

Sample Handling and Analysis Method for Chemical Warfare Agents in Soils Contaminated with Chemical and/or Biological Warfare Agents

P. A. D'Agostino, C. L. Chenier and J. R. Hancock
DRDC Suffield

Defence R&D Canada – Suffield

Technical Memorandum

DRDC Suffield TM 2003-025

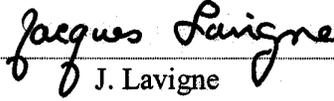
April 2003

Author



P. A. D'Agostino

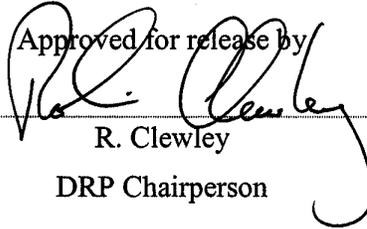
Approved by



J. Lavigne

H/CBDS

Approved for release by/



R. Clewley

DRP Chairperson

Abstract

A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated using three soil samples spiked at the 80 : g/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure. This sample handling and analysis method for soil (or other) samples will form a cornerstone of the chemical/biological warfare agent identification strategy being developed at DRDC Suffield to meet National commitments.

Résumé

Une méthode pour manipuler et analyser les échantillons a été mise au point et vise à identifier les agents chimiques de guerre (comme composés intacts ou comme les produits de leur hydrolyse) dans des échantillons suspects de contenir des agents de contamination biologique ou chimique de guerre. La méthode, basée sur une extraction aqueuse, la stérilisation en autoclave d'un extrait aqueux et une analyse CPL- IPE- SM (chromatographie en phase liquide – ionisation par pulvérisation d'électrons -spectrométrie de masse), a été évaluée en utilisant trois échantillons de sols semés à un niveau de 80 : g/g avec du phosphate d'éthyle, de l'acide isopropyle méthylphosphonique ou du thiodiglycol. Ces trois composés ont été facilement extraits et identifiés, après l'analyse CPL-IPE-SM, avec le phosphate d'éthyle subissant une hydrolyse durant la procédure de stérilisation en autoclave. Cette méthode de manipulation et d'analyse d'échantillons pour les sols (ou autres) sera une étape primordiale dans la stratégie d'identification d'agent de guerre chimique et biologique qui est mise au point à RDDC Suffield et vise à répondre aux engagements souscrits par le pays.

This page left intentionally blank.

Executive summary

Introduction: The Canadian Forces (CF) may be called on to perform peacekeeping or battlefield operations in regions of the world where there is a significant threat of chemical/biological (CB) warfare agent use. To operate effectively in these theatres the CF must be able to identify the CB agent used. Mass spectrometry (MS), is a powerful analytical technique for the identification of both known and unknown compounds and DRDC Suffield is currently investigating this instrumental technique in fulfillment of CF detection and identification requirements.

Results: A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated using three soil samples spiked at the 80 : g/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure.

Significance: A sample handling and analysis method for soil (or other) samples was developed as part of a chemical/biological warfare agent identification strategy for the G8 Summit held in Kananaskis (2002). This sample handling and analysis method for soil (or other) samples will form a cornerstone of the CB warfare agent identification strategy being developed at DRDC Suffield to meet CF and National commitments and will be incorporated into the Standard Operating Procedures of the DRDC Suffield Chemical Biological Forensic Reference Laboratory.

Future Plans: The reported method will be a valuable addition to the present methods for the identification of chemical warfare agents and their hydrolysis products in samples collected by the Canadian Forces, RCMP or in support of Chemical Weapons Convention challenge inspections. The application of tandem mass spectrometry to sample containing CB contamination is anticipated upon installation of a new instrument at DRDC Suffield.

D'Agostino, P.A., Chenier, C.L., Hancock, J.R. 2003. Sample Handling and Analysis Method for Chemical Warfare Agents in Soils Contaminated with Chemical and/or Biological Warfare Agents. DRDC Suffield TM 2003-025.

Sommaire

Introduction: Les Forces canadiennes (FC) peuvent être appelées à entreprendre des opérations de maintien de la paix ou de champ de bataille dans des régions du monde où existe une menace importante d'utilisation d'agents chimiques et biologiques de guerre (CB). Pour être capable d'opérer efficacement dans ces théâtres, les FC doivent être capables d'identifier les agents CB utilisés. La spectrométrie de masse (SM) est une technique analytique puissante pour l'identification des composés connus et inconnus et RDDC Suffield examine actuellement cette technique qui est instrumentale pour satisfaire aux besoins de détection et d'identification des FC.

Résultats : Une méthode pour manipuler et analyser les échantillons a été mise au point et vise à identifier les agents chimiques de guerre (comme composés intacts ou les produits de leur hydrolyse) dans des échantillons suspects de contenir des agents de contamination de guerre biologique ou chimique. La méthode, basée sur une extraction aqueuse, la stérilisation en autoclave d'un extrait aqueux et une analyse CPL-IPE-SM, a été évaluée en utilisant trois échantillons de sols semés à un niveau de 80 : g/g avec du phosphate d'éthyle, de l'acide isopropyle méthylphosphonique ou du thiodiglycol. Ces trois composés ont été facilement extraits et identifiés selon CPL- IPE- SM avec le phosphate d'éthyle subissant une hydrolyse durant la procédure de stérilisation en autoclave. Cette méthode de manipulation et d'analyse d'échantillons pour les sols (ou autres) sera une étape primordiale dans la stratégie d'identification d'agents de guerre chimiques et biologiques qui est mise au point à RDDC Suffield et vise à répondre aux engagements souscrits par le pays.

