of MDLs and CRLs is reported elsewhere (Grant et al. 1988), but we observed an interesting comparison here. For analytes where CRLs were established over a range of homogeneous variance that 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 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 elsewhere. MDL and CRL estimates for tetryl were about twice those for other analytes with similar absorptivities. This was due to the lack of sonic bath temperature control for these tests.

SUMMARY AND CONCLUSIONS

The results presented in this report, together with those from Cragin et al. (1985), Jenkins and Leggett (1985), Jenkins and Walsh (1987) and Jenkins et al. (1988b), provide a thorough presentation of the development of a method to determine nitroaromatics and nitramines in soil. Since the experimental evidence to support each methodological step is spread among these five reports, a summary reviewing this body of information is appropriate. Below we will present a quick overview of the method and a discussion of each methodological step, its rationale, and our experience using the method with over 100 explosives-contaminated soil samples from over 10 states.

Overview of the method

Soil samples are air dried to constant weight out of direct sunlight. The soil is then ground with a mortar and pestle to a fine powder and homogenized by placing it in a closed container and shaking it thoroughly. A 2.00-g subsample is then placed in a glass 6-dram vial with a Teflon-lined cap.

A 10.0-mL aliquot of acetonitrile is added, the soil is dispersed by vortex mixing for 1 minute, and the solution is extracted in an ultrasonic bath for 18 hours. The bath is maintained at ambient temperature by passing cooling water continuously through cooling coils immersed in the bath during the period of extraction.

The vial is removed from the bath and allowed to settle for 30 minutes. A 5.00-mL aliquot is removed, placed in a glass scintillation vial and combined with 5.00 mL of aqueous $CaCl_2$ (5 g/L). The vials are shaken and allowed to stand for at least 15 minutes to allow flocculation.

A 5-mL aliquot is filtered through a 0.5-µm Millex SR filter into a clean scintillation vial.

The extract is analyzed by RP-HPLC on an LC-18 column (25 cm × 4.6 mm, Supelco) eluted with 1.5 mL/min of 1:1 water–methanol. If peaks with retention times corresponding to analytes of interest are observed, a confirmation determination is made on an LC–CN column (Supelco) using an identical eluent and flow rate.

Air drying

Cragin et al. (1985) discussed the merits of various drying procedures for soil to be extracted and analyzed for explosives. They preferred freeze drying but recommended air drying at room temperature in a fume hood as a practical alternative.

Jenkins et al. (1988b) studied photodegradation during air drying in direct sunlight. Up to 11% of the TNT was lost under worst-case conditions. Keeping the soil out of direct sunlight during drying is recommended.

The major reason for drying soil prior to extraction is the difficulty of homogenizing wet soils in order to obtain a representative subsample. Cragin et al. (1985) reported, however, that extraction was efficient even using wet soils.

Our experience indicates that 1–2 days are generally sufficient to air-dry soils. We spread the soil uniformly in a 9-inch aluminum pie pan out of direct light in a fume hood.

Soil grinding

Generally the laboratory will receive a several-hundred-gram sample of soil for analysis. The procedure we describe uses a 2.00-g subsample. Grinding is used to reduce particle size and facilitate representative subsampling. During method development we initially sieved the ground soil to remove rocks and other plant debris but problems were encountered with analyte carryover on the sieve from soils with very high explosive concentrations. We recommend manually removing large stones and plant material and grinding the soil to a fine powder. Some extraction differences were observed by Jenkins et al. (1988b) when different particle sizes were used. These effects were minor, however, compared to the potential for carryover if sieves are not thoroughly cleaned with solvent between samples.

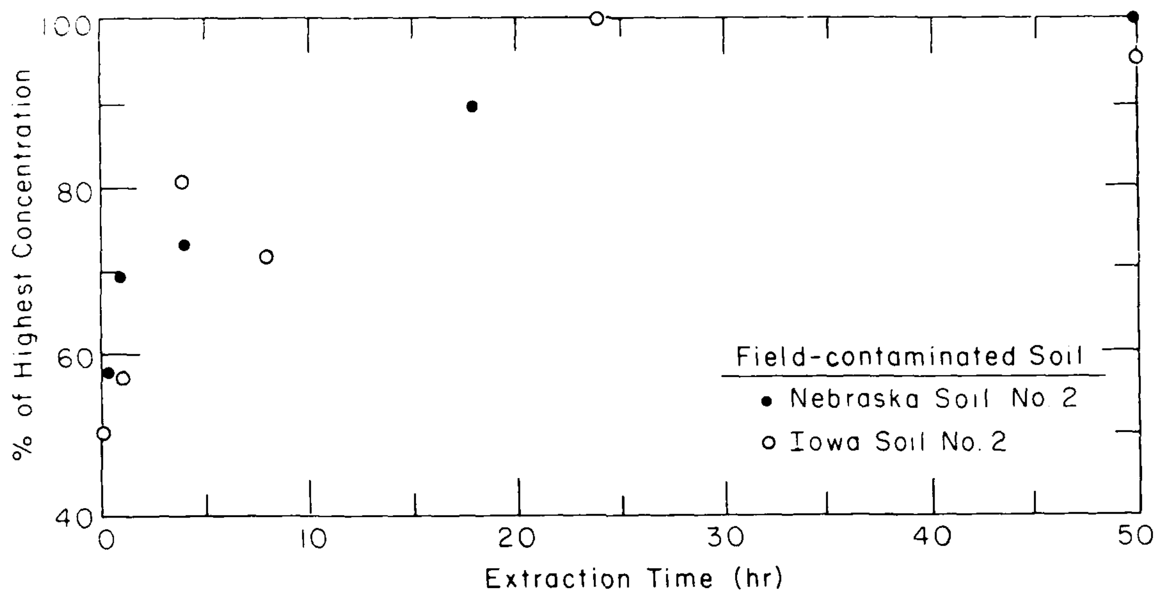
Extraction

Jenkins and Leggett (1985) compared a number of extraction techniques and solvents and con-

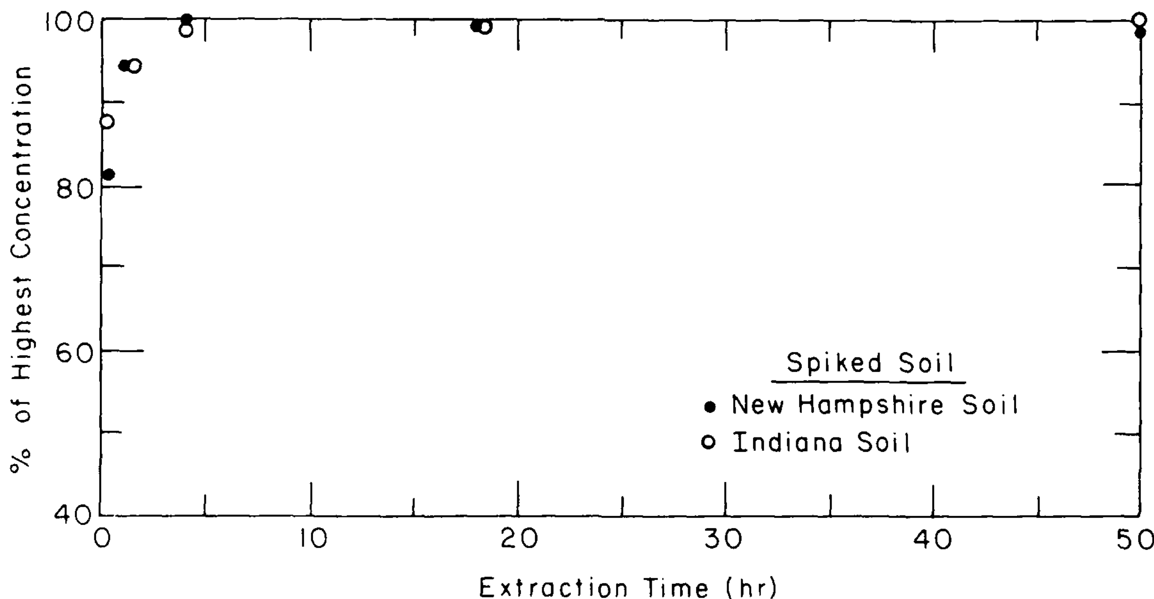
cluded that a sonic bath extraction using acetonitrile was optimum. The soil-to-solvent ratio was varied and found to have a minimal effect on extraction efficiency (Jenkins et al. 1988b). The maximum ratio tested (2 g and 10 mL) was chosen to lower detection limits while providing sufficient extract for both primary analysis and secondary analyte confirmation.

Extraction time was studied by Jenkins and Leggett (1985), Jenkins and Walsh (1987) and Jenkins and Grant (1987). The time required to reach maximum values depends on the specific soil and analyte. An 18-hour period is recommended (Jenkins and Walsh 1987).

Other laboratories using sonic bath extraction have used much shorter extraction times based on results for recovery of analytes spiked into soil. Unfortunately any analyte that has been in contact with soil for years under field conditions does not extract as rapidly as a spiked analyte (Fig. 9), and short extraction periods can seriously underestimate analyte concentrations.



a. Field-contaminated soils.



b. Spiked soils.

Figure 9. Kinetics of extraction of TNT from soils.

The recommended method requires vortex mixing prior to sonic bath extraction. Manual shaking was also found to be acceptable (Jenkins et al. 1988b), but a short period of vortex mixing is a very effective way to quickly disperse the soil in the extraction solvent.

The number of samples that can be extracted in the sonic bath simultaneously was studied by Jenkins et al. (1988b). The results indicated that extraction efficiency was not affected by processing as many as 36 samples simultaneously.

Flocculation

Prior to injection onto the HPLC column, the soil extracts must be diluted with water to change the solvent strength to a level similar to that of the mobile phase, and they must be filtered to remove particles.

Because 18-hour extraction periods are used in the sonic bath, soil is dispersed into very fine particles. When these suspensions were filtered, the 0.5-µm membranes rapidly plugged. Centrifugation helped to a degree, but many soil extracts were still very difficult to filter. Often extracts were lost as filter holders ruptured when excessive pressure was used.

We observed, however, that adding aqueous $CaCl_2$ to the extracts resulted in rapid flocculation of suspended matter (Jenkins et al. 1988b). The flocculated material quickly settled, and the supernatants were easily filtered in almost all cases. Careful investigation indicated that, with the exception of TNB, analyte concentrations were not affected by the flocculation step. A small reduction in TNB was observed, which we initially attributed to the formation of a TNB complex, which was found to break down if the extracts were allowed to stand at room temperature overnight (Jenkins et al. 1988b). Subsequently we found that the concentration of TNB we observed was being influenced by a slow degradation of 2,4,6-trinitrobenzaldehyde (TNBA), which forms TNB by decarbonylation (Jenkins et al. (in press). The addition of $CaCl_2$ appears to stabilize TNBA in the extract, resulting in a more accurate result for TNB.

The flocculation step has dramatically improved the overall usability of the method. High-speed centrifugation can probably be used effectively, but the sample preparation time will be longer.

Filtration

Extracts were filtered through 0.5-µm Millex SR filters prior to RP-HPLC analysis. Jenkins et al. (1987) and Walsh et al. (1988) found that while loss of analyte can occur when water is filtered through several types of disposable filter membrane, no analyte loss was observable when solutions composed of 1:1 water–organic solvent were filtered.

RP-HPLC analysis

Jenkins and Walsh (1987) evaluated a number of RP-HPLC column–eluent combinations. The best separation for the major analytes (HMX, RDX, TNB, DNB, TNT, tetryl and 2,4-DNT) was achieved using an LC-18 column (Supelco) eluted with 1:1 water–methanol (Fig. 10). This separation has proven to be very successful with a wide range of soil extracts. The analytes most often observed in contaminated soil are TNT, 2-aminodinitrotoluene, TNB, 2,4-DNT and RDX, all of which are adequately separated by the column–eluent combination described above. A listing of the retention times of major analytes and a number of potential interferences is presented in Table 22.

When potential analytes are identified in the primary analysis based on retention items, we analyze the extract on a second column. The LC-CN column, also eluted with 1:1 water–methanol, has proven to be very successful for analyte confirmation. While the LC-18 column provides a separa-

Table 22. Retention times and capacity factors for primary analytes and potential interferences on LC-18 and LC-CN columns.

	Retention time (min)		*Capacity factor (k)**	
Substance	*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 1.71 min on LC-18 and 2.00 min on LC-CN.

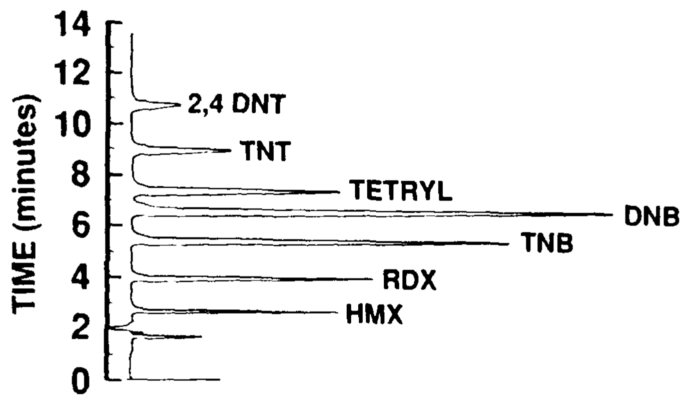
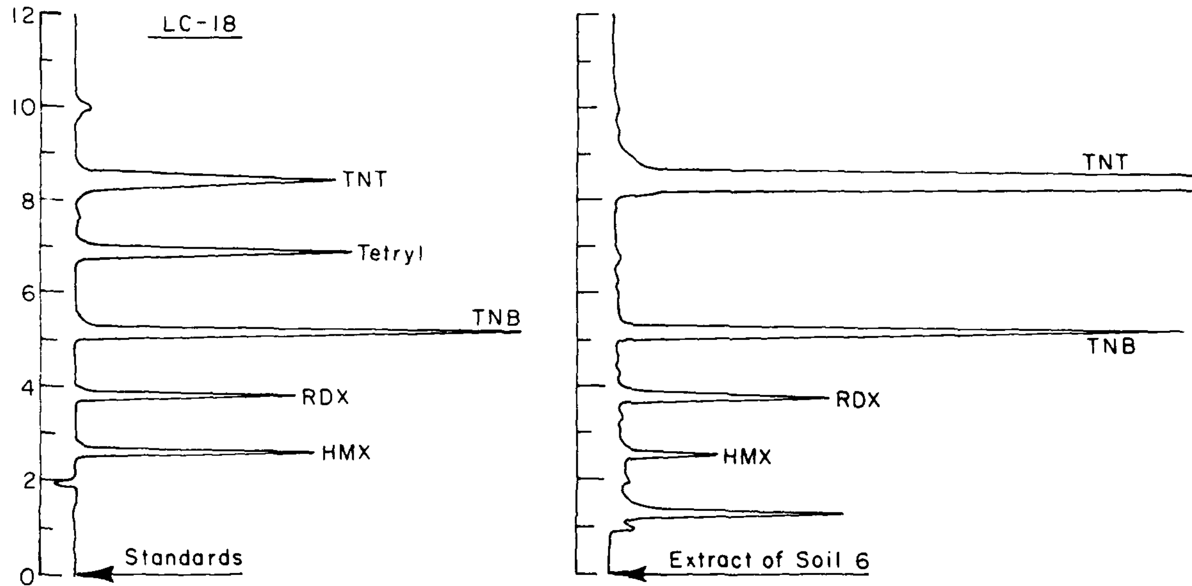
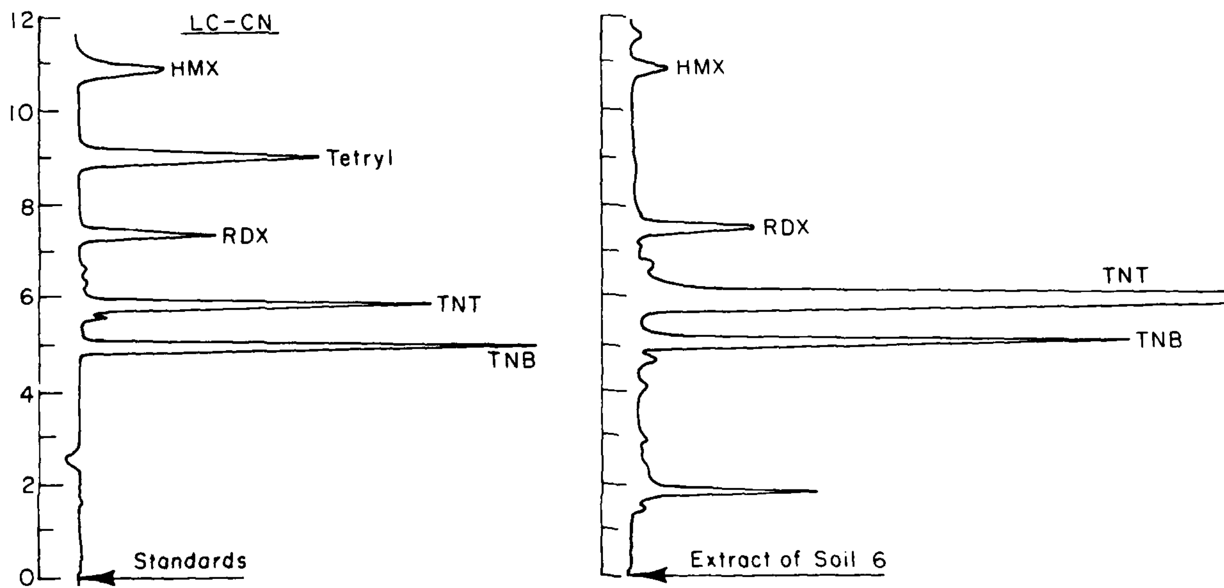


Figure 10. Chromatogram of standards for seven primary analytes on an LC-18 column eluted with 1.5 mL/min of 1:1 methanol–water.



a. LC-18.



b. LC-CN.

Figure 11. Separation on two columns.

Table 23. Summary of method performance characteristics.

Analyte	CRL* (µg/g)	MDL† (µg/g)	Accuracy (%)	Analytical precision Intralaboratory (% rsd)	Interlaboratory (% rsd)
HMX	2.2	1.3	95.4**	3.7***	5.7***
RDX	1.0	0.74	96.8**	2.3***	4.3***
TNB	0.24	0.29	92.0**	4.4***	6.8***
DNB	0.12	0.11	93.0**	4.0***	6.9***
Tetryl	0.65	0.12	74.7**	17.9***	30.7***
TNT	0.24	0.08	96.8**	3.5***	4.7***
2,4-DNT	0.07	0.03	96.0**	3.4***	4.4***
2-Am-DNT	0.11	0.03	91.5**	2.4†††	—
2,6-DNT	0.16	0.07	108.1††	2.2†††	—
NB	0.11	0.08	100.3††	1.3†††	—
o-NT	0.24	0.07	92.2††	4.3†††	—
p-NT	0.22	0.07	103.7††	4.3†††	—
m-NT	0.25	0.07	101.1††	2.9†††	—

* Certified reporting limit obtained using the method of Hubaux and Vos (1970).

† Method detection limit obtained as described in the *Federal Register* (1984).

** Obtained from the regression analysis of edited data for spiked soils in the collaborative test.

†† Obtained from the regression analysis of analyte recovery from spiked soils over a four-day period at CRREL.

*** Obtained from the collaborative test results on spiked soils.

††† Obtained from the pooled results of the four-day recovery test using spiked soils at CRREL.

tion that parallels the order of octanol–water partition coefficients of the analytes (K_{ow}), the order of elution on the LC-CN column is very different (Table 22). HMX, which elutes first on the LC-18, elutes last among the primary analytes. RDX also elutes much later on LC-CN, and the retention times for the aminodinitrotoluenes and tetryl are also quite diagnostic (Fig. 11). The LC-CN column, however, does not provide adequate separation of TNT and the DNTs to allow confirmation of a small amount of the DNTs in the presence of a large amount of TNT. If TNT is present in abundance, though, 2,4-DNT is generally present at a much reduced concentration. In the unusual circumstance where the DNTs were present in the absence of TNT, confirmation on LC-CN is possible.

Overall method performance

A summary of the overall performance characteristics is provided in Table 23. Accuracy and precision estimates for HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT were obtained from the results of our collaborative test. CRL and MDL estimates for all analytes, as well as accuracy and precision for the remaining analytes, were obtained from data collected at CRREL.

We have used the method described in this report for soil samples from a number of present and former Army sites. It has proven to be dependable in routine use. Analytes elute from the primary analytical column in order of increasing K_{ow}. The K_{ow} values range from 1.3 for HMX (Jenkins, in prep.) to 263 for m-nitrotoluene (Hansch and Leo 1979). For other interfering compounds to elute within the range of retention times for the analytes of interest, the K_{ow} must be within this range and the substance must absorb at 254 nm. Very few environmental contaminants satisfy these criteria, so we have observed little interference for soil extracts. The LC-CN column has proven to be very useful for analyte confirmation.

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APPENDIX A: ANALYTE CONCENTRATIONS (µg DRY SOIL) REPORTED BY COLLABORATORS.

Tables A1 to A7 are for spiked soils; Tables A8 to A14 are for real soils. The format for each table is identical. Each lab is a row; each sample, a column. Duplicate samples are adjacent. The data are annotated to identify outliers according to how they were discovered and whether they were eliminated. Where nonuniform variance was found, samples were grouped into homogeneous ranges.

Table A1. HMX Results for Collaborative Study: Spiked Soils, Unedited Data.

O LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7- spike C	5 samp6- spike D	6 samp9- spike D	7 samp11- spike A	8 samp15- spike A
1	44.51	45.89	38.65	38.65	96.05	94.87	9.07	8.68
3	(R)43.13	(R)49.60	37.45	37.03	97.19	95.47	9.36	12.47
4	39.93	43.52	38.99	34.43	96.61	D 87.52	8.18	6.76
5	47.80	49.20	38.00	38.80	94.60	96.40	9.18	9.24
6	L 54.00	L 61.50	L 48.90	L 44.70	L 127.00	L 127.00	L 9.45	L 12.70
7	D 32.60	43.10	38.40	D 52.20	D 62.60	D 69.60	8.50	D 18.80
8	50.00	49.30	41.00	41.40	104.00	102.00	11.00	11.10

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

( ): Outlier but not eliminated

Table A2. RDX Results for Collaborative Study: Spiked Soils, Unedited Data.

O LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7- spike C	5 samp6- spike D	6 samp9- spike D	7 samp11- spike A	8 samp15- spike A
1	47.73	46.58	98.33	96.34	87.92	85.18	9.28	8.83
3	46.98	49.28	99.68	98.89	87.35	88.20	6.66	7.14
4	45.83	46.01	99.63	94.40	87.84	83.62	10.28	8.91
5	47.90	49.20	91.60	92.70	85.70	87.20	9.81	9.55
6	L 49.50	L 56.00	L 112.00	L 105.00	L 102.00	L 102.00	L 9.75	L 11.80
7	L 31.10	L 38.40	L 71.70	L 63.80	L 56.90	L 63.80	L 7.22	L 11.00
8	50.20	49.50	101.00	101.00	93.50	91.50	10.20	9.97

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

( ): Outlier but not eliminated

Table A3. TNB Results for Collaborative Study: Spiked Soils, Unedited Data.

O LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7 spike C	5 samp6- spike D	6 samp9- spike D	7 samp11- spike A	8 samp15- spike A
1	11.52	11.07	50.26	48.52	1.74	1.61	9.20	9.20
3	10.47	11.04	46.91	45.93	0.00	0.00	8.76	D 6.98
4	10.35	11.25	48.61	42.81	1.86	1.09	10.31	9.88
5	11.50	D 7.89	46.80	47.00	1.73	1.81	9.24	9.14
6	10.10	11.30	44.30	40.90	D 2.80	D 2.72	10.50	(D)11.70
7	10.20	10.30	43.60	42.80	1.46	1.21	10.00	9.83
8	11.90	11.30	48.60	49.80	1.68	1.69	9.30	9.16
		V1		V2		V1		V1

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A4. DNB Results for Collaborative Study: Spiked Soils, Unedited Data.

O LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7- spike C	5 samp6- spike D	6 samp9- ·spike D	7 samp11- spike A	8 samp15- spike A
1	5.77	5.77	4.85	4.81	0.92	0.97	2.77	2.70
3	5.20	5.69	4.77	4.63	1.11	1.24	2.73	D 2.14
4	5.86	6.05	4.65	4.89	0.95	0.82	2.98	2.90
5	5.60	5.72	4.00	4.68	0.88	0.92	2.69	2.75
6	5.55	5.65	4.59	4.72	1.10	0.97	2.71	2.68
7	(L) 5.21	(L) 5.03	(L) 4.19	(L) 4.21	(L) 0.78	(L) 0.93	(L) 1.84	(L) 2.50
8	5.66	5.92	5.07	4.90	0.83	0.96	2.92	2.72

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A5. TETRYL Results for Collaborative Study: Spiked Soils, Unedited Data.

LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7- spike C	5 samp6- spike D	6 samp9- spike D	7 samp11- spike A	8 samp15- spike A
1	13.40	8.26	17.86	14.98	2.16	0.38	24.13	27.53
3	(L)24.09	(L)23.73	(L)19.21	(L)20.02	(L) 4.93	(L) 4.55	(L)35.25	(L)39.13
4	7.33	12.79	16.80	11.27	2.45	0.41	26.86	17.23
5	23.30	24.20	18.60	18.80	4.52	4.56	37.70	30.78
6	L 2.74	L 3.45	L 4.19	L 4.13	L 1.02	L 0.66	L 8.10	L 11.70
7	11.30	18.50	14.40	13.60	0.94	0.14	32.30	22.10
8	22.70	22.10	17.80	16.70	0.00	0.00	35.70	32.50

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

( ): Outlier but not eliminated

Table A6. TNT Results for Collaborative Study: Spiked Soils, Unedited Data.

LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7- spike C	5 samp6- spike D	6 samp9- spike D	7 samp11- spike A	8 samp15- spike A
1	4.46	6.37	25.02	24.67	28.90	28.75	72.61	72.25
3	2.93	2.68	24.40	23.99	27.47	27.49	70.19	70.17
4	(L) 7.78	(L) 5.15	(L)25.14	(L)25.77	(L)29.49	(L)30.35	(L)75.40	(L)79.06
5	2.88	2.92	25.40	24.80	29.80	30.60	75.20	70.98
6	6.25	7.10	D 16.10	D 15.20	D 16.90	D 15.20	D 51.50	D 54.00
7	7.43	2.19	23.70	25.20	27.50	28.80	73.10	73.30
8	4.86	2.01	27.10	26.30	27.00	30.00	75.40	75.00

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

( ): Outlier but not eliminated

Table A7. DNT Results for Collaborative Study: Spiked Soils, Unedited Data

O LAB #	1 samp1- spike B	2 samp10- spike B	3 samp5- spike C	4 samp7- spike C	5 samp6- spike D	6 samp9- spike D	7 samp11- spike A	8 samp15- spike A
1	0.99	0.99	3.77	3.76	4.73	4.78	9.93	9.70
3	1.07	0.00	4.27	3.64	(D) 4.22	4.84	9.41	9.48
4	0.97	0.88	4.01	3.80	4.83	5.02	10.16	10.12
5	0.82	0.94	3.75	3.76	4.71	4.76	9.53	9.51
6	0.91	D 1.47	3.67	3.70	5.10	4.79	9.35	9.30
7	0.95	0.96	3.85	3.76	4.59	4.74	9.65	9.32
8	1.05	1.23	4.20	3.91	5.15	4.69	9.71	9.72

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A8. HMX Results for Collaborative Study: Field Contaminated Soils, Unedited Data.

O LAB #	1 samp2- tenn	2 samp4- tenn	3 samp3- louis	4 samp13- louis	5 samp8- neb	6 samp12- neb	7 samp14- iowa	8 samp16- iowa
1	22.80	28.63	225.64	223.99	2.79	3.13	D 163.23	79.65
3	21.95	27.31	218.90	257.60	6.49	6.15	60.13	91.11
4	(L) 21.51	(L) 19.42	(L)186.20	(L)193.60	(L) 1.83	(L) 1.80	(L) 41.83	(L) 60.27
5	21.90	19.70	210.00	194.00	2.15	2.34	90.10	43.90
6	31.90	33.30	299.00	349.00	2.96	2.96	61.00	74.00
7	20.80	18.30	208.00	184.00	D 22.30	7.48	75.40	58.30
8	22.40	24.80	268.00	236.00	2.15	2.54	52.50	100.00
		V1		V2		V1		V2

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A9. RDX Results for Collaborative Study: Field Contaminated Soils, Unedited Data

O LAB #	1 samp2- tenn	2 samp4- tenn	3 samp3- louis	4 samp13- louis	5 samp8- neb	6 samp12- neb	7 samp14- iowa	8 samp16- iowa
1	144.5	148.70	820.55	816.64	0.85	1.56	71.29	74.94
3	116.3	158.54	880.00	910.20	3.28	0.00	91.60	75.23
4	123.6	128.00	858.70	848.90	0.64	0.54	55 63	74.13
5	144.0	132.00	841.00	784.00	0.00	1.46	55 70	64.30
6	L 169.00	L 175.00	L 1000.00	L 1160.00	L 1.10	L 1.23	L 85.50	L 83.00
7	104.0	111.00	1000.00	970.00	0.56	0.52	57.90	82.70
8	159.0	168.00	932.00	859.00	0.00	0.00	80.60	89.50
	V1		V2		BD		V1	

BD: median below detection limit; eliminated

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A10. TNB Results for Collaborative Study: Field Contaminated Soils, Unedited Data.

O LAB #	1 samp2- tenn	2 samp4- tenn	3 samp3- louis	4 samp13- louis	5 samp8- neb	6 samp12- neb	7 samp14- iowa	8 samp16- iowa
1	4.67	4.68	1.85	1.84	2.23	2.42	68.14	66.82
3	4.40	4.38	1.52	1.89	2.40	2.21	72.59	78.64
4	4.72	4.86	1.43	1.55	1.99	2.12	91.48	74.43
5	4.70	4.92	1.73	1.73	2.86	2.27	66.50	69.70
6	L 5.90	L 5.25	L 2.52	L 4.47	L 2.50	L 2.52	L 81.00	L 104.00
7	4.37	4.44	1.50	1.44	2.03	2.09	79.40	74.70
8	(L)4.31	(L)4.13	(L)1.72	(L)1.52	(L)2.40	(L)1.95	(L)57.50	(L) 66.30
	V1		V1		V1		V2	

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A11. DNB Results for Collaborative Study, Field Contaminated Soils, Unedited Data

O LAB #	1 samp2-tenn	2 samp4-tenn	3 samp3-louis	4 samp13-louis	5 samp8-neb	6 samp12-neb	7 samp14-iowa	8 samp16-iowa
1	1.51	1.45	0.18	0.26	0.05	0.24	0.72	0.77
3	0.00	1.52	0.00	0.00	0.00	0.00	0.00	0.00
4	(D) 1.89	1.56	0.10	0.15	0.00	0.00	0.39	0.41
5	1.63	1.35	0.00	0.00	0.00	0.00	0.55	0.59
6	1.37	1.30	0.52	0.11	0.00	0.00	0.71	0.72
7	1.42	1.55	0.11	0.10	0.00	0.00	0.53	D 2.84
8	1.26	1.35	0.00	0.00	0.00	0.00	0.00	0.00
			BD			BD		

BD: median below detection limit; eliminated

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A12. TETRYL Results for Collaborative Study: Field Contaminated Soils, Unedited Data.

O LAB #	1 samp2-tenn	2 samp4-tenn	3 samp3-louis	4 samp13-louis	5 samp8-neb	6 samp12-neb	7 samp14-iowa	8 samp16-iowa
1	2.28	1.80	2.49	2.58	0.43	0.87	2.25	2.76
3	0.00	2.20	1.88	2.48	0.00	0.00	0.00	0.00
4	2.88	1.90	2.19	2.42	0.20	0.16	1.54	2.56
5	2.06	1.92	2.30	1.87	0.00	0.00	1.85	1.91
6	L 2.76	L 2.80	L 4.38	L 4.72	L 0.63	L 0.68	L 4.29	L 4.44
7	3.10	3.46	2.60	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
						BD		

BD: median below detection limit; eliminated

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

(): Outlier but not eliminated

Table A13. TNT Results for Collaborative Study: Field Contaminated Soils, Unedited Data

D LAB #	1 samp2- tenn	2 samp4- tenn	3 samp3- louis	4 samp13- louis	5 samp8- neb	6 samp12- neb	7 samp14- iowa	8 samp16- iowa
1	7.47	7.32	13.19	13.65	0.90	0.99	683.82	712.85
3	6.18	7.95	D 3.48	0.00	0.00	0.00	706.60	705.50
4	9.15	9.18	9.99	9.78	0.30	0.06	602.10	574.70
5	(R) 8.99	(R) 6.18	10.80	11.20	0.67	0.81	694.00	753.00
6	5.35	4.92	11.70	11.80	0.48	0.79	590.00	660.00
7	8.15	9.18	11.10	10.50	0.82	0.68	746.00	648.00
8	7.41	7.18	9.29	9.06	0.00	0.00	720.00	569.00
		V1		V1		V1		V2

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

( ): Outlier but not eliminated

Table A14. DNT Results for Collaborative Study: Field Contaminated Soils, Unedited Data.

D LAB #	1 samp2- tenn	2 samp4- tenn	3 samp3- louis	4 samp13- louis	5 samp8- neb	6 samp12- neb	7 samp14- iowa	8 samp16- iowa
1	0.52	0.55	1.25	1.25	0.50	0.70	0.84	0.99
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	(R) 2.20	(R) 0.24	0.30	0.31	0.36	0.37	1.11	0.29
5	(R) 0.37	(R) 1.62	0.52	0.60	0.48	0.50	1.22	0.00
6	(L) 0.93	(L) 0.95	(L) 3.13	(L) 2.56	(L) 0.64	(L) 0.60	(L) 1.57	(L) 1.92
7	0.62	0.96	2.56	3.28	0.74	0.38	0.00	0.00
8	0.00	0.00	1.52	1.06	0.00	0.00	0.00	0.00

L: Lab Rank Outlier
D: Dixon Outlier
R: Range Outlier
V: Homogeneous Variance Group

( ): Outlier but not eliminated

APPENDIX B: STANDARD DEVIATIONS FOR INTRALABORATORY AND INTERLABORATORY COMPARISONS FOR EDITED DATA (OUTLIERS ELIMINATED).

These data were derived from a one-way ANOVA on each set of duplicate samples. The value of F for the statistical test for differences among laboratories is also listed. Each analyte has its own table. The formats are identical. The top rows are spiked soils and the bottom are real soils. Within each group, rows are sequenced from lowest to highest average found concentration.

Table B1. Summary characteristics of laboratory performance: HMX, edited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk A	9.41	1.09	11.57	3.19	1.58	16.75	1.45
spk C	38.44	1.48	3.84	2.22	1.87	4.87	1.27
spk B	46.00	2.43	5.29	2.90	3.39	7.38	1.40
spk D	97.47	1.20	1.23	* 13.8	3.28	3.37	2.73
neb	3.44	0.19	5.43	*211.12	1.93	55.97	10.30
tenn	23.91	2.48	10.36	* 6.52	4.80	20.08	1.94
iowa	68.32	22.64	33.14	0.31	18.35	26.86	0.81
louis	232.42	20.55	8.84	* 10.2	48.63	20.92	2.37

* significant at 95% probability level

Table B2. Summary characteristics of laboratory performance: RDX, edited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk A	9.06	0.49	5.44	*13.14	1.31	14.46	2.66
spk B	47.92	0.94	1.96	4.96	1.62	3.38	1.73
spk D	87.80	1.80	2.05	4.61	3.01	3.43	1.67
spk C	97.36	1.82	1.87	* 6.67	3.57	3.66	1.96
iowa	72.79	10.77	14.79	1.71	12.53	17.22	1.16
tenn	136.47	13.21	9.68	4.10	21.10	15.46	1.60
louis	876.75	29.58	3.37	* 9.34	67.28	7.67	2.27

* significant at 95% probability level

Table B3 Summary characteristics of laboratory performance: TNB, edited data

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk D	1.59	0.26	16.60	0.92	0.26	16.28	0.98
spk A	9.71	0.37	3.86	* 7.92	0.79	8.15	2.11
spk B	10.95	0.51	4.68	1.66	0.59	5.40	1.15
spk C	46.20	1.91	4.14	3.81	2.97	6.43	1.55
louis	1.64	0.13	7.76	2.61	0.17	10.42	1.34
neb	2.25	0.23	10.30	1.40	0.25	11.28	1.10
tenn	4.55	0.09	2.06	*13.44	0.25	5.55	2.69
iowa	72.18	6.05	8.38	3.24	8.80	12.20	1.46

* significant at 95% probability level

Table B4. Summary characteristics of laboratory performance: DNB, edited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk D	0.96	0.08	8.58	3.79	0.13	13.28	1.55
spk A	2.75	0.07	2.71	4.57	0.12	4.53	1.67
spk C	4.64	0.20	4.41	3.65	0.31	6.73	1.52
spk B	5.62	0.17	3.01	* 5.20	0.30	5.30	1.76
iowa	0.61	0.05	8.71	*13.57	0.14	23.51	2.70
tenn	1.47	0.14	9.20	2.04	0.17	11.34	1.23

* significant at 95% probability level

Table B5. Summary characteristics of laboratory performance: Tetryl, edited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk D	2.50	0.90	35.98	* 9.66	2.08	83.06	2.31
spk C	16.67	1.86	11.15	3.16	2.68	16.09	1.44
spk B	17.64	3.02	17.11	* 9.24	6.83	38.73	2.26
spk A	30.10	4.84	16.09	2.98	6.83	22.71	1.41
iowa	2.15	0.47	21.73	0.96	0.46	21.52	0.99
louis	2.31	0.28	11.90	1.06	0.28	12.07	1.01
tenn	2.40	0.41	17.05	3.26	0.60	24.89	1.46

* significant at 95% probability level

Table B6. Summary characteristics of laboratory performance: TNT, edited data.

0 r1[B	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk B	4.64	1.83	39.43	1.59	2.08	44.83	1.14
spk C	25.12	0.57	2.28	* 4.86	0.98	3.90	1.71
spk D	28.85	1.00	3.48	2.17	1.26	4.38	1.26
spk A	73.56	1.62	2.20	* 4.45	2.68	3.64	1.65
neb	0.65	0.14	21.81	* 8.12	0.30	46.57	2.13
tenn	7.47	0.94	12.57	3.77	1.45	19.40	1.54
louis	11.01	0.26	2.40	*63.15	1.50	13.60	5.66
iowa	668.97	55.02	8.22	1.65	63.38	9.47	1.15

* significant at 95% probability level

Table B7. Summary characteristics of laboratory performance: DNT, edited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk B	0.98	0.07	7.57	2.78	0.10	10.40	1.37
spk C	3.85	0.20	5.08	0.89	0.19	4.93	0.97
spk D	4.78	0.23	4.86	0.90	0.23	4.74	0.98
spk A	9.64	0.11	1.15	*12.58	0.29	2.99	2.61
neb	0.53	0.13	24.86	1.24	0.14	26.33	1.06
tenn	0.90	0.74	82.93	0.23	0.58	65.14	0.79
iowa	1.13	0.37	32.54	3.00	0.52	46.03	1.41
louis	1.53	0.30	19.46	*28.21	1.14	74.37	3.82

* significant at 95% probability level

APPENDIX C: STANDARD DEVIATIONS FOR INTRALABORATORY AND INTERLABORATORY COMPARISONS FOR UNEDITED DATA (OUTLIERS ELIMINATED).

Table C1. Summary characteristics of laboratory performance: HMX, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk A	10.32	3.03	29.36	0.87	2.93	28.39	0.97
spk C	40.61	4.05	9.98	1.95	4.92	12.11	1.21
spk B	46.72	4.01	8.59	* 4.95	6.93	14.82	1.73
spk D	96.49	3.20	3.31	*62.51	18.02	18.68	5.64
neb	4.79	3.96	82.76	2.81	5.47	114.17	1.38
tenn	23.91	2.48	10.36	* 6.52	4.80	20.08	1.94
iowa	75.10	30.63	40.79	1.04	30.91	41.16	1.01
louis	232.42	20.55	8.84	*10.20	48.63	20.92	2.37

* significant at 95% probability level

Table C2. Summary characteristics of laboratory performance: RDX, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk A	9.31	1.22	13.12	1.98	1.49	16.02	1.22
spk B	46.73	2.73	5.84	* 8.75	6.03	12.90	2.21
spk D	85.91	2.39	2.78	*56.06	12.76	14.85	5.34
spk C	94.69	3.28	3.47	*31.12	13.16	13.90	4.01
iowa	74.43	9.99	13.43	2.03	12.30	16.53	1.23
tenn	141.55	12.34	8.72	* 6.29	23.55	16.64	1.91
louis	905.79	50.78	5.61	* 7.22	102.95	11.37	2.03

* significant at 95% probability level

Table C3. Summary characteristics of laboratory performance: TNB, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk D	1.78	0.24	13.56	* 8.73	0.53	29.90	2.21
spk A	9.51	0.59	6.19	* 5.77	1.08	11.39	1.84
spk B	10.73	1.08	10.02	0.69	0.99	9.21	0.92
spk C	46.20	1.91	4.14	3.81	2.97	6.43	1.55
iouis	1.91	0.53	28.01	3.55	0.81	42.27	1.51
neb	2.29	0.21	9.38	1.79	0.25	11 08	1.18
tenn	4.70	0.19	4.14	*10.60	0.47	9.96	2.41
iowa	75.09	8.31	11.07	3.13	11.95	15.92	1.44

* significant at 95% probability level

Table C4. Summary characteristics of laboratory performance: DNB, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk D	0.96	0.08	8.58	3.79	0.13	13.28	1.55
spk A	2.65	0.24	9.26	2.24	0.31	11.79	1.27
spk C	4.64	0.20	4.41	3.65	0.31	6.73	1.52
spk B	5.62	0.17	3.01	* 5.20	0.30	5.30	1.76
iowa	0.83	0.73	87.88	0.91	0.72	85.99	0.98
tenn	1.47	0.14	9.20	2.04	0.17	11.34	1.23

* significant at 95% probability level

Table C5. Summary characteristics of laboratory performance: Tetryl, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk D	2.23	0.83	37.23	*10.47	1.99	89.15	2.39
spk C	14.88	1.72	11.56	*18.18	5.33	35.80	3.10
spk B	15.56	2.80	18.00	*16.65	8.32	53.47	2.97
spk A	27.22	4.59	16.85	* 8.32	9.90	36.37	2.16
tenn	2.47	0.37	14.83	3.61	0.56	22.52	1.52
iowa	2.70	0.41	15.08	*15.70	1.18	43.58	2 89
louis	2.72	0.27	9.88	*23.60	0.94	34.65	3.51

* significant at 95% probability level

Table C6. Summary characteristics of laboratory performance: TNT, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk B	4.64	1.83	39.43	1.59	2.08	44.83	1.14
spk C	23.77	0.58	2.45	*79.55	3.70	15.55	6.35
spk D	27.02	1.03	3.83	*45.41	4.98	18.45	4.82
spk A	70.58	1.64	2.33	*49.43	8.25	11.69	5.02
neb	0.65	0.14	21.81	* 8.12	0.30	46.57	2.13
tenn	7.47	0.94	12.57	3.77	1.45	19.40	1.54
louis	10.43	0.26	2.54	*177.28	2.50	23.94	9.44
iowa	668.97	55.02	8.22	1.65	63.38	9.47	1.15

* significant at 95% probability level

Table C7 Summary characteristics of laboratory performance: DNT, unedited data.

0 sample	1 mean found	2 intra stdev	3 %rsd intra	4 F lab	5 inter stdev	6 %rsd inter	7 inter/ intra
spk B	1.02	0.18	17.22	0.85	0.17	16.56	0.96
spk C	3.85	0.20	5.08	0.89	0.19	4.93	0.97
spk D	4.78	0.23	4.86	0.90	0.23	4.74	0.98
spk A	9.64	0.11	1.15	*12.58	0.29	2.99	2.61
neb	0.53	0.13	24.86	1.24	0.14	26.33	1.06
tenn	0.90	0.74	82.93	0.23	0.58	65.14	0.79
iowa	1.13	0.37	32.54	3.00	0.52	46.03	1.41
louis	1.53	0.30	19.46	*28.21	1.14	74.37	3.82

* significant at 95% probability level

APPENDIX D: COMPARISONS OF UNEDITED AND EDITED DATA.

The tables list the ratios of the data in Appendix C to those in Appendix B.

Table D1. Ratio Comparison of Unedited to Edited HMX Data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk A	1.10	2.78	1.86	3	14
spk C	1.06	2.75	2.63	3	14
spk B	1.02	1.65	2.04	3	14
spk D	0.99	2.66	5.49	5	14
neb	1.39	21.18	2.84	1	14
tenn	1.00	1.00	1.00	0	14
iowa	1.10	1.35	1.68	1	14
louis	1.00	1.00	1.00	0	14

Table D2. Ratio comparison of unedited to edited RDX data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk A	1.03	2.48	1.14	4	14
spk B	0.98	2.91	3.72	4	14
spk D	0.98	1.33	4.24	4	14
spk C	0.97	1.80	3.69	4	14
iowa	1.02	0.93	0.98	2	14
tenn	1.04	0.93	1.12	2	14
louis	1.03	1.72	1.53	2	14

Table D3. Ratio comparison of unedited to edited TNB data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk D	1.12	0.92	2.06	2	12
spk A	0.98	1.57	1.37	1	14
spk B	0.98	2.10	1.67	1	14
spk C	1.00	1.00	1.00	0	14
louis	1.16	4.19	4.71	2	14
neb	1.02	0.93	1.00	2	14
tenn	1.03	2.07	1.85	2	14
iowa	1.04	1.37	1.36	2	14

Table D4. Ratio comparison of unedited to edited DNB data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk D	1.00	1.00	1.00	0	14
spk A	0.96	3.28	2.50	2	14
spk C	1.00	1.00	1.00	0	14
spk B	1.00	1.00	1.00	0	14
iowa	1.37	13.77	4.99	1	10
tenn	1.00	1.00	1.00	0	13

Table D5. Ratio comparison of unedited to edited Tetryl data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk D	0.89	0.92	0.95	2	12
spk C	0.89	0.93	1.99	2	14
spk B	0.88	0.93	1.22	2	14
spk A	0.90	0.95	1.45	2	14
tenn	1.15	0.79	1.20	2	11
iowa	1.17	1.48	4.22	2	8
louis	1.13	0.66	1.58	2	11

Table D6. Ratio comparison of unedited to edited TNT data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk B	1.00	1.00	1.00	0	14
spk C	0.95	1.02	3.77	2	14
spk D	0.94	1.03	3.95	2	14
spk A	0.96	1.01	3.08	2	14
neb	1.00	1.00	1.00	0	10
tenn	1.00	1.00	1.00	0	14
louis	0.95	1.00	1.67	1	13
iowa	1.00	1.00	1.00	0	14

Table D7. Ratio comparison of unedited to edited DNT data.

0 ratio of:	1 mean conc	2 intralab std dev	3 interlab std dev	4 number rejected	5 number original
spk B	1.04	2.36	1.65	1	13
spk C	1.00	1.00	1.00	0	14
spk D	1.00	1.00	1.00	0	14
spk A	1.00	1.00	1.00	0	14
neb	1.00	1.00	1.00	0	10
tenn	1.00	1.00	1.00	0	10
iowa	1.00	1.00	1.00	0	7
louis	1.00	1.00	1.00	0	12

APPENDIX E: REVERSED-PHASE HPLC METHOD FOR THE DETERMINATION OF EXPLOSIVE RESIDUES IN SOIL

I. SUMMARY

A. ANALYTES. The following analytes can be determined using this method: HMX, RDX, 135TNB, 13DNB, TETRYL, 246TNT, 24DNT, 26DNT, NB, 2NT, 3NT, 4NT, 2ADNT.

B. MATRIX. This method is suitable for determination of nitramine and nitroaromatic residues in soil and sediment.

C. GENERAL METHOD. This method involves extraction of soil using acetonitrile in a sonic bath followed by determination using reversed-phase HPLC - UV 254 nm. Research supporting the establishment of this method is contained in references 4–6.

II. APPLICATION

A. TESTED CONCENTRATION RANGE. For a 2.00-g soil sample extracted with 10 mL of acetonitrile in which a 100 µL aliquot of 1/1 acetonitrile–aqueous $CaCl_2$ was injected, this method was found to be linear over the concentration ranges shown in Table E1.

Table E1. Range of linearity for various analytes.

Analyte	*Linear range (µg/g)*
HMX	2.2–50
RDX	1.0–50
135TNB	0.24–25
13DNB	0.12–25
TETRYL	0.65–50
NB	0.11–5.3
246TNT	0.24–25
2ADNT	0.11–5.0
26DNT	0.16–5.1
24DNT	0.07–25
2NT	0.24–5.1
4NT	0.22–5.0
3NT	0.25–5.1

Table E2. Sensitivity at 254 nm for 100-µL injection volumes.

	Sensitivity	
Analyte	(Absorbance units/µg)	(Absorbance units at CRL)
HMX	7.39×10^{-2}	3.25×10^{-3}
RDX	5.57×10^{-2}	1.11×10^{-3}
135TNB	1.00×10^{-1}	5.00×10^{-4}
13DNB	1.18×10^{-1}	5.90×10^{-4}
TETRYL	5.53×10^{-2}	7.19×10^{-4}
NB	7.06×10^{-2}	3.67×10^{-4}
246TNT	6.67×10^{-2}	3.34×10^{-4}
2ADNT	5.92×10^{-2}	2.96×10^{-4}
26DNT	4.19×10^{-2}	2.18×10^{-4}
24DNT	7.21×10^{-2}	3.61×10^{-4}
2NT	3.04×10^{-2}	1.52×10^{-4}
4NT	2.55×10^{-2}	1.28×10^{-4}
3NT	2.73×10^{-2}	1.37×10^{-4}

B. SENSITIVITY. The response of the UV detector at 254 nm for the various analytes using a 100-µL injection volume is given in Table E2.

C. REPORTING LIMITS. Certified reporting limits (CRLs) for the following analytes were determined over a four-day period using the method of Hubaux and Vos (1). These values are reported along with method detection limits obtained according to the *Federal Register* (2) in Table E3.

Table E3. Certified reporting limits (CRLs) and method detection limits (MDLs) for various analytes.

Analyte	CRL (µg/g)	MDL (µg/g)
HMX	2.2	1.3
RDX	1.0	0.74
135TNB	0.25	0.29
13DNB	0.25	0.11
TETRYL	0.65	0.12
NB	0.26	0.08
246TNT	0.25	0.08
2ADNT	0.25	0.03
26DNT	0.26	0.07
24DNT	0.25	0.03
2NT	0.25	0.07
4NT	0.25	0.07
3NT	0.25	0.07

Table E4. Retention times for primary analytes and potential interferences.

	Retention time (min)	
	LC-18	LC-CN
Primary analytes		
HMX	2.4	8.4
RDX	3.7	6.2
135TNB	5.1	4.1
13DNB	6.2	4.2
TETRYL	6.9	7.4
NB	7.2	3.8
246TNT	8.4	5.0
2ADNT	9.1	5.7
26DNT	9.8	4.6
24DNT	10.1	4.9
2NT	12.3	4.4
4NT	13.3	4.4
3NT	14.2	4.5
Potential interferences		
NG	7.7	6.0
4ADNT	8.9	5.1
SEX	2.4	5.1
TAX	2.8	3. 7
245TNT	8.4	5.9
24DANT	3.2	4.2
26DANT	2.4	3.7
C6H6	11.2	3.5
MEC6H5	23.0	3.9

The limits shown refer to a 100-µL injection volume of a 1:1 dilution of the acetonitrile soil extract with 5-g/L aqueous $CaCL_2$. No extract preconcentration was used.

D. INTERFERENCES

Retention times for the primary analytes and most likely interferences are presented in Table E4 for both the primary analytical column (LC-18) and the confirmation column (LC-CN). Experience with soils from a wide variety of sites indicates that only rarely have analytes been tentatively identified on the primary column that were not confirmed on the confirmation column.

Because of the similarity of retention times between 24DNT and 26DNT, the ability to quantitate both in a single sample is poor. It is possible, however, to identify both analytes if they are present in similar concentrations.

E. ANALYSIS RATE. We estimate that 24 samples can be extracted and analyzed over a two-day period if stock solutions have been prepared in advance.

F. SAFETY INFORMATION. Use the normal safety precautions appropriate for any flammable organic solvents.

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 - 10 mL volumetric, glass
5 mL, volumetric, glass, 1 per sample
4. 6-dram glass vials with Teflon-lined caps, 1 per sample
5. Scintillation vials - 20 mL, glass, 2 per sample
6. Disposable syringes - Plastipak, 3 mL, 1 per sample
7. Autosampler vials (optional), 1 per sample.

B. INSTRUMENTATION

1. Minimum requirements include an isocratic HPLC pump, a 100-µL sample loop injector and a fixed- or variable-wavelength 245-nm UV detector
2. Strip chart recorder - full-scale range appropriate to detector
3. Digital integrator - HP 3393 (or equivalent)
4. Autosampler (optional)
5. Vortex mixer
6. Ultrasonic bath, temperature controlled to less than 25°C
7. LC-18 (Supelco) RP-HPLC column, 25 cm × 4.6 mm (5-µm particle size)
8. Balance (±0.0001 g)
9. LC-CN (Supelco) RP-HPLC column, 25 cm × 4.6 mm (5-µm particle size)

C. ANALYTES

1. HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), boiling point: decomposes, solubility in water at 22.5°C: 5.0 mg/L, octanol/water partition coefficient: 1.3, CAS #2691-41-0.
2. RDX (hexahydro-1,3,5-tetranitro-1,3,5-triazine), boiling point: decomposes, melting point: 203.5°C, solubility in water at 25°C: 60 mg/L, octanol/water partition coefficient: 7.5, CAS #121-82-4.
3. 135TNB (1,3,5-trinitrobenzene), octanol/water partition coefficient: 15, CAS #99-35-4.
4. 13DNB (1,3-dinitrobenzene), octanol/water partition coefficient: 31, CAS #99-65-0.
5. TETRYL (methyl-2,4,6-trinitrophenylnitramine), octanol/water partition coefficient: 43, CAS #479-45-8.
6. 246TNT (2,4,6-trinitrotoluene), boiling point: explodes at 280°C, melting point: 80.1°C, solubility in water at 25°C: 130 mg/L, octanol/water partition coefficient: 68, CAS #118-96-7.
7. 24DNT (2,4-dinitrotoluene), boiling point: decomposes at 300°C, melting point: 70°C, solubility in water: 300 mg/L, octanol/water partition coefficient: 95, CAS #121-14-2.
8. 26DNT (2,6-dinitrotoluene), melting point: 66°C, octanol/water partition coefficient: 97, CAS #606-20-2.
9. NB (nitrobenzene), boiling point: 211°C (flashpoint: 88°C), melting point: 5.7°C, solubility in water: 2 g/L, octanol/water partition coefficient: 71, CAS #98-95-3
10. 2ADNT (2-amino-4,6-dinitrotoluene), octanol/water partition coefficient: 80, CAS #35572-78-2.
11. 2NT (o-nitrotoluene), boiling point: 225°C, melting point: 54°C, octanol/water partition coefficient: 171, CAS # 88-72-2.
12. 4NT (p-nitrotoluene), boiling point: 238°C, melting point: 54°C, octanol/water partition coefficient: 202, CAS #99-99-0.
13. 3NT (m-nitrotoluene), boiling point: 231°C, melting point: 15°C, octanol/water partition coefficient: 263, CAS #99-08-1.

D. REAGENTS AND SARMS

1. HMX – SARM quality
2. RDX – SARM quality
3. 135TNB – SARM quality
4. 13DNB – SARM quality
5. TETRYL – SARM quality
6. 246TNT – SARM quality
7. 24DNT – SARM quality
8. 26DNT – SARM quality
9. NB – SARM quality
10. 2ADNT – SARM quality
11. 2NT – Reagent grade
12. 4NT – Reagent grade
13. 3NT – Reagent grade
14. Methanol – HPLC grade
15. Acetonitrile – HPLC grade
16. Water – Reagent grade
17. CaC_2 – Reagent grade

IV. CALIBRATION

A. INITIAL CALIBRATION

1. Preparation of Standards. Standards for each analyte were dried to constant weight in a vacuum desiccator in the dark. About 0.25 g of each dried SARM was weighed out to the nearest 0.1 mg, transferred to individual 250-mL volumetric flasks and diluted to volume with acetonitrile. Stock solutions are stored in a refrigerator at 4°C in the dark. Stock standards are good for periods up to a year after the date of preparation.

Two combined analyte stock standards (A and B) are prepared as follows: For stock standard A, 5.0 mL of the HMX, RDX, 135TNB, 13DNB, TETRYL, 246TNT and 24DNT stock standards are combined in a 250-mL volumetric flask and diluted to volume with acetonitrile. Stock standard B is prepared in an identical manner using the NB, 2ADNT, 26DNT, 2NT, 4NT and 3NT stock standards.

To prepare the calibration standards, 5.00 mL of stock standards A and B are placed in separate 100-mL volumetric flasks and diluted to volume with acetonitrile. These standards, C-100 and D-100, contain each analyte at approximately 1000 µg/L. Further dilutions of C-100 are obtained as shown in Table E5. Dilution for D-100 are obtained separately in an identical manner. All standards are diluted 1:1 with 5 g/L of aqueous $CaCl_2$ before injection.

2. Instrument Calibration. Duplicate injections of each standard over the concentration range of interest are sequentially injected into the HPLC in random order. Peak areas or peak heights are obtained for each analyte. Retention times for the various analytes are shown in Table E4.

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

B. DAILY CALIBRATION. Standards C-100 and D-100, as described above, are used for daily calibration. These standards can be used for a period of 30 days after preparation. Each is analyzed in triplicate at the beginning of the day, singly after the midpoint of the run, and

Table E5. Preparation of calibration standards.

Standard	Volume of standard C (mL)	Size of volumetric flask (mL)	Approximate solution conc. (µg/L)
C-100	Straight	—	1000
C-50	25	50	500
C-20	10	50	200
C-10	10	100	100
C-5	5	100	50
C-2	2	100	20
C-1	1	100	10
C-0.5	0.5	100	5

singly after the last sample of the day. Response factors for each analyte are obtained from the mean peak areas or peak heights obtained over the course of the day and compared with the response factor obtained for the initial calibration. The mean response factors for daily calibration must agree within ±25% of the response factors obtained for the initial calibration for the first seven calibrations. Subsequently response factors must agree within two standard deviations of the initial calibration. If these criteria are not met, a new initial calibration must be obtained.

V. CERTIFICATION TESTING

A. PREPARATION OF SPIKING SOLUTIONS

Individual stock analyte spiking solutions are prepared in an identical manner to that described for the stock calibration standards. Two combined analyte spiking standards are prepared as follows. For combined stock spiking solution A, 2.00 mL of the HMX, RDX, 135TNB, 13DNB, TETRYL, 246TNT and 24DNT are combined in a 200-mL volumetric flask and diluted to volume with acetonitrile. The analyte concentrations in this solution (X-100) are about 10 µg/L. Diluted spiking solutions are prepared as shown in Table E6.

Table E6. Preparation of spiking solutions.

Standard	Volume of combined stock spiking solution (mL)	Size of Volumetric flask (mL)	Approximate concentration (µg/L)
X-100	Straight	—	100
X-50	50	100	5
X-20	20	100	2
X-10	10	100	1
X-5	5	100	0.5
X-2	2	100	0.2
X-1	1	100	0.1
X-0.5	1	200	0.05

B. PREPARATION OF CONTROL SPIKES. Spiked soil samples are prepared by placing a series of 2.00-g subsamples of USATHAMA Standard Soil in individual 6-dram glass vials. Each tube is spiked by addition of 1.00 mL of one of the spiking standards described above, and the tubes are allowed to stand uncapped for 18 hours prior to addition of the extraction solvent.

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

VI. SAMPLE HANDLING AND STORAGE

A. STORAGE. All soil samples are stored in a refrigerator at 4°C in the dark until extracted. Samples should be air-dried and processed as soon as possible after receipt and always within 7 days.

B. SOIL DRYING/HOMOGENIZATION. Soil samples are air-dried* to constant weight prior to extraction. Care must be taken to ensure that the soil is not exposed to direct sunlight during the drying period.

Dried soil is ground thoroughly and homogenized on a roller mill or by manual shaking in a closed container. Care must be taken to solvent-clean the mortar and pestle between samples.

C. CONTAINERS. All containers used to store wet or dried soil should be cleaned according to procedures specified in the USATHAMA QA Manual (1987) and rinsed with acetonitrile.

