Executive Summary of *Chemical Warfare Agents and their Hydrolysis Products from the US EPA Standardized Analytical Methods* and *GC-MS Analytical Method for the Analysis of Chemical Warfare Agent Degradation Products Listed in the EPA Standardized Analytical Methods*
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ABSTRACT

The analysis of Chemical Warfare Agents (CWA) and their associated degradation products has been left to the United States defense agencies until the threat of terrorism in the US became a real issue. The method to detect, and quantify CWA's and their associated degradation products will be under control of the US EPA for any site contaminated with these agents used during a homeland security incident. The concentrations of the degradation products will need to be quantified in water, soil, and air to ensure that they do not present any health and welfare issues. The Gas Chromatography/Mass Spectrometer (GC-MS) method is based on EPA method 8270D. The degradation products of the blister agents can be directly analyzed; no derivatization is required. The sample preparation required for any of the soil and water matrices would be to extract the compounds from the matrix, derivatize the organophosphate compounds/nerve agents, prepare the GC-MS solutions with appropriate internal standards, and analyze the mixture. The list of compounds the EPA will focus efforts on comes from the EPA document, *Standardized Analytical Methods for Environmental Restoration following Homeland Security Events,* 3rd edition.

HISTORY OF THE NERVE AGENTS

The history of nerve agents began on 23 December 1936, when Dr. Gerhard Schrader of the I. G. Farbenindustrie laboratory first prepared Tabun (ethyl dimethylphosphoramidocyanidate, GA). Schrader had been in charge of a program to develop new types of insecticides since 1934, working first with fluorine-containing compounds such as acyl fluorides, sulfonyl fluorides, fluoroethanol derivatives, and fluoroacetic acid derivatives. In 1935, he prepared dimethylphosphoramidofluoridic acid as a continuation of the previous line of research. He obtained patents for this compound in Germany, the United Kingdom, Switzerland, and the United States, and began to systematically investigate the dimethylphosphoramides, leading to the preparation of Tabun. Schrader found that Tabun was extremely potent against insects; 5 ppm of Tabun killed all the leaf lice he used in his initial experiment. In January 1937, he was the first to observe the effects of nerve agents on human beings when he and a laboratory assistant began to experience meiosis (contraction of the pupils of the eyes) and shortness of breath because of their exposure to Tabun vapor in the laboratory. As Harris and Paxman noted, Schrader and his assistant "were lucky to escape with their lives."

In 1935, the Nazis had passed a decree which required all inventions of possible military significance to be reported to the Ministry of War. A sample of Tabun was sent to the chemical warfare (CW) section of the Army Weapons Office at Berlin-Spandau in May 1937, and Schrader was summoned to Berlin to give a demonstration. At that time Schrader's patent application was made secret. Colonel Rödriger, head of the CW section, ordered the construction of new laboratories for the further investigation of Tabun and other organophosphate compounds. Schrader soon moved to a new laboratory at Wuppertal-Elberfeld in the Ruhr valley.

MANUFACTURE OF TABUN BY NAZI GERMANY

In 1939, a pilot plant for Tabun production was set up at Munster-Lager, on Luneberg heath near the German Army proving grounds at Raubkammer. In January 1940, the Germans began construction of the full scale plant, code named *Hochwerk*, at Dyernfurth-am-Oder (now Brzeg Dolny in Poland), on the Oder River 40 km from Breslau (now Wroclaw) in Silesia. The plant covered an area 1.5 by 0.5 miles and was completely self-contained, synthesizing all intermediates as well as the final product, Tabun. The facility had an underground plant for filling munitions, which were then stored at Krappitz (now Krapowice) in Upper Silesia. An IG Farbenindustrie subsidiary, Anorgana GmbH, operated the Tabun plant, as well as all other CW agent production plants in Germany.

The plant took an extraordinarily long period, from January 1940 until June 1942, to become operational. This was due primarily to the difficult nature of the production process. Certain intermediates were so corrosive that the Germans were forced to run all reactions in quartz- or silver-lined vessels. The extreme toxicity of Tabun required that the final production units be enclosed in double glass-lined walls, with a stream of pressurized air circulating between the walls. All units were periodically decontaminated with steam and ammonia.

The Dyernfurth workforce numbered 3,000, all German nationals. Workers were equipped with respirators and clothing made from a rubber/cloth/rubber sandwich; the clothing was discarded after the tenth wearing. Despite these precautions, over 300 accidents occurred before production began, and at least 10 workers were killed during the 2.5 years of operation. Some examples of incidents:

- Four pipe fitters had liquid Tabun drain onto them and die before their rubber suits could be removed.
- A worker had 2 liters of Tabun pour down the neck of his rubber suit and died within 2 minutes.
- Seven workers were hit in the face with a stream of Tabun of such force that the liquid was forced behind their respirators; only two survived despite heroic resuscitation measures.

SARIN: THE SECOND NERVE AGENT

In 1938, a second potent organophosphate nerve agent was discovered. This agent, Sarin (1-isopropyl methylphosphonofluoridate, GB) was named for its four discoverers: *S*chrader, *A*mbros, *R*ödriger, and van der L*in*de. In June 1939, the formula for Sarin was passed to the CW section of the Army Weapons Office at Berlin-Spandau along with a sample of the compound.

All the synthetic routes for Sarin investigated at that time required the use of hydrogen fluoride, which caused severe corrosion problems. Pilot plants were constructed at Spandau, Münster Lager, on Luneberg heath, and pilot production of Sarin was conducted in Building 144 in Dyernfurth. The Dyernfurth Sarin plant is listed as having a capacity of 40 or 100 tons per month. A 500-ton per month production plant was under construction at Falkenhagen, southeast of Berlin, at the end of World War II. Estimates vary for the total Sarin production from 500 kg to 10 tons.

The United States began producing Sarin in the early 1950s and ended regular production in 1956.

THE SECRET OF TABUN GETS OUT

On 11 May 1943, the British captured a German chemist who had worked at the main Army CW research laboratory in Spandau. The prisoner told the British the code name for Tabun (Trilon 83), the chemical reactions by which it was produced, its effects, and methods of use and defense against Tabun. This was compiled into an MI-9 intelligence report of 3July1943. Following the war, the Allies contended that they first became aware of Tabun in April 1945, when a German ammunition dump was captured and a shell containing Tabun was shipped to the United Kingdom for analysis. However, the record appears to show that the 1943 report was ignored.

THE END OF THE WAR

At the end of 1944, Germany had produced 12,000 tons of Tabun: 2,000 tons loaded into projectiles and 10,000 tons loaded into aircraft bombs. These munitions were stored at Krappitz (Krapowice) in Upper Silesia as well as in abandoned mine shafts in Lausitz and Saxony. Some stocks were also transported to Bavaria in anticipation of a last ditch defensive stand by the Nazis.

In August 1944, as the Red Army approached Silesia, and the Western Allies began the race for the German border, the Nazis began systematically destroying documentation of the research on and the manufacture of Tabun and Sarin. In early 1945, Dyernfurth was to be abandoned and tons of liquid nerve agents were simply poured into the Oder. The plant was rigged for demolition, but the Russians surrounded the plant before it could be destroyed. The Luftwaffe was then ordered to bomb the plant, but they also failed to destroy it. It is believed that the Soviets captured both the full-scale Tabun plant and the pilot Sarin plant intact. The Soviets later captured the nearly complete full-scale Sarin plant at Falkenhagen. It has been reported that production at Dyernfurth resumed in 1946 under Russian control.

SOMAN

Richard Kuhn discovered Soman (1,2,2-trimethylpropyl methylphosphonofluoridate, GD) in the spring of 1944, while working for the German Army on the pharmacology of Tabun and Sarin. The documents detailing the discovery were buried in a mineshaft 10 miles east of Berlin, where they were discovered by the Soviets and removed. The Soviets produced and stockpiled Soman during the Cold War.

VX

Several chemical companies and other scientists working independently discovered the potency of a class of organophosphate esters of substituted 2-aminoethanethiols in 1952 and 1953. Almost simultaneously in 1954:

- ICI brought Amiton (*O,O*-diethyl-*S*-[2-(diethylamino)ethyl] phosphorothiolate) to market
- R. Ghosh and J. F. Newman of ICI submitted a manuscript containing the details of this class of compounds (*A New Group of Organophosphate Pesticides*, *Chemistry and Industry*, **1955**, 118)
- Schrader, now at Farbenfabriken Bayer AG, prepared *S*-[2-(diethylamino)ethyl]-*O*-isopropyl methylphosphonothioate

- Tammelin at the Swedish government's chemical warfare defense laboratory prepared *S*-[2-(diethylamino)ethyl]-*O*-ethyl methylphosphonothiolate and *S*-[2-(dimethylamino)ethyl]-*O*-isopropyl methylphosphonothiolate
- Ghosh at ICI prepared *S*-[2-(diethylamino)ethyl]-*O*-ethyl ethylphosphonothiolate

A Soviet team from the I. M. Sechenov Institute in Leningrad had already predicted the anticholinesterase activity of S-2-dialkylaminoethyl phosphono- and phosphorothiolates. The British CW laboratory at Porton began investigating this class of compounds, and notified the US CW laboratory at Edgewood, which began a systematic investigation of the entire class. In 1958, the US selected VX (*S*-[2-[bis(1-methylethyl)amino]ethyl]-*O*-ethyl methylphosphonothiolate) for manufacture. Construction of the production plant began in 1959; production ran from 1961 through 1968.

An interesting footnote concerns the chemical structure of VX, which the US government classified as secret until the early 1970s. The Soviets had learned of the toxicity of the class of compounds. They had either learned the molecular formula ($C_{11}H_{26}NO_2PS$) or they had obtained a garbled version of the structure; as a result, the Soviet V-gas has the structure *S*-[2-(diethylamino)ethyl]-*O*-ethyl isobutylphosphonothiolate, a slightly different compound from VX with the same formula. It is also interesting to note that the Stockholm International Peace research Institute (SIPRI) report, published in 1971, states that the formula for VX is secret, but a table in the report with a speculative structure correctly identifies VX.

PHYSIOLOGICAL ACTION OF NERVE AND BLISTER AGENTS

Production and distribution of Chemical Warfare Agents for the purpose of producing mass casualties is an easy task for any individual or group with just basic chemistry skills. The nerve agents– GA (Tabun), GB (Sarin), GD (Soman), and VX –are potent esterase inhibitors. One of the more important interactions of nerve agents is the inhibition of acetylcholinesterase. Normally, this enzyme degrades (hydrolyzes) Acetylcholine (2-(acetyloxy)-N,N,N-trimethylethanaminium) found in the synapses between nerves (Figure 1).

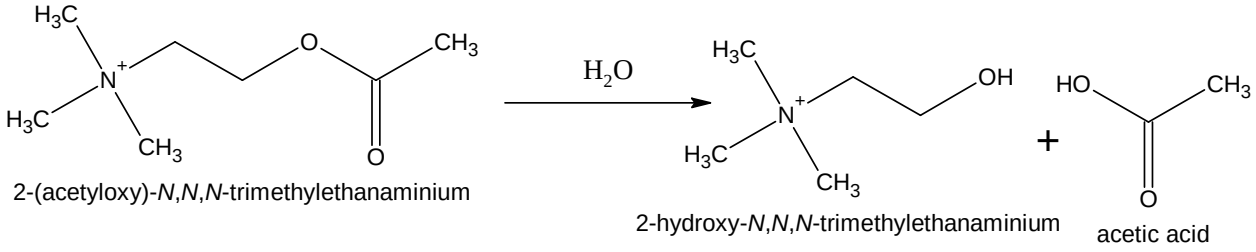


Figure 1. Acetylcholine Hydrolysis

Nerve agents combine with the hydroxyl group of a serine residue of the enzyme, giving an inactive phosphonylated form of the enzyme (shown for GB in Figure 2).

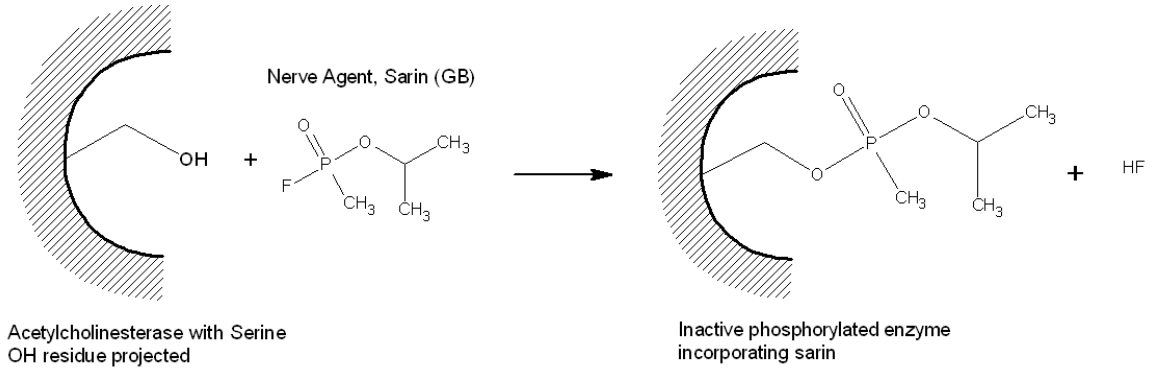


Figure 2. Inactivation of Acetylcholinesterase by the Nerve Agent Sarin

The complex prevents the action of acetylcholinesterase on Acetylcholine and its subsequent breakdown into acetic acid and choline. If Acetylcholine is not broken down, it will continue to fire the postsynaptic receptors, creating constant neuromuscular spasms.

The vesicants, or blister agents, are good alkylating agents, reacting with nucleic acids and various enzymes. The sulfur mustards are bifunctional alkylating agents reacting mainly at the N-7 site on guanine residues, disrupting the normal function of DNA (Figure 3).

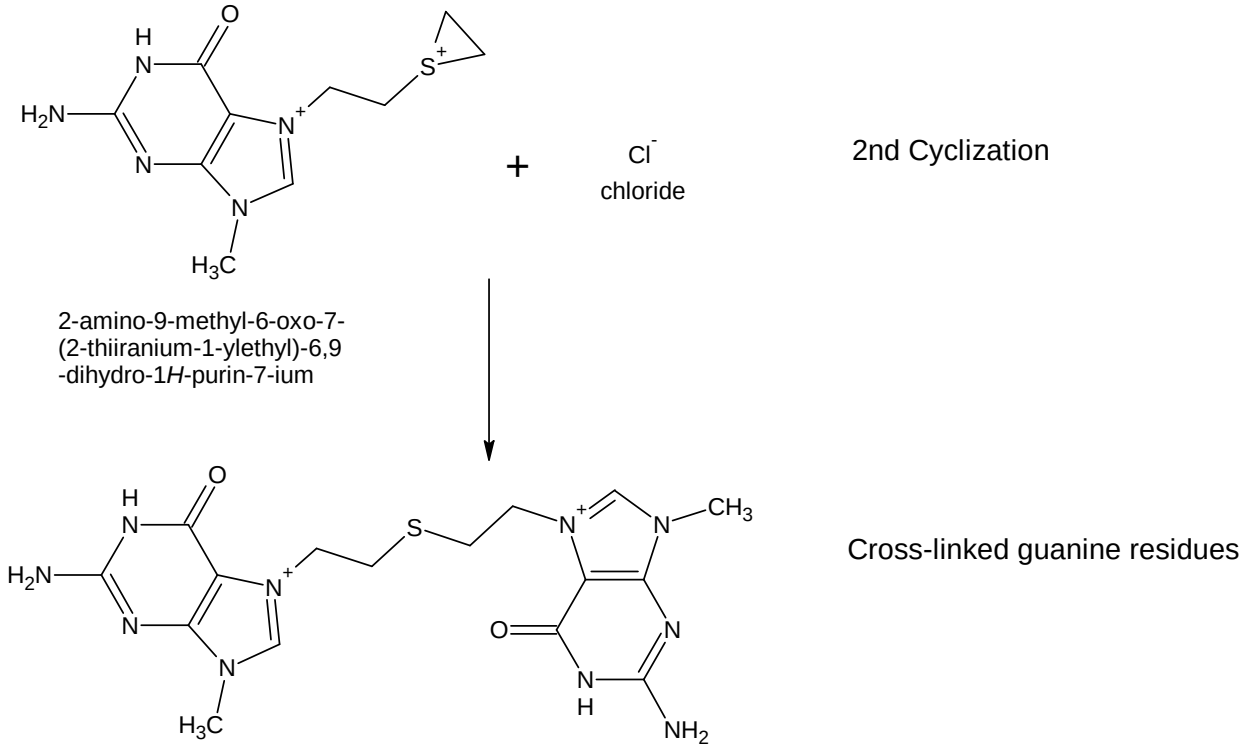


Figure 3. Reaction of Sulfur Mustard with Guanine

About 25% of the alkylations lead to interstrand cross-linking of the DNA (Figure 4).

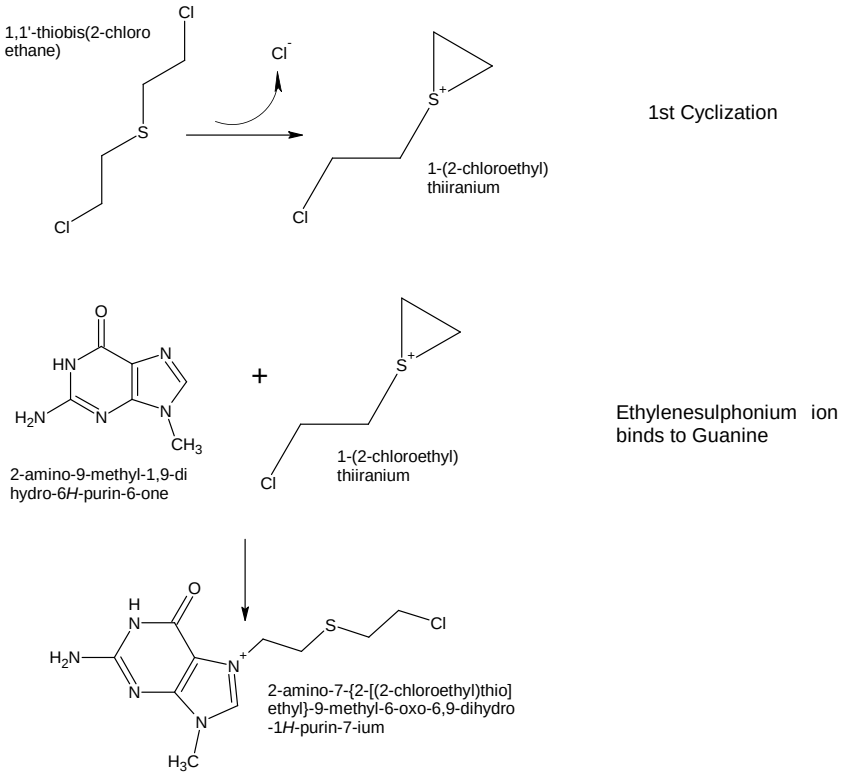
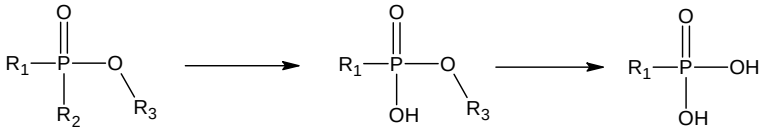


Figure 4. Sulfur Mustard Cross-Linking Guanine Residues

Lewisite, being an Arsenic-containing blister agent is capable of reaction with thiol groups forming –S-As-S- structures. Lewisite binds irreversibly with enzymes containing thiol groups, suggesting that some sort of enzyme inhibition produces the toxic effects noted with Lewisite. Other work has suggested that the toxicity of Lewisite may be some other inherent property not present with inorganic arsenic.

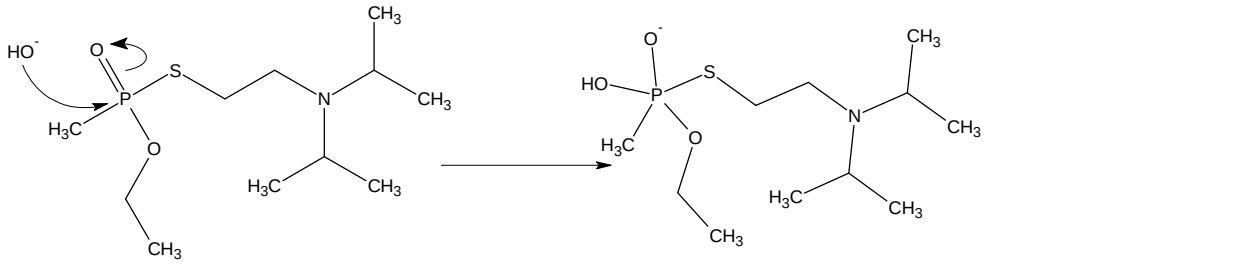
The degradation in the environment of the nerve and blister agents will most likely be from hydrolysis. The G series nerve agents all hydrolyze in a similar manner, shown in Figure 5.



Agent	R_1	R_2	R_3
GA, Tabun	$N(CH_3)_3$	CN	CH_2CH_3
GB, Sarin	CH_3	F	$CH(CH_3)_2$
GD, Soman	CH_3	F	$CH_2(CH_3)C(CH_3)_3$
GF, Cyclosarin	CH_3	F	C_6H_{11}

Figure 5. General Hydrolysis Scheme for the G Series Nerve Agent

The nerve agent VX undergoes hydrolysis by forming a penta-coordinate phosphorus intermediate (Figure 5a).

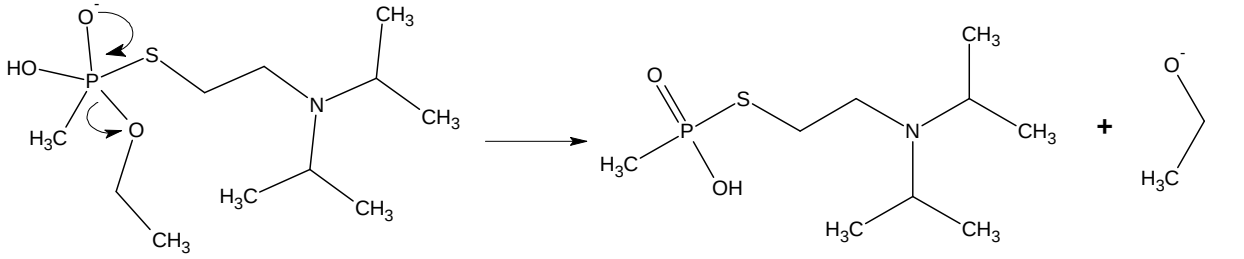


S-[2-(diisopropylamino)ethyl] *O*-ethyl methylphosphonothioate

4-hydroxy-8-isopropyl-4,9-dimethyl-3-oxa-5-thia-8-aza-4λ^5-phosphadecan-4-olate

Figure 5a. VX, Formation of Penta-Coordinate Phosphorus Intermediate

This intermediate can decompose by three pathways, all pH dependant: P-O (Figure 5b), P-S (Figure 5c), or C-S (Figure 5d) cleavage. Below pH 6.5, the P-S cleavage is the only decomposition noted. A pH in the range of 7-10 results in all three decompositions. A pH greater than 10 results in P-S cleavage only. In distilled water the proportions of each cleavage has been examined and shown to be P-S 34-37%, P-O 42-50%, and ~10% due to C-S cleavage.

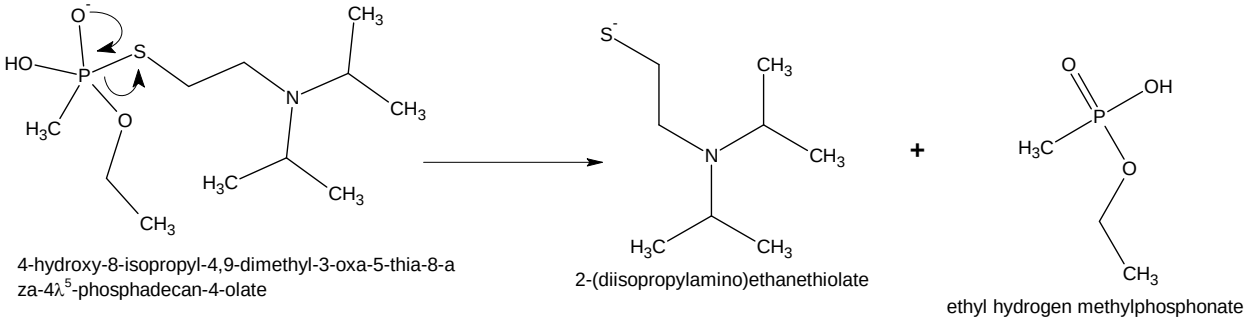


4-hydroxy-8-isopropyl-4,9-dimethyl-3-oxa-5-thia-8-aza-4λ^5-phosphadecan-4-olate

S-[2-(diisopropylamino)ethyl] hydrogen methylphosphonothioate, EA 2192

ethanolate

Figure 5b. VX P-O Cleavage

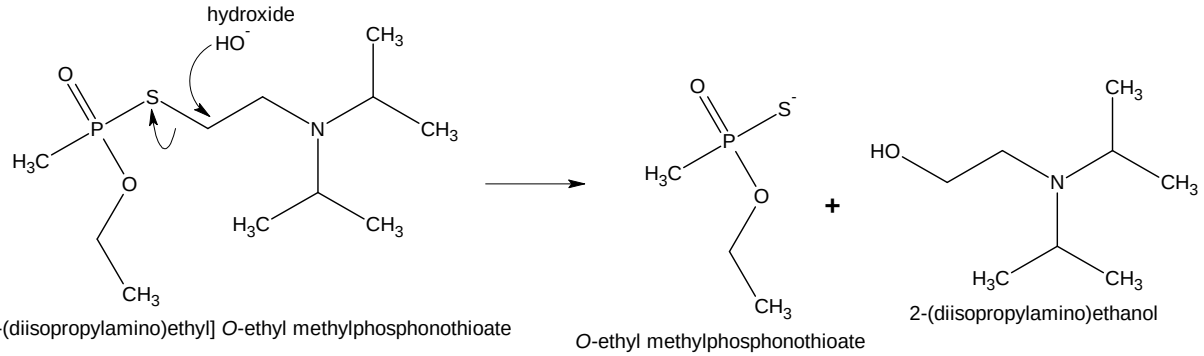


4-hydroxy-8-isopropyl-4,9-dimethyl-3-oxa-5-thia-8-aza-4λ^5-phosphadecan-4-olate

2-(diisopropylamino)ethanethiolate

ethyl hydrogen methylphosphonate

Figure 5c. VX P-S Cleavage



S-[2-(diisopropylamino)ethyl] *O*-ethyl methylphosphonothioate

O-ethyl methylphosphonothioate

2-(diisopropylamino)ethanol

Figure 5d. VX C-S Cleavage

The sulfur blister agents undergo hydrolysis by forming a cyclic intermediate and by expelling a chloride ion (Figure 6).

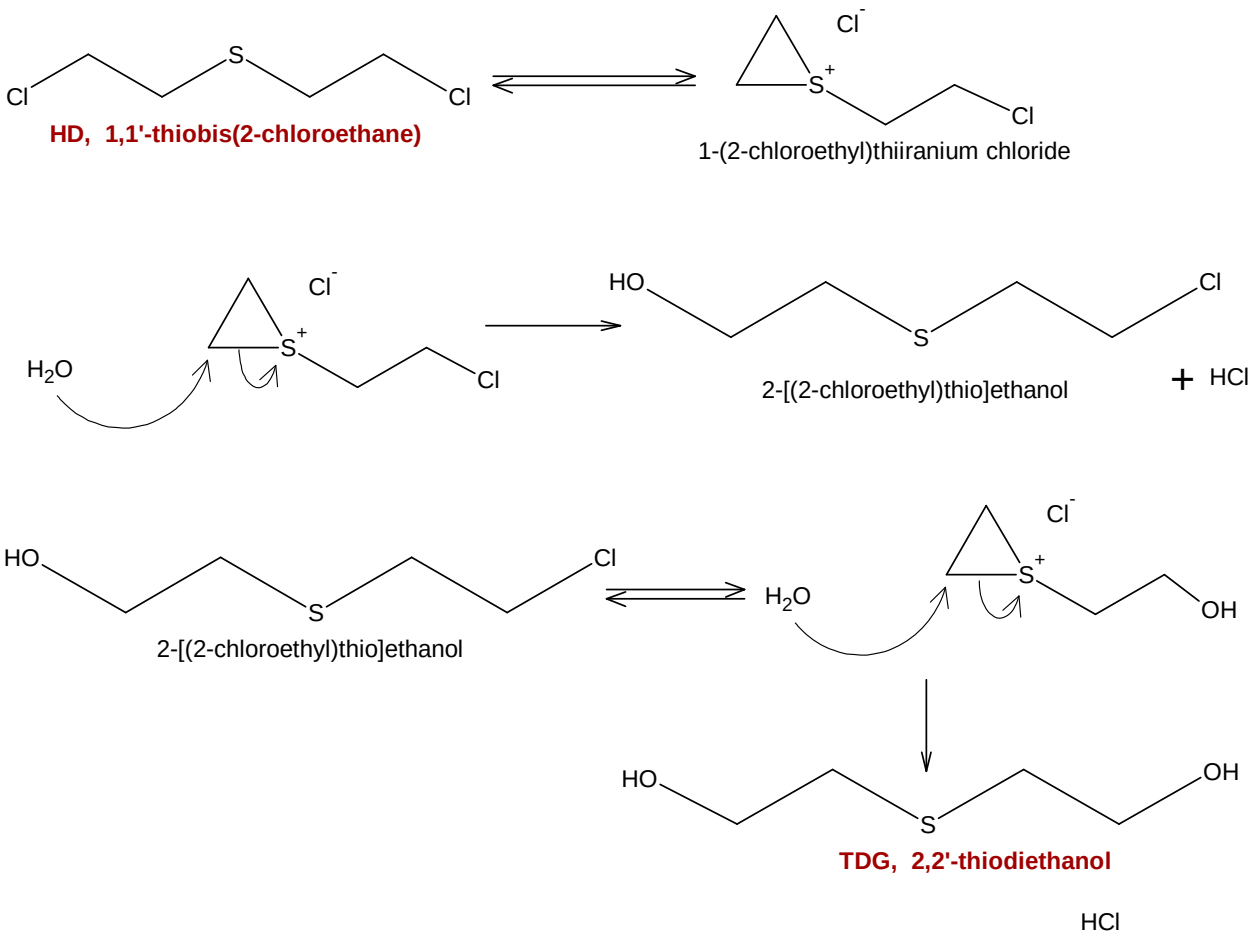
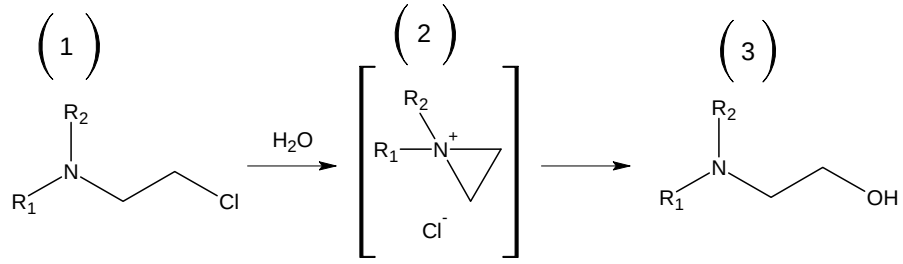


Figure 6. Hydrolysis of Sulfur Mustard

The nitrogen-based mustard agents also undergo hydrolysis by a similar method as the sulfur analogs (Figure 7).



	R_1	R_2	**Nitrogen Mustard**
HN1	C_2H_5	CH_2CH_2Cl	Steps 2-3 repeated for each CH_2CH_2Cl group.
HN2	CH_3	CH_2CH_2Cl	
HN3	CH_2CH_2Cl	CH_2CH_2Cl	

Figure 7. Hydrolysis of Nitrogen Mustard Agents

Lewisite undergoes a quantitative hydrolysis to 2-chlorovinylarsonous acid with subsequent hydrolysis to Lewisite oxide (Figure 8).

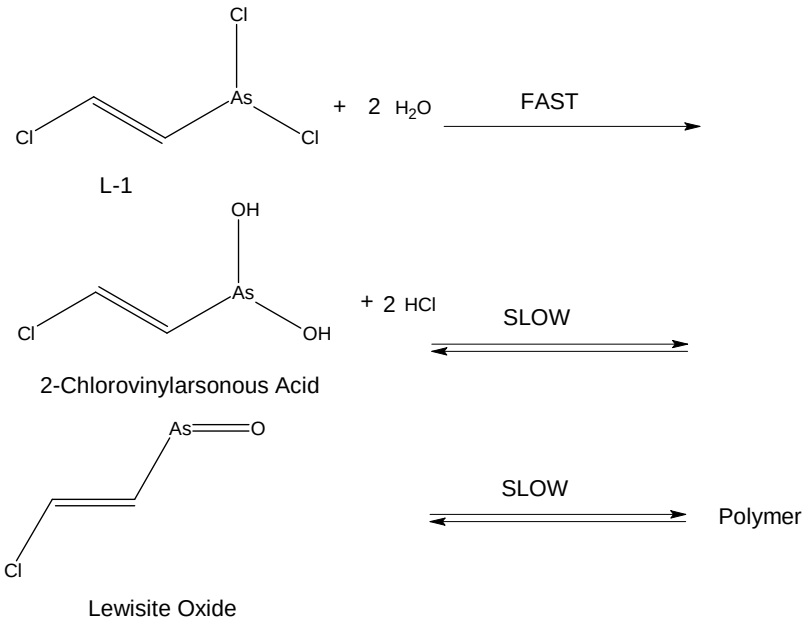


Figure 8. Hydrolysis of Lewisite

The compounds of interest generated from these hydrolysis processes are shown in Table 1 with their corresponding CWA. In the event of a homeland security incident involving any of these compounds, the US EPA will be the federal agency tasked with site remediation.

Table 1. CWA Hydrolysis Compounds

Degradation Product	Original CW Agent	CAS #	Vendor/Source	Part Number
Dimethylphosphoramidic Acid	GA	33876-51-6	ECBC	
Ethyl Hydrogen Dimethylamidophosphate, Sodium Salt	GA	2632-86-2	Cerilliant	ULM-6091-1.2
Diisopropyl methylphosphonate	GB	1445-75-6	Cerilliant	ERD-083
Isopropyl methylphosphonic Acid (IMPA)	GB	1832-54-8	Cerilliant	ERI-015
Isopropyl methylphosphonic Acid (IMPA) Neat	GB	1832-54-8	Cerilliant	SCI-006
1,2-Dichloroethane	HD	107-06-2	Aldrich	284505-100ML
1,4-Dithiane	HD	505-29-3	Aldrich	D217700-5G
Thiodiglycol	HD	111-48-8	Aldrich	166782-100G
1,4-Thioxane	HD	15980-15-1	Aldrich	131970-5G
2-Chlorovinyl Arsonous Acid	Lewisite	85090-33-1	ECBC	
Lewisite Oxide	Lewisite	1306-02-1	ECBC	
EA2192	VX	73207-98-4	ECBC	
Ethyl methylphosphonate	VX	1832-53-7	Aldrich	386561-1G
MethylPhosphonic Acid	VX, GB, GD	993-13-5	Aldrich	289868-1G
Pinacolyl methyl phosphonate	GD	616-52-4	Aldrich	386588-5G

TOXICITY

The first symptoms of nerve agent poisoning, at exposure as low as 1ppm/10 minutes, are the muscarine-like effects:

- miosis (eye pupil constriction, resulting in dimmed vision)
- frontal headache, eye pain
- runny nose
- anorexia (loss of appetite)
- nausea
- excessive sweating
- tightness in the chest, heartburn

The sequence in which these symptoms appear may vary with route of exposure. If the exposure is greater than 1ppm per 10 minute exposure, these effects are followed by other muscarine-like symptoms typical of more severe exposure:

- abdominal cramps
- vomiting
- profuse sweating
- dyspnea (shortness of breath)
- diarrhea
- tenesmus (painful, ineffective straining to urinate or defecate)
- drooling and tearing
- urinary frequency
- involuntary urination or defecation
- excessive bronchial secretion

Shortly after the onset of moderate muscarine-like effects, a number of nicotine-like effects ensue:

- fatigue
- mild generalized weakness
- twitching, jerking, and staggering
- cramps
- pallor (paleness)

Exposure to sublethal doses (Ct <14.6 mg min/m^3) produce these central nervous system symptoms:

- tension
- anxiety
- jitteriness
- restlessness

- emotional lability
- giddiness
- insomnia

More extensive exposure leads to the following symptoms:

- