

EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR 403

HPLC DETERMINATION OF HEXANITROHEXAAZAISOWURTZITANE (CL-20) IN SOIL AND AQUEOUS MATRICES

J. Steven Anthony
Emily A. Davis
Mark V. Haley
Jan E. Kolakowski
Carl W. Kurnas
Carlton T. Phillips
Michael Simini
Roman G. Kuperman
Ronald T. Checkai

RESEARCH AND TECHNOLOGY DIRECTORATE

November 2004

Approved for public release;
distribution is unlimited.

20050124 081

ABERDEEN PROVING GROUND, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE (DD-MM-YYYY) XX-11-2004		2. REPORT TYPE Final		3. DATES COVERED (From - To) Dec 2002 - Jan 2004	
4. TITLE AND SUBTITLE HPLC Determination of Hexanitrohexaazaisowurtzitane (CL-20) in Soil and Aqueous Matrices				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Anthony, J. Steven; Davis, Emily A.; Haley, Mark V.; Kolakowski, Jan E.; Kurnas, Carl W.; Phillips, Carlton T.; Simini, Michael; Kuperman, Roman G.; and Checkai, Ronald T.				5d. PROJECT NUMBER 62262255200	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) DIR, ECBC, ATTN: AMSRD-ECB-RT-TT, APG, MD 21010-5424				8. PERFORMING ORGANIZATION REPORT NUMBER ECBC-TR-403	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Strategic Environmental Research and Development Program (SERDP CP-1254), 901 North Stuart Street, Suite 303, Arlington, VA 22203				10. SPONSOR/MONITOR'S ACRONYM(S) SERDP	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Hexanitrohexaazaisowurtzitane (CL-20) is a novel energetic material being investigated as a potential replacement for currently used explosive and propellant formulations. The principal objective of this study was to focus on the quantitative chemical analysis of freshly amended (0.01-100 mg/kg) and weathered/aged (0.01-10000 mg/kg) CL-20 in Sassafra Sandy Loam (SSL) soil extracts. Using modified U.S. Environmental Protection Agency (USEPA) Method 8330A, the instrumental limit of detection was 0.01 mg/L. In acetonitrile extracts, the limits of detection for fresh and weathered/aged CL-20 in soils were 0.08 mg/kg and 0.10 mg/kg, respectively, while in ATCLP (water) extracts, the limits of detection for fresh and weathered/aged CL-20 in soils were 0.1 mg/kg and 0.25 mg/kg, respectively. At all amended concentrations, paired t-tests were used to show that the recovery percentages for freshly amended and weathered/aged CL-20 in soil were statistically different. The water extract recoveries were lower due to CL-20's reduced solubility in water. Higher concentrations of CL-20 in soil (≥ 1000 mg/kg) did not show a decrease in recovery. The CL-20 (1.5-100 mg/L) was also directly amended to aquatic test media, and the percentage of recoveries were determined. An interlaboratory study was used to validate the modified method and verify experimental results.					
15. SUBJECT TERMS					
CL-20	Environmental fate	HPLC	Energetic material	HMX	
Environmental transport	Chemical analysis	Quantitation	Nitramines	RDX	
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson
U	U	U	UL	26	19b. TELEPHONE NUMBER (include area code) (410) 436-2914

Blank

PREFACE

The work described in this report was authorized under Project No. 62262255200 and supported by the Strategic Environmental Research and Development Program (SERDP) (Arlington, VA). The work was started in December 2002 and completed in January 2004.

Records are maintained in official U.S. Army Edgewood Chemical Biological Center (ECBC) notebooks in the Life Sciences Official Archives and/or in the Technical Library. Studies were conducted under, and in compliance with, current good laboratory practice standards, and they were reviewed periodically by either the quality assurance coordinator or his designee. The performance of this study was consistent with the objectives and standards in "Good Laboratory Practices for Nonclinical Laboratory Studies" (21 CFR 58, Food and Drug Administration, U.S. Department of Health and Human Services, April 1988).

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Acknowledgments


This research was supported by the U.S. Department of Defense through the Strategic Environmental Research and Development Program. The authors thank ATK Thiokol Propulsion (Brigham City, UT) for providing the CL-20 material, and Jacqueline Scotto, ECBC, for quality assurance assistance.

QUALITY ASSURANCE

This study (Project SERDP-CP-1254) was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

<u>Phase Inspected</u>	<u>Date</u>	<u>Reported</u>
Analytical procedures	14 Jan 04	14 Jan 04
Data and Final Report	4 Jan 05	4 Jan 05

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.



DENNIS W. JOHNSON
Quality Assurance Coordinator
Toxicology, Aerosol Sciences and
Obscurants Senior Team
Research and Technology Dir.

CONTENTS

1.	INTRODUCTION	9
2.	MATERIALS AND METHODS.....	10
2.1	CL-20	10
2.2	Soil	10
2.3	Analytical Methods	10
2.4	Standard Preparation	11
2.5	Sample Preparation	11
2.5.1	ACN Extractions from Soil.....	11
2.5.2	Water Extractions from Soil	11
2.5.3	Aquatic Test Media.....	11
3.	RESULTS	12
3.1	Analytical Figures of Merit.....	12
3.2	CL-20 Extractions from SSL Soil.....	12
3.2.1	ACN Extractions.....	12
3.2.1.1	Freshly Amended CL-20 in SSL Soil	12
3.2.1.2	Weathered/Aged Amended CL-20 in SSL Soil	12
3.2.2	Water (ATCLP) Extractions	13
3.2.2.1	Freshly Amended CL-20 in SSL Soil	13
3.2.2.2	Weathered/Aged CL-20 in Amended SSL Soil	13
3.3	CL-20 Extractions from Aquatic Test Samples	13
3.4	Interlaboratory Study	13
4.	DISCUSSION.....	14
4.1	Recovery of CL-20 in ACN Extracts.....	14
4.2	Recovery of CL-20 Using Water (ATCLP) Extractions.....	15
4.3	Interlaboratory Study	15
5.	CONCLUSIONS.....	15
	LITERATURE CITED	25

FIGURES

1.	Chemical Structures of the Cyclic Nitramine Compounds RDX, HMX, and CL-20	16
2.	UV Spectrum of CL-20 with $\lambda_{\text{max}} = 230 \text{ nm}$	16
3.	Calibration Curve of CL-20 from 0.05 to 25 ppm with 50- μL Injection Volume	17
4.	Chromatograms of CL-20 Standards 1, 0.25, and 0.05 ppm with 50- μL Injection Volume	17
5.	Chromatogram of 0.05 ppm CL-20 Standard with its Associated S/N.....	18
6.	Recovery Percentages Determined from Amended SSL Soil ACN Extracts.....	18
7.	Recovery Percentages of Four Different Concentrations of CL-20 Amended to SSL Soil and Subjected to a 20-Week Weathering/Aging Process	19
8.	Recovery Percentages Determined from ATCLP (Water) Extractions of Amended SSL Soil	19
9.	Recovery Percentages Determined for Directly Amended Aquatic Media Tests 1.5-, 3.1-, 6.2-, 12.5-, 25-, 50-, and 100-mg/L Samples Extracted with Acidified ACN.....	20
10.	Recovery Percentages Determined from Several Split SSL Soil Samples Amended with Seven Different CL-20 Concentrations for Comparison of Analytical Results Between Three Different Laboratories: ECBC, Biotechnology Research Institute (BRI), and Pacific Northwest National Laboratory (PNNL).....	20