VII. PROCEDURE

A. EXTRACTION/DILUTION WITH AQUEOUS CaCL2. A 2.00-g subsample of each dried soil is placed in individual 6-dram glass vials. A 10.0-mL aliquot of acetonitrile is added to each vial, the vials are capped with Teflon-lined caps, and the suspensions are subjected to vortex mixing for 1 minute and placed in an ultrasonic bath for 18 hours. If tetryl is an analyte of interest, the sonic bath should be maintained less than 25°C.

The samples are removed from the sonic bath and allowed to cool and settle for 30 minutes. A 5.00-mL aliquot of the supernatant is removed and combined with a 5.00-mL aliquot of aqueous $CaCl_2$ solution (5 g/L) in a glass scintillation vial. The vials are shaken and allowed to stand for 15 minutes. The supernatant is placed in a Plastipak syringe and filtered through a 0.5-µm Millex SR filter membrane. The first 2–3 mL are discarded and the remainder is retained for analysis.

B. DETERMINATION. Determination of analyte concentrations in the diluted extracts is obtained by RP-HPLC on a 254-nm UV detector. A 100-mL loop is overfilled by injecting 500 µL of the acetonitrile–$CaCl_2$ sample solution through the loop and injecting it onto an LC-18 column eluted with 1.5 mL/min of 1/1 (V/V) methanol–water. Retention times for the analytes of interest and a number of potential interferences are given in Table E4 for both LC-18 (the primary analytical column) and LC-CN (the column used to confirm analyte identity). A chromatogram obtained for the seven primary analytes is shown in Figure E1.

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* Wet samples can be analyzed as well, but it is very difficult to obtain a homogeneous subsample without air drying. If wet soils are used, a separate subsample is used for moisture determination.

VIII. CALCULATIONS

A. RESPONSE FACTORS. Since a linear calibration curve with zero intercept is to be expected, calculation of results on a daily basis is obtained using response factors calculated for each analyte. The mean response ($\bar{R}$) for each analyte from repeated determination of Standards C-100 and D-100 are obtained in either peak area or peak height units. The response factor (*RF*) for each analyte is then obtained by dividing the mean response by the known solution concentration (*C*) in units of µg/L:

$$RF = \frac{\bar{R}}{C}. \qquad \text{(E1)}$$

B. ANALYTICAL CONCENTRATION: The concentrations (µg/L) of the analyte (C_a) are obtained by dividing the response for each analyte (R_a) by the appropriate response factor (RF_a)

$$C_a = \frac{R_a}{RF_a}. \qquad \text{(E2)}$$

Concentration in soil (X_a) on a µg/g basis is then obtained by multiplying solution concentrations by the volume of extraction solvent (0.01 L), and dividing by the actual mass of dry soil extracted (*M*):

$$X_a = \frac{C_a \cdot (0.01)}{M}. \qquad \text{(E3)}$$

IX. DAILY QUALITY CONTROL

A. CONTROL SPIKES: Spiked soil samples are prepared as described for Class 1 methods in the USATHAMA QA Program (2nd Edition, March 1987). This requires the use of a method blank, a single spike at two times the certified reporting limit, and duplicate spikes at 10 times the certified reporting limit for each analytical lot. Control spikes are prepared using the appropriate spiking solution in an identical manner as described in Section V.

B. CONTROL CHARTS. The control charts required are described for Class 1 methods in USATHAMA QA Program (2nd Edition, March 1987). This will require use of standard Shewhart $\bar{X}$ and *R* charts for the duplicate high spike and moving average $\bar{X}$ and *R* charts for the single low spike. Details on the charting procedures required are specified in USATHAMA QA Program (2nd Edition, March 1987).

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APPENDIX F

PROTOCOL FOR

INTERLABORATORY STUDY OF A REVERSED PHASE

HPLC METHOD FOR THE DETERMINATION OF

MUNITIONS RESIDUES IN SOIL

(February 17, 1988)

I. INTRODUCTION

A. Objective

The goal of this study is to assess the capabilities of this HPLC method for the determination of HMX, RDX, TNB, DNB, Tetryl, TNT and DNT* residues in soils.

B. Overview

Reversed phase HPLC will be used to determine the levels of the seven analytes in acetonitrile extracts of several different soils. Some are field contaminated soils and others are spiked standard soils. Analytes are extracted for 18 hrs in an ultrasonic bath. The extract is flocculated with aqueous calcium chloride and filtered before HPLC analysis. Strict adherence to the analytical protocol is essential in order for the statistical analysis of results to provide unbiased estimates of method performance. Bias in the intralaboratory precision can lead to the conclusion that laboratories differ systematically when they really do not. For instance, bias is introduced by discarding selected results and repeating analyses on an arbitrary basis.

Careful attention to detail is necessary to assure proper evaluation of the capabilities of the method for two reasons. Participation in this study represents the investment of a large amount of time and money by the organizers and the participating laboratories. Furthermore, if this method develops into a national regulatory method, a biased evaluation has much greater financial implications than just the cost of this interlaboratory study.

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* HMX: octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
RDX: hexahydro-1,3,5-trinitro-1,3,5-triazine
TNB: 1,3,5-trinitrobenzene
DNB: 1,3-dinitrobenzene
Tetryl: methyl-2,4,6-trinitrophenylnitramine
TNT: 2,4,6-trinitrotoluene
DNT: 2,4-dinitrotoluene

II. PREPARATIONS

A. Analyst

One analyst will be selected by the lab manager to oversee all aspects of this study including receipt of materials through data analysis. If normal lab policy is that one group performs sample preparation and another performs analysis, this is acceptable. There are several places where unsolved problems may call a halt to the protocol and require contacting Tom Jenkins* at CRREL. Further work cannot be performed until the problem is solved. Reasonable attempts to resolve problems should be made before calling.

B. Record Keeping

One notebook should be used exclusively for this study and should be labeled appropriately. Carbon or photo copies of notebook pages should be made. The original notebook must be submitted with the analytical report; the lab retains the copy. Complete documentation of experimental work and calculations is essential to help trace the sources of problems that may be discovered after data are returned to the coordinating laboratory.

C. Receipt of Materials

The following materials will be supplied by CRREL:

Sixteen, glass test tubes each containing about 2 grams of soil, sealed with teflon-lined caps.

One glass test tube containing specified concentrations of the seven analytes in a soil sample.

One LC18 column. One LC-CN column.

Sufficient syringes, syringe filters, and sample vials.

The following standard materials supplied by USATHAMA:

One vial each of DNT, TNT, TNB, DNB, and Tetryl (all 200 mg neat) and RDX and HMX (200 mg under isopropanol).

Arrangements should be made to notify the analyst immediately when these materials arrive. Upon receipt, the analyst will log each sample container

*Phone: 603-646-4385 or 603-646-4462, U.S. Army Cold Regions Research and Engineering Laboratory, Hanover, NH

into the project notebook. Each entry should contain identification number, name, date of arrival, and description of condition. Inspect each container for damage. Broken, cracked, or leaking containers should be reported immediately to Tom Jenkins at CRREL, who will send replacements.

D. Storage of Materials

Soil samples must be stored in the dark in a refrigerator or cold room (temperature around 4°C, not below 0°C) immediately after receipt. The SARM's should be stored in a freezer ($\leq$ 0°C).

III. ANALYSIS

A. Overview

The analytical work will be performed in two steps. The analyst first will spend some time becoming familiar with the test procedure. During this period working curves for each of the seven analytes will be prepared and steps will be taken to establish that they are linear and pass through the origin. Then a sample whose composition is specified (provided by CRREL) will be analyzed. This experience should help to uncover potential systematic errors and allow the analyst to correct the causes. If uncorrected, these errors could cause a laboratory's results to be excluded from the statistical analysis at the end of the study.

The second portion of the work consists of analysis of sixteen soil samples. These samples represent a range of matrices to which the HPLC method being tested should be applicable. Some amount of the seven analytes (HMX, RDX, TNB, DNB, Tetryl, TNT, DNT) may be present.

The effort should be spread over five days, broken down conveniently as follows:

Day 1:	Extraction of Test sample (18 hours) in sonic bath Monitor sonic bath temperature	
Day 2:	Conditioning of HPLC column Performance test Flocculate, filter, store test sample filtrate	Add solvent to soil samples Start extraction (to run overnight)
Day 3:	Establish calibration curves Analyze test sample	Stop extraction, remove samples from bath Flocculate, filter, store filtrates
Day 4:	Verify calibration Analyze filtrates	
Day 5:	Calculations	

B. Experimental

1. Instrumentation

Chromatograph: The HPLC instrument should consist of a single high pressure pump and a 254 nm fixed wavelength ultraviolet absorption detector. If a fixed wavelength detector is not available, then a multi-wavelength detector set to 254 nm may be used. A complete description of the instrument will be required in the report.

Strip chart recorder (if integration does not provide chromatogram): Full scale capacity should be compatible with the UV detector used. The trace is necessary to provide permanent record of experimental results. Computer storage of chromatograms is permissible if that is standard practice for the laboratory. These records should be retained by the participating laboratories unless requested by the coordinating laboratory.

Integrator: Calculates peak areas; may be a stand-alone digital integrator or computer-controlled integrator; mechanical or analog integrators may not be substituted without authorization. Peak height measurement may be substituted as long as it is done consistently for a given analyte (for all samples and the interspersed calibration standards).

Sample loop injector: Nominal 100 microliter volume; syringe injection of 100 µL into a larger loop is permissible, but less desirable. Specify which option used in report.

2. Operating Parameters

Column: LC18 (Supelco) reversed phase, 25 cm x 4.6 mm; shipped from CRREL filled with methanol. Until this study has been completed, the column may not be used for any other purpose.

Column temperature: Room temperature; record hourly during analysis ($\pm$ 1°C).

Solvent system: 50% water, 50% methanol by volume. Prepare using graduated cylinders, not volumetric flasks (because of solution contraction upon mixing). Prepare in 500 mL portions, then vacuum filter through a solvent-washed Whatman glass micro-fiber filter to remove particulate matter and to degas the solvent. Fresh solvent should be prepared daily. Other methods of solvent degassing are permissible.

Flow rate: 1.5 mL/min.

Detector: 254 nm.

Integrator: Threshold set low enough to avoid negative intercept in working curve and high enough to avoid positive intercept (see section III.E).

Recorder/Integrator Chart Speed: 0.5 in/min.

3. Hardware/Glassware

Vortex Mixer: Any commercial unit.

Sonic Bath: Any commercial unit capable of holding at least 16 test tubes (25 x 200 mm). Tubes must be held in rack suspended in bath (resting on bottom inhibits coupling of RF energy into bath). (CRREL uses a rubber coated, 36-place test tube rack set onto basket accessory for sonic bath. Beakers are not acceptable because sonic power at samples is damped.) Fill with distilled water to level recommended by manufacturer. Overnight operation during extraction of soils causes substantial heating of bath. Cooling must be provided if temperature exceeds 50°C. This problem may be diagnosed and corrected while the test sample is being extracted. Some evaporative loss of bath water will occur but not enough to affect results.

HPLC syringe: Any liquid-tight syringe of capacity 0.5 to 1.0 mL (e.g. Hamilton 750).

Syringe Filter: 0.5 µm Millex SR disposable filter, 25 mm diameter, CRREL supplied.

Sample filtration syringe: 3 mL, Pla[illegible]ak (Becton, Dickinson and Co.); CRREL supplied (sterile - no further cleaning necessary).

Volumetric flasks and pipets: Glass, class A or B; make sure condition is good (e.g. pipet tips not broken).

Scintillation vials: 20 mL glass with polyethylene cap insert (not aluminum); CRREL supplied (sterile; no further cleaning necessary).

Cleaning of volumetric glassware: Soak overnight in detergent, scrub briefly, rinse well with hot tap water, rinse with reagent-grade acetone, rinse with deionized water, oven dry at 105°C; rinse with acetonitrile before filling.

Reagents: Methanol, acetonitrile; HPLC grade.
Water; in-house reagent grade.

Calcium chloride solution: 500 mL of aqueous 10.0 g/L $CaCl_2$; using in-house reagent grade water and reagent grade calcium chloride.

C. Calibration Standards.

1. Individual stock standards for HMX, RDX, TNB, DNB, Tetryl, TNT, and DNT. These solutions must be used for the entire study.