Portée des résultats : Une méthode de manipulation et d'analyse d'échantillons pour les sols (ou autres) a été mise au point comme faisant partie d'une stratégie d'identification d'agents de guerre chimiques et biologiques proposée au Sommet du G8 qui s'est tenu à Kananaski, en 2002. Cette méthode de manipulation et d'analyse d'échantillons pour les sols (et autres) sera une étape importante dans la stratégie d'identification des agents de guerre CB. Cette stratégie est mise au point à RDDC Suffield ; elle vise à répondre aux engagements souscrits par le pays et sera incorporée dans le mode opératoire normalisé du Laboratoire de référence chimique et biologique médico-légal du RDDC Suffield.

Planification future : Cette méthode apporte un complément important aux méthodes actuelles en ce qui concerne l'identification des agents chimiques de guerre et des produits de leur hydrolyse en échantillons recueillis par les Forces canadiennes ou la GRC ainsi qu'aux inspections par mise en demeure de la Convention sur les armes chimiques. On prévoit d'appliquer la spectrométrie de masse en tandem aux échantillons contaminés par des agents CB dès l'installation d'un nouvel instrument à RDDC Suffield.

D'Agostino, P.A., Chenier, C.L., Hancock, J.R. 2003. Sample Handling and Analysis Method for Chemical Warfare Agents in Soils Contaminated with Chemical and/or Biological Warfare Agents. DRDC Suffield TM 2003-025.

Table of contents

Abstract.....	i
Résumé	i
Executive summary	iii
Sommaire.....	iv
Table of contents	v
List of figures	vi
Acknowledgements	vii
Introduction	1
Experimental.....	3
Sample handling	3
Biocontainment level 3 (BL-3) sample handling equipment list.....	3
LC-ESI-MS analysis.....	4
Results and discussion.....	5
Conclusions	10
References	11

List of figures

- Figure 1. LC-ESI-MS total-ion-current (90 to 200 Da) chromatograms obtained for the autoclaved aqueous extracts of the Ottawa sand samples spiked at the 80 : g/g level with a) triethyl phosphate (peak # 2), b) isopropyl methylphosphonic acid (peak # 3) and c) thiodiglycol (peak # 4). Diethyl hydrogen phosphate (peak # 1), the initial hydrolysis product of triethyl phosphate, was formed during autoclaving. 7
- Figure 2. Typical ESI-MS data (sampling cone: 20V) obtained for a) diethyl hydrogen phosphate and b) triethyl phosphate during LC-ESI-MS analysis of the autoclaved aqueous extracts of the spiked Ottawa sand samples. 8
- Figure 3. Typical ESI-MS data (sampling cone: 20V) obtained for a) isopropyl methylphosphonic acid and b) thiodiglycol during LC-ESI-MS analysis of the autoclaved aqueous extracts of the spiked Ottawa sand samples. 9

Acknowledgements

The authors would like to acknowledge Ms. Lori McLaws and Ms. Laurel Negrych for their autoclaving assistance and Dr. Bill Kournikakis and Mr. Doug Bader for their helpful discussions on safe biological warfare agent sample handling.

This page left intentionally blank.

Introduction

More than 140 States Parties have ratified the Chemical Weapons Convention (CWC) and agreed not to develop, produce, stockpile, transfer or use chemical weapons and agreed to destroy their own chemical weapons and production facilities. The CWC has reduced the likelihood of chemical weapons use by States Parties, but there remains a serious concern that other parties may make use of these weapons against civilian or military targets. Methods need to be developed to ensure that suspect samples collected under these scenarios can be analysed for the presence of chemical warfare agents in a timely manner. These analytical demands are being actively addressed by DRDC Suffield through the development and application of new mass spectrometric (MS) methods for the detection and identification of chemical warfare agents in a variety of samples.

Gas chromatography (GC) has been used extensively for the separation and identification of chemical warfare agents, with GC-MS being used frequently for the characterization of these compounds [1,2]. GC-MS, while suitable for the direct analysis of organophosphorus chemical warfare agent in organic extracts, is usually not preferred for the direct analysis of aqueous samples or extracts since these samples normally require additional sample handling steps and derivatization prior to analysis. LC-ESI-MS is being used increasingly, as electrospray mass spectrometric data may be used to directly identify chemical warfare agents, degradation products and related compounds in collected aqueous samples or extracts.

