EPA 600/R-13/044 | May 2013 | www.epa.gov/ord

United States
Environmental Protection
Agency

Stability Study for Ultra-Dilute Chemical Warfare Agent Standards

Office of Research and Development
National Homeland Security Research Center

EPA/600/R-13/044
May 2013

Stability Study for Ultra-Dilute Chemical Warfare Agent Standards

U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Disclaimer

The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under Interagency Agreement DW-89-92328201 and DW-89-92261601 with the U. S. Department of Energy (DOE). It has been subjected to Agency's administrative review and approved for publication. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the United States government or Lawrence Livermore National Security, LLC. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Neither the United States government nor Lawrence Livermore National Security, LLC, nor any of their employees makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights.

Auspices Statement

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344; the Lawrence Livermore National Laboratory report number is LLNL-TR-573732. The research team was comprised of Roald Leif, Carolyn Koester and Heather Mulcahy.

Questions concerning this document or its application should be addressed to:

Romy Campisano (EPA Project Officer)
U.S. Environmental Protection Agency
National Homeland Security Research Center
26 W. Martin Luther King Drive, MS NG16
Cincinnati, OH 45268
513-569-7016
Campisano.Romy@epa.gov

Acknowledgments

The research team wish to acknowledge the support of all those who helped plan and prepare this report. The contributions of Don MacQueen, Lawrence Livermore National Laboratory, who performed statistical analyses, are greatly appreciated.

Abbreviations/Acronyms

BSTFA – N,O-bis(trimethylsilyl)trifluoroacetamide

CWA – Chemical Warfare Agent. The CWAs of interest in this report are HD, GB, GD, GF, and VX.

DBT – dibenzothiophene

DCM – dichloromethane

$(DES)_2$ – bis[2-(diisopropylamino)ethyl] disulfide, formula $C_{16}H_{36}N_2S_2$

DESH – 2-(N,N-diisopropylamino)ethanethiol, formula $C_8H_{19}NS$

EMPA – ethyl methylphosphonic acid

EPA – United States Environmental Protection Agency

ERLN – Environmental Response Laboratory Network

FPD – flame photometric detector

GB – Sarin, O-isopropyl methylfluorophosphonate, formula $C_4H_{10}FO_2P$

GC – gas chromatograph

GC-FPD – gas chromatography coupled with a flame photometric detector

GC/MS – gas ghromatography/mass spectrometry

GD – Soman, O-pinacolyl methylphosphonofluoridate, formula $C_7H_{16}FO_2P$

GF – Cyclosarin, cyclohexyl methylphosphonofluoridate, formula $C_7H_{14}FO_2P$

HD – Distilled sulfur mustard, bis(2-chloroethyl)sulfide, formula $C_4H_8Cl_2S$

Hex – hexane

IMPA – isopropyl methyl phosphonic acid

LLNL – Lawrence Livermore National Laboratory

MS – mass spectrometer

NMR – nuclear magnetic resonance (spectroscopy)

OPCW – Organisation for Prohibition of Chemical Weapons

ppm – parts-per-million, equivalent to µg/mL or ng/µL

Pyro A – O,O-diethyl dimethylpyrophosphonate, formula $C_6H_{16}O_5P_2$

Pyro B – O-ethyl, O-isopropyl dimethylpyrophosphonate, formula $C_7H_{18}O_5P_2$

TMP – trimethylphosphate

VX – O-ethyl-S-(2-diisopropylaminoethyl) methylphosphonothioate, formula $C_{11}H_{26}NO_2PS$

Executive Summary

The purpose of this project was to determine the stability over time of the ultra-dilute chemical warfare agent (CWA) analytical standard solutions in dichloromethane and hexane for the five CWAs under normal conditions of storage and use. Ultra-dilute (10 ppm) CWA standards (i.e., analytical standard solutions) are being synthesized by Lawrence Livermore National Laboratory (LLNL) and supplied to the Environmental Response Laboratory Network (ERLN) laboratories. The Environmental Protection Agency established the national ERLN to support large scale environmental responses for chemical, biological, and radiological threats during nationally significant incidents. These standards are provided as authentic standards for the analyses of CWA contaminants that could remain in the aftermath of a terrorist attack, for the unambiguous identification and quantification of CWAs, and for analytical method development by the ERLN laboratories. Currently, data on the long-term stability of the ultra-dilute standards are lacking. The shelf-life data collected in this study is intended to be used to estimate reliable shelf lives for the ultra-dilute standards.

CWAs in single-component and five-component standard solutions, containing 5–10 ppm each CWA in hexane and dichloromethane (DCM), were studied. The CWAs studied included sarin (GB), soman (GD), cyclohexylsarin (GF), sulfur mustard (HD), and O-ethyl-S-(2-diisopropyl-aminoethyl) methylphosphonothioate (VX). The specific objectives were the following:

1. Measure the stability, over the course of 12 months, of single-component ultra-dilute (10 ppm) CWA standards that were stored at 4 °C in the dark
2. Measure the stability over the course of 12 months of multiple-component ultra-dilute (5–10 ppm) CWA standards that were stored at 4 °C in the dark
3. Determine if CWA stability differs in single- and multiple-component mixtures,
4. Determine if solvent choice (hexane versus DCM) affected shelf life
5. Compare the stabilities of CWA standards stored in flame-sealed amber glass ampoules versus CWA standards stored in amber glass vials closed with Teflon®-lined, silicone septa screw caps that were opened and closed periodically

After the CWA standards were prepared in the desired solvent, 1-mL aliquots were placed in flame-sealed, amber glass ampoules. A predetermined number of these ampoules were set aside for the stability study (i.e., the "sealed ampoule standards"). A predetermined number of the ampoules were immediately cracked open and transferred to screw-cap vials. Duplicate sealed ampoules were sampled at predetermined times and then discarded. Duplicate vials were sampled at predetermined times, resealed, and stored for the next sampling. All ampoules and vials were stored at 4 °C ± 2 °C in a refrigerator. CWA concentrations were measured using a gas chromatograph coupled with a flame photometric detector. CWA concentrations were plotted as a function of time over the course of one year. Selected samples were also analyzed by gas chromatography/mass spectrometry

(GC/MS) to identify contaminants and degradation products.

Estimated shelf lives for CWA standards under different conditions were determined using Dunnett's Test (Hsu 1996) to identify the time point before which a statistically significant decrease in analyte concentration occurred; see Table 1. Results for individual standards stored in sealed ampoules showed that the CWA standards made in DCM were stable for a year. Individual standards of CWAs stored in hexane and in sealed ampoules showed varying stabilities; GD and GF were stable for a year, HD was stable for six months, VX was stable for three months, and GB was stable for only two months (although at six months, GB concentration had decreased by only 15% of its initial value).

Table 1. Summary of stability study data and estimated shelf lives for standards stored at 4 ºC.

Estimated Shelf Lives* for CWAs in Single-Component Solutions (months)				
	Dichloromethane		Hexane	
	Screw-cap Vial	Sealed Ampoule	Screw-cap Vial	Sealed Ampoule
GB	5	12	4	2
GD	12	12	12	12
GF	12	12	12	12
HD	6	12	4	6
VX	2**	12	5	3
Estimated Shelf Lives* for CWAs in Multiple-Component Solutions (months)				
	Dichloromethane		Hexane	
	Screw-cap Vial	Sealed Ampoule	Screw-cap Vial	Sealed Ampoule
GB	9	12	0.3	9
GD	12	6	0.7	9
GF	12	12	12	12
HD	6	6	3	6
VX	0.2	≤1**	12	≤1**

Notes: * Estimated shelf life is defined as the time point prior to that for which a statistically significant decrease in concentration was detected by Dunnett's Test.

** Because large variabilities between replicate analyses were noted (relative standard deviation >50%), shelf-life was based on best judgement rather than the results of the Dunnett's Test.

Data for individual standards stored in vials that were periodically reopened showed that most CWAs were stable in both DCM and hexane solutions for six months.

Exceptions to this period of stability were GB, which was stable for only four months in hexane and five months in DCM, and VX, which degraded after five months in hexane and after two months in DCM. Overall, the CWA standards were more stable in the unopened ampoules when compared to the screw capped vials.

Based on the results of Dunnett's Test, multiple-component standards stored in sealed ampoules showed that all CWAs in DCM and hexane were stable for at least six months. However, the research team believes that the statistical analyses overestimate the stability of VX because of the higher variabilities in the concentrations measured for sample replicates (>50% relative standard deviations amongst replicate measurements in some cases). In these cases, VX stability may be less than two months in hexane and DCM.

Multiple-component standards stored in opened vials showed decreased stabilities for most CWA. GD and GF in DCM were stable for 12 months. In hexane, GF was also stable for 12 months, but GD was stable for only 0.7 months (although at one month, the measured GD concentration had decreased by only 10% of its initial value).

GB in hexane was not stable for two weeks (although GB in DCM was stable for nine months). HD in DCM stored in an opened vial was stable for six months, while HD in hexane was only stable for three months. Multiple-component standards in DCM and hexane showed estimated stability times for VX of 0.2 and 12 months, respectively. Overall, the CWA standards were more stable in the unopened ampoules when compared to the screw-capped vials.

Because VX was most prone to degradation, its breakdown was examined in some detail. The breakdown of VX in the VX-only standards was initiated by the presence of ethyl methylphosphonic acid (EMPA); O,O-diethyl dimethylpyrophosphonate was formed by the reaction of EMPA with VX. In the multiple-component standards, the breakdown of VX was initiated by the presence of isopropylmethylphosphonic acid (IMPA), a trace contaminant present in the GB stock solution. VX reaction with IMPA was observed to produce O-ethyl, O-isopropyl dimethylpyrophosphonate. As the IMPA impurity provided another pathway for VX degradation, VX stability in multiple-component standards was less than that observed when VX was present as a single component in a solvent.

This shelf life study demonstrates that the stabilities of the CWAs vary greatly between compound classes (e.g., G-, H- and V-agents), and VX stability can be affected by the presence of other CWAs. Currently, CWA standards are being supplied to the ERLN laboratories as two solutions – the first, 10 ppm VX in DCM and the second, a mixture of GB (10 ppm), GD (5 ppm), GF (10 ppm), and HD (5 ppm) in DCM. Based on the results of this study, the research team recommends that, for convenience in planning and as a simple rule of thumb, all CWA standards, prepared in DCM, in sealed ampoules be used within six months of receipt and that, once opened and mixed into five-component solutions containing GB, GD, GF, HD, and VX, all CWA standards be kept for no longer than one week. The team further recommends that future work be performed to determine how VX can be stabilized in the ultra-dilute standards. Such stabilization strategies could include the removal of EMPA and IPMA from the standard solution, implementation of a stringent water removal strategy and the use of a stabilizer to prevent VX degradation.

Table of Contents

List of Figures

Introduction

The purpose of this project was to determine the stability over time of the ultra-dilute CWA analytical standard solutions in dichloromethane and hexane for the five chemical weapons agents (CWAs) under normal conditions of storage (i.e., 4 ºC) and use. Ultra-dilute (10 ppm) chemical warfare agent (CWA) standards are being supplied by Lawrence Livermore National Laboratory (LLNL) to the Environmental Response Laboratory Network (ERLN) laboratories to allow the use of authentic standards to assist in analyses required in remediation scenarios involving CWAs. The Environmental Protection Agency established the national ERLN to support large scale environmental responses for chemical, biological, and radiological threats during nationally significant incidents. It is critical for the ERLN laboratories to be able to work with authentic CWA standards to allow the unambiguous identification and quantification of CWAs. The ultra-dilute standards are synthesized by LLNL for use in analytical method development by the ERLN laboratories. However, data regarding the long-term stability of the ultra-dilute standards are lacking. The data collected in this study are intended to be used to determine reliable shelf lives for the ultra-dilute standards.

Shelf life for analytical standards is defined as the length of time a standard can be stored, from initial preparation to final use, without significant changes to the original analyte concentrations. The shelf life may be affected by solvent type and storage conditions. Analytical standards are used to create the calibration curves that are used to determine the concentrations of target analytes in authentic samples. To generate accurate calibration curves (and, therefore, measure target analyte concentrations), it is essential that the exact concentrations of analytes in ultra-dilute standards be known. If one adheres to the observed shelf lives and conditions, one can reasonably expect that the analyte concentrations in a standard will remain at their specified values. For this reason, it is important to have guidelines to indicate how long and under what conditions an analytical standard can be stored and used before its concentration changes significantly, adversely affecting the ability to provide valid data.

The ultra-dilute CWA standards studied were sarin (GB), soman (GD), cyclosarin (GF), sulfur mustard (HD), and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). These analytes were studied as single-component standards and in a multiple-component standard solution containing GB, GD, GF, HD, and VX. The five-component standard is desirable as a working standard because it minimizes the number of separate CWA standard solutions needed for the quantitation of multiple CWA analytes. For the ERLN laboratories, these five compounds were initially combined in the same working standard at concentrations of 5 to 10 µg/mL (ppm). As part of this study, the chemical stabilities of these five CWAs were investigated in solutions prepared in both dichloromethane (DCM) and hexane.