For each material:

a. Vacuum dry SARMs at ambient temperature to constant weight (within 1 mg); a vacuum desiccator or vacuum oven attached to a water aspirator or vacuum pump will suffice. Keep SARMs in darkness as much as possible during handling. For RDX and HMX,

remove most of the isopropanol by means of a Pasteur pipet, air dry for several hours, then vacuum dry. Store dried SARM s in a desiccator over dry calcium chloride or Drierite and place in the dark when not in use.

b. Accurately weigh about 0.1 g of each dried SARM onto weighing paper (e.g. VWR or Fisher-brand "Weighing Paper"); transfer carefully into separate 100 mL volumetric flasks. Reweigh weighing paper. Record mass to 0.1 mg.

c. Dissolve and dilute to volume with acetonitrile.

d. Wrap the stoppered joint with Parafilm as an added protection against evaporation.

e. Calculate concentrations exactly in mg/L and label flasks.

f. Store in dark in refrigerator at about 4°C (not below 0°C).

2. Combined-Analyte Working Stock Standard

a. Remove the stock standards from the refrigerator and allow to warm to room temperature (at least 30 min; but not overnight).

b. Invert flasks several times to mix.

c. Into a 100.0 mL volumetric flask, pipet 10.0 mL each of HMX, RDX, TNT, and TNB stock solutions and 5.00 mL each of DNT, DNB, and Tetryl stock solutions. Dilute to volume with acetonitrile and invert several times to mix.

d. Pipet 10.00 mL of the solution from (c) into a 100.0 mL volumetric flask, fill to the mark with acetonitrile, and invert to mix. This standard (the combined analyte working stock) will contain about 10 mg/L HMX, RDX, TNT, and TNB and 5 mg/L DNT, DNB, and Tetryl.

e. Calculate the concentrations exactly in mg/L, label the flasks, and date them.

f. Wrap the stoppered joints with Parafilm and store the flasks in refrigerator when not in use. This standard is stable for 28 days when properly stored.

3. Working Standards

a. To be prepared fresh on <u>each</u> analysis day as instructed. (All on calibration day; high standard only on analysis day).

b. Remove the combined-analyte working stock standard from the refrigerator and allow to warm to room temperature (at least 30 min., but not overnight).

c. Invert flask several times to mix.

d. Pipet the following aliquot volumes into the indicated volumetric flask: 2.00 mL into 500.0 mL, 5.00 mL into 500.0 mL, 10.00 mL into 250.0 mL, 10.00 mL into 100.0 mL, and 10.00 mL into 25.00 mL.

e. Fill to mark with acetonitrile. Stopper and invert ten times to mix.

f. Calculate the concentrations exactly in µg/L, label and date the flasks.

4. Injected Standards

a. For each standard, pipet 10.0 mL into a scintillation vial.

b. Add 10.0 mL of 10.0 g/L $CaCl_2$ by means of a pipet.

c. Affix cap and shake to mix.

d. Prepare blank by combining 10.0 mL of acetonitrile mixture with 10.0 mL of the $CaCl_2$ solution in vial. Affix cap and mix.

e. Label all vials appropriately.

The solutions that result represent the following concentrations in an aliquot of extract:

Dilution ratio of working stock standard	Approximate concentrations (µg/L)	
	For DNT, DNB, Tetryl	For HMX, RDX, TNT, TNB
2/500	20	40
5/500	50	100
10/250	200	400
10/100	500	1000
10/25	2000	4000
stock	5000	10000

Note that these values represent the concentrations before addition of the $CaCl_2$ solution. (The actual concentrations after dilution are half as large.) This can be done because the samples are treated similarly: a one-to-one dilution is made by adding 10.0 mL of $CaCl_2$ to 10.0 mL of acetonitrile extract. Thus, the analytical results derived from the working curve need not

be corrected for this extra dilution. Care must be taken in this step to pipet these 10 mL volumes accurately since experience has indicated that a significant error at this stage is compounded when peak areas are measured.

D. HPLC Procedure

1. Initial conditioning (Day 2)

The HPLC column is new. Consequently, conditioning with the mobile phase and a test of performance are required before using the column for analysis.

a. Conditioning: Follow the procedure below (section III.D.2) for instrument warm-up except pass at least 30 void volumes (about 60 mL) of mobile phase through the column. Continue until the UV detector baseline is level when set to its greatest sensitivity.

b. Performance test (calculation of plate number)

(1) Taking a 1 mL aliquot from the combined-analyte working stock standard, dilute to 100 mL in a volumetric flask with acetonitrile. Take a 10 mL aliquot, add 10 mL of 10 g/L $CaCl_2$ in a vial. Cap and shake to mix.

(2) Use the proper sample injection procedure described in section III.D.3 below to obtain a chromatogram. All seven analytes should elute within 12 minutes. Use the conditions described in section III.B.2 above, but select a chart speed that spreads the peaks out abnormally wide (such that widths at half height are at least 2.0 cm). Measure the peak width at half height for TNT and RDX to the closest millimeter.

(3) Calculate the number of plates (N) on the column from each peak using the equation

$$N = 5.54 \left(\frac{t_r}{t_{0.5}}\right)^2$$

where t_r is the retention time and $t_{0.5}$ is the width of the peak at half height, both in minutes.

(4) Average the results for the two analytes.

(5) If the average value is less than 3,000 plates, carefully recheck the calculation. If there is no error, allow another 30 void volumes of mobile phase to wash through the column and repeat the experiment. If the calculated value of N still does not exceed 3,000, the column is not performing up to its specification. If used it may invalidate results from this laboratory. Notify Tom Jenkins at CRREL immediately if this occurs.

2. Normal Warm-up Procedure

   a. Turn on all electronic equipment and allow to warm-up for at least 30 min.

   b. Pass at least 15 void volumes of mobile phase through the column (20 min. at 1.5 mL/min) and continue until the UV detector baseline is level when set to its greatest sensitivity.

   c. Make certain the pumps are not experiencing vapor lock as indicated by large pressure fluctuations.

   d. Check system thoroughly for leaks.

3. Sample Injection Procedure

   a. Fill the analytical syringe with acetonitrile and discharge into a waste beaker.

   b. Repeat twice more to remove traces of previous sample.

   c. Rinse syringe three times with the sample.

   d. Fill syringe with sample to at least 500 µL and inject most of this through sample loop; avoid introducing air bubbles. Overfilling the loop in this manner assures that the sample injected is not diluted by solvent in the loop.

   e. If an autosampler is used, follow your usual procedure but assure sufficient flushing is achieved to preclude carryover.

E. Preliminary Experiments

(Day 1)

Before beginning the analyses of the soil extracts, the analyst should become familiar with the analytical procedure. For this purpose a test tube containing soil spiked with the seven analytes has been included as a test sample. This sample should be extracted following instructions for the real samples (see Section III.F. Days 2 and 3). Store filtrate overnight at room temperature (necessary for accurate TNB results). If not then analyzed, refrigerate. Analyze on the calibration day (Day 3).

(Day 3)

Follow the instrument warm-up and column conditioning instructions (see section III.D.2). Prepare the working standards and blank as specified in sections III.C.3 and III.C.4. Using the procedure described in section III.D.3, inject low and high standards and blank into the HPLC at least once. Ascertain the detector range that provides sizable but on-scale peaks so that a good chromatographic record results. Make certain that integration is occurring properly.

1. Carefully prepare calibration curves for the seven analytes. These curves will be the basis of all the remaining quantitative work; consequently, it is essential that systematic errors be avoided.

a. Obtain chromatograms of the working standards and blank in duplicate (14 injections total). Sequence the injections randomly (see Appendix A).

b. Plot peak area versus concentration for each of the seven analytes.

Do not average the duplicates before plotting. Inspect the plot for gross deviations from linearity -- a set of duplicates wildly off line, or a large degree of curvature. Significant deviation from linearity is evidence for systematic bias. Whereas it is possible to make analytical determinations with a nonlinear working curve, it is preferable that the systematic error be found and corrected before beginning the interlaboratory test measurements.

Once gross errors have been corrected and the plot looks reasonably linear, more rigorous statistical tests must be applied. (If obvious curvature still exists and you have the appropriate computational facilities, inspect the residuals as an aid in diagnosing the problem; otherwise, contact Tom Jenkins).

c. Calculate the regression analysis tables for each analyte using both the model through the origin and the model with an intercept (see Appendix B).

d. Test the model with an intercept for lack of fit for each analyte (see Appendix B). (Comparison of correlation coefficients alone is insufficient.)

If a significant lack of fit exists for any of the analytes, plot the regression line on top of the data points. Inspect for wild points or curvature. (If you have the appropriate computational facilities, inspect the regression residuals.) Try to resolve the source of nonlinearity. If the problem cannot be resolved. contact Tom Jenkins.

e. Test the hypothesis that the intercept equals zero (see Appendix B).

The daily calibration routine is simplified if the zero-intercept hypothesis is accepted. Each day, a complete working curve need not be constructed. Instead, several replicates of the most concentrated standard are analyzed.

If it is found that an intercept is not zero, the most likely reason is that the integrator "zero" has been set too high (negative intercept) or too low (positive intercept). Adjust the integrator and repeat steps a through d. If this

repetition fails to provide zero intercepts, search for other causes. If the problem cannot be resolved, contact Tom Jenkins.

2. Inject the test sample (if refrigerated allow to warm to room temperature) into the HPLC (see Section III.D.3).

3. Determine the concentrations of all seven analytes in the test sample using the working curves. Compare results with the specified values. The determined values should be within 15% of the specified values. If not, attempt to resolve discrepancies and inject another aliquot. If all analytes are within 15% of the specified values, proceed to section F; if not, contact Tom Jenkins.

F. Analysis of Soil Samples

Day 2 (Extraction)

1. Arrange the sixteen test tubes containing soil samples in random sequence as determined by Appendix C.

2. Pipet 10.00 mL of acetonitrile into each tube.

3. Vortex each tube one minute.

4. Place all tubes into rack in sonic bath for 18 hours. (Plan this to run overnight.) Record tube arrangement in rack in case labels are eradicated during extraction. Solvent level in tubes need not be below water level in bath. (In all previous work about one third of solvent was above water level.) Keep out of direct sunlight.

   Note: Shorter extraction may be adequate for some materials. However, because of the variability of sonic bath efficiencies (because of age, temperature, dimensions, manufacturers), a long extraction overrides these effects, thus minimizing interlaboratory variability.

Day 3 (Flocculation, filtration)

1. Remove tubes from bath. Wait 30 min. to allow them to cool and for soil particles to settle.

2. From each tube, transfer a 5.00 mL aliquot using a pipet into a clean, labeled vial. Add 5.00 mL of 10 g/L $CaCl_2$ solution using a pipet.

3. Cap tightly and shake briefly. Wait at least 15 minutes while suspended soil particles flocculate and settle.

4. Using the 3 mL Plastipak syringe, withdraw a portion of the supernatant and filter through a 0.5 micrometer Millex SR filter to waste. Withdraw a second portion and filter through the same filter unit, collecting the filtrate in a clean labelled scintillation vial.

5. Cap tightly, label appropriately, and store in dark at room temperature overnight. (An overnight delay is required for accurate TNB results.) If additional storage is needed, refrigerate.

Day 4 (Analysis)

1. Remove the combined-analyte working stock standard (and samples, if necessary) from refrigerator and allow to warm to room temperature (at least 30 min., but not overnight). If samples were refrigerated overnight, allow to warm and then shake vigorously to ensure that mixing is complete. Because of the presence of 10 mg/L calcium chloride, acetonitrile has occasionally salted out at refrigeration temperatures, forming two layers.

2. Warm up instrument and condition HPLC column (see section III.D.2).

3. Calibration

   If working curves with zero intercepts were obtained during the preliminary experiments, daily calibration only requires analysis of the most concentrated working standard. Proceed as follows:

   a. Obtain the combined-analyte stock standard.

   b. Prepare one vial of this standard for injection (see section III.C.4 a to c. Keep this vial tightly capped when not in use.

   c. Obtain chromatograms of this standard in triplicate (see section III.D.3).

   d. Calculate the mean and standard deviation of the peak areas for each of the seven constituents.

   e. For each analyte, compare this mean with the response expected from the working curves already established (see Appendix D).

   f. If the test indicates no differences for any of the analytes, skip to instruction 4.

   g. If the test is significant for any of the analytes, there may be a systematic preparation error, or instrumental response has drifted. To distinguish between these possibilities, carefully repeat steps a through e.

   h. If the tests against the working curves (Appendix D) still indicate significant difference, test for equivalence between the two sets of triplicates run today (see Appendix E).

   i. If the test in h indicates no difference, skip to instruction 4.

   j. If the test in h indicates significant difference, either the instrument is subject to strong short term drift or noise or there is insufficient reproducibility in the analyst's technique of solution preparation. Call Tom Jenkins before proceeding further.

4. Each of the sixteen extracts will be injected once into the HPLC instrument. In addition, the standard prepared in step 3b above will be injected five times. Consequently, a total of 21 injections will be made. The sequence of processing and injection must be randomized. Determine the order of injection of samples (see Appendix F). Then using proper procedure (see section III.D.3), inject each solution into the HPLC in this sequence.

5. You can expect an occasional analyte to be above the calibration range. If you find an analyte present at a level more than three times the highest standard:

   a. Pipet 1.00 mL of sample into a 50.00 mL volumetric and dilute to the mark with premixed 1:1 acetonitrile-10 g/L $CaCl_2$. Analyze this dilution for the overrange analyte.

   b. Reanalyze the sample following the overrange sample in the original sequence. Report these data as the primary data. Also report the original run data. This reanalysis protects against carry-over from the overrange sample.

G. Data Analysis

1. Determine calibration curves for each of the seven analytes:

   a. Calculate the mean peak area ($\bar{y}$) for the five replicates of the standard.

   b. Solve the equation $\bar{y}/x_{HI} = b_1$ where x_{HI} is the known concentration of the highest standard and b_1 is the slope of the working curve.

2. Substitute the value for the slope into the working curve equation $y = b_1x$. Calculate the concentrations (x) for the 16 injections of soil extract using individual peak areas (y).

3. Convert µg/L to µg/g: Multiply by 0.0100L and divide by the mass of soil sample (data provided by CRREL). Report values to three or four significant figures but not past 0.01 µg/g.

H. Reporting of Results

An example of the format for reporting re[illegible] is given in Appendix G.

APPENDIX A

Random Injection Sequence for Working Curve

The samples consist of a blank and five standards, each of which will be injected in duplicate (1 and 2). The sequence of injection of these 14 trials must be random. Use computer generated random numbers, random number tables, or pull slips of paper numbered 1 to 14 from a hat. Record the resulting sequence in the following table and in the notebook, then use this table to keep track of the order of injections.

Standard Concentration (µg/L nominal)			
DNT,DNB, Tetryl	RDX,HMX TNT,TNB	Replicate	Sequence
0	0	1	
0	0	2	
20	40	1	
20	40	2	
50	100	1	
50	100	2	
200	400	1	
200	400	2	
500	1000	1	
500	1000	2	
2000	4000	1	
2000	4000	2	
5000	10000	1	
5000	10000	2	

APPENDIX B

REGRESSION ANALYSIS*

Previous testing has demonstrated that chromatographic peak area (y) should be a linear function of analyte concentration (x). Two models may be tested, the model through the origin: $\hat{y} = b_1 x$, and the model with an intercept: $\hat{y} = b_o + b_1 x$. The coefficients for these models can be calculated as follows:

For model through origin:

$$b_1 = \frac{\Sigma xy}{\Sigma x^2} \qquad b_o = 0$$

For model with intercept:

$$b_1 = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \qquad b_o = \bar{y} - b_1 \bar{x}$$

where $\bar{y}$ and $\bar{x}$ represent respective mean values, n is the number of data points, and $\hat{y}$ is the value of y predicted by the regression equation.**

Regression analysis tables are used to determine whether the data fit the linear models well enough and which linear model is more applicable. The tables must be calculated as follows:

Table for Model with Intercept

	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-ratio (F)
Residual	$\Sigma y^2 - \left[\frac{(\Sigma y)^2}{n}\right] - b_1^2 \left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]$	10	$\frac{\text{resid. SS}}{10}$	----
Error	$\frac{\Sigma d^2}{2}$	6	$\frac{\text{SS error}}{6}$	----
Lack of fit (LOF)	Residual SS - Error SS	4	$\frac{\text{SS LOF}}{4}$	$\frac{\text{MS LOF}}{\text{MS error}}$

*Do not round off intermediate numbers in calculations. Carry through at least six digits to avoid round off errors, even though in the final results less than six digits will be significant.

**The two replicate analyses of the blank (zero analyte) are <u>not</u> used to obtain regression equations; they are meant as indicators of contamination.

where n is the number of data points and d is the difference between the peak areas of duplicates. For the model through the origin, the table is:

Table for Model through Origin

	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-ratio (F)
Residual	$\left[\Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2}\right]$	11	$\frac{\text{resid. SS}}{11}$	----
Error	$\frac{\Sigma d^2}{2}$	6	$\frac{\text{SS error}}{6}$	----
Lack of fit (LOF)	Residual SS - Error SS	5	$\frac{\text{SS LOF}}{5}$	$\frac{\text{MS LOF}}{\text{MS error}}$

Computer software statistics packages may be used to derive regression residuals. However, it is rare that the residuals are split into error and lack-of-fit components.

Test for lack of fit: For the model with an intercept, the critical value is $F_{.95}(4,6) = 4.53$. If the F-ratio calculated in the right hand column of the regression analysis table exceeds the critical value, there is a significant lack of fit; i.e., the working curve is not linear. Steps as suggested in the text must be taken to correct this problem. If the calculated value is less than 4.53, the linear model is satisfactory. It is not necessary to test the model through the origin for linearity.

After establishing linearity, the intercept must be tested to determine whether it is significantly different from zero. Calculate the F ratio:

$$F = \frac{\text{(resid. SS for model through origin)} - \text{(resid. SS of model with intercept)}}{\frac{\text{(residual SS of model with intercept)}}{10}}$$

where the "residual SS" are in the tables. This can be done only after LOF has been shown to be insignificant. The critical value is $F_{.95}(1,10) = 4.96$. If the calculated value exceeds the critical value, the intercept is significantly different from zero. Steps as suggested in the text must be taken to correct this problem. If the problem cannot be resolved, contact Tom Jenkins. If the calculated value is less than 4.96, the intercept is zero.

APPENDIX C

Random Handling Sequence for Soil Samples

The samples consist of sixteen soil samples. The sequence of processing must be random. Use computer generated random numbers, random number tables, or pull slips of paper numbered 1 to 16 from a hat. Record the resulting sequence in the following table and in the notebook, then use this table to keep track.

Sample	Sequence
A	
B	
C	
D	
E	
F	
G	
H	
I	
J	
K	
L	
M	
N	
O	
P	

APPENDIX D

Daily check of instrument calibration is achieved by measuring the detector responses for the seven analytes in the most concentrated standard. This is performed before beginning the analysis of a number of samples.

The statistical test is based on comparing the mean of triplicate peak area measurements of the standard with the confidence intervals around the working curve which was established during the preliminary experiments. The equations used to perform the comparison are as follows:

$$s_{yp} = \left[\frac{(n_{wc} - 1)\, s_{wc}^2 + (n - 1)\, s^2}{(n_{wc} - 1) + (n - 1)}\right]^{1/2} \quad (1)$$

$$PI = y_{HI} \pm t_{CRIT}\, s_{yp} \left(\frac{1}{n} + \frac{x_{HI}^2}{\Sigma x^2}\right)^{1/2} \quad (2)$$

$$t_{CRIT} = t_{.95}\ (df = 13) = 2.16 \quad (3)$$

where

n: equals 3, the number of data points in set to be compared with working curve

n_{wc}: equals 12, the number of measurements used to calculate working curve

s: standard deviation of triplicates

s_{wc}: square root of residual mean square from regression analysis table for model through origin,

s_{yp}: pooled standard deviation

PI: prediction interval

y_{HI}: peak area predicted for high standard by working curve

x_{HI}: known concentration of high standard

Σx^2: summation over all of the standard concentrations squared (remember that each is used twice; value should be about 234,343,200 for HMX)

df: degrees of freedom, equals 13; 11 for working curve, 2 for triplicates

Notes on use:

a. Standard deviations of triplicates are most easily calculated by means of:

$$s = \left(\frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}{n - 1} \right)^{1/2}$$

b. Example:

(1) Given: slope = 2.5 concentration = 10000 µg/L HMX

$s_{wc} = 500$

$y_{HI} = 2.5 \times 10000 = 25,000$

(2) At start of day, 3 replicates are run. Mean area is $\bar{y}$ = 24,000 with s = 450.

(3) Use equation 1:

$$s_{yp} = \left(\frac{11(500)^2 + 2(450)^2}{11 + 2} \right)^{1/2} = 492.6381$$

(4) Use equation 2:

$$PI = 25,000 \pm 2.16(492.6381) \left(\frac{1}{3} + \frac{10000^2}{234,343,200} \right)^{1/2}$$

Thus PI = 25,000 ± 928 = [24,072; 25,928]

(5) Is the mean of the triplicates within the PI? No, since prediction interval is [24,072; 25,928] and y = 24,000 is outside interval.

APPENDIX E

Comparison of Two Sets of Triplicate Standards

Use the following equations:

$$S_{yp} = \sqrt{\frac{s_1^2 + s_2^2}{2}} \quad (1)$$

$$t = \frac{(n)^{1/2} |\bar{y}_1 - \bar{y}_2|}{(2)^{1/2} s_{yp}} \quad (2)$$

$$t_{.95} \text{ (df = 4)} = 2.78 \quad (3)$$

where

$\bar{y}_1$ and $\bar{y}_2$ are the means of the two sets of triplicates

s_1 and s_2 are the corresponding standard deviations

$n = 3$

if $t < t_{.95}$, then the hypothesis that $\bar{y}_1 = \bar{y}_2$ is accepted.

APPENDIX F

Random Injection Sequence for Analyses of Soil Extracts

The soil extracts will be injected once each into the HPLC; the standard, five times. The sequence of 16 sample injections for each soil sample must be random with the standards being injected at fixed points in this sequence (see table below). Use computer generated random numbers, random number tables, or pull slips of paper number 1 to 16 out of a hat. Record the results in table below, and also in the notebook.

Sample	Replicate	Sequence
Standard	1	-----------------First--------------------
Standard	2	--------between 4th and 5th positions-------
Standard	3	--------between 8th and 9th positions-------
Standard	4	--------between 12th and 13th positions-----
Standard	5	-----------------Last---------------------
Sample 1		
Sample 2		
Sample 3		
Sample 4		
Sample 5		
Sample 6		
Sample 7		
Sample 8		
Sample 9		
Sample 10		
Sample 11		
Sample 12		
Sample 13		
Sample 14		
Sample 15		
Sample 16		

APPENDIX G
Format of Final Report

FINAL REPORT
on HPLC Determination of Ordinance Materials in Water

Sponsor Laboratory: USACRREL

Participating Laboratory:

Laboratory Manager:
Analyst(s):

Checklist of items to be included in report:

laboratory manager's profile of analyst ________

original project notebook ________

complete description of HPLC instrument and integrator ________

Preliminary Experiments

Plate count of HPLC column: ____________

Analyte	SARM mass(g)	Analyte Retention Time	Composition of Test Sample (μg/g)	Working Curves in form (area) = b_1 (concentration)
HMX				
RDX				
TNB				
DNB				
Tetryl				
TNT				
DNT				

Analytical Results   Date of Analysis: ____________________

	Determined Concentrations (μg/g)*						
Sample	HMX	RDX	TNB	DNB	Tetryl	TNT	DNT
A							
B							
C							
D							
E							
F							
G							
H							
I							
J							
K							
L							
M							
N							
O							
P							

*Report values to three significant figures, but not past 0.01 μg/g.

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Bauer, Christopher F.

Development of an analytical method for the determination of explosive residues in soil: Part III. Collaborative test results and final performance evaluation / by Christopher F. Bauer, Thomas F. Jenkins, Stephan M. Koza, Patricia W. Schumacher, Paul H. Miyares, and Marianne E. Walsh. Hanover, N.H.: U.S. Army Cold Regions Research and Engineering Laboratory; Springfield, Va.: available from National Technical Information Service, 1989.

vi, 98 p., illus., 28 cm. (CRREL Report 89-9.)

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1. Chemical analysis. 2. Collaborative tests. 3. Explosives. 4. Soils. 5. Soil contamination. 6. Soil extraction.

I. Jenkins, Thomas F. II. Koza, Stephan M. III Schumacher, Patricia W. IV. Miyares, Paul H. V. Walsh, Marianne E. VI. United States Army. VII. Corps of Engineers. VIII. Cold Regions Research and Engineering Laboratory. IX. Series: CRREL Report 89-9.