Researchers have developed atmospheric pressure ionization (e.g., electrospray (ESI), ionspray and atmospheric pressure chemical ionization) methods for the characterization of polar pesticides [3], organophosphate esters [4], and chemical warfare agents and/or their degradation products [5-25]. These ionization modes have been interfaced to liquid chromatography (LC) and capillary electrophoresis (CE), with LC-MS [9-12, 14, 15, 18-24] and CE-MS [5, 13] methods being reported for the identification of lower volatility chemical warfare agent hydrolysis products. Recently, DRDC Suffield published a number of LC-ESI-MS papers on the simultaneous identification of organophosphorus chemical warfare agents and their hydrolysis products in aqueous (or snow) samples [14-17, 20, 23-25] and aqueous extracts of contaminated soil samples [21, 22]. The ESI-MS data acquired during these and other analyses have been compiled into an ESI-MS database [26] that may be used for identification purposes.

A particularly challenging problem from a sample handling and analysis viewpoint has been the development of a safe procedure for the analysis of chemical warfare agents in samples where biological warfare agent contamination is also suspected. Analysis for chemical warfare agent contamination can only take place after the sample has been deemed free of biological warfare agent contamination. Unfortunately, the time required for culturing experiments to prove the absence of biological activity may take up to two weeks. A more rapid sample handling and analysis method on these types of unknown samples is required.

DRDC Suffield's recent experiences with aqueous samples and extracts, and their analyses by LC-ESI-MS [11-12, 14-17, 20-26] enabled the development of a new sample handling and analysis method for samples where the chemical/biological contamination was unknown. Soil, a typical environmental sample, was selected to evaluate this analytical approach which

utilizes aqueous extraction, autoclaving to free the sample of biological activity and LC-ESI-MS for compound confirmation. Ottawa sand samples were spiked at the 80 : g/g level with triethyl phosphate (a chemical warfare agent simulant that is resistant to hydrolysis), isopropyl methylphosphonic acid (the initial hydrolysis product of sarin) or thidiglycol (the hydrolysis product of mustard). This spiking level was below typical battlefield contamination levels, estimated to be in the 100 to 1000 µg/g range, based on a contamination density of 1 to 10 g/m² (soil density about 1 g/cm³ and a 1 cm sampling depth) and considered typical of soil contamination levels that might be expected hours to days after an attack. Soil samples were extracted with water using ultrasonic vibration and a portion of the aqueous extract was removed and autoclaved to eliminate biological activity. This aqueous extract was centrifuged and analysed by LC-ESI-MS to confirm compound identity.

Experimental

Sample handling

Three Ottawa sand samples (3.0 g) were each weighed into 15 x 125 mm screw-capped Teflon-lined glass culture tubes. The sand samples were then spiked with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol (25 : L aliquot of a 10 mg/mL solution in water) and allowed to stand at room temperature for one hour to simulate collected contaminated soil samples.

The spiked samples were ultrasonically extracted with water (10 mL) in the glass culture tubes for 10 minutes and then centrifuged in the same tubes at 2000 rpm for 10 minutes to settle out most of the sand. An aliquot of the aqueous layer (6 to 7 mL) was removed and transferred into a screw-capped Teflon-lined 20 mL glass scintillation vial. The lid was left finger-tight and the vial was autoclaved for two hours at 121°C at 15 psi (liquid cycle). The sterilized aqueous extract was allowed to cool and an aliquot (1 mL) was removed, transferred into a 1.5 mL plastic microcentrifuge tube, and centrifuged for 10 min at 10,000 rpm. A portion of the resulting extract was removed and stored in a 1.8 mL screw-capped Teflon-lined glass sample vial prior to LC-ESI-MS analysis.

An aliquot (1 mL) from the initial aqueous soil extracts was taken prior to autoclaving for comparative purposes. It was transferred into a 1.5 mL plastic microcentrifuge tube, and centrifuged for 10 minutes at 10,000 rpm. A portion of the resulting extract was removed and stored in a 1.8 mL screw-capped Teflon-lined glass sample vial prior to LC-ESI-MS analysis.

Biocontainment level 3 (BL-3) sample handling equipment list

1. analytical balance
2. Teflon-lined glass culture tubes
3. disposable scoopulas
4. water (extraction solvent)
5. 5 and 10 mL disposable pipettes and bulb
6. ultrasonic bath (or vortex)
7. Teflon-lined glass scintillation vials
8. lab marker
9. chemical waste jar
10. methanol/KOH decontamination solution

LC-ESI-MS analysis

LC separations were performed with a MicroTech 150 mm x 0.32 mm i.d fused-silica capillary column packed with Zorbax C₁₈ SB (5 µm particle size). The sample was introduced onto the column with a Rheodyne 8125 manual injector equipped with a 5 µL sample loop. The following solvent compositions were prepared for the mobile phase: Solvent A (0.1% trifluoroacetic acid (TFA) in water) and Solvent B (0.1% TFA in acetonitrile/water, 95:5). Chromatographic separations were performed with an Applied Biosystems model 140B dual syringe pump using a 5% to 75%B gradient over 30 minutes. In order to minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 150 µL/min and split prior to the injector such that the flow through the column was 10 µL/min.

LC-ESI-MS data were acquired using a Micromass LCT time-of-flight mass spectrometer equipped with the Z-spray electrospray interface. The electrospray capillary was operated at 3.2 kV with a sampling cone voltage of 20 volts. Nitrogen desolvation gas was introduced into the interface (80 °C) at a flow rate of 480 L/h. Nitrogen nebulizer gas was introduced at a flow rate of 66 L/h. ESI-MS data were acquired from 70 to 700 Da (1 sec) in the continuum mode with a resolution of 5000 (50% valley definition).