TABLES

1.	Physical and Chemical Characteristics of SSL Soil	21
2.	Reproducibility of Calibration Curve Slopes Over Four Months.....	21
3.	Recovery Percentages Determined for ACN Extractions of CL-20 Amended Concentrations in Fresh and Weathered/Aged SSL Soils.....	22
4.	Scheduled Monitoring. Recovery Percentages for all CL-20 Weathered/Aged in SSL Soils Extracted with ACN.....	22
5.	Recovery Percentages Determined for ATCLP (Water) Extractions of CL-20 from Freshly Amended and Weathered/Aged Amended SSL Soil	23
6.	Recovery Percentages Determined from CL-20 Directly Amended Aquatic Test Media Samples.....	23
7.	Recovery Percentages of CL-20 in Amended SSL Soil Samples Extracted with ACN (QA/QC Interlaboratory Study).....	24

Blank

HPLC DETERMINATION
OF HEXANITROHEXAAZAISOWURTZITANE (CL-20)
IN SOIL AND AQUEOUS MATRICES

1. INTRODUCTION

Nitramine explosives, such as 1,3,5,7-tetranitro-1,3,5,7-tetraaza-cyclooctane (HMX) and 1,3,5-trinitro-1,3,5-triazadiazocyclohexane (RDX) are toxic and possibly carcinogenic.^{1,2} They not only present a health hazard to military personnel who handle them but also pose an additional environmental hazard.¹ Explosive materials enter the environment through manufacturing, testing, detonation, and disposal, allowing them to contaminate soil and groundwater. Due to their toxicity and potential carcinogenic properties, it becomes important to identify and detect trace levels of explosives that may be present in the environment.^{1,2} The costs of managing large volumes of hazardous waste associated with energetic materials prompts the Department of Defense (DoD) to develop new materials that will have either equal or superior performance characteristics with less environmental impact.³

Hexanitrohexaazaisowurtzitane (CL-20) is a novel, energetic material, synthesized by Nielsen et al.^{4,5} and adopted by ATK Thiokol Propulsion (Brigham City, UT), that is being investigated as a potential replacement for currently used explosive and propellant formulations. CL-20 is a thermodynamically stable, highly energetic, polycyclic nitramine (N-NO₂) compound that is structurally similar to other monocyclic nitramine compounds [e.g., HMX and RDX (Figure 1)].^{6,7} However, CL-20 contains several unique chemical and structural properties including larger molecular size, unique caged structure, higher crystal density, and higher heat of formation that give CL-20 significant performance advantages over HMX and RDX.^{2,6,7,8} Structurally, because CL-20 does not contain halogens, subsequent studies may find combustion products to be environmentally more acceptable,³ however, the environmental impact of the CL-20 parent compound and the combustion/breakdown products have not yet been determined.

Recent investigations by Trott et al.² show that CL-20 biodegrades in laboratory soil microcosms. This biodegradation is different compared to other nitramine compounds (e.g., RDX). In addition, aerobic and anaerobic abiotic degradation reactions can occur in CL-20.⁹

The objectives of this project were to investigate the fate and transport of CL-20 through the vadose zone, and to investigate the environmental effects of CL-20 on terrestrial plants, soil organisms, and aquatic species. Fate and transport was investigated using a standardized soil-core microcosm system. In addition to freshly amended soil, the effects of weathering/aging were incorporated into the experimental design to better reflect the real-world potential of CL-20 undergoing biotic/abiotic degradation and transformation processes. The principal objective of this report was to focus on the quantitative chemical analysis of CL-20 in amended natural sandy loam soil and in aquatic test media. The quantitation of CL-20 has been performed using a method, developed by the Biotechnology Research Institute (BRI) of the National Research Council (NRC), Canada,¹⁰ which is a modification of the modified

U.S. Environmental Protection Agency (USEPA) Method 8330A.¹¹ This modified method was applied to artificially amended soil and aquatic samples because CL-20 has not been used in full production, and is not known to have been released into the environment yet.

2 MATERIALS AND METHODS

2.1 CL-20.

The ϵ -polymorph ($\geq 95\%$ by Fourier Transform Infrared Spectroscopy)* of CL-20 (2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane) used in these experiments (lot number 2180150) was obtained from ATK Thiokol Propulsion. The lot number is actually a grind number of combined nitration lots 590N-01-0002, -0003, -0004, -0005, -0006, -0008, -0009, -0010, -0011, -0012, -0013, and -0014 with a purity of 99.3% as determined by High Performance Liquid Chromatography (HPLC) analysis.* CL-20 is an odorless, white crystalline solid¹² that has a low solubility in water (4.8 mg/L)^{2,8} compared to RDX and HMX (38.4 mg/L and 6.6 mg/L, respectively).²

2.2 Soil.

The soil chosen for this research was Sassafras Sandy Loam [SSL (fine-loamy, siliceous, mesic typic hapludult)] collected from a grassy field (M-Field) at the Edgewood Area, Aberdeen Proving Ground, MD. The physical and chemical characteristics of this soil (low clay and organic matter) support a relatively high level of CL-20 bioavailability. Therefore, this soil is a fitting type for assessing stability, chemical/biochemical degradation, transport, and toxicity of CL-20. The physical and chemical characteristics of the soil are given in Table 1.¹³

In this report, analytical determinations for freshly amended soils were amended with a known amount of CL-20, hydrated to a specified percentage of the water holding capacity (WHC), and extracted following a 24-hr equilibration period. The WHC of SSL soil is 18% of the dry soil mass. Weathered/aged CL-20 in soils refers to soils with a known amount of CL-20 added that were subjected to alternate wetting/drying cycles either in a greenhouse or in the controlled environmental chamber over a specified period of time.

2.3 Analytical Methods.

The instrumentation used for detecting CL-20 was an Agilent (Wilmington, DE) 1100 Series HPLC equipped with a Supelcosil LC-CN column (25 cm x 4.6 mm x 5 μ m). An isocratic 70:30 methanol:water mobile phase was used with a flow rate of 1.0 mL/min and a 50 μ L injection volume. The autosampler was set at 10 °C. Detection of CL-20 was accomplished with a Diode Array Detector (DAD) set at 230 nm (λ_{max}). The UV spectrum for CL-20 is shown in Figure 2 as provided by Larson, et. al.¹⁴

* Spaulding, K. Thiokol Propulsion, Brigham City, UT. Personal communication, 2001.

2.4 Standard Preparation.

All standards were prepared using HPLC grade acetonitrile (ACN). Initially, a primary stock solution (10,000 ppm CL-20) was prepared and diluted to make solutions of 50, 20, 2, 0.5, and 0.1 ppm CL-20/ACN. To make the calibration standards, further dilutions were made with acidified water (sodium bisulfate) solution (50:50) to yield standards of 25, 10, 1, 0.25, and 0.05 ppm CL-20/ACN/H₃O⁺.

2.5 Sample Preparation.

2.5.1 ACN Extractions from Soil.

Sassafras Sandy Loam soils were amended with nominal CL-20 concentrations of 0.01 to 100 mg/kg (CL-20/soil). Ten milliliters of ACN (5 mL for amendments <0.5 mg/kg) were added to a 2 g soil sample, vortexed for 1 min, and sonicated in an ultrasonic bath at 20 °C for 18 ± 2 hr. The samples were then centrifuged at 2700 rpm for 30 min. Five milliliters of the supernatant was removed and combined with 5 mL of CaCl₂/NaHSO₄ (5 and 0.2 g/L, respectively) aqueous solution. For amendments <0.5 mg/kg, 3 mL of the supernatant was mixed with 3 mL of CaCl₂/NaHSO₄. The solution was then vortexed for 10-15 s and allowed to stand for 30 min. The supernatant was filtered using 10 mL disposable syringes (Becton Dickinson, Franklin Lakes, NJ) equipped with 0.45 µm Millipore PTFE filters. The first 3 mL of filtrate were discarded before transferring 1 mL of the extract to an HPLC autosampler vial. This extraction technique was applied to freshly amended and weathered/aged CL-20 in SSL soils.

2.5.2 Water Extractions from Soil.

The SSL soil samples were extracted using an Adapted Toxicity Characteristic Leaching Procedure (ATCLP).¹⁵ For terrestrial toxicity tests, ATCLP-based estimates were used to measure the CL-20 concentrations in soil that was presumed to be immediately available to either terrestrial plants or soil invertebrates. The modification involved substituting the CO₂ saturated American Society for Testing and Materials (ASTM) Type I water for acetic acid, better simulating soil-water conditions due to respiration by soil biota.

Soils were amended with nominal CL-20 concentrations of 0.01 - 100 mg/kg (CL-20/soil). Extractions were performed similarly to the ACN extractions, except 20 mL of CO₂-water (10 mL for amendments <0.5 mg/kg) was used to extract the CL-20 from the soil (5.5-g sample), and an acidified ACN solution (H₃O⁺/ACN) was added after the supernatant was removed instead of the CaCl₂/NaHSO₄ solution. For amendments <0.5 mg/kg, 5 mL of the supernatant was mixed with 5 mL of acidified ACN solution. This extraction technique was applied to freshly amended and weathered/aged CL-20 in SSL soils.

2.5.3 Aquatic Test Media.

The aquatic test media solutions were prepared by adding solid CL-20 (100 mg) with a known volume of well water. The solution was shaken, sonicated, and mixed for 10 min.

Serial dilution was performed followed by extraction. To extract the CL-20 from aquatic samples, 5 mL of the sample was added to a vial followed by 5 mL of acidified ACN. Further sample preparation was similar to that followed for the soil samples.

3 RESULTS

3.1 Analytical Figures of Merit.

A five-point calibration curve was created from standards (0.05, 0.25, 1.0, 10, and 25 ppm) with excellent linearity ($r^2 > 0.99999$). Figure 3 shows an example of a calibration curve with its associated correlation coefficient (r^2 value) and equation of the line. The reproducibility of the slope over a 4-month period was determined to be 149.0 ± 5.0 with a % RSD of 3.4 ($n = 14$, Table 2). Figure 4 shows representative chromatograms for three of the five standards (1, 0.25, and 0.05 ppm). The lowest standard used (0.05 ppm) was nearly four times greater than the instrument's limit of detection (LOD) of 0.01 ppm ($S/N = 3$) as given in Figure 5.

3.2 CL-20 Extractions from SSL Soil.

Three extracts were prepared at each amended concentration for subsequent data analysis and statistical interpretation. The HPLC analysis was used to determine the analytical recovery percentages as compared against the nominally amended concentrations. According to the modified USEPA Method 8330A (NRC), calibration curves were run before and after each set of soil sample extracts each day to assure that the detector response was consistent ($\pm 3\%$). Standards were also interspersed among sample sequences to assure reproducibility.

3.2.1 ACN Extractions.

3.2.1.1 Freshly Amended CL-20 in SSL Soil.

Fifteen freshly amended CL-20 soil concentrations of 0.01 to 100 mg/kg were extracted with ACN and analyzed by HPLC. The LOD, in freshly amended soil, was 0.08 mg/kg. At this amended soil concentration, as calculated from the equation below, the corresponding CL-20 solution concentration was 0.04 mg/L. This concentration is four times greater than the instrument's detection limit of 0.01 mg/L. All amended concentrations at or above 0.08 mg/kg had recoveries $>97\%$ (Figure 6, Table 3).

$$\text{mg CL-20 /kg soil} = \frac{\text{mg/L} \times \text{extraction volume (l)}}{\text{dry soil weight (kg)}}$$

3.2.1.2 Weathered/Aged Amended CL-20 in SSL Soil.

The same 15 amended CL-20 concentrations (0.01 - 100 mg/kg) used in the freshly amended soil ACN extractions were then subjected to CL-20 amended soil, which had been subjected to a 5-month weathering/aging process. The soil samples were again extracted

with ACN and analyzed by HPLC. The LOD in weathered/aged CL-20 in soils was 0.1 mg/kg, which corresponds to a CL-20 solution concentration of 0.02 mg/L (previous equation). This concentration is two times greater than the instrument LOD of 0.01 mg/L. All amended concentrations at or above 0.1 mg/kg had recovery percentages ranging from 45 to 73% (Figure 6, Table 3).

Separate soil samples with CL-20 amended concentrations of 0.5, 1, 10, and 100 mg/kg were used to further investigate the weathering/aging process. Monitoring involved extracting six samples of each concentration over a 5-month period. Recovery percentages from these ACN soil extractions were determined for all amended concentrations (Figure 7, Table 4).

3.2.2 Water (ATCLP) Extractions.

3.2.2.1 Freshly Amended CL-20 in SSL Soil.

Fourteen freshly amended CL-20 soil concentrations of 0.01 - 50 mg/kg were extracted with water and analyzed by HPLC. The LOD in soil determined from the ATCLP extractions was 0.1 mg/kg. This corresponds to an analytical concentration (solution) of approximately 0.02 mg/L (previous equation). All amended concentrations at or above 0.1 mg/kg had recovery percentages ranging from 20 to 59% (Figure 8, Table 5).

3.2.2.2 Weathered/Aged CL-20 in Amended SSL Soil.

The same 14 amended CL-20 concentrations (0.01 - 50 mg/kg) used in the freshly amended soil ATCLP extractions were then subjected to a weathering/aging process over a 5-month period. The soil samples were then extracted with water (ATCLP) and analyzed by HPLC. The LOD in weathered/aged soils was 0.25 mg/kg, which corresponds to a CL-20 solution concentration of 0.02 mg/L (previous equation). This concentration is two times greater than the instrument LOD of 0.01 mg/L. All amended concentrations at or above 0.25 mg/kg had recovery percentages ranging from 17 to 31% (Figure 8, Table 5).

3.3 CL-20 Extractions from Aquatic Test Samples.

The aquatic test media was directly amended with seven CL-20 concentrations (1.5 - 100 mg/L). Recovery percentages >75% were observed for five amended concentrations ranging from 6.2 to 100 mg/L. Lower recovery percentages were observed for 1.5 and 3.1 mg/L. All recovery percentages are shown in Figure 9 with the data summarized in Table 6.

3.4 Interlaboratory Study.

An interlaboratory quality control/quality assurance (QC/QA) study was performed on split samples of CL-20 amended SSL soil to compare analytical results of the three research groups (ECBC, BRI, and PNNL) working on the CL-20. The purpose of using three separate laboratories was to provide evidence of the reliability of the modified analytical method

for CL-20. Four freshly amended soil samples with CL-20 concentrations ranging from 0.1 to 10 mg/kg, and three amended soil samples (undergoing weathering/aging) ranging from 100 to 10000 mg/kg were extracted with ACN and analyzed by HPLC (Figure 10, Table 7).

4 DISCUSSION

4.1 Recovery of CL-20 in ACN Extracts.

The recovery percentages from freshly amended and weathered/aged amended SSL soils extracted with ACN are graphed versus their amended concentrations (Figure 6). The 5-month weathered/aged amended SSL soil concentrations had much lower recovery percentages compared to the freshly amended soil concentrations. The data from freshly amended and weathered/aged amended CL-20 SSL soil ACN extracts were statistically different at the 95% confidence level (CL) for all amended concentrations. The LODs of CL-20 in freshly amended and weathered/aged CL-20 amended SSL soils also reflect this trend (0.08 mg/kg freshly amended and 0.10 mg/kg weathered/aged amended). Decreases in CL-20 concentrations in amended soils subjected to the weathering/aging procedures suggest that the chemical environment of CL-20 amended soil can be altered during weathering/aging, similar to changes that can occur in vadose zone soil environments in the field. Specific mechanisms of such decrease are not known and require additional investigations. Information available suggests that biotic and abiotic degradation of the parent compound as well as sorption to soil constituents are among the factors contributing to CL-20's fate in soils.^{2,9}

Yinon performed electron ionization (EI) mass spectrometry (MS) on HMX and RDX¹⁶ to determine degradation products and identify possible reaction schemes. The EI mass spectrometric decomposition of explosives can parallel the decomposition of energetic materials by explosive shock.¹⁶ Many of the products are formed either from reduction of the nitro groups or from ring cleavage/rearrangement. With CL-20, it is thought that cage opening occurs through breaking a particular C-C bond to form an aromatic 3-ring compound and losing several nitro groups. Further loss of nitro groups forms a 2-ring aromatic compound. Liquid chromatography/MS/MS (LC/MS/MS) has been used to help identify some of CL-20's degradation products⁹ along with investigating additional pathways that might form under low pH conditions with small amounts of water present.

The CL-20 concentrations in SSL soil amended with 0.5, 1, 10, and 100 mg/kg nominal concentrations were monitored during a 20-week weathering/aging procedure (Figure 7). The recovery percentages in all concentrations on week 1 were statistically different from the recovery percentages in the same concentrations on week 20. The CL-20 concentrations begin to decrease between week 6 and week 15; but, the recovery percentages were statistically equivalent at all amended concentrations from weeks 1 to 6 and weeks 15 to 20 (95% CL). This decrease could be attributed to either degradation of the parent compound or sorption-to-soil constituents previously discussed.

Soil samples amended at concentrations of 1000 and 10000 mg/kg CL-20 were also subjected to a weathering/aging process (data not shown) for 5 and 15 months. Recoveries >90% were determined at months 5 and 15 and were statistically equivalent (95% CL). These results show that at or above 1000 mg/kg, CL-20 is more persistent compared with lower amended concentrations and can pose a greater risk of environmental impacts in case of accidental release.

4.2 Recovery of CL-20 Using Water (ATCLP) Extractions.

The recovery percentages from water extracts of freshly amended and weathered/aged CL-20 amended SSL soils using the ATCLP method are graphed versus their amended concentrations (Figure 8). This figure shows that CL-20 weathered/aged soils had lower recovery percentages than did the freshly amended soils. The data from the weathered/aged and freshly amended soils extracted using the ATCLP method were analyzed using the paired t-test and were statistically different at all concentrations (95% CL). Due to CL-20's low solubility in water, recovery percentages of freshly and weathered/aged amended CL-20 in SSL soil using ATCLP water-extracted soils were considerably lower than the recovery percentages from the corresponding freshly and weathered/aged amended SSL soil in ACN extracted soils.

4.3 Interlaboratory Study.

The recovery percentages for all amended concentrations determined from the three participating laboratories are presented in Figure 10. Paired t-tests were performed and comparisons made between the three laboratories. The recovery percentages determined by BRI and PNNL were consistently higher than the recovery percentages determined by ECBC except for the 0.5 mg/kg amendment. The data from ECBC and BRI at the 99% CL showed that there were no significant differences in the results at all amended concentrations. The data from ECBC and PNNL showed that there were no significant differences in the amended concentrations except for the 100 and 1000 mg/kg (99% CL). Comparisons between BRI and PNNL showed that there were no significant differences in the results (95% CL).

5 CONCLUSIONS

The high performance liquid chromatography analysis has been successfully used to quantitate CL-20 concentrations using the modified U.S. Environmental Protection Agency Method 8330A. The method has been used to determine amended concentrations of CL-20 to soil and aquatic test media in support of ongoing environmental projects. The decrease in CL-20 recovery from weathered/aged CL-20 in soil has been observed. Analytical results have been validated by conducting an interlaboratory study for quality control/quality assurance results. Determinations of CL-20 concentrations in soil and aquatic media confirmed the validity of the modified method.

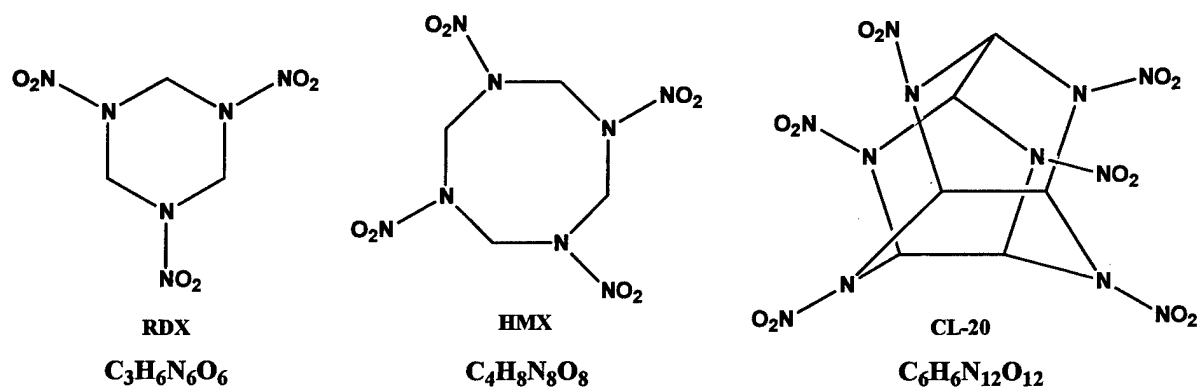


Figure 1. Chemical Structures of the Cyclic Nitramine Compounds RDX, HMX, and CL-20.

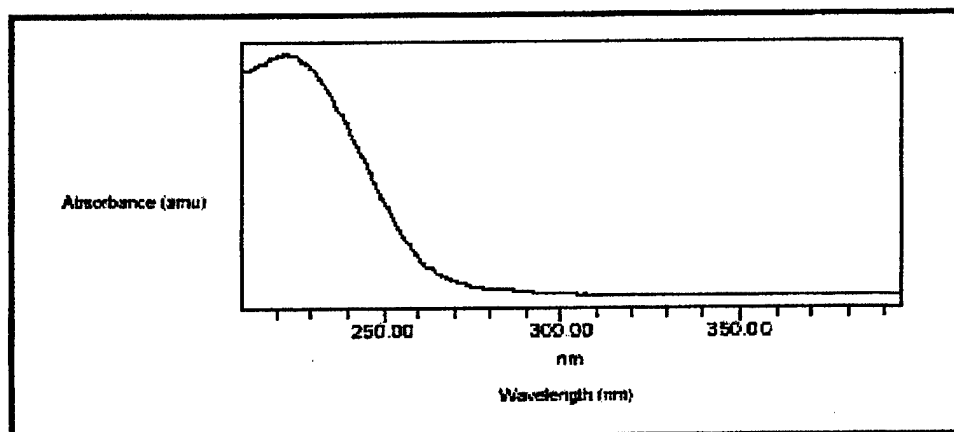


Figure 2. UV Spectrum of CL-20 with $\lambda_{\max} = 230$ nm (Reference 15).

