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**Analytical Methods for Environmental Sampling
of Chemical Warfare Agents and Their
Degradation Products**

**Proceedings of a Conference
Aberdeen, Maryland
September 20-21, 1994**

Date Published - June, 1995

**Sponsored by
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Analytical Methods for Environmental
Sampling of Chemical Warfare Agents and
Their Degradation Products

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PREFACE

The first technical Conference on Analytical Methods for Environmental Sampling of Chemical Warfare Agents and Their Breakdown Products was held on September 20-21, 1994, at the Holiday Inn-Chesapeake House, Aberdeen Md. Organization was provided by staff of the Health Sciences Research Division at Oak Ridge National Laboratory (ORNL; Oak Ridge, Tennessee) and the U.S. Army Center for Health Promotion and Preventive Medicine (Aberdeen Proving Ground, Maryland) with support by the Chemical Stockpile Emergency Preparedness Program, the Army Environmental Center, and the Army Chemical and Biological Defense Command (all of the Aberdeen Proving Ground, Maryland). Production assistance for this volume of *Proceedings* was provided by Ms. Beverly Norton of the Health Sciences Research Division, ORNL, with support by Ms. Joan Carrington. Details and logistics of conference management were capably performed by staff of the ORNL Conference Office (Finance and Business Management Division), particularly Ms. Joy Lee.

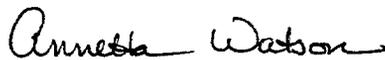
Standardization of analytical protocols to reliably detect chemical warfare agents and their degradation products in soil, water and other complex environmental media is needed to support the various chemical weapons disposal and emergency preparedness programs, Chemical Weapons Convention (CWC) treaty compliance, installation restoration and base closure decisions. The principal purpose of the Conference was to foster exchange among investigators and end users on these issues. Approximately 140 persons registered and attended all or most of the Conference sessions, which addressed 5 major topics:

- Implementation for Treaty Compliance, Installation Restoration and Stockpile Disposal Decisions (Session I)
- Existing Analytical Methods (Session II)
- Practical Applications of Existing Analytical Techniques (Session III)
- Immunoassay Technologies (Session IV)
- Environmental and Biological Fate of Agents and Their Degradation Products (Session V)

General Walter Busbee (Commander of the U.S. Army Chemical Materiel Destruction Agency at Aberdeen Proving Ground) was the keynote speaker, and summarized a number of environmental monitoring issues related to chemical weapons disposal. Although the research described was funded or partially funded by U.S. federal agencies, individual papers do not necessarily reflect the official view of these agencies; thus, no endorsement should be inferred.

Papers published in these *Proceedings* are organized by session, and in the order they were presented. Cross-referencing is accomplished by an author index. A glossary of technical terms is also provided.

During final editing of these *Proceedings*, a deliberate release of suspected nerve agent sarin took place (20 March, 1995) in the Tokyo subway system. The ensuing investigation made use of the analytical procedures documented in several *Proceedings* papers, as well as the expertise of their authors. This topic has turned out to be far more timely than the editors could have possibly imagined.



Annetta Watson, Editor
Health Sciences Research Division, ORNL



Stephen Kistner, Editor
U.S. Army Center for Health Promotion and Preventive Medicine, APG

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**SESSION I IMPLEMENTATION FOR TREATY COMPLIANCE,
INSTALLATION RESTORATION, AND STOCKPILE DISPOSAL
DECISIONS**



The Role of the AMC Treaty Lab in
International Chemical Weapon Treaties
by
Stephen Lawhorne and Lynn Hoffland
AMC Treaty Lab

The multilateral Chemical Weapons Convention has been signed by more than 150 nations and is expected to enter into force in 1995. The Wyoming Memorandum of Understanding and the Bilateral Destruction Agreement are bilateral agreements between Russia and the United States, and bilateral inspections were initiated with a United States trial challenge in Russia in August 1994. These multilateral and bilateral agreements include provisions for sampling and analysis to verify compliance. Those provisions may come into play for former chemical weapons (CW) production sites, destruction sites; and, by challenge inspection, potentially any Army Materiel Command (AMC) or AMC contractor site.

The AMC Charter for the Executive Agent for Chemical Treaty Compliance (CTC) dated 15 January 1993, directs the establishment of "an accredited AMC laboratory for the purposes of sample analysis to support implementation of bilateral and multilateral chemical treaties." In January 1993, the Commander of the Chemical and Biological Defense Agency directed the Executive Agent for CTC to develop a comprehensive proposal for establishing a laboratory to support chemical treaty operations. In September 1993, the proposal was presented to the Commanding General, Chemical and Biological Defense Command, and guidance was given to proceed with the establishment of a Treaty Laboratory (TL) with the central management at the Edgewood Area of Aberdeen Proving Ground.

The broad mission of the Treaty Laboratory was originally to provide services and expertise for the protection of AMC and Army interests. This mission has been expanded recently to include support to other DoD facilities. The laboratory will provide analytical results, advice and assistance to management, inspectors, and negotiators. The laboratory may be responsible for the analysis of samples. Laboratory personnel must be prepared to conduct, assist in, or train others to perform on-site sampling and analysis at inspection sites. Because of the potential national and international consequences of the laboratory's efforts, it must make a significant and continuing investment in activities which cause its products to be accepted with intuitive credibility, e.g., quality management, peer relations with similar laboratories, training, and elaborate information management. Support is expected to include activities such as:

- Provision of credible, laboratory based technical support and consultation to management, inspectors and negotiators;
- Conduct of quality assurance, quality control, and information management for sampling and analysis functions;
- Technical assurance of optimal on-site sampling and analysis;

- Advice in the collection of samples and analytical support relevant to occupational safety and health of inspector personnel;
- Conduct of activities necessary for credibility of the laboratory. These activities include initial and repetitive efforts needed for national and international accreditation; participation in "round robin" blind sample analyses to compare results with other international laboratories and joint development and validation of analytical methods.

It is anticipated that the laboratory will respond to the following requirements:

- analysis of numerous "routine" samples per year, with results expected within hours or days;
- analysis of relatively fewer, highly demanding "one-of-a-kind" samples with results expected within days to weeks;
- take inspection samples at all AMC sites;
- assessment of equipment, training of personnel and sustainment training of teams to conduct or assist in on-site sampling and analysis;
- maintenance of certifications and similar credentials;
- modifications, development, validation and trial of analytical methods;
- maintenance of chain-of-custody information and historical data necessary for sampling and analysis credibility.

To accomplish this list of varied missions, the AMC Treaty Laboratory will actually be a system of laboratories consisting of a central coordinating laboratory and a set of collaborating laboratories which conduct validation analyses, make available special skills, and aid in coping with surges in workload. This plan encompasses the collaborating laboratory arrangements as well as the development of the coordinating laboratory.

An interim AMC treaty laboratory was established from existing resources and will perform AMC treaty laboratory functions until a fully functioning treaty laboratory is established. The coordinating laboratory is being approached with the intent of conserving Government employee manpower and using that manpower in the most efficient manner. Routine tasks will be primarily accomplished by contract, and sudden surges in workload will be managed by matrix from within ERDEC as well as contract effort.

The interim coordinating laboratory has been established in the JA Wing of building E-3330 in the Edgewood Area of Aberdeen Proving Ground. The area includes several laboratories, of which half are operated by contractor personnel. These facilities are approved

for only dilute chemical agents; no surety work can be done there. Current activities focus on finalizing methodology for all of the potential analytes expected to be encountered during the upcoming challenge inspections, thorough training of all operators on all of the equipment, design and implementation of a complete data management system, development of the standard and internal operating procedures, and development of a standards program for items other than agents.

In the meantime, dedicated facilities are being prepared for the AMC Treaty Laboratory in building E-5100. (See figure 1.) These facilities will allow surety work, and are being designed to accept unknowns. There are separate office areas for Government and contractor personnel, but the analytical instruments will be accessible to both. The facility is designed such that unknowns will enter from the north, where the greatest negative pressure will be maintained. Here the samples can be screened and classified. An irradiation capability is being considered for unknown samples. Sample storage is designed to accommodate neat agent if necessary. As the hazards of the sample are characterized, the appropriate sample preparation can be performed in the next area, before the final analyses are performed in the southern area of the laboratory. Analytical capabilities include gas chromatography and liquid chromatography methods with a variety of detectors (flame photometric, flame ionization, mass spectroscopy, mass selective detection, atomic emission detection). Nuclear Magnetic Resonance (NMR), supercritical fluid extraction, and inductively coupled plasma/mass spec capabilities will also be installed. We will begin moving into this new facility in March 1995.

The collaborating laboratories were selected to augment the chemical and biological warfare related analytical expertise available at ERDEC's coordinating laboratory to adequately cover any capabilities to handle samples that might be generated under any of the various treaties. The arrangements with the collaborating laboratories have been made via formal memoranda of understanding addressing the general requirements as best as we can anticipate. Memoranda of understanding are already in place with the Centers for Disease Control and Prevention, Lawrence Livermore National Laboratory, and the National Institute of Standards and Technology. Drafts have been sent to Dugway Proving Ground and the Food and Drug Administration. We just recently discussed questions with the US Army Medical Research Institute for Chemical Defense and the US Army Medical Research Institute for Infectious Diseases which we hope will allow us to finalize a Memorandum of Agreement with them. We feel confident that with this network of laboratories we can analyze any sample in any media.

The different treaties have different potential tasks for the AMC Treaty Laboratory. Under the Wyoming Memorandum of Understanding there is a potential for on-site sampling and analysis during challenge inspections. Allowable samples could include air and environmental samples. Accordingly, we have assembled a portable analysis capability. The portable system includes a gas chromatograph with dual flame photometric detector and mass selective detector as the primary analytical instrument. Electrical generator, gas generators, laptop computer, and data management software are all included in the system. The instruments are packaged in a shipping container that can protect the equipment against the rigors of freight shipments to the required sampling and analysis locations. Separate sample collection and preparation kits complete the portable capabilities. We expect to use this equipment at any U.S.

Trace Analyze
Sample Prep &
Routine Trace
Analysis Lab
(DFPD, FID TCD)

High Tech Trace
Analysis
Lab

Data
Management
Sys

Contract
Personnel

Routine
Trace
Analyze Lab
(MSD AED)

Operation Officer/
Tech Coord
Conf Room/Library

Director
Secretary
Clerk Typist

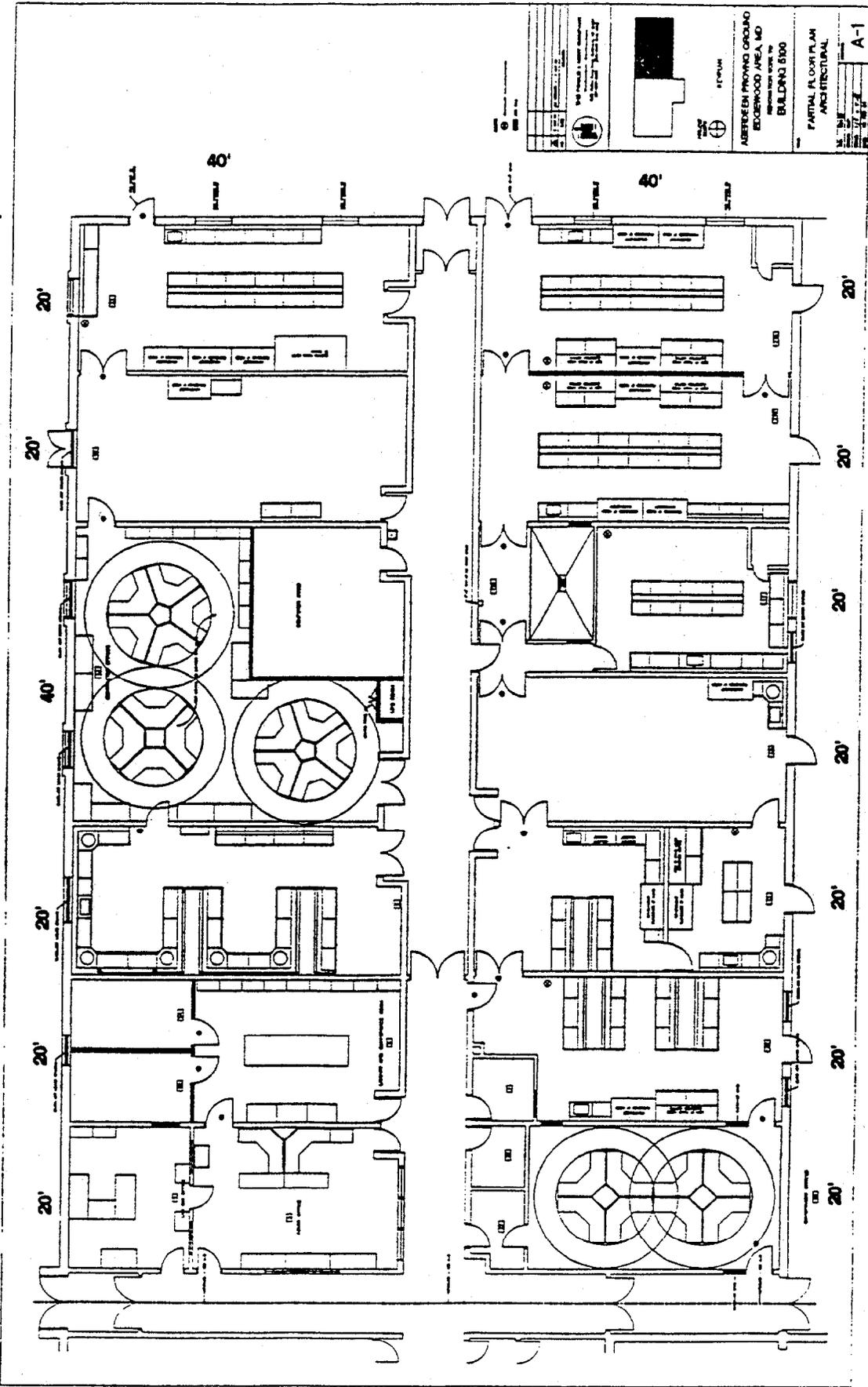


Figure 1 Schematic Floor Plan of E5100

facility being challenged by the Russians, if the final negotiations for that inspection include any on-site sampling and analysis.

Under the Bilateral Destruction Agreement between the United States and Russia sampling and analysis requirements also exist. The agreement pertains to the mutual destruction of existing stockpiles of chemical warfare materials in both countries, and it includes provisions for on-site inspection and analysis of feed streams and effluents at the destruction sites. Laboratory facilities will be built at each destruction facility. The laboratory will include facilities for both the US and Russian inspectors to analyze separate splits of any samples collected during the destruction process. The AMC Treaty Laboratory's input to the destruction facility laboratories include advice on layout, equipment requirements, methodology, standards support, and data management.

In all cases, the AMC Treaty Laboratory will provide the expertise and analytical capabilities to protect DoD interests. If there are any inconsistencies or anomalies identified during the analysis of samples under any of these treaties, the AMC Treaty Laboratory will provide authoritative, validated results that will clarify the earlier results.

The ability to provide authoritative, validated results that will meet the requirements of the scientific and legal community is an essential ingredient of our operations. These capabilities require an extensive investment in training as well as strict adherence to operational standards. Some of the major pieces of the AMC Treaty Laboratory program include Quality Assurance, Control, and Management; Laboratory Certification; Information Management; and a Standards Program.

Quality Assurance, Control, and Management encompass all of the traditional areas, such as a formal quality manual or QA plan; ISO quality systems training; integrity of the sample collection, handling, delivery, and analysis process; certification of the analytical methods and the personnel performing them; and process controls. The QA plan will include those areas addressed in ISO Guide 25 in addition to a formal certification under ISO 9001. Laboratory personnel must be trained in the overall quality system as well as specific training on analytical methods using specific analytical instruments. Analytical results will not be accepted if the operator does not have current training on the instrument and method. Laboratory personnel must also be trained in the use of the information management system, the usual safety and security issues, and hazardous waste management.

Laboratory certification is a formal process where a third party "registrar" will perform an audit of our operations. Prior to the audit, we must fully implement our QA procedures, train our people, and fully document all of our procedures. We will first perform an internal audit and management review. We anticipate being ready for the internal audit at the end of calendar year 1995. We are confident we will be ready for this audit based upon the dedication and resolution of our staff and the unequivocal support of CBDCOM and ERDEC management.

Information management is an integral part of the laboratory operation. All of the sample preparation and analysis is only as good as the data collected and reported. We are

currently contracting for a customized laboratory information management system that will take information directly from the analytical instrument, through the review and approval process within the laboratory, to storage in a validated data management system. The system will include auditing of all changes, version implementations of methods, and a carefully constructed access system that allocates permissions or authorities commensurate with the task. The system will include the ability to identify children and parents of samples (i.e., identify all samples and analyses performed on splits or extracts from a single original sample), present QA/QC reports, and perform some data validation tasks, such as assuring that operators have appropriate training on instruments and methods before accepting data. The data management system will have other modules to track chemical inventories, track personnel training records, and track equipment maintenance records. The data management system will operate on a network server to be installed in building E-5100. Different schemes are currently being evaluated for collaborating laboratory access. One approach is for satellite licenses for specialized software subsets; others include dial-up access from the remote locations and simple, formatted data files submitted via diskette.

A formal standards program is being established for materials other than agents. Our Chemical Agent Standard Analytical Reference Material (CASARM) program will provide standards for agents. Standards must be developed and maintained for other analytes, such as degradation products, intermediates, and nonstandard agents. We anticipate a combination of in-house and contract effort on the standards program. The coordinating laboratory would then be the source of standards for the collaborating laboratories.

One other aspect of demonstrating and gaining acceptance of our analytical capabilities is to participate in international round robins. We participated in the most recent round robin, and did extremely well. We will continue to participate in these round robins, perhaps expanding them to our collaborating laboratories.

The exact requirements of the AMC Treaty Laboratory continue to change as negotiations continue under the bilateral agreements with Russia. The CWC has not yet entered into force, but we anticipate that role in protecting DoD interests will continue under that multilateral treaty.

Screening Methods for Chemical Warfare Agents in Environmental Samples at the Edgewood Area of Aberdeen Proving Ground, Maryland

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F. G. Lattin, US Army Edgewood Research, Development and Engineering Center
J. Wrobel, US Army Directorate of Safety, Health and Environment, Aberdeen Proving Ground, Maryland

I. Introduction

The U. S. Army Edgewood Research, Development and Engineering Center, the U. S. Army Aberdeen Proving Ground Support Activity, Directorate of Safety, Health and the Environment and SciTech Services Inc., an independent contractor, have developed an approach for screening environmental samples for the presence of chemical warfare agents. Since 1918, the Edgewood area of Aberdeen Proving Ground has been a research and testing ground for toxic agent compounds. Since these materials are considered highly toxic, screening for their presence in environmental samples is necessary for safe shipment to contract laboratories for testing by EPA guidelines. The screening ensures worker safety and maintains US Army standards for transportation of materials potentially contaminated with chemical warfare agents.

Chemical warfare agent screening involves two procedures: (1) head space analysis of the sample for time weighted average (TWA) levels; and, (2) extraction of the sample with dichloromethane followed by analysis with gas chromatography/flame photometric detection (GC-FPD). This method has been used to simultaneously screen for the presence of chemical warfare agents GB, GD, HD and VX. A high pressure, liquid chromatography method (HPLC) is used to screen for the hallucinogenic material BZ. A third method, using a GC-Mass Spectrometer (MS) or a GC-Atomic Emissions Detector (AED) is used to screen simultaneously for agents HD and lewisite, a combination that is often found at World War I period sites.

II. Experimental

A. GC-FPD Screening Methods for Chemical Warfare Agents in Environmental Samples - Head Space Analysis

The purpose of the head space screen is to ensure that gross contamination is not present on any environmental samples. Head space is measured by collecting an air sample onto a tenax-filled tube at a predetermined flow rate and sample time. The air sample is subsequently analyzed by GC-FPD for the presence of agent compounds (sulphur and phosphorus peaks at specified retention times) at TWA levels.

The samples are collected on Depot Area Air Monitoring System or DAAMS tubes for each soil sample, using a flow of approximately 0.2 L/minute for 120 minutes. The tubes are inserted into the double-bagged samples across the opened lid of the sample container. One tube from each site is screened for agent compounds. The

assay uses a gas chromatograph with a very sensitive dual flame photometric detector (FPD) (sulphur and phosphorus sides). The dual FPD simultaneously detects all the major CW agents (GA, GB, GD, GF, VX, and HD). Positive results (greater than 1 TWA) are confirmed by either GC-AED or GC-MS.

1. Head Space Analysis by GC-FPD

The instruments include a Hewlett-Packard Gas Chromatograph (GC), Dynatherm Inc. ACEM 900 and related accessories. The instrumentation desorbs the sample from these tubes on to a capillary column. The column separates the agents and delivers them to a dual FPD that, in turn, detects quantitatively their presence. All instrument calibrations are checked daily, and preoperational checklists are completed to ensure all systems are operating at prescribed parameters.

One calibration standard is prepared to approximate 0.25 TWA for each agent in one solution. This standard is injected at incremental levels. The levels are 0.25, 0.50, 0.75, 1.0 and 1.25 TWA. The combination standard is used for routine operations. However, there are occasions where any combinations of standards may be used in establishing the standard curves. The general procedures are as follows:

- a. Agent standards are removed from cold storage and allowed to reach room temperature.
- b. A conditioned tenax-filled sorbent tube is placed into a Dynatherm tube conditioner with a flow no less than 200 milliliters per minute (ml/min).
- c. A 10-microliter (μ l) syringe is used to withdraw one (1) μ l of agent.
- d. The tube is spiked in the tube conditioner by depressing the plunger on the syringe and allowed to aspirate for three minutes.
- e. The tube is placed into the Dynatherm and the calibration procedure is initiated.
- f. The calibration procedure is repeated with increasing agent volumes by one (1) μ l up to a final volume of five (5) μ l.

Normal sample analysis begins after suitable standard curves are generated for the desired agents. Chromatography conditions were as follows:

Tube conditions of ACEM:	Heat for 3 min at 300°C
Trap conditions of ACEM:	Heat for 3 min at 300°C, cool 1 min
ACEM valve:	250°C
ACEM interface:	280°C
Column:	DB-5 ¹ , 30m x 0.53 mm I.D., 1.5 μ m film

¹ 5%-diphenyl, 95%-dimethylsiloxane

	thickness
Carrier:	Nitrogen at 35 cm/sec
Oven:	65°C for 1.5 min
	65-100°C at 20°C/min
	100-280°C at 35°C/min
	280°C for 7.4 min
Detector:	Dual FPD at 260°C

Lewisite with or without HD is assayed as the methyl mercaptan (methylthiol) derivative by GC-AED or GC-MS. An injection device developed by Dynatherm (model LTD 940) derivatizes samples automatically. The system introduces a fixed amount of methyl mercaptan onto the sample sorbent bed. An automated rotary valve in the injector controls the filling and purging of a sample loop containing three (3) cc methyl mercaptan. The LTD 940 is used to prepare both samples and calibration standards. Chromatography conditions are the same as above for the general screening except that the sample is assayed by GC-AED or GC-MS instead of FPD. The GC-FPD method for lewisite is still under development.

2. Data Storage and Evaluation

The data collected from each GC-FPD are processed and stored on a Hewlett-Packard LIMS (Laboratory Information Management System) which has a weekly tape backup routine. The chromatogram consists of peak area, peak height, retention time, and other related information. Results of analyses are checked for validity. The integrator reports the results in nanograms (ng) on column. These values are evaluated based on sampling time and rate of air flow during sampling. For example, the TWA level for HD is 72 ng on column if the sample is taken for 120 minutes at 0.2 liters per minute air flow. If any chromatogram indicates higher than TWA level of any agent in any of the samples, a duplicate DAAMS tube is assayed using either a GC-AED (atomic emission detector), a GC-FPD with a column of a different polarity, or a GC-MS to confirm the result.

3. Quality Control of Air Samples

A Quality Plant (QP) sample tube, spiked with a known volume of agent standard, is periodically analyzed as a control sample. This QP sample, having laboratory air aspirated through it at 0.2 L/minute for 120 minutes, validates the ability of the GC-FPD to detect the agent in field samples. A Quality Laboratory (QL) sample (DAAMS tube spiked with three [3] µl of dilute agent standard at the 0.25 TWA level) is assayed every 10 samples. QL samples are used at the beginning and at the end of each day to ensure that instruments are calibrated. All quality control procedures and methods are specified in the ERDEC Monitoring Branch's QA/QC plan.

B. GC-FPD Screening Methods for Chemical Warfare Agents in Environmental Samples - Soil Sample Analysis

1. Soil Sample Preparation

The soil sample method consists of extraction of the samples with dichloromethane and subsequent direct injection into the GC. Each sample is prepared by weighing two (2) grams (g) of soil in a 10 mL screw-top test tube. The sample is then extracted, using a solution of two (2) mL dichloromethane modified with 0.015% of 2-diisopropylaminoethanol (improves VX recovery), and filtered into an autoinjector vial using a 0.45 µm syringe filter on a three to five mL syringe. Reagent blanks are analyzed along with quality control samples including matrix spikes.

For lewisite testing, a 5 g soil sample is added to 3 mL of water modified with propane dithiol (0.1% volume). The mixture is then extracted with dichloromethane (2 mL), filtered into a vial using a 0.45 µm filter. The extract is analyzed by GC-MS or GC-AED using the conditions listed below for confirmation.

GC-FPD Conditions

A Hewlett-Packard 5890 GC

Column: DB-5, 30m x 0.53 mm I.D., 1.5 µm film thickness
Carrier: Nitrogen at 35 cm/sec
Oven: 65°C for 1.5 min
65-100°C at 20°C/min
100-280°C at 35°C/min
280°C for 7.4 min
Injector: 1-5 µL of extract Splitless, 280°C
Detector: Dual FPD

2. Confirmation of Positive Soil Samples

Confirmation of positive screen results is accomplished by analysis of the soil extract by GC-AED or GC-MSD (model 5971). Typical conditions for confirmation by GC-MS or GC-AED are as follows:

Column: DB-5, 30m x 0.25 mm I.D., 1.0 µm film thickness
Carrier: Helium at 30cm/sec
Oven: 60°C for 3 min
60-100°C at 20°C/min
100-280°C at 35°C/min
280°C for 7.4 min
Injector: Splitless, 250°C
Interface: 280°C (MSD)
Detector: MSD in selected ion mode
AED in sulfur, chlorine, arsenic, or phosphorus mode

III. Discussion

The overriding goal of this program is to establish a systematic approach to screening and reporting the results of trace analysis of chemical warfare agents in environmental samples. Two issues were present at the initiation of this work. The first

issue concerned method validation and certification. Presently, standard certification procedures are not applicable to chemical warfare agent screening since certification agencies such as the EPA consider these methods within the realm of the Army. The Army, on the other hand, has not fully set up a certification program for environmental analysis since this requirement has been a recently-mandated mission.

Currently, the method is based on performance-based validation with precision and accuracy data whenever method changes are begun. Method reliability is based on percent of spiked quantities recovered from a Standard Analytical Reference Material (SARM). In its pre-conditioned state, SARM consists of a soil matrix scrubbed with solvent to remove any organic interferences specific to the matrix. Once received in the laboratory, the matrix is further treated by heating for 24 hours to remove any interferences from the solvent and then spiked with various amounts of agent to be assayed. The spiked SARM sample validates the method's ability to recover the agent from the matrix. In the laboratory, these samples are known as Quality Plant (QP) samples. In this way the variable matrix effects are considered. SARM matrices for these analyses are distributed by the U.S. Army Environmental Center (AEC).

The most important indicator of method reliability in the soil analysis is the recovery of the agent from matrix spikes. Soils can vary greatly in composition, especially in water content. If agent is not recovered from the matrix spikes of a sample then the analysis is not considered valid and steps are taken to correct the problems if possible. Recoveries can appear poor because of interferences or degradation of the agent. In the laboratory, the matrix spikes are known as Quality Internal Recovery Control (QIRC) samples.

To date, percent recoveries have fallen within following representative means and standard deviations:

AGENT	SARM (QP)		MATRIX (QIRC)	
	MEAN (%) RECOVERY	STD. DEVIATION	MEAN (%) RECOVERY	STD. DEVIATION
GB	76	18	55	27
GD	78	22	70	29
HD	110	46	106	35
VX	49	23	55	11

Linear regression plots of recoveries over a two-year period show that results fall within 95% confidence limits established for this method. Chromatographic data in Figure 1 shows discernible peaks at retention times assigned for the target agents (the agents detected on the phosphorous side of the dual flame photometric detector).

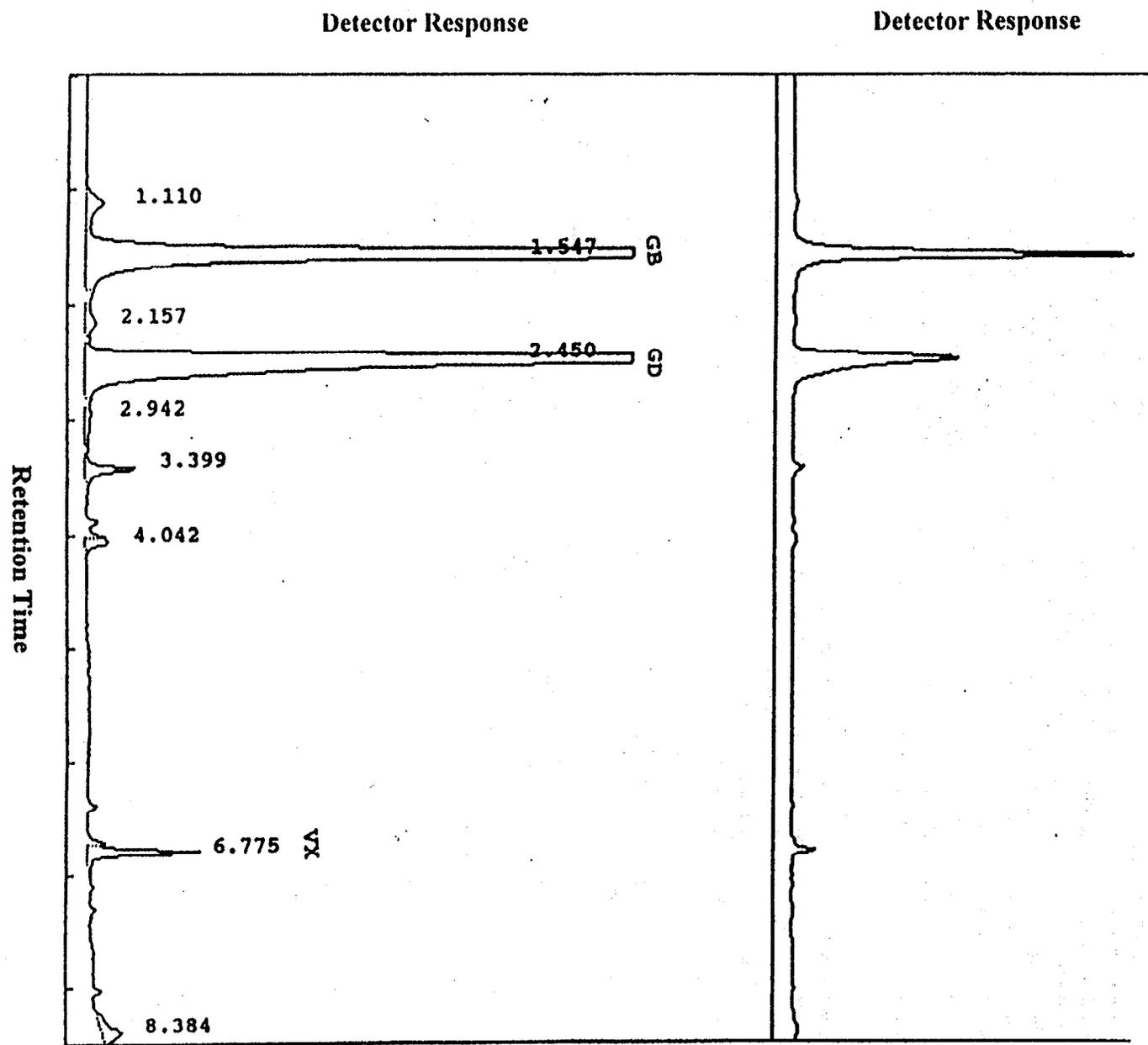


Figure 1. Sample Gas Chromatogram for Phosphorus Detector Response of a GB, GD and VX Sample Spike.

The method detection limit is based on the recovery data achieved in the reference material and matrix spikes. The method detection limit is the lowest target concentration of each agent for which a percent recovery was determined within 95% confidence bounds calculated from the recovery data for each agent (target versus found concentration). Future plans include certification by the EPA as a standard method for screening environmental samples.

The second issue concerned the levels at which environmental samples would be considered releasable, i.e., no longer subject to surety restrictions in handling and transportation. Although tested concentrations are below agent drinking water levels, found in Department of the Army Technical Bulletin 557², these levels are used as an upper limit standard for reporting the presence of these materials in environmental samples.

Agent drinking water standards are set by the Department of the Army for short-term consumption (7 consecutive days or less) under field conditions when troops do not have access to drinking water that meets long-term consumption standards. The short-term drinking water standards for chemical agents are shown below:

Chemical Agents

lewisite	2.0 mg/L
mustard	0.2 mg/L
nerve agents	0.02 mg/L

These levels dictate sample clearance for shipment to off-site laboratories.

Figure 2 also shows the decision-tree for samples which do not meet initial screening parameters. In these instances, samples are assayed with GC/AED or GC/MS to enhance specificity or selectivity.

IV. Conclusions and Future Work

Results of routine environmental samples assayed over the past two years have shown that agent amounts have not been detected at the drinking water level limits. However, evidence of the presence of agent below drinking water levels has been seen. Confirmation is not always conclusive because suspected agent at very low levels is subject to large amounts of interference.

Because of this information void, we are investigating the use of data qualifiers to show uncertain or unconfirmed results at very low levels (below quantitation from current instrumentation). The study will focus on equating data qualifiers used by the Environmental Protection Agency to report inconclusive results from samples analyzed for priority pollutants.

² Full citation: Headquarters, Department of the Army Technical Bulletin, Occupational and Environmental Health, Sanitary Control and Surveillance of Field Water Supplies, TB MED 577, March 1986.

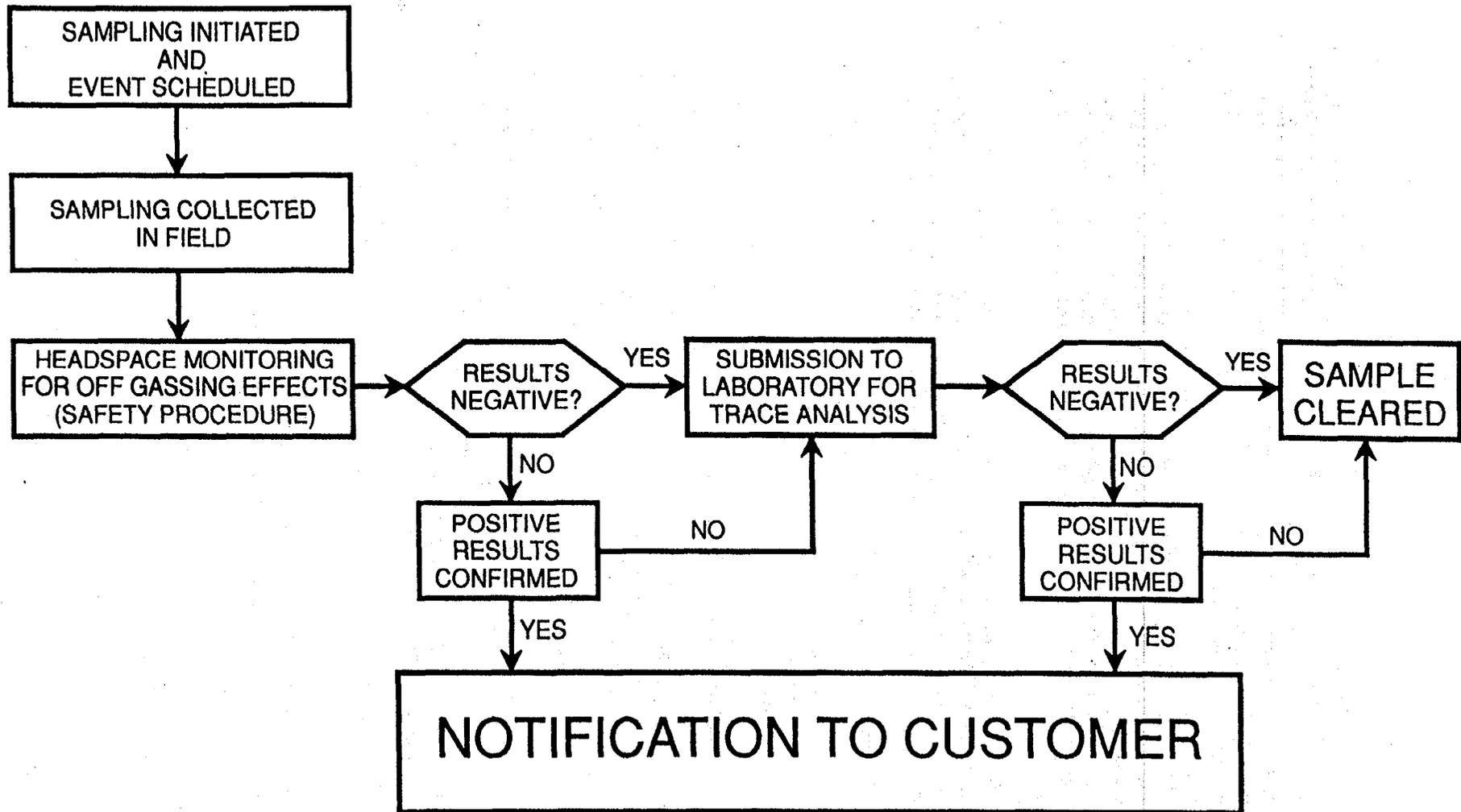


Figure 2. Process for Sample Clearance

A second area of investigation is the development and use of methods to detect and quantify lesser known agents, some of which were used during the World War I period. These agents, and some of their degradation products, have become an area of concern for the Army which is currently embarking on an intensive effort to remediate sites that were formerly used as chemical warfare test and research centers prior to World War II. A full range of analytical methods that can be used to routinely test for the presence of all compounds of interest will significantly enhance the Army's ability to detect and characterize potential contamination and meet the increasing demands for site remediation.

U.S. ARMY CHEMICAL DEMILITARIZATION AND REMEDIATION ACTIVITY NON-STOCKPILE MONITORING APPROACH

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1. The Non-Stockpile Chemical Materiel Program

In Section 176 of Public Law (PL) 102-484, the 1993 Defense Authorization Act, Congress directed the U.S. Army to submit a report identifying the locations, types, and quantities of non-stockpile chemical materiel (NSCM). As part of that report, published in the Survey and Analysis Report for the Non-Stockpile Chemical Materiel Program¹, five categories of NSCM were addressed: buried chemical warfare materiel (CWM); recovered chemical weapons; and miscellaneous CWM. To better define the scope of CWM burial sites, four separate types of sites were discussed: chemical agent identification set (CAIS) burials, small CWM burial sites with no explosives, small CWM burial sites with explosives, and large CWM burial sites (with and without explosives). A total of 215 potential CWM burial sites, distributed throughout 33 states, the U.S. Virgin Islands, and the District of Columbia, were identified.

The U.S. Army Program Manager for Non-Stockpile Chemical Materiel (PMNSCM) is responsible for the disposal of all chemical warfare materiel (CWM) at these sites. This mission begins with receipt of the recovered CWM from the U.S. Army Corps of Engineers (USACE) or another responsible organization³, continues through CWM transportation, interim storage, disposal, and concludes with closeout of the disposal operations. To support this mission, the PMNSCM is currently developing a series of mobile systems to provide onsite characterization and disposal of recovered CWM in a safe, secure, and environmentally acceptable manner. One mobile system currently under design is the Rapid Response System (RRS) for disposal of recovered CAIS. The RRS and the RRS monitoring system and procedures illustrate the PMNSCM monitoring approach and serve as the focus of this discussion.

2. The Rapid Response System

The RRS system consists of two vehicles: an operations trailer and a utilities trailer. Each of these vehicles can be transported by C-130 military aircraft and/or driven to

¹ U.S. Army Chemical Demilitarization and Remediation Activity; formerly the U. S. Army Chemical Materiel Destruction Agency

² Science Applications International Corporation

³ USACE is responsible for Formerly Used Defense Sites (FUDS). Other agencies may assume these roles at other cleanup sites.

burial sites. The operations trailer houses a system of coupled negative-pressure gloveboxes with three stations for characterization and disposal of CAIS. A Raman spectrophotometer with fiber optic probe is used to nonintrusively identify the chemical contents of the CAIS². The operations trailer also houses a heating and air conditioning system that provides temperature control and clean air from outside the trailer, a carbon filter ventilation system that purifies exhaust air from the glovebox, and near real-time air monitoring systems for detection of airborne chemical materials. The utilities trailer houses the electrical generator system, the air compressor system, and other required equipment. In the field, the two trailers are parked close to each other.

2.1 CAIS Characterization and Disposal

CAIS characterization and disposal is accomplished in several distinct steps. Metal shipping containers, known as pigs, and overpacks containing CAIS are transported to the RRS from the interim storage area or the excavation site. One by one, the pigs and overpacks are loaded into the operations trailer glovebox through an airlock, which provides access through the trailer door from the outside. At the glovebox unpack station, a glovebox operator opens one pig or overpack at a time, and unpacks the CAIS ampules and bottles. At the next glovebox station, a glovebox operator uses the Raman spectrophotometer and fiber optic probe to identify the chemicals contained in each CAIS ampule and bottle. Once the material is identified, the operator labels and segregates the ampules and bottles according to the chemical contents.

Chemical warfare material, such as sulfur mustard (H, HS, or HD), lewisite (L), and nitrogen mustards (HN-1 or HN-3), are chemicals that are regulated by Public Laws 91-121 and 91-441; they are neutralized separately in a closed reactor system at the third glovebox station. If required, the neutralant solutions are characterized by a laboratory to confirm chemical material destruction to the required levels.

CAIS items identified as industrial compounds, such as phosgene (CG), cyanogen chloride (CK), and chloropicrin (PS), are chemicals which, although used for chemical warfare, are commonly used in industry, and do not need to be neutralized before shipment to a commercial disposal facility. Ampules containing CG, CK, PS, or GA-simulant (a mixture of nontoxic compounds that combine to simulate the odor of GA and its affect on detector paper), and bottles containing solid PS, chloroacetophenone (CN), adamsite (DM), or triphosgene are packaged in accordance with Department of Transportation (DOT) regulations for transportation to an approved hazardous waste treatment facility with neutralization or other treatment.

2.2 Monitoring During RRS Operations

Monitoring for chemical hazards during RRS operations ensures the safety of operators, the environment, and the surrounding communities. Field monitoring procedures rely on four specific types of monitoring:

- a. *Near Real-Time Air Monitoring.* Five MINICAMS® are used to monitor the workspace inside the RRS operations trailer. Set to alarm at 80-percent of the time-weighted average (TWA) hazard level for each of the vaporous chemical materials, the MINICAMS® systems provide early, rapid warning of airborne exposure hazards inside the operations trailer. These systems are also used to monitor the midbed of the carbon filter ventilation system at the 80-percent TWA level, indicating any need for replacing the used charcoal filter banks before contamination reaches the filter bank exhaust. The MINICAMS® are also used as needed to verify that the glovebox system has been decontaminated, and for monitoring waste drums.
- b. *Confirmation Air Monitoring.* MINICAMS® alarms for HD⁴, HN-1 and HN-3 are confirmed by the Depot Area Air Monitoring System (DAAMS); bubbler samples are used to confirm MINICAMS® alarms for L. The DAAMS and bubbler samples are analyzed at an offsite or mobile onsite laboratory. MINICAMS® for PS, CG, CK, and chloroform are confirmed with colorimetric gas detection tubes.
- c. *Historical Air Monitoring.* Historical methods are used to monitor the charcoal filter exhaust. Historical monitoring at the TWA level utilizes DAAMS methods for HD, HN-1, and HN-3, and bubbler methods for L.
- d. *Monitoring Solid and Liquid Wastes.* Solid and liquid wastes are collected and sealed in impermeable containers. Samples of liquid wastes and solid powdery residues may be collected and sent for laboratory analysis as required. Solid and liquid wastes are packaged in accordance with DOT regulations, and shipped to a hazardous waste treatment facility. Final field sampling procedures will be promulgated after testing at Tooele Army Depot.

2.3 Monitoring Standards

Contamination of the ambient air with HD, HN-1, HN-3, L, CK, CG, PS, or chloroform vapors may present a hazard if the concentration of airborne contaminants rises above the TWA levels indicated in Table 1. The TWA values for each chemical are established by the Surgeon General as the permissible exposure level for workplace activities, and indicate the level at which exposure to that chemical for 8 hours a day, 40 hours per week, indefinitely, will not cause adverse effects.

⁴ For the remainder of this document, discussions of HD encompass H, HS, and HD.

Table 1. Workplace Exposure Limits for CAIS Compounds

Chemical	Workplace Time-Weighted-Average (mg/m ³)
Mustard (HD)	0.003 ^{a,b}
Nitrogen mustard (HN)	0.003 ^c
Lewisite (L)	0.003 ^{a,b}
Cyanogen chloride (CK)	0.6 ^{c,d,e}
Phosgene (CG)	0.4 ^d
Chloropicrin (PS)	0.67 ^f
Chloroform	9.7 ^d

NOTES:

- a Department of the Army, 1992³
- b U.S. Department of Health and Human Services, 1988⁴
- c U.S. Army Toxic and Hazardous Materials Agency, 1983⁵
- d Occupational Safety and Health Administration, 1993⁶. This standard is below the threshold limit value listed by the American Conference of Governmental Industrial Hygienists (ACGIH).
- e Ceiling value, not to be exceeded at any time during working day.
- f Threshold Limit Values and Biological Exposure indices for 1993-1994, ACGIH (1993)⁷.

Investigation-derived wastes may also be contaminated with chemical materiel and are screened for contamination before transport to waste treatment facilities. Residual chemical materiel contamination of liquid wastes must be below the established decontamination limits, currently under development. The levels must also be below the permitted levels for each specific hazardous waste facility before the waste may be received.