Results and discussion

Samples that may be contaminated with a combination of chemical and/or biological warfare agents pose a special problem to chemical and biological specialists tasked with determining the presence of chemical or biological warfare agents. Such a sample would initially be received into biocontainment level 3 (BL-3) at DRDC Suffield where biological identification may be safely carried out. Under normal circumstances, a sample extract requiring removal from BL-3 for use in a BL-2 or chemical laboratory would be sterilized by 0.22 μm filtration. A sterility check of the filtered extract would be conducted in BL-3, a process that may take up to two weeks. During this time chemical detection within BL-3 would be limited to devices such as the Chemical Agent Monitor. A more rapid sample handling and analysis method that could determine the presence (or absence) of chemical warfare agent contamination would be valuable to the military or during crime scene investigations. In addition, rapid determination of the absence of chemical warfare agent would benefit those working in BL-3 as the level of precautions required could be reduced.

The most rapid and effective means of sterilizing a sample contaminated with biological warfare agents that allows removal of the sample from BL-3, without a sterility check, involves autoclaving the sample for 2 hours. Any sample undergoing this process is necessarily exposed to water vapour at a high temperature, making the likelihood of chemical warfare agent hydrolysis high. An analytical method for chemical warfare agent identification must therefore be able to identify the principal hydrolysis products of the common chemical warfare agents. LC-ESI-MS may be used for this purpose and has the added benefit of being able to also detect and identify intact organophosphorus chemical warfare agents and many related compounds in aqueous sample extracts [26].

The developed sample handling method involves aqueous extraction of the sample (e.g., soil) in BL-3, followed by sterilization of the aqueous extract in the autoclave between BL-3 and the rest of the building. The sterilized container and aqueous contents can then be safely manipulated in the chemical analysis laboratory and analysed for the presence or absence of chemical warfare agents, their hydrolysis products or related compounds.

Three compounds were selected for evaluation of the proposed sample handling method, based in part on DRDC Suffield experiences with aqueous extraction and analysis of contaminated soil samples [21, 22]. Triethyl phosphate, an organophorous compound that has been used as a nerve agent simulant, was selected to investigate the extent of hydrolysis during autoclaving, as this compound is much more resistant to hydrolysis than the common organophosphorus chemical warfare agents. Isopropyl methylphosphonic acid was selected as a typical organophosphorus chemical warfare agent hydrolysis product and to investigate the likelihood of hydrolysis to methylphosphonic acid. Finally thiodiglycol was selected since this product would be expected following mustard hydrolysis. It should be noted that mustard cannot be readily detected by LC-ESI-MS, but hydrolyses readily in the presence of water to thiodiglycol, a compound that may be detected by LC-ESI-MS.

Figure 1 illustrates typical chromatograms obtained during LC-ESI-MS analysis of the three spiked soil samples. All three spiked compounds were readily extracted from the Ottawa sand

(recovery efficiencies were not estimated) at the 80 : g/g level. Triethyl phosphate underwent some hydrolysis (about 10-20%) to diethyl hydrogen phosphate, suggesting that hydrolysis of organophosphorus chemical warfare agents to their initial acids would be significant following autoclaving. Hydrolysis of isopropyl methylphosphonic acid to methylphosphonic acid, the common hydrolysis product for many of the organophosphorus chemical warfare agents, was not observed and no additional products were observed in the aqueous extract containing thiodiglycol. Figure 2 and 3 illustrate typical ESI-MS data obtained for each of the spiked compounds and diethyl hydrogen phosphate with a lower sampling cone voltage.

The chromatograms obtained for the aqueous extracts that did not undergo autoclaving were similar with two exceptions. The concentration of the spiked analyte was noticeably lower in these samples since the aqueous extracts underwent a 20% volume reduction during autoclaving. Peak area measurement comparisons suggested that there was little loss of analyte during the autoclave step. Finally, no hydrolysis of triethyl phosphate was found to occur in the spiked aqueous extracts that did not undergo autoclaving, a finding that was consistent with prior experiences.