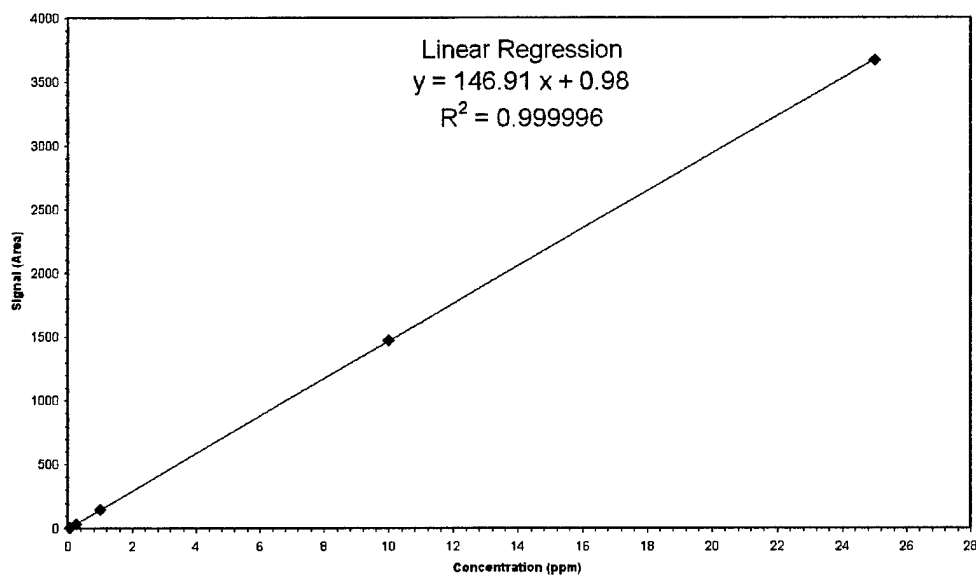


Figure 3. Calibration Curve of CL-20 from 0.05 to 25 ppm with 50- μ L Injection Volume. Separation as discussed in Section 2.3.

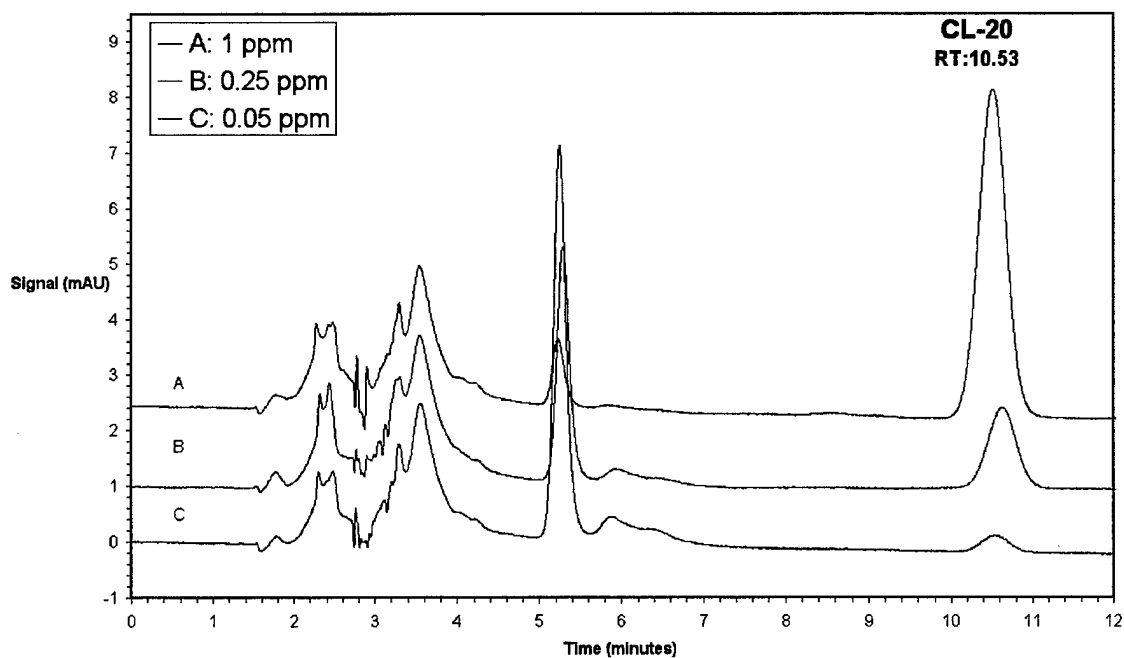


Figure 4. Chromatograms of CL-20 Standards 1, 0.25, and 0.05 ppm with 50- μ L Injection Volume. Separation as discussed in Section 2.3.

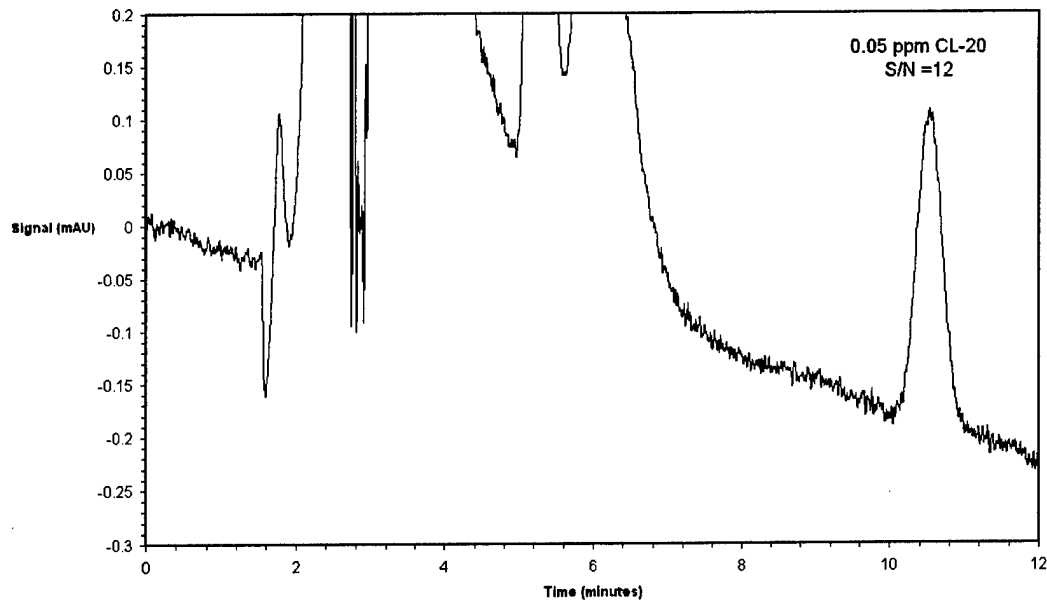


Figure 5. Chromatogram of 0.05 ppm CL-20 Standard with its Associated S/N. Separation as discussed in Section 2.3.

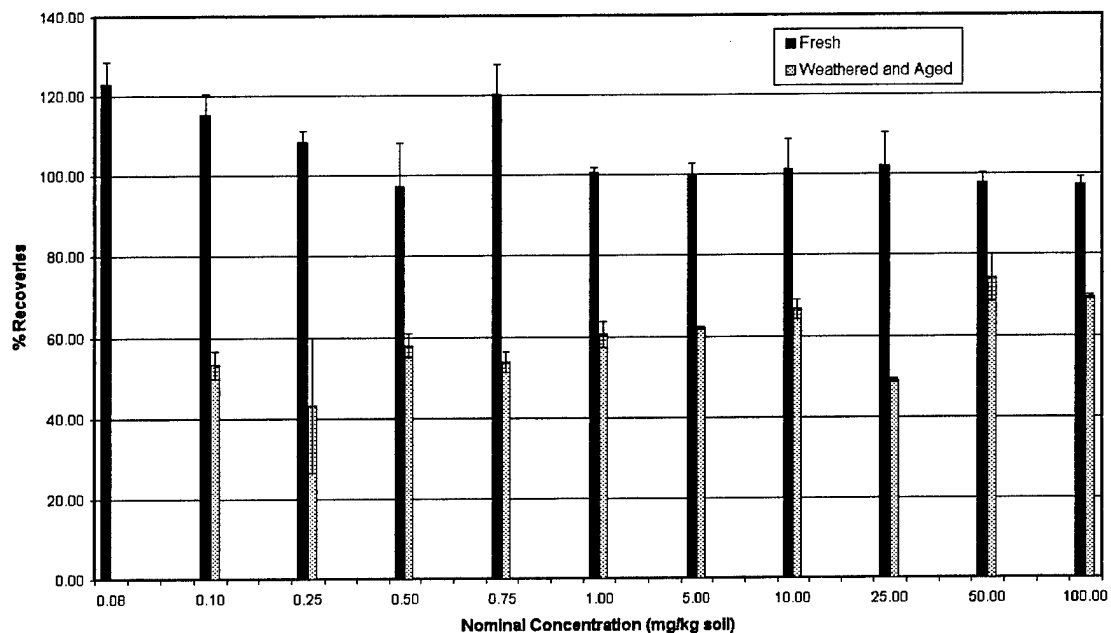


Figure 6. Recovery Percentages Determined from Amended SSL Soil ACN Extracts. ACN extractions on freshly amended soils at 15 different CL-20 concentrations (dark bars). Recovery percentages determined from the same CL-20 amended soils after a 5-month weathering/aging process are also given (light-colored bars). Error bars represent the standard deviation of the mean (three replicates).

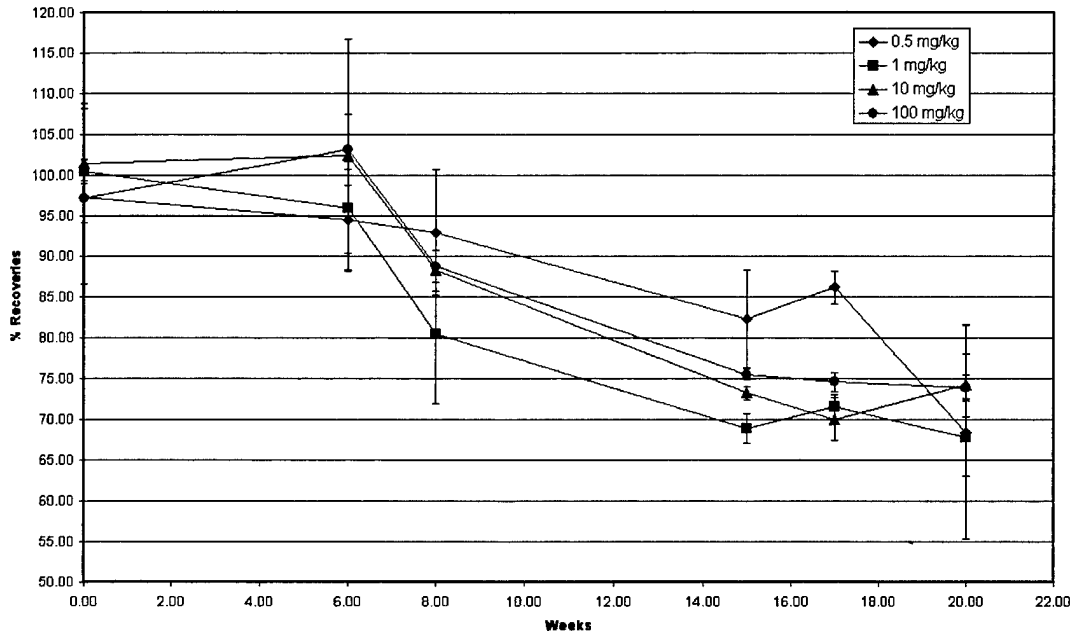


Figure 7. Recovery Percentages of Four Different Concentrations of CL-20 Amended to SSL Soil and Subjected to a 20-Week Weathering/Aging Process. Soils were extracted six different times during a 20-week period. Error bars represent the standard deviation of the mean (three replicates).

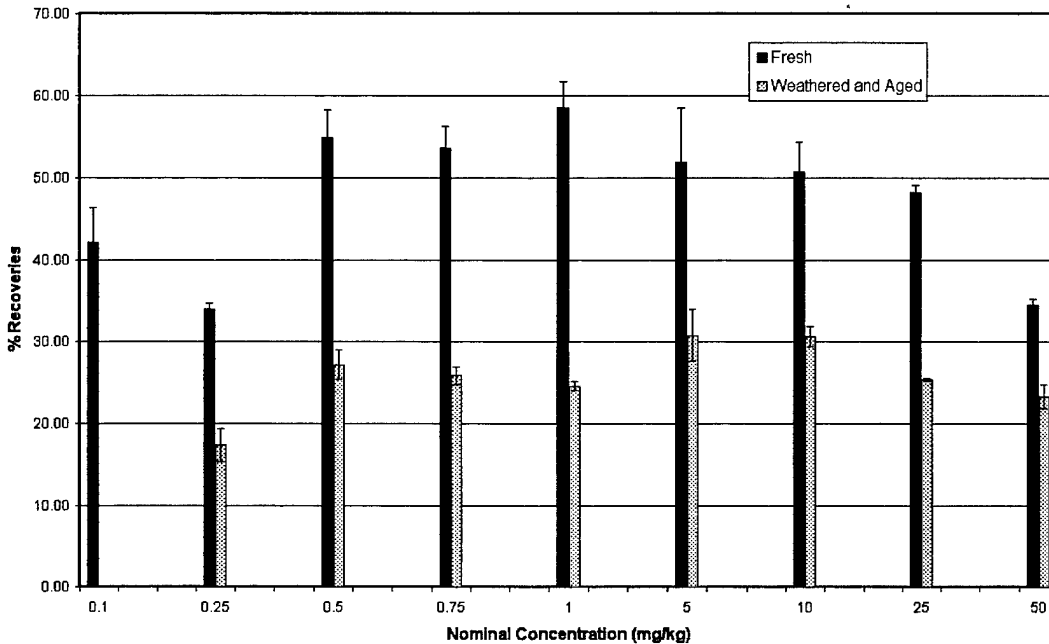


Figure 8. Recovery Percentages Determined from ATCLP (Water) Extractions of Amended SSL Soil. Water extractions applied to 15 different freshly amended CL-20 concentrations (dark bars). Recovery percentages determined from the same soils after a 5-month weathering/aging process are also given (light-colored bars). Error bars represent the standard deviation of the mean (three replicates).

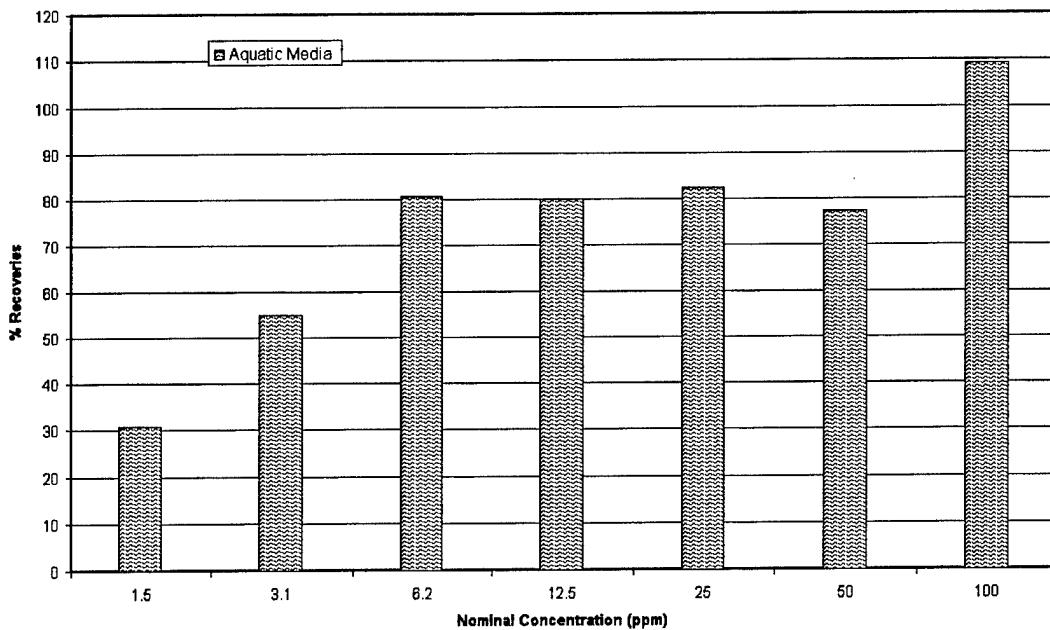


Figure 9. Recovery Percentages Determined for Directly Amended Aquatic Media Tests 1.5-, 3.1-, 6.2-, 12.5-, 25-, 50-, and 100-mg/L Samples Extracted with Acidified ACN.

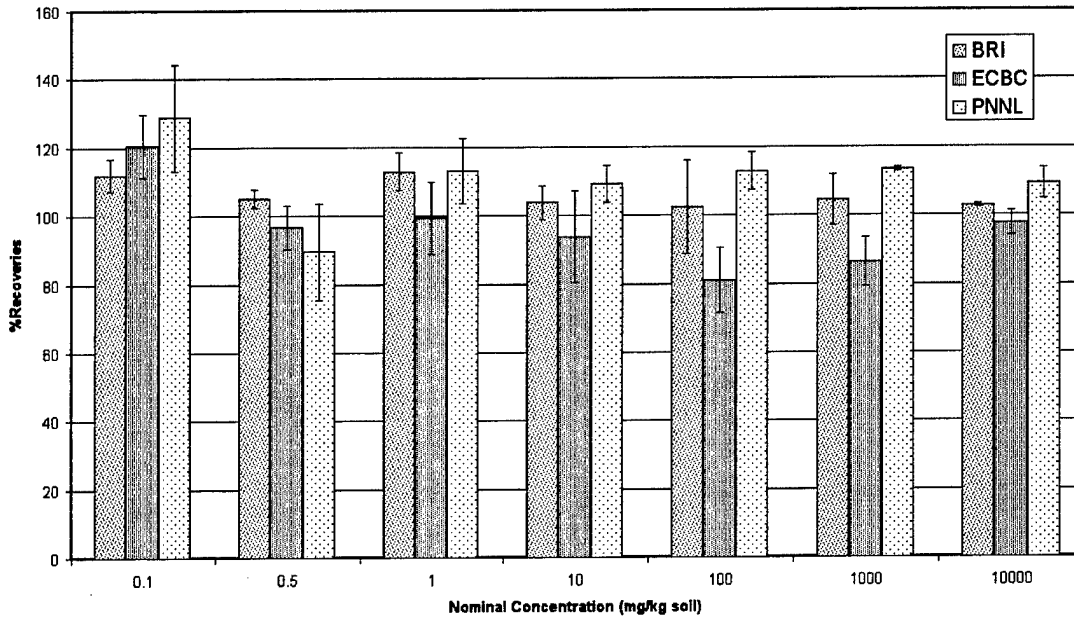


Figure 10. Recovery Percentages Determined from Several Split SSL Soil Samples Amended with Seven Different CL-20 Concentrations for Comparison of Analytical Results Between Three Different Laboratories: ECBC, Biotechnology Research Institute (BRI), and Pacific Northwest National Laboratory (PNNL). Extraction was accomplished with ACN. Error bars represent the standard deviation of the mean (number of replicates equal to three).

Table 1. Physical and Chemical Characteristics of SSL Soil.

Soil Parameter	<i>Sassafras Sandy Loam</i>
Sand %	70
Silt %	13
Clay %	17
Texture %	Sandy loam
CEC cmol kg ⁻¹	5.49
Organic matter %	1.3
pH	5.2

Table 2. Reproducibility of Calibration Curve Slopes Over 4 Months.

<i>Calibration Curve</i>	<i>Slope (m)</i>	<i>Date taken</i>
1	144.54	052203
2	143.67	060203
3	144.70	060603
4	147.30	061703
5	150.84	062603
6	157.92	071803
7	157.58	072403
8	157.76	073103
9	146.96	081803
10	146.27	091103
11	147.18	091603
12	148.13	092203
13	146.48	092603
14	146.48	092603
Average	149.00	
Stdev	5.0	
%RSD	3.4	

Table 3. Recovery Percentages Determined for ACN Extractions of CL-20 Amended Concentrations in Fresh and Weathered/Aged SSL Soils.

<i>Nominal (mg/kg)</i>	<i>Average % Recovery Fresh</i>	<i>SD*</i>	<i>Average % Recovery W/A</i>	<i>SD</i>
0.01	N/A**	N/A	N/A	N/A
0.02	N/A	N/A	N/A	N/A
0.03	N/A	N/A	N/A	N/A
0.06	N/A	N/A	N/A	N/A
0.08	122.96	5.8	N/A	N/A
0.10	115.25	5.2	53.20	3.4
0.25	108.37	2.9	43.18	16.8
0.50	97.37	10.8	57.93	2.9
0.75	120.19	7.6	53.76	2.6
1.00	100.51	1.4	60.55	3.1
5.00	99.87	3.0	62.05	0.5
10.00	101.45	7.4	66.65	2.4
25.00	102.10	8.4	49.00	0.6
50.00	97.91	2.5	74.21	5.8
100.00	97.23	2.2	69.46	0.6

* Not applicable

** Standard Deviation (amended soil concentration was below instrumental LOD)

Table 4. Scheduled Monitoring. Recovery Percentages for all CL-20 Weathered/Aged in SSL Soils Extracted with ACN.

<i>Week</i>	<i>0.5 mg/kg % Recovery</i>	<i>SD</i>	<i>1 mg/kg % Recovery</i>	<i>SD</i>	<i>10 mg/kg % Recovery</i>	<i>SD</i>	<i>100 mg/kg % Recovery</i>	<i>SD</i>
0	97.37	10.8	100.51	1.4	101.45	7.4	97.23	2.2
6	94.52	6.3	96.01	5.7	102.41	14.3	103.13	4.3
8	92.95	7.7	80.49	8.5	88.24	2.5	88.81	2.0
15	82.34	6.0	68.92	1.9	73.25	0.8	75.53	0.7
17	86.16	2.1	71.54	1.5	70.01	2.6	74.60	1.2
20	68.47	13.1	67.78	4.8	74.24	3.9	73.88	1.6

Table 5. Recovery Percentages Determined for ATCLP (Water) Extractions of CL-20 from Freshly Amended and Weathered/Aged Amended SSL Soil.

<i>Nominal Concentration (mg/kg)</i>	<i>Average % Recovery Fresh</i>	<i>SD</i>	<i>Average % Recovery W/A</i>	<i>SD</i>
0.01	N/A	N/A	N/A	N/A
0.02	N/A	N/A	N/A	N/A
0.03	N/A	N/A	N/A	N/A
0.06	N/A	N/A	N/A	N/A
0.08	N/A	N/A	N/A	N/A
0.10	42.15	4.2	N/A	N/A
0.25	34.04	0.7	17.41	2.0
0.50	54.88	3.3	27.17	1.9
0.75	53.68	2.6	25.85	1.1
1.00	58.53	3.3	24.55	0.5
5.00	51.90	6.6	30.78	3.2
10.00	50.80	3.6	30.62	1.3
25.00	48.27	0.9	25.32	0.2
50.00	34.44	0.8	23.29	1.4

Table 6. Recovery Percentages Determined from CL-20 Directly Amended Aquatic Test Media Samples.

<i>Nominal Concentration (mg/L)</i>	<i>Average % Recoveries</i>
1.5	30.67
3.1	54.84
6.2	80.65
12.5	80.00
25	82.40
50	77.40
100	109.15

Table 7. Recovery Percentages of CL-20 in Amended SSL Soil Samples Extracted with ACN (QA/QC Interlaboratory Study).

<i>Nominal Concentration (mg/kg)</i>	<i>Average % Recoveries BRI</i>	<i>SD</i>	<i>Average % Recoveries ECBC</i>	<i>SD</i>	<i>Average % Recoveries PNNL</i>	<i>SD</i>
0	0	0.0	0.00	0.0	0.0	0.0
0.1	111.82	4.7	120.52	9.3	128.70	15.6
0.5	105.07	2.8	96.67	6.4	89.56	14.1
1	112.86	5.5	99.33	10.5	113.10	9.4
10	103.81	5.0	93.87	13.3	109.23	5.3
100	102.44	13.6	80.92	9.5	112.89	5.4
1000	104.44	7.5	86.39	7.2	113.41	0.7
10000	102.85	0.3	97.73	3.6	109.15	4.5

LITERATURE CITED

1. Yinon, J. *Forensic and Environmental Detection of Explosives*; John Wiley and Sons, Ltd: New York, 1999; pp 186-187.
2. Trott, S.; Nishino, S.F.; Hawari, J.; Spain, J. Biodegradation of the Nitramine Explosive CL-20. *Appl. Environ. Microbiol.* **2003**, *69*(3), pp 1871-1874.
3. <http://www.serdp.org/02SON/PPSON-02-01.html> (accessed December 2000).
4. Nielsen, A.T.; Christian, S.L.; Moore, D.W.; Gilardi, R.; George, C.F. Synthesis of 3,5,12-Triazawurtzitanes (3,5,12-Triazatetracyclo[5.3.1.1^{2,6}.0^{4,9}] Dodecanes). *J. Org. Chem.* **1987**, *52*(9), pp 1656-62.
5. Nielsen, A.T.; Nissan, R.A.; Vanderah, D.J.; Coon, C.L.; Gilardi, R.D.; George, C.F.; Flippen-Anderson, J. Polyazapolycyclics by Condensation of Aldehydes with Amines. 2. Formation of 2,4,6,10,12-Hexabenzyl-2,4,6,8,10,12-Hexaazatetracyclo-[5.5.0.0^{5,9}.0^{3,11}] Dodecanes from Glyoxal and Benzylamines. *J. Org. Chem.* **1990**, *55*(5), pp 1459-66.
6. Bazaki, H.; Kawabee, S.; Miya, H.; Kodama, T. Synthesis and Sensitivity of Hexanitrohexaazaisowurtzitanane (HNIW). *Propellants, Explos., Pyrotech.* **1998**, *23*, pp 333-336.
7. Nielsen, A.T.; Chafin, A.P.; Christian, S.L.; Moore, D.W.; Nadler, M.P.; Nissan, R.A.; Vanderah, D.J.; Gilardi, R.D.; George, C.F.; Flippen-Anderson, J.L. Synthesis of Polyazapolycyclic Caged Polynitramines. *Tetrahedron.* **1998**, *54*, pp 11793-11812.
8. <http://www.cee.stevens-tech.edu/ResProj.html> (accessed June 2002).
9. Szecsody, J.E.; Girvin, D.C.; Devary, B.D.; Campbell, J.A.; Fredrickson, H.L.; Crocker, F.H.; Qasim, M.M.; Riley, R.A.; Gamerdinger, A.P.; Lemond, L.A. *Factors Effecting the Fate and Transport of CL-20 in the Vadose Zone and Groundwater: Progress Report for 2003*; SERDP Annual Report CP-1255; Pacific Northwest National Laboratory: Richland, WA, 2003.
10. Hawari, J.; Beaulieu, C.; Deschamps, S.; Paquet, L.; Monteil-Riviera, F. *CL-20 Analysis By High Performance Liquid Chromatography (HPLC)*; National Research Council: Canada, 2002.
11. <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8330a.pdf> (accessed June 2002).
12. CL-20; Materials Safety Data Sheet. Thiokol Propulsion, Brigham City, UT, 2001.

13. Kuperman, R.; Checkai, R.; Simini, M.; Phillips, C.; Anthony, J.S; Haley, M.; Kolakowski, J.; Chester, N.; Kurnas, C.; Davis, E. *Environmental Fate and Transport of a New Energetic Material CL-20*; SERDP Annual Report CP-1254; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2003.

14. Larson, S.; Felt, D.; Escalon, L.; Davis, J.; Hansen, L. *Analysis of CL-20 in Environmental Matrices: Water and Soil*; ERDC/EL TR-01-21; U.S. Army Corps of Engineers Engineer Research and Development Center: Vicksburg, MS, 2001.

15. Haley, M.V.; Checkai, R.T.; Kurnas, C.W.; Wentzel, R.S.; Nwanguma, R.O.; Sadusky, M. *Toxicity Determination of Explosive Contaminated Soil Leachates to Daphnia magna Using an Adapted Toxicity Characteristic Leaching Procedure*; ERDEC-TR-030; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1993; UNCLASSIFIED Report (AD-A270 410).

16. Yinon, J. Mass Spectrometry of Explosives: Nitro Compounds, Nitrate Esters, and Nitramines. *Mass Spectrom. Rev.* **1982**, *1*, pp 257-307.