3. Non-Stockpile Monitoring Program

USACDRA is also developing monitoring methods, techniques, and strategies for use during other PMNSCM activities at small CWM burial sites, including transportation, storage, and use of other disposal systems. Guidance for monitoring during these operations is provided in the PMNSCM Monitoring Concept Plan for Buried Chemical Materiel, Types 1 and 2⁸.

The PMNSCM monitoring program currently focuses on the 21 chemicals listed in Table 2. Additional chemicals may be added upon recommendation by the Non-Stockpile Monitoring Steering Committee. This committee, currently directed by USACDRA, is being formed to address questions that arise during development of the Non-Stockpile program. The committee will involve USACDRA and other Army organization and outside agencies in the evaluation of analytical methods, procedures, instruments, and other monitoring requirements. It is hoped that this committee will assist in integration of the agencies' efforts, and ensure that decisions made regarding monitoring at these sites are consistent between, and supported by, the Non-Stockpile community.

Table 2. Non-Stockpile Chemical Materiel

Chemical Materials	Industrial Chemicals
Levinstein mustard (H)	Adamsite (DM)
Lewisite (L)	Bromobenzyl cyanide (CA, also known as BBC)
Sulfur Mustard (HD)	Chloroacetophenone (CN)
Sulfur Mustard-lewisite mixture (HL)	Chloroacetophenone in benzene and carbon tetrachloride (CNB)
Sulfur Mustard-T mixture (HT)	Chloroacetophenone and chloropicrin in chloroform (CNS)
Nitrogen mustards (HN)	Chloropicrin (PS)
Sarin (GB)	Cyanogen chloride (CK)
Soman (GD)	Hydrogen cyanide (AC)
Tabun (GA)	Phosgene (CG)
VX	White phosphorous (WP)
	3-Quinuclidinyl benzilate (BZ) ^a

NOTES:

a This compound was predominantly used by the military; however, it is commercially available, may be commercially distributed, and is classified non-lethal (DA, Memorandum DAMO-FDB, 13 June 1994)⁹.

4. REFERENCES

1. U.S. Army Chemical Materiel Destruction Agency (USACMDA). Survey and Analysis Report for the Non-Stockpile Chemical Materiel Program. 1993.
2. U.S. Army Chemical Disposal and Remediation Activity (USACDRA). ERDEC Raman Test. Test Report. December 1994.
3. Department of the Army (DA). The Army Chemical Agent Safety Program, Army Regulation (AR) 385-61. 3 November 1992.
4. U.S. Department of Health and Human Services. Final Recommendations for Protecting the Health and Safety Against Potential Adverse Effects of Long-Term Exposure to Low Doses of Agents: GA, GB, VX, Mustard Agent (H, HD, T), and Lewisite (L). Centers for Disease Control. Federal Register 53: 8504-8507. 1988.
5. U.S. Army Toxic and Hazardous Materials Agency. Disposal of Chemical Agent Identification Sets at Rocky Mountain Arsenal, Colorado. Final Report. Report No. DRXTH-IS-FR 83203. August 1983.
6. Occupational Safety and Health Administration, Code of Federal Regulations, 29 CFR 1910.1000. Occupational Safety and Health Standards for Toxic and Hazardous Substances. 1992.
7. American Conference of Government Industrial Hygienists (ACGIH). Threshold Unit Values and Biological Exposure Indices for 1993-1994. 1993.
8. U.S. Army Chemical Materiel Destruction Agency (USACMDA). Program Manager for Non-Stockpile Chemical Materiel (PMNSCM) Monitoring Concept Plan (MCP). Buried Chemical Materiel Types 1 and 2. July 1994.
9. Department of the Army (DA). Memorandum DAMO-FPD. 13 June 1994.

SESSION II EXISTING ANALYTICAL METHODS



ANALYTICAL TECHNIQUES FOR THE DETECTION AND IDENTIFICATION OF CHEMICAL WARFARE MATERIALS FROM ENVIRONMENTAL SAMPLES

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INTRODUCTION

The detection and identification of chemical warfare (CW) material in diverse and complex matrices has become increasingly important to support the environmental clean-up of military and industrial sites that were historically used in the research, production, use, storage and/or demilitarization of chemical weapons. Reliable and defensible identification of hazardous materials (HM) is necessary to comply with the increasingly stringent regulations imposed by local, state, and federal agencies which govern handling, treatment, storage, and disposal of HM. In addition, before sites can be reutilized, existing HM must be properly identified so that the proper methods of removal, treatment and disposal can be determined.

The samples collected from a given site may be extremely varied and can include soil, water, vegetation, common construction debris, air, or neat chemicals. As a result, sample preparation and analytical techniques must be flexible, yet complete, to characterize a given sample. A variety of chromatographic and spectroscopic techniques are applied to each sample, with information gained from one technique often guiding the selection of subsequent techniques. In many cases, it is necessary to collect complementary data from multiple techniques so that unknowns can be identified in the absence of verifiable authentic standards. The analytical techniques employed include nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC) with multiple detectors, high performance liquid and ion chromatography (HPLC/IC), mass spectrometry (MS), atomic absorption spectroscopy (AA), inductively coupled plasma emission spectro-photometry (ICP) and infrared (IR) spectroscopy. An overview of sample preparation and analytical techniques employed for the detection and identification of CW materials is presented below.

ANALYTICAL PROCEDURES

Sample Preparation. Each sample is prepared for analysis according to a set protocol depending on the type of sample. Neat (without solvent) liquids are examined as received, and/or after concentration, in addition to being extracted in the same way as soil/debris samples (see Figure 1). Soil/debris samples are generally extracted with both organic and aqueous solvent (Figure 2). The solvents used for extraction vary according to the analysis to be performed but in general include chloroform (CHCl_3), methanol (CH_3OH), methylene chloride (CH_2Cl_2), tetrahydrofuran (THF), hexane (C_6H_{12}) or their deuterated analogues.

In general, each of the samples or sample/solvent combinations is homogenized using a vortex mixer for 30 s; sonicated in ice for at least 10 min; and phase separated using a centrifuge followed by decanting. Depending on the protocol, samples may be concentrated by

Figure 1. Sample preparation for aqueous samples.

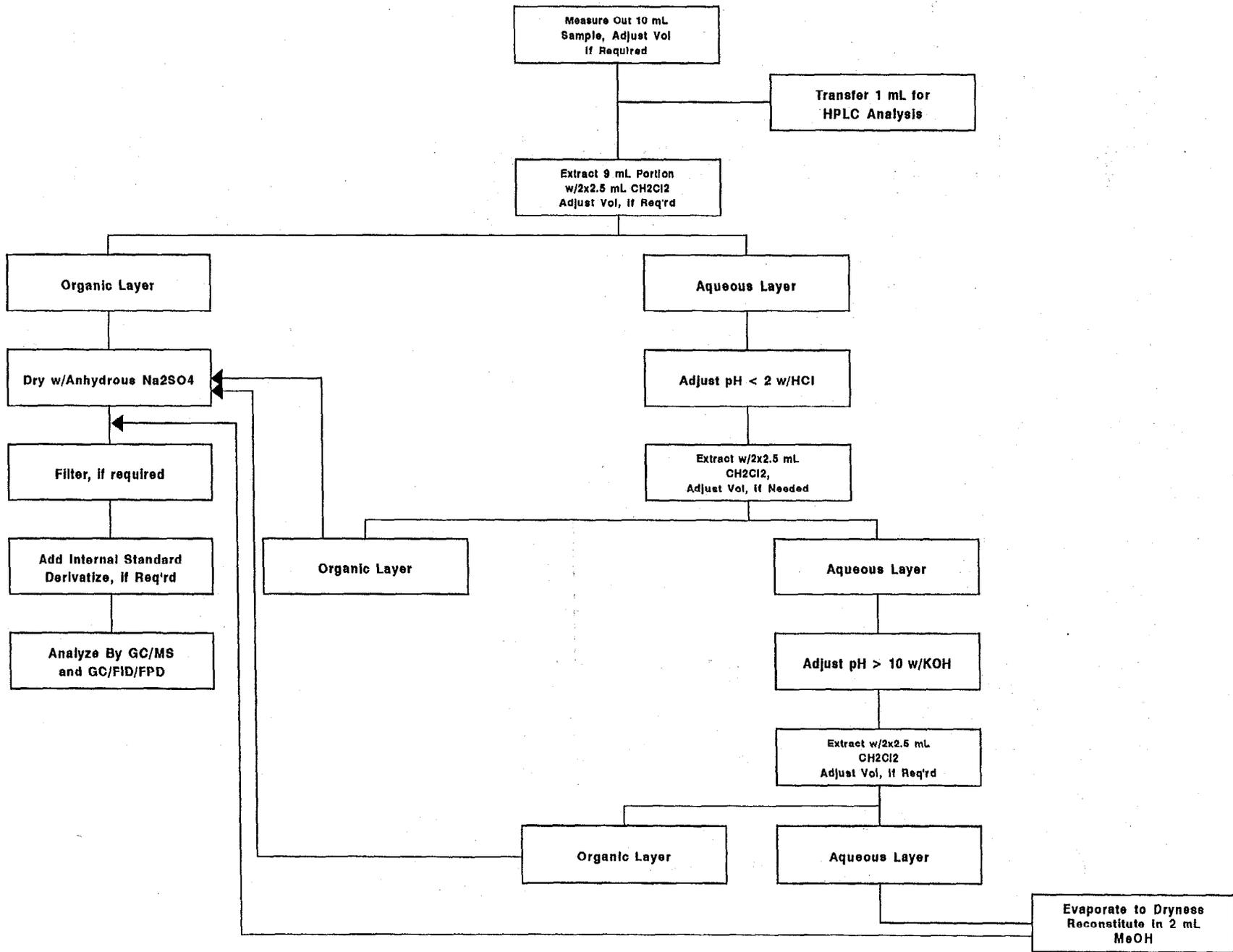
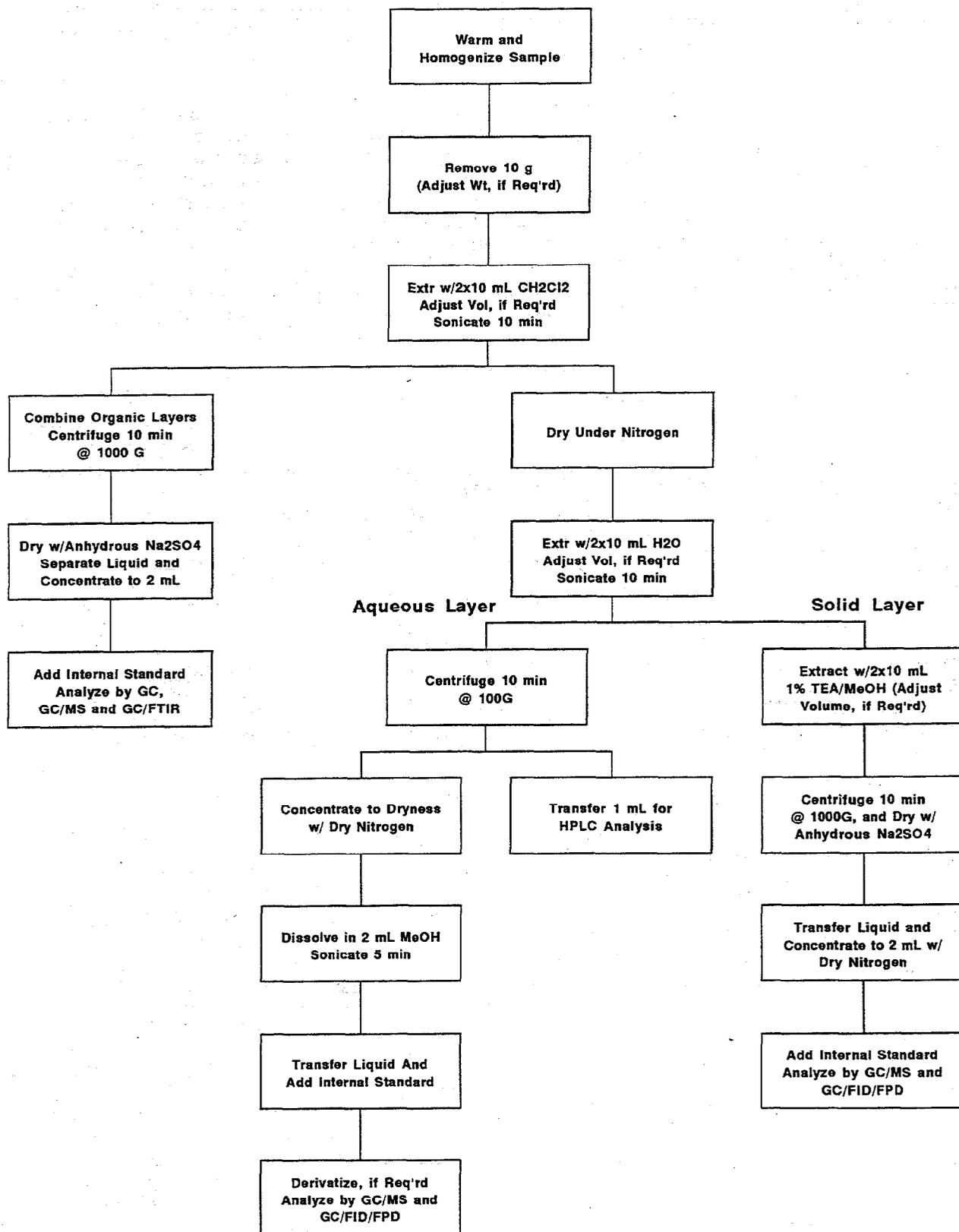


Figure 2. Sample preparation for soil/debris samples.



passing a stream of nitrogen over the liquid; or derivatized, converting analytes which do not chromatograph readily (e.g. alkyl-phosphonic acids) into forms amenable to column separation. All sample weights, sample extraction volumes, recovery volumes, etc. are measured and recorded. The extracts are then distributed for analysis (Figure 3).

Chromatography. Chromatographic techniques are used to separate the components of a sample before detection. The separation is based on a physical partitioning of the components between two phases; normally one of these phases is fixed, or stationary and the other is mobile. When the mobile phase is a gas, either a liquid or a solid phase can be used as a stationary phase and the technique is generally termed gas chromatography (GC). Once separated, the individual components are detected and quantitated using a variety of specific and non-specific detector systems. High performance liquid and ion chromatography (HPLC/IC) is similar to GC, using a liquid as the mobile phase.

(1) GC

In GC, the stationary phase is contained in a column through which the mobile phase is continually swept. Samples are introduced at the inlet to the column, and the individual components pass through the column at a rate determined by their relative affinity for either the mobile or stationary phase. Components with a high affinity for the stationary phase take longer to pass through the column than those with a low affinity. Components leave the column at different times and are individually detected using a variety of detector systems, the selection of which depends on the type of sample, the sensitivity required, the agents suspected to be present and the protocol that is being followed. These can include: thermal conductivity (TC), flame ionization (FID), flame photometric (FPD), electron capture (ECD), ion trap (ITD), mass selective (MSD), sulfur chemiluminescence (SCD) and nitrogen/phosphorus (NPD). Instruments can be equipped with auto injectors or thermal desorption inlets as required.

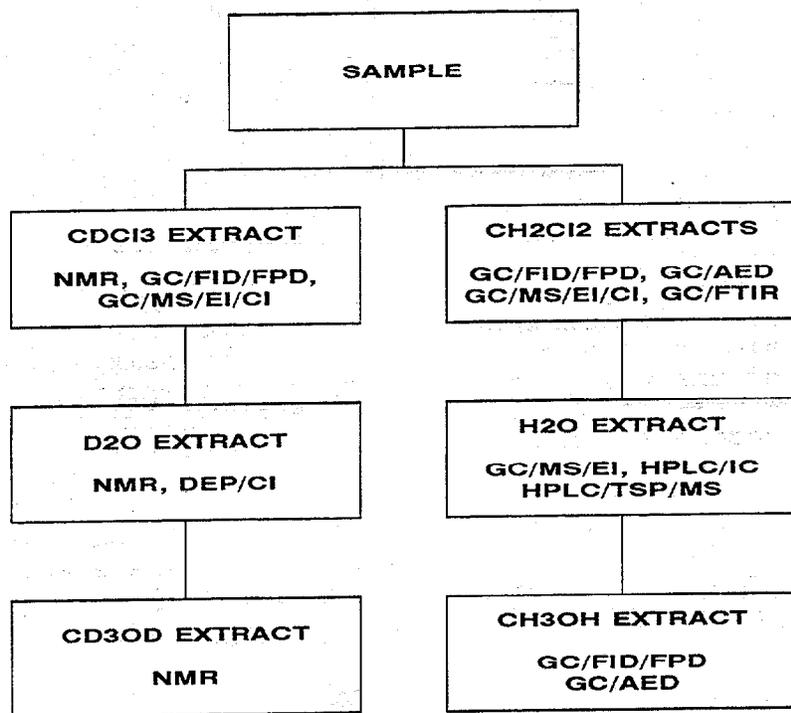
(2) HPLC/IC

High performance liquid and ion chromatography are analytical techniques whereby (1) samples are injected into a pump driven liquid mobile phase stream; (2) the analytes of interest are separated from the sample matrix downstream on a solid stationary phase contained in a column; and (3) the separated analytes of interest are instrumentally detected at the column outlet. Ion-exchange, ion-exclusion and reverse phase mechanisms operate to separate the CW materials and degradation products on the column. Detection is normally accomplished by ultra violet (UV), electrochemical (EC), or conductivity detectors.

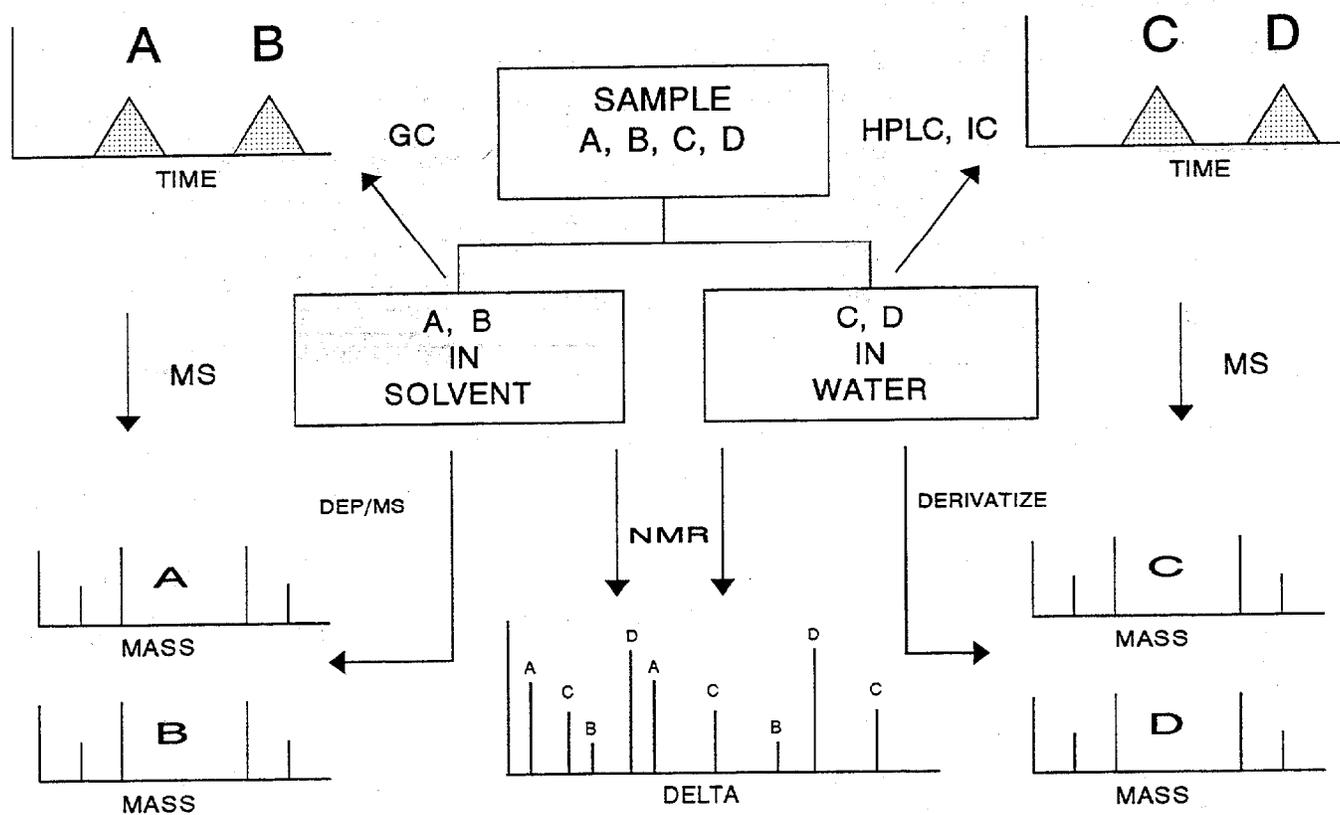
Spectroscopy. Spectroscopic techniques are used to probe the chemical composition and structure of individual components of a sample mixture. These techniques are used for compound identification and, in most cases, quantitation. When combined with chromatography for analyte separation, they provide powerful detection, identification and quantitation tools.

Figure 3. Distribution of samples for analysis.

(a) Distribution of sample extracts to analytical techniques.



(b) Analyte separation and identification from a mixed sample of analytes A, B, C, and D.



(1) NMR

Nuclear magnetic resonance spectroscopy is a technique used to characterize the molecular structure of chemical compounds. Samples, usually neat liquids or solids dissolved in appropriate solvents, are placed into a strong, homogeneous magnetic field and irradiated at characteristic radio frequencies to probe the nuclei comprising the analyte. The information collected is specific to a given nucleus. For example, a ^1H NMR spectrum consists of signals for all the ^1H nuclei in the sample. Information for a given nucleus (i.e., ^{13}C , ^{31}P , ^{19}F , etc.) can be collected separately (1D NMR), or in conjunction with other nuclei (2D NMR). The chemical structure is determined from the resulting spectrum, or in complex cases, from multiple spectra. Because the signals observed are directly proportional to the amount of a given nucleus in the sample, NMR is quantitative and the mole ratio of compounds present can be determined. However, NMR is not inherently as sensitive as GC or MS and requires pre-concentration to reach a sensitivity of 1 ppm.

Spectra are interpreted manually and assignments confirmed by comparison with spectra of authentic samples from an in-house database. For those compounds not in the database, structure elucidation by NMR is complemented by spectroscopic data from GC/MS, HPLC/IC and/or Fourier Transform Infrared Spectroscopy (FTIR) analyses.

(2) MS

Mass spectrometry provides for the identification of compounds of interest based on molecular weight and fragmentation patterns. Liquid samples or extracts are commonly introduced to the mass spectrometer directly (direct exposure probe, DEP), or after chromatographic separation (i.e., GC, LC, etc.). Analytes that do not chromatograph readily by GC are derivatized before analysis. Vapor samples are first concentrated by collection on an adsorbent material such as Tenax, then desorbed, separated, and directed into the mass spectrometer. The fragmentation/ionization of the analyte is then accomplished in the spectrometer in several ways, two of which, electron impact (EI) and chemical ionization (CI), are commonly used to detect CW related materials. Samples are analyzed either neat or as a solvent extract. The mass range is normally scanned from 60 to 550 atomic mass units (amu) at 1 scan/sec. Compound identification is accomplished via computer assisted matching of the resulting spectra with those maintained in standard databases or by direct comparison with spectra obtained from authentic reference compounds.

(3) FTIR

Fourier transform infrared spectroscopy is also used to characterize the molecular structure of chemical compounds. Samples are prepared as liquids or solids and irradiated with infrared radiation. Signals are obtained which represent the chemical bonds and functional groups in the molecule. Identification of compounds is based on comparison with library reference spectra and by interpretation in conjunction with the other spectroscopic techniques (NMR and MS).

(4) ICP

Inductively coupled plasma emission spectrophotometry is used to perform quantitative elemental analyses and is usually set to screen for approximately 40 elements. Samples are prepared by digestion in nitric acid with microwave heating. Detection limits vary for the elements but typically range from 0.01 - 2 ppm.

(5) Graphite Furnace Atomic Absorption (GFAA) Spectrophotometry

Graphite furnace atomic absorption spectrophotometry is a technique commonly used to detect arsenic in complex matrices. Prior to analysis, the samples are digested with nitric acid and hydrogen peroxide at 95 °C in order to convert organically bound arsenic to an inorganic form. A nickel nitrate solution is added to each sample to serve as a matrix modifier preventing arsenic volatilization during sample heating. Standard arsenic solutions are prepared from traceable standards and used for quantitation. The detection limit for arsenic determined in this manner is 0.005 ppm (5 ppb).

APPLICATIONS

NMR. Normally, NMR is used primarily as a tool for determining the chemical structure of concentrated samples of relatively high purity. However, recently it has proven to be a useful technique for screening samples for CW materials and degradation products. It is best used for the screening of neat liquids, aqueous samples and decontamination solutions for the breakdown products of G, V agents and sulfur mustard (H). Neat liquids can be identified quickly, often before sample preparation begins for the other techniques. Sample preparation and further analysis can then be modified to best utilize available techniques.

NMR is particularly valuable for screening aqueous samples for G and V degradation products. For example, the alkylphosphonic acids, which are difficult to chromatograph, are easily detected using ^{31}P and ^1H NMR in most solvent systems. Analyte detection levels of 10-20 ppm are often obtained without concentration, and levels of 1-5 ppm after concentration. Special solvent suppression NMR techniques can be employed to reach a detection limit of 1 ppm or lower.

^1H NMR is used to screen CDCl_3 , D_2O , and CD_3OD extracts. If evidence for CW materials containing phosphorus is found, then a longer term screen using ^{31}P NMR is carried out as confirmation. If evidence for H or lewisite (L) related materials is found, confirmation is not done by NMR since these materials contain only ^{13}C as an active NMR nucleus (at least 100 times less sensitive than ^1H or ^{31}P NMR).

GC. Trace level screening for chemical agents and their degradation products is normally performed by GC using a flame photometric detector (GC/FPD) operating in the phosphorus mode for organophosphorus-based G and V agents, or in the sulfur mode for H or related compounds. A portion of the questionable matrix (i.e. water,

soil, etc.) is spiked with a mixture of chemical agents. The spiked sample and a similar non-spiked portion are then separately extracted with chloroform. The sample extracts are then instrumentally compared with each other and with standard calibration curves. This allows for quantitatively determining agent content, if present, and for determining the extraction efficiency for recovery of the agents from the matrix in question. The detection limits are typically 0.02 ug/mL (20 ppb) for organophosphorus agents and 0.1 ug/mL (100 ppb) for sulfur compounds.

Identification of unknown components in a sample can be accomplished using GC coupled with an ion-trap detector (GC/ITD). The GC/ITD technique is usually performed on organic extracts from a suspect matrix and allows for separation of non-ionic organic compounds and the generation of mass spectra for each component. Pre-screening, using GC with an FID detector, is usually performed to establish working concentration levels necessary to avoid saturation of the ITD detector and to establish optimum separation parameters for the GC. The identity of a component can be determined by computer assisted matching of the mass spectrum against library databases of known compounds or by comparison with spectra obtained from authentic compounds. This technique is sensitive to the 1 ppm level or better without concentration of the sample.

HPLC. These techniques are utilized on aqueous samples to search for breakdown products of CW agents. The breakdown products of G and V type agents (i.e., phosphonic acids) are separated using an ion-exchange column based on their relative acid dissociation constants (detection limits ~1 ppm). Mustard (H) and L degradation products are analyzed by reverse phase techniques based on their respective lipophilicity with detection limits ranging from 100 ppb to 1 ppm. Compounds such as thiodiglycol (TDG), thiodiglycolsulfoxide (TDGO), thiodiglycolsulfone (TDGO₂), arsenite (AsO₂⁻), and arsenate anions (AsO₄⁻²) are best separated using ion-exclusion methods and are detectable down to 1 ppm.

MS. GC/MS/CI(EI). Samples are separated using conventional GC techniques and introduced through an interface into the mass spectrometer. For CI, the reagent gas is usually methane, although ammonia is also used. Neat samples are injected in a split mode to avoid saturation and overload; extracts are injected in the splitless mode.

DEP/CI(EI). Solid samples and extracts are analyzed as required by placing the sample on a direct exposure probe tip. After evaporation of the solvent at room temperature, the probe is placed at the inlet to the mass spectrometer and rapidly heated, introducing the sample into the spectrometer. The mass spectrum is then recorded under CI or EI conditions. Unlike the GC technique above, there is no separation of components, and the spectrum acquired is a composite of all the components present. Mass to charge ratios (m/z) are reported for each compound identified.

Quantitation can be accomplished by GC/MS using single ion monitoring (SIM) or mass chromatography. The peak areas of specific ions are used to calculate the analyte concentration against a standard calibration curve.

Additional Techniques. Other techniques, such as FTIR, ICP, and GFAA are used to supplement the data acquired from the primary techniques described above. Functional group identification and structure determination by FTIR augments data obtained by MS and NMR. Sample composition determined by elemental analysis (ICP, GFAA) provides valuable information on elements such as arsenic, sulfur, phosphorus, chlorine, iron, and aluminum that are not detected by other techniques.

SUMMARY

Sample preparation and analytical techniques must be flexible, yet complete, to characterize samples containing the wide variety of CW agents and their degradation products that exist. A variety of chromatographic and spectroscopic techniques are necessary, with information gained from one technique supplementing and often guiding the selection of subsequent techniques. In many cases, it is necessary to collect complementary data from multiple techniques so that unknowns can be identified in the absence of verifiable authentic standards. The analytical techniques employed include nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC) with multiple detectors, high performance liquid and ion chromatography (HPLC/IC), mass spectrometry (MS), atomic absorption spectroscopy (AA), inductively coupled plasma (ICP) emission spectrophotometry and infrared (IR) spectroscopy.

Rapid Detection of Chemical Agents Using Direct Sampling Ion Trap Mass Spectrometry

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ABSTRACT

Advances in ion trap mass spectrometry have led to the development of highly sensitive analytical instruments which are versatile, simple, and relatively easy to operate¹⁻⁴. In addition, dramatic reductions in the size and weight of ion trap mass spectrometers have facilitated the modification of these devices for use in the field⁵. These combined features have been exploited for the development of direct thermal desorption mass spectrometry methods for the rapid determination of trace levels of chemical warfare agents and precursors in air and other media. These analytical methods generally require only minimal sample preparation and utilize little or no chromatographic separation of the analytes prior to mass analysis. This provides fast results and a high sample throughput. Compound specificity is achieved through the combined use of selective chemical ionization (CI) and collision induced dissociation multi-stage mass spectrometry (MS/MS)⁶⁻⁸ making it possible to discriminate against many common chemical interferences such as hydrocarbons. The thermal desorber inlet for the ion trap was designed to be used with standard 3 inch x 0.25 inch sorbent tubes (DAAMS tubes) packed with either Tenax or Chromosorb 106. In addition to the analysis of compounds collected on sorbent tubes, the thermal desorber can also be used as a heated injector for the direct analysis of microliter aliquotes of liquid samples, such as groundwater and extracts of plant material. Detection limits are typically in the range of 50 pg of analyte adsorbed onto a sorbent tube and sample turnaround time is approximately 2-3 minutes. This technology has been evaluated using GB, G-analog of VX, and HD as well as simulants for each of these compounds. Work is also currently in progress in our laboratory to evaluate the capabilities of various other ion trap mass spectrometers and inlet configurations for real-time determination of CW agents and precursors which may be encountered in a variety of scenerios.

BACKGROUND

Direct Sampling Ion Trap Mass Spectrometry (DSITMS) was originally developed in the late 1980's as an analytical tool for confirming airborne releases of GB, VX, and HD at chemical munition demilitarization facilities⁹. Because of the highly toxic nature of these agents, the analytical detection limits for these compounds must be below the levels which present both acute and chronic health hazards (see Table 1) to those working at the demilitarization facilities. In addition, stringent stack emission levels have been established to help protect the general public from exposure to accidental releases of hazardous

TABLE 1
Monitoring Levels and Detection Limits for GB, VX, HD^a

2-Hour Time Weighted Average (2 Hr TWA)^b				
Agent	TWA Limit	Flow Rate	Sample Time	0.125 of TWA
GB	1x10 ⁻⁴ mg/m ³	200 mL/min	120 min	300 pg
HD	3x10 ⁻³ mg/m ³	200 mL/min	120 min	9,000 pg
VX	1x10 ⁻⁵ mg/m ³	1,000 mL/min	120 min	50 pg
12-Hour Time Weighted Average (12 Hr TWA)^b				
Agent	TWA Limit	Flow Rate	Sample Time	0.125 of TWA
GB	1x10 ⁻⁴ mg/m ³	200 mL/min	720 min	1,800 pg
HD	3x10 ⁻³ mg/m ³	200 mL/min	720 min	54,000 pg
VX	1x10 ⁻⁵ mg/m ³	200 mL/min	720 min	180 pg
Allowable Stack Concentration (ASC)^c				
Agent	ASC Limit	Flow Rate	Sample Time	0.125 of ASC
GB	3x10 ⁻⁴ mg/m ³	250 mL/min	60 min	563 pg
HD	3x10 ⁻² mg/m ²	250 mL/min	60 min	5,625 pg
VX	3x10 ⁻⁴ mg/m ⁴	250 mL/min	60 min	56 pg
General Population Limit (GPL)^c				
Agent	GPL Limit	Flow Rate	Sample Time	0.125 of GPL
GB	3x10 ⁻⁶ mg/m ³	200 mL/min	720 min	54 pg
HD	1x10 ⁻⁴ mg/m ³	200 mL/min	720 min	1,800 pg
VX	3x10 ⁻⁶ mg/m ³	1,000 mL/min	720 min	270 pg
Immediate Danger to Life and Health (IDLH)^b				
Agent	IDLH Limit	Flow Rate	Sample Time	0.125 of IDLH
GB	2x10 ⁻¹ mg/m ³	200 mL/min	1 min	5,000 pg
HD	4x10 ⁻¹ mg/m ³	200 mL/min	1 min	10,000 pg
VX	4x10 ⁻¹ mg/m ³	200 mL/min	1 min	10,000 pg

^aThe quantities shown in this table indicate the required analytical detection limits for chemical agents collected and concentrated on DAAMS tubes under the indicated conditions of flow, sampling time, and required monitoring levels. The TWA, ASC, GPL, and IDLH levels shown in mg/m³ are the instantaneous analytical detection limits for real-time continuous monitoring.

^bProgram Manager for Chemical Demilitarization, Aberdeen Proving Ground, MD, personal communications to M. Wise, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN. 1987.

^cDepartment of Health and Human Services, Centers for Disease Control, "Final Recommendations for Protecting the Health and Safety Against Potential Adverse Effects of Long-Term Exposure to Low Doses of Agents: GA, GB, VX, Mustard Agent (H, HD, T) and Lewisite (L)." *Fed. Reg.* 53:8504-8507. (Mar 15, 1988).

materials. In order to satisfy the need for occupational exposure monitoring, the analytical response time must be fast enough provide a real-time or near real-time indication of the presence of CW agents at or above levels which present an immediate danger to workers, such as might occur from as a result presence of leaking munitions or storage containers. Instrument sensitivity and reliability must be sufficient so that false negative indications will not occur, especially at concentrations of agents in air which produce acute toxic effects. Further, in order to minimize the chances of false positives which could cause needless and costly facility down-time, the specificity of the analytical method must be sufficient to allow discrimination against commonly encountered interferences. This paper describes the basic direct sampling ion trap instrumentation, the general method of direct thermal desorption, and overall performance of the analytical method.

ION TRAP MASS SPECTROMETRY INSTRUMENTATION

Because of the need for very high sensitivity, fast response, and the capability to respond to a variety of different types of compounds, mass spectrometers are a logical choice for real-time or near real-time detection of CW agents and related precursors or breakdown products¹⁰⁻¹². Ion trap mass spectrometers in particular have unique features which make them especially useful for this application. These include: 1) enhanced sensitivity due to the ability to store and accumulate ions of targeted analytes, 2) enhanced selectivity due to the ability to utilize a wider range of chemical ionization reagents than is possible with conventional chemical ion sources, 3) enhanced specificity by using MS/MS techniques, and 4) easy conversion to direct sampling operation without the need for additional vacuum pumping hardware. Furthermore, the mechanical simplicity of ion traps and their ability to operate at higher background pressures of air and water than most other types of mass spectrometers makes them especially rugged and reliable for direct sampling and field analysis.

At the time of our initial involvement in the development of the instrumentation for use at chemical demilitarization facilities, ion trap mass spectrometers were in an early stage of commercialization with only two models available, both manufactured by Finnigan MAT Corporation (San Jose, CA). These were the benchtop IonTrap Detector (ITD) which was sold as a GC detector with limited analytical capabilities, and the much larger Ion Trap Mass Spectrometer (ITMS) which was a research instrument equipped with EI, CI, selective ion storage, and MS/MS capability. Because the instrument which was being developed was to be installed in a laboratory setting at the demilitarization facility, physical size of the instrument was not an important consideration. For this reason, the Finnigan ITMS was selected as the platform for the development of the direct thermal desorption methods.

Most of the results described in this paper were generated using a direct sampling ITMS instrument. Although the Finnigan ITMS is no longer available, there are presently several other ion trap mass spectrometer products have become available from Finnigan and other vendors (see Table 2). In fact, significant advances in electronic and computer technology during the last 3 to 4 years have resulted in ion trap instruments which have

superior analytical capabilities, yet are considerably smaller than the original ITMS. One of these ion trap instruments in particular has been developed for the Edgewood Research and Development Engineer Center (ERDEC) by Bruker Franzen (Germany) specifically for the detection of chemical and biological weapons¹³⁻¹⁴. Other instruments such as the Finnigan Magnum and the Teledyne 3-DQ are sold commercially as general purpose mass spectrometers and GC detectors, they have been successfully modified in our laboratory for direct sampling and for on-site field applications. These instruments have performed well for environmental monitoring applications and the analytical methods which were originally developed using the laboratory-based ITMS have transferred easily to these newer instruments with little or no modification.

Table 2
Commercial Ion Trap Instruments

<u>Manufacturer</u>	<u>Instrument</u>	<u>Capabilities</u>
Bruker-Franzen ^a	CBMS	EI, MS/MS, 512 amu Fieldable
Finnigan MAT	Magnum ^b	EI, CI, 650 amu
	Magnum Upgrade	Negative ions, MS/MS Selected Ion Monitoring 800 amu
Teledyne	3-DQ ^b	EI, CI, MS/MS, 650 amu Selective Ion Storage Selective ion monitoring Enhanced sensitivity
Varian	Saturn ^b	EI, CI, MS/MS, 650 amu Selective ion monitoring

^a The CBMS was developed for the US Army ERDEC by Bruker-Franzen.

^b Indicates that this model of instrument has been successfully modified and tested with the ORNL-developed direct sampling inlet system.

DIRECT SAMPLING INLET SYSTEM FOR ION TRAP MASS SPECTROMETERS

The direct sample inlet system has been designed to be compatible with all commercially available ion trap mass spectrometers which have a 1 inch ID O-ring compression fitting for the installation of the sample transfer line. A typical ion trap configuration for direct sampling is shown in Figure 1.

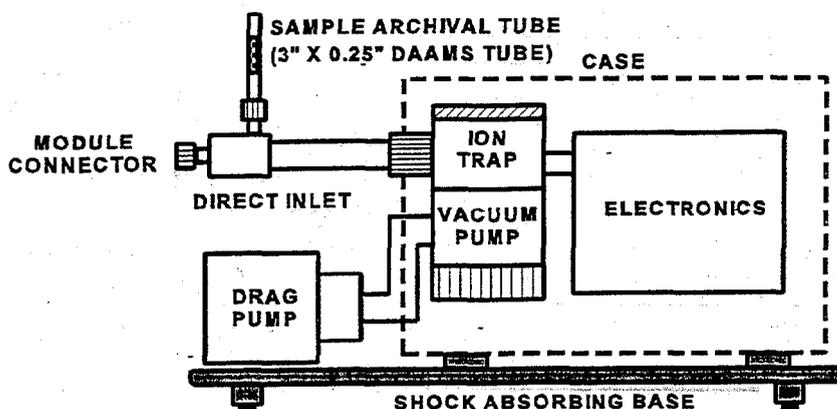


Figure 1. Typical ion trap mass spectrometer configured with a direct sampling inlet.

The hardware consists of a universal interface and several readily interchangeable sample inlet modules for the real-time analysis of air, purge analysis of water and soil-slurries, and thermal desorption of sorbent tubes as shown in Figure 2. The universal interface consists of fixed-orifice splitter connected to a heated capillary restrictor transfer line (12 inches x 100 microns ID). This interface is a direct replacement for most of the standard GC interfaces which are provided with the commercial ion traps. The sample inlet modules connect directly with the open/split interface by means of a quick connect fitting and a single electrical cable which mates with a receptacle on the ion trap chassis. These features enable the ion trap to be converted from one sampling mode to another in less than 1 minute without the need for breaking vacuum on the mass spectrometer. An important added feature is that the vent port of the splitter is configured to accept 1/4 inch diameter sorbent tubes. These sorbent tubes can be used to collect and archive up to 99% of the analytes from a sample while the remaining 1% are analyzed with the ion trap. The archived samples can be analyzed at a later date using conventional thermal desorption GC/MS or other techniques for quality assurance or confirmation of results. This can be a particular benefit when dealing with unique samples such as might be collected as the result of sporadic transient events.

DIRECT THERMAL DESORPTION ION TRAP ANALYTICAL METHODS

Although real-time direct air monitoring as well as direct analysis of water and soil samples are all being investigated for their applicability to the rapid detection low part-per-billion levels of CW agents, precursors, and breakdown products, most work to date has involved the direct thermal desorption of DAAMS tubes. The use of DAAMS tubes for sample collection provides a convenient means of measuring integrated TWA samples and also

provides sufficient preconcentration capacity to enable sub part-per-billion detection limits to be achieved for targeted analytes in air. For these experiments, the thermal desorber was attached directly to the open-split interface on the ion trap. The method which was developed for CW agents involved flash heating a DAAMS tube for 10 seconds with 450-500 watts of power while purging the sorbent bed with 40 to 60 mL/min of helium. The compounds which were desorbed from the DAAMS tube were transported into the ion trap through the capillary restrictor which was heated to 200 C. The flow rate through the restrictor into the ion trap was approximately 1 standard mL/min and the flow rate through the split vent port was 39 to 59 standard mL/min. This means that approximately 97% or more of the analytes could be recollected and archived on a DAAMS tube attached to the split vent if desired.

In all cases for GB, HD, VX and their simulants, the ion trap was operated using proton transfer chemical ionization in order to minimize fragmentation of the analyte molecules and to provide enhanced selectivity against possible interferences such as hydrocarbons. Isobutane (99.9%) was used as the chemical ionization reagent and was selected because it has a proton affinity which is greater than that of most hydrocarbons and less than that of the chemical warfare agents and simulants. The isobutane was admitted into the ITMS by means of the batch inlet which was equipped with a metering valve and was typically

maintained at a nearly constant pressure of 5×10^{-5} torr (uncorrected ionization gauge reading). In order to achieve maximum sensitivity and specificity for the CW agents and simulants, the ion trap was always operated using MS/MS. The objective was to form a protonated molecular ion by means of the chemical ionization reaction followed by collision induced dissociation MS/MS of these ions to form a characteristic fragment ion spectrum.

The specific CI MS/MS scan functions (instrument control software routines) were written for each of the CW agent simulants using the experiment editor provided with the commercial software. In each case, the isobutane was typically ionized for 5-10 msec with the electron beam to create the isobutane reagent ions. Following this step, all ions above mass-to-charge (m/z) 60 were ejected from the ITMS, allowing the isobutane reagent ions to remain in the ion trap cell while electron ionization products resulting from other compounds were eliminated. The reagent ions were then allowed to react for 50-100 msec in order to generate $(M+H)^+$ ions for the analyte of interest. For each of the CW agents and simulants, isobutane efficiently formed $(M+H)^+$ with very few fragment ions. Following the chemical ionization reaction event, the $(M+H)^+$ ion for the analyte of interest was isolated in the ITMS cell through a combination of rf sweeps and applied dc potentials. Typically, all of the ions at m/z values less than that of the $(M+H)^+$ ion for the simulant were ejected by ramping the rf level to a point just below the stability of this ion. Following this step, all ions at higher m/z values were ejected by applying a dc potential to the cell at the same time in order to create a "notch filter" in which only the $(M+H)^+$ ions remained stable. Using this technique, it was possible to cleanly eject ions within 1 amu above and below the desired analyte ion without significantly affecting the intensity of the analyte ion.

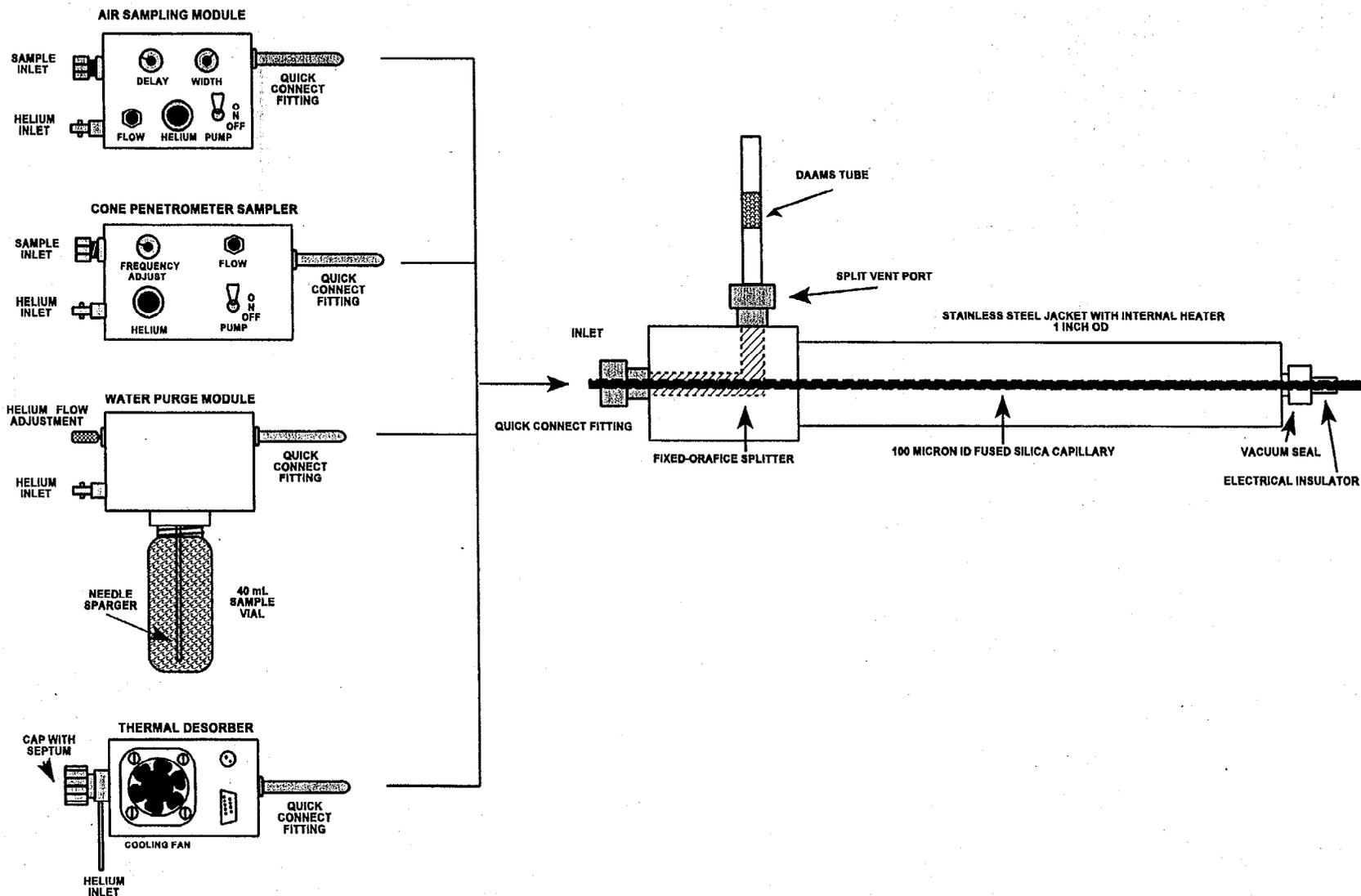


Figure 2. Direct sample introduction modules and capillary restrictor inlet system for the ion trap mass spectrometer.

Collision induced dissociation (CID) MS/MS of the $(M+H)^+$ ion was performed by irradiating the isolated ions with a low amplitude rf signal at a frequency equal to the natural frequency of motion of ions within the ITMS cell. This resonant excitation resulted in an absorption of power and an increase in the kinetic energy of the ions. Collisions between the kinetically excited ions and neutral gas molecules (helium and isobutane) resulted in collisional activation of the ions and subsequent fragmentation by means of low energy pathways. Using CW simulants as examples, the CID MS/MS spectrum of the $(M+H)^+$ ion of DIMP (m/z 181) resulted in fragment ions at m/z 97 and m/z 139. The CID MS/MS spectrum of m/z 125 for DMMP showed major fragment ions at m/z 93, m/z 95, and m/z 111. Finally, the CID MS/MS spectrum for dibutyl-sulfide (DBS) showed major fragment ions at m/z 57, m/z 91, and m/z 117.

Under the conditions described above, the data output using the direct thermal desorption CI/MS/MS ion trap method is a temporal plot 1 to 2 minutes in width which is comprised of a series of spectra collected at a rate of 1 per second. From this series of spectra, the ion current response for characteristic MS/MS fragment ions corresponding to the targeted analyte can be extracted and plotted as shown in Figure 3.

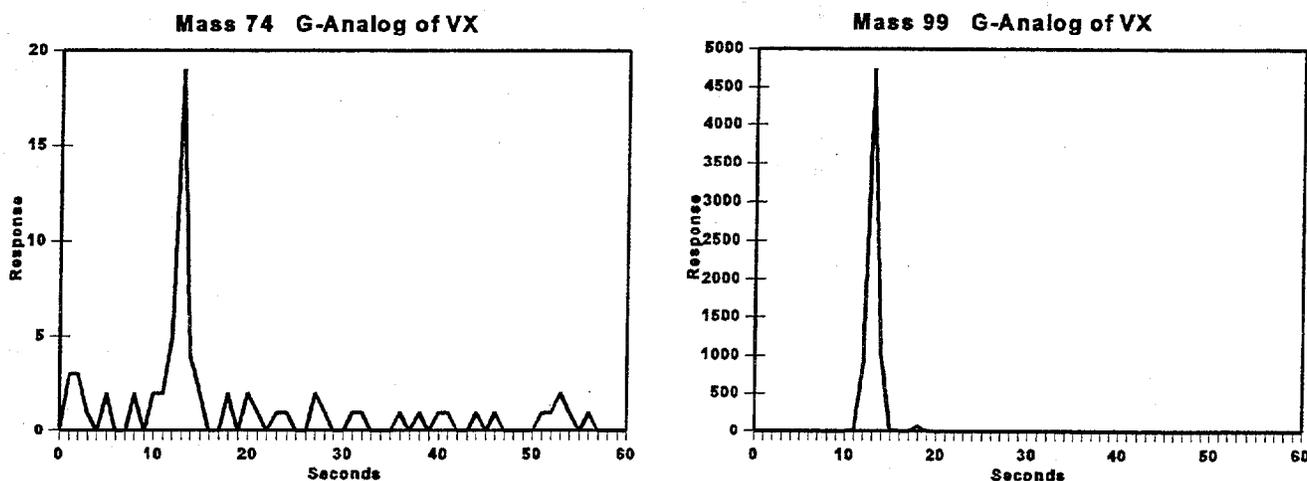


Figure 3. Direct thermal desorption CI/MS/MS plots showing the extracted ion current for m/z 74 and m/z 99 resulting from the fragmentation of $(M+H)^+$ ions (m/z 141) generated from 1 ng of the G-analog of VX spiked onto a DAAMS tube.

For both the simulants and the CW agents, thermal desorption profiles ranged from approximately 5 seconds to 30 seconds in width. The integrated area beneath the thermal desorption profile is directly proportional to the concentration of an analyte on a DAAMS tube provided that analytes are fully desorbed from the sorbent tube during the heating cycle. For quantification purposes, a working curve would be generated by thermally desorbing a series of DAAMS tubes spiked with known amounts of analyte.

RESULTS AND DISCUSSION

Initial experiments were conducted to determine if the detection limit and linear dynamic range requirements could be met for the CW agent simulants under thermal desorption CI/MS/MS conditions. For each simulant, a series of CI/MS/MS spectra were generated at the required detection limit (Table 1) and working curves were generated over 2 to 3 orders of magnitude in concentration above the required detection limits. The working curves were found to be linear over the range of concentrations investigated. A representative CI/MS/MS spectrum of 50 pg of DMMP (simulant for GB) is shown in Figure 4 which clearly shows the characteristic fragment ions with good signal-to-noise. This would correspond to the lowest required detection limit for GB at 0.125% for the ASC and GPL levels.

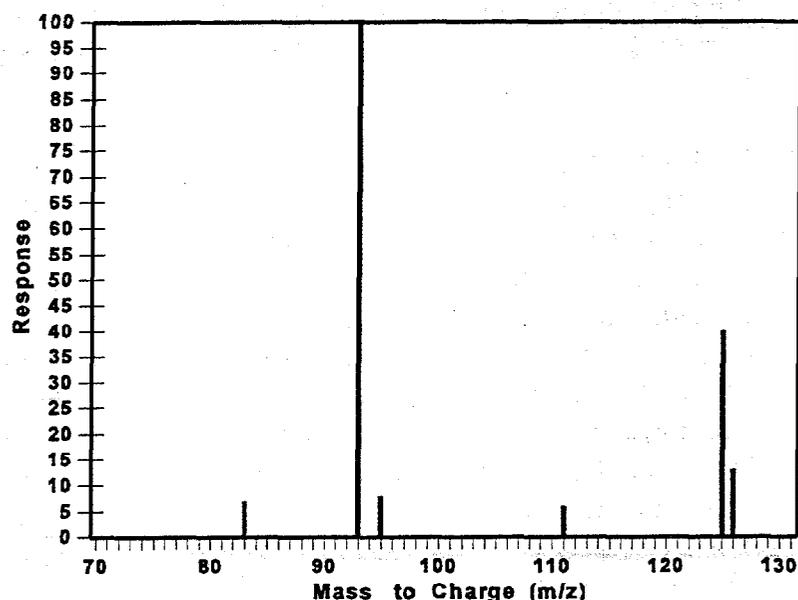


Figure 4. CI/MS/MS mass spectrum generated from the collisional dissociation of the protonated molecular ion for DMMP (m/z 125). This spectrum was produced using by injecting 50 pg of DMMP into the thermal desorber. Characteristic fragment ions are readily observed at m/z 91, m/z 93, and m/z 111. Undissociated protonated molecular ions are shown at m/z 125.

The reproducibility of quantitative measurements using the thermal desorber in conjunction with the ITMS was examined by performing repeated measurements at the lowest required detection limits for each of the CW agent simulants (approximately 100 pg for DIMP and DMMP and 1 ng for DBS). Experiments in which a liquid sample was injected directly into the thermal desorber typically showed a reproducibility of approximately 10% at the 95% confidence limit. In contrast, experiments in which a sample was desorbed from a DAAMS tube showed reproducibility ranging from 10-25%, suggesting that careful sorbent tube conditioning and spiking are also important.

For each of the CW agent simulants studied, precision and accuracy (P&A) testing had to be performed before acceptance of the methods for further testing with actual CW agents. These tests involved 4 consecutive days of testing for each of the CW simulants with two sets of samples run each day at each of the following GPL levels: blank, 0.125, 0.250, 0.50, 0.80, 1.0, and 1.5 (see Table 1). The GPL limits were used for this study because they represented the lowest required detection limits and therefore the most stringent challenge for the analytical method. A range analytical of standards were prepared for each of the CW agent simulants in methanol. A 1 uL aliquot of the lowest concentration standard corresponded to the lowest required detection limit for each simulant. The DAAMS tubes were conditioned for 1-2 hours by heating them to a temperature of 200°C while simultaneously purging with helium at a flow of 100-200 mL/min. The conditioned tubes were then spiked with CW agent simulants using an on-column capillary injection syringe to deposit liquid simulant (in methanol) approximately 1 centimeter above the sorbent material. Simultaneously, air was drawn through the DAAMS tube at a flow rate of 400 mL/min to evaporate the liquid and deposit it homogeneously onto the sorbent bed.

The results of the P&A testing demonstrated that the detection limits (in GPL units) for all three CW agent simulants were readily achieved with signal to noise ratios of at least 30 to 1. These signal to noise ratios were obtained using approximately 125 picograms (pg) of DIMP or DMMP. Instrument response was linear over the required range of 0.12 to 1.50 GPL. Analytical precision was determined by generating six point calibration curves for each simulant using a minimum of three replicates per GPL level (Figure 5). In most cases, three fragment ions were used for testing the precision and accuracy for each simulant. Check standards were used to test the quantitative accuracy of the method by determining targeted concentrations verses found values. All of experimental P&A testing data for the simulants was subjected to statistical analysis by the Army and found to meet their criteria for method acceptance. Further P&A testing was then conducted by another contractor at the Tooele Army Depot demilitarization facility using GB, HD, and the G-analog of VX. These data were similar to those generated from the simulants and also passed method acceptance.

CONCLUSIONS

The technology of direct thermal desorption MS/MS has been demonstrated to be useful for the rapid determination of low levels of chemical warfare agents and simulants which have been collected on DAAMS tubes. The use of selective chemical ionization and MS/MS reduces the potential for interferences from background chemical contamination which can hinder other types of analytical devices. The degree of selectivity achieved with CI and MS/MS has also enables the elimination chromatographic separation of the

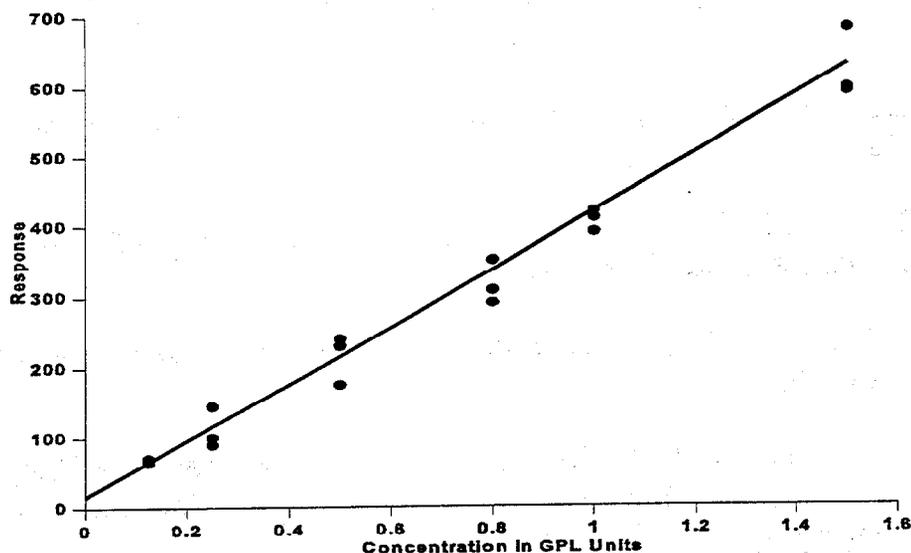


Figure 5. Working curve for DIMP generated for P&A testing. This curve is representative of the detection limits, and precision that can be expected for direct thermal desorption ion trap methods.

analytes in a complex matrix, greatly reducing sample handling and speeding up the overall analysis time. Precision and accuracy tests which were conducted using CW agent simulants and actual agents passed the criteria established method acceptance. Although the results presented in this paper are concerned with the analysis of DAAMS tubes for the determination of CW agents in air, modifications of the the thermal desorption method are proving useful for the rapid analysis of CW agents and related compounds in extracts of other media. In addition, as ion traps have evolved from large laboratory-based instruments into much smaller fieldable instruments, work in our lab has included investigating the utility of direct air monitoring for real-time analysis applications. Improved electronics and new operating modes are likely to provide real-time detections limits in the sub-part-per-billion range, possibly eliminating the need to preconcentrate samples on sorbent tubes for many applications.

REFERENCES

1. R.E. March, "Ion Trap Mass Spectrometry", *Int. J. Mass Spectrom. Ion Proc.*, **118/119**, 71-135 (1992).
2. R.G. Cooks, G.L. Glish, S.A. McLuckey, R.E. Kaiser, "Ion Trap Mass Spectrometry", *Chem. and Eng. News*, **69**, 26-41 (1991).
3. J.F.J. Todd, "Ion Trap Mass Spectrometry, Past, Present, and Future?", *Mass Spectrom. Rev.* **10**, 3-52 (1991).
4. B.A. Eckenrode, S.A. McLuckey, and G.L. Glish, "Comparison of Electron Ionization and Chemical Ionization Sensitivities in Ion Trap Mass Spectrometry", *Int. J. Mass Spectrom. Ion Proc.*, **106**, 137-157 (1991).
5. M. B. Wise, C.V. Thompson, M.V. Buchanan, R. Merriweather, and M. R. Guerin, "Direct Sampling Ion Trap Mass Spectrometry", *Spectroscopy*, **8**(5), 14-22, 1993.
6. S.A. McLuckey, G.L. Glish, and G.J. Van Berkel, "Multi-stages of Mass Spectrometry in Quadrupole Ion Traps", *Int. J. Mass Spectrom. Ion Proc.*, **106**, 213-235 (1991).
7. J.V. Johnson, R.A. Yost, P.E. Kelley, and D.C. Bradford, "Tandem in Space and Tandem in Time Mass Spectrometry - Triple Quadrupole and Quadrupole Ion Traps", *Anal. Chem.*, **62**, 2162-2172 (1990).
8. J.N. Louris, R.G. Cooks, J.E.P. Syka, P.E. Kelley, G.C. Stafford, Jr., and J.F.J. Todd, "Instrumentation, Applications, and Energy Deposition in Quadrupole Ion Traps", *Anal. Chem.* **59**, 1677-1685 (1987).
9. M.B. Wise, R.H. Ilgner, and M.V. Buchanan, "Detection and Quantification of Trace Organics in Air by Direct Thermal Desorption Ion Trap Mass Spectrometry", *Proceedings of the 38th ASMS Conference on Mass Spectrometry and Allied Topics*, Tucson, June 3-8, 1481-1482 (1990).
10. E. R. J. Wils, "Mass spectral data of precursors of chemical warfare agents", *Fresenius J. Anal. Chem.*, **338**, 2-27 (1990).
11. S.N. Ketkar, S.M. Penn, and W.L. Fite, "Real-Time Detection at Parts per Trillion Levels of Chemical Warfare Agents in Ambient Air Using Atmospheric Pressure Ionization Tandem Quadrupole Mass Spectrometry", *Anal. Chem.*, **63**, 457-459 (1991).
12. J. A. Syage, "Real-Time Detection of Chemical Agents using Molecular Beam Laser Mass Spectrometry", *Anal. Chem.*, **62**, 505A-509A (1990).

13. S.A. Lammert, R. Merriweather, M.B. Wasserman, "Detection of Chemical Weapons Convention Treaty Compounds Using a Field-Portable Ion Trap Mass Spectrometer", Presented at the Third International Conference on On-Site Analysis, Montgomery, TX 22-25 January, 1995.

14. E.W. Sarver, M.B. Wasserman, R. Merriweather, S.A. Lammert, "Detection of Chemical Agents, Precursors, and By-products Using Ion Trap Technology", Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, 7 March, 1995.

USE OF NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY FOR THE
ANALYSIS OF CHEMICAL WARFARE AGENTS AND THEIR DEGRADATION
PRODUCTS IN ENVIRONMENTAL SAMPLES

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INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful analytical techniques for elucidating the molecular structure of organic compounds. The sample (usually a liquid or a solid dissolved in an appropriate deuterated solvent) is placed within a strong, homogeneous magnetic field. Nuclei possessing magnetic moments within the sample align themselves with or against the direction of the static field. The sample is then irradiated with radio frequency (RF) electromagnetic radiation, causing these nuclei to resonate and flip orientation with respect to the magnetic field. This phenomenon creates a signal that ultimately results in an NMR spectrum - a plot of resonance frequency vs peak intensity. The RF frequency used to irradiate the sample at a particular magnetic field strength dictates which nuclei will be observed in the NMR spectrum. Thus, for a magnetic field strength of 9.4 Tesla, protons (^1H) will be observed at 400 MHz, fluorine (^{19}F) nuclei at 376 MHz, phosphorus (^{31}P) nuclei at 162 MHz, and carbon (^{13}C) nuclei at 100 MHz.

In environmental samples, the identification and detection of chemical warfare (CW) related compounds is best accomplished using high field (>400 MHz) high resolution NMR. For these samples, the three NMR parameters of interest are: (1) the chemical shift or delta (δ in ppm) value, which gives information about the immediate environment of the nuclei of interest; (2) the coupling constant or J value (in Hz) which gives information about the number and types of neighboring magnetic nuclei; and (3) the intensity of the resonance, which is directly proportional to the number of nuclei experiencing a particular magnetic environment. With this information, supplemented by complementary mass spectrometric (MS) and infrared (IR) data, NMR can be used to detect and confirm the presence of CW compounds, their precursors and degradation products. Furthermore, these NMR parameters can be used to elucidate the structures of unknown organic components that may also be of interest.

EXPERIMENTAL PROCEDURES

Sample Preparation. For NMR analysis, separate samples are usually prepared using deuterated solvents. If there is only enough of the environmental sample to prepare one set of samples, the NMR procedures are used, and the same set of samples is then analyzed by all appropriate techniques.

The soil/debris samples and unknown liquid/aqueous samples are prepared for NMR as outlined in Schemes 1 and 2, respectively. Each sample is placed into a clean, dry 5-mm Pyrex NMR tube. The tube is

capped with a pressure cap, and the top of the tube is wrapped with Parafilm. Multinuclear NMR spectra are obtained for each sample, as required.

Instrumental Procedure. For detecting CW-related compounds in environmental samples, an NMR spectrometer of at least 200 MHz is recommended. Our laboratory uses a Varian Unity Plus 400 MHz multinuclear Fourier transform (FT) NMR system. Since most QA/QC plans require the analyst to document that the instrumentation is in good working condition before analyses proceed, the spectrometer is tuned, the homogeneity (i.e., resolution) adjusted, and the signal-to-noise ratio (S/N) measured using test samples for the particular observe nuclei. The test spectra for these nuclei are recorded, and certain criteria (e.g., stability, sensitivity, and resolution) must be met before continuing with the unknown samples.

For each unknown sample, the spectrometer is tuned and the homogeneity adjusted. Data for each nucleus of interest are then accumulated until the desired signal-to-noise level has been achieved (5 min to > 16 hrs). After data accumulation, the NMR spectra are stored on hard disk and hard copies plotted out. The NMR chemical shifts and coupling constants for each nucleus observed are determined and compared with spectra in the database or with spectra taken of authentic compounds. Results are correlated with other analytical techniques (GC/MS, IR and HPLC), and record sheets are completed and submitted for inclusion in a final formal report.

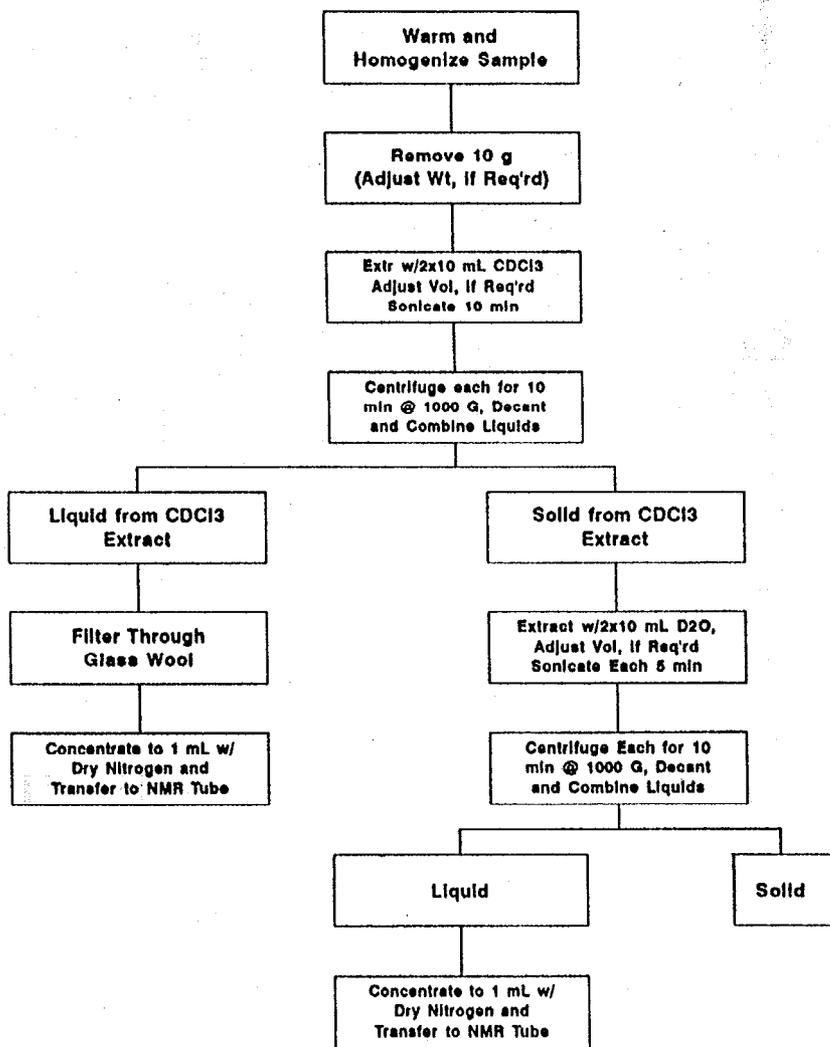
Data Validation. Compound identifications are validated by comparison to spectra in a database library or to spectra of the authentic compounds that may be acquired during the investigation. For the same solvent and the same concentration, the chemical shift values for the unknown should be within ± 0.1 ppm of those in the database and the coupling constants should be within ± 0.5 Hz. When unknown compounds (not found in the database) are present, the NMR experiments are planned so as to obtain the structural information needed as a complement to MS, IR and other techniques. In these cases, compound identification is validated when all of the spectroscopic data available are consistent with the proposed structure.

Quantitation. After identification of an unknown analyte, quantitation is accomplished by preparing a standard solution of known concentration of the compound in the same solvent as the unknown. The NMR spectrum of the standard sample is then obtained using the same experimental parameters as those used to obtain the spectrum of the unknown sample. The absolute integral values obtained for the unknown are then compared with the absolute integral values obtained for the standard solution to arrive at an approximate concentration ($\pm 30\%$) for the unknown analyte.

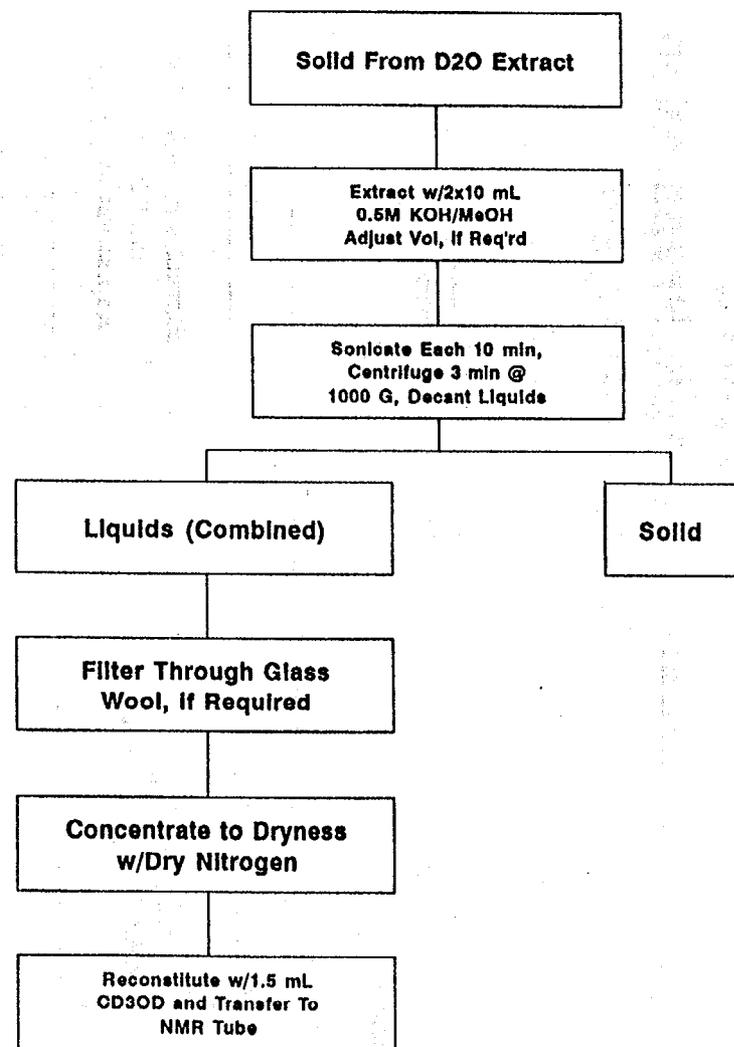
DISCUSSION

When one considers analyzing environmental samples for organic contaminants, sensitive chromatographic techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC), often coupled with mass spectrometry (MS), are first to come to mind. NMR

Scheme 1 Soil/Debris Sample Preparation for NMR Analysis

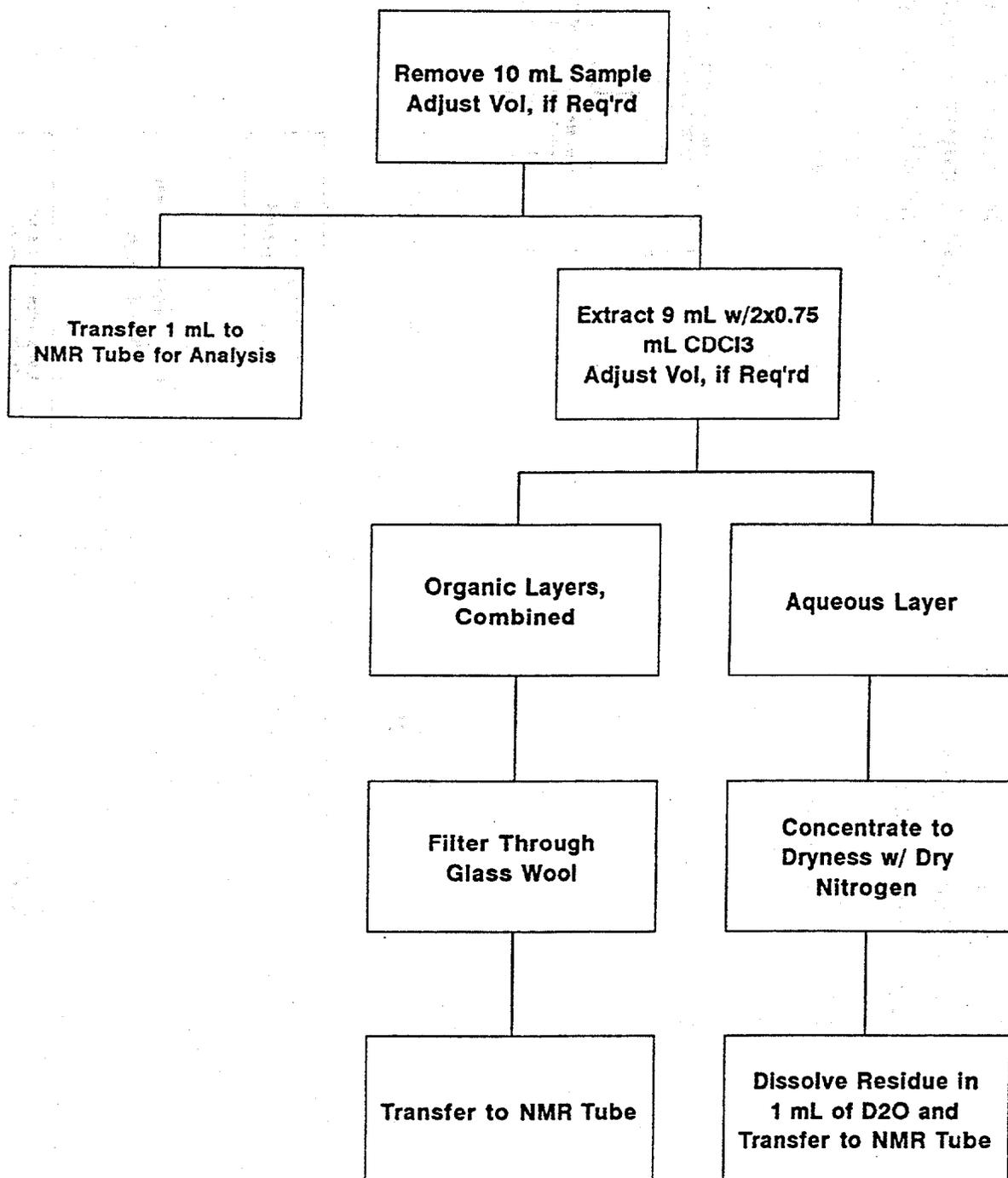


Scheme 1 (Con't) Soil Sample Preparation for NMR Analysis



Scheme 2

Aqueous Sample Preparation for NMR Analysis



spectroscopy usually is not considered a primary method for the analysis of these types of samples for several reasons:

(1) Sensitivity: Compared to GC, HPLC and MS which can have detection limits in the low parts per billion (ppb) range, the detection limits for NMR are usually parts per thousand (ppt), or at best, tens of parts per million (ppm).

(2) Time: To detect compounds in the ppm range, the NMR experiment time may range from a few hours per sample to over 60 hrs per sample (i.e., an over-the-weekend run).

(3) Interferences: NMR is a non-discriminatory technique. That is, there is no physical separation of the analyte from the background/matrix compounds. Thus, the signals (i.e., resonances) from all organic compounds present in the sample will be observed, and small resonances from the compounds of interest may be obscured.

(4) Sample Size/Sample Preparation: The NMR experiment requires at least 0.75 mL of liquid sample. Liquids can be used directly (if there is a sufficient amount available) but solids and small amounts of liquids are usually dissolved in deuterated solvents for NMR analysis.

(5) Difficulty with Reference Compounds: The chemical shift parameter is temperature, concentration and pH dependent. This makes using reference compounds to validate the presence of acidic and basic degradation products difficult since the solvent system for the reference spectrum must closely match that of the unknown in order for the NMR parameters to be within experimental error.

(6) Expense: The minimum cost of an NMR spectrometer system suitable for analyzing environmental samples is approximately \$150K (200 MHz, ^1H , ^{13}C and ^{31}P capability) and can easily exceed \$1M for state-of-the-art, high field, multinuclear instruments (600 and 750 MHz). Sample preparation is also more expensive than other techniques because the deuterated solvents required are much more expensive than their protonated analogs, ranging in cost from \$18/100 g of chloroform-d to \$60/100 g of methanol-d₄.

Recently, the Analytical Chemistry Team, ERDEC, has begun routinely using multinuclear NMR spectroscopy as a primary method for the analysis of environmental samples. NMR is being used as a complementary method to the usual GC, HPLC and MS procedures for detecting CW agents and their degradation products in various environmental matrices and has proven to have several advantages:

(1) Sensitivity: Using larger samples for extraction and concentrating liquids by 10-100 fold, the detection limit for certain compounds can be extended down to 1 ppm or less. This detection limit is comparable to that obtained for polar CW degradation products by ion chromatography (IC), HPLC and derivatization GC/MS.

(2) Time: For unknown liquids, the NMR experiment can be as short as 1 min. A quick, initial characterization of an unknown as

greater than 99% water or as an oil or other organic compound greatly simplifies the Team's planning for further sample preparation and analyses. For NMR experiments that require long accumulation times in order to see small amounts of an analyte, the spectroscopist can run the sample unattended overnight or even over the weekend.

(3) Interferences: It has been found that naturally occurring ions in water and soil (e.g., Na^+ , Ca^{+2} , Mg^{+2} , Cl^- , SO_4^{-2} , etc.) tend to interfere with the detection of CW-related degradation products by HPLC/IC. Their presence also appears to adversely affect the derivatization reactions required for GC/MS analysis of these polar compounds. The NMR experiment, on the other hand, is not affected by the presence of these compounds, and the CW-related degradation products are readily detected. Furthermore, environmental samples have little, if any, background phosphorus-containing compounds making ^{31}P NMR an excellent method for detecting the organophosphorus CW-related compounds.

(4) Sample Preparation: Little preparation is required for samples to be analyzed by NMR. Liquids can be run neat (without solvent), and aqueous samples can be run neat or concentrated by gentle evaporation for increased sensitivity. Solids, including soils and debris, require only straightforward extractions with deuterated organic solvents and deuterated water. Many quality assurance/quality control (QA/QC) plans recommend or require the preparation of duplicate samples. Thus, the preparation of separate NMR samples provides the Team with a duplicate set of samples for analysis. Since NMR is a non-destructive technique, the samples can be analyzed by other methods after NMR characterization has been completed.

(5) Reference Compounds: NMR provides information about the molecular structure of the analytes. This allows the spectroscopist to ascertain the identity of the compound without the need for a reference standard. This is extremely important for determining the presence of unexpected analytes or unknowns for which no standard exists in the database.

The Analytical Chemistry Team recognized the advantages of NMR for detecting CW-related compounds and currently uses it as the primary method for characterizing all unknown samples so that strategies for further analyses, if required, can be developed. In our laboratory, NMR has been used successfully to detect CW-related compounds in a variety of environmental samples. Some examples follow:

(1) Incinerator Test Burn Samples. To test a contractor's design for an incinerator to be used for the destruction of organophosphorus compounds, a test burn was conducted using methylphosphonic acid (MPA) as the feedstock. Samples of the feedstock, the quench tank waters, and the venturi liquids were obtained, and ^{31}P NMR was used to determine if complete destruction of the C-P bond (the organophosphorus compound) had been accomplished. Using standard samples, it was determined that the detection limit for MPA would be 1 ppm for an overnight ^{31}P run.

Figure 1 is the ^{31}P NMR spectrum of a sample of quench tank water which shows some organophosphorus compounds still present. Any resonances downfield of $\delta 10$ represent compounds containing the C-P bond whereas resonances upfield of $\delta 10$ usually represent inorganic phosphorus compounds (no C-P bond). Thus, the presence of the peak at $\delta 21.6$, identified as MPA, and the peak at $\delta 17.4$, identified as dimethyldiphosphonic acid, indicate that complete destruction was not accomplished. Digital integration of the peak areas shows that 0.22% of the phosphorus in this sample still contains the $\text{CH}_3\text{-P}$ moiety. A coupled ^{31}P NMR spectrum (inset) confirms the presence of the C-P bond for the resonance at $\delta 21.6$. Knowing the total weight of phosphorus in this sample, the absolute concentration of the C-P compounds can be calculated.

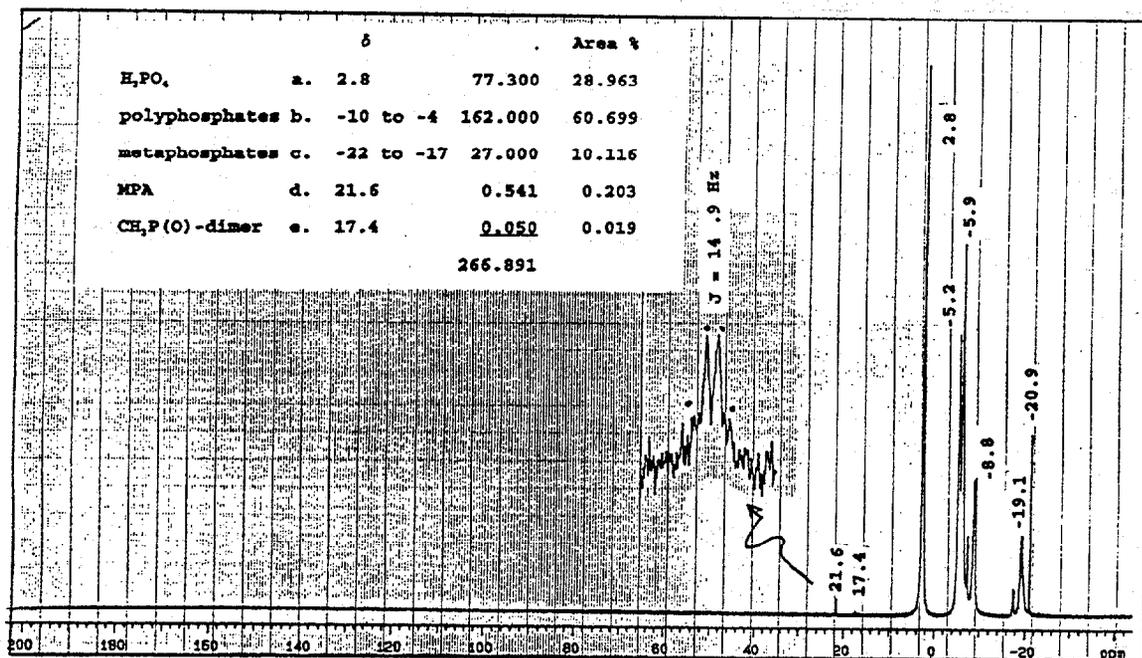
(2) Unknown Samples Found on Post. Unknown samples found/or unearthed on Post and environmental samples suspected of containing CW-related chemicals are routinely submitted to the Analytical Chemistry Team for characterization. Figure 2 shows the NMR spectra of one such sample submitted recently by a contractor performing site remediation work at an APG-EA dump site. The sample was a clear liquid contained in a screw-capped polyethylene bottle. The ^1H NMR spectrum (Figure 2a) shows that the sample is primarily water, but that it contains some ethyl methylphosphonic acid (EMPA), a CW-related degradation product. The ^{31}P spectrum (Figure 2b) confirms the presence of EMPA ($\delta 27.6$) and also shows that a smaller amount of methylphosphonic acid (MPA, $\delta 22.1$) is also present. The ^{19}F NMR spectrum (Figure 2c) showed a small amount of fluoride ion present ($\delta -108.9$) indicating that these acids may have come from the degradation of ethyl methylphosphonofluoridate, an analog of the nerve agent GB. For NMR analysis, no sample preparation was required, and the identification took less than 30 min. Since these two compounds are degradation products of both G and V nerve agents, the NMR results led to further testing of this sample for low levels of related G and V compounds.

(3) Operation Safe Removal. On 5 Jan 93, laboratory debris and munitions from WWI CW agent studies at American University were discovered in the Spring Valley development in Washington, D.C. Ninety-eight separate items/samples (i.e., soil and debris) were delivered to ERDEC for chemical characterization. These samples were analyzed by the Analytical Chemistry Team over a two month period, and thirty-three compounds were identified and confirmed.¹

Figure 3 shows the ^1H NMR spectrum of the chloroform-d extract of a typical sample which consisted of broken glass and jars. The spectrum shows the usual hydrocarbon background ($\delta 0.9$ and 1.25) as well as several peaks in the $\delta 6.3\text{-}7.6$ region indicative of trans-vinyl moieties similar to Lewisite (L). Three compounds were observed; based on literature values and spectra of authentic L, L-2 and L-3, it was concluded that no Lewisite, itself, was present. The compound at $\delta 6.72$ and 6.87 was identified as bis(2-chlorovinyl)arsine, L-2, and the compound at $\delta 6.38$ and 6.48 was identified as tris(2-chlorovinyl)arsine, L-3. The major component ($\delta 6.98$ and 7.56) was not conclusively identified, but the low field chemical shift values for the two protons indicate that the arsenic in this compound is probably oxidized. The presence of L-2 and L-3 in this sample was confirmed by GC/MS and direct exposure probe (DEP) MS.

Figure 1. ^{31}P NMR Spectra of an Incinerator Test Burn Sample.

(a) Entire ^{31}P Spectrum and Coupled Spectrum of Resonance at δ 21.6.



(b) ^{31}P Spectrum, Expanded, Showing Integrated Regions.

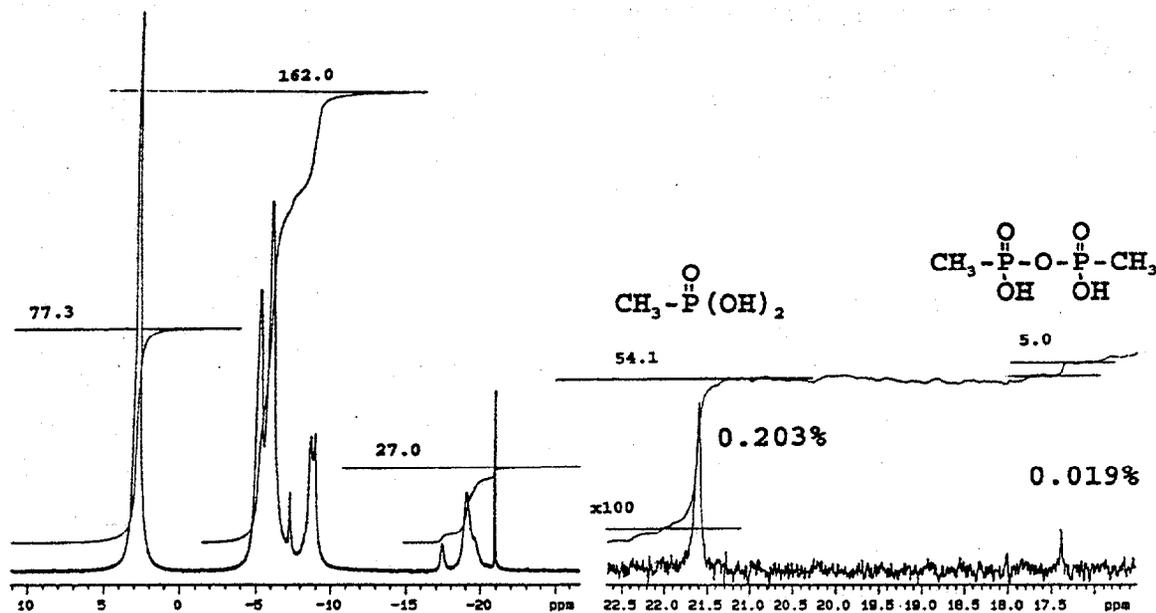
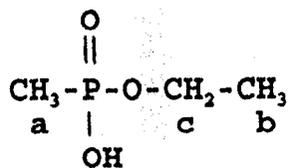
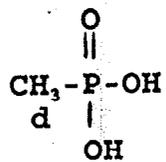


Figure 2. (a) ^1H NMR Spectrum of an Unknown Found On Post.