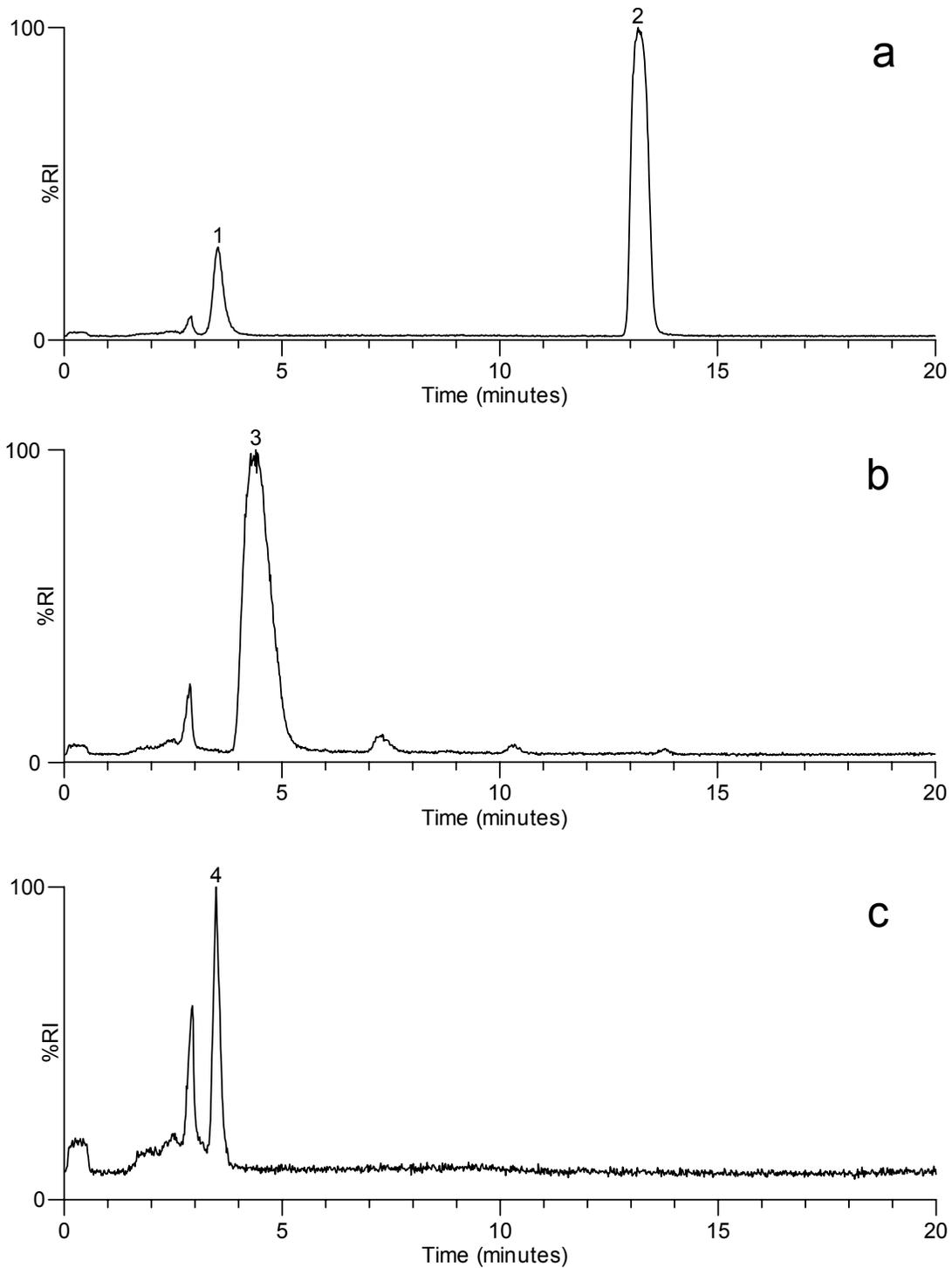


Figure 1. LC-ESI-MS total-ion-current (90 to 200 Da) chromatograms obtained for the autoclaved aqueous extracts of the Ottawa sand samples spiked at the 80 : g/g level with a) triethyl phosphate (peak # 2), b) isopropyl methylphosphonic acid (peak # 3) and c) thiodiglycol (peak # 4). Diethyl hydrogen phosphate (peak # 1), the initial hydrolysis product of triethyl phosphate, was formed during autoclaving.