As noted above, the primary goal of this study was to collect CWA stability data to provide guidance for establishing shelf lives for the ultra-dilute CWA standards, so that the ERLN laboratories could establish expiration dates for their ultra-dilute CWA standards. Individual standards and combined standard solutions kept at a storage temperature of 4 °C were evaluated and the concentrations were measured for a period of one year. Standards were stored in both sealed ampoules and in screw-capped vials to assess the effect of reopening the vials on the stability of the standards. A secondary goal was to determine if CWA standards could be provided to the ERLN

laboratory as multiple-component standards or if they needed to be provided as single-component standards. To that end, both single- and multiple-component standards were tested for stability.

Materials and Methods

To determine CWA stability and shelf lives, aliquots of single- and multiple-component standards were analyzed at various time intervals and the concentration of each CWA in the solutions was quantified.

Standard Preparation and Storage

CWAs were synthesized at LLNL. The standards were checked for purity by nuclear magnetic resonance spectroscopy (NMR) and by gas chromatography/mass spectrometry (GC/MS). Stock solutions of each agent, at a concentration of 1000 µg/mL, were gravimetrically prepared from the purified neat agents in both dichloromethane, DCM (Riedel-de Haen, GC-grade, Lot # 7284M, with amylene as a stabilizer) and hexane (Fluka, >99.0% purity, GC-grade, Lot # 1351600, Filling Code 1407326). Working standards were prepared in both DCM and hexane from the respective stock solutions by volumetric dilution. The final concentrations of CWA in solution reflected those currently shipped to the ERLN laboratories. The concentration of CWA in each single-component standard was 10 µg/mL. The concentrations of CWAs in the mixed standards were 10 µg/mL for GB, 5 µg/mL for GD, 10 µg/mL for GF, 5 µg/mL for HD, and 10 µg/mL for VX. These concentrations were derived from initial experiments which considered the analyte responses of the CWAs when analyzed by GC/MS. All standards were flame-sealed in ampoules two days after preparation; this allowed a day to perform analyses to verify that the concentrations of the CWAs were correct and that no significant contaminants were present in the standard solutions.

Standards were stored either in flame-sealed, amber glass ampoules (P/N 176796, Wheaton Science Products, Millville, NJ) or amber glass vials, closed with Teflon®-lined, silicone septa screw caps (P/N 5182-0556, Agilent Technologies, Santa Clara, CA). For the sealed ampoule standards, aliquots of the prepared standards were transferred to amber glass ampoules and flame-sealed; the procedure is described in the following section. The t=0 ampoules were then immediately analyzed and the remaining ampoules were immediately refrigerated. A sufficient number of standards in ampoules were prepared such that duplicate sealed ampoules could be sampled at predetermined times and then discarded.

As indicated previously, all standards, including those that would be stored in screw-capped vials, were initially transferred to flame-sealed ampoules. It was deemed to be important to first transfer the standards to flame-sealed ampoules to accurately reproduce the procedure by which the standards were prepared and shipped to the ERLN laboratories (i.e., all standards are placed in flame-sealed ampoules prior to shipping to the laboratories and being opened for the first time). In addition, the attempt was made to reproduce any changes that might occur to the standards during the flame-sealing process (e.g., introduction of water into the standards). As soon as a batch of ampoules were flame sealed, the ampoules were opened and the aliquots of standards were immediately transferred to screw-cap vials. As soon as practical, the t=0 vials were analyzed and the remaining vials were immediately refridgerated until later analysis. The vials were sampled at predetermined times, resealed, and stored for the next sampling.

All standards were refrigerated (4 ºC ± 2 ºC) until analysis to mimic storage conditions expected to be used by the laboratories. The refrigerator was equipped with a thermometer (Item # 20700T, H-B Instrument Company, Collegeville, PA), which was visually checked periodically during the course of the study. Had the temperature of the refrigerator exceeded the range of 2 ºC to 6 ºC at any point during the study, the standards would have been moved to a different refrigerator. Such temperature excursions did not occur.

Ampoule Sealing

One-milliliter aliquots of the working standards were transferred to 2-mL, prescored, amber, borosilicate ampoules using a variable volume pipettor tipped with a long Pasteur pipet. The ampoules were used as received from the vendor. The ampoules were loosely covered with a septum, while their headspace was flushed with argon. The ampoules were then placed in liquid nitrogen (to freeze the solvent and prevent its evaporation) prior to flame sealing. Flame sealing was done using an Ampulmatic® automated ampoule sealing device (Bioscience, Inc., Allentown, PA), using a propane/oxygen flame. The procedures used to seal the ampoules were identical to those used by LLNL to seal ampoules of ultra-dilute standards prior to sending them to the ERLN laboratories.

Instrumentation

The analyses of the CWAs were performed by gas chromatography coupled with a flame photometric detector (GC-FPD) using an Agilent 6890 gas chromatograph (GC) equipped with an HP-5ms column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The GC oven was heated using the following program: isothermal for 1 min at 40 ºC, 15 ºC/min to 300 ºC, and held isothermal for 1 min, with the injector and detectors at 250 ºC, and helium at 1.4 mL/min as carrier gas. The detector, a dual wavelength FPD, enabled both sulfur (S) - and phosphorus (P) - containing analytes to be quantified in the same GC run. Instrument check samples consisting of malathion, trimethylphosphate (TMP), and dibenzothiophene (DBT) were analyzed to assess GC-FPD response and to perform calibrations. This calibration standard has been used for several years to test GC-FPD operation during Organisation for Prohibition of Chemical Weapons (OPCW) proficiency tests and has been determined to have a long shelf-life (> 5 years). TMP and malathion were used to test the response of the P-channel and DBT and malathion were used to test the response of the S-channel.

Analytical Procedure

Direct analysis of the 10 µg/mL standards could not be done on the GC-FPD because the detectors would saturate at this level, so the standards were diluted to a suitable level prior to injection. For all of the individual standard solutions, aliquots of the original solutions were transferred to new vials and diluted with equal volumes of the same solvent prior to analysis. Because the five-component mixes were prepared with components at both 5 and 10 µg/mL, the multiple-component standard mixes were analyzed both undiluted (to quantify the components originally present at 5 µg/mL) and diluted with equal volumes of the appropriate solvent (to allow the analysis of components originally present at concentrations of 10 µg/mL).

Individual standards were analyzed on at the start of the experiment (Day/Month 0) and on Days 14 (Month 0.5), 27 (Month 1), 56 (Month 2), 82 (Month 3), 111 (Month 4), 140 (Month 5), 171 (Month 6), 273 (Month 9), and 365 (Month 12).

Multiple-component standards were analyzed on at the start of the experiment (Day/Month 0) and on Days 9 (Month 0.3), 22 (Month 0.7), 37 (Month 1), 65 (Month 2), 93 (Month 3), 124 (Month 4), 148 (Month 5), 177 (Month 6), 285 (Month 9), and 386 (Month 13).

The timeline and sequence of events for standard preparation, ampoulation or ampoulation followed by transfer into a glass vial, and initial analysis is shown in Figure 1.

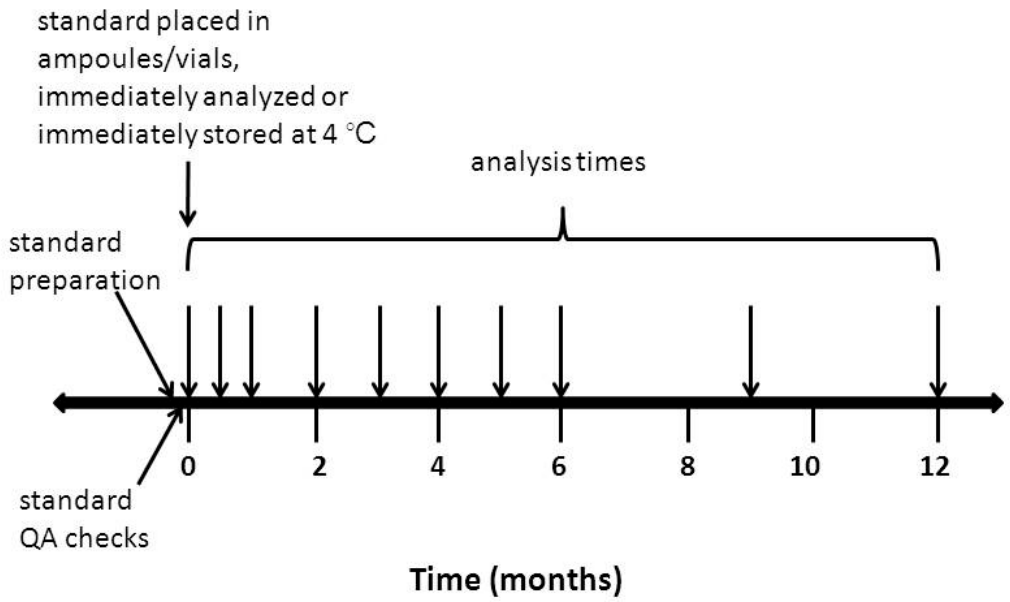


Figure 1. Timeline for standard preparation, ampoulation or ampoulation followed by transfer into a glass vial, and analysis.

Duplicate or triplicate ampoules/vials were analyzed on each sampling day. Each analysis that was performed over the duration of the study for standards in sealed ampoules was accomplished using a new, freshly-opened ampoule. Analyses for vials were performed using aliquots that had been repeatedly opened and closed during the course of the study; this was done to simulate how standards would be stored in vials and used by the CWA laboratories. The specific numbers of replicates that were analyzed during each time series are provided in the paragraphs discussing Figures 2–10.

Quantitation of Target Analytes

Quantitation was performed by external standard method, using a seven-point calibration curve. The calibration compounds used for this study were TMP, DBT, and malathion. These calibration compounds are also part of a multi-component standard that has been used for over five years for instrument evaluation in

support of another LLNL project and found to be stable during this time. As these compounds were known to be stable in that test solution, it was assumed that the calibration standard would be stable for the one-year duration of this study. A stock solution (2 mg/mL each analyte) of calibration standard was made, divided into aliquots, and the aliquots were sealed in multiple ampoules at the beginning of this study. Then, at the designated sampling points, the required dilutions for the calibration solutions were made from newly-opened calibration stock solutions. Because analyte responses obtained by GC-FPD for standards in hexane and responses obtained for standards in DCM had been demonstrated to be different during the initial phase of this study, calibration curves were made using the solvent system of the samples to be measured. TMP, DBT, and malathion were selected for use as external standards, permitting the analytes of interest to have chromatographic retention times of 0.70 to 1.70 relative to one of the external standards. GB, GD and GF, which all contain a phosphorus atom, were analyzed by P-FPD and were quantified using the TMP calibration curve. Also using P-FPD, VX was quantified using the malathion calibration curve. HD, which contains a sulfur atom, was detected by the S-FPD and was quantified using the DBT calibration curve.

Seven calibration levels were used for quantitation, covering a range from 0.5 µg/mL to 6 µg/mL (ppm) so that the analyte concentrations would fall within the calibration range. All CWA standards to be measured were analyzed at 5 µg/mL (assuming 100% recovery of their known t=0 concentration). The response of the FPD was linear in the phosphorus mode. The G-agents and VX were therefore quantified using linear regression calibration curves. In sulfur mode, the response of the FPD was non-linear. A power series fit was therefore used to characterize the S-FPD response for the given calibration range. R-squared values for all calibration curves were >0.99 and continuing calibration standards showed that the standard responses remained within ±20% of their expected values during analyses.

Results and Discussion

The concentrations of CWAs were measured at various times for single-component and multiple-component standards stored at 4 ºC ± 2 ºC to determine whether degradation of the CWAs occurred during the course of the study. The standards were made in both hexane and DCM. DCM was one solvent of interest because ultra-dilute CWA standards are currently prepared and shipped to the ERLN laboratories in DCM. Standards were also prepared in hexane, as hexane may be a solvent of choice for future use. Standards were stored in both sealed, amber glass ampoules and in amber glass vials with Teflon-lined, silicone septa screw caps. The sealed glass ampoules represent how the ultra-dilute CWA standards are currently shipped to the ERLN laboratories and how the standards would be stored prior to opening. Screw-capped vials represent the storage conditions of working solutions in use by laboratories.

The initial concentration of CWA in each single-component standard was 10 µg/mL. The initial concentrations of CWAs in the mixed standards were 10 µg/mL for GB, 5 µg/mL for GD, 10 µg/mL for GF, 5 µg/mL for HD, and 10 µg/mL for VX. These concentrations (referred to as "storage concentrations") were derived from initial experiments which considered the analyte responses of the CWAs when analyzed by GC/MS. Before analysis, the standards were diluted to ensure that the linear range of the GC-FPD was not exceeded. All single-component standards were diluted by a factor of two prior to analysis (i.e., yielding an initial concentration of 5 ppm; the result of this dilution is referred to as the "analysis concentration"). The multi-component standards were analyzed twice – the first time they were analyzed without dilution to measure GD and HD concentrations and the second time they were analyzed at a twofold dilution to quantify GB, GF, and VX. The results of the analyses are presented in Figures 2–10 as graphs of concentration versus time for each CWA under varying conditions. Note that the starting concentration for each of the CWAs is 5 ppm, the target concentrations (t=0) at which all of the CWAs were analyzed. Data were displayed as analysis concentrations, rather than storage concentrations, so that all data could be displayed with the same y-axis. Decreasing trends in CWA concentrations with time will be apparent regardless of the absolute magnitude of the concentrations plotted.