EMPA



MPA

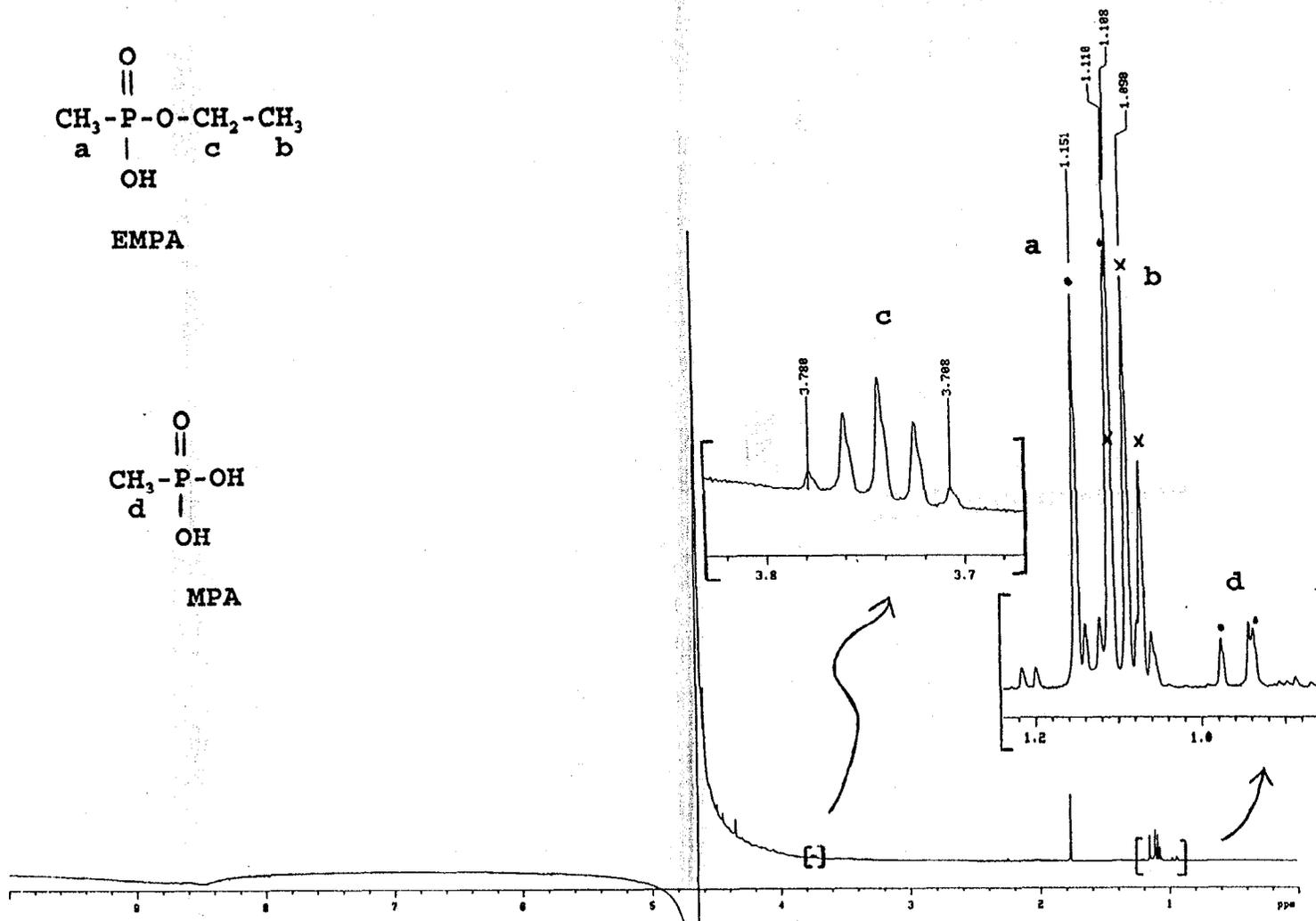
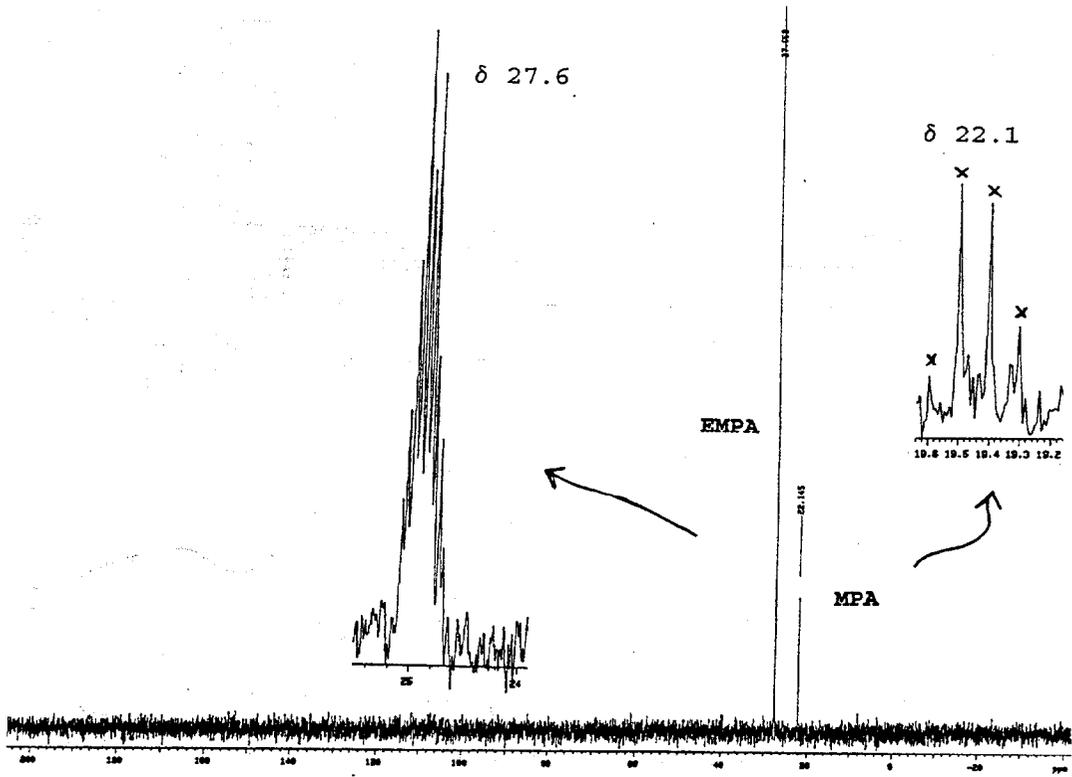


Figure 2. (b) ^{31}P NMR Spectrum of an Unknown Found On Post.



(c) ^{19}F NMR Spectrum of an Unknown Found On Post.

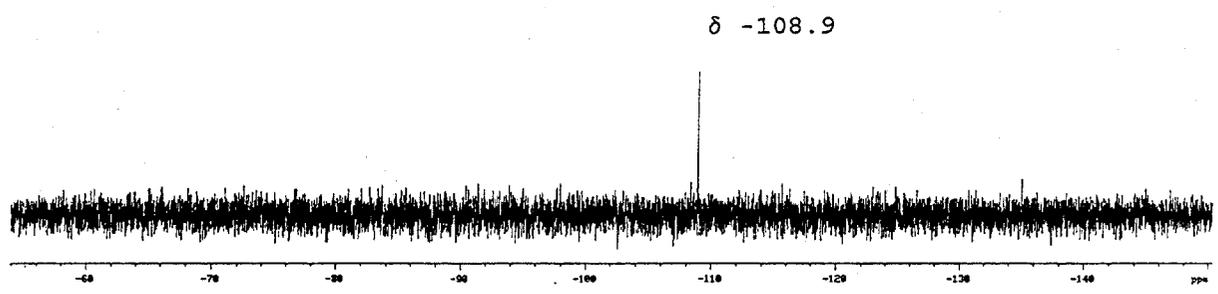


Figure 3. ^1H NMR Spectrum of an Operation Safe Removal/Spring Valley Sample.

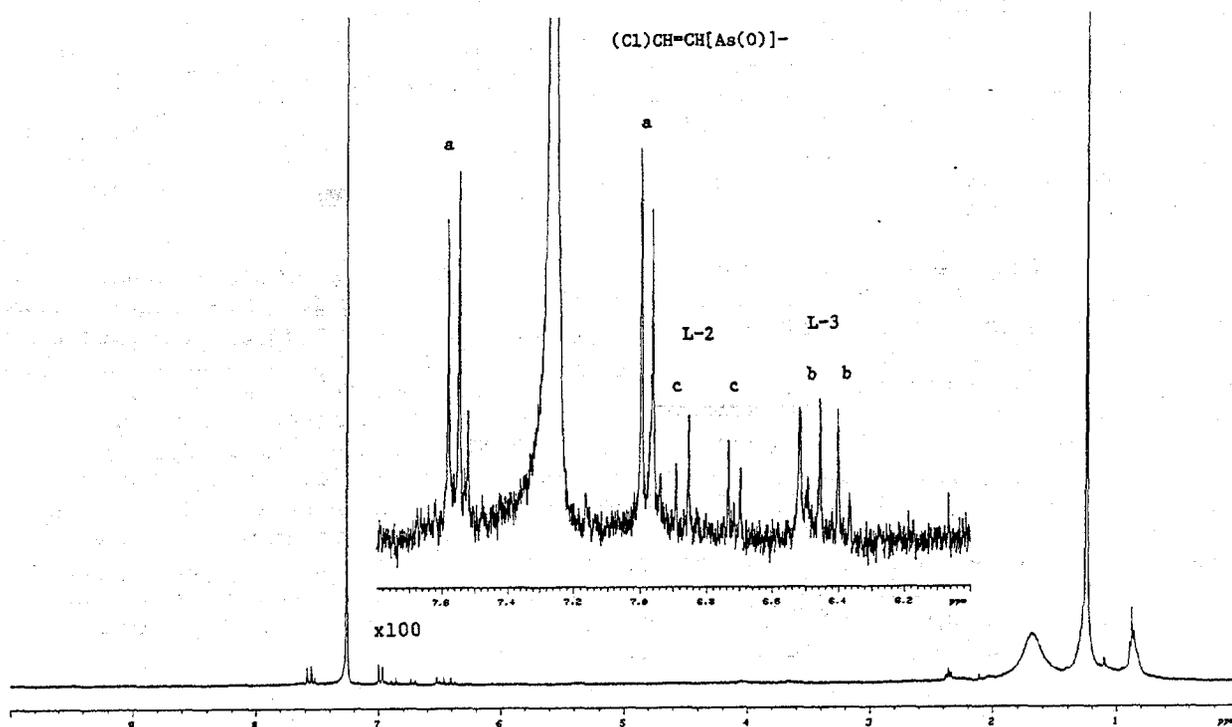
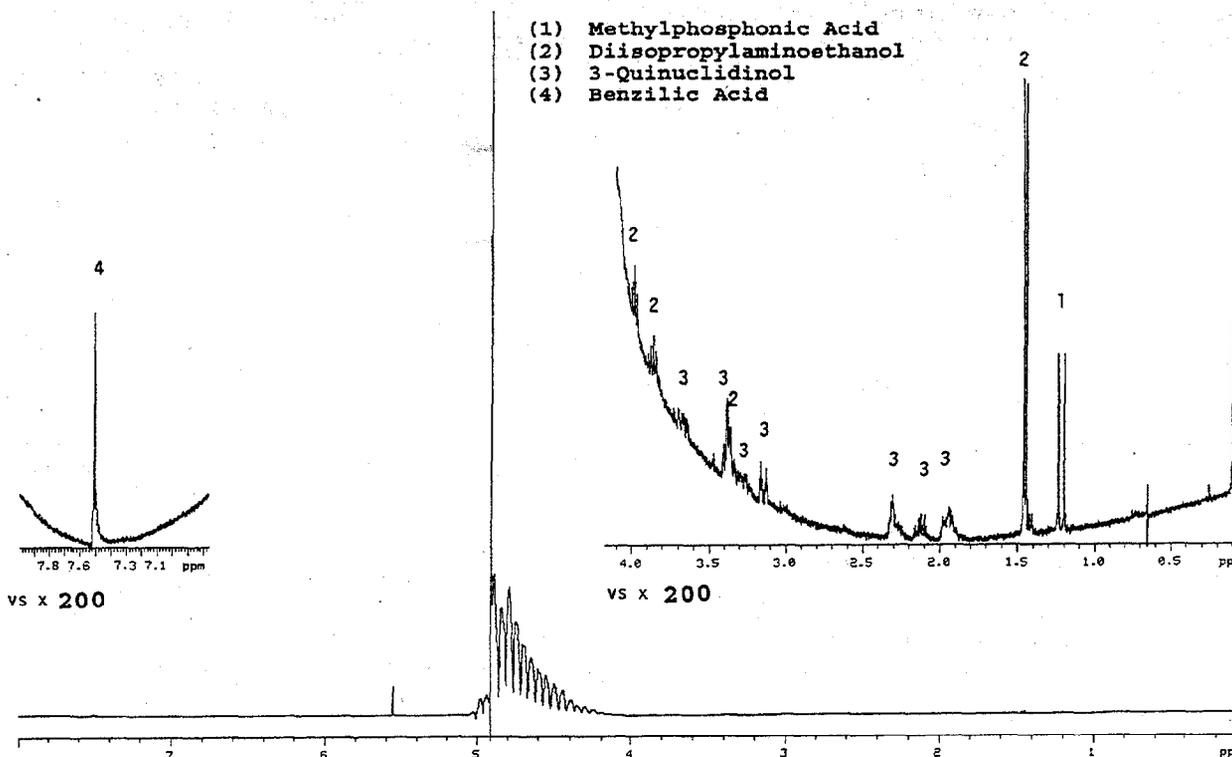


Figure 4. ^1H NMR Spectrum of an Aqueous Round Robin 4 Sample.



(4) Treaty Verification Samples. For the past four years, the Analytical Chemistry Team has supported ERDEC's participation in the International Interlaboratory Comparison Tests (Round Robins) that have been initiated to develop recommended operating procedures (ROP's) for evaluating samples under the Chemical Weapons Convention (CWC) soon to be in effect. While not considered a primary analytical technique for the evaluation of treaty samples, NMR has proven itself to be invaluable in detecting the CW-related degradation products spiked into the soil and water samples.²

Figure 4 shows the ¹H NMR spectrum of an aqueous sample from Round Robin 4 (Mar 93). All of the spiked compounds were observed and were immediately identified by interpretation. The spiked compounds were methylphosphonic acid, MPA (10 ppm); diisopropylaminoethanol, KB (10 ppm); 3-quinuclidinol, 3-Q (20 ppm); and benzoic acid, BA (10 ppm). The sample was run neat (no sample preparation), and the spectrum was acquired overnight, unattended. A second sample was prepared by gently evaporating nine mLs of the water and reconstituting it with one mL D₂O. This sample required only one hr of data accumulation to obtain an excellent spectrum. This concentrated sample, together with standards of known concentrations, was used to quantitate the spiked compounds. The NMR results (MPA, 9 ppm; KB, 8 ppm; 3-Q, 18 ppm; and BA, 8 ppm) closely matched the theoretical levels expected for these compounds.

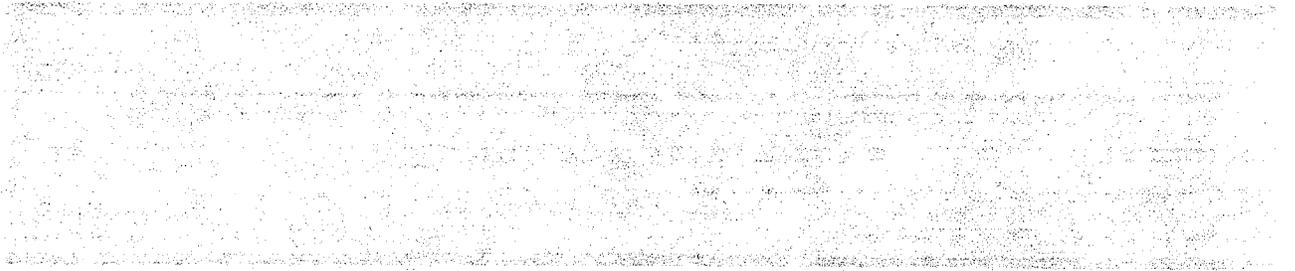
CONCLUSION

In spite of its inherently low sensitivity and its non-discriminatory nature, NMR spectroscopy has proven to be an invaluable analytical tool for determining the presence of chemical warfare agents and their degradation products in environmental samples. The Analytical Chemistry Team, ERDEC, has begun routinely using multi-nuclear NMR spectroscopy as a complementary method to gas chromatography, liquid chromatography and mass spectrometry for detecting CW-related compounds in various environmental matrices and for characterizing all unknown samples so that strategies for handling and further analyses can be developed.

REFERENCES

1. Brooks, M.E., Beaudry, W.T., Bossle, P.C., Herd, R.E., Lochner, J.M., Pleva, S.G., Reeder, J.H., Rohrbaugh, D.K., Rosso, T.E., Szafraniec, L.J., and Szafraniec, L.L. "Operation Safe Removal: Spring Valley, Washington, D.C. Analytical Results: January-February 1993." Edgewood Research, Development and Engineering Center Special Publication (ERDEC-SP-008), July 1993. Unclassified Report.
2. Rohrbaugh, D.K., Beaudry, W.T., Bossle, P.C., Ellzy, M.W., Janes, L.G., Lochner, J.M., Pleva, S.G., Reeder, J.H., Rosso, T.E., Szafraniec, L.J., and Szafraniec, L.L. "ERDEC Contribution to the 1993 International Treaty Verification Round Robin Exercise IV." Edgewood Research, Development and Engineering Center Technical Report (ERDEC-TR-176), July 1994. Unclassified Report.

**SESSION III PRACTICAL APPLICATIONS OF EXISTING
ANALYTICAL TECHNIQUES**



RESULTS OF THE FIRST PROVISIONAL TECHNICAL SECRETARIAT
INTERLABORATORY COMPARISON TEST

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1. Introduction

The principal task of this laboratory in the first Provisional Technical Secretariat (PTS) Interlaboratory Comparison Test was to verify and test the extraction and preparation procedures outlined in the Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament (1993 Edition) in addition to our laboratory extraction methods and our laboratory analysis methods. Sample preparation began on 16 May 1994 and analysis was completed on 12 June 1994. The analytical methods used included NMR (^1H and ^{31}P), GC/AED, GC/MS (EI and methane CI), GC/IRD, HPLC/IC, HPLC/TSP/MS, MS/MS (Electrospray), and CZE.

2. Analysis Team Personnel

The following personnel from ERDEC contributed to the first PTS test:

Lynn Hoffland	(Team Leader, Sample Custodian and Preparation)
Kenneth Sumptner	(Sample Preparation)
Eugene Vickers	(Sample Preparation)
Tom Rosso	(Sample Preparation)
Linda Szafraniec	(NMR)
William Beaudry	(NMR)
Paul Bossle	(HPLC/IC)
Dennis Johnson	(QA/QC)
Ron Checkai	(Soil Expert)

The following personnel from EAI Corporation, Abingdon, MD contributed:

Tom Albro	(Team Leader, Sample Preparation, GC/AED, GC/MS EI and CI)
Jack Stuff	(Sample Custodian, GC/AED)

Robert Warren	(Sample Preparation)
William Creasy	(HPLC/TSP/MS)
Richard Cheicante Jr.	(CZE)
Barry Williams	(GC/IRD)
Mike Smith	(GC/MS EI and CI)

And the following personnel from GeoCenters Corporation, Aberdeen Proving Ground, MD contributed:

Phil Smith	(ESI-MS, ESI-MS/MS)
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3. Receipt and Storage of Samples

Samples for the First PTS interlaboratory comparison test were received from Lawrence Livermore National Laboratory on 5 May 1994 by the sample custodian. Inspection by the QA/QC coordinator revealed that the containers were in excellent condition and were stored at 6°C in a locked refrigerator, with access only by the sample custodian until 13 June 1994 when the soil samples were homogenized by the soil expert, and the water sample extraction was begun. Additional samples were obtained from the sample custodian as needed.

Eight samples from two soils, a water and a decontamination solution were received and were identified as follows:

SL-15	Spiked soil sample
SLB-15	Soil blank for SL-15
SC-15	Spiked soil sample
SCB-15	Soil blank for SC-15
WC-15	Spiked water sample
WCB-15	Water sample blank for WC-15
DS-15	Spiked Decon solution
DSB-15	Decon solution blank for DS-15

50 grams of each soil sample and 50 ml each of water and decontamination solution were provided.

4. Chain-of-Custody Procedures

All samples were logged in by the sample custodian using a sample receipt form. Samples were stored in a locked refrigerator at 4 °C. PTS test sample transfers were documented using a Sample Control Record Form.

5. Sample Preparation

On 13 May 1994, the soil samples were homogenized by the soil expert and the water extraction begun. Additional samples were obtained from the sample custodian as needed over the course of the exercise.

Water and decontamination samples were prepared as outlined in the Recommended Operating Procedures (ROP) [1]. The soil samples were placed in a glove bag. The bag was sealed and then inflated with nitrogen. A solid sorbent tube attached to a sampling pump was used to puncture the bag. The soil sample was opened and the atmosphere in the bag was drawn through the tube. The tubes were then thermally desorbed into a gas chromatograph interfaced to an atomic emission detector. The soil samples were then prepared as outlined in the ROP. The ROP for preparation of aqueous samples is outlined in Figure 1. The ROP for soil sample preparation is outlined in Figure 2. An abbreviated method for the soil and water was also performed with no additional information being generated from the subsequent analysis. NMR has its own procedures which are a modification of the recommended procedures.

6. Instrumentation

Instrumentation used in this study is listed in Figure 3. All instruments were calibrated prior to use. The procedures and calibration data were documented.

7. Analytical Approach

NMR, again this year, was used extensively for sample screening. Some analytes were first detected by NMR and then confirmed by another technique. Part of the reason for this quick response was that both the water and decontamination samples were analyzed "as received" in addition to some preparation methods (i.e., solvent extraction). This approach allowed analytical procedures to begin before all the extractions were completed. Many times, this first "quick look" provided information that could be used by other analysts.

As soon as samples were ready, a "shot gun" approach was used by giving the extractions to as many analysts as possible and reviewing the results as soon as they came in to provide "heads up" information to all analysts. Table 1, "Summary of Analytical Methods", lists the analytical methods used by compound detected.

8. Authentic Standards

Authentic reference standards for all five identified compounds were available in our laboratory for unambiguous identification. The source of the compounds was as follows:

Name	CAS#	Cat. No.	Vendor
Thiodiglycol (2,2'-thiodiethanol)	111-48-8	16,678-2	Aldrich
2-hydroxyethane sulfonic acid (isethionic acid)	156-00-1	22,007-8	Aldrich
thiodiglycol sulfoxide (2,2'-sulfonyl diethanol)	3085-45-8	S43063-3	Sigma- Aldrich
diisopropyl methylphosphonate	1445-75-6	30301	Alfa
n-butanephosphonic acid	3321-64-0	10317	Lancaster
methylphosphonic acid	993-13-5	30357	Johnson Matthey
O-pinacolyl methylphosphonate	616-52-4	N/A	prepared in- house
diisopropylamine	108-18-9	11,001-9	Aldrich
isopropyl alcohol (2-propanol)	67-63-0	27,049	Aldrich

9. QA/QC Procedures

For each method of analysis, blanks were used to eliminate false positives, procedures for instrument calibration were documented, and test mixes were used to monitor instrument performance.

Separate extractions using non-deuterated and deuterated solvents and two methods of extractions, abbreviated and full ROP, in effect, result in the preparation of three parallel samples (two are recommended for QA/QC in sample preparation). Each set of extractions was performed by separate individuals concurrently and were well sealed before storage to prevent cross contamination between different sets of samples. This provides an independent check if a particular sample in one set is suspected of contamination.

10. Quantitation Methods

Quantitation was performed using a number of techniques. The techniques selected for quantitation were, for the most part, techniques which analyzed the sample in as close to its original form as possible. In this regard, techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy, Ion Chromatography (IC), Capillary Zone Electrophoresis (CZE) and High Pressure Liquid Chromatography - Thermospray - Mass Spectrometry (HPLC/TSP/MS) were used to look at the water sample directly. In this manner, extraction and derivatization efficiencies are eliminated giving a more direct reading of sample components. In the case of the soil samples, these techniques were used to look at samples one step removed from the original material. Here, organic extracts such as the methylene chloride layers in the ROP preparations or chloroform as in the case of the NMR preparations were analyzed by techniques suitable for this type of matrix, specifically gas chromatography techniques (AED, EI-SIM etc..) as well as NMR. Aqueous extracts were analyzed like the water samples by direct analysis except by those techniques susceptible to ion (salt) interferences. In these cases, the sensitivity of the technique was either severely diminished or eliminated completely. The results, expressed in ug of analyte per gram of sample, were as follows;

Compound	WC-15	DS-15	SC-15	SL-15
Methylphosphonic acid	450	250	70	100
O-pinacolyl methylphosphonate	--	--	300	--
Diisopropyl methylphosphonate	--	--	--	300

Thiodiglycol sulfoxide	--	*	--	160
n-Butylphosphonic acid	350	--	--	--

* = Tentative result

11. Results

All five identified compounds were detected in the samples using the recommended operating procedures. A summary of the application of each method of analysis follows.

11.1 Ion Chromatography (IC)

Ion chromatography (IC) is a useful method for the detection of ionic species. In this exercise, IC was used for the detection of n-butyl phosphonic acid and methylphosphonic acid in the water (WC-15) and thiodiglycol sulfoxide (TDGO) in the soil water extract (SL-15). Both were identified and quantitated in spiked samples. Ion chromatography was also used to screen for ethyl methylphosphonic acid, isopropyl methylphosphonic acid, and pinacolyl methylphosphonic acid. None of these compound was detected with IC.

11.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

Again, in this year's interlaboratory comparison test, NMR demonstrated it's utility, not only as a screening tool, but also as stand-alone spectroscopic technique. The positive attributes of non-destructive analysis, high sensitivity, relatively low matrix interference, specific detection (C vs H vs P) and spectroscopic identification and interpretation makes NMR both a universal and, in some cases, a definitive method of detection.

All of the compounds identified in this report, except one, were detected using this technique.

11.3 Gas Chromatography - Mass Spectrometry (GC/MS)

Mass Spectrometry, both low resolution electron impact (EI) and low resolution chemical ionization (CI), was used throughout this study. A number of compounds were confirmed by one or both of these techniques. EI analysis of the sample extracts showed detection of all the phosphonic acids in all the samples. Additionally EI was used to confirm the presence of the

phosphonate (o-PMP) and diester compound (DIMP). CI confirmed EI's findings in all cases except for the phosphonic acids in the two soil samples.

Both techniques were used to elucidate the structure of an unknown sulfur containing compound in the DS sample. Whereas the analysis was not definitive, the information provided by these techniques plus the AED and IRD data allowed the assignment of a tentative structure.

11.4 High Pressure Liquid Chromatography - Thermospray - Mass Spectrometry (HPLC/TSP/MS)

This was the first year this technique has been fully utilized by this laboratory in conjunction with analyses of this type. Because of the success by other laboratories in past international round robins as well as the ability to analyze most liquid samples directly, a large level of effort was placed in developing our HPLC/TSP/MS capabilities. The results show our efforts have paid off in that many of the compounds reported were either discovered using this technique or confirmed by it.

11.5 Capillary Zone Electrophoresis (CZE)

CZE was a second technique added to this year's list of available methods. Besides offering the advantages of fast analysis times (less than 10 min.) and relatively low detection limits, the CZE offers the additional advantages of injection volumes which are so low (on the order of a few nanoliters), the technique is effectively non-destructive. Utilizing this technique, we were able to observe both the methylphosphonic acid and n-butylphosphonic acid in the water sample within minutes of the start of interlaboratory comparison test.

11.6 Electrospray Interface - Mass Spectrometry - Mass Spectrometry (MS/MS)

Direct sample analysis is also an advantage of MS/MS. This technique was used to look at the water sample as well as organic and aqueous extracts of the soils. The ionic content of the soil extracts prohibited detection of trace level contaminants due to interference in the resulting spectrum. This was also the case but on a much larger scale for the decontamination sample. Here, the ionic concentration was so high it prevented detection of any of the spiked compounds.

11.7 Gas Chromatography - Atomic Emission Detector (GC/AED)

The GC/AED was used as the screening tool in place of a number of other detectors such as the NPD, ECD and FPD. It showed excellent sensitivity to the phosphorous derivatives and was critical in the discovery and structure elucidation of the sulfur compound found in the decontamination sample. Additionally, because of the compound independent calibration capability of the system, the AED was calibrated on phosphorous and was used to quantitate all phosphorous compounds observed.

11.8 Gas Chromatography - Infrared Detector - Mass Spectrometer (GC/IRD/MS)

GC/IRD/MS was used sparingly during this test. Many of the samples, due to either the concentration of the spiked component, the complexity of the sample (and therefore chromatogram), or both, allowed only the analysis of the diisopropyl methylphosphonate in the soil sample SL. More help was provided by this technique in trying to understand the structure of the sulfur compound observed in as well as the chemistry surrounding the DS sample.

12. Findings

Three Schedule 2 [2] compounds which are hydrolysis products of Schedule 1 [2] compounds are reported. The compound thiodiglycol (TDG) sulfoxide was also found. It is not scheduled; however, it is known to be an oxidation product of thiodiglycol (Schedule 2) and a degradation product of S-mustard (schedule 1). One unscheduled compound, not known to be a degradation product of a scheduled compound, was observed. That compound, n-butylphosphonic acid, was reported due to the similar structure to scheduled compounds.

Four of five compounds reported had supporting documentation from four or more separate analytical techniques. The remaining compound, thiodiglycol sulfoxide (TDGO) reported in sample SL-15, was supported by the tentative result from NMR of thiodiglycol. In addition, NMR tentatively reported 2-hydroxyethane sulfonic acid in sample DS-15, with supporting evidence from GC/MS-EI, GC/MS-CI and GC/AED indicating that the bleach DS-15 contained decomposition products from either TDG, TDGO or the sulfone (results of spiking experiments). It was difficult to determine the spiked compounds because the extracts reacted in the methylene chloride over a period of three days to form chlorine-

saturated byproducts. In summary, TDG, or a hydrolysis or oxidation product was definitely present in DS-15 and SL-15.

Use of NMR also tentatively identified diisopropylamine in WC-15 and 2-propanol in DS-15; no supporting results were obtained. This does lead to a lesson learned from this exercise in that better methods need to be developed for the detection of volatile compounds.

One other observation is that better methods need to be developed to eliminate salts in the extracts, as the high salt contents in the soils and decontamination solutions provided a high background in several techniques (HPLC/TSP/MS, IC, CZE and ESI-MS/MS).

13. Conclusions

13.1 Stated Purpose

The stated purpose of the exercise was to provide further validation of the recommended operating procedures and to start the process of establishing proficiency testing for the designation of analytical laboratories under the Technical Secretariat.

A judgement on the suitability of the ROPs or of the ability of this laboratory to execute those and other analytical methods cannot be made without knowing the contents of the sample. However, a few conclusions can be made.

13.2 Level of Effort

First, the amount of labor expended by this laboratory to complete the exercise is clearly in excess of what will be reasonable for a routine sample load in support of implementing the CWC. This is partially because the samples coming from routine inspections, challenge and even allegations of use inspections would have specific questions related to the aims of the inspection for which the Technical Secretariat was seeking answers. It would be reasonable to assume that questions related to the aims of most inspections would not entail analysis for all scheduled chemicals and their degradation products.

Second, this laboratory, and presumably others, views this test and previous international round robins as a means of validating their laboratory's procedures and capabilities. Because all the assets of the lab are subject to validation, all are used in the execution of the analysis regardless of the utility of the information in writing the final report. Under

more realistic circumstances, where more clearly defined purposes for performing analysis are given with the request for analysis of samples, some value judgements will certainly be exercised concerning the cost/benefit of using various techniques toward answering those specific questions.

13.3 Shortcomings of Techniques and Future Research Indicated

In the execution of this sample analysis exercise, several shortcomings of the ROPs and the modification of those procedures as practiced in this laboratory became apparent. Those are listed below along with this laboratory's current plans for addressing those shortcomings.

Volatile Compounds: The current procedures for identifying volatile compounds are clearly not fully suitable. This laboratory will explore thermal stripping of soil and other solid samples; as well as purge and trap sampling of liquids. We also see merit in exploring extraction of samples followed by solid sorbent trapping of volatile components during sample blowdown.

Highly Polar Compounds: It appears that modifications to the ROPs have resulted in significant improvements in the ability to detect phosphonic acids, especially methyl phosphonic acid, when compared to the results of last round robin. However, the difficulty experienced by this laboratory in confirming the presence of oxidation and other reaction products of TDG is worth noting. There is evidence in this report that TDG had oxidized to the sulfone, the sulfoxide and 2'-hydroxyethylsulfonic acid. There is further evidence that, upon extraction of the samples, perchlorinated products of those oxidation products were either isolated or produced. Further research both to determine the chemistry of thiodiglycol in decontamination solutions and to further develop analytical procedures to determine the products of decontamination is clearly indicated.

The problem with identifying highly polar degradation products of scheduled materials in decontamination solutions is certainly a larger task than expected, as illustrated by this exercise. Many non-compliance scenarios would have the site under control of the host for several days following the allegation of non-compliance. One would reasonably expect that the host would make an effort to remove evidence of non-compliance prior to the inspection and that one means of removing such evidence would be liberal application of standard decontamination solutions. For the analytical chemist, the problem will be finding scheduled

compounds or their degradation products in a matrix that has a high ionic content, possibly high or low pH, and possible high levels of oxidizers such as hypochlorite ion. Future research for this laboratory will include methods for stabilizing such samples both on-site and on receipt at the laboratory as well as sample preparation methods that selectively remove reactive and interfering components of the sample matrix. We are also interested in new separation methods suitable for direct analysis (i.e. no derivitization of analytes) for target compounds in aqueous samples.

As mentioned before, a number of new analytical methods were used by this laboratory for the first time in such an exercise. These methods displayed high utility and will, therefore, be further developed. Among these were electrospray interface MS/MS, capillary zone electrophoresis and gas chromatography/atomic emission detection.

References

1. "RECOMMENDED OPERATING PROCEDURES FOR SAMPLING AND ANALYSIS IN THE VERIFICATION OF CHEMICAL DISARMAMENT," Marjatta Rautio, ed., The Ministry for Foreign Affairs of Finland, Helsinki, 1993.
2. "Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and Their Destruction," United States Arms Control and Disarmament Agency, October 1993.

"Solving Practical Problems in Environmental Sampling for Chemical Agents and their Degradation Compounds"

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Abstract

The analyses of environmental samples for chemical agent degradation products were conducted using analytical test methods designed for evaluation of solid waste samples. All methods are found in the 3rd Edition of EPA's compendium of analytical methods (SW-846) dated July 1992. These EPA methods are recommended for compliance testing required by the Resource Conservation and Recovery Act (RCRA) and are routinely used for the analysis of environmental samples. In the past several years, these same methods were used to support the survey of areas suspected of having chemical agent or chemical agent degradation compound contamination.

An overview is presented of the U.S. Army Center for Health Promotion and Preventive Medicine's (previously the U.S. Army Environmental Hygiene Agency) involvement with the analysis of samples for chemical agents and their degradation compounds collected from sites such as Tooele Army Depot, Rocky Mt. Arsenal, Newport Army Depot, Johnston Island, and Spring Valley, (a residential site near American University in Washington D.C.)

Discussed are practical problems encountered during a quick response of a non-surety laboratory to analyze environmental samples for agents and or their degradation compounds. These include the early establishment of data quality objectives, establishment of points of contact, the need for a status report, obtaining reference standards, transport of samples from the site to the laboratories, handling and storage of samples to include chain of custody, handling split samples for screening, meeting sample holding time requirements, the need for surety training for those in the laboratory, training in sampling techniques, and the disposal of waste materials and samples found to be contaminated.

Introduction

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), formerly the U.S. Army Environmental Hygiene Agency (USAEHA), is committed to assuring that chemical demilitarization facilities are in full compliance with all applicable Federal, State, Local, and Army regulations, and to rendering any technical assistance necessary to protect the health and safety of workers, as well as to preserve the environment. In fulfilling this mission, our engineers and scientists, over the past twenty years, have gained a wealth of information regarding the testing of hazardous materials. Familiar topics include the analysis of chemical agents, chemical agent simulants, and chemical agent degradation compounds from a variety of matrices.

In its demilitarization program to dispose of obsolete and retrograde chemical agents and munitions, the U.S. Army is often faced with unique engineering and analytical requests. Many of these analytical requests coming to USACHPPM are received within the Directorate of Laboratory Sciences (DLS) and require methods development or modification of existing analytical methods to address the health related issues. To exemplify USACHPPM's participation in the analysis of chemical agent degradation products and/or chemical agent simulants, a brief overview of some of our past involvement is in order.

FIELD IMPLEMENTATION

During the mid 1970's and into the 80's, our engineers and scientists were involved with a variety of field studies to assure that disposal facilities were not posing a health problem. Because of the unique properties of many of these hazardous compounds, the development of analytical methods or the adaptation of currently used methods was required to provide the necessary analytical support. Stack samples of the riot control agent O-chlorobenzylidene malononitrile (CS) and its degradation compounds was accomplished by sampling incinerator stack emissions of deactivation furnaces located at Tooele Army Depot (TEAD). The incinerators used for this disposal were rotary kiln prototypes designed in support of the Chemical Agent Munition Disposal System (CAMDS) program. The DLS scientists were given the tasks of developing analytical methods¹ capable of quantitating CS, hydrogen cyanide (HCN), cyanogen chloride (CK), hydrogen chloride (HCl), and phenolic compounds in both incinerator stack emissions and caustic scrubbers. Also in the 70's, we were involved with the testing and evaluation of direct reading instrumentation² and the development of analytical methods³ for the collection and analysis of concentrations of carbonyl chloride (phosgene) as well as conducting efficiency studies of the isopropyl methylphosphono-fluoridate (GB) neutralization process using a caustic scrubber⁴ at Rocky Mt. Arsenal (RMA). Computer modeling using key punch cards for air dispersion and tracer studies was also conducted for Rocky during this time.

In 1985 our engineers and scientists were involved with the incineration of a simulant for phosphonothioic acid, methyl-, S-(2-bis(1methylethylamino)ethyl) O-ethyl ester (VX) at the Newport Army Ammunition Plant. Again the USAEHA labs were given the tasks to develop a sampling train capable of collecting the VX simulant, bis(2-ethylhexyl) hydrogen phosphite (BIS), and an analytical procedure for its quantitation. The ensuing laboratory spiking study using BIS and bench testing of an analytical procedure developed in DLS for explosives analysis⁵ of incinerator emissions provided quantitative recoveries. Field testing was accomplished when concentrations of BIS were added to the incinerator fuel supply and burned during the operation of a deactivation furnace. The analysis of BIS was achieved using a gas chromatograph (GC) equipped with a nitrogen/phosphorus detector (NPD).

In 1989, after CAMDS was in full operation, USAEHA was asked to review the air pollution, ground water, solid waste, hazardous waste, and waste water management programs⁶ at the south area of Tooele Army Depot (TEAD). Resulting data obtained from environmental samples provided to the DLS laboratories played an important part in this evaluation of the Tooele facility.

During the 1990's many factors (including our past engineering and analytical accomplishments, the fact that we are a full service EPA-certified laboratory, and the increased number of military installation closures) greatly influenced our increased involvement in conducting analysis of environmental samples collected from non-stockpile chemical sites.

The USACHPPM laboratories conducted a background study of samples collected from the Johnston Atoll facility. These data include concentrations of organophorous (OP), volatile and semi-volatile organic compounds found in samples of a variety of matrices (coral, mollusk shells, sea sand and water).

Recently a validation of procedure was conducted for the analysis of Di-isomethylphosphanate (DIMP) a degradation product of the chemical agent GB (Sarin) in a variety of vegetation species for RMA. Samples were extracted with methyltertbutyl ether (MTBE) and analyzed using a GC with a nitrogen-phosphorus detector. Analytical procedures for isopropylmethyl phosphonic acid (IMPA), methylphosphonic acid (MPA) and fluoroacetic acid (FC2A) were also validated in water using ion chromatography⁷ in the USACHPPM laboratories.

The USACHPPM laboratories are currently involved with the U.S. Army Chemical Materiel Destruction Agency in developing and evaluating procedures for the analysis of surrogate compounds suitable for use during the incineration of organophosphorus (OP) chemical agents. We are in the preliminary stages of this investigation, but use of OP pesticides holds great promise for surrogate use since they exhibit many similar chemical properties to the OP agents.

Our scientists and engineers worked with the Chemical and Biological Defense Command (CBDCOM) when high explosive rounds and unexploded chemical ordnance rounds were unearthed in a utility trench at the Spring Valley housing development located in the suburbs of Washington, DC on 15 Jan. 1993. This was the first emergency situation involving the known presence of chemical agents with which our Agency was involved. The target analytes included phosgene, cyanogen chloride, chloropicrin, and bromobenzyl cyanide in addition to the SW-846 list of semi-volatiles, volatile compounds, and metals.

In the past 6 months, USACHPPM laboratories have analyzed a number of samples where there were concerns with the possible presence of chemical agents.

- Thiodyglycol was analyzed from soil and water samples collected from the US Army Defense Distribution Depot at Ogden, Utah.
- "Orange ooze" coming from a burial pit located at the former Black Hills Army Depot near Provo, South Dakota was analyzed and identified as iron deposits. This material was suspect in the reported high death rate of lambs on neighboring ranches.
- Analysis of the contents of steel drums that caused skin rashes to workers removing them from a bunker located on Spangdahlem Air Base in Germany was identified as tricalcium arsenate.
- USACHPPM labs identified calcium hydroxide in "irritating soil" removed from utility trenches at an U.S. Army installation at Sagami, Japan.

In each of these cases we provided the chemical analysis to resolve questions concerning the chemical composition of these samples.

SAMPLE MATRIX

As previously stated, our engineers and chemists are constantly confronted with problems arising from a variety of sample matrices including air, water, soil, and biota. From an analytical viewpoint, sample matrix is one of the most critical factors to consider when trying to identify target and unknown compounds exposed to environmental conditions. Compound stability and its degradation products are of primary concern.

Of the sample matrices that are submitted to an environmental laboratory including air, water, soil and biota, atmospheric sampling is the least problematic. Ambient air is a cleaner matrix of known background composition, therefore rendering it interference-free and less difficult to analyze. However, products of incomplete combustion that are present in the background of acidic incinerator stack emission samples certainly cause sample analysis to be more challenging.

There are more validated procedures for the analysis of water for its chemical composition than any other matrix. Generally, the analysis of water exhibits fewer analytical interferences than soil and biota. However, water analysis, due to the solubility and hydrolysis of compounds, can present complex analytical problems due to analyte instability.

A soil matrix presents additional challenges to the analysts. The analysis becomes more complex because soil is a natural mixture of both organic and inorganic materials. It is not homogeneous, as are air and water, and it can contain organisms that digest organic material. These properties present both extraction and analysis problems even when the sample is free of contamination. The humic acids, decomposed organic matter, and other naturally occurring compounds make soils much more difficult to analyze than air or water. Analysis is made even more complex with contamination from solvents, fuels and lubricants. In many instances the soils' ionic properties cause them to be more difficult to extract. The acidity or alkalinity of a soil also influences its ease of extraction, as found with the Johnston Atoll shell aggregate samples.

The last and most difficult matrix is biota. The complexities of the analysis of plant and animal tissues are just beginning to be realized. USACHPPM scientists have found that fatty acids, proteins, chlorophyll and many other cellular components caused the need for additional analytical cleanup procedures to be included in currently used methods. Gel permeation and the use of silica gel or solid sorbent sample prep are examples of effective means of sample cleanup for tissue analysis.

ANALYTICAL METHODS

Many of the analytical methods that are currently used in the USACHPPM labs for environmental assessment of solid waste and/or soil analysis are those found in the USEPA Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846 Compendium of Analytical Methods⁸. These SW-846 methods include analytical procedures for both water and soil samples and can be easily modified for biota samples. Organics analyzed by these methods include those compounds referred to as volatile (boiling under 200°C), semi-volatile, the OP and chlorinated pesticides, and inorganic compounds. The inorganic methods include soil, water and biological tissues analysis.

The methods used for ambient air analysis include those EPA Toxic Organics methods that are known as methods TO-1 through TO-14. These include analysis for volatile and semi-volatile organic compounds ranging from vinyl chloride and benzene to polynuclear aromatic hydrocarbons and pesticides.

The volatile and semi-volatile organic sampling trains (VOST & SEMI-VOST) are EPA-recommended sampling trains that are routinely used for the analysis of incinerator emissions. These methods are also found in the EPA SW-846 Compendium of Methods.

Specific methodologies have been validated by the US Army Environmental Center (USAEC) for the analysis of agent degradation compounds and use analytical instrumentation normally found in a well equipped environmental chemistry laboratory. These methods are used by most environmental laboratories that routinely screen for agent degradation compounds.

Compound degradation products that are normally screened or analyzed for can be grouped into several general categories. Thiodiglycol and organosulfur compounds which are associated with sulfur mustard manufacturing and hydrolysis⁹ are detected in water at the low part per billion (ppb) level ranging from 1 to 20 ppb. In soil, detection of these sulfur mustard products ranges from 1-15 ppm.¹⁰ These are analyzed by GC using a flame photometric detector (FPD) operating in the sulfur mode.

Phosphonates that are degradation products of GB (Sarin) are other indicator compounds and can be identified and quantified at the low ppb levels in both water and a soil matrix using a GC/FPD operating in the phosphorus mode¹¹.

The organoacids such as IMPA and MPA, are agent hydrolysis products associated with agents GB and VX. Chloroacetic acid and fluoroacetic acid are suspected hydrolysis products of sulfur mustard manufacture. These compounds can be analyzed in water from 25 to 200 ppb.¹² IMPA, chloroacetic acid and fluoroacetic acid can be detected in soil to 50 ppb.¹³ All of these organoacids are analyzed using ion chromatography (IC) procedures.

On the inorganic analytical list appear such compounds as arsenic, phosphates, hydrogen cyanide, and fluoride. These are all degradation products that could indicate possible chemical agent contamination.

Analytical instruments that are routinely used in the analysis of environmental samples for chemical agent analysis, include gas chromatograph with a mass spectrometer detector (GC/MS), gas chromatograph with flame photometric detector (GC/FPD), ion chromatograph with a conductivity detector (IC), inductively coupled plasma with a mass spectrometer detector (ICP/MS), remote sensing fourier transform infrared spectrometers (FTIR) and gas chromatography with an atomic emissions detector (GC/AED). Methods using the last two instruments systems for agent analysis are currently in the developmental stages.

PROBLEMS ENCOUNTERED

Last, are the problems that are associated with supporting projects that involve chemical agent degradation analysis. One of the major problems in providing quick response support to an emergency situation is functioning with the lack of established Data Quality Objectives (DQO) or having time to plan an approach to resolve a specific problem. In many instances, DQO tend to evolve during the project rather than at the onset. These DQO include analyte lists, required detection limits, responsiveness, critical exposure levels, quality control procedures and health risk factors so that methods can be selected to meet those objectives. As with any quick response, there are compromises that are made to provide immediate sample results. These could include less QC, the use of non-validated methodologies, or less time spent on the data review process.

The remaining problems are separated into two major categories of logistical and technical problems. Keep in mind these are problems that are encountered by a non-surety laboratory.

Logistical problems include being able to respond quickly to situations and being able to provide samples to the laboratory in a timely manner. To insure responsiveness, USACHPPM instituted a quick response SOP¹⁴ that allows rapid and effective response to requests for assistance to large scale environmental and health emergencies. In this SOP, the responsibilities and functions of the involved programs within the organization were identified.

Organic samples analyzed by the EPA SW-846 methods have a seven-and/or a fourteen-day holding time and require refrigeration during shipping and storage. With the time required for shipping to the laboratory, in addition to the time required for a chemical agent screen from Chemical and Biological Defense Command (CBDCOM), this holding time could easily be exceeded. One alternative would be to collect duplicate samples and have one set go to the CBDCOM agent screening laboratory and the other come to the DLS laboratory to be placed in a holding area until the agent screen was completed. Another alternative would be to permit the volatile organic¹⁵ screen to be conducted using a tare-weighed sample vial. This vial is not opened because the analysis is accomplished by an autosampler piercing the sample vial septa and injecting the sample. This could be accomplished before the agent screen from CBDCOM was completed. This approach would only occur after there were no positive detects observed by Tech Escort during the field screening for agents. A volatile screen could be accomplished concurrently with the CBDCOMs laboratory agent screen.