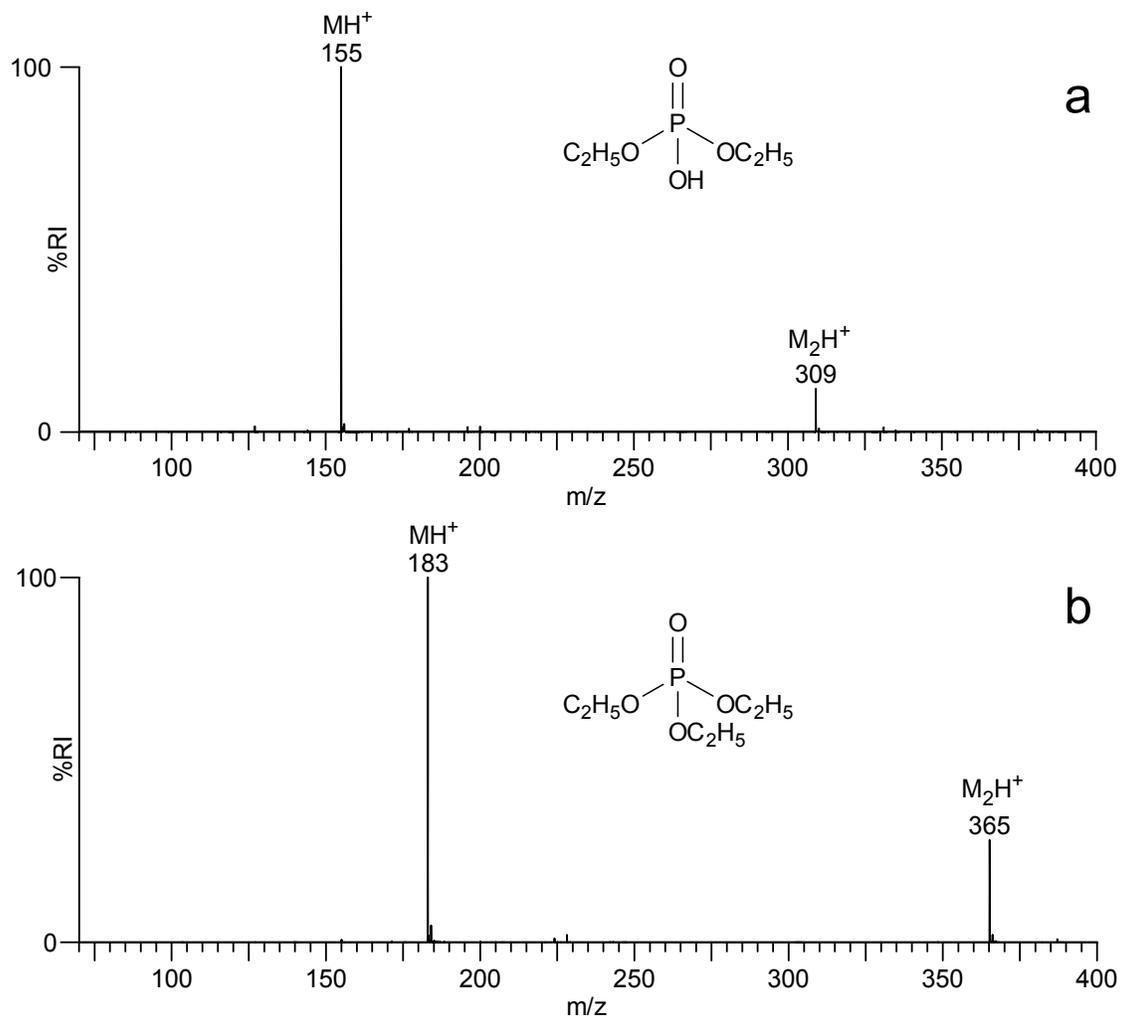


Figure 2. Typical ESI-MS data (sampling cone: 20V) obtained for a) diethyl hydrogen phosphate and b) triethyl phosphate during LC-ESI-MS analysis of the autoclaved aqueous extracts of the spiked Ottawa sand samples.

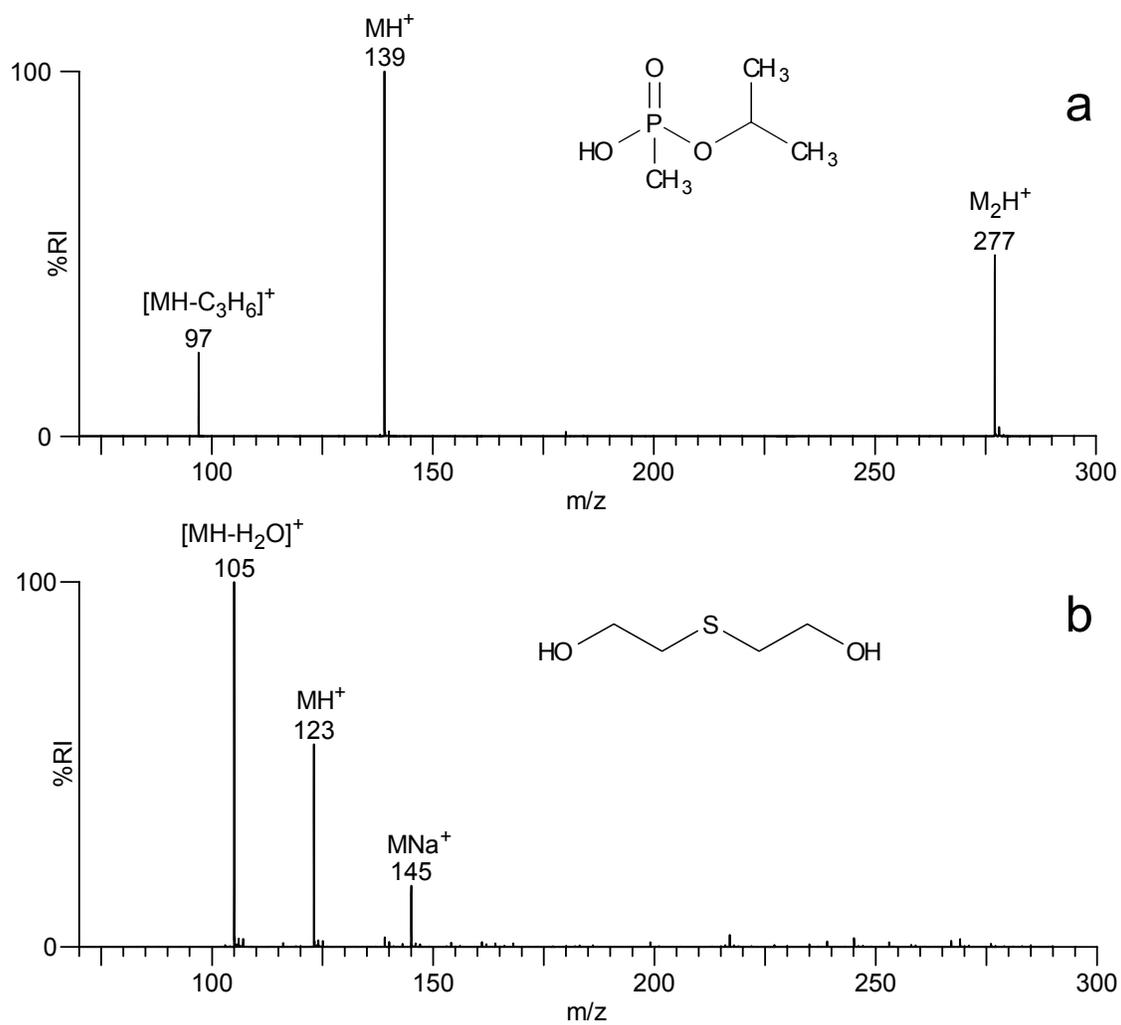


Figure 3. Typical ESI-MS data (sampling cone: 20V) obtained for a) isopropyl methylphosphonic acid and b) thiodiglycol during LC-ESI-MS analysis of the autoclaved aqueous extracts of the spiked Ottawa sand samples.

Conclusions

A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated with three soil samples spiked at the 80 : g/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three spiked compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure. The sample handling and analysis method for soil (or other) samples was developed as part of a chemical/biological warfare agent identification strategy for the G8 Summit held in Kananaskis (2002). This method is now being incorporated into the Standard Operating Procedures of the DRDC Suffield Chemical Biological Forensic Reference Laboratory which will begin operation in 2003.

References

1. Kientz, Ch. E. (1998). Chromatography and mass spectrometry of chemical warfare agents, toxins and related compounds: state of the art and future prospects. *J. Chromatogr. A*, 814, 1-23.
2. Witkiewicz, Z., Mazurek, M. and Szulc, J. (1990). Chromatographic analysis of chemical warfare agents. *J. Chromatogr.*, 503, 293-357.
3. Slobodnik, J., van Baar, B. L. M. and Brinkman, U. A. Th. (1995). Column liquid chromatography-mass spectrometry: Selected techniques in environmental applications for polar pesticides and related compounds. *J. Chromatogr. A*, 703, 81-121.
4. Bell, A. J., Despeyroux, D., Murrell, J. and Watts, P. (1997). Fragmentation and reactions of organophosphate ions produced by electrospray ionization. *Int. J. Mass Spectrom. Ion. Proc.*, 165/166, 533-550.
5. Kostiainen, R., Bruins, A. P. and Hakkinen, V. M. A. (1993). Identification of degradation products of some chemical warfare agents by capillary electrophoresis-ion spray mass spectrometry. *J. Chromatogr.*, 634, 113-118.
6. D'Agostino, P. A., Provost, L. R. and Hancock, J. R. (1994). Mass spectrometric identification of chemical warfare agent precursor and degradation products scheduled by the chemical weapons convention, In *Proceedings of the 42 nd Annual Conference on Mass Spectrometry and Allied Topics*, Chicago, Illinois, 275-276.
7. Borrett, V. T., Colton, R. and Traeger, J. C. (1995). The electrospray mass spectra of phosphonic acid, methyl phosphonic acid and its alkyl esters, and their complexes with alkali and alkali earth metal ions. *Eur. Mass Spectrom.*, 1, 131-140.
8. Borrett, V. T., Mathews, R. J., Colton, R. and Traeger, J. C. (1996). Verification of the United Nations chemical weapons convention: The application of electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.*, 10, 114-118.
9. Black, R. M. and Read, R. W. (1997). Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, and tandem mass spectrometry, to the analysis and identification of degradation products of chemical warfare agents. *J. Chromatogr. A*, 759, 79-92.
10. Black, R. M. and Read, R. W. (1998). Analysis of degradation products of organophosphorus chemical warfare agents and related compounds by liquid chromatography-mass spectrometry using electrospray and atmospheric pressure chemical ionization. *J. Chromatogr. A*, 794, 233-244.

11. D'Agostino, P. A., Provost, L. R. and Hancock, J. R. (1998). Packed Capillary Column Electrospray Mass Spectrometry and Tandem Mass Spectrometry of Hydrolysed HT and HQ, Suffield Report No 691, 23 pages.
12. D'Agostino, P. A., Provost, L. R. and Hancock, J. R. (1998). Analysis of mustard hydrolysis products by packed capillary liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A*, 808, 177-184.
13. Mercier, J.-P., Chaimbault, P., Morin, Ph., Dreux, M. and Tambute, A. (1998). Identification of phosphonic acids by capillary electrophoresis-ion spray mass spectrometry. *J. Chromatogr. A*, 825, 71-80.
14. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (1999). Packed Capillary LC-ESI-MS Analysis of O-Ethyl S-[2-(diisopropylamino)ethyl] Methylphosphonothiolate (VX), Suffield Report No 706, 28 pages.
15. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (1999). Analysis of O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) and its degradation products by packed capillary liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A*, 837, 93-105.
16. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (1999). Analysis of GA, GB, GD and GF in Aqueous Samples by Packed Capillary LC-ESI-MS, DRES TM 1999-047, 16 pages.
17. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (1999). Packed capillary liquid chromatography-electrospray mass spectrometry analysis of organophosphorus chemical warfare agents. *J. Chromatogr. A*, 840, 289-294.
18. Read, R. W. and Black, R. M. (1999). Rapid screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography-mass spectrometry and atmospheric pressure chemical ionization. *J. Chromatogr. A*, 862, 169-177.
19. Hooijschuur, E. W., Kientz, C. E., Hulst, A. G. and Brinkman, U. A. Th. (2000). Determination of hydrolysis products of sulfur mustard by reversed-phase microcolumn liquid chromatography coupled on-line with sulfur flame photometric detection and electrospray ionization mass spectrometry using large-volume injections and peak compression. *Anal. Chem.* 72, 1199-1206.
20. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (2000). Analysis of Tabun and Related Compounds by Packed Capillary Liquid Chromatography-Electrospray Mass Spectrometry (LC-ESI-MS), DRES TM 2000-004, 25 pages.
21. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (2000). Determination of Organophosphorus Chemical Warfare Agents and their Hydrolysis Products in Soil by Packed Capillary Liquid Chromatography-Electrospray Mass Spectrometry, DRES TR 2000-075, 19 pages.

22. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (2001). Determination of sarin, soman and their hydrolysis products in soil by packed capillary liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A*, 912, 291-299.
23. D'Agostino, P. A., Chenier, C. L. and Hancock, J. R. (2001). Identification of sarin and related compounds in snow by packed capillary liquid chromatography-electrospray mass spectrometry. DRES TM 2001-044, 24 pages, UNCLASSIFIED.
24. D'Agostino, P. A., Hancock, J. R. and Provost, L. R. (2001). Electrospray mass spectrometry of chemical warfare agents. In Gelpi E. (Ed), *Advances in Mass Spectrometry*, 15, 297-316. John Wiley and Sons Ltd., New York.
25. D'Agostino, P. A., Chenier, C. L. and Hancock, J. R. (2002). Packed capillary liquid chromatography-electrospray mass spectrometry of snow contaminated with sarin. *J. Chromatogr. A*, 950, 149-156.
26. D'Agostino, P. A., Chenier, C. L. and Hancock, J. R. (2002). Electrospray mass spectrometry of chemical warfare Agents, degradation products and related compounds. DRES TR 2002-028, 85 pages, UNCLASSIFIED.

UNCLASSIFIED

SECURITY CLASSIFICATION OF FORM
(highest classification of Title, Abstract, Keywords)

DOCUMENT CONTROL DATA (Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)		
1. ORIGINATOR (the name and address of the organization preparing the document. Organizations for who the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in Section 8.) Defence R&D Canada – Suffield PO Box 4000, Station Main Medicine Hat, Alberta T1A 8K6	2. SECURITY CLASSIFICATION (overall security classification of the document, including special warning terms if applicable) Unclassified	
3. TITLE (the complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title). Sample Handling and Analysis Method for Chemical Warfare Agents in Soils Contaminated with Chemical and/or Biological Warfare Agents		
4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.) D'Agostino, Paul A., Chenier, Claude L., Hancock, James R.		
5. DATE OF PUBLICATION (month and year of publication of document) April 2003	6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc) 23	6b. NO. OF REFS (total cited in document) 26
7. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.) Technical Memorandum		
8. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)		
9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)	9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)	
10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.) DRDC Suffield TM 2003-025	10b. OTHER DOCUMENT NOS. (Any other numbers which may be assigned this document either by the originator or by the sponsor.)	
11. DOCUMENT AVAILABILITY (any limitations on further dissemination of the document, other than those imposed by security classification) <input checked="" type="checkbox"/> Unlimited distribution <input type="checkbox"/> Distribution limited to defence departments and defence contractors; further distribution only as approved <input type="checkbox"/> Distribution limited to defence departments and Canadian defence contractors; further distribution only as approved <input type="checkbox"/> Distribution limited to government departments and agencies; further distribution only as approved <input type="checkbox"/> Distribution limited to defence departments; further distribution only as approved <input type="checkbox"/> Other (please specify)		
12. DOCUMENT ANNOUNCEMENT (any limitation to the bibliographic announcement of this document. This will normally corresponded to the Document Availability (11). However, where further distribution (beyond the audience specified in 11) is possible, a wider announcement audience may be selected).		

UNCLASSIFIED

SECURITY CLASSIFICATION OF FORM

UNCLASSIFIED
SECURITY CLASSIFICATION OF FORM

13. ABSTRACT (a brief and factual summary of the document. It may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall begin with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (S), (C) or (U). It is not necessary to include here abstracts in both official languages unless the text is bilingual).

A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated using three soil samples spiked at the 80 : g/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure. This sample handling and analysis method for soil (or other) samples will form a cornerstone of the chemical/biological warfare agent identification strategy being developed at DRDC Suffield to meet National commitments.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifies, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus-identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

Chemical warfare agents
Organophosphorus compounds
Sarin
G-agents
Liquid chromatography
Mass spectrometry
Electrospray

UNCLASSIFIED
SECURITY CLASSIFICATION OF FORM