The data were examined using Dunnett's Test (Hsu, 1996). Dunnett's Test allows the comparison of the means of several experimental groups with the mean of a control group in an analysis of variance setting. In this study, one key experimental factor is elapsed time, for which the outcome of interest is a decrease in concentration relative to the initial concentration. Because there was no *a priori* basis for any particular pattern of decrease (e.g., linear, logarithmic) as a function of time, the initial measurements (t=0) were considered to be a control group, and Dunnett's Test was used to examine whether any subsequent time points had lower average concentrations (a one-sided statistical test of the null hypotheses that all subsequent time point averages are greater than or equal to that at the initial time). This test was performed separately within each combination of agent, solvent, storage container (e.g., opened vial or sealed ampoule), and standard type (i.e., single- or mixed-component). Within each such combination, Dunnett's Test was performed at a significance level of alpha = 0.05, so

that the probability of incorrectly declaring any statistically significant differences among the multiple comparisons with control was 5% overall. In the subsequent discussion, the phrase "statistically significant" refers to rejection of the null hypothesis under the conditions described above.

Single-Component Standards

Single-component standards in dichloromethane, stored in screw-capped vials.

Figure 2 shows the concentrations (ppm) as a function of time (months) for the individual CWA standards that were prepared in DCM, sealed in ampoules, then reopened and stored at 4 ºC in vials that were closed with Teflon-lined screw-caps. Each point represents the average measured concentration from two different vials (only one vial was analyzed on t=0.5 months, or 14 days), and the error bars represent plus/minus (±) the standard deviation of the measurements. No statistically significant loss in GB was observed until midway through the study, at Month Six, when GB was measured at 89% of its original concentration. A gradual drop in GB concentration was observed at the nine- and twelve-month time points; the average GB concentration was 66% of its starting concentration after twelve months of storage in a screw-capped vial. No losses were observed for GD and GF during the one-year period. HD was found to relatively stable. The final average concentration of HD represented an 81% recovery after one year. Low concentrations for HD (that were statistically significant) were measured at the nine-month time point. These abnormally low values were consistent with chromatographic problems experienced at that time and may be outliers due to poor chromatographic conditions in the GC-FPD that adversely affected the response of HD. VX did not show a statistically significant concentration decrease until Month Nine. However, by Month Three, the average concentration of VX dropped to 74% of its initial concentration and continued to decrease for the remainder of the study.

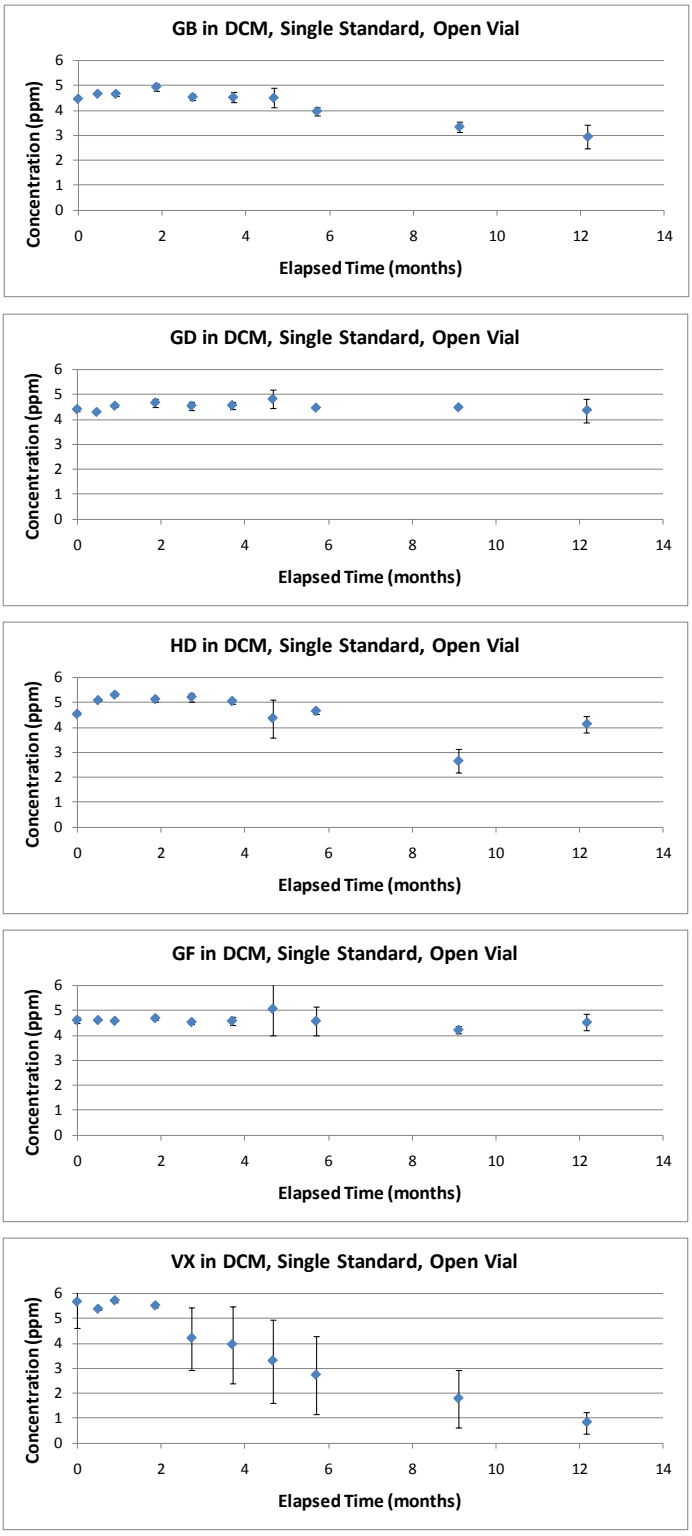


Figure 2. Concentration (ppm) over time (months) for chemical warfare agent single-component standards in dichloromethane in reopened vials

Examination of the data in Figure 2 shows large standard deviations in the average VX measurements made for many of the duplicate samples. Such large variabilities in the data (approximately ±50% for the last six timepoints of the study) hinder the ability of the statistical analysis to discern decreases in VX concentrations (i.e., decreases in VX concentration over time actually occurred, but could not be detected by Dunnett's Test). Possible reasons for the large variations in measured VX concentrations for replicate samples are discussed later in this section (refer to discussion of Figure 8).

Single-component standards in dichloromethane stored in sealed ampoules.
Figure 3 shows the concentrations as a function of time for the individual CWA standards that were prepared in DCM, sealed in ampoules, stored at 4 ºC, then opened and analyzed at the designated time. Each point represents the average measured concentration from two different ampoules and the error bars represent ± the standard deviation of the measurements. Overall, no statistically significant losses of CWAs were observed in any of these individual standards. As observed in the previous data set, low concentrations for HD were measured at the nine-month time point; this low concentration was attributed to chromatographic problems experienced at the time of analysis and, thus, this data point was deemed to be an outlier.

Single-component standards in hexane, stored in screw-capped vials.
Figure 4 shows the concentrations as a function of time for the individual CWA standards that were prepared in hexane, sealed in ampoules, then opened, analyzed, and stored at 4 ºC, in vials closed with Teflon-lined screw caps. Each point represents the average measured concentration from two different vials (only one vial was analyzed at t=nine days) and the error bars represent ± the standard deviation of the measurements. The concentration of GB was relatively stable, with no statistically significant loss detected until Month Five. After this time point, the concentration of GB steadily decreased to 40% of its initial value after one year. No statistically significant losses were observed for GD and GF over the period of one year. No statistically significant loss of HD occurred until Month Five, when the HD concentration was approximately 80% of its initial value. For VX, no statistically significant change in concentration was observed until Month Six. The final measured VX concentration at Month 12 represents approximately 50% of the initial concentration.

Single-component standards in hexane, stored in sealed ampoules.
Figure 5 shows the concentrations as a function of time for the individual CWA standards that were prepared in hexane, sealed in ampoules, stored at 4 ºC, then opened and analyzed at the designated times. Each point represents the average measured concentration from two different ampoules and the error bars represent ± the standard deviation of the measurements. The trends observed for this set of samples match those of the previous set of opened hexane standards shown in Figure 4. For GB, a significant loss in concentration was observed by Month Three (although the decrease in GB concentration was only 20%). By one year, the GB concentration dropped 26% from its initial value. No statistically significant losses were observed for GD and GF over the one year time period. HD was stable for six months. A steady and statistically significant decrease in VX concentration was observed, beginning at Month Four. The final VX concentration at Month 12 was 2.69 µg/mL, representing a 47% drop from the starting concentration.

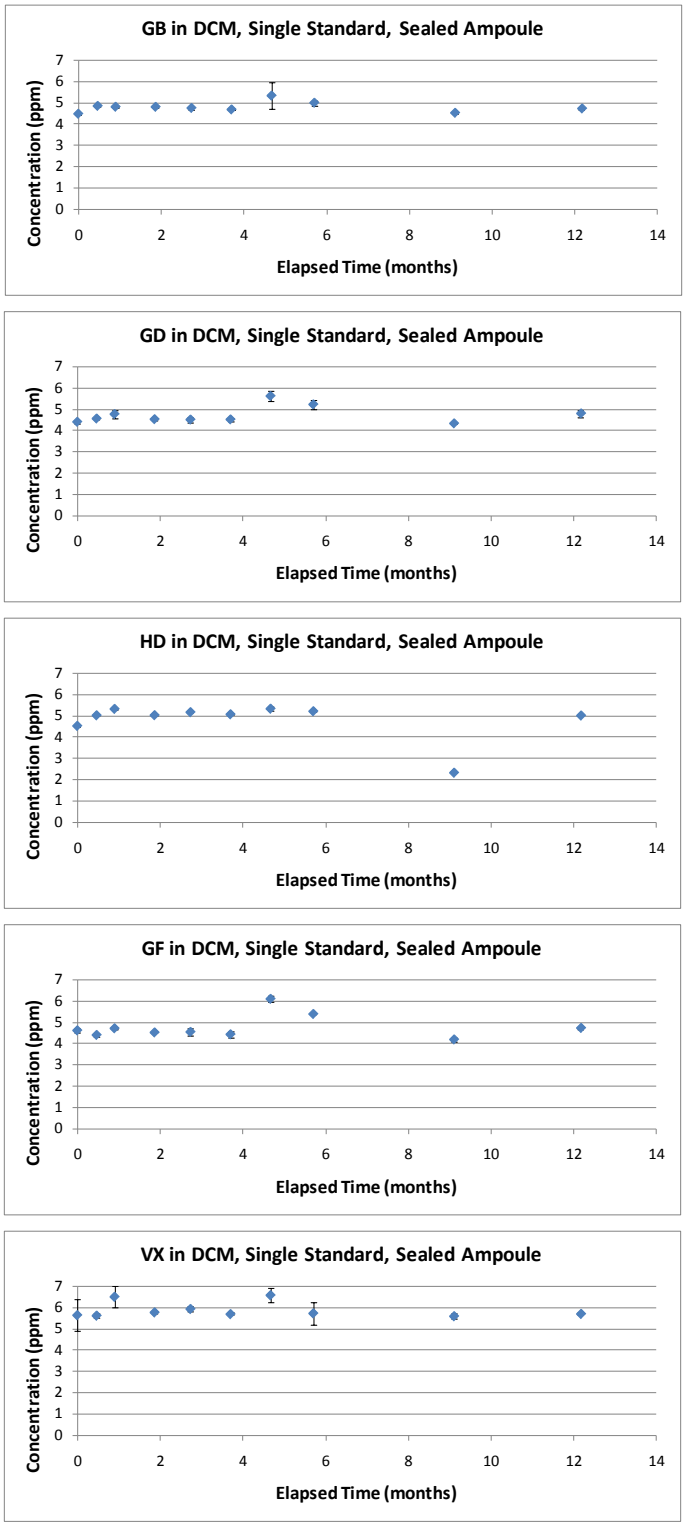


Figure 3. Concentration (ppm) over time (months) for chemical warfare agents in dicloromethane as a single-component standard in sealed ampoules.

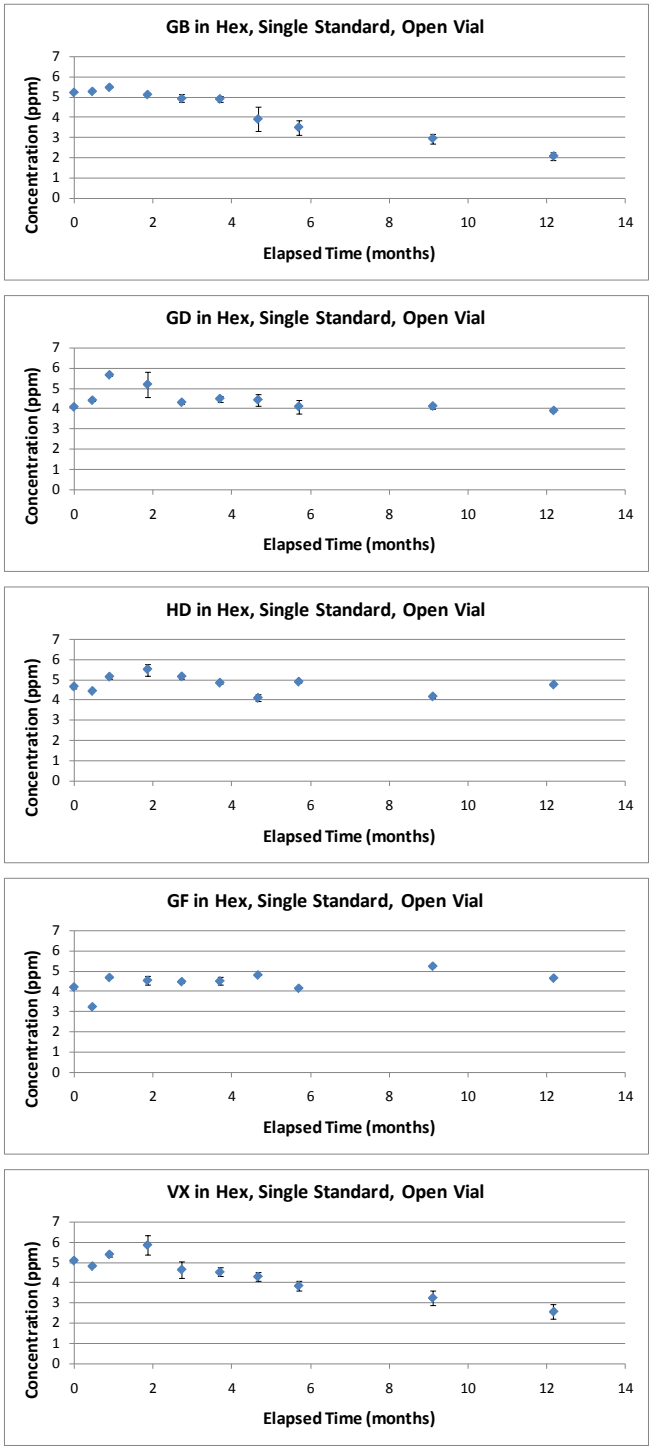


Figure 4. Concentration (ppm) over time (months) for chemical warfare agents present in hexane as a single-component standard, in reopened vials.

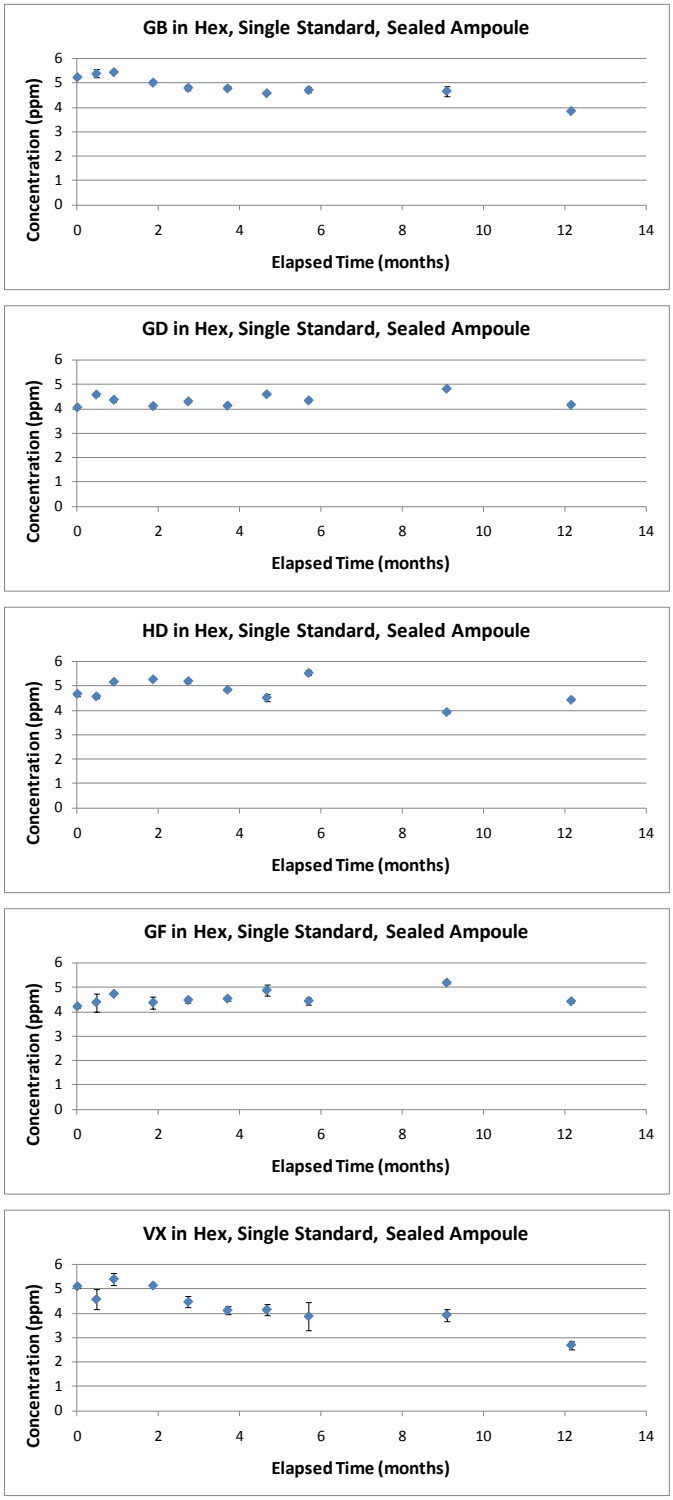


Figure 5. Concentration (ppm) over time (months) for chemical warfare agents present in hexane as a single-component standard in sealed ampoules.

Multiple-Component Standards

Multiple-component standards in dichloromethane stored in screw-capped vials.

Figure 6 shows CWA concentrations as a function of time for the ultra-dilute CWA standard mix, containing GB, GD, GF, HD, and VX, that was prepared in DCM, sealed in ampoules, then opened and stored at 4 ºC in vials sealed with Teflon-lined screw caps. Each point represents the average measured concentration from three different vials (only two vials were analyzed at t=0) and the error bars represent ± the standard deviation of the measurements. No statistically significant loss in GB was observed until the twelve-month time point, where only 72% of the original concentration of GB remained. No statistically significant losses were observed for either GD or GF for the duration of the year-long study. For HD, only 44% of the original concentration remained after one year. A gradual statistically significant decrease in HD began after Month Six. VX was found to be quite reactive when combined with the other CWAs and stored in screw-capped vials. Almost 20% of the VX was lost after only nine days. VX continued to degrade throughout the study period, with approximately 95% of the VX lost after one year.

Multiple-component standards in dichloromethane stored in sealed ampoules.

Figure 7 shows the CWA concentrations as a function of time for the ultra-dilute CWA standard mix that was prepared in DCM, sealed in ampoules, stored at 4 ºC, and opened immediately prior to analysis. Each point represents the average measured concentration from two different ampoules (three ampoules were analyzed at t = nine days) and the error bars represent ± the standard deviation of the measurements. No statistically significant losses were observed for either GB or GF for the duration of the one year period. GD showed a statistically significant loss after nine months and HD was stable for only the first six months of the study.

No statistically significant VX losses were observed over the course of the study. However, when the VX concentrations for duplicate samples were averaged, large error bars were observed and the power of the statistical test to detect concentration decreases was diminished (refer to previous discussion). Upon examination of the individual data points representing VX concentrations, some of the ampoules contained VX at its original concentration while others contained lower concentrations of VX (with decreasing VX concentrations as study time increased); see Figure 8. For example, at the four month time point, the two replicate samples happened to be two ampoules where VX did not degrade. At the six month time point, the two replicate samples happened to be two ampoules where VX did degrade. The other time points, with the exception of the initial measurement at t = 0, consist of one ampoule containing undegraded VX and the other ampoule containing VX that had undergone a substantial amount of loss. The distribution between ampoules containing degraded VX and those containing degraded VX appears to be evenly split and random. Because of the good precision of the VX measurements at t=0 and t=4 months and because the calibration curves for VX were successfully constructed during the course of this study, the VX concentration differences between ampoules were attributed to VX degradation and were not attributed to inherent problems in the reproducibility of the measurements.

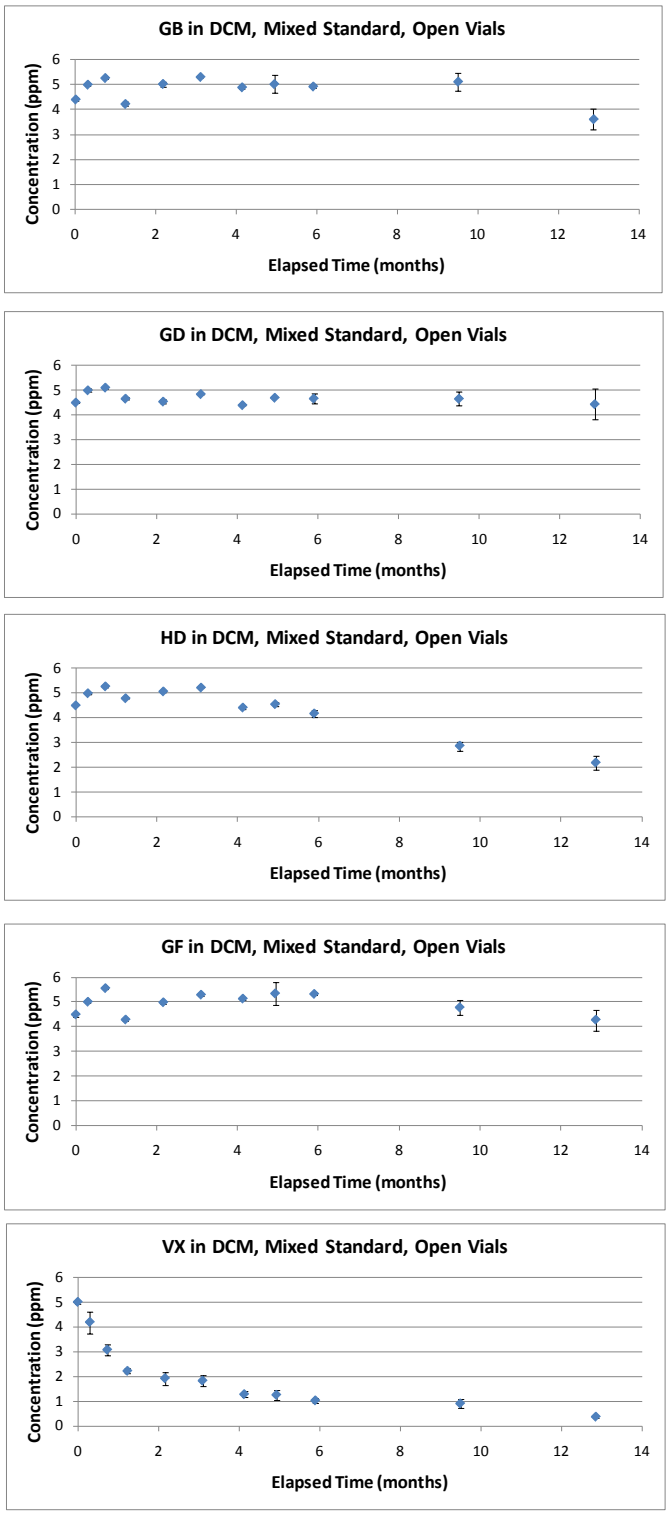


Figure 6. Concentration (ppm) over time for chemical warfare agents present in dichloromethane as a multiple-component standard, in reopened vials.

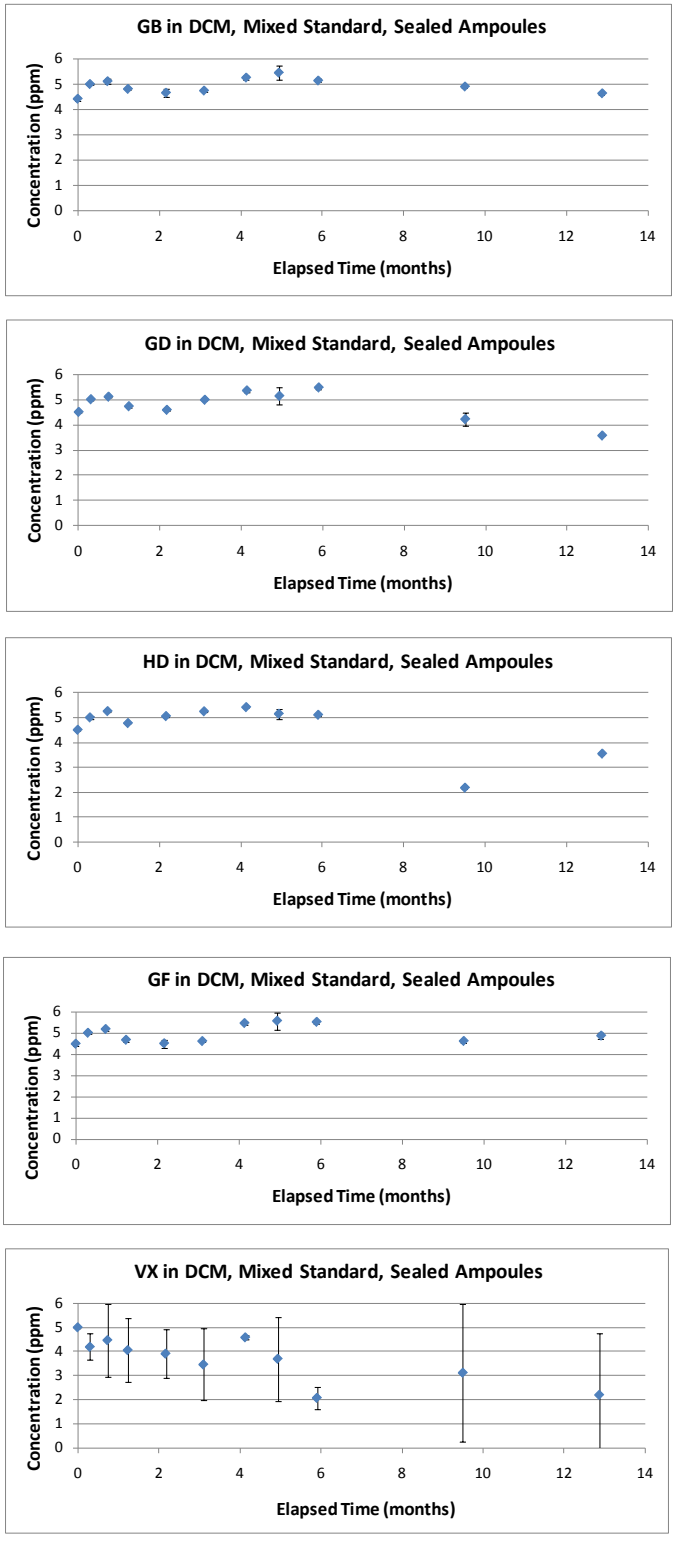


Figure 7. Concentration (ppm) over time for chemical warfare agents present in dichloromethane as a multiple-component standard in sealed ampoules.

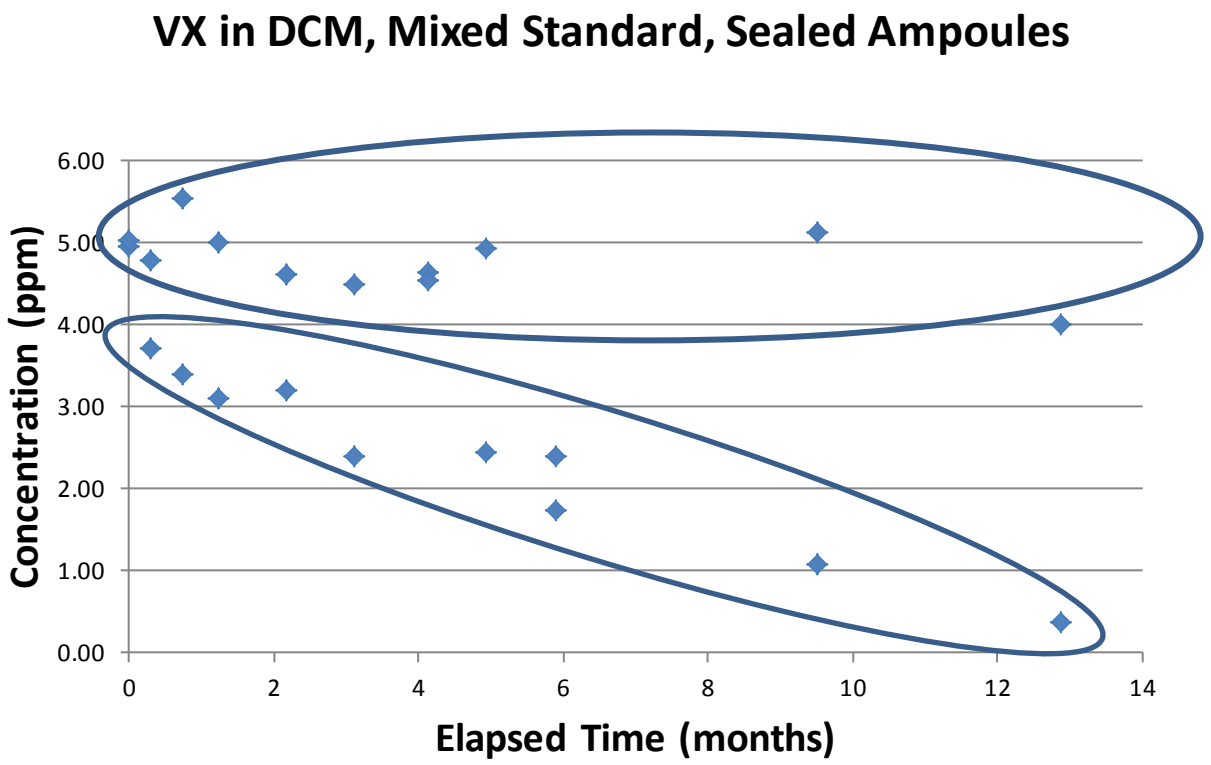


Figure 8. Concentration (ppm) over time (months) for VX in a multiple-component standard in dichloromethane, in sealed ampoules.

Multiple-component standards in hexane stored in screw-capped vials.

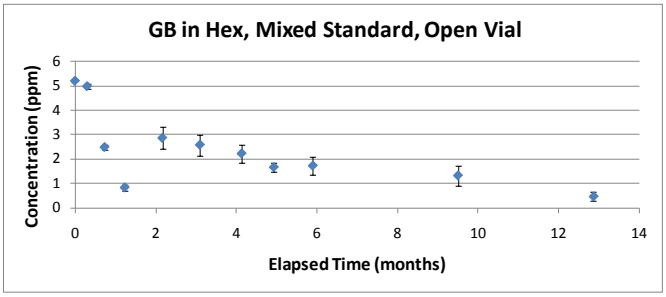
Figure 9 shows the CWA concentrations as a function of time for the ultra-dilute CWA standard mix that was prepared in hexane, sealed in ampoules, opened, then stored at 4 ºC in vials sealed with Teflon-lined screw caps. Each point represents the average measured concentration from three different vials (two vials were analyzed at t = 0) and the error bars represent ± the standard deviation of the measurements. GB was not stable in this sample set. By three weeks, statistically significant decreases in GB concentrations were detected and subsequent data collected at other time points revealed a steady loss of GB. Only 10% of the GB remained in the mixed standard in hexane after a year. GD showed a significant decrease in concentration at Month One. However, at this time point, only an 8% decrease in the initial GD concentration was observed. No statistically significant loss of GF was observed for the duration of the one-year period. HD was stable for first three months, but approximately 40% of the HD was lost during the remainder of the study. No statistically significant VX degradation occurred during this study; 80% of the initial VX remained after twelve months.

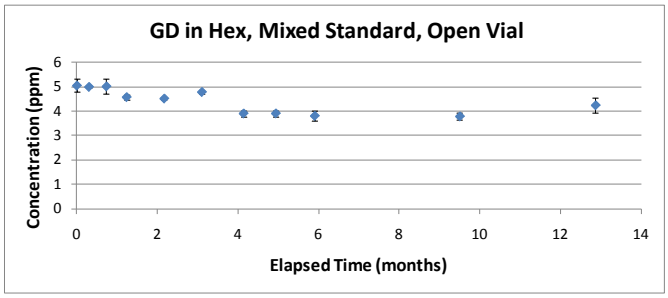
Multiple-component standards in hexane, stored in sealed ampoules.

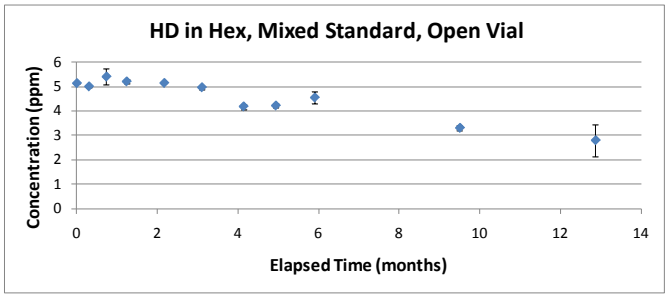
Figure 10 shows CWA concentrations as a function of time for the ultra-dilute CWA standard mix that was prepared in hexane, sealed in ampoules, stored at 4 ºC, and opened immediately prior to analyses. Each point represents the average measured concentration from two different ampoules (three vials were analyzed at t = 9 days) and the error bars represent ± the standard deviation of the measurements. No statistically significant losses were observed for either GB or GD for the first nine months, but by the one year time point a 50% drop in GB concentration occurred and a 30% drop in GD concentration occurred. GF was stable during the course of the study and no loss was detected. No statistically significant loss was observed for HD during first six months of the study, but a 30% loss of HD had occurred by the one-year mark. Loss of VX became statistically significant after Month Six, with a 60% loss of VX measured at the study's end.

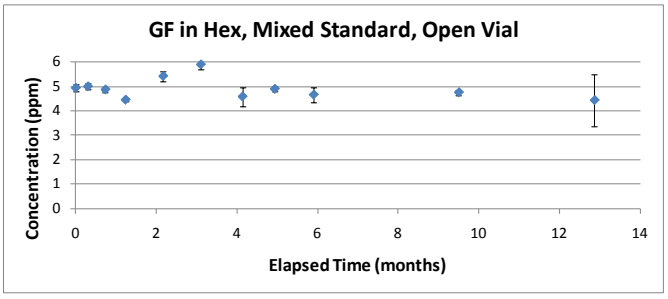
Comment on VX Stability (with regards to the ultra-dilute standards in dichloromethane)

VX was the most susceptible CWA to loss during storage. This loss was attributed to chemical degradation. VX was also the analyte that the EPA's CWA laboratories had found most prone to degradation (from discussions during several of EPAs CWA Protocol Teleconferences). Initially, LLNL had supplied VX to EPA's CWA labs as a multiple-component standard containing GB, GD, GF, HD, and VX in DCM. After the first CWA laboratories began working with the multiple-component standards, they too observed VX losses. LLNL therefore began shipping VX to the CWA laboratories as a single-component solution in DCM. GB, GD, GF, and HD were shipped to the CWA laboratories as a four-component mixture, also in DCM. Below is a discussion of the degradation issues that were observed with ultra-dilute VX standards. The discussion focuses on the VX standard in DCM because the ultra-dilute VX standard that is currently being shipped to the ERLN laboratories is diluted in this solvent.









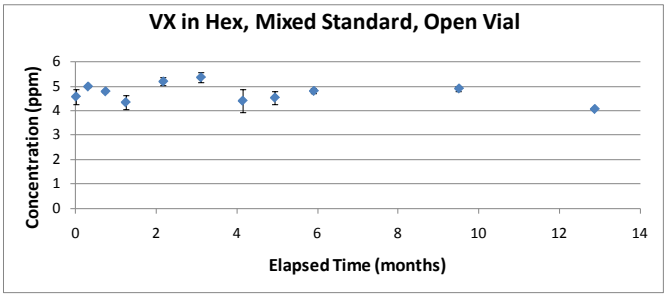


Figure 9. Concentration (ppm) over time (months) for chemical warfare agents present in hexane as a multiple-component standard, in reopened vials.

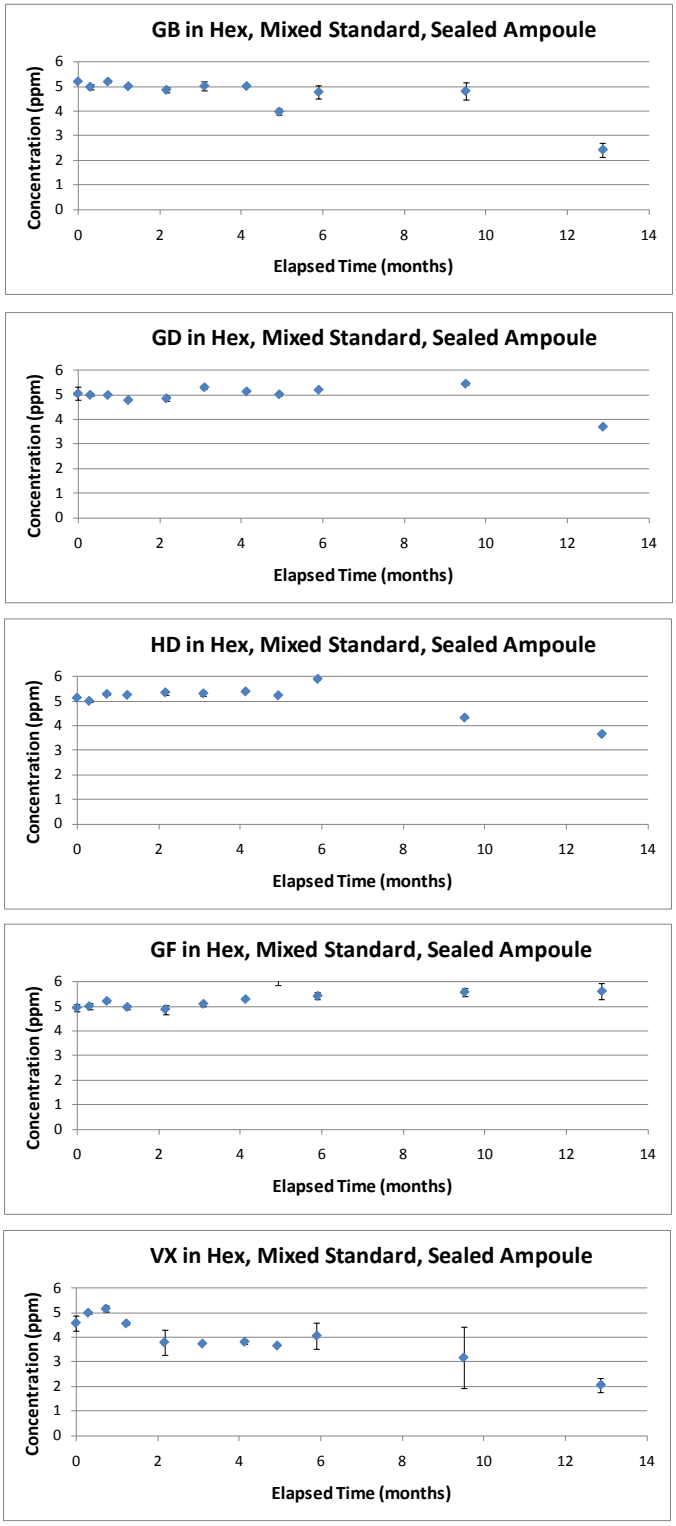


Figure 10. Concentration (ppm) over time (months) for CWAs present in hexane as a multiple-component standard in sealed ampoules.

Some of the individual solutions of 10 ppm VX in DCM that were stored in sealed ampoules were stable for the duration of the study (see Figure 3). This same VX solution, when stored in screw-capped vials and subjected to periodic openings at the designated analysis times, experienced statistically significant loss of VX after nine months (see Figure 2). Using gas chromatography/mass spectrometry (GC/MS), the main breakdown products observed in the ultra-dilute VX standards were 2-(diisopropylamino)ethanethiol (DESH), O,O-diethyl dimethylpyrophosphonate (Pyro A), and *bis*[2-(diisopropylamino)ethyl] disulfide [$(DES)_2$], an oxidation product formed by dimerization of two DESH molecules. Derivatization of the VX standard using N,O-*bis*(trimethylsilyl)-trifluoroacetamide (BSTFA) confirmed the presence of ethyl methylphosphonic acid, another compound produced from the hydrolysis of VX (Buckles et al., 1977; Yang et al., 1996; Yang, 1999). Mass spectra of the VX degradation products have been compiled in Appendix A.

The GC-FPD chromatograms of Figure 11 show the presence of the Pyro A degradation product (doublet at 16.7 min) of VX (peak at 21.1 min) in one-year-old standards. Under the separation conditions used (temperature program of 40 ºC for 3 min, ramped at 8 ºC/min to 300 ºC, and held at 300 ºC for 3 min, with helium as a carrier gas at a constant flow of 1.4 mL/min, with HP-5ms GC column, 30 m x 0.25 mm i.d. x 0.25 µm film thickness), the Pyro A was chromatographically resolved into two peaks, each representing stereoisomers which arise because of the two stereogenic phosphorous centers (Benschop and De Jong, 1988). While other groups have also observed this analyte as a VX degradation product, Pyro A is usually reported as a single peak in the literature because of the particular GC conditions used. Separation strategies used by other workers (e.g., larger diameter GC columns, faster oven temperature ramps) have produced separations with lowered chromatographic resolution. Other experimenters were therefore not able to observe the stereoisomers (e.g., Brevett et al., 2008; D'Agostino et al., 1987; Rohrbaugh, 2000). Symmetrical pyrophosphonates are known to be formed through the breakdown of VX (Yang et al., 1996) and present as impurities of and/or formed during the breakdown of nerve agents (Kumar et al., 2008).

Pyro A was formed by the reaction of ethyl methylphosphonic acid (EMPA) with VX; see Scheme 1. EMPA is a well-known hydrolysis product of VX (Rohrbaugh 1998). It was confirmed, experimentally, that Pyro A was produced by reaction of VX and EMPA by preparing a DCM solution containing both VX and EMPA, each at 50 ppm and then monitoring the solution composition over the course of several days. Pyro A was observed to be produced in this reaction mixture.

Although Pyro A (doublet at 16.7 min) appears in the chromatogram of the individual VX standards that experienced some degradation, another doublet (at 16.9 min), representing a second VX degradation product, appeared in the five-component solutions; see Figure 12. (GC conditions for this analysis were: 40 ºC for 3 min, ramped at 8 ºC/min to 300 ºC and held at 300 ºC for 3 min, with helium as a carrier gas at a constant flow of 1.4 mL/min, with a HP-5ms GC column, 30 m x 0.25 mm i.d. x 0.25 µm film thickness.) This compound was identified as O-ethyl, O-isopropyl dimethylpyrophosphonate (Pyro B) — a pyrophosphonate formed by the reaction of VX with isopropyl methyl phosphonic acid (IMPA), a contaminant present in the GB solution. This unsymmetrical pyrophosphonate was unique to the multiple-component standard and was not present in the standards that contained VX

only. Scheme 2 shows the reaction pathway for the production of Pyro B.

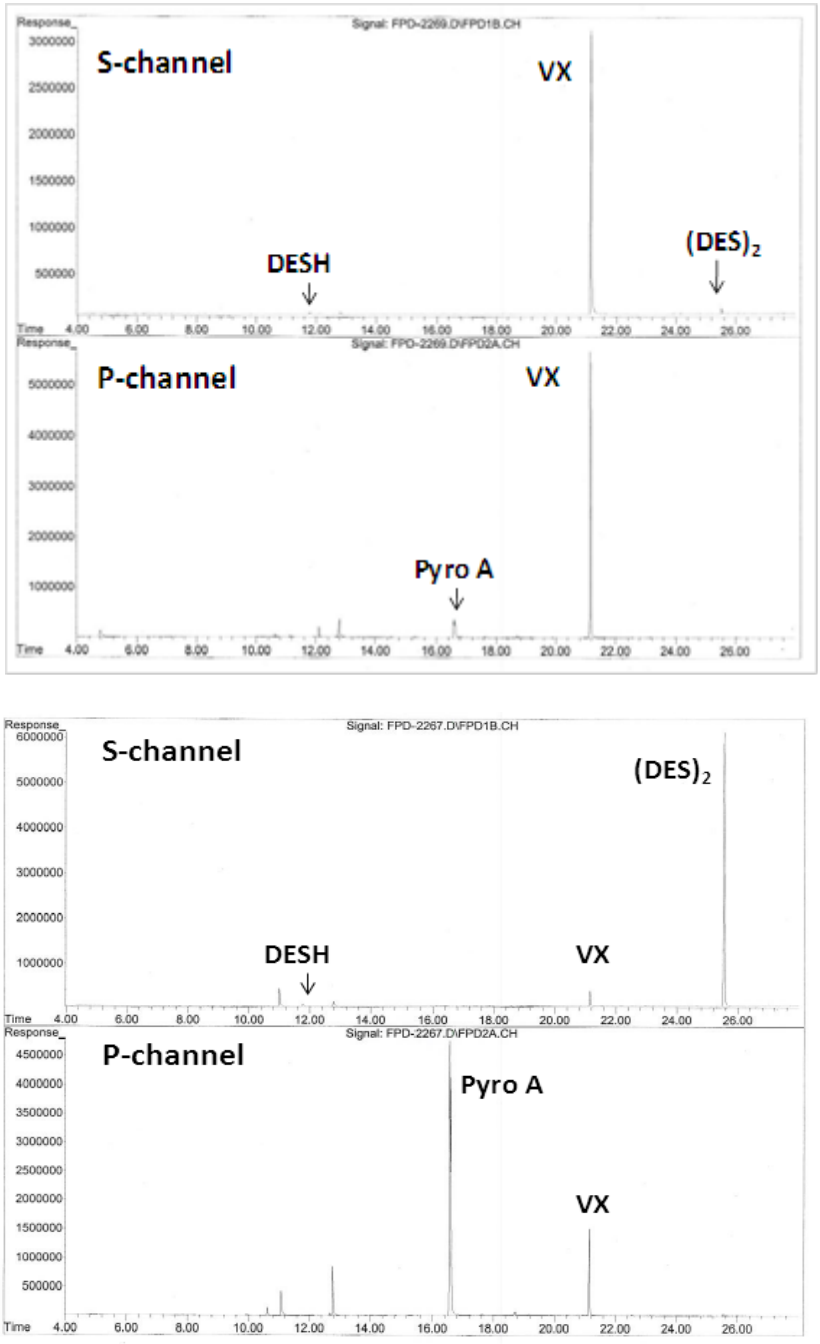
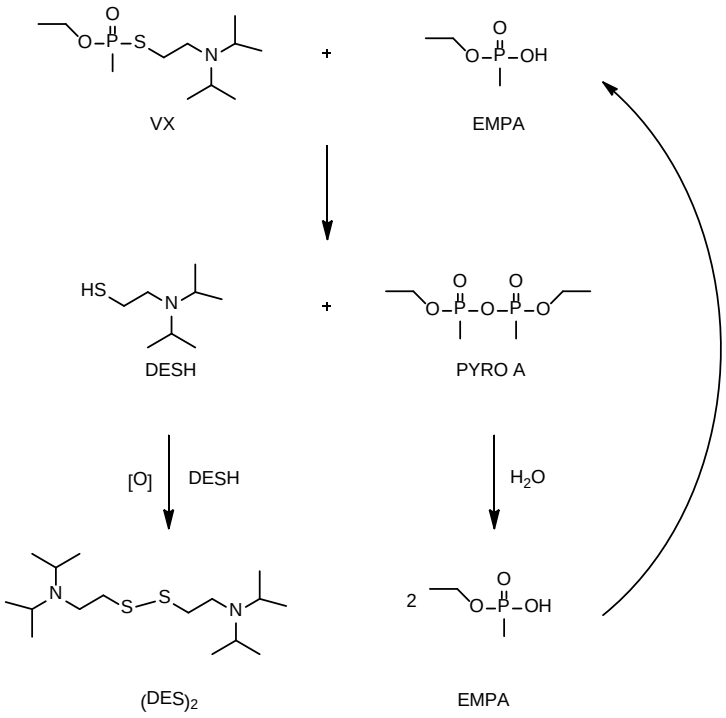
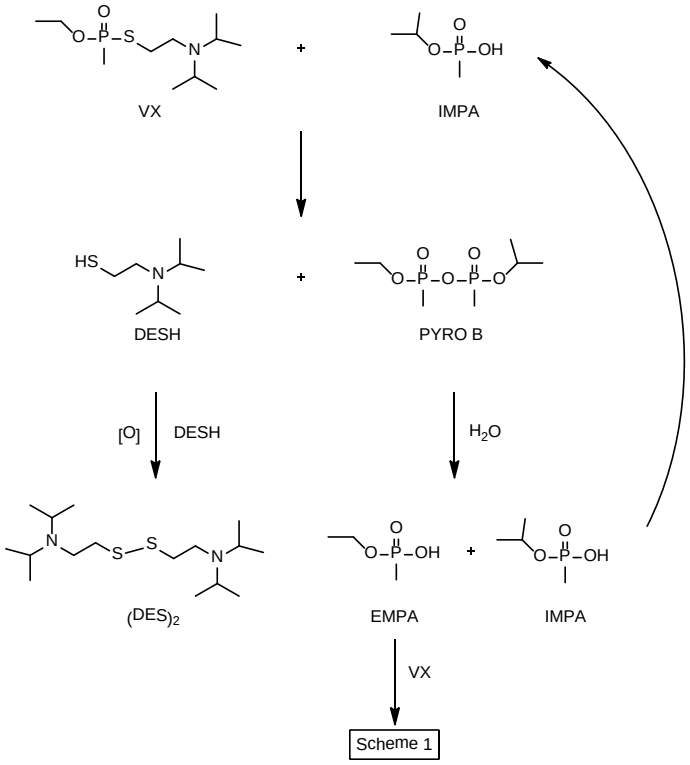


Figure 11. GC-FPD chromatograms (both S and P channels) of single-component VX standards in dichloromethane after one year of storage, at 4 ºC, in a sealed ampoule (top) and in a Teflon-lined, septum-capped vial (bottom).

Scheme 1. Autocatalytic degradation mechanism for VX initiated by EMPA.



Scheme 2. Autocatalytic degradation mechanism for VX initiated by IMPA.



EMPA – Ethyl methylphosphonic acid
IMPA – Isopropyl methylphosphonic acid
Pyro A – O,O-Diethyl-dimethylpyrophosphonate
Pyro B – O-Ethyl, O-isopropyl-dimethylpyrophosphonate
DESH – 2-(N,N-diisopropylamino)ethanethiol
$(DES)_2$ – *bis*[2-(diisopropylamino)ethyl] disulfide

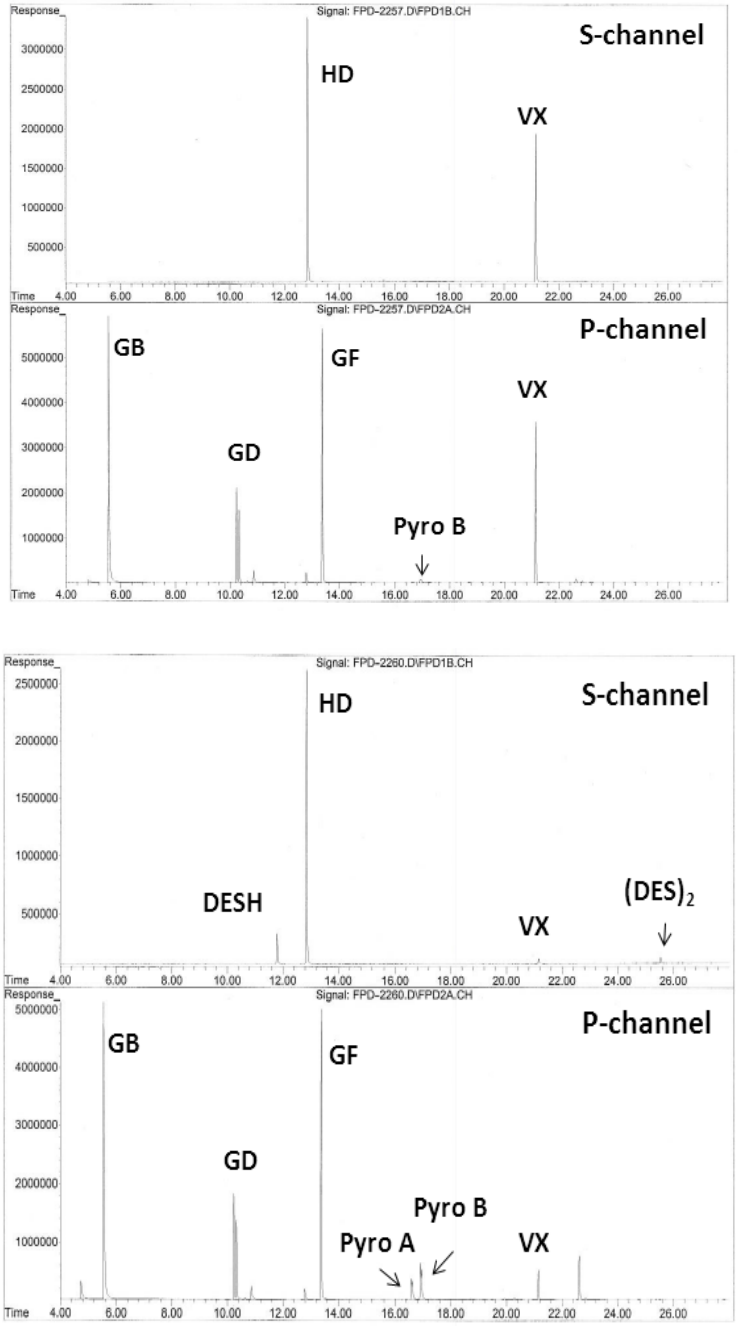


Figure 12. GC-FPD chromatograms of multiple-component chemical warfare agent standards in dichloromethane after one year of storage, at 4 ºC, in a sealed ampoule (top) and in a Teflon-lined, septum-capped vial (bottom).

It was confirmed that the breakdown of VX in the multiple-component standards was initiated by the presence of IMPA by performing a simple experiment in which VX and IMPA, both at 50 ppm in DCM, were allowed to react, at room temperature, over the course of several days. Pyro B was produced by this reaction. This reaction provides an explanation for the faster degradation of VX in the multiple-component standards. In the multiple-component standards, the initial attack of VX by IMPA initiates the autocatalytic breakdown cycle of VX (e.g., Yang et al., 1996) and, as this cycle proceeds, the production of both pyrophosphonate compounds occurs; see bottom chromatogram of Figure 12 and also Scheme 2. A review of the GC/MS data of the freshly-prepared (t = 0), five-component standard in DCM showed traces of Pyro B; however, Pyro A, formed through the autocatalytic breakdown cycle of VX (Buckles et al., 1977; Yang et al., 1996), did not appear until after the formation of Pyro B.

The above discussion helps explain the observations from this study. IMPA was not present in the VX-only standard and, therefore, VX was stable in a sealed ampoule. However, the individual VX standards exhibited loss of VX after being opened to the atmosphere, perhaps through hydrolysis from trace amounts of water introduced during storage and handling. The solvents used for the preparation of all the standards were ultrapure solvents but not anhydrous, and no additional water removal was done prior to standard preparation. Because these CWA standards contained analytes at 5 or 10 µg/mL (ppm), the trace water present in the solvents was likely present at concentrations much higher than those of the CWAs and, therefore, at a stoichiometric excess. For the solvents used in this study, vendor labeling gave an upper limit of 500 ppm water in hexane and 200 ppm water in the DCM (determined by Karl Fischer titration). In such a situation, hydrolytic degradation, once initiated, may proceed to completion (Yang et al., 1996; Brevett et al., 2009).

This study suggests that there are several factors affecting the stability of VX in dilute solutions. As discussed above, VX can react with impurities and water present in the standards. As the standard solutions age, increasing amounts of water might also be introduced into the solution from the ambient environment, shortening the shelf life of the standard. Clearly, potential factors affecting the stability of VX should be systematically studied and strategies for providing ultra-dilute standards with longer shelf lives should be studied.

Conclusions

Statistically significant changes (as determined by Dunnett's Test and described in the third paragraph of the "Results and Discussion" section) in concentrations occurred with some CWAs during refrigerated storage (4 ºC ± 2ºC) and the stabilities of the CWAs were compound-dependent. Results for individual standards showed that all analytes (GB, GD, GF, HD, and VX) were stable for 12 months when prepared in DCM and stored in sealed ampoules. GD and GF, as individual standards, were stable for 12 months under all of the conditions studied (i.e., both in hexane and DCM in sealed ampoules and screw-capped vials). Overall, the CWA standards were more stable in the sealed ampoules, when compared to the screw-capped vials. Table 2 summarizes the study findings and provides estimated analyte shelf life. The estimated analyte shelf life is defined as the time point before which the decrease in analyte concentration was determined to be statistically significant, by Dunnett's Test, from its initial concentration and was followed by other statistically significant decreases in concentration in subsequent month(s). For compounds that showed no degradation, 12 months, which represented the project duration, is used as the default shelf life.

During the course of this study, VX exhibited the most degradation. Anecdotal evidence also suggested that the ERLN laboratories observed VX degradation in the CWA standards that were shipped to them. These observations illustrate the reactivity of VX and the potential difficulty of preparing VX standards that will remain stable over an extended period of time. VX stability depends on the presence and, presumably, also the concentrations, of impurities (some of which can arise from other CWAs). Future work could be performed to determine how VX can be stabilized in the ultra-dilute standards. Stabilization strategies might include: (a) the removal of EMPA and IMPA from the solution, which would prevent the mechanisms shown in Schemes 1 and 2, (b) implementation of a stringent water removal strategy, and (c) the use of a stabilizer to prevent VX degradation (Buckles et al., 1977). Of these strategies, the last two might be the most feasible to implement because the research team has detected EMPA in newly-synthesized VX, even after a washing process should have removed it; (IMPA is an impurity of GB and only an issue if VX is shipped in a mixture containing GB).

The results of this study may be used to guide the procurement and replacement schedules of ultra-dilute CWA standards used by the ERLN Laboratories. Currently, all ultra-dilute CWA standards shipped to the ERLN laboratories are made in DCM. VX, at 10 ppm, is made as a single-analyte solution in DCM; data from this study suggest that VX in this standard remains stable for one year. Once opened and/or combined with other CWAs (as is frequently done when GC/MS calibration curves are made), this VX standard should be used or replaced within one week. The remaining CWAs, including GB (10 ppm), GD (5 ppm), GF (10 ppm), and HD (5 ppm), are shipped as multiple-component standards, also in DCM, to the ERLN laboratories. While the multiple-component CWA standard used in this study, which contained VX, is different from the multiple-component CWA standard currently being shipped to the ERLN laboratories, the data collected may still be used to provide stability guidance. The reasonable expectation is that the multiple-component CWA standards will remain stable for six months in sealed or opened ampoules. However, if these standards were shipped as

single-component solutions in DCM, they could be kept for one year. For convenience in planning and as a simple rule of thumb, the research team recommends that all CWA standards in sealed ampoules be used within six months of production and that, once opened, all CWA standards that do not contain VX be kept for no longer than six months and VX-containing mixed standards be used within a week (individual VX standards stored in screw capped vials can be stable for up to six months).

Table 2. Summary of stability study data and estimated shelf lives when standards are stored at 4 ºC.

Estimated Shelf Lives[a,b] for CWAs in Single-Component Solutions (months)				
	Dichloromethane		**Hexane**	
	Screw-cap Vial	**Sealed Ampoule**	**Screw-cap Vial**	**Sealed Ampoule**
GB	**5** (0%)	**12** (+6%)	**4** (–6%)	**2** (–4%)
GD	**12** (0%)	**12** (+9%)	**12** (–4%)	**12** (–9%)
GF	**12** (0%)	**12** (+2%)	**12** (–11%)	**12** (–9%)
HD	**6** (+3%)	**12** (+11%)	**4** (+4%)	**6** (+18%)
VX	**2** (–3%)[c]	**12** (+1%)	**5** (–15%)	**3** (–13%)
Estimated Shelf Lives[a,b] for CWAs in Multiple-Component Solutions (months)				
	Dichloromethane		**Hexane**	
	Screw-cap Vial	**Sealed Ampoule**	**Screw-cap Vial**	**Sealed Ampoule**
GB	**9** (+16%)	**12** (+5%)	**0.3** (–4%)	**9** (–8%)
GD	**12** (+1%)	**6** (+21%)	**0.7** (–0.6%)	**9** (+8%)
GF	**12** (–5%)	**12** (+9%)	**12** (–10%)	**12** (+14%)
HD	**6** (–7%)	**6** (+13%)	**3** (–3%)	**6** (+15%)
VX	**0.2** (–16%)	≤**1** (–11%)[c]	**12** (–11%)	≤**1** (0%)[c]

Notes:
[a] Estimated shelf life is defined as the time point prior to that for which a statistically significant decrease in concentration was detected by Dunnett's Test.
[b] Numbers in parentheses give the percent change in concentration from t = 0 to the concentration measured at the estimated shelf life time; note that, on average, the percent standard deviation for replicate measurements made at the shelf life time agreed within 5% (range of 0.4% to 24%, excluding VX datapoints indicated with "c").
[c] Because large variabilities between replicate analyses were noted (relative standard deviation >50%), shelf-life was based on best judgement rather than the results of the Dunnett's Test.

References

Benschop H. P. and De Jong L. P. A. (1988) *Nerve agent stereoisomers: Analysis, isolation and toxicology*. Acc. Chem. Res. 21(10), 368-374.

Brevett C. A. S., MacIver B. K., Sumpter K. B. and Rohrbaugh D. K (2008) *SSMAS NMR Study of HD, GD, and VX on Carbon Fiber Textiles for Wipes. Report ECBC-TN-035*, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD.

Brevett C. A. S., Sumpter K. B., Pence J., Nickol R. G., King B. E., Giannaras C. V. and Durst H. D. (2009) *Evaporation and degradation of VX on silica sand*. J. Phys. Chem. 113(16), 6622-6633.

Buckles L. C., Lewis S. M. and Lewis F. E. (1977) *S-(2-diisopropylamino-ethyl) O-ethyl methylphosphonothiolate stabilized with soluble carbodiimides*. United States Patent 4,012,464.

D'Agostino P. A., Provost L. R. and Visentini J. (1987) *Analysis of O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) by capillary column gas chromatography-mass spectrometry*. J. Chromatogr. 402, 221-232.

Hsu, J. C. (1996) *Multiple Comparisons, Theory and Methods* (Chapter 3), Chapman & Hall, NY (ISBN 0 412 98281 1).

Kumar R., Pardasani D., Mazumder A. and Dubey D. K. (2008) *Microwave induced synthesis of O,O-dialkyl dialkylpyrophosphonates under solvent free conditions: Markers of nerve agents*. Aust. J. Chem. 61, 476-480.

Rohrbaugh D. K. (1998) *Characterization of equimolar VX-water reaction product by gas chromatography-mass spectrometry*. J. Chromatogr. 809, 131-139.

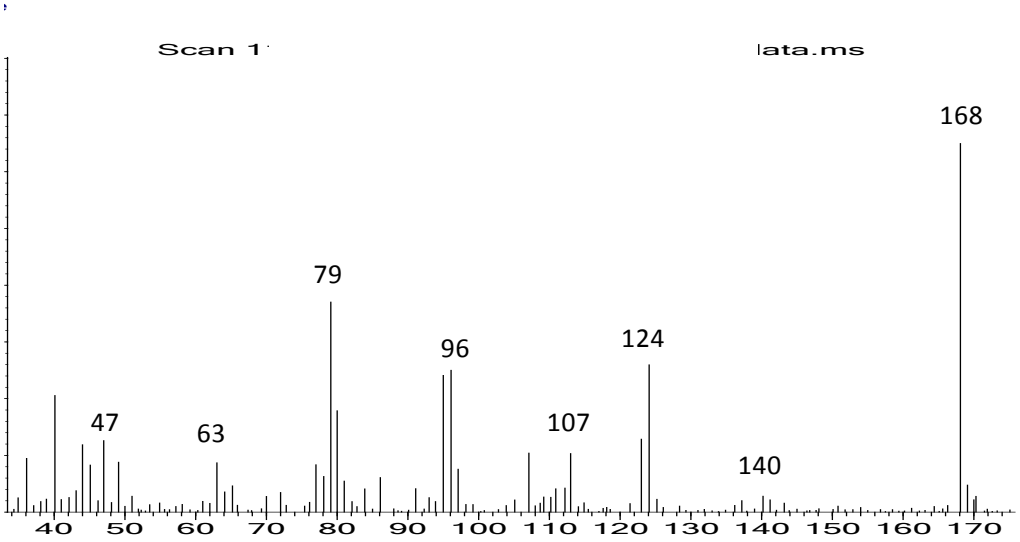
Rohrbaugh D. K. (2000) *Methanol chemical ionization quandrupole ion trap mass spectrometry of O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) and its degradation products*. J. Chromatogr. A 893(2), 393-400.

Yang Y.-C. (1999) *Chemical detoxification of nerve agent VX*. Acc. Chem. Res. 32(2), 109-115.

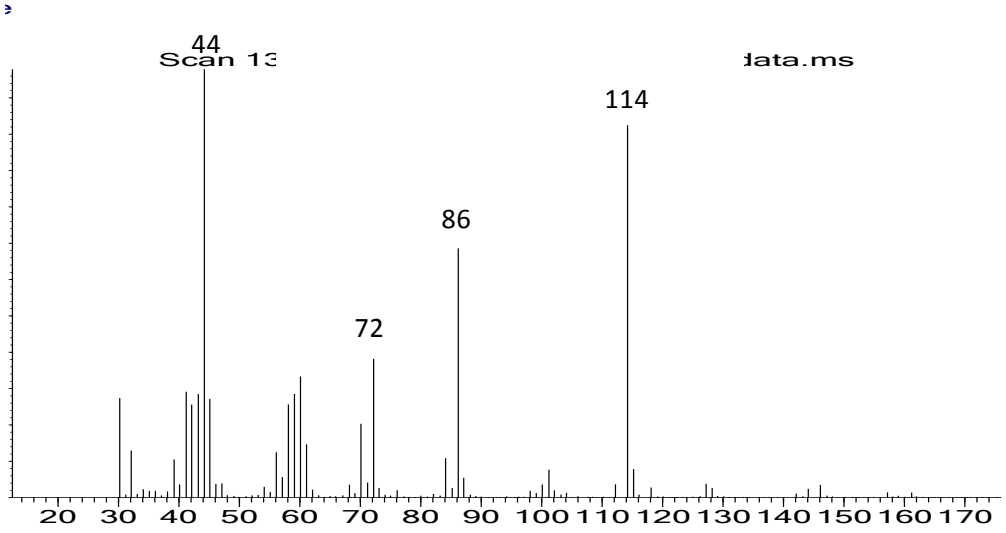
Yang Y.-C., Szafraniec L. L., Beaudry W. T., Rohrbaugh D. K., Procell L. R. and Samuel J. B. (1996) *Autocatalytic hydrolysis of V-type nerve agents*. J. Org. Chem. 61(24), 8407-8413.

Appendix A: Mass Spectra of VX Degradation Products

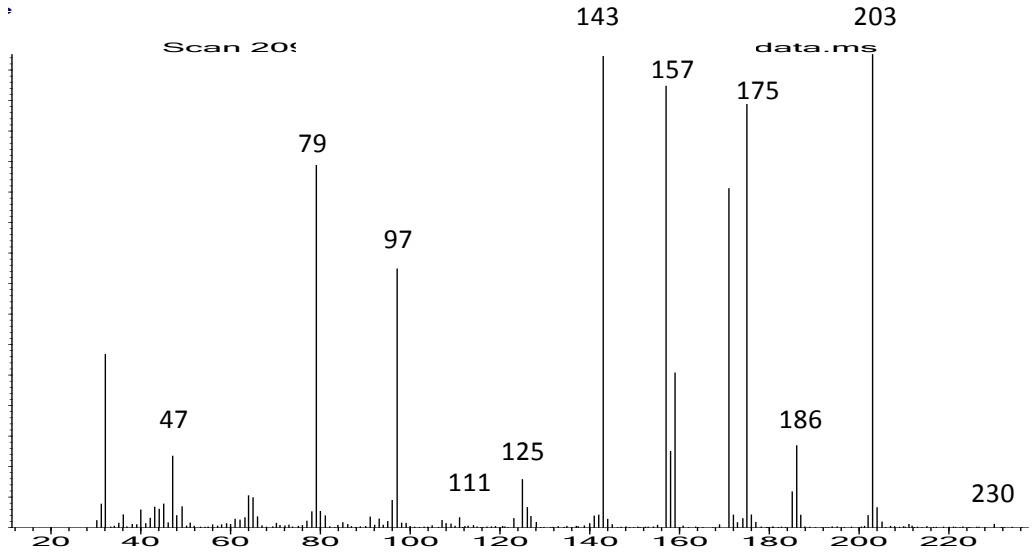
The mass spectra and retention times presented here were collected using an HP-5ms column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). GC conditions for this analysis were: 40 ºC for 3 min, ramped at 8 ºC/min to 300 ºC, and held at 300 ºC for 3 min, with helium as a carrier gas at a constant flow of 1.4 mL/min. Retention times (RT) and monoisotopic molecular weights (MW) are provided for reference.



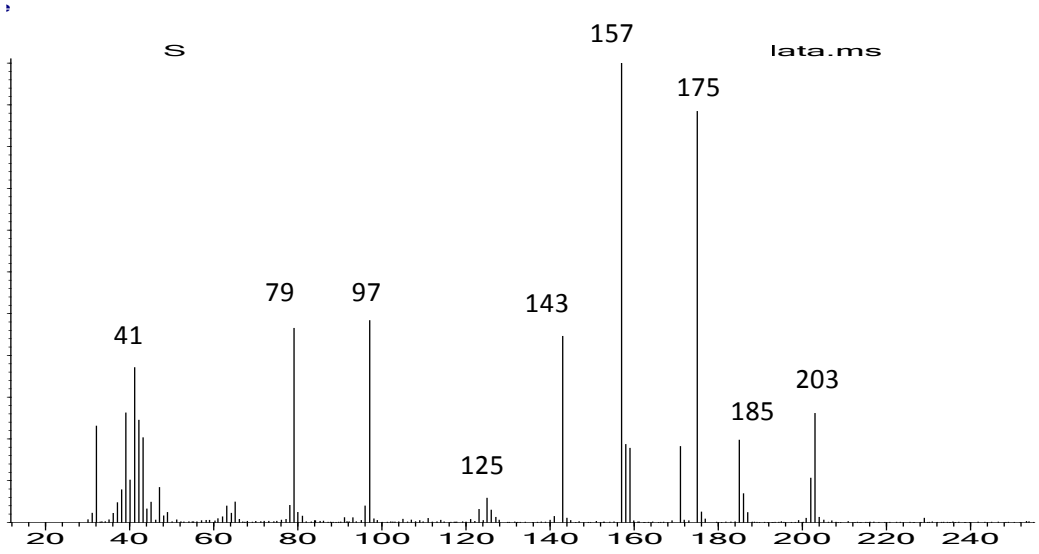
Diethyl methylphosphonothioate. RT = 10.6 min. MW= 168.



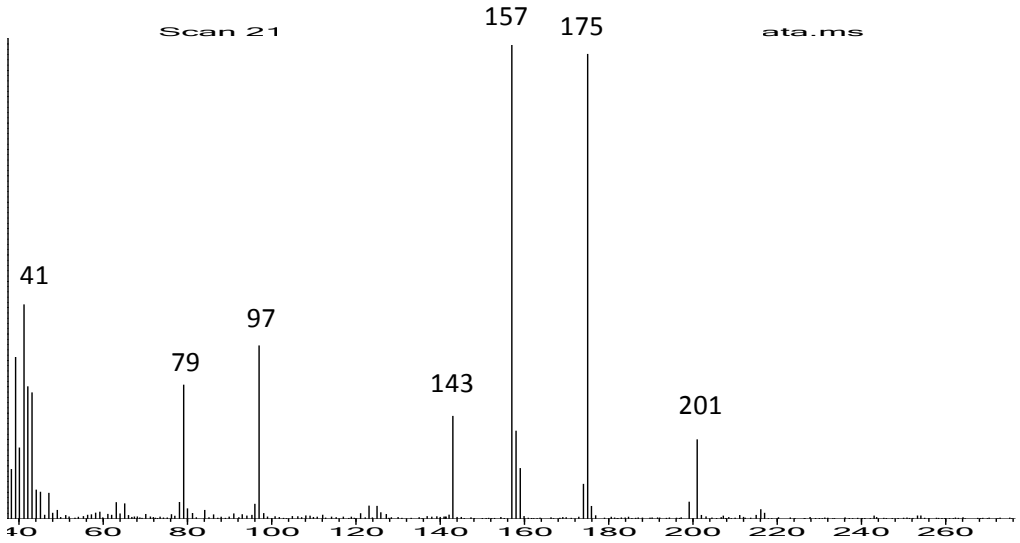
2-(Diisopropylamino)ethanethiol (DESH). RT = 11.7 min. MW= 161.



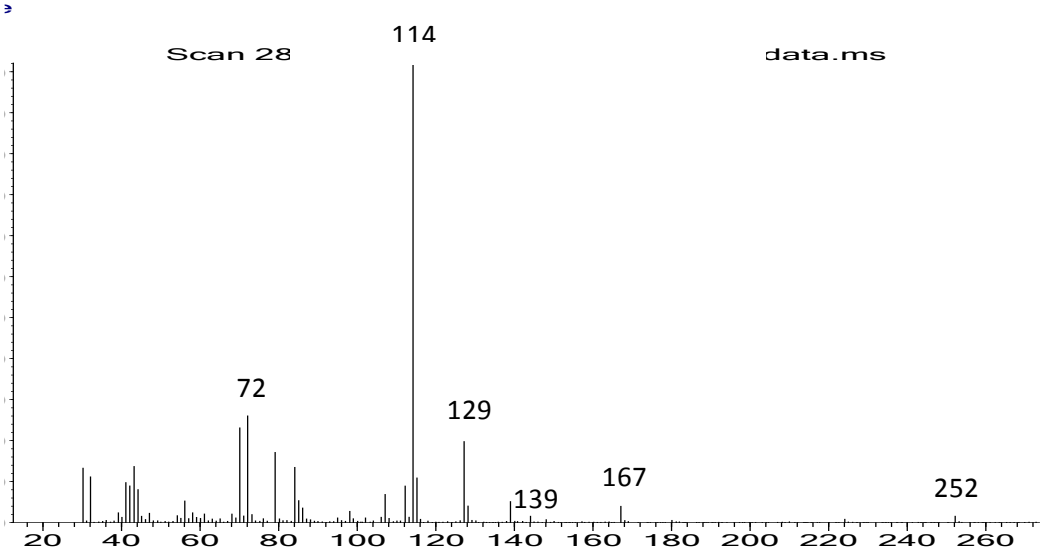
***O,O*-Diethyl dimethylpyrophosphonate (Pyro A)**. RT = 16.6 min. MW= 230.



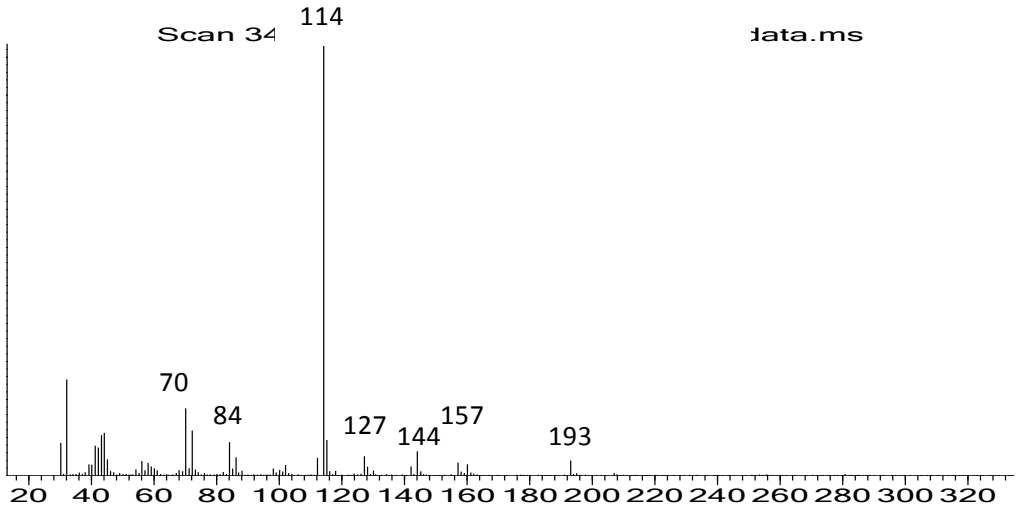
***O*-Ethyl, *O*-isopropyl dimethylpyrophosphonate (Pyro B)**. RT = 17.0 min. MW= 244.



Diisopropyl dimethylpyrophosphonate. RT = 17.3 min. MW= 258.



VX. RT = 21.2 min. MW= 267.



Bis(diisopropylaminoethyl)disulfide (DESH-dimer). RT=25.7 min; MW=320.

EPA
United States
Environmental Protection
Agency

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT NO. G-35

Office of Research and Development (8101R)
Washington, DC 20460

Official Business
Penalty for Private Use
$300