Since the USACHPPM labs are not in the US Army's surety program and do not maintain an inventory of chemical agent degradation compounds, problems were encountered in obtaining these compounds to use as standard reference materials during the "Quick Response" required for the Spring Valley project. One of the primary causes for this was the lack of an on-post chemical registry. One of the compounds, bromobenzyl cyanide, was investigated in the early 1900s but a supply was unavailable for reference purposes. It was only through other Agencies located at the Edgewood Area of Aberdeen Proving Ground that archived mass spectra were provided allowing this problem to be resolved. Quicker data turn-around-time could potentially be achieved if a registry or master chemical inventory of all chemical agent degradation compounds on hand at the different Agencies located at U.S. Army installations could be on file and be made readily available to those laboratories that would potentially be involved in the assessment of an agent investigation.

Another solution for alleviating the problem of obtaining reference standards would be to generate detector responses of all chemical agents and their degradation compounds. The responses could be compared to the detector responses obtained for internal standard compounds which are routinely analyzed by an environmental laboratory during sample analysis. If these relative responses were archived and provided to laboratories participating in agent degradation analysis, a screen could proceed without actually having the compounds in the laboratory to use as standards. This technique would give all analytical laboratories in DOD that currently run SW-846 methods the capability to estimate the sensitivity and detection limits for chemical agents and their degradation compounds.

Training, including proper sampling techniques and the importance of maintaining sample chain of custody, should be provided to all those in the surety program. Special emphasis should be given to those individuals who come in direct contact with samples. All individuals who potentially would provide sample analysis should be in the Chemical Agent Surety Program and be provided the annual Chemical Surety Briefing and the annual agent physical.

To keep pertinent project information current, a periodic status report needs to be made available. This could easily be accomplished by use of electronic mail system.

From a laboratory perspective, most of the technical problems revolve around the interaction of the analyte with the sample matrix and the interferences produced. In analysis for HCN in incinerator stack emissions at TEAD following CS incineration, the high levels of background CO₂ reacted with the NaOH impinger solutions. Production of the reaction product, Na₂CO₃, required the use of standard additions for accurate analyte quantitation. In the case of shell aggregate samples collected from Johnston Atoll, the highly alkaline nature of these samples caused a reaction with the acetone extracting solvent. The formation of 4-hydroxy-2-pentanone interfered with the chromatography and made it necessary to modify sample extraction procedures to include a solvent exchange. Thereafter, acetone was solvent-exchanged with methylene chloride. In many instances compounds can be derivatized to a more stable compound. This is always recommended where possible, as with the collection of phosgene in air¹⁶ at 0.1 ppb using an aniline impinger solution to form carbanilide.

Preserving the integrity of the sample is another major problem. A data set is only as good as the collection procedures used and the storage and handling the sample received. Contamination can be averted through proper training and the presence of knowledgeable individuals providing guidance at the sample site. Samples suspected to contain chemical agents are always screened by CBDCOM before receipt at USACHPPM.

More stringent QC needs to be provided with all sample types. This includes travel blanks that are blank sample QC matrix that are sent to the field with the sampling media and are opened at the sampling site, sample blanks which are samples collected in the field from an uncontaminated area, and spiked matrix blanks that are prepared in the laboratory and taken to the field and returned to be analyzed along with samples.

SUMMARY

The urgency of a situation helps set the requirements for a site assessment and causes greater demands to be placed on all involved. As the urgency increases, so do the problems that prevent the DQO from being achieved. The demands for quick response, field screening and data turn around time (QST), cause a magnification of all the problems discussed. A well-equipped non-surety analytical laboratory can contribute greatly in achieving QST.

REFERENCES

1. Williams, K. "Evaluation of Sampling Techniques for Cyanide Emissions." *Am. Ind. Hyg. Assoc. J.* 39:832-835 (1978).
2. "Air Pollution Special Study No. 99-041-75/76, Phosgene Measurement, Rocky Mountain Arsenal, Denver, Colorado, 22 Sept.-3 Oct. 1975," U.S. Army Environmental Hygiene Agency (USAEHA), 29 Apr. 1976.
3. "Air Pollution Engineering Special Study No. 99-041-75/76, Phosgene Measurement." Rocky Mountain Arsenal, Denver, Colorado, 19-30 April 1976, USAEHA, 5 Aug. 1976.
4. Kistner, S. D. Lillian, J. Ursillo, N. Smith, K. Sexton, M. Tuggle, G. Esposito, G. Podolak and S. Mallen, "A Caustic Scrubber System for the Control of Phosgene Emissions: Design, Testing, and Performance." *Air Pollution Control Association Journal*, Vol. 28, No. 7, 673-676 (1978).
5. Van Slyke, S., K. Williams, M. Sheely, L. Clark and S. Scheibler "Sampling and Analytical Techniques for Air Pollution Source Testing of Incinerators for Explosive Materials." 78 Annual Air Pollution Control Association Meeting, Detroit, Michigan, 16-21 June 1985.
6. "Executive Summary Final Report Environmental Program Review NO. 37-26-1013-90, Chemical Agent Munitions Disposal System, Tooele AD, Tooele, Utah." May, 1989.
7. U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), SOP# UT02: "Ion Chromatographic Analysis of Isopropylmethyl Phosphonic Acid (IMPA), Methylphosphonic Acid (MPA) and Fluoroacetic Acid (FC2A) in Soil." Aberdeen Proving Ground (APG) MD. Mar. 1990.
8. USEPA "Test Methods for Evaluating Solid Waste." USEPA SW-846; 3rd Edition. Washington, DC. 1992.
9. "Draft of the Site Monitoring Concept Study.", U.S. Army Chemical Material Destruction Agency, APG, MD, June, 1993.
10. USATHAMA. SOP#s UL09 and AAA8, "The Analysis of Thiodiglycol and Organosulfur Compounds in Soil." APG, MD. Dec. 1993/Jan. 1988.

11. U.S. Army Environmental Center (USAEC), SOP#s UK11 and LK09, "The Gas Chromatographic Analysis of GB Degradation Compounds." APG, MD. Dec. 1993.
12. USAEC, SOP# UT04, "The Analysis of Chloroacetic Acid and Fluoroacetic Acid in Water." APG, MD. Dec. 1993.
13. USAEC, SOP# LT04, "The Analysis of Chloroacetic Acid and Fluoroacetic Acid in Soil." APG MD. Dec. 1993.
14. USAEHA, Quick Response Task Force SOP, 30 APR 1993.
15. Black, Robin M., "Application of Headspace Analysis, Solvent Extraction, Thermal Desorption and GC/MS to the Analysis of Chemical Warfare Samples Containing Sulfur Mustard and Related Compounds." *J. Chrom.*, 637 (1993) 71-80.
16. USEPA Air Toxic Methods #TO-6, "Method for the Determination of Phosgene In Ambient Air Using High Pressure Liquid Chromatography." 1st Revision, Sept. 1986.

The Chemical Agent Experience at Rocky Mountain Arsenal

by

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ABSTRACT

Rocky Mountain Arsenal (RMA) was constructed and commissioned in 1942 for the production of sulfur mustard and other chemical munitions for possible use in World War II. RMA also became a production site for Lewisite and Sarin, including synthesis and munition filling. Other chemical agents such as Phosgene were routinely handled, filled into munitions and demilitarized. During the 1970's and the early 1980's, RMA served as a primary demilitarization facility for the destruction of chemical agents. Throughout its chemical weapons history, RMA generated waste materials from production, neutralization, decontamination and testing. These operations led to the possibility of chemical agent contamination in soils, process equipment and structures that have required special attention as part of the overall Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) environmental cleanup operations being conducted by the Program Manager Rocky Mountain Arsenal (PMRMA). Adjusting normal sampling operations associated with CERCLA-type activities for the special Army regulations covering chemical agents has been a difficult task. This presentation will describe the evolution of chemical agent related efforts and operations as they pertain to RMA environmental cleanup activities, to include field sampling requirements, analytical methods, commercial laboratory use and the role of the on-site PMRMA laboratory.

A COPY OF OVERHEAD SLIDES USED IN THE CONFERENCE PRESENTATION OF THIS MATERIAL FOLLOWS.

Chemistry

The Chemical Agent Experience at Rocky Mountain Arsenal

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Presented by:
Gregory B. Mohrman
Chief, Laboratory Support Division
Analytical Methods for Environmental Sampling of
Chemical Warfare Agents and Their Degradation Products, 20-21 September 94

Program Manager Rocky Mountain Arsenal

Chemistry

HISTORICAL

- **Rocky Mountain Arsenal established in 1942**
- **Synthesized chemical warfare agents, filled and demilitarized munitions**
- **Shell Oil Co. use of process facilities for pesticide production**
- **Program Manager's Office formed in 1985 with the sole mission of performing the environmental cleanup**
- **RMA is an inactive installation with no active military mission other than the environmental program**

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RMA CHEMICAL AGENT HISTORY

- **Synthesized GB, HD & L**
- **Stored VX (Bulk)**
- **Various munitions were filled, stored and demilitarized on the post**
- **Environmental contamination resulted from waste disposal activities and burial**
- **Special projects included phosgene, cyanogen chloride and Adamsite disposal and demilitarization**
- **In early 1983, RMA completed the demilitarization of all known agent identification tests (I.D. Kits)**
- **RMA no longer stores or handles any chemical munitions**

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TERMS

- SURETY
- RDT & E DILUTES
- POTABLE WATER STANDARDS
- NEUTRALENTS
- CASARMS
- NERVE AGENTS
- BLISTER AGENTS
- INCAPACITATING AGENTS
- BLOOD AGENTS
- FORTUITOUS SAMPLING

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SURETY VS. NON-SURETY

- Surety materials as defined in AR 50-6
- RMA is no longer a surety facility
- The on-site laboratory was designed to be a non-surety facility
- Special precautions are taken to prevent the possibility of introducing "surety" materials into the facility
- Suspect "fortuitous" samples are field diluted to reach RDT & E levels

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AGENT BY-PRODUCTS AND DEGRADATION PRODUCTS

- **Representative compounds were selected early in the program**
- **Basis for selection included:**
 - **expected prevalence**
 - **expected persistence**
 - **availability of neat materials for standards**
 - **ease of analytical techniques**
- **Examples of primary agent degradation target analytes**
 - **GB – DIMP, DMMP, IMPA, Fluoride, (Fluoroacetic acid)**
 - **VX – EMPA**
 - **HD – Thiodiglycol, 1,4 Oxathiane, Dithiane**
 - **L – Total arsenic, Lewisite Oxide**

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ENVIRONMENTAL MEDIA OF CONCERN

- AIR
- SOIL
- WATER
- SEWERS
- PROCESS EQUIPMENT
- STRUCTURES
- UNKNOWNNS
- BIOTA (Arsenic only)

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FIELD OPERATIONS

- **AIR**
 - M8 (GB & VX only)
 - M18
 - CAM
 - DAAMS
 - RTAP (Minicams, HP-Dynatherm)
- **STRUCTURES/PROCESS EQUIPMENT**
 - DAAMS
 - Minicams
 - RTAP (HP-Dynatherm)
- **SOIL**
 - M8
 - M18
 - Recent SVRP
 - Real-Time Minicams
 - DAAMS

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LABORATORY OPERATIONS

- **Only authorized laboratories can store and use reference materials**
- **Value of an on-site facility is demonstrated**
- **Screening versus quantitative analysis**
- **Turnaround time**
- **Confirmation**
- **Sample release to commercial laboratories**
- **CASARMS**
- **Quality Assurance (air versus other media)**
- **Technology versus health based criteria**

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FORTUITOUS SAMPLES

- Process equipment
- UXO
- Treaty compliance
- Sample dilution kit
- Lots of coordination between field and laboratory

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OTHER CONSIDERATIONS

- **SAMPLING**
 - Discrete versus composite samples
 - Wipe samples
 - Ambient temperature
 - Sample transport
- **REFERENCE MATERIALS**
 - CASARMS
 - Transportation
 - Access and control
- **DECONTAMINATION**
 - 3X/5X Requirements
 - Waste management

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AGENT DEGRADATION PRODUCTS

- **Several compounds, e.g., DIMP and DMMP are commercially available**
- **SARM**
- **Commercial laboratory use**
- **Standard array of GC, GC/MS and IC methods are applicable to the various compounds**
- **Sample transport is not an issue with this set of compounds**

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SOIL VOLUME REFINEMENT PROGRAM (SVRP)

- **Field effort conducted March – September 1993**
- **Objective was to refine soil volume estimates for various contaminants to improve remedial cost estimates**
- **Secondary objective was to closely examine "suspect " agent contamination areas to confirm or deny the existence of these analytes**
- **Effort included GB, VX, HD and L as target analytes**
- **Specifically included fluoroacetic acid (compound 1080)**

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SVRP DATA QUALITY OBJECTIVES

- Previous methods used for the Remedial Investigation were technology based limits of 5.0 ug/g in soil for the various agents
- For the SVRP, these were lowered to 0.5 ug/g as target reporting limits
- No definitive health based criteria were, or have been established for the agents in soil
- For safety concerns at commercial laboratories, typical extraction procedures were used to calculate the final concentration of agent in an extract if 0.5 ug/g were present in the field sample
- Values were compared to the AR 50-6 specifications for concentration in mg/ml and for total mg content in the raw sample

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COMPARISON OF CHEMICAL AGENT CONCENTRATION TO RELATED STANDARDS AND REGULATIONS

- AR 50-6 limits for RDT & E quantities;

<u>Agent</u>	<u>Solvent Concentration (mg/ml)</u>	<u>Total Amount (mg)</u>
GB	2.0	20
HD	10	100
VX	1.0	10
L	5.0	50

- Total content of 1000g soil sample
- 10g soil sample extract taken to 0.5 ml final volume

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SVRP Continued...

FIELD EFFORT

- On-site, near real time air monitoring for GB, HD and VX
- Lewisite by M18 kit
- Soil samples from discrete depths
- Field agent detections required laboratory confirmation for positive identification
- Mobile laboratory for on-site air monitoring, including the Minicams, was provided as GFE to the sampling contractor
- Prior to field mobilization, all contractor personnel were trained in the handling and storage of agent standards and were required to demonstrate analysis proficiency

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SVRP Continued...

PMRMA LABORATORY EFFORT

- All SVRP soil samples (approximately 1300 total) were analyzed for GB, HD, VX and L prior to releasing samples off post
- The laboratory provided 24-hr. turnaround for all soils in order to minimize impact on sample hold times for commercial laboratories
- Positive detections underwent second column or GC/MS confirmation
- All samples with positive detections were provided to the laboratory for decontamination

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SVRP Continued...

COMMERCIAL LABORATORY EFFORT

- **Samples were sent to various commercial laboratories for VOC, SVOC, pesticide and agent degradation analysis**
- **No incidents of inappropriate sample submission occurred**

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SUMMARY

- The Army has not established health or risk based residual levels for agents in soil
- Limits at RMA continue to be technology based but have not been pushed to extreme levels
- The State of Colorado through the Colorado Water Quality Commission has proposed a state-wide drinking water standard of 8.0 ug/L (ppb) for DIMP
- The current EPA health advisory for DIMP is 600 ppb

Program Manager Rocky Mountain Arsenal

SESSION IV IMMUNOASSAY TECHNOLOGIES



Pesticide Reentry Problems and Relationships to CSEPP

by

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ABSTRACT

Federal and state agencies have almost 50 years experience with the "Reentry Problem" which is the toxic effects to fieldworkers caused by exposure to pesticide residues applied to agricultural areas. The most common means to avoid these effects is to establish "Reentry Intervals". Unfortunately, such an interval is not feasible if a chemical warfare agent is inadvertently released. Use of a "Reentry Level" is preferred for such a case. This use requires that an analytical method be established that will be fast, specific, and sensitive. Ideally, it could be used on-site in the field. An enzyme linked immuno specific assay or a similar procedure may be the best solution to these limitations.

The Development of Immunoassays for Detection of Chemical Warfare Agents
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ABSTRACT

With the advent of enzyme linked immunoabsorbant assays (ELISA) and monoclonal antibodies in the last two decades, there has been considerable effort devoted to the development of antibodies to detect and quantify low molecular weight toxic substances in environmental or biological fluids. Polyclonal antibodies against paraoxon (the toxic metabolite of parathion) were reported as capable of detecting paraoxon in body fluids at a level of 10^{-9} M (~260 pg/mL) when used in a competitive inhibition enzyme immunoassay (CIEIA). Monoclonal antibodies developed against a structural analogue of the chemical warfare agent soman were capable of detecting soman in buffer solutions at a level of 10^{-6} M (~180 ng/mL). In addition, these antibodies were highly specific for soman even in the presence of its major hydrolysis product. Subsequent studies with antisoman monoclonal antibodies reported an extension of the level of sensitivity to ~80 ng/mL. Furthermore these antibodies did not cross react with other chemical warfare nerve agents such as sarin or tabun. In all cases, the time for a confirmatory test was two hours or less. Immunoassays for T-2 micotoxins have also been reported with a minimal detection range of 2 pg/assay to 50 ng/assay for the polyclonal and monoclonal T-2 antibodies respectively. These antibodies offer a sensitive, rapid and low cost approach to the diagnosis or detection of the presence of toxic chemical substances.

BACKGROUND

The standard methods of analysis for detection of organophosphorus chemical warfare agents either require time-consuming isolation and cleanup procedures and expensive analytical equipment such as gas chromatography (GC) or gas chromatography-mass spectrometry (GC/MS), or they rely on rather nonspecific color reactions that result from changes in the activity of the enzyme acetylcholinesterase. The former procedures are very quantitative, slow and expensive; while the latter rapid approach is qualitative or semi-quantitative but can give ambiguous results. What would be most desirable is a method that could detect a specific chemical warfare agent in a rapid, semi-quantitative manner. In addition, the results should be subject to minimal interference from hydrolysis products or structurally related compounds.

One approach that provides many of the features of such an analytical method is

based on the use of antibodies specific for the analyte of interest. This has shown considerable applicability in the analysis of insecticides in environmental samples (1-6). For example, Hunter and Lenz reported that paraoxon, the active metabolite of parathion, could be detected at a level of 1 nM in biological fluids (3).

More recent efforts have expanded this approach and applied it to developing antibodies against various of the organophosphorus chemical warfare agents. Initially, polyclonal and subsequently monoclonal antibodies were developed against the highly toxic chemical warfare agent soman (7-9). These proved to be useful in the development of several immunoassays capable of quantitatively determining the amount of soman present in aqueous solution (7) as well as mammalian serum, milk and water (10, 11). In all cases a competitive immunoassay was the method of choice for quantifying the amount of soman present in solution. More recently both polyclonal (12) and monoclonal (13) antibodies have been developed against another chemical warfare agent, VX, and polyclonal antibodies have been developed against sulfur mustard (14). Besides the development of antibodies against the organophosphorus chemical warfare agents, polyclonal (15) and monoclonal (16, 17) antibodies have been reported against the trichothecene mycotoxin T-2 also.

CONSIDERATIONS FOR DEVELOPMENT OF AN ASSAY

An immunoassay, to be most effective, should be designed to detect the analyte of interest (the hapten) and, ideally, nothing else. While this may seem obvious, it must be remembered that other analytical approaches such as GC or GC/MS often detect a host of species from which the analyte of interest must then be uniquely identified. As with most analytical techniques, it is useful to estimate the expected concentration of the analyte in the milieu chosen for analysis. In the case of immunoassays, if reasonable estimates can be made regarding the concentration and binding constant characteristics of the antibody being developed, then theoretical calculations can be carried out to determine if the analyte will be detectable under the conditions of the assay.

Since most small molecules (<1000 Daltons) are not capable of eliciting an immune

response that would lead to antibody production, the hapten (analyte) must be attached to a protein carrier molecule to make an immunogen. In carrying out the reaction covalently joining the hapten to the protein carrier, care must be taken to ensure that the structural features of the hapten that are the most important, or unique, are retained after the immunogen is synthesized. Unfortunately, the strongest immune response will be to the protein carrier molecule, rather than to the hapten attached to it. To identify those antibodies that are specific for the hapten, the hapten must be covalently attached to a second protein carrier molecule that is not related to that used for the immunogen. This second protein-hapten molecule is often called an antigen and is used as the test compound in an enzyme-linked immunoassay (EIA) to ensure that the antibodies identified are specific for the hapten of interest and not for the carrier protein.

It is also useful to consider if polyclonal or monoclonal antibodies will be required. If the intent is to qualitatively determine the presence of some particular molecule, polyclonal antibodies may not only be acceptable, but they may be preferable. What polyclonal antibodies lack in specificity they can often make up for in sensitivity since these antibodies have a range of binding constants. Contrariwise, if a more quantitative assay is required then monoclonal antibodies capable of binding only to the molecule of interest would be a requisite. In this case while some sensitivity may be sacrificed, selectivity will be increased.

Lastly, a decision must be made with respect to the test hapten that will be used in screening the antibodies produced by hybridoma cells when developing monoclonal antibodies. If high specificity is desired, then the test hapten for screening the antibody producing cells should be the specific analyte itself. If that compound is unavailable or unstable under the condition of the assay, then a compound that has maximum structural similarity with the analyte should be chosen. Since the antibodies being produced at this point represent a wide repertoire of binding affinity and specificity, it is incumbent upon the scientist to limit the possibilities in order to enhance specificity; otherwise, the resultant antibody may be ill-suited to the analytical task at hand. To obtain some quantitative estimate of the amount of analyte present, a competitive inhibition enzyme immunoassay (CIEIA or CIA) is often the method of choice.

**PROPERTIES OF CURRENTLY
AVAILABLE ANTIBODIES AGAINST
CHEMICAL WARFARE AGENTS**

In the early 1980's Hunter et al. (7) developed monoclonal antibodies against the chemical warfare agent soman. They immunized animals with a soman analogue that had been covalently attached to either keyhole limpet hemocyanin (KLH) or to bovine serum albumin (BSA) (Figure 1). The resultant monoclonal antibodies bound to the test antigen and this binding was inhibited in a competitive manner by free soman in solution (Figure 2). Further studies with these antibodies allowed for the elucidation of their structural and stereochemical specificity (9). The antisoman antibodies did not cross react either with sarin, another toxic chemical warfare agent, or with hydrolyzed soman wherein the fluorine had been replaced by a hydroxyl group. Based on their studies Hunter et al. (7) concluded that these antibodies could be used to quantitate levels of soman at 1 μ M (200 ppb). Using an assumed soman LD50 of 6 μ g/kg in humans with all of the soman

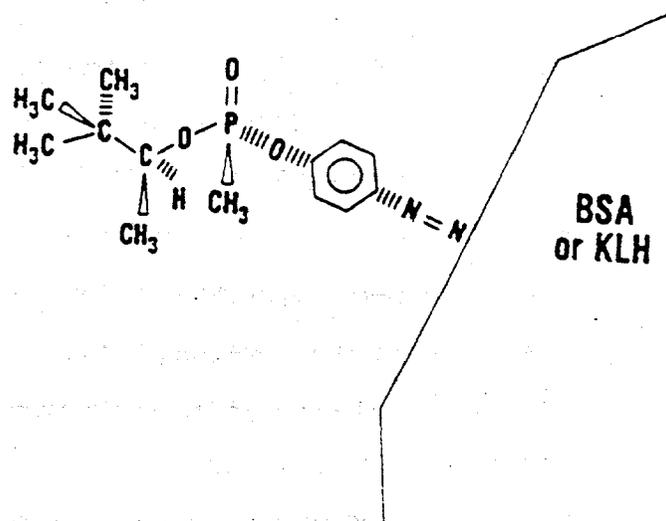


Fig. 1. Structure of soman-protein conjugates employed for immunization and/or immunoassay.

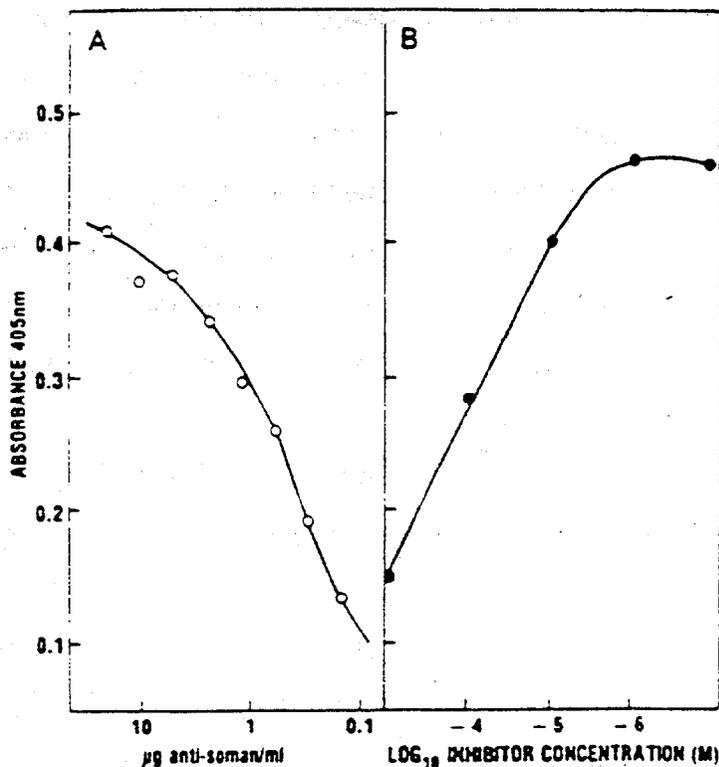


Fig. 2. (A) Titration of the binding of anti-soman to soman-KLH. (B) Inhibition of the binding of anti-soman antibody to soman-KLH by various concentrations of free soman.

distributed in a blood volume of five liters, the circulating concentration of soman equivalent to an LD50 would be 0.47 μM , or below the limits of detection with that antibody. Subsequent work by Erhard et al. (10, 11) led to the development of additional anti-soman monoclonal antibodies that were capable of detecting pure soman in solution at a level of 0.5 μM , which is within the range estimated for a median lethal dose of soman in humans (*vide supra*). The same antibodies were found to be capable of detecting soman in mammalian serum at a level of 1.3 μM (11) in good agreement with the levels of detection reported by Hunter et al. (7). Clearly, if monoclonal antibodies are to be used in an immunochemical detection system for serum samples, an increase in the binding constant of at least one order of magnitude, and preferably two, will be required.

In addition to soman, antibodies specific for VX, another highly toxic organophosphorus chemical warfare agent, have been developed. Initially, polyclonal anti-VX antibodies were developed by Rong and Zhang (12) using an undefined VX analogue. These antibodies, raised in rabbits, afforded the immunized animals full protection against the lethal effects of an LD95 dose of VX at both 7 days post immunization and again at 31 days post immunization. When the serum from immunized rabbits was administered to mice in a passive immunization, the mice were afforded protection against an LD95 dose of VX for up to 10 days after the immunization (12). More recently, Grognet et al. (13) reported the development of monoclonal anti-VX antibodies against a series of structurally similar VX analogues. Of the monoclonal antibodies produced, most were specific for VX, with only low cross reactivity with soman, sarin or tabun. Two of the haptens, however, did elicit antibodies with affinity for soman or sarin in the micromolar range. While these antibodies could neutralize the inhibition of acetylcholinesterase by VX *in vitro*, they afforded no *in vivo* passive protection, even though they had affinity constants of 9 nM (13). The authors reported that they are currently working on the development of an immunoassay for VX using these antibodies.

Other antibodies have been developed against sulfur mustard analogues (Lieske et al., 14) and the trichothecene mycotoxin T-2 (15-17). The polyclonal antibodies against sulfur mustard had a lower limit of detection of 5 μM (14). The polyclonal anti T-2 toxin antibodies had a reported lower limit of detection of about 2 pg per assay. The monoclonal

antibodies developed by Hunter et al. (16) when used in a CIEIA, could detect 50 ng/assay. Further studies with the monoclonal antibodies showed that they could reverse the intracellular toxicity of T-2 toxin (17).

SUMMARY

Current efforts have demonstrated that antibodies, both monoclonal and polyclonal, can be produced that have specific affinity for two of the chemical warfare organophosphorus nerve agents, soman and VX. Only the anti-soman antibodies have been investigated to a sufficient degree to determine if they could be used in an immunoassay to detect the presence of soman in an aqueous sample. The detection limits were in the micromolar (ppb) range which, while fairly sensitive, was about twice the estimated concentration of the median lethal dose of soman in humans. The antibodies for VX have not yet been utilized in an immunoassay, but to be useful they should also be capable of detecting VX in the sub micromolar range. In most of the reports to date, the total assay time was two hours or less with an incubation time of the antibody with the nerve agent of 10 to 30 minutes. Both the anti-soman and anti-VX antibodies had the requisite specificity, neither exhibiting a strong cross reaction with the metabolites or hydrolysis products of the respective nerve agents, soman and VX.

The results to date clearly show that antibodies can be developed against various chemical warfare agents. These antibodies have been used to demonstrate that immunoassays capable of detecting chemical nerve agents in the micromolar (ppb) range are quite feasible. While the current antibodies do not have the requisite affinity for detecting nerve agents at a nanomolar concentration, which would be below the estimated human median lethal dose range, there are no technical barriers to developing antibodies of higher affinity. Given that several reports of immunoassays for nerve agents have already been published, there is every reason to believe that future efforts will result in the development of immunoassays for nerve agents or their metabolites with the required sensitivity, selectivity and speed of response.

REFERENCES

1. Hammock, B. D. and Mumma, R. O. (1980). Potential of immunochemical technology for pesticide analysis. in *Pesticide Analytical Methodology*, (Harvey, J., Zweig, G., Eds.): American Chemical Society: Washington, DC, pp 321-352.
2. Brimfield, A. A., Lenz, D. E., Graham, C. and Hunter, Jr., K. W., *J. Agr. Food Chem.* (1985). Mouse monoclonal antibodies against paraoxon: potential reagents for immunoassay with constant immunochemical characteristics. **33**, 1237-1242.
3. Hunter, Jr, K. W. and Lenz, D. E., (1982). Detection and quantification of the organophosphate insecticide paraoxon by competitive inhibition enzyme immunoassay. *Life Sci.* **30**, 355-361.
4. Al-Rubae, A. Y. (1978). The enzyme-linked immunosorbent assay, a new method for the analysis of pesticide residues. *PhD Dissertations*, The Pennsylvania State University, University Park, PA
5. Van Emon, I., Seiber, I., and Hammock, B. (1987). Application of an enzyme-linked immunosorbent assay (ELISA) to determine paraquat residues in milk, beef and potatoes. *Bull. Environ. Contam. Toxicol.* , **39**, 490-497.
6. Heldman, E., Balan, A., Horowitz, O., Ben-Zion, S., and Torton, M., (1985). A novel immunoassay with direct relevance to protection against organophosphate poisoning. *FEBS Lett.* , **180**, 243-248.
7. Hunter, Jr., K. W., Lenz, D. E., Brimfield, A. A., and Naylor J. A., (1982). Quantification of the organophosphorus nerve agent soman by competitive inhibition enzyme immunoassay using monoclonal antibody. *FEBS Lett.* **149**, 147-151.
8. Lenz, D. E., Brimfield, A. A., Hunter, Jr., K. W., Benschop, H. P., de Jong, L. P. A., van Dijk, C., and Clow, T. R., (1984). Studies using a monoclonal antibody against soman. *Fund. Appl. Toxicol.*, **4**, S156-S164.
9. Brimfield, A. A., Hunter, K. W., Lenz, D. E., Benschop, H. P., van Dijk, C., and de Jong, L. P. A., (1985). Structural and stereochemical specificity of mouse monoclonal antibodies to the organophosphorus cholinesterase inhibitor soman. *Mol. Pharmacol.* , **28**, 32-39.

10. Erhard, M. H., Schmidt, P., Kuhlmann, R., and Losch, U. (1989). Development of an ELISA for detection of an organophosphorus compound using monoclonal antibodies. *Arch. Toxicol.* , **63**, 462-468.
11. Erhard, M. H., Kuhlmann, R., Szinicz, L., and Losch, U., (1990). Detection of the organophosphorus nerve agent soman by an ELISA using monoclonal antibodies. *Arch. Toxicol.* , **64**, 580-585.
12. Rong, K.-T. and Zhang, L.-J., (1990). Immunologic protection against VX intoxication in experimental animals. *Pharmacology and Toxicology* , **67**, 255-259.
13. Grognet, J.-M., Ardouin, T., Istin, M., Vandais, A., Noel, J.-P., Rima, G., Satge, J., Pradel, C., Sentenac-Roumanou, H., and Lion, C., (1993). Production and characterization of antibodies directed against organophosphorus nerve agent VX. *Arch. Toxicol.*, **67**, 66-71.
14. Lieske, C. N., Klopčic, R. S., Gross, C. L., Clark, J. H., Dolzine, T. W., Logan, T. P. and Meyer, H. G., (1992). Development of an antibody that binds sulfur mustard. *Immuno. Let.*, **31**, 117-122.
15. Peters, H., Dierich, M. P., and Dose, K., (1982). Enzyme-linked immunosorbent assay for detection of T-2 toxin. *Hoppe-Seyler's Z. Physiol. Chem.*, **363**, 1437-1441.
16. Hunter, Jr., K. W., Brimfield, A. A., Miller, M. A., Finkelman, F. D., and Chu, S. F., (1985). Preparation and characterization of monoclonal antibodies to the trichothecene mycotoxin T-2. *Appl. Environ. Microbiol.* **49**, 168-172.
17. Hunter, Jr., K. W., Brimfield, A. A., Knower, A. T., Powell, J. A., Feuerstein, G. Z., (1990). Reversal of intracellular toxicity of the trichothecene mycotoxin T-2 with monoclonal antibody. *J Pharm. Exper. Ther.*, **256**, 1183-1187.

THE APPLICATION OF IMMUNOASSAY-BASED ANALYTICAL METHODS TO THE MEASUREMENT OF ENVIRONMENTAL CONTAMINANTS

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Abstract

The recent development of several low-cost, rapid field analytical tests based on immunoassay technology has provided on-site tools for the cost-effective assessment of soil and water contamination. Immunoassay-based tests have the advantages of being sensitive and analyte-specific, while still being easy to use under field conditions. Extensive application of immunoassay-based field analytical methods for pentachlorophenol, polychlorinated biphenyls, polyaromatic hydrocarbons, and petroleum hydrocarbons have shown them to be accurate, rugged, and reliable.

The immunoassay method is based on the specific detection properties of an antibody, a molecule of biological origin that can be made to chemically "recognize" small organic molecules, similar to many environmental contaminants. A colorimetric "readout" is coupled to the antibody-based detection system to form the basis for a field-ready analytical system. The principles of operation and the practical implementation of these field tests will be described.

Introduction

The clean-up of hazardous waste sites typically proceeds in phases with analytical testing being performed during every phase. In the first phase of site assessment, a relatively small number of samples are collected and analyzed to identify hazardous compounds. Sites characterized as hazardous ultimately require mapping of the site, remediation, and on-going closure monitoring. Initial testing is performed in off-site laboratories using chromatographic and spectroscopic instrumentation (GC, GC/MS, HPLC) and accepted reference methodology. These methods are performed by trained technicians and are able to effectively identify and quantify multiple contaminants within a test sample when performed with appropriate quality control (QC) and quality assurance (QA).

The complexity and sequential nature of laboratory-based testing increases the cost of analysis and limits sample throughput. Test results often take weeks to obtain at a cost of hundreds of dollars per sample. These methods, while effective for the low volume testing associated with the first phase of site assessment, are relatively inefficient for the high volume testing required during mapping, remediation, and monitoring. Analysis of the larger number of samples collected under these circumstances by laboratory-based methods imposes a substantial time and expense penalty on environmental projects, due to sample throughput limitations. Properly applied field analytical methods, however, can reduce the time required to define the extent of site contamination, identify hot spots and clean areas, monitor the progress of remediation, and verify clean-up prior to closure. The use of field testing in conjunction with confirmatory laboratory-based testing can result in savings of both time and money, while still meeting project data quality objectives for each phase of a project where field testing is applied. To be effective, such methods need to be simple, compatible with ambient site conditions, inexpensive, and provide the ability to process large numbers of samples rapidly while producing data that correlates with reference methodology. Immunoassay-based field analytical methods possess these characteristics.

Immunoassay Technology

The immunoassay technique relies on a molecule referred to as an antibody that is developed to have a high degree of affinity for the target analyte. The high specificity and high affinity of the antibody is coupled with a very sensitive colorimetric reaction that provides

visualization of the result. All of this chemistry is accomplished with a small number of solutions that are applied to the processed sample or a dilution thereof. Either soil or water samples can be analyzed using immunoassays. Soil samples require a simple extraction step and subsequent filtration of the extractant, whereas water samples need only pH normalization and filtration. A wide range of analyte concentration in samples is accommodated through conventional serial dilutions. Extraction, normalization, and sample dilutions can all be preformatted for ease of use in the field.

The attributes that make immunoassay tests ideal for field screening include:

- immunoassay-based tests are extremely specific;
- they are accurate and precise;
- they are easy to use;
- immunoassay-based tests are rapid (<30 minutes);
- immunoassay-based tests are not significantly affected by the composition of the sample (soil or water) or the presence of other compounds.

Several studies have shown that results from immunoassay-based analytical tests correlate well with those obtained with accepted analytical reference methods [1,2]. For example, the results of a field trial application of an immunoassay-based soil test for petroleum hydrocarbons (EnSys PETRO RIS[®] Soil Test) are shown in Table 1.

Table 1
Field Test Trial with Gasoline Contaminated Soil

Sample ID	IR Method (ppm)	100 ppm Test		1000 ppm Test	
		Result	Evaluation	Result	Evaluation
AST-01	<20	< 100	•	< 1000	•
AST-02	520	≥ 100	•	≥ 1000	FP
AST-03	1700	≥ 100	•	≥ 1000	•
AST-04	130	≥ 100	•	< 1000	•
AST-05	20	≥ 100	FP	< 1000	•
AST-06	40	≥ 100	FP	< 1000	•
AST-07	400	≥ 100	•	< 1000	•
AST-08	640	≥ 100	•	< 1000	•
AST-09	1600	≥ 100	•	≥ 1000	•

• - Immunoassay and IR agree

FP - false positive

A construction equipment staging yard was found to be contaminated with gasoline. An above ground storage tank (AST) containing gasoline and a dispensing pump had previously been located on the property. As contaminated soil was removed from the ground using a backhoe, it was tested using the field method, and a split sample sent to the laboratory for analysis by the standard infrared (IR) method, EPA Method 418.1. High correlation between the IR method and the immunoassay-based field test was observed for these gasoline-contaminated soil samples. Overall, only three false positives results were recorded for eighteen analyses (83% correlation). The absence of a significant number of false negative results is a highly desirable trait for a field analytical method, while the appearance of a moderate number of false positives is not viewed as a disadvantage, because normally they would all be verified by further laboratory-based testing.

The EnSys PETRO RIS[®] Soil Test was used to evaluate the level of diesel fuel contamination in soils and sludges from a railroad soil and wastewater impound. Samples of contaminated soils and sludges were collected from a temporary on-site storage area, a manhole, and a wastewater retention pond. Each sample was split following homogenization for laboratory analysis by EPA Methods 8015 (diesel range extractibles by GC) and 418.1, as well as being analyzed on-site using the PETRO RIS[®] Soil Test.

The results reported for Method 8015 agreed conclusively with the field analysis for 10 of the 14 samples (71%) tested (Table 2). False positive results were recorded for the four results

that did not agree. The results of analysis by Method 418.1 agreed conclusively with the immunoassay-based field results for 10 of the 14 samples (71%). Three of the samples for which agreement was not obtained yielded false negative results. The remaining sample yielded a false positive result with the field test.

It is interesting to note that there was good agreement (within +100%, -50%) between the standard EPA methods for only six samples out of 14 (43%).

Table 2
Field Test Trial with Diesel Contaminated Soil

Sample ID	GC extractables (ppm)	IR hydrocarbons (ppm)	75 ppm Test			750 ppm Test		
			Result	Evaluation		Result	Evaluation	
				GC	IR		GC	IR
1-B	5720	20800	≥ 75	•	•	≥ 750	•	•
2-A	610	14700	≥ 75	•	•	≥ 750	FP	•
2-B	370	6800	≥ 75	•	•	≥ 750	FP	•
2-C	2270	1950	≥ 75	•	•	≥ 750	•	•
3-B	4870	18600	≥ 75	•	•	≥ 750	•	•
3-C	760	1180	≥ 75	•	•	< 750	FN*	FN
4-A	66	4100	≥ 75	FP*	•	< 750	•	FN
4-B	303	2100	≥ 75	•	•	< 750	•	FN
5-A	20400	29600	≥ 75	•	•	≥ 750	•	•
5-B	26300	28600	≥ 75	•	•	≥ 750	•	•
5-C	267	330	≥ 75	•	•	≥ 750	FP	FP
6-B	550	22700	≥ 75	•	•	≥ 750	FP	•
8	59300	64400	≥ 75	•	•	≥ 750	•	•
9	26500	12900	≥ 75	•	•	≥ 750	•	•

- - Immunoassay and GC or IR results agree
- FP - False positive
- FN - False negative
- FP* - False positive but within 25% of GC or IR results
- FN* - False negative but within 25% of GC or IR results

Finally, the U.S. Environmental Protection Agency (EPA) has evaluated and approved three immunoassay field methods for inclusion in their solid waste analytical methods manual [3]. EPA field screening methods for pentachlorophenol in soil and water (Method 4010), polychlorinated biphenyls in soil (Method 4020), petroleum hydrocarbons in soil (Method 4030), and polyaromatic hydrocarbons in soil (Method 4035) are now available.

Application of Immunoassay-Based Tests in the Field

Immunoassay-based field tests have numerous applications related to hazardous waste site characterization, remediation, and closure. One of the situations where field testing works to best advantage is where a single analyte is driving the assessment and clean-up process and many samples need to be collected and analyzed for this analyte to characterize its presence on a site. Frequently, site assessment of soil contamination is conducted to determine the extent in both the lateral and vertical directions. On a large site with non-uniform contamination, immunoassay field testing conducted at several concentration levels on samples collected on a systematic grid can be used to construct a map of contamination on the site. Such a map can be invaluable for determining the actual site boundaries, defining clean areas and hot spots, optimizing further sample collection, estimating the volume of contaminated soil, establishing a remediation approach, and accurately assessing remediation costs and timeframe. The specificity, high sensitivity and rapid throughput of immunoassay-based field tests give them a distinct advantage in this application.

The extensive use of field analytical tests in site characterization can actually result in an

increased level of information about the site with no sacrifice in data quality or increase in cost. Although many view the quality of data obtained using field test methods with mild suspicion, the application of a properly designed QA/QC plan can ensure that data obtained in the field meet project data quality objectives. A QA/QC plan for field analysis usually contains a component that relates to the application of quality control in the field in the form of duplicate analyses, blanks, performance evaluation sample testing, etc., and a component that specifies confirmatory laboratory testing (QA) of a portion of the samples analyzed in the field.

The cost savings associated with immunoassay-based field testing arise from two sources. First, the analysis of samples in the field is usually more inexpensive on a direct basis than laboratory-based analysis. Savings typically range from 10% to 50%, depending on the project. But a more important economic benefit of field testing results from accomplishing site assessment work in one trip to the site, whereas the delays inherent in conventional analytical laboratory-based testing require multiple trips on all but the smallest projects. The need for resampling due to lost samples and missed holding times and the need for more samples to further characterize hot spots and unexpected contamination all result in the mobilization of personnel and equipment that is unnecessary when data are acquired in "real-time" through the use of immunoassay-based field testing.

Field testing can provide cost-effective, timely in-process evaluation of soil as it is being excavated and removed or remediated. The expense of treatment or removal of soil can be minimized with the application of timely monitoring. The need for monitoring is particularly acute for soil contaminated with compounds like pentachlorophenol and PCBs, which are extremely costly to clean up. The economic incentive for cleaning or removing only soil that is contaminated above the action level is great. The high specificity of immunoassay-based tests leads to few false negative results, making them a valuable tool for remediation monitoring.

Once contaminated soil has been cleaned or removed, field tests can be used to verify the effectiveness of the remediation phase of the project and guide the collection of samples required for closure permitting purposes.

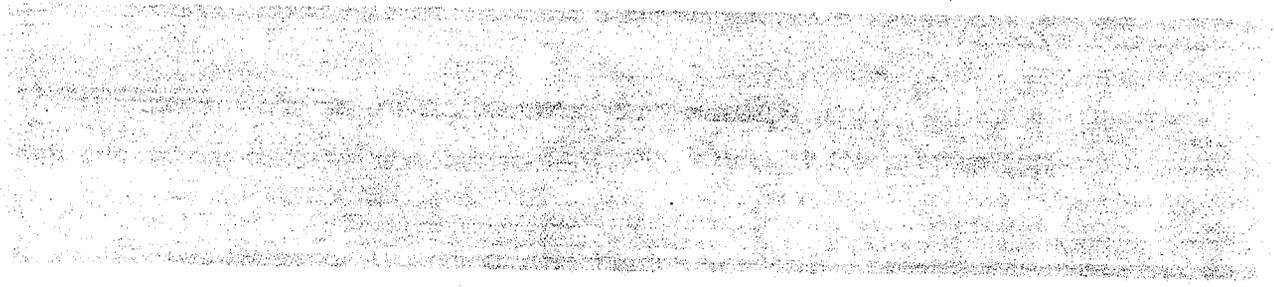
Conclusions

Field analytical methods based on immunoassay technology possess the characteristics necessary to provide valid on-site data for hazardous waste site assessment, remediation monitoring, and clean-up verification prior to closure. They have proven to correlate well with accepted reference analytical methodology for the on-site testing of soil and water and have been approved by the U.S. EPA. The use of immunoassay-based field tests can result in significant savings of time and money with no sacrifice in data quality.

References

- [1] van Emon J. M. and Lopez-Avila V.: Immunochemical Methods for Environmental Analysis. *Analytical Chemistry* 64 pp.79-88 (1992).
- [2] Mapes J. P., McKenzie K. D., McClelland L. R., Movassaghi S., Reddy R. A., Allen R. L., Friedman S. B., Penta RISC Soil - A Rapid, On-Site Screening Test for Pentachlorophenol in *Soil. Bull. Environ. Contam. Toxicol.* 49 p.334 (1992).
- [3] Office of Solid Waste, United States Environmental Protection Agency. Test Methods for Evaluating Solid Waste, SW-846, 3rd edition, (1986).

**SESSION V ENVIRONMENTAL AND BIOLOGICAL FATE OF
AGENTS AND THEIR DEGRADATION PRODUCTS**



ENVIRONMENTAL CHEMISTRY OF CHEMICAL WARFARE AGENTS

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INTRODUCTION

This paper summarizes the approach used in the preparation of a Handbook for the Corps of Engineers, Huntsville Division, on the environmental chemistry of chemical warfare agents. The agents GB and HD will be used to illustrate the type of information in the report. Those readers interested in the full report should contact Mr. Arkie Fanning, Huntsville Corps of Engineers at (505) 955-5256.

The U.S. Army Corps of Engineers (ACE) has identified approximately 7,200 formerly used defense sites (FUDS) in the United States, some of which are suspected to be contaminated with chemical warfare agents (CWA). The ACE has responsibility for environmental clean-up of FUDS, including site characterization, evaluation and remediation of the site. Thirty-four FUDS and 48 active DOD installations that may contain CWA were identified in an Interim Survey and Analysis Report by the USACMDA Program Manager for Non-Stockpile Chemical Material (NSCM). The chemical agents listed include sulfur mustard (H), lewisite (L), tabun (GA), sarin (GB), VX, hydrogen cyanide (AC), cyanogen chloride (CK), phosgene (CG), BZ, and CS^{1,2}.

ORGANOPHOSPHORUS AGENTS

The organophosphorus (OP) agents were first developed by German scientists prior to World War II. Biologically speaking, the OP agents act by inhibiting acetylcholinesterase (AChE), a critical chemical link in the transmission of nerve impulses. The first OP selected by the German scientists as a chemical warfare agent was Tabun (GA), ethyl N,N-dimethylphosphoramino-cyanidate. GA was followed in rapid succession by the more toxic OPs Sarin (GB), isopropyl methylphosphonofluoridate, and Soman (GD), pinacolyl methylphosphonofluoridate³.

GB is a colorless or amber, odorless liquid soluble in water and many organic solvents. GB is very volatile with a vapor pressure of 2.9 mm Hg at 25 °C. GB vapors are readily absorbed by most materials; however, the high volatility results in almost complete desorption in a few hours after removal from the source. This ready uptake and release presents a potential hazard if contaminated materials are placed in closed spaces where lethal concentrations could build up.

Hydrolysis of the fluorophosphonates like GB occurs first through the loss of a fluorine, then more slowly through the loss of the alkoxy group. The hydrolysis products, the corresponding phosphoric acids, are generally non-toxic. The hydrolysis pathways for GB and other phosphonofluoridates are shown in Figure 1.

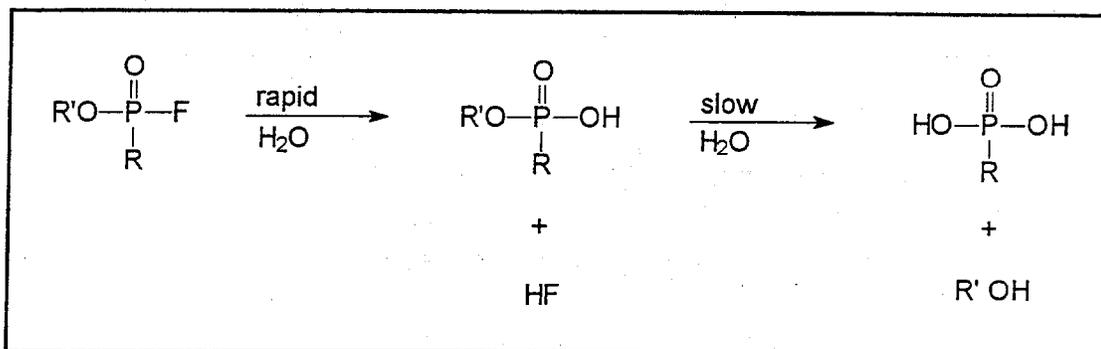


Figure 1. Hydrolysis Pathways for GD and GB.

In most reported studies on the decomposition of GB in soils, the parent and the primary and secondary hydrolysis products, O-isopropyl methyl phosphonate and methylphosphonic acid, were identified. In addition, a GB impurity, diisopropyl methyl phosphonate has also been found⁴. The hydrolysis products are acids or, in alkaline media, the corresponding salts. While the original phosphonofluoridates can be extracted into organic solvents for analysis, the byproducts are much more hydrophilic and are not quantitatively extractable.

The hydrolysis rate of GB is a function of the temperature and pH of the medium, with the rate being a minimum between 4 and 6. Figure 2 illustrates the effect of pH on the hydrolysis half-life of GB over the pH range of 2 to 10, as reported by several investigators⁵⁻¹⁵. It must be noted that $t_{1/2}$ is the time to achieve 50% decomposition of the agent. It would take approximately 6.6 times the $t_{1/2}$ for 99% reduction of the parent compound. Figure 5 illustrates the time necessary ($20 \times t_{1/2}$) to achieve a 1×10^6 reduction of GB as a result of hydrolysis. This would result in the neat agent being degraded to below 1 ppm.

For unbuffered systems above pH 6, the GB hydrolysis reaction may be self-limiting due to the production of isopropyl methylphosphonic acid ($\text{pK}_a = 1.96$) and HF ($\text{pK}_a = 3.14$), both weak acids, which will reduce the pH into the 4-6 range where the hydrolysis is at a minimum. Below the neutral region, the reaction will be accelerated by the production of these acid byproducts and the resulting lowered pH of the system^{6,16}. Shih and Ellin¹⁷ measured the production of acid from the hydrolysis of GB and showed that in unbuffered systems the initial agent concentration will affect the hydrolysis rate. They were able to predict the concentration of GB remaining by measuring the pH of the final solution.

The other two important variables in the hydrolysis rate of GB are temperature and the type and concentration of dissolved ionic species. Figure 3 illustrates the effect of temperature on GB half-life for solutions with a pH of 7.0. Epstein⁶ derived the following equation to predict the half-life as a function of pH and temperature:

$$\log t_{1/2} = \frac{5039}{T(^{\circ}\text{K})} - 8.035 - \text{pH}$$

There is approximately a four-fold increase in the hydrolysis rate per 10° increase in temperature in more basic solutions ($> \text{pH } 6.5$), and a two-fold increase in acidic solutions ($< \text{pH } 4$)⁶.

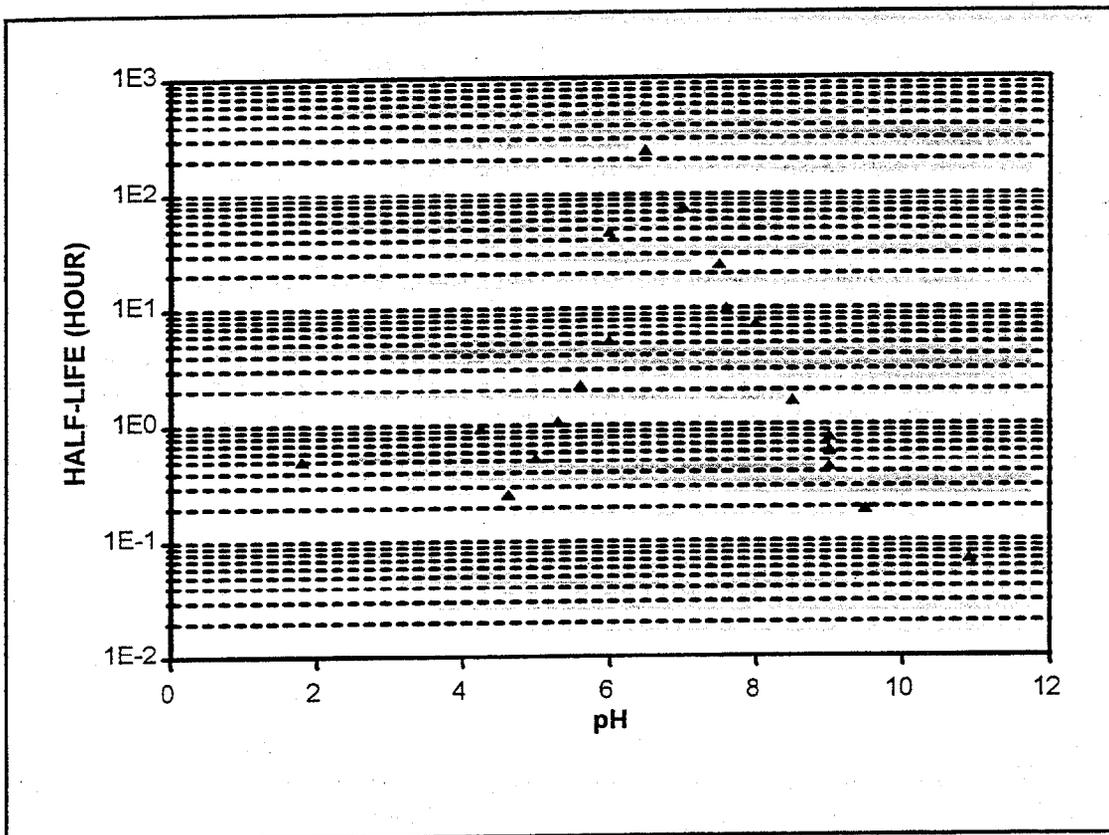


Figure 2. GB Hydrolysis VS pH

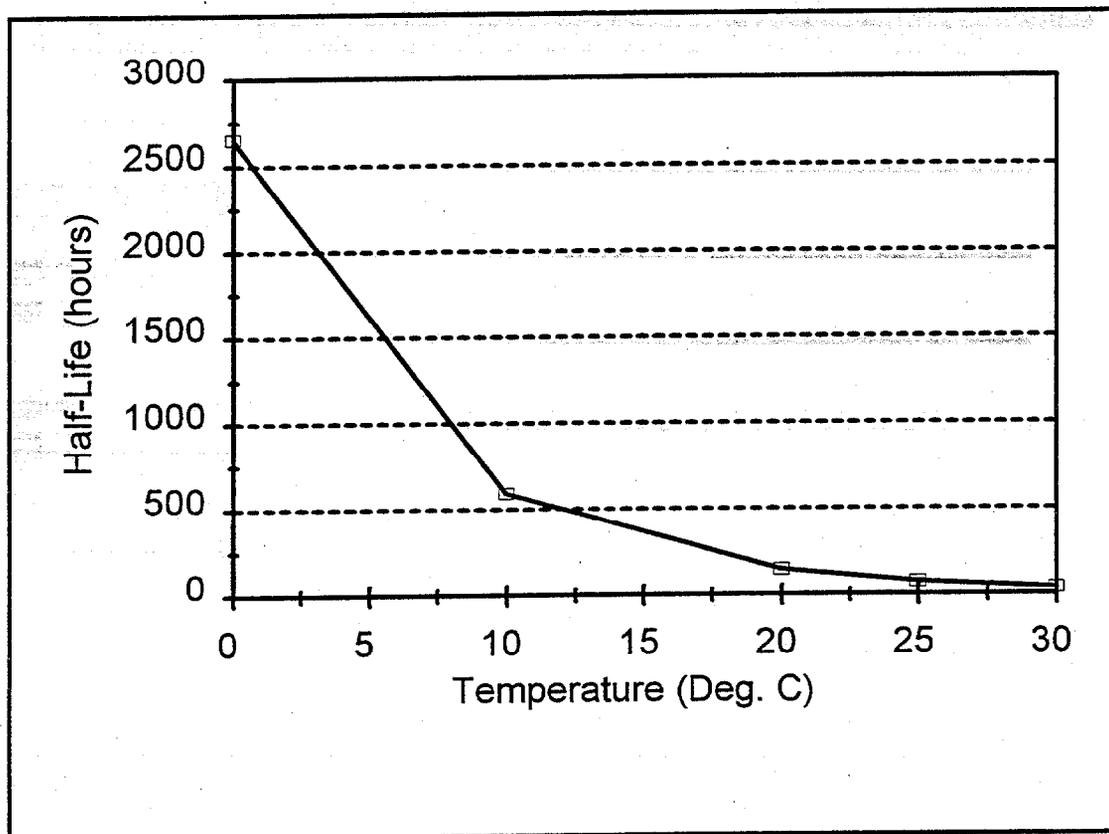


Figure 3 Effect of Temperature on GB Half-Life

Many metals such as magnesium, copper, cobalt, manganese, cerium, aluminum and calcium have the ability to accelerate the hydrolysis of GB¹⁵. The impact of dissolved constituents is complicated by the effect of pH on the hydrolysis of the metals that catalyze the hydrolytic decomposition of GB. Interestingly, Epstein^{6,7} determined that the hydrolyzed metal cation (MeOH⁺) catalyzes the reaction rather than the unhydrolyzed, free metal species (Me⁺⁺). It is postulated that this results from the electrophilic reaction of the metal hydroxo ion, which indirectly catalyzes the nucleophilic attack of hydroxide ion on the P atom, increasing the polarity of the fluorophosphonate¹⁵.

For the three divalent metals investigated by Epstein, the order of catalytic effectiveness copper, manganese and magnesium, reflects the order of their first hydrolysis products (pK₁) of 8, 10.6 and 11.4, respectively. At pH values below the pK₁, the unhydrolyzed species (Me⁺⁺) predominates; and at pH values above the pK₁, the majority of the total concentration of the metal exists as the hydrolyzed species (MeOH⁺). This has also been observed with magnesium and calcium at very high pHs, and uranyl ion in dilute solutions at low pHs¹⁸. The effect of copper on the half-life of GB at pHs below 7 can be estimated by the following equation⁶:

$$t_{1/2} = \frac{5.7 \times 10^3 (H^+)}{(Cu_{tot})}$$

Above pH 7, the low solubility of copper hydroxide reduces the catalytic effect to a level where it is probably insignificant compared to the effect of pH alone. The effect of trace dissolved ions can also be seen by comparing the hydrolysis rate in seawater and distilled water. At pH 7.9 in seawater, t_{1/2} was 0.4 hour compared to approximately 7.5 hours at pH 8.0 in distilled water⁵. Particularly in groundwater systems, the presence of metals such as iron may participate in catalytic reactions and accelerate the hydrolysis of the organophosphorous agents. This catalytic effect is also observed with metal-organo chelates. This effect has been exploited for detoxification, particularly for protecting the skin¹⁵.

MUSTARD AGENTS

The sulfur mustard family of vesicants was one of the first first chemical warfare materials used in World War I. In the US and England, the bis(2-chloroethyl)thioether or bis(chloroethyl)sulfide is called "mustard gas" because of its odor. After its use near Ypres France, it was identified as "Yperite" in some early publications. Commercial sulfur mustard (H) is normally a mixture of a large number of homologs with a ClC₂H₄S- moiety, which is more toxic than distilled mustard (HD). Oxygen mustard (T), 1,2 bis(chloroethylthio)ether, is the other important sulfur mustard agent identified as a possible contaminant at FUDS¹. HT is a mixture of 60% HD and 40% T, has a greater persistence, lower freezing point and is more stable than HD⁹.

Pure sulfur mustard is a colorless, odorless, oily liquid; however, the commercial product has a yellow/brown color with a sweet odor due to contaminants¹⁵. Heating mustard to its boiling point results in thermal degradation and the formation of products with a strong garlic and mustard odor. HD is classified as a persistent agent due to its low vapor pressure (0.165 mm Hg, 25 °C). HD has a low solubility in aqueous solutions, but it dissolves readily in organic solvents. Mustards can penetrate into many materials including rubbers, plastics, wood and concrete and still retain their toxic properties. Therefore, unless decontaminants can penetrate into the materials, the hazard of vapor exposures and skin contact will still remain.

Because of the low aqueous solubility of mustards, the rate of hydrolysis is dependent on the rate of solvation. This will be controlled by the surface area exposed to the solvent which in turn is a function of particle size and turbulence. Complicating the picture is the tendency, in quiescent

conditions, of mustards to polymerize at the mustard/solution interface, further shielding the bulk of the agent from hydrolysis reactions. Experience has demonstrated that mustards can remain stable under water for years if there is little turbulence or mixing^{15,19,20}. Small²¹ calculated it took 867 hours for a 1-cm diameter HD droplet in quiescent water at 18 °C to decrease by one half. Demek, et al.²² determined that the dissolution rate for HD in flowing seawater would be approximately ten times as high as in quiescent seawater. The effect of temperature on the solution rate (S) of mustard in distilled water can be calculated from the following equation²³.

$$S = 233.7 \times e^{-\frac{6215}{T}}$$

The hydrolysis of H is pH dependent, with reversible reactions taking place in acidic solutions and decomposition accelerated in neutral and basic mediums. The hydrolysis of mustard takes place in two stages¹⁵. In the first stage, a heterocyclic sulfonium cation is formed (Figure 4). These onium compounds are highly reactive, and their interaction with enzymes, DNA and proteins is the basis for the skin toxicity of mustards²⁰. As illustrated in Figure 5, in the second stage the sulfonium cation initiates a series of reactions between water and intermediate products.

Formation of the first intermediate is the rate-limiting step in hydrolysis; however, the overall decomposition is still controlled by the dissolution rate. Anything that increases the solution rate, i.e., mixing or addition of a cosolvent such as alcohol or acetone, will increase the apparent hydrolysis rate. The maximum rate of hydrolysis is reported to be 104 mg/min/L at 25 °C for mustard gas in equilibrium with water¹².

The rate of HD hydrolysis is difficult to predict due to the dependence on the rate of solvation. Although mustard in solution can undergo a relatively rapid hydrolysis, the slow solvation can inhibit the observed decomposition in natural environments. The relative amounts of water also affect the distribution of the hydrolysis byproducts. In dilute aqueous solutions, thiodiglycol (TDG) is the dominant byproduct; whereas, in cases of limited water, the TDG reacts with the intermediates to form the toxic intermediates HD-TDG, HD-2TDG and CH-TDG²³. In the absence of sufficient mixing, the TDG formed would concentrate at the mustard/water interface and form sulfonium salts with the dissolving mustard. These products and the other oligomers would shield the bulk mustard and reduce solvation²⁰. These linear polymers are biologically active and display much of the same vesicant properties as HD.

There is significant variation in the reported hydrolysis rates of mustard in aqueous solution, as would be expected with the dependence on the mixing rate and concentration. In his review, Small²¹ reported half-lives from 7.4 to 15.8 minutes for 20 °C, and from 3.9 to 8 minutes for 25 °C. Sanchez, et al.¹⁸ summarized half-life data versus temperature from Ward and Seiders²⁴ and from Finnish studies for both the first and second hydrolysis products. At 21 °C, the $t_{1/2}$ for the first hydrolysis product was 6 minutes and for the second hydrolysis product it was 15 minutes. Figure 6 shows the half-life versus temperature as reported by Franke¹⁴. Sides, et al.²⁵ reported a half-life for HD of 4.9 minutes at pH 9.5 at 25 °C.

As would be expected from Figure 4, increased chloride inhibits the formation of the sulfonium cation. This effect of chloride on the hydrolysis rate was investigated by Bartlett and Swain²⁶, who showed that the intermediate sulfonium ion preferably reacts with a nucleophilic reagent such as Cl⁻ to reform mustard. This is observed as an apparent reduction in the hydrolysis rate. Thus, the calculated hydrolysis rate in freshwater is 2.5 times faster than in seawater²³.

Two other common products that have been identified on surfaces and groundwater at Rocky Mountain Arsenal are 1,4-dithiane and 1,4-oxathiane¹⁹. These are formed due to the dechlorination of mustard and the half-mustard. 1,4-dithiane is a thermal degradation product, and 1,4-oxathiane is a principal contaminant found on concrete contaminated with H. The half-life for 1,4-oxathiane is reported to be 1,747 hours¹⁹. The compounds of environmental interest for mustard are listed in Table 1.

SOIL SYSTEMS

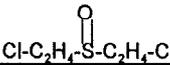
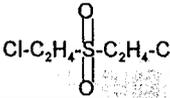
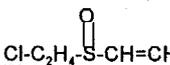
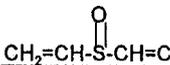
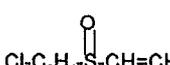
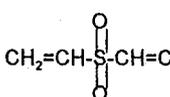
The fate and migration of chemical agents and their decomposition products in the subsurface soil environment will be controlled by many factors, including the chemical properties and concentration of the agent, time since the agents were buried, presence of other chemicals such as decontaminants, depth, temperature cycles, location of groundwater table, annual precipitation, soil type, pH, trace inorganic constituents, organic content, and microbial populations. Because each site will have a unique combination of these factors, and most of the required information for environmental fate models will not exist, predictions will, at best, be rough approximations.

Soil environments, except in arid regions, can be expected to have relative humidities in excess of 90% and, therefore, most chemical agents can be expected to undergo hydrolysis reactions. While data are generally available on the hydrolysis rates of chemical agents in pure water, these data are not appropriate for the heterogeneous and diverse soil environment. The data on the fate of chemical agents in soil are generally limited to studies that investigated the fate of agents on the ground surface or in "closed-containers." These studies were designed to understand the persistence of agents applied under realistic wartime situations, and have minimal relevancy to understanding the fate of agents buried several meters below ground. In addition, the relative agent-to-soil ratios studied have been in the range of mg per g of soil, rather than the high concentrations that might be expected for burial sites of partially decontaminated munitions.

Studies on the decomposition of GB on γ -alumina were done by Kuiper, et al²⁷. GB was strongly adsorbed to the γ -alumina and the hydrolysis was promoted by the basic surface sites. The hydrolysis product was identified as isopropyl methylphosphonic acid. The half-life of GB adsorbed to soil was reported to be 4 hours (20 °C)²⁸. Using a dynamic flow system, moist air was passed over soil contaminated with GB, and 15% of the GB was hydrolyzed after 20 minutes. Assuming the pH of the soil was 6-7, this is considerably less than the 170 hours reported by Epstein⁶.

No studies were located that addressed the decomposition rate of HD in soil. The majority of studies involving the fate of HD in soil systems was focused on tactical issues concerned with persistence²⁹⁻³¹. At the soil surface, the predominant mechanism controlling the persistence of HD is evaporation, which it is expected to be minimal for subsurface contamination or for agents inside partially ruptured munitions. The role of vaporization as a mechanism of migration through soils is unknown; however, it has been observed that clays are unsuccessful as a barrier to HD vapor²³. Although HD dissolved in water readily hydrolyzes, numerous instances of spilled or buried HD remaining intact over many tens of years have been reported²¹. As noted earlier, unless there is adequate mixing, TDG, HD

TABLE 1. ENVIRONMENTAL CHEMISTRY OF MUSTARD

Symbol	Name	Structure	Reg #	Source
HD	Sulfur mustard	Cl-C H -S-C H -Cl	505-60-2	Agent
CH	Hemi-mustard	Cl-C H -S-C H -OH	693-30-1	Hydrolysis
HT	2,2-Bis(2-Chloroethyl thioethyl) ether	Cl-C H -S-C H -O-C H -S-C H -Cl	63918-89-8	Agent
TDG	Thiodiglycol	HO-C H -S-C H -OH	111-48-8	Hydrolysis
CVS	2-Chloroethyl vinyl sulfide	Cl-C H -S-CH=CH	81142-02-1	Dechlorination of HD
DVS	Divinyl sulfide	CH =CH-S-CH=CH	627-51-0	Dechlorination of HD
HO	Mustard sulfoxide	 Cl-C ₂ H ₄ -S-C ₂ H ₄ -Cl	5819-08-9	Oxidation of HD
HO ₂	Mustard sulfone	 Cl-C ₂ H ₄ -S-C ₂ H ₄ -Cl	471-03-4	Oxidation of HD
CVSO	2-Chloroethyl vinyl sulfoxide	 Cl-C ₂ H ₄ -S-CH=CH ₂	40709-82-8	Dechlorination of HD
DVSO	Divinyl sulfoxide	 CH ₂ =CH-S-CH=CH ₂	1115-15-7	Dechlorination of HD
HVS	2-Hydroxyethyl vinyl sulfide	HO-C H -S-CH=CH	3090-56-0	Dechlorination of CH
CVSO ₂	2-Chloroethyl vinyl sulfone	 Cl-C ₂ H ₄ -S-CH=CH ₂	7327-58-4	Dechlorination of HO
DVSO ₂	Divinyl sulfone	 CH ₂ =CH-S-CH=CH ₂	77-77-0	Dechlorination of HO
HD-TDG	Bis(2-hydroxyethyl)-2-(2-chloroethylthio) ethyl-sulfonium	Cl-C ₂ H ₄ -S-C ₂ H ₄ -S ⁺ -(C ₂ H ₄ OH) ₂	64036-91-5	Hydrolysis of HD
HD-2TDG	Bis-2-(bis(2-hydroxyethyl)-sulfonium ethyl) sulfide	S-C ₂ H ₄ -S ⁺ -(C ₂ H ₄ OH) ₂ -C ₂ H ₄ -S ⁺ -(C ₂ H ₄ OH) ₂	64036-79-9	Hydrolysis of HD
CH-TDG	Bis(2-hydroxyethyl)-2-(2-hydroxyethylthio) ethyl-sulfonium chloride	HO-C ₂ H ₄ -S-C ₂ H ₄ -S ⁺ -(C ₂ H ₄ OH) ₂	107327-27-5	Hydrolysis of HD
DT	1,4-Dithiane	S-C H -S-C H -	505-29-3	Thermal
HDLP	HD Linear polymer	Cl-C H -(S-C H) -S-C H Cl		

polymers and/or other TDG-sulfonium salts would concentrate at the surface of HD and inhibit the dissolution of HD and subsequent hydrolysis. Particularly in soil systems where water slowly diffuses through the soil matrix, hydrolysis of bulk HD would be expected to be reduced. For situations where the HD is not in droplets, but dispersed or absorbed by the soil, hydrolysis would be expected to proceed if there is sufficient water present.

The intermediates and byproducts of HD hydrolysis are water soluble and, therefore, would be expected to migrate away from the initial site of contamination. Since many of the intermediates such as CH hydrolyze at a faster rate than HD, their presence at a site would indicate the presence of HD²¹.

BIODEGRADATION

Bacterial degradation of organic compounds is one of the most important mechanisms for the natural attenuation of pollutants in soil and aquatic systems. Microbial metabolism of pesticides is well documented, including those pesticides such as parathion that have structures similar to the organophosphorus agents³².

Actual data on the microbial degradation of CWAs are limited¹⁹. Mustards are cell poisons and would, therefore, be expected to inhibit bacterial growth. If bacterial degradation or oxidation did occur through exoenzymes, it would be a minor factor compared to other oxidative and/or hydrolytic reactions.

Organophosphonates have been shown to undergo complete metabolisms to alcohol, alkane and phosphate^{33,34}. *Pseudomonas testosteroni* degraded O-alkyl alkylphosphonates, such as sarin and soman, by first cleaving the alkoxy group to yield the alcohol and divalent alkylphosphonate. The latter is further degraded to yield the alkane and inorganic orthophosphate. This degradation occurred under aerobic conditions only when the agents were the sole and limiting phosphorous source. This was the first report of microbial cleavage of the C-P bond, and the same organism could not break other carbon-heteroatoms such as arsenates, sulfonates and mercurials. In addition, the organism was not able to degrade an alkylphosphonothioate such as VX.

In another study, Cook, et al.³⁵ evaluated the ability of *Pseudomonas*, isolated from soils and sewage, to degrade phosphonates. They found that multiple strains of *Pseudomonas* could use 13 ionic alkylphosphonates or O,O-dialkylphosphonates, including MPA and the sodium salt of IMPA as a phosphorous source, but only 2-aminoethylphosphonic could be used as a carbon source.

Daughton, et al.³⁴ investigated the microbial degradation and soil retention of MPA and other O-alkylmethylphosphonic acid esters. The soils of interest were a Spodosol (pH 3.9, 35% organic) a silty loam (pH 6.5, 5.5% organic) and three clays. As would be expected from the high organic content, the Spodosol retained 95.4% of the MPA in solution, 42% of IMPA and 32% of PMPA. The silty loam only retained 11% of MPA in solution. The other alkylphosphonates and thiophosphates were not bound to the Spodosol. The observed retention of MPA is higher than would be expected from simple adsorption to the organic matter in the soil based on the K_{oc} of 1.4²¹.

When Spodosol was added to cultures of *P. testosteroni* the Spodosol inhibited the microbe's ability to use inorganic phosphorous and MPA as P sources. The Spodosol did not effect the degradation of IMPA, however. This inhibition reflected the ability of the soil to strongly bind inorganic phosphate and MPA, but not IMPA, and make them inaccessible for microbial growth. It was observed that these products of organophosphorus hydrolysis would normally be accessible to microbial degradation, since they are water soluble. Since the phosphorous in phosphonates is only used if other more readily available P is not available, if inorganic phosphate is prevalent, little degradation would be

predicted. However, where P is limiting, nutrient degradation of phosphonates may occur through bacterial action.

ENVIRONMENTAL FATE

Prediction of the environmental fate of buried CWA is complicated by a general lack of knowledge on if, or how, the agents were decontaminated, the configuration of the agent/munitions and the site-specific subsurface geology and hydrogeology. Although it is not possible to make definitive predictions of the form and migration of buried agents without detailed information on past activities and site-specific information there are numerous chemical and physical properties and calculated parameters that are useful in predicting the relative behavior of chemical compounds in the environment. These properties and parameters can be used with other data gathered during preliminary assessments in planning remedial investigations and prioritizing sites for remediation. The key properties associated with predicting the chemical fate of organic chemicals include water solubility, vapor pressure, octanol-water partition coefficient, and half-life.

The chemical and physical properties of some of the agents and their decomposition products are shown in Table 2. Calculated environmental transport properties are shown in Table 3, also modified from Small²¹. Although many of the physical properties and environmental transport indices are estimated from empirical substituent relationships from Hansch and Leo³⁶ and Lyman, et al.,³⁷ they can be used to assess the relative effects of environmental factors on the fate of the agents and their decomposition products at small burial sites. The use of these estimated factors is illustrated below. A computer search was made of numerous databases including Chemical Abstracts, Beilstein, and the National Library of Medicine Hazardous Substances Data Base. For most compounds, there were little or no data of environmental significance.

It is evident from a review of these tables that the hydrolysis of the agents in a soil system will markedly effect their environmental transport. If not hydrolyzed immediately, GB with a relatively high water solubility and medium log K_{ow} and K_{oc} , would be leached by precipitation or transported by groundwater as would their degradation products. The hydrolysis of GB would reduce the potential for vapor migration and/or evaporation as indicated by the lower volatility potential (VP) and air-soil partition coefficients (R_o).

HD, for instance, has a log K_{ow} of 1.37 and K_{oc} of 133, indicating that it would be much more attracted to soil organics and clays than would the hydrolysis products, which all have much lower K_{ow} and K_{oc} values. This is also reflected in the retardation factors. For example, HD would be expected to take over 6 times longer than TDG to migrate the same distance through soil. The opposite holds true for volatility. TDG has a significantly lower vapor pressure (P_v) and volatility potential. In low moisture soils, such as found in arid regions, HD may migrate as vapor through the soil interstices.

TABLE 2. CHEMICAL AND PHYSICAL PROPERTIES OF AGENTS AND AGENT DECOMPOSITION PRODUCTS
(Modified From Small, 1984)

Compound Name or Abbreviation	log K _{ow}	C _{sol} mg/L	K _{oc}	P _o mm Hg	MW
GB	0.72	>>	59	2.9	140
IMPA	-0.54	4.8 X 10 ⁴	12	3.4 X 10 ⁻³	140
HD	1.37	1.0 X 10 ³	133	1.0 X 10 ⁻¹	159
TDG	-0.77	7.8 X 10 ⁴	9.1	1.9 X 10 ⁻⁵	118
CVS	1.11	1.4 X 10 ³	96	5.8	122.5
DVS	0.85	2.5 X 10 ³	69	6.0 X 10 ¹	86
HO	-0.85	9.3 X 10 ⁴	8.2	6.5 X 10 ⁻¹	175
HO ₂	-0.51	1.1 X 10 ⁴	13	9.6 X 10 ⁻¹	191
CVSO	-1.11	1.6 X 10 ⁵	5.9	6.4 X 10 ⁻²	138.5
DVSO	-1.37	2.8 X 10 ⁵	4.3	9.2 X 10 ⁻¹	102
HVS	0.53	5.0 X 10 ³	46	3.8	102
CVSO ₂	-0.77	7.8 X 10 ⁴	9.1	2.3 X 10 ⁻²	154.5
DVSO ₂	-1.03	1.4 X 10 ⁵	6.6	9.0 X 10 ⁻²	118
HD-TDG	Unstable				
HD-2TDG	Unstable				
CH-TDG	Unstable				
DT	0.77	3 X 10 ³	63	8.0 X 10 ⁻¹	120
OT	-0.26	2.7 X 10 ⁴	17		

TABLE 3. CALCULATED ENVIRONMENTAL TRANSPORT INDICES FOR AGENTS AND AGENT DECOMPOSITION PRODUCTS
(Modified From Small, 1984)

Compound Name or Acronym	LI	VP	R	H	R _o
GB	3.7	4.9 X 10 ⁻⁸	5.1	5.4 X 10 ⁻⁷	1.3 X 10 ⁻⁵
IMPA	1.4	5.8 X 10 ⁻⁹	1.8	1.3 X 10 ⁻⁸	1.6 X 10 ⁻⁶
HD	7.2	7.6 X 10 ⁻⁷	10	2.1 X 10 ⁻⁵	2.3 X 10 ⁻⁴
TDG	1.2	2.1 X 10 ⁻¹²	1.6	3.0 X 10 ⁻¹²	4.8 X 10 ⁻¹⁰
CVS	5.5	4.2 X 10 ⁻⁵	7.7	6.5 X 10 ⁻¹	9.9 X 10 ⁻³
DVS	4.2	3.5 X 10 ⁻⁴	5.8	2.7 X 10 ⁻³	5.8 X 10 ⁻²
HO	1.2	8.5 X 10 ⁻⁷	1.6	1.6 X 10 ⁻⁶	2.9 X 10 ⁴
HO ₂	1.9	6.9 X 10 ⁻⁶	1.9	2.2 X 10 ⁻⁵	2.5 X 10 ⁻³
CVSO	1	6.8 X 10 ⁻⁸	1.4	7.3 X 10 ⁻⁸	1.8 X 10 ⁻⁵
DVSO	0.91	7.7 X 10 ⁻⁷	1.3	1.0 X 10 ⁻⁷	1.5 X 10 ⁻⁴
HVS	3.1	1.7 X 10 ⁻⁵	4.2	1.0 X 10 ⁻⁴	3.3 X 10 ⁻³
CVSO ₂	1.2	3.2 X 10 ⁻⁸	1.6	6.0 X 10 ⁻⁸	9.5 X 10 ⁻⁶
DVSO ₂	1.1	9.7 X 10 ⁻⁸	1.5	1.0 X 10 ⁻⁷	2.2 X 10 ⁻⁵
HD-TDG					
HD-2TDG					
CH-TDG					
DT	3.9	4.3 X 10 ⁻⁶	5.4	4.2 X 10 ⁻⁵	9.9 X 10 ⁻⁴
OT					

SUMMARY

The significant potential for chemical warfare agent contamination at FUDS, and on active DoD installations, makes it important that USA professionals involved with site assessment and remediation be aware of the environmental chemistry of the chemical agents and their degradation products. This report summarizes data on the fate and transport of these compounds from the professional chemistry and environmental literature, USA technical reports, and some foreign documents. This report this paper summarizes was written for use by non-chemists in planning and executing the cleanup of these contaminated sites. Those environmental characteristics of a site that may affect the level or the extent of contamination were analyzed for the agents of interest. Trends are displayed in graphs for ease of use, since trends and relative rates are more relevant in extrapolating laboratory data to field situations. The reader is referred to the original references for more detailed information.

There are many gaps in the chemical and environmental data, particularly for decomposition products. Especially lacking are field data from actual contaminated sites. As the USA non-stockpile chemical materiel program generates such data, it should be used to update the information in this document.

REFERENCES

1. USACMDA, Program Manager for Non-Stockpile Chemical Materiel, "Non-Stockpile Chemical Materiel: Survey and Analysis Report," November 1993a.
2. USACMDA, Program Manager for Non-Stockpile Chemical Materiel, "Site Monitoring Concept Plan," June 1993b.
3. Siegfried, Major F., Textbook of Military Chemistry, Volume 1, USAMIIA-HT-039-82, 1977.
4. Agency for Toxic Substances and Disease Registry (ASTDR), "Health Assessment for Rocky Mountain Arsenal, Commerce City, Adams County, Colorado, Region 8," NTIS PB90-118258, 1988.
5. Epstein, J., "Rate of Decomposition of GB in Seawater," *Science*, Vol. 170, pp. 1396-1397, 1970.
6. Epstein J., "Properties of GB in Water," *Journal of American Waterworks Assoc.*, 66, pp. 31-37, 1974.
7. Epstein, J., and Mosher, W.A. "Magnesium Ion Catalysis of Isopropyl Methylphosphonfluoride. The Charge Effect in Metal Ion Catalysis," *Jor. Phys. Chem.*, Vol. 72(2), pp. 622-625, 1968
8. Epstein, J., V.E. Bauer, M. Saxe, and M.M. Demek, "The Chlorine-catalyzed Hydrolysis of Isopropyl Methylphosphonofluoridate (Sarin) in Aqueous Solution," *Journal of the American Chemical Society*, 78, pp. 4068-4071, 1956.
9. Wolinski, J., and K. Sawicki, *Roczniki Chemii*, Vol. 38, p. 745, 1964.
10. Department of the Army (DA), "Military Chemistry and Chemical Compounds," FM 3-9, 1975.
11. Britton, K.B., "Low Temperature Effects on Sorption, Hydrolysis and Photolysis of Organophosphates," USA COE SP-86-38, AD B114618, 1986.
12. Forsman, N., H. Frostling, O. Hertzberg, B. Jansson, L. Larsson, J. Lundin, A. Meyerhoffer, G. Persson, J. Santesson, B. Sorbo, B. and Ostman, "C-Weapons (Characteristics and Defense)," USAMIIA-HT-010-79, USA Medical Intelligence and Information Agency, Fort Detrick, MD, AD AO5722, 1979.
13. Larsson, L., "The alkaline hydrolysis of isopropoxy-methyl-phosphoryl fluoride (sarin) and some analogues," *Acta Chem. Scand.*, Vol. 11(7), pp. 1131-1142, 1957.
14. Larsson, L., "The alkaline hydrolysis of two sarin analogues and of tabun," *Acta Chem. Scand.* Vol. 12(4) pp. 783-785, 1958.
15. Franke, S., Textbook of Military Chemistry Volume I, USAMIIA-HT-039-82, AD B062913, 1982.
16. Buckles, L.C., "The Hydrolysis Rates of G Agents," TCIR 393, AD-B966 236, AD B966236, 1947.

17. Shih, M.L. and Ellin, R.I.; "Stability of Aqueous Solutions of Sarin and Soman: Influence of Concentration and an Equation for Determining Concentration," *Bull. Environ. Contam. Toxicol.*, Vol. 3, pp. 1-5, 1984.
18. Epstein, J. and D.H. Rosenblatt, "Kinetics of Some Metal Ion-catalyzed Hydrolyses of Isopropyl Methylphosphonofluoridate (GB) at 25°", *Journal of American Chemical Society*, Vol. 80, pp. 3596-3598, 1958.
19. Sanchez, M.L., C.R. Russell, and C.L. Randolph, "Chemical Weapons Convention (CWC) Signature Analysis," DNA-TR-92-73, AD B171788, 1993.
20. Trapp, R., "The Detoxification and Natural Degradation of Chemical Warfare Agents," Stockholm International Peace Research Institute, 1985.
21. Small, M.J., "Compounds Formed From the Chemical Decontamination of HD, GB, and VX and Their Environmental Fate," USA MRDC TR-8304, AD A149515, 1984.
22. Demek, M.M., Davis, G.T., Dennis, W.H., Hill, A.L., Farrand, N.P., Musselman, Mazza, R.J., Levine, W.D., Rosenblatt, D.H., and Epstein, J., "Behavior of Chemical Agents in Seawater", EATR 4417, US Army Edgewood Arsenal research Laboratory, AD-873 242L, 1970
23. Rosenblatt, D.H., T.A. Miller, J.C. Dacre, M. Illar, and R.R. Cogley, "Problem Definition Studies on Potential Environmental Pollutants II. Physical, Chemical Toxicological and Biological Properties of 16 Substances," USA Medical Research and Development Command TR 7509, AD A030428, 1975.
24. Ward, J.R., and Seiders, "On the Activation Energy for the Hydrolysis of bis-(2-chloroethyl) Sulfide," *Thermochimica Acta*, Vol. 81, pp. 343-348, 1984.
25. Sides, G.D., Dismukes, E.D., and Spafford, R.B., "Evaluation of Decontamination Formulations," ARCSL-CR-81050, US Army Armament Research and Development Command, Chemical Systems Laboratory, AD A106385, 1981.
26. Bartlett, P.D., and Swain, C.G., "Kinetics of Hydrolysis and Displacement Reactions of β,β -Dichlorodiethyl Sulfide (Mustard Gas) and of β -Chloro β -hydroxydiethyl Sulfide (Mustard Chlorohydrin)", *Journal of the American Chemical Society*, (71), 1406 - 1415, 1973
27. Kuiper, A.E.T, van Bokhaven, J.J.G.M., and Medema, J., "The role of heterogeneity in the kinetics of a surface reaction. I. Infrared characterization of the adsorption structures of organophosphates and their decomposition", *Jor. Catalysis*, Vol 43, pp. 154-157, 1976
28. Sinkensen, D.V., "Investigation into the fate of GB dispersed on various surfaces", Porton Technical paper No. 270, Chemical Defence Experimental Establishment, Porton, Wilts, United Kingdom, 1952
29. Penski, E.C., "I: The Puzderliński Model and Applications to Plastic Surfaces," *Surface Evaporation, Penetration, Hydrolysis, and Degradation of Hazardous Chemicals Under a Variety of Conditions*, CRDEC-TR-126, January 1990a.

30. Penski, E.C., "II: Soils Contaminated with Sarin and Mustard," *Surface Evaporation, Penetration, Hydrolysis, and Degradation of Hazardous Chemicals Under a Variety of Conditions*, CRDEC-TR-162, May 1990b.
31. Puzderliski, A., "Persistence of Drops of Sarin and Yperite in Soil (Serbo-Croatian)," *Naucno-Tehnicki Pregled*, AD-A124201, 30(5), 1980.
32. Khan, S. U., Pesticides in the Soil Environment, Elsevier Scientific Pub. Co., Amsterdam, New York, 1980.
33. Daughton, C.G., A.M. Cook, and M. Alexander, "Bacterial Conversion of Alkylphosphonates to Natural Products via Carbon-Phosphorus Bond Cleavage," *Journal Agric. Food Chem*, 27(6), pp. 1375-1382, 1979a.
34. Daughton, C.G., A.M. Cook, and M. Alexander, "Phosphate and soils binding: factors limiting bacterial degradation of ionic phosphorous-containing pesticide metabolites," *Appl. Environ. Microbiol.*, 37, pp. 605-609, 1979b.
35. Cook, A.M., Daughton, C.G., and Alexander, M., "Phosphate Utilization by Bacteria," *J. Bacteriology*, 133(1), pp. 85-90, 1978.
36. Hansch, C., and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, John Wiley, New York, 1979.
37. Lyman, W.J., W.F. Reehl, and D.H. Rosenblatt, Handbook of Chemical Property Estimation Methods, McGraw Hill Book Company, D.H., eds., New York, NY. 1981.

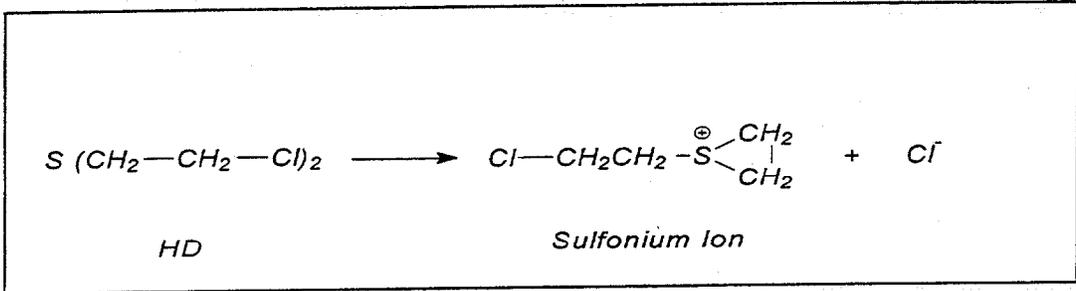


Figure 4. Formation of Sulfonium Ion in First Stage of HD Hydrolysis.

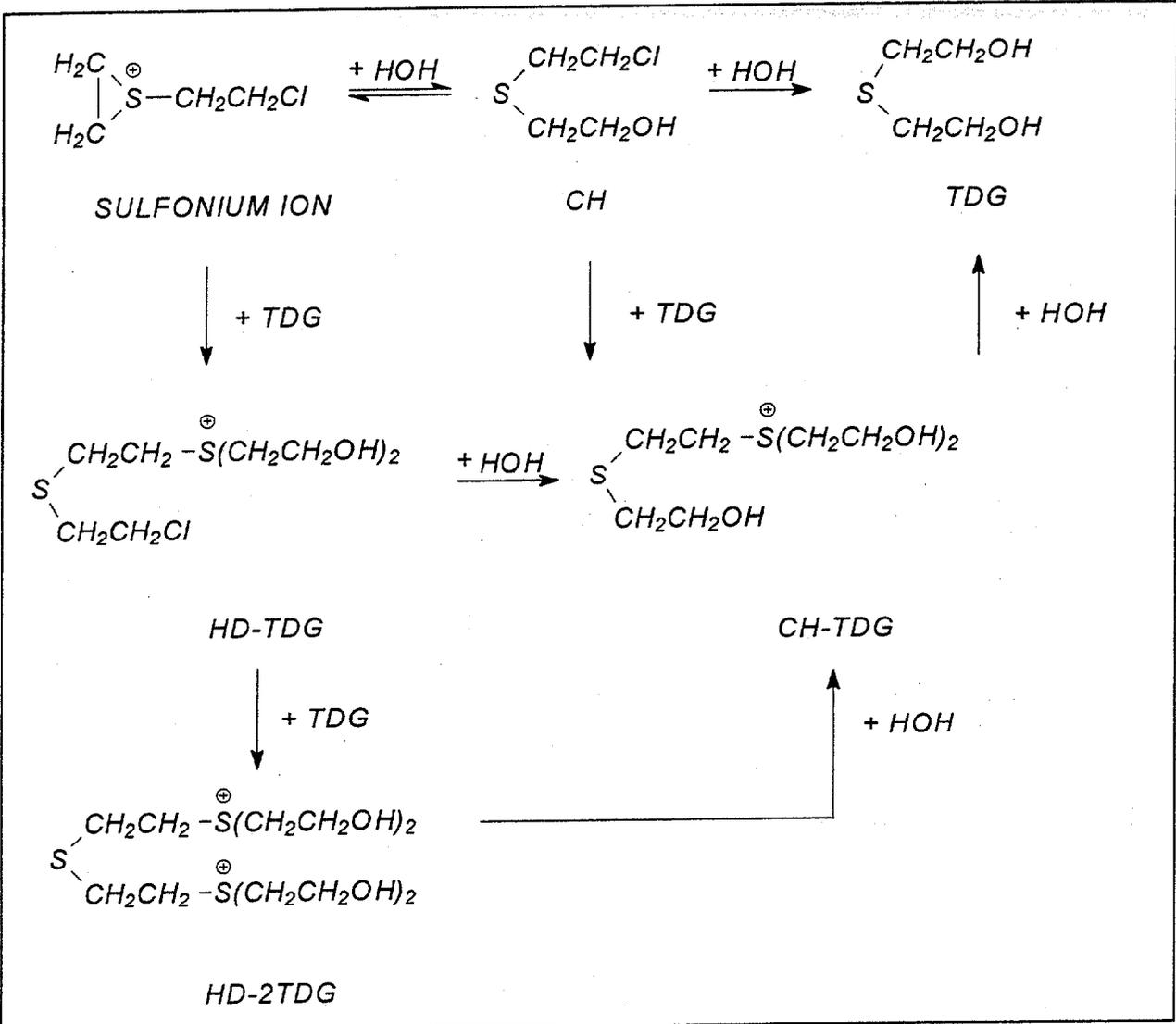


Figure 5. Second Stage of HD Hydrolysis.

Methodology and Biological Monitoring of Exposure to Chemical Warfare Agents

by

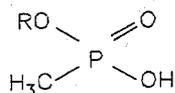
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Aberdeen Proving Ground, MD

INTRODUCTION

In the past few years, our institute has developed several GC/MS methods for the detection of the breakdown products of toxic organophosphonates (soman, sarin, GF) and vesicant sulfur mustard in biological samples. Recently we developed a modified GC/MS method for VX and are continually working on the methodology for lewisite and tabun. The purpose is to have an analytical tool to verify the exposure of chemical warfare agents in humans. Analytical procedures for quantitating the hydrolyzed phosphonic acids from nerve agents in environmental samples have been reported by many analysts (1-5). For more complex matrices such as biological samples, there is not yet a method reported. To make these polar acids amenable to gas chromatographic analysis a prior derivatization is needed. We found the pentafluorobenzyl ester derivatives of the phosphonates are suitable for verification and pharmacokinetic studies in biological samples (6-7). This method may also serve as an alternative method for confirmation purposes in environmental samples.

MATERIALS AND METHODS

Isopropyl methylphosphonic acid (IMPA) and pinacolyl methylphosphonic acid (PMPA) were synthesized by L. J. Szafraniec (U.S. Army Edgewood Research Development and Engineering Center, APG, MD) and their methyl deuterated analogs by Chemsyn Science Laboratories (Lenexa, KS). Cyclohexylmethylphosphonic acid (CMPA) was obtained by hydrolyzing GF in base as described in the literature (8). Their respective structures are shown below:



COMPOUND	R
IMPA	isopropyl
PMPA	pinacolyl
CMPA	cyclohexyl

Deuterated PMPA was used as an internal standard for both PMPA and CMPA, and deuterated analog for IMPA.

The sample preparation and chromatographic conditions are listed in the following paragraphs.

Experimental

- Add 100 ng deuterated analog to 1 mL urine as internal standard.
- Acidify the sample with concentrate HCl to pH 1.
- Pass through a C18 solid-phase cartridge to extract the phosphonic acid.
- Elute with 1 mL methanol to a vial containing 20 mg K_2CO_3 .
- Evaporate the methanol eluant to dryness.
- Add 1 mL methylene chloride containing 3 mg 18-crown-6.
- Add 10 uL pentafluorobenzyl bromide and derivatize at 50°C for one hour.
- Evaporate the organic supernatant to dryness.
- Reconstitute with 100 uL carbon tetrachloride and inject 1 uL to GC.

Chromatography

- Column: DB-17, 0.25 mm ID, 30 m length.
- Carrier: Helium at 1 mL/min.
- Injector Temp: 180°C.
- Oven Temp: 60°C x 1 min, 20°C/min to 200°C, 200°C x 4 min, 30°C/min to 260°C.
- Transfer Line Temp: 280°C.
- Inj. Vol.: 1 uL.
- Internal STD: d_3 -IMPA, d_3 -PMPA.
- Mass Selective Detector.
- EI (HP5970) m/z 256, 259.
- CI (HP5971) m/z mass plus one for each specific phosphonic acid (reagent gas: isobutane).

RESULTS AND DISCUSSION

The following Figure (1) shows the retention times of the perfluorobenzyl ester of the various phosphonic acids and the deuterated analogs. The retention time increased proportionately as the lipophilicity of the alkyl side chain of the methylphosphonic acid increased. The pinacolylmethylphosphonic acids were resolved into two pairs of diastereomer separated by 0.23 minutes.

The electron ionization (EI) mass spectra of all three derivatized acids shared several characteristics. A molecular ion was absent for all three derivatized acids. The base peak for all three compounds was at m/z 181, a non-specific ion of the PFB derivatizing reagent. The relative abundance of the major ions observed in the EI mass spectra are summarized in Table I and Figure 2. The ready loss of the entire R group under EI conditions produced two fragmentation pathways designated as class specific. Both pathways produced abundant ions for all three compounds.

Compound specific fragmentation pathways where the R group was only partially fragmented or remained attached in whole to the phosphonyl backbone varied greatly among the three compounds in their relative importance. Derivatized PMPA produced several compound specific ions, but the derivatized CMPA produced virtually none. Loss of a methyl group from -R alkyl chain was observed for the pinacolyl and isopropyl compounds producing ions at m/z 345 and 303 for PMPA and IMPA, respectively. The ion abundance was very small for the pinacolyl group but very prominent from the isopropyl group. An additional compound specific pathway existed for the derivatized PMPA due to the tendency of the pinacolyl moiety to lose an isobutene group. Loss of the isobutene produced an ion at m/z 303 with further loss of the derivatizing group producing a m/z 123 ion. The methyl deuterated label attached directly to the phosphorus atom remained intact in all class and compound specific ions, indicating that the methyl group was not affected by the ionization process.

Under isobutane positive chemical ionization (CI) conditions, all three derivatized acids produced a strong MH^+ ion with a relative abundance of at least 93%. This ion was the base peak for both IMPA and CMPA. Other compound specific ions observed resulted from the loss of the PFB derivatizing group with protonation of both oxygens. This process was observed for CMPA and IMPA, but the corresponding m/z 181 ion for PMPA was nearly absent (Table II and Figure 3). Isobutane adduct ions were also observed for all three compounds. A prominent class specific m/z 277 fragment ion was also observed for all three compounds. While the protonation of the oxygen under EI conditions also produced a m/z 277 ion, the ready availability of protons under CI conditions appeared to make this a much more predominant pathway and produced the base peak for PMPA.

Table I. EI mass spectra

<u>m/z</u>	<u>proposed structure</u>	Relative Abundance (%)		
		<u>PMPA</u>	<u>IMPA</u>	<u>CMPA</u>
A) Compound Specific Ions				
345	M-CH ₃ (PMPA)	1	0	0
303	M-CH ₃ (IMPA) / M-C(CH ₃) ₃ (PMPA)	20	14	0
123	M-C(CH ₃) ₃ -PFB+H (PMPA)	42	0	0
B) Class Specific Ions				
277	M-R+2H	25	3	15
276	M-R+H	4	10	3
256	M-RF	54	66	32
97	CH ₃ P(OH) ₃	23	9	9
80	CH ₃ P(OH) ₂	13	44	22
C) Non-specific Ions				
181	PFB	100	100	100
161	PFB-HF	8	10	

Table II. Positive CI Mass Spectra

<u>m/z</u>	<u>proposed structure</u>	Relative Abundance (%)		
		<u>PMPA</u>	<u>IMPA</u>	<u>CMPA</u>
A) Compound Specific Ions				
399	[M+C ₃ H ₃] ⁺ (PMPA)	7	0	0
397	[M+C ₃ H ₃] ⁺ (CMPA)	0	0	6
361	[M+H] ⁺ (PMPA)	93	0	3
359	[M+H] ⁺ (CMPA)	1	0	100
357	[M+C ₃ H ₃] ⁺ (IMPA)	0	1	1
319	[M+H] ⁺ (IMPA)	6	100	9
179	[M-PFB+2H] ⁺ (CMPA)	0	1	72
139	[M-PFB+2H] ⁺ (IMPA)	0	23	4
B) Class Specific Ions				
277	[M-R+2H] ⁺	100	12	65
256	[M-RF] ⁺	4	3	9
C) Non-specific Ions				
181	[PFB] ⁺	2	7	9

CONCLUSIONS

Monitoring of the compound specific ions in EI and CI spectra along with the retention time difference from the deuterated internal standard allows positive identification of each of these metabolites in body fluids of alleged victims. The presence of closely separated diastereomer pairs produced a distinctive ion chromatogram for PMPA.

REFERENCES

1. Verweij, A., C. E. A. M. Degenhardt and H. L. Boter, The Occurrence and Determination of PCH_3 -Containing Compounds in Surface Water, *Chemosphere*, 8, 115 (1979).
2. Schiff, L.J., S. G. Pleva and E.W. Sarver, Analysis of Phosphonic Acids by Ion Chromatography in *Ion Chromatographic Analysis of Environmental Pollutants*, Vol. 2, ed. by J. D. Mulik and E. Sawicki, p. 329. Ann Arbor Science Publishing, Ann Arbor (1972).
3. Bossle, P.C., J. J. Martin, E. W. Sarver and H. Z. Sommer, High-Performance liquid Chromatography Analysis of Alkylmethylphosphonic Acid by Derivatization, *J. Chromato*, 267, 209 (1983).
4. Wils, E.R.J. and A. G. Hulst, Determination of Organophosphorus Acids by Thermospray Liquid Chromatography-Mass Spectrometry, *J. Chromato*, 454, 261 (1988).
5. Tornes, J.A. and B. A. Johnsen, Gas Chromatographic Determination of Methylphosphonic Acids by Methylation with Trimethylphenylammonium Hydroxide, *J. Chromato*, 467, 129 (1989).
6. Shih, M.L., J. R. Smith, J. D. McMonagle, T. W. Dolzine and V.C. Gresham, Detection of Metabolites of Toxic Alkylmethylphosphonates in Biological Samples, *Biol. Mass Spec*, 20, 717, (1991).
7. Shih, M.L., J. D. McMonagle, T. W. Dolzine and V. C. Gresham, Metabolite Pharmacokinetics of Soman, Sarin and GF in Rats and Biological Monitoring of Exposure to Toxic Organophosphorus Agents, *J. Appl. Tox.*, 14(3), 195-199, (1994).
8. Harris, L.W., L.M. Braswell, J. P. Fleisher and W. J. Cliff, Metabolites of Pinacolyl Methylphosphonofluoridate (Soman) after Enzymatic Hydrolysis *In Vitro*, *Biochem. Pharmacol*, 13, 1129 (1964).

Chemical Warfare Detectors Worldwide

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The purpose of this presentation is to provide information on the principal technologies used to detect and identify chemical warfare (CW) agents. Detectors of harmful CW agents may be grouped into three major categories based on their operating principles. The three technologies involved are biochemical, ion mobility spectrometry (IMS), and flame photometry (FP). Once a chemical agent has been detected by one of these means, the presence must often be "confirmed" by a second test that is based on a different technology. Two major means of confirming the presence of a chemical agent are mass spectrometry (MS) and "classical" wet chemistry.

Biochemical-Based Detectors. Biochemical-based detectors monitor the activity of a cholinesterase enzyme that has been exposed to an air sample. Enzyme activity is typically monitored photometrically; inactivation of the enzyme indicates the presence of a cholinesterase inhibitor. The targeted analytes for the biochemical-based detectors are nerve agents; e.g., G- and V-type agents. The threshold sensitivity level for this technology is quite low, approximately 0.001 to 0.01 mg/m³. There are limited interference problems and the response time is typically several minutes.

Ion Mobility Spectrometry-Based Detectors. IMS-based detectors contain a radioactive isotope. Air samples collected by the detector pass through an ionization chamber, where the radioactive source ionizes the various constituents of the sample. The resultant ions are directed down an ion drift tube toward a collection electrode. Each ionization species will travel toward the electrode with a different velocity (depending on the individual product of mass and charge), and the species separate over time as they arrive at the electrode. The air sample's composition is then determined when the collection signal is processed by the detector electronics. Targeted analytes for IMS-based systems are nerve agents and mustard. The threshold sensitivity level (approximately 0.01 to 0.1 mg/m³) for this technology is not quite as low as the biochemical method. There are some interference problems associated with IMS (e.g., various smokes and engine exhaust), and the response time is typically measured in seconds.

Flame Photometry-Based Detectors. In FP-based detectors, the air sample is burned in a hydrogen-rich flame. The constituents of the air, when burned, emit light of characteristic wavelengths. A photomultiplier tube then monitors wavelengths of light unique to targeted analytes; e.g., sulfur-containing (mustard) and phosphorus-containing (organophosphorus nerve agents) chemicals. The capabilities of an FP-based detector are virtually the same as of an IM-based detector. The targeted analytes are nerve agents and mustard, and the threshold sensitivity level is approximately 0.01 to 0.1 mg/m³. There are some interference problems (non-CW agents containing phosphorus or sulfur), and the response time is typically measured in seconds.

Confirmation. Mass spectrometry is a sensitive, reliable, and quite versatile approach to identifying/characterizing CW agents. Some countries would use MS to confirm the presence of a CW agent. Ironically, despite the tremendous advances in analytical techniques -- e.g., MS -- the use of decades-old chemical reactions to confirm the presence of chemical agents is still commonplace in many countries. For example, a number of chemical reactions can be used to confirm the presence of mustard. Some detection kits do so by using the chemical 3,3'-dimethylphenolphthalein. Mustard alkylates the carboxyl group of this phenolphthalein derivative, causing a recognizable color change. Another example of the use of wet chemistry -- or, in this case, actually biochemistry -- is a simple test for nerve agents. The cholinesterase-inhibition assay is a powerful test for confirming the presence of a nerve agent (G- and V-type) following an initial alarm from an IMS- or FP-based detector.

NEUTRALIZATION/BIODEGRADATION OF HD

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ABSTRACT

The reaction of sulfur mustard (2,2'-dichlorodiethyl sulfide, HD) with NaOH was investigated with respect to the potential utilization of this reaction for the demilitarization of HD stockpiles. Initial studies with Chemical Agent Standard Analytical Reference Material (CASARM) and [¹³C]HD defined the essential parameters of the HD/NaOH reaction with respect to the effects of temperature and NaOH concentration. A temperature increase from 30°C to 70°C resulted in a greater than 28-fold increase in the hydrolysis rate, corresponding to an enthalpy of activation value of 17.9 Kcal/mol. NaOH requirements were essentially stoichiometric (0.528 g NaOH per g HD).

The effects of varied HD concentrations on the product yield were investigated. At lower HD concentrations, thiodiglycol (TDG) was the major product. As HD concentrations increased, the relative yield of ether and thioether products increased with a concomitant decrease of TDG.

Material balance was performed by ¹³C NMR to determine the overall product distribution. Approximately 35% of the carbon from HD formed TDG, 60% formed ether-alcohol compounds and 5% formed thioxane and elimination products. Under typical conditions, hydrolysis was complete (no HD or chlorinated organics remained) as determined by both ¹³C NMR and GC/MS.

In order to determine if the process would have application to partially degraded samples which are frequently encountered in demilitarization operations, 64% HD recovered from a buried munition was tested. No chlorinated compounds were detectable in the hydrolysate and the basic distribution of products was similar to that seen with CASARM and munitions-grade material.

Biodegradation experiments with hydrolyzed [¹⁴C] HD as the sole source of carbon for growth demonstrated mineralization by the evolution of CO₂.

INTRODUCTION

In a recent report to Congress,¹ the U.S. Army recommended a research, development, testing and evaluation program to pursue two different chemical demilitarization alternative technologies. The recommended technologies were chemical neutralization as a stand-alone technology (neutralization to acceptable final products) and neutralization followed by biodegradation. This report details the initial studies of HD (2,2'-dichlorodiethyl sulfide) hydrolysis as a neutralization step to produce a biodegradable effluent.

Advantages of hydrolysis include low cost, favorable stoichiometry (2 moles or 80 g NaOH required per one mole or 159 g HD), complete dechlorination and products which are promising candidates for subsequent biodegradation.

The mechanism of mustard hydrolysis at ambient temperature has been investigated previously.²⁻⁶ In dilute solutions of HD ([Cl] ~0) and in the presence of a small amount of polar organic solvent (i.e. 5 vol % acetone), the two-step hydrolysis rate constants² are $k_1 = 2.35 \times 10^{-3} \text{ s}^{-1}$ and $k_2 = 4.33 \times 10^{-3} \text{ s}^{-1}$ at 25°C. These rates represent short half-lives (~5 min) in a homogeneous solution. However, as the HD

concentration increases, the rate of hydrolysis in pure water is limited by the rate of mass transfer. This is due to the insolubility of HD in aqueous solution and the fact that dissolution and reaction take place simultaneously.⁵ In a previously-studied two phase system, dissolved and unreacted HD could not be detected in pure water.⁵ HD is hydrolyzed at the interface, and the hydrolysis products then dissolve in water. Therefore, agitation is a critical parameter, and any measurements of the hydrolysis rate must account for this. Both the rate of mass transfer and the rate of hydrolysis can be accelerated at elevated temperatures which are practical for large-scale destruction of mustard stockpiles. In this study, mustard hydrolysis was investigated at elevated temperatures (up to 90° C) in a two-phase system.

Biodegradation studies were performed with [¹⁴C] HD which was first hydrolyzed and subsequently biologically mineralized by bacteria which utilized it as their sole source of carbon for growth. In order to meet international treaty requirements,⁷ it is necessary to not only detoxify chemical agents but to destroy them irreversibly. It is also necessary to consider the method for final disposal since liquids cannot be landfilled. For these reasons, biological treatment offers a potentially attractive option to produce a product which meets treaty requirements and is acceptable for disposal.

MATERIALS AND METHODS

CASARM (Chemical Agent Standard Analytical Reference Material) **HD** (lot # HD-U-2325-CTF-N) was used as received and was greater than 97% pure by NMR. [¹³C] **HD** was custom synthesized by the Illinois Institute of Technology Research Institute (IITRI). ¹³C NMR analysis showed it to be 99.8% pure chemically. The isotopic purity was 98.7% on the alpha carbon and 98.5% on the beta carbon. [¹⁴C] **HD** was also synthesized by Illinois Institute of Technology Research Institute and was 98.7 % pure by Gas Chromatography (GC). Carbons were uniformly labeled and the specific activity was 5 mCi/mmol. **Munitions-grade HD** was obtained directly from a one-ton storage container from the Aberdeen Proving Ground stockpile. It was 89.2% pure by GC/Mass Spectrometry (MS).

GC/Flame Photometric Detection analysis of HD, GC/MS, Thermomixer experiments, Nuclear Magnetic Resonance experiments and thiodiglycol (TDG) High Pressure Liquid Chromatography (HPLC) analyses were all performed as described.⁸

Scintillation counting was performed by absorbing 100 ul of the liquid onto a paper filter disk which was then dried and placed in the bottom of a 20 ml scintillation vial. The vial was filled with 20 ml of Ready Safe liquid scintillation cocktail (Beckman, Fullerton, CA) and counted in a Packard 1900 TR Liquid Scintillation Analyzer. Counting efficiency was measured at 95.7 %.

RESULTS

Effect of Temperature on the Rate of HD Hydrolysis. Because HD dissolution and hydrolysis are essentially simultaneous, the rate of hydrolysis is dependent on agitation. Therefore, absolute hydrolysis rates cannot be measured in this system. The observed rate, expressed as HD disappearance over time, is a function of both the rate of dissolution and the rate of hydrolysis. Therefore, when agitation is controlled and temperature is varied, the relative observed hydrolysis rates can be obtained by monitoring the disappearance of HD over time.

8 mM CASARM HD was hydrolyzed in 0.5 ml 20 mM NaOH for varying lengths of time at 30, 40, 50, 60, 70, 80 and 90° C. Agitation and temperature were controlled by conducting separate reactions in a Thermomixer. This precluded the need to withdraw aliquots from a two-phase reaction system. CHCl₃ extracts of each entire reaction were analyzed by GC/FPD. The square roots of the peak areas were plotted versus time (Figure 1) and the slopes of these lines were plotted

versus temperature (Figure 2), then as an Arrhenius plot (Figure 3). The hydrolysis rates at 80° and 90° were too fast to measure by this method but the rate at 70° was more than 28 times the rate at 30° C. The enthalpy of activation for the HD hydrolysis reaction was calculated as 17.9 Kcal/mol.

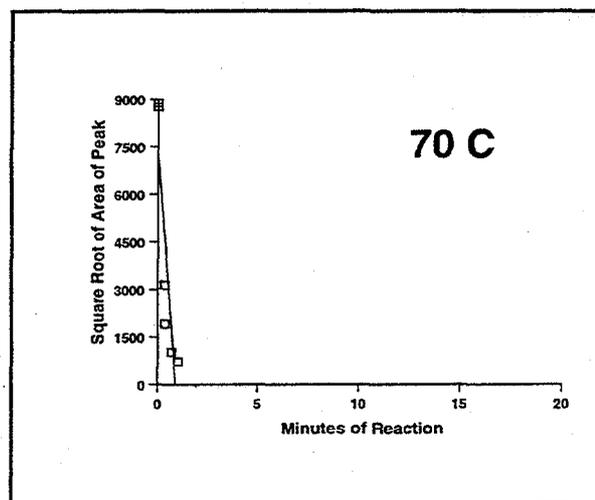
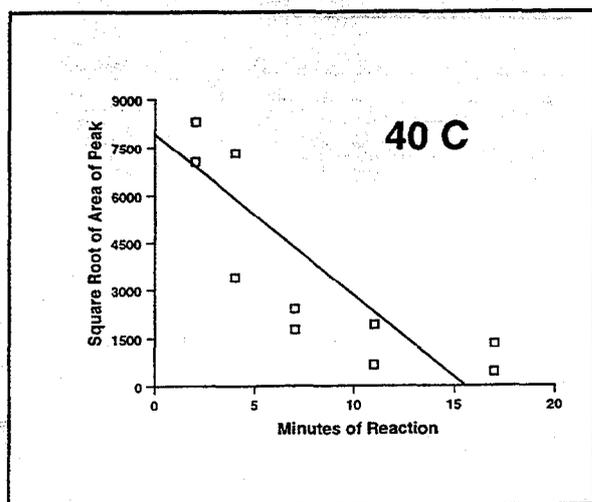
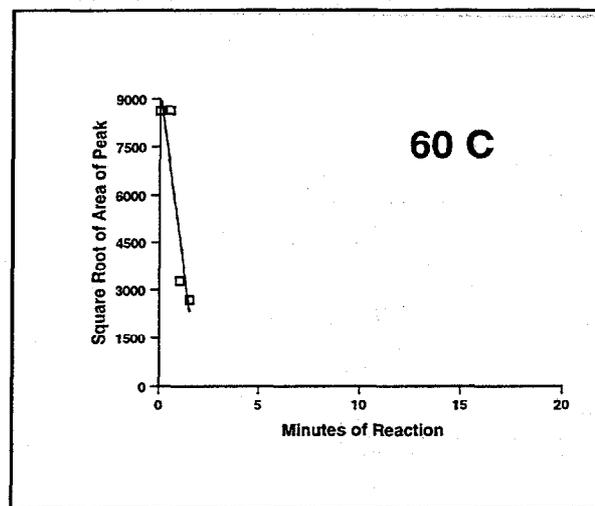
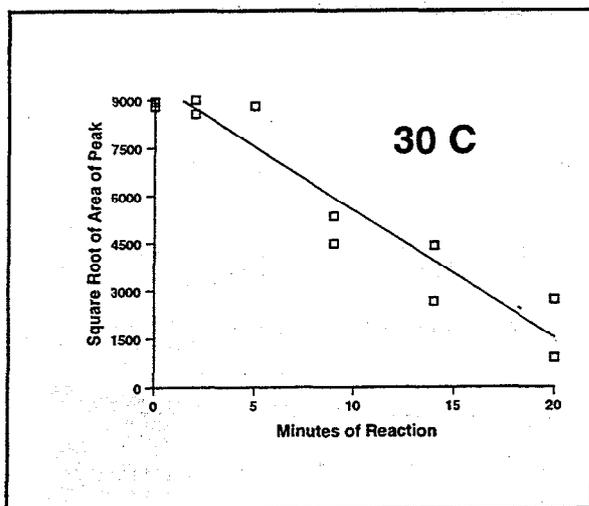
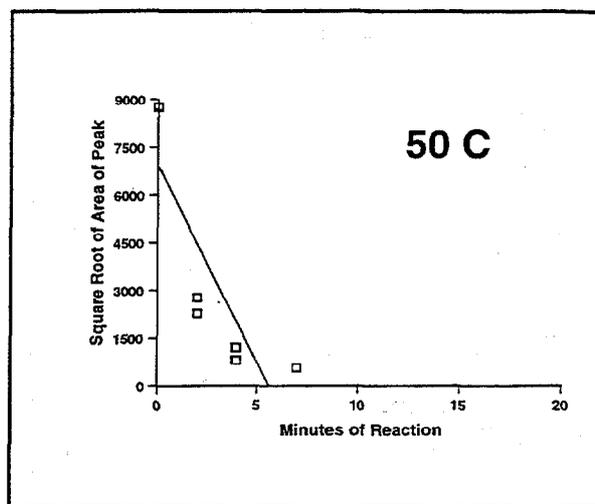


Figure 1. HD concentration versus time at 30, 40, 50, 60 and 70° C.

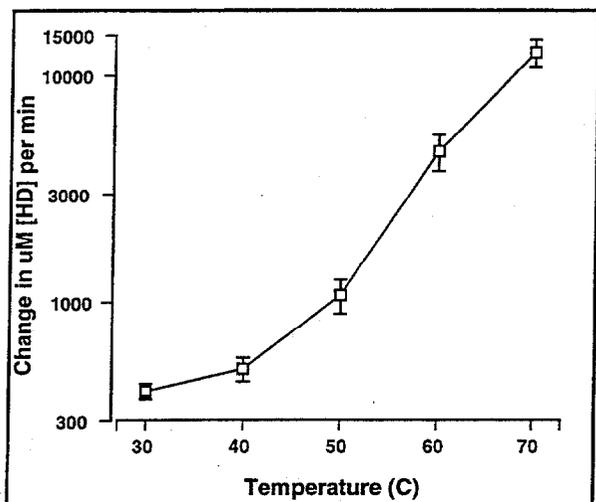


Figure 2. Slope of HD hydrolysis versus temperature. Error bars represent one standard deviation.

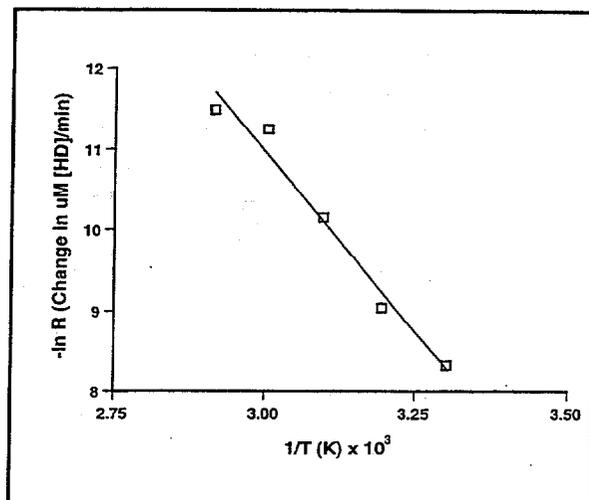
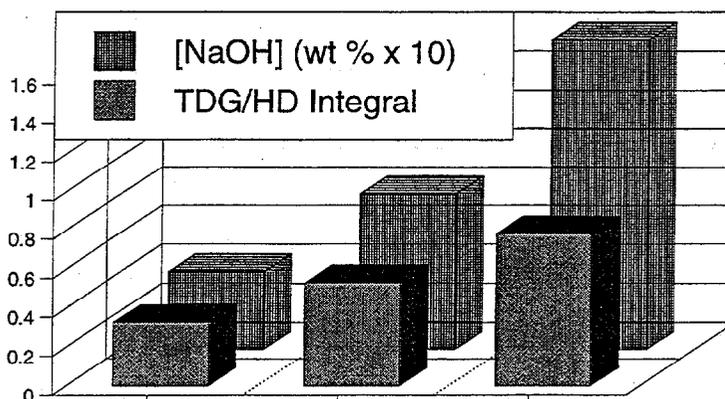


Figure 3. HD hydrolysis Arrhenius plot. $r = 0.9806$, $r^2 = 0.9615$.

Effect of NaOH Concentration on the Rate of HD Hydrolysis.

Using NMR analysis of separate reactions of 8 mM ¹³C HD in 10 mM, 20 mM and 40 mM (.04, .08 and .16 %) NaOH at 50° C for 10 min followed by CDCl₃ extraction, the amount of TDG product and the amount of HD remaining were determined. The TDG/HD integral provides an indication of the extent of the reaction which is proportional to the rate at intermediate time points in the reaction profile. Results shown in Figure 4 show that an increase in NaOH excess from 25% to 150% (10 mM to 20 mM) yielded 38% less HD and 15% more TDG. A 400% NaOH excess (40 mM) yielded 44% less HD and 34% more TDG as compared to a 25% NaOH excess. These data indicate some rate enhancement with increasing NaOH concentration but the change in the rate of the hydrolysis reaction was proportionally much less than the corresponding increase in the NaOH concentration. Other data¹³ have shown that over a wider range of NaOH concentration, the hydrolysis rate eventually decreases as the NaOH concentration increases, presumably due to viscosity effects.

Figure 4. TDG/HD Integrals vs. Incomplete Hydrolysis: 50 C for 10



From a process standpoint, the salt yield will increase with increasing NaOH concentration. Consequently, the relatively minor rate enhancement realized by increasing the NaOH concentration may not be worth the increased salt yield.

Material Balance of CASARM HD Hydrolysis: 50 ml of CASARM HD was reacted with 200 ml 16.7% (5% stoichiometric excess) NaOH at 90° C for five hours. This reaction yielded a two-phase product containing primarily TDG in the aqueous phase and primarily ethers and thioethers in the bottom organic phase. No solid precipitate was formed. The two phases were quantitated separately using ¹H NMR in order to determine the overall material balance. Results shown in Figure 5 indicate that at this HD concentration and temperature the ether products comprised approximately 55% of the total products, TDG comprised approximately 35% and elimination products and thioxane were minor components. Total proton recovery was 104% of theoretical.

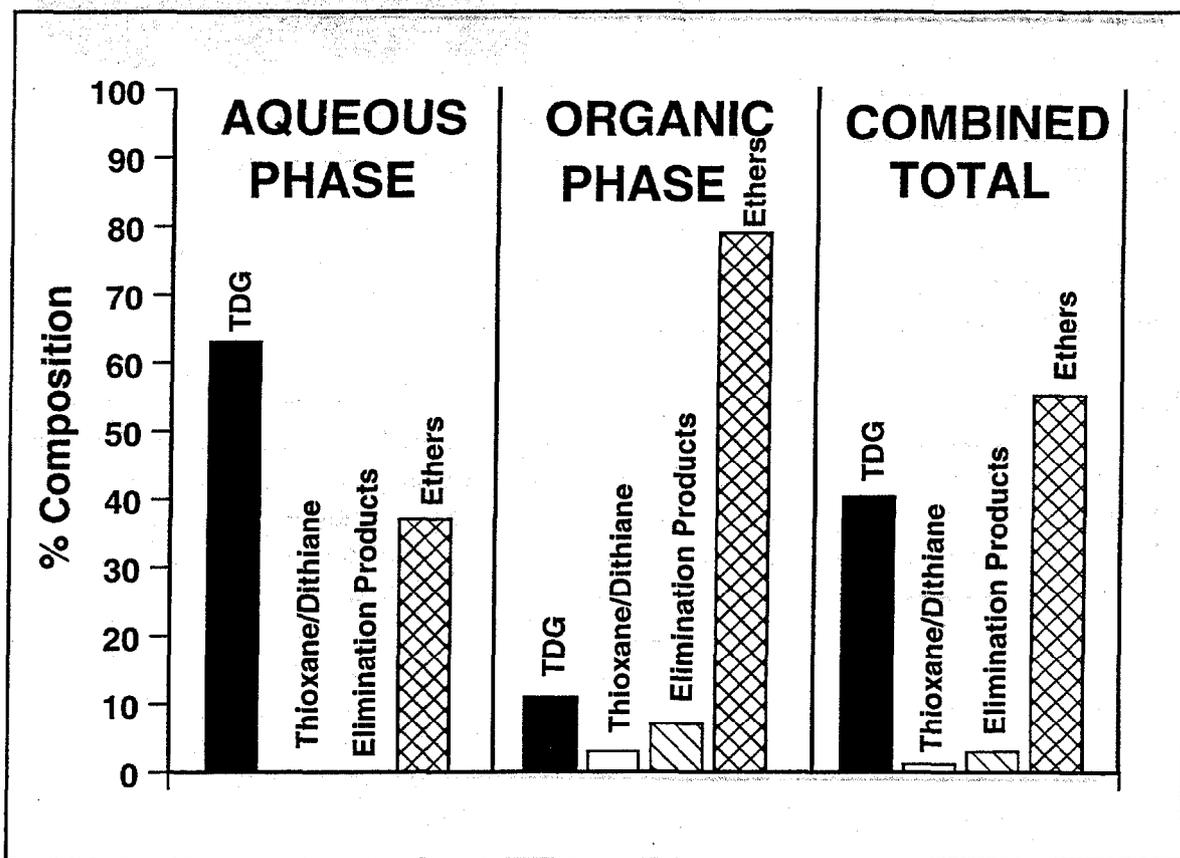


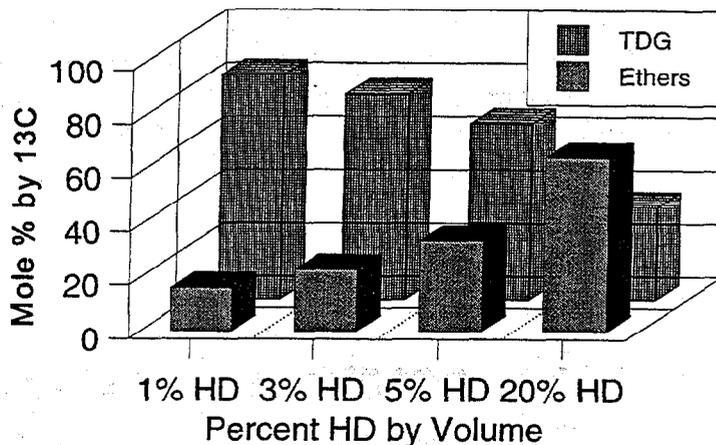
Figure 5. Results of material balance on reaction of CASARM HD with 5% excess NaOH (¹H NMR data).

GC/MS Analysis of Munitions-Grade HD. HD was removed from a ton-container from the Aberdeen Proving Ground stockpile and analyzed by GC/MS. It was found to contain 89.2% HD. The major impurity (4.7%) was (1,2-bis [2-chloroethylthioethane]), also known as compound Q or sesquimustard. The second most predominant impurity was dichloroethane (2.4%) which is probably formed as a thermal decomposition product from the HD dimer. The next most predominant products (2.0%) were the combined isomers of $\text{ClCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}$ which are believed to be thermal decomposition products of the H-2TG and CH-TG sulfonium ions by way of reaction with chloride ion.⁹

Effects of HD Concentration.

Munitions-grade HD was reacted to completion with a 5% stoichiometric excess of NaOH (2.1 moles of NaOH per mole of HD) at HD concentrations of 1,3,5 and 20% (vol/vol) at 90° C. The total ¹³C NMR integrals of TDG vs. ethers/thioethers were compared. Results in Figure 6 show an increase in the yield of ether/thioether products with an increase in HD concentration. The reactions at 1,3 and 5% HD all produced an aqueous product with some flocculent precipitate (apparently iron complexes, see reference 8 for analytical data), whereas the 20% reaction yielded a product which contained a bottom organic phase (primarily ether/thioether compounds) as well as the aqueous phase which contained primarily TDG.⁸

Figure 6. [HD] VS. PRODUC



Hydrolysis of Degraded Spring Valley HD. A major consideration for any demilitarization process is its capacity to detoxify degraded agent. In actual demilitarization operations, HD samples are sometimes encountered which are significantly solidified¹⁰. These samples are frequently referred to as "polymerized" or "gelled" mustard although the actual identity may be unknown.

An HD sample which had been removed from a munition excavated from a 75 year-old burial site at Spring Valley, Washington DC was used as a worst case sample. It had previously been analyzed as being 64.2% pure.¹¹ The Spring Valley HD was of a much thicker consistency than the munitions-grade HD obtained from the ton-container but similar in appearance (dark brown).

A reaction was conducted with 20 ml Spring Valley HD in 16.7% NaOH in a 100 ml (total) reaction (5% excess NaOH, based on the stoichiometry and specific gravity of pure HD) with an argon purge at 90° C. After five hours of agitation, a sample was extracted with CHCl₃ and analyzed by GC/MS. No HD and no chlorinated organics were detected. The GC/MS products detected were similar in structure and distribution to those seen with the CASARM and munitions-grade HD.⁸

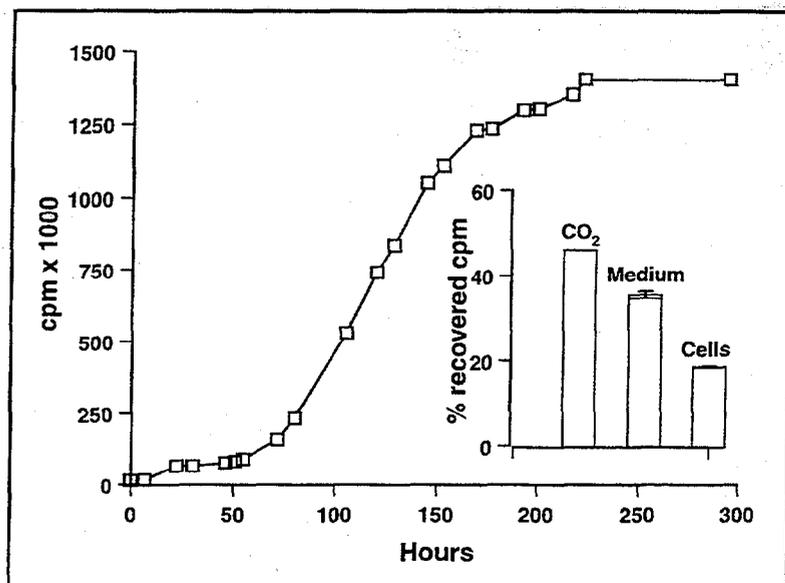


Figure 7. Biomineralization of hydrolyzed ¹⁴C HD.

Biodegradation of Hydrolyzed ¹⁴C HD. Although hydrolysis is adequate for the dechlorination of HD, the single most abundant product, TDG, is a HD precursor and a Schedule 2 compound under the Chemical Weapons Convention Treaty.⁷ Therefore, it is necessary to further degrade and preferably mineralize the TDG. Previous studies¹² have shown that *Alcaligenes xylosoxidans* ssp. *xylosoxidans* strain SH42 is capable of growth on TDG as the sole carbon source. However, mineralization has not previously been demonstrated directly (i.e. quantitation of CO₂ evolution).

¹⁴C labeled HD was hydrolyzed in a 25% excess of NaOH overnight and mixed at a ratio of 1 : 10 with similarly hydrolyzed unlabeled CASARM HD. This mixture was diluted to 30 mM in mineral salts medium and the pH adjusted to 9.2. The medium was added to a biometric flask with a NaOH trap for CO₂ and inoculated with SH42. Incubation was at 30° C with shaking. Hydrolyzed HD was the only source of carbon for growth and therefore the only source of CO₂ which was trapped in the NaOH solution as carbonate. 100 ul samples of NaOH were removed periodically to measure radioactivity. From these data, a mineralization curve was constructed representing CO₂ production (mineralization) over time (Figure 7). The bars represent the final distribution of radioactivity; 46% of the recovered radioactivity had been converted to CO₂, 35.5% was found in the medium (unmineralized substrate, lysed cells, secreted proteins, etc.) and 18.5 % was found in the cells. The total recovery of radioactivity was 90.5 % of theoretical.

DISCUSSION

Temperature is a critical factor in the rate of HD hydrolysis. At 70° C, the rate is more than 28 times as great as the rate at 30° C. The calculated enthalpy of activation value is 17.9 Kcal/mol.

As the HD concentration increases, the yield of ethers and thioethers increases and the yield of thiodiglycol decreases.

Variations in the mustard feedstock yielded relatively minor differences in products. The basic distribution of alcohols, ether-alcohols, thioether-alcohols, etc. occurred regardless of whether the starting material was munitions-grade HD, degraded HD (Spring Valley sample) or HT. With all the samples tested it proved possible to achieve complete hydrolysis (no chlorinated organics remaining) at concentrations of at least 20% mustard with only a 5% stoichiometric excess of NaOH. Although the toxicities of the product compounds have generally not been established, the dechlorination of the organics is clearly important from the standpoint of detoxification.

In experiments described elsewhere,⁸ this same process was also demonstrated with another stockpile agent, HT, yielding a similar product distribution.

In subsequent biodegradation experiments using hydrolyzed [¹⁴C] HD as the sole source of carbon for growth, it was possible to quantitate the evolution of CO₂ and the final distribution of radioactivity to determine the extent to which the hydrolyzed HD was mineralized by *Alcaligenes xylosoxidans* ssp. *xylosoxidans* strain SH42. 46% of the radioactivity was released as CO₂, 36% remained in the medium (unmineralized substrate, components of lysed cells, secreted proteins, etc.) and 19% was found in the cells. The radioactivity must have been distributed throughout all the cell components since the hydrolyzed [¹⁴C] HD was the only source of carbon available.

REFERENCES

1. Chemical Demilitarization Alternative Technology Report to Congress, April 1994.

2. Bartlett, P.D. and Swain, C.G. 1949, Kinetics of hydrolysis and displacement reactions of β,β' -dichlorodiethyl sulfide (mustard gas) and of β -chloro- β' -hydroxydiethyl sulfide (mustard chlorohydrin) *J. Am. Chem. Soc.* **71**, 1406- 1415.
3. McManus, S.P., Neamati-Mazrach, N., Hovanes, B.A., Paley, M.S. and Harris, J.M. 1985, Hydrolysis of mustard derivatives. Failure of the Raber-Harris probe in predicting nucleophilic assistance. *J. Am. Chem. Soc.* **107**, 3393-3395.
4. Yang, Y.-C., Szafraniec, L.L., Beaudry, W.T. and Ward, J.R. 1987, Direct NMR measurements of sulfonium chlorides produced from the hydrolyses of 2-chloroethyl sulfides. *J. Org. Chem.* **52**, 1637-1638.
5. Yang, Y.-C., Szafraniec, L.L., Beaudry, W.T. and Ward, J.R. 1988, Kinetics and mechanism of the hydrolysis of 2-chloroethyl sulfides. *J. Org. Chem.* **53**, 3293-3297.
6. Yang, Y.C., Ward, J.R., Wilson, R.B., Burrows, W. and Winterle, J.S. 1987, On the activation energy for the hydrolysis of Bis-(2-chloroethylethyl) sulfide. II. *Thermochim. Acta.* **114**, 313-317.
7. Report of the Conference on Disarmament to the General Assembly of the United Nations, CD/1173, Sept. 1992, Appendix I, Annex 1, Schedules of Chemicals.
8. Harvey, S. P., Beaudry, W.T., Bossle, P.C., Kolakowski, J.E., Procell, L.R., Rohrbaugh, D.K., Sorrick, D.C., Stroup, A.N., Szafraniec, L.L., Wagner, G.W. and Yang, Y.C. Agent Neutralization. I. Hydrolysis of Sulfur Mustard, Report to the Office of the Program Manager, Chemical Demilitarization, March, 1994.
9. Rohrbaugh, D.K., Yang, Y.-C., and Ward, J.R. 1989, The characterization of sulfonium chlorides by gas chromatography/mass spectrometry and the degradation of 2-chloroethyl sulfide derivatives. *Phosphorus, Sulfur and Silicon* **44**:17-25.
10. Alternative Technologies for the Destruction of Chemical Agents and Munitions. National Academy Press, Washington D.C., June, 1993.
11. Brooks, M.E., Beaudry, W.T., Bossle, P.C., Herd, R.E., Lochner, J.M., Pleva, S.G., Reeder, J.H., Rohrbaugh, D.K., Rosso, T.E., Szafraniec, L.J., Szafraniec, L.L. 1993, Operation Safe Removal: Spring Valley, Washington D.C. Analytical Results: Jan-Feb 1993.
12. Harvey, S.P. and DeFrank, J.J. Biodegradation of Chemical Warfare Agents: Demilitarization Applications *in Army Science: The New Frontiers, Military and Civilian Applications*. Kamely, D., Bannister, K.A. and Sasmor, R.M., eds. Borg Biomedical Services, Saratoga, WY, 1993.
13. Beaudry, W.T., Bossle, P.C., Harvey, S.P., Kolakowski, J.E., Procell, L.R., Rohrbaugh, D.K., Sorrick, D.C., Stroup, A.N., Szafraniec, L.L., Yang, Y.C. Neutralization of HD to Biodegradable Components, Proceedings of 1994 Army Science Conference, in press.

Characterization and Fate of Alkyl Methylphosphonates in Standard Soils Used in the Provisional Technical Secretariat Inter-Laboratory Comparison

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ABSTRACT: Soil properties are shown to have profound effects on both the analytical approach used and the interpretation of analytical results, particularly in soils. Alkyl methylphosphonates are used as indicator compounds for the detection of organophosphonate nerve agents in both site remediation and Chemical Weapons Treaty verification activities. The environmental fate of alkyl methylphosphonates is extremely dependant on the physico-chemical characteristics of the soil matrix. This study presents the soil characteristics and alkyl methylphosphonate fate properties of three standard soils used in the Provisional Technical Secretariat Inter-Laboratory Comparison Testing Program. Alkyl methylphosphonates are transformed in soils by a variety of mechanisms, and degradation rates can vary tremendously between soil types. Sorption, chemical hydrolysis, and biological activity are shown to be critical processes. Environmental fate parameters must be used in the consideration of target compounds, technique, and data interpretation.

Introduction

Analysis and verification of Chemical Warfare Agent (CWA) related materials in environmental samples is critical to the success of the Chemical Weapons Treaty. The Provisional Technical Secretariat has the responsibility to ensure that the laboratories of the participating countries can produce reliable and interchangeable results. The most recent inter-laboratory comparison yielded mostly successful compound identifications, but quantitative results were widely scattered¹.

The alkyl methylphosphonates are degradation products of organophosphonate CWA and some of their simulants. This laboratory is currently evaluating the environmental fate properties of alkyl methylphosphonates in a variety of soil systems. Adsorption, desorption, and hydrolysis rates and extent can be critically

influenced by the chemical and physical properties of the soils. Analytical methods which seek to quantify CWA in soils must consider these properties in order to compare the effects of method and technique.

This project characterized three soils used in the interlaboratory testing program by measuring a wide range of soil properties. This baseline data will be useful in evaluating the results of future interlaboratory comparisons as well as indicating potential modifications to existing methods of both testing and analysis. The characterization data has also been applied to the evaluation of some environmental fate experiments for the alkyl methylphosphonates in these soil systems.

Materials and Methods

Reagents

Type I deionized water was obtained from a Barnstead NANOpure reagent water system fed by a Corning still. Acetonitrile (ACN) was Fisher Optima grade. NaOH solutions were prepared using 50% w/w Fisher certified reagent. Regenerant solution was prepared from trace metal grade H₂SO₄. Phosphate (PO₄) was a primary reference standard (KH₂PO₄, Fisher). Methylphosphonic Acid (MPA) (>98%) was obtained from Alfa. Isopropyl methylphosphonic acid (IMPA) and ethyl methylphosphonic acid (EMPA) (>97%) were supplied by the U.S. Army Environmental Center (USAEC) as Standard Analytical Reference Materials (SARMs). All other chemicals used were ACS grade or reagent grade.

Soils

Treaty soil #1 (TS1, labelled "Sandy Loam") and #2 (TS2, labelled "Clay Loam") were provided directly to our laboratory from EAI, Inc. (Abingdon, MD) in amber glass bottles containing approximately 5 kg of each soil. Both soils had been previously sieved to 2 mm and air dried. No other information was provided.

Treaty soil #3 (TS3) was collected by this laboratory in a wooded area of Union County, New Jersey. The soil is identified on U.S. Department of Agriculture (USDA) soil maps as the Boontown soil series, A horizon. The sampling area was first cleared of forest debris. An exploratory hole revealed that the A horizon was only 10-15 cm thick, but the boundary of the B horizon was evident by a sharp color change from very dark brown to light brown. During collection, an effort was made to collect only the A horizon. The soil was returned to the laboratory, sieved to 2 mm, homogenized, and air-dried. The chaff (>2 mm) and the sieved soil were then weighed separately.

Other soils referenced for comparison are a series of fifteen New Jersey soils² and four U. S. Army reference soils³. These soils have been characterized similarly by the procedures described here.

Methods

Routine soil tests were performed by the University of Delaware Soil Testing Laboratory⁴. Triplicate samples of each soil were randomly labeled and submitted separately. A brief summary of the methods used follows for convenience. Soil pH was measured using a 1:1 (v:v) soil:solution slurry with deionized water (DIW, ASTM Type 1), and a Adams-Evans buffer solution (buffer-pH). Soil organic matter content (OM%) was estimated using the Walkley-Black method. Phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), zinc (Zn), sodium (Na), and copper (Cu) were extracted using the double acid method (Mehlich 1) and analyzed by colorimetry (P) or atomic adsorption spectrophotometry (AAS) (K, Mg, Ca, Mn, Zn, Na, Cu). Trace metals (Cd, Cr, Ni, Pb, As) were determined using acid digestion (EPA method 3050) followed by analysis by inductively coupled argon plasma spectroscopy (EPA SW-846). Sulfate-Sulfur was determined using an acidic monocalcium phosphate extract analyzed turbidimetrically. Total soil nitrogen (Total-N), ammonium (NH₃-N), and nitrate (NO₃-N) were determined by the macro-Kjeldahl method of Bremner. Effective cation exchange capacity (CEC) was defined as the sum of exchangeable bases (Ca, Mg, and K by AAS) extracted by 1N ammonium acetate. Exchangeable acidity (Exch Acid) was determined by extraction with 1M potassium chloride quantified by titration of the extract. Total nitrogen (Total-N), total carbon (Total-C), and total sulfur (Total-S), were determined using a CNS elemental analyzer. Particle size analysis (sand, silt, clay) were estimated using the modified Bouyoucos hydrometer method. Soil textural (USDA) class was estimated by the hand texture method ("feel"), but should be used with caution since processed (sieved and stored) soils may give different results than soils *in situ*.

Soil surface area was determined using the N₂-BET method on a Quantasorb BET analyzer (Model QS-7). Soils were degassed for at least 24 hours (135 °C). The inert carrier was He. Triplicate measurements were made for each soil.

The quantities of three metal oxides, Al₂O₃, Fe₂O₃, and MnO₂, in each soil were determined by three different extraction methods: (1) perchloric - nitric acid digestion (acid method)⁵ (2) sodium citrate - bicarbonate - dithionate extraction (CBD method)⁶, and (3) ammonium oxalate extraction (oxalate method)⁷. Triplicate measurements were made for each soil. After extraction, the soluble metal was determined on a Perkin-Elmer 5000 Atomic Absorption Spectrophotometer. Each extraction method is designed to extract a different fraction of the metal oxides present in the soils, thereby empirically representing the crystallinity and availability of each fraction. The oxalate method is the weakest, extracting only amorphous oxides. The CBD method extracts amorphous and some crystalline oxides, and the acid method is designed to extract all available oxides. In general, each extraction subsumes the previous one (e.g., Fe_{ox} < Fe_{cbd} < Fe_c). The metal oxides are indicators of the availability of specific (inner-sphere) adsorption sites⁸.

Analysis of the alkyl methylphosphonates was performed by ion chromatography (Dionex 4500i, 4 mm OmniPAX column, with conductivity detection) using the method we have published previously⁹. Phosphate (isotherms) and major anions were also determined by ion chromatography (Dionex DX-300, 2 mm AS-11

column, with conductivity detection) using simple hydroxide gradients adapted from the column operating manual¹⁰.

Fluoride sorption was measured by titration^{11,12} using a fluoride specific electrode (Fisher 13-620-522), a Ag/AgCl double junction reference electrode (Orion 90-02), and a Tanager (IDE-8800) automatic titrator. The reaction vessel was a polypropylene sleeve inside a double-walled glass beaker cooled to 21 °C. The soil solution (1 g soil:25 mL solution, mixed for 24 h at 50 rpm) was titrated separately from 25 mL of supernatant (filtered from a 2 g:50 mL mixture). An equal amount (25 mL) of acetate/CDTA buffer (pH 5.5) was added after mixing to prevent hydroxide and aluminum interferences. Fluoride additions were made at 5 m intervals over a 12 h period. The amount of fluoride sorbed was calculated by difference between the suspension and the supernatant.

For the isotherm and hydrolysis experiments, soil suspensions (1 g soil:25 mL solution) were mixed in 30 mL Polyalloy centrifuge tubes (Nalgene) on a Fisher Model 224 reciprocating water bath shaker set at 25 °C and 50 rpm. Isotherm experiments used a 24 h mixing time. The soil suspensions were immediately filtered upon removal from the bath using 0.45 µm FP-450 membranes (Gelman) which have been previously shown to halt hydrolysis and any apparent sorption of the alkyl methylphosphonates³. The isotherms for IMPA and EMPA used initial concentrations of 0, 0.1, 0.2, 0.4, 1.0, 2.0, and 4.0 mg/L; for MPA and phosphate, the initial concentrations were 1, 4, 10, 40, 100, 400, 1000 mg/L. Hydrolysis experiments were all conducted using an initial concentration of 4 mg/L.

Microbiological reactions were studied as enrichment cultures. Media was prepared according to the "BMM" recipe of Schowanek and Verstraete¹³ except that chloride salts were used so that chloride could be removed prior to alkyl methylphosphonate analysis. The initial inoculation was 500 µL from the corresponding soil/compound hydrolysis experiment added to 10 mL of media with 200 µL of 2000 mg/L stock solution of the appropriate alkyl methylphosphonate (as the sole phosphate source) in pre-sterilized culture tubes (Fisher). The media and stock solutions were filter sterilized (0.2 µm) prior to use. The culture tubes were kept at 28 °C in a incubator and hand mixed daily. Subsequent inoculations (at intervals of 6, 6, and 11 days) were made with 500 µL of the previous batch. Controls were made similarly, except for inoculation, for each chemical in each batch.

Results

Soil Characterization

The soil characterization results are presented in tabular form (Tables 1-6). For the most part, they are not particularly unusual and represent an excellent range for the testing of laboratory protocol. TS1 is a neutral, sandy soil, TS2 is a more basic, slightly calcareous clay, and TS3 is typical of an acidic, east coast forest soil.

However, TS1 and TS2 have been clearly modified with fertilizer, and possibly other amendments common to soils in agricultural use. We have confirmed this

result; TS1 and TS2 were collected from farmlands near Lawrence Livermore National Laboratory in California¹⁴.

Table 1. Physical characteristics of the soils. In the parenthesis is the SD for the last significant figure given (n = 3).

Soil	% > 2 mm	% Sand	% Silt	% Clay	m ² /g N-BET
TS1	Unk ^a	88 (2)	4 (2)	8 (4)	1.72 (6)
TS2	Unk ^a	46 (0)	28 (6)	26 (6)	9.98 (4)
TS3	10.6	54 (4)	35 (3)	10 (1)	4.04 (9)

^aThe sieving data for TS1 and TS2 are not available.

Table 2. Aggregative chemical properties.

Soil	USDA Class ^a	pH	Buffer-pH	OM %	CEC meq/g	Exch Acid meq/g
TS1	Loamy Sand	6.6 (0)	7.83 (2)	0.4 (0.5)	4.04 (14)	0.01 (0)
TS2	Sandy Clay Loam	7.9 (0)	7.81 (2)	2.6 (0.5)	22.75 (7)	<0.05
TS3	Loam	4.8 (0)	7.02 (3)	12.1 (0)	29.5 (6)	3.53 (6)

^aEach soil had one (of three) USDA Classification which was different:
TS1 - Sand, TS2 - Loam, and TS3 - Sandy Loam.

Table 3. Nutrient chemical characteristics.

Soil	PO ₄ -P mg/kg	NH ₄ -N mg/kg	NO ₃ -N mg/kg	Total N %	Total C %	SO ₄ -S mg/kg	Total S %
TS1	47 (3)	3.3 (2)	13 (2)	0.032 (3)	0.41 (2)	7.7 (3)	0.018 (3)
TS2	76 (1)	2.9 (2)	68 (6)	0.182 (3)	1.66 (3)	23.0 (4)	0.035 (2)
TS3	7.1 (4)	71 (7)	17 (2)	0.46 (2)	6.9 (5)	30.5 (5)	0.058 (2)

Table 4. Major cations. All values in mg/kg.

Soil	K	Ca	Mg	Mn	Zn	Na	Cu
TS1	62 (4)	600 (30)	109 (8)	16 (1)	5.5 (4)	14.0 (6)	2.5 (2)
TS2	156 (2)	2280 (35)	479 (8)	23 (1)	7.3 (1)	109 (2)	1.05 (1)
TS3	42 (1)	287 (2)	41 (1)	33 (1)	17.9 (6)	7.9 (9)	5.5 (2)

Table 5. Trace metals. All values in mg/kg.

Soil	Cd	Cr	Ni	Pb	As
TS1	1.5 (2)	16 (3)	14 (1)	36 (7)	206 (12)
TS2	2.54 (2)	21.5 (5)	20.8 (3)	35 (3)	424 (30)
TS3	2.08 (4)	15.1 (3)	17.7 (3)	172 (6)	413 (18)

Table 6. Al₂O₃, Fe₂O₃, and MnO₂ in wt% by sequential extractions.

Soil	Al _{ox}	Al _{cbd}	Al _{ac}
TS1	0.36 (1)	1.7 (2)	2.36 (5)
TS2	0.66 (2)	3.8 (2)	4.8 (2)
TS3	0.96 (5)	3.2 (4)	4.46 (9)
	Fe _{ox}	Fe _{cbd}	Fe _{ac}
TS1	0.28 (1)	0.91 (12)	3.4 (2)
TS2	0.51 (1)	1.94 (6)	6.1 (9)
TS3	0.73 (5)	1.86 (8)	2.7 (3)
	Mn _{ox}	Mn _{cbd}	Mn _{ac}
TS1	0.009 (0.5)	0.013 (0.5)	0.024 (2)
TS2	0.031 (0)	0.035 (1)	0.064 (3)
TS3	0.099 (4)	0.121 (4)	0.218 (3)

Sorption

Establishment of a mass balance is critical to an understanding of environmental fate properties. Our approach has been to work backwards through transformation pathways (e.g., GB→IMPA→MPA→PO₄) to maintain accountability. This is particularly important for soils which can degrade alkyl methylphosphonates relatively quickly. The fluoride data can be used as both a control, and to estimate surface coverage¹².

As noted in the methods section above, isotherm experiments were mixed for 24 h prior to extraction. It must be emphasized that for phosphate and MPA, 24 h may not be enough time to reach equilibrium on some soils^{3,15}. However, these results can be very useful for calibration and modelling¹⁶.

All isotherms were evaluated using a Langmuir model which assumes a maximum surface coverage. This approach is suitable for the ionic compounds in the

study¹⁷. The constants were estimated by non-linear regression using a modified Levenberg-Marquardt algorithm¹⁸ in MATLAB (The Mathworks, Inc.).

TS1 and TS2 had a high phosphate background which significantly affected results. The initial, water-extractable phosphate concentrations were estimated by a loading experiment using blanks of 1 g/25 mL, 2 g/25 mL, and 5 g/25 mL soil/DIW for TS1 and TS2, and back-calculated using a mass balance approach¹⁶. The amount of phosphate "added" was then modified prior to estimation of the Langmuir constants (Table 7). The fluoride (F⁻) isotherms were determined in a similar manner.

Table 7. Phosphate and fluoride isotherms.

Soil	PO ₄		F ⁻	
	Q (ug/g) ^a	b (L/ug)	Q (ug/g)	b (L/ug)
TS1	495 (7)	3.7 × 10 ⁻³ (5)	147 (5)	5.7 × 10 ⁻⁵ (3)
TS2	1230 (10)	3.4 × 10 ⁻³ (7)	256 (11)	5 × 10 ⁻⁵ (1)
TS3	3140 (30)	3.5 × 10 ⁻⁵ (7)	1390 (150)	2.8 × 10 ⁻⁵ (5)

^aas ug PO₄-P/g soil

Results of the alkyl methylphosphonate sorption experiments are typical of the soils that we have tested previously. Maximum sorption (Q) constants have ranged from 38-8500 ug/g for MPA (19 soils, Figure 1), 3.3-40 ug/g for IMPA (11 soils), and 0.6-13.9 ug/g for EMPA (10 soils)^{9,16}. TS1 and TS2 are both at the lower end of the range, and TS3 is among the highest.

Table 8. Methylphosphonate sorption.

Soil	MPA		IMPA		EMPA	
	Q (ug/g)	b (L/ug)	Q (ug/g)	b (L/ug)	Q (ug/g)	b (L/ug)
TS1	53 (3)	5 × 10 ⁻⁴ (1)	3.3 (5)	1.0 × 10 ⁻³ (3)	1.08 (5)	6 × 10 ⁻³ (1)
TS2	52 (3)	5 × 10 ⁻⁴ (1)	5.3 (7)	9 × 10 ⁻⁴ (3)	1.06 (7)	4.0 × 10 ⁻³ (9)
TS3	1670 (70)	3.9 × 10 ⁻⁵ (5)	11.7 (9)	6 × 10 ⁻³ (2)	13.9 (9)	5 × 10 ⁻³ (1)

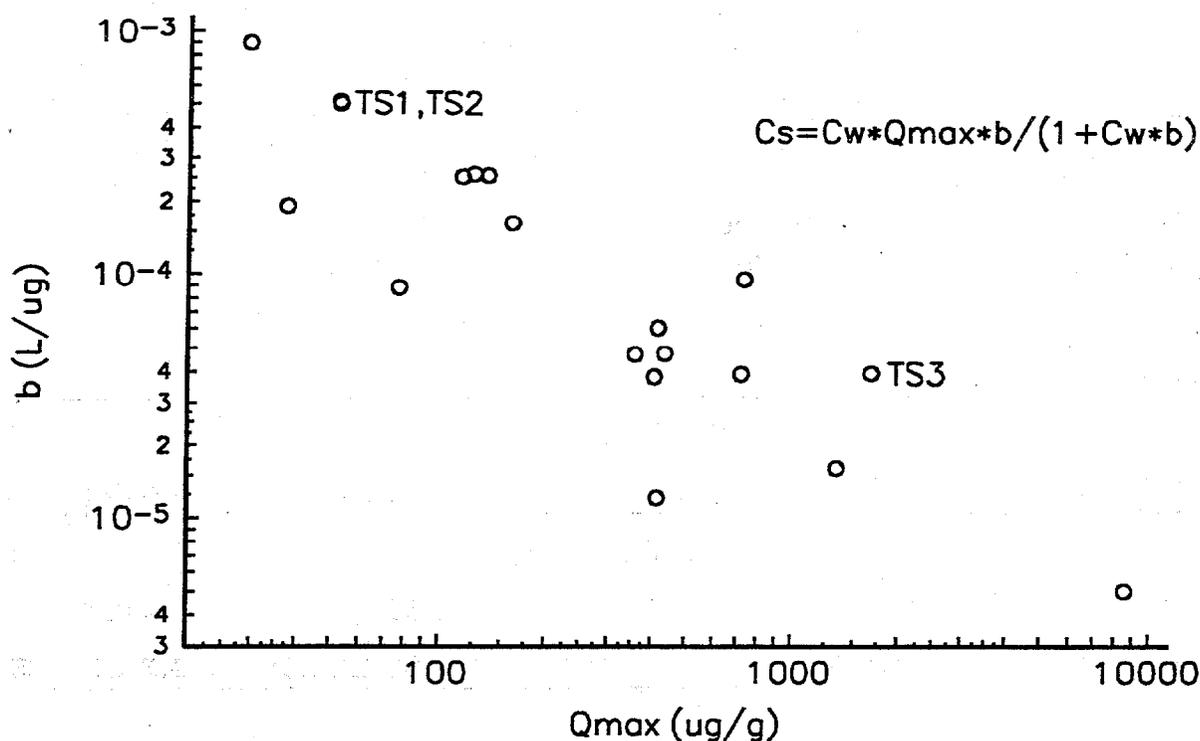


Figure 1. Langmuir constants for MPA on 19 soils.

Alkyl Methylphosphonate Hydrolysis

An initial hydrolysis experiment was conducted using 4 mg/L IMPA (1 g soil/25 mL solution) to determine sampling times. After 96 h, TS1 and TS2 exhibited no degradation, while TS3 had completely transformed the IMPA to MPA. Therefore, the sampling times for TS1 and TS2 were set at approximately weekly intervals for 6 weeks. TS3 was sampled on an approximately logarithmic schedule (0.5, 1, 1.5, 2, 4, 6, 9, 12, 18, 24, 53.5, and 84 h).

TS3 produced results consistent with our prior experience with alkyl methylphosphonate hydrolysis on soils^{3,16}. Sorption of IMPA was rapid and complete prior to the first sample. The rate was first order in the solution concentration (C_w) of IMPA (or EMPA) (Figure 2). The rate of EMPA hydrolysis was faster than that of IMPA. When compared to previous soils tested, the magnitude of the rate was correlated to soil pH (lower is faster) and exchangeable acidity (higher is faster). MPA is evidently undergoing a slow sorption process similar to that in some other soils³.

TS1 and TS2 hydrolyzed IMPA to MPA at a much slower rate, but still first order in the solution concentration (Table 9). However, we were surprised to see that EMPA was almost completely converted to MPA on TS2 after only 150 h (first sample), and similarly on TS1 after 344 h (second sample). An examination of the data revealed a noticeable lag time for TS2 (Figure 3). This suggested biological activity, and enrichment cultures for all three soils in IMPA, EMPA, and MPA were initiated.

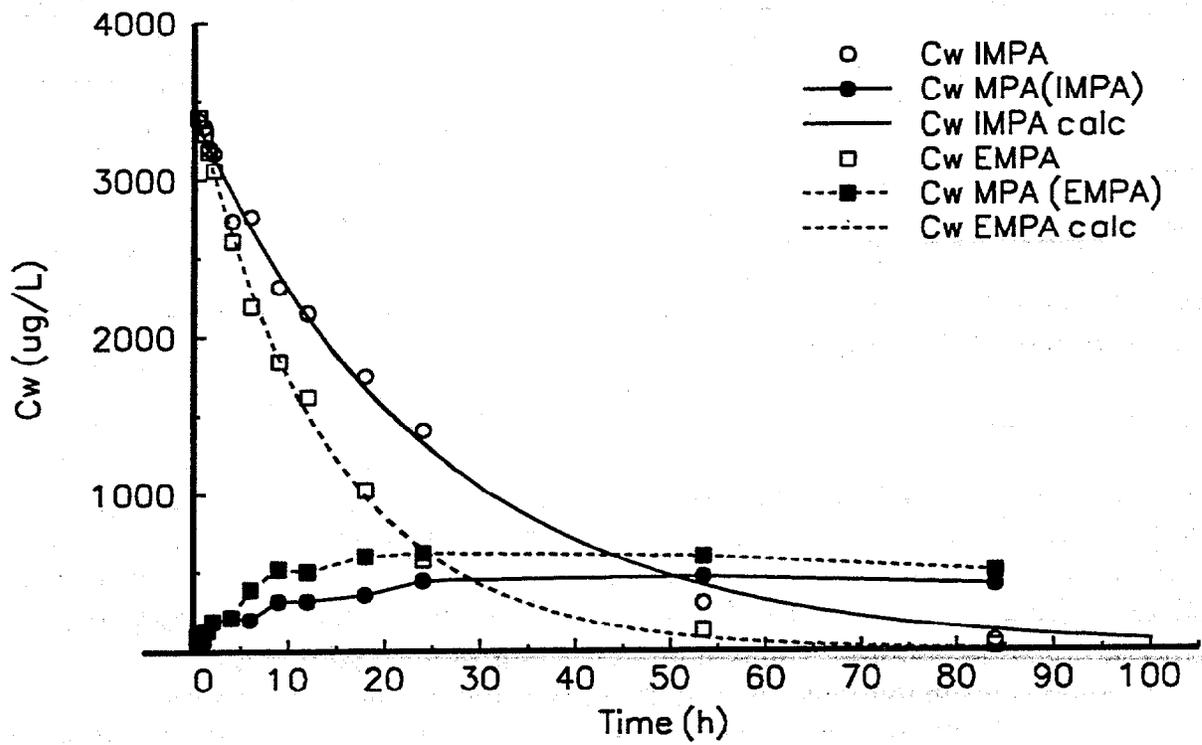


Figure 2. Hydrolysis of IMPA and EMPA on TS3.

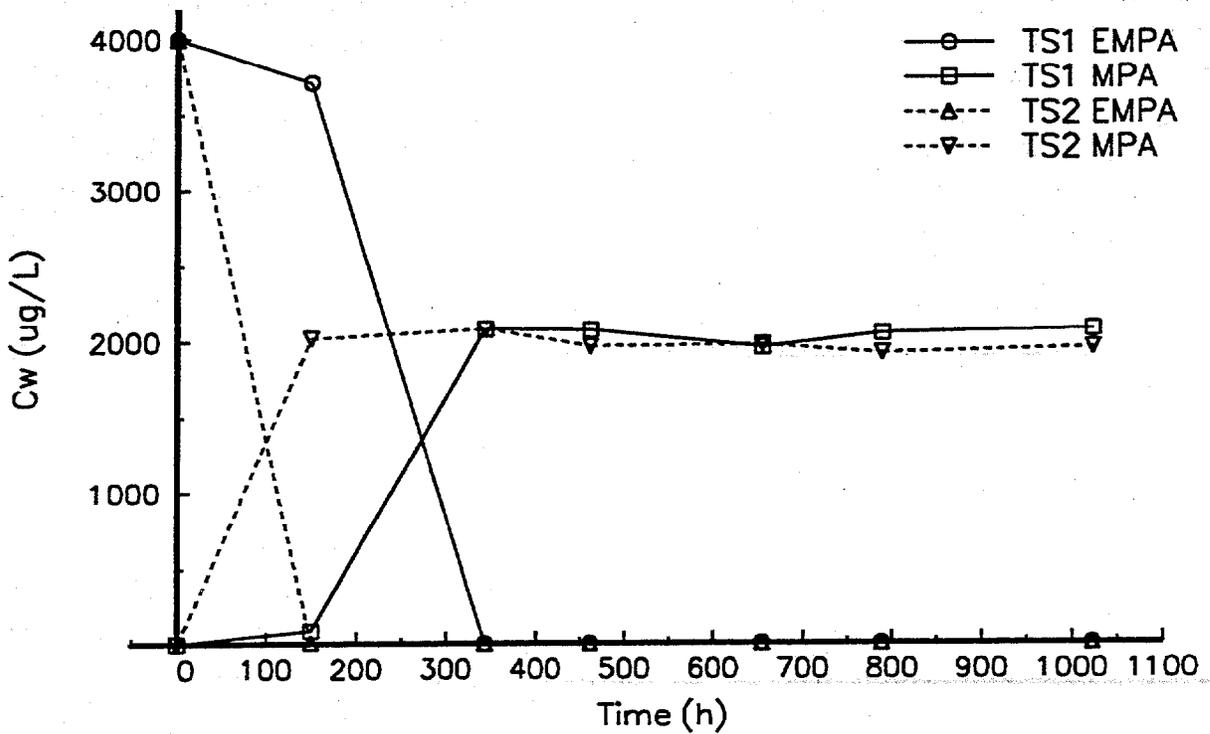


Figure 3. Results of EMPA hydrolysis experiment on TS1 and TS2.

Table 9. First order rate constants (h^{-1}) for hydrolysis of IMPA and EMPA to MPA.

Soil	IMPA to MPA	EMPA to MPA
TS1	4.5×10^{-5} (6)	Unk ^a
TS2	1.2×10^{-4} (2)	Unk ^a
TS3	4.0×10^{-2} (2)	7.1×10^{-2} (2)

^aRate constants for EMPA on TS1 and TS2 could not be determined because of biological activity (see text).

Biological Study

Enrichment cultures were prepared as described in the methods section above. Samples were evaluated for alkyl methylphosphonates and also for common anions because our previous work showed that nitrification can occur in C-P lyase positive cultures³. Total phosphonate-P was determined by mass balance, including transfer from the previous culture. The experiment is currently continuing.

The rate of appearance of visible growth (white, filamentous masses) slowed with each enrichment, and the relative visible mass between compounds was $\text{MPA} > \text{EMPA} > \text{IMPA}$. No visible growth on the IMPA cultures could be observed at the end of the third enrichment.

No evidence of phosphonate-P consumption could be demonstrated within experimental error (in our previous work, for MPA, this was on the order of 10^{-4} h^{-1}). However, biologically mediated hydrolysis was clearly occurring, and at very high rates (Figures 4 and 5). Our previous study found that the highest rate of biologically mediated hydrolysis exceeded the rate of phosphonate consumption only by a factor of 10. The rate of hydrolysis decreased with each enrichment, and was not observed after the third enrichment for TS1/IMPA, TS3/IMPA, and TS3/EMPA. No reaction was observed in any of the controls.

We suspect that the organism(s?) responsible for this biologically mediated hydrolysis may not be able to utilize phosphonate-P as a phosphate source, although many C-P lyase positive organisms can hydrolyze phosphonate esters (many authors, first by Daughton et al.¹⁹. This work is not yet complete, and we hope to be able to isolate the organism(s?) soon.

Conclusions

These three soils represent a variety of challenges to researchers participating in the Inter-Laboratory Comparison Testing Program. The chemical and physical characteristics of the soils span a wide range in almost every parameter measured.

All three of the soils were capable in some way of transforming alkyl methylphosphonates. This is true of most of the soils we have tested, with the exception of highly calcareous soils. By extension, it must be expected that soil

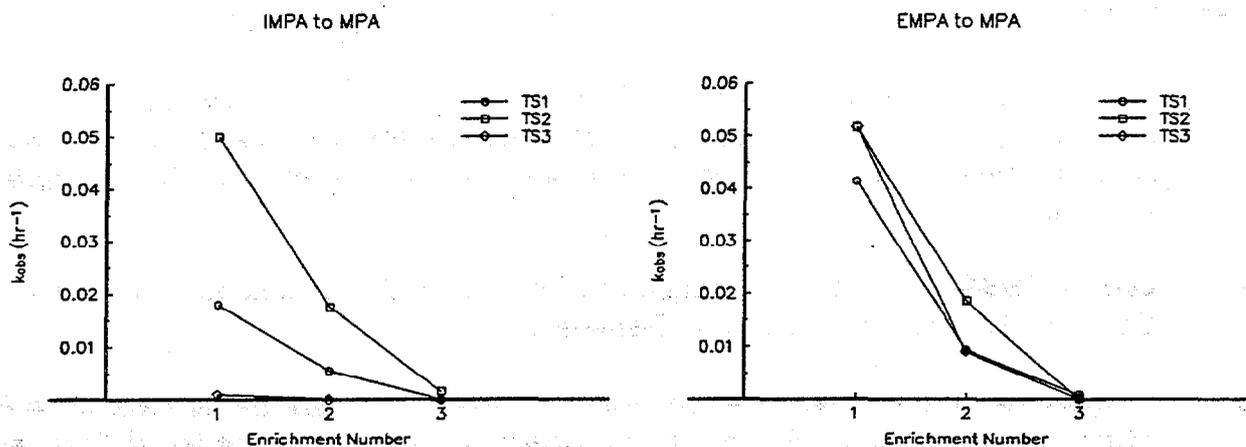


Figure 4. Biologically mediated hydrolysis of IMPA in enrichment cultures.

Figure 5. Biologically mediated hydrolysis of EMPA in enrichment cultures.

samples taken during field sampling would react in similar ways. These fate characteristics must be taken into account during the interpretation of analytical results, both from a methodological and verification perspective.

Limitation of biological activity after sampling is clearly critical. Spiking, holding, and extraction times will significantly affect inter-laboratory results. For the soils we have tested, soils capable of hydrolyzing alkyl methylphosphonates have also been capable of hydrolyzing dialkyl methylphosphonate simulants, such as DIMP. This may confound verification results.

We are currently attempting to develop a more predictive model describing alkyl methylphosphonate fate in soils. The fate of other compounds related to CWA are likely to behave in a similarly varied way in heterogeneous soil systems. Therefore, this laboratory is also investigating the fate of thiodiglycol, a mustard degradation product, in soils. Research into other CWA degradation products is also needed.

Acknowledgement

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References

1. Ministry for Foreign Affairs of Finland (MFAF). 1993. *International Inter-Laboratory Comparison (Round-Robin) Test for the Verification of Chemical Disarmament. F4: Validating of Procedures for Water and Soil Samples*. Helsinki.
2. Lee, S. 1994. The Fate and Transport of Inorganic Contaminants in New Jersey Soils. Ph.D. diss., University of Delaware.
3. Kingery, A. F., and H. E. Allen. March 1994a. *Environmental Fate of Alkyl Methylphosphonates Arising from Chemical Surety Material (CSM) and Potential Non-CSM Sources in Soils and Aqueous Media. Final Report to USAEC*. Newark, DE: University of Delaware.
4. Sims, J. T., and S. E. Heckendorn. 1991. *Methods of Soil Analysis*. Newark DE: University of Delaware Soil Testing Lab.
5. Hesse, P. R. 1972. Total (elemental) analysis and some trace elements. *A Textbook of Soil Chemical Analysis*, 2nd ed. New York: Wiley.
6. Mehra, O. P., and M. L. Jackson. 1960. Iron oxide removal from soils and clays by a dithionate-citrate system buffered with sodium bicarbonate. *Clays Clay Miner.* 7: 317-327.
7. Iyenger, S. S., L. W. Zelazny, and D. C. Martens. 1981. Effects of photolytic oxalate treatment on soil hydroxy interlayered vermiculites. *Clays Clay Miner.* 29: 429-433.
8. Bohn, H. L., B. L. McNeal, and G. A. O'Connor. 1985. *Soil Chemistry, 2nd Ed.* New York: Wiley.
9. Kingery, A. F., and H. E. Allen. 1994b. Ion chromatographic separation of closely related nerve agent degradation products using an organic modifier to provide selectivity. *Anal. Chem.* 66: 155-159.
10. DIONEX Corporation. 1993. *Instructions for the AS-11 Analytical Column*. Sunnyvale, CA.
11. APHA, AWWA, and WPCF. 1993. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. Washington: American Public Health Association.

12. Sigg, L., and W. Stumm. 1981. The interaction of anions and weak acids with the hydrous goethite (α -FeOOH) surface. *Colloid Surf.* 2: 101.
13. Schowanek, D., and W. Verstraete. 1990. Phosphonate utilization by bacterial cultures and enrichments from environmental samples. *Appl. Environ. Microbiol.* 56 (4): 895-903.
14. Stuff, J. 1994. EAI, Inc. *Personal Communication* to A. Kingery, University of Delaware, Department of Civil Engineering, Sept. 21, 1994.
15. Barrow, N. J. 1992. The effect of time on the competition between anions for sorption. *J. Soil Sci.* 43: 421-428.
16. Kingery, A. F., and H. E. Allen. January 1993. *Extraction and Chromatographic Development of Selected Organophosphorus Compounds from Soils and Aqueous Media. Final Report to USAEC.* Newark, DE: University of Delaware.
17. Travis, C. C., and E. L. Etnier. 1981. A survey of sorption relationships for reactive solutes in soil. *J. Environ. Qual.* 10: 8-17.
18. Shrager, R. I., and A. Jutan. undated. Levenberg-Marquardt non-linear regression program in MATLAB (The Mathworks Inc., Natick, MA).
19. Daughton, C. G., A. M. Cook, and M. Alexander. 1979. Bacterial Conversion of alkylphosphonates to natural products via carbon-phosphorus bond cleavage. *J. Agric. Food Chem.* 27: 1375-1382.

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GLOSSARY OF TECHNICAL ACRONYMS

3-Q	3-quinuclidinol
AA	atomic absorption spectroscopy
AAS	atomic absorption spectrophotometry
AC	a blood agent; hydrogen cyanide; prussic acid (CAS No. 74-90-8)
ACE	U.S. Army Corps of Engineers
AChE	acetylcholinesterase; an enzyme normally present in human blood and which inactivates the neurotransmitter, acetylcholine; nerve agents combine with AChE to prevent it from inactivating acetylcholine
ACN	acetonitrile
AED	atomic emission detector
AEHA	Army Environmental Hygiene Agency (now, U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD)
AMC	Army Materiel Command, Alexandria, VA
amu	atomic mass units
AR 50-6	Army Regulation 50-6 <i>Chemical Surety</i> ; addresses controls for recovery of chemical weapons related material discovered during environmental remediation programs or by chance during other operations
AST	above-ground storage tank
BA	benzilic acid
BBC	a crowd control (tearing) agent; CA; bromobenzyl cyanide; camite; bromobenzene- cyanide; obsolete since the 1920's
BIS	bis (2-ethylhexyl) hydrogen phosphite
BSA	bovine serum albumin
BZ	a hallucinogenic, incapacitating, chemical warfare agent; 3-quinuclidinyl benzilate
CA	a crowd control (tearing) agent; BBC; bromobenzyl cyanide; camite; bromobenzene- cyanide; obsolete since the 1920's
CAIS	chemical agent identification set

CAM	chemical agent monitor; for atmospheric concentrations of mustard and nerve agent
CAMDS	chemical agent munition disposal system; at Tooele Army Depot, Utah
CASARM	chemical agent standard analytical reference material
CBD	sodium citrate-bicarbonate-dithionate extraction of metal oxides in soils
CBDCOM	U.S. Army Chemical and Biological Defense Command, Aberdeen Proving Ground, MD
cc	cubic centimeter; cm ³
CDTA	calcium disodium tetraacetate; calcium EDTA; buffer used in analytical chemistry procedures
CG	a choking agent; phosgene; carbonyl chloride (CAS No. 75-44-5)
CH	hemi-mustard; product of agent HD hydrolysis; CAS 693-30-1
CH-TDG	toxic intermediate product of agent HD hydrolysis
CHPPM	U.S. Army Center for Health Promotion and Preventive Medicine, APG, MD (formerly, Army Environmental Hygiene Agency)
CI	chemical ionization
CID	collision-induced dissociation
CIEIA	competitive inhibition enzyme immunoassay
CK	a blood agent; chlorine cyanide; cyanogen chloride (CAS No. 506-77-4)
CMPA	cyclohexyl methylphosphonic acid, a nerve agent hydrolysis product
CN	a crowd control (tearing) agent; "tear" gas; Chemical Mace; chloroacetophenone (CAS No. 532-27-4)
CNB	a crowd control (tearing) agent; chloroacetophenone in benzene and carbon tetrachloride; obsolete
CNS	a crowd control (tearing) agent; chloroacetophenone and chlorpicrin in chloroform; obsolete
CS	O-chloro benzylidene malononitrile; "pepper spray"; a riot control agent with irritant properties for the eye, throat and skin
CTC	Chemical Treaty Compliance
CW	chemical warfare

CWA	chemical warfare agent
CWC	Chemical Weapons Convention; the multilateral chemical weapons control treaty
CWM	chemical warfare material
CZE	capillary zone electrophoresis
DAAMS	Depot Area Air Monitoring System
DEP	direct exposure probe (usually coupled with a mass spectrometer)
DC	direct current
DIMP	di-isopropyl methyl phosphonate; simulant for nerve agent GB
DIW	de-ionized water
DLS	Directorate of Laboratory Sciences (of CHPPM)
DM	a crowd control (vomiting) agent; the organic arsenic compound adamsite; univerrally banned in the 1930's by Western nations for use against civilian populations
DMMP	di-methyl methyl phosphonate; simulant for nerve agent VX
DNA	deoxyribonucleic acid; major constituent of chromatin
DoD	Department of Defense
DOT	U.S. Department of Transportation
DQO	data quality objectives
DSITMS	direct sampling ion trap mass spectrometry
EC	electrochemical
ECD	electron capture detector
EI	electron impact; electron ionization
EI-SIM	electron impact-single ion monitoring
EIA	enzyme-linked immunoassay
ELISA	enzyme-linked immunoabsorbant assay
EMPA	ethyl methylphosphonic acid

ERDEC	Edgewood Research Development and Engineering Center, Aberdeen Proving Ground, MD
FC2A	fluoroacetic acid
FID	flame ionization detector
FPD	flame photometric detector
FT	Fourier transform
FTIR	Fourier Transform Infrared Spectroscopy
FUDS	formerly used defense sites
GA	a volatile nerve agent; Tabun; ethyl ester of N,N-dimethyl phosphoramidocyanidate (CAS No. 77-81-6)
GB	a volatile nerve agent; Sarin; isopropyl methylphosphonofluoridate (CAS No. 107-44-8)
GC	gas chromatograph or chromatography
GC/MS	gas chromatography/mass spectrometry
GC/AED	gas chromatography/atomic emission detector
GC/IRD	gas chromatography/infrared spectroscopy detector
GC/ITD	gas chromatography/ion-trap detector
GD	a volatile nerve agent; soman; methyl-1,2,2-timmethylpropyl ester of pinacolyl methyl phosphonofluoridate (CAS No. 96-64-0)
GF	an experimental nerve agent that was a derivative of agent GB and of similar toxicity; less stable than GB and never adopted for production by major powers
GFAA	Graphite Furnace Atomic Absorption Spectrophotometry
H	a vesicant ("blister") agent; Levinstein sulfur mustard; an unstable mixture of 70% bis (2-chloroethyl) sulfide and 30% sulfur impurities produced by the Levinstein process; (CAS No. 471-03-4)
HD	a persistent vesicant ("blister") agent; distilled sulfur mustard; bis (2-chloroethyl sulfide) (CAS No. 505-60-2)
HD-2TDG	toxic intermediate product of agent HD hydrolysis
HD-TDG	toxic intermediate product of agent HD hydrolysis

HM	hazardous materials
HN-1	a vesicant ("blister") agent; Nitrogen Mustard One; the nitrogen mustard compound 2,2-dichlorotriethylamine
HN-2	a vesicant ("blister") agent; Nitrogen Mustard Two; mustine; the nitrogen mustard compound 2,2-dichloro-N-methylethylamine
HN-3	a vesicant ("blister") agent; Nitrogen Mustard Three; 2,2,2-trichlorotriethylamine
HPLC/IC	high performance (pressure) liquid chromatography/ion chromatography
HPLC	high performance (pressure) liquid chromatography
HPLC/TSP/MS	high performance (pressure) liquid chromatography/thermospray/mass spectrometry
HS	a vesicant ("blister") agent; an early formulation of sulfur mustard used in WWI
IC	ion chromatography
ICP/MS	inductively coupled plasma/mass spectrometer detector
ICP	inductively coupled plasma emission spectrophotometry
IMPA	isopropyl methylphosphonic acid, a nerve agent hydrolysis product
IMS	ion mobility spectrometry
IR	infrared
ISO	International Standards Organization
ITD	ion trap detector
ITMS	ion trap mass spectrometer
KB	di-isopropyl amino ethanol
KLH	keyhole limpet hemocyanin
L	a vesicant ("blister") agent; Lewisite; the organoarsenic compound dichloro (2-chlorovinyl) arsine (CAS No. 541-25-3)
L-2	bis (2-chlorovinyl) arsine; a Lewisite derivative
L-3	tris (2-chlorovinyl) arsine; a Lewisite derivative
LIMS	Laboratory Information Management System

μM	micro Mole; 10^{-6} of a compound's molecular weight (usually expressed in grams)
M18	a portable test kit for detecting selected nerve and blister agents in the field; used by technical escort teams and in military depots
M8	detector paper for the surface determination of gross quantities of liquid mustard or nerve agent; can not discriminate between mustard or nerve agents
MHz	a measure of radiofrequency; 10^6 hertz (a hertz is equal to 1 cycle per second)
MINICAMS	miniature chemical agent monitor; a hand-held air monitor for nerve and mustard chemical warfare agents
MPA	methylphosphonic acid; a hydrolysis product of nerve agent GB (sarin)
MS	mass spectrometry
MS/MS	electrospray interface mass spectrometry/mass spectrometry; multi-stage mass spectrometry
MSD	mass spectrographic detector
MTBE	methyl tertbutyl ether, a solvent
ng	nanogram; 10^{-9} gram
nM	nano Mole; 10^{-9} of a compound's molecular weight
NMR	nuclear magnetic resonance spectroscopy
NPD	nitrogen phosphorous detector
NSCM	non-stockpile chemical materiel
OM	organic matter, usually expressed as a percent (%)
OP	organophosphorous
PFB	perfluorobenzyl ester of various phosphonic acids; a derivatized form of the acid
pg	picogram; 10^{-12} gram
PMNSCM	Program Manager for Non-Stockpile Chemical Materiel
PMPA	pinacolyl methylphosphonic acid, a nerve agent hydrolysis product
PMRMA	Program Manager, Rocky Mountain Arsenal, Colorado
ppb	parts per billion; 1 part in 10^9

ppm	parts per million; 1 part in 10^6
ppt	parts per thousand; 1 part in 10^3
PS	chloropicrin
PTS	Provisional Technical Secretariat (of the Chemical Weapons Convention)
QA/QC	Quality assurance/Quality control
QIRC	Quality internal recovery control
QL	Quality Laboratory
QP	Quality Plant
QST	Quick response, field Screening and data Turn-around time
RDT&E	Research, development, test and evaluation
RF	radiofrequency
RMA	Rocky Mountain Arsenal (Colorado)
ROP	recommended operating procedures
ROPS	recommended operating procedures
RRS	Rapid Response System (for disposal of recovered chemical agent identification sets)
RTAP	real time analytical platform
S/N	signal to noise ratio; a parameter used in calibrating NMR spectrometers
SARM	Standard Analytical Reference Material
SCD	sulfur chemiluminescence detector
SEMI-VOST	semi-volatile organic sampling train
SIM	single-ion monitoring
SOP	standard operating procedure
SVOC	semi-volatile organic compounds
SVRP	soil volume refinement program (at RMA)

T	oxygen mustard; added to vesicant agent HD to lower the freezing point of the mixture, which is then known as agent HT; 1,2 bis (2-chloroethylthio) ether (CAS No. 63918-89-8)
T-2	trichothecene mycotoxin
TC	thermal conductivity
TDG	thiodiglycol
TDGO	thiodiglycol sulfoxide
TEAD	Tooele Army Depot (Utah)
TL	treaty laboratory
TWA	time weighted average; the average air concentration of a chemical substance for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect (ACGIH 1995)
USACE	U.S. Army Corps of Engineers
USACMDA	U.S. Army Chemical Materiel Destruction Agency, Aberdeen Proving Ground, MD
USAEC	U.S. Army Environmental Center, Aberdeen Proving Ground, MD
UV	ultraviolet
UXO	unexploded ordnance
VOST	volatile organic sampling train
VX	a persistent nerve agent; methyl-S-[2-(bis(1-methylethyl)amino)ethyl] O-ethyl ester of phosphonothioic acid (CAS No. 50782-69-9)
WP	white phosphorus; used in the manufacture of smoke bombs, incendiary shells and tracer bullets

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH). 1994-1995. *Threshold limit values for chemical substances and physical agents and biological exposure indices*. ACGIH, Kemper Woods Center, 1330 Kemper Meadow Drive, Cincinnati, OH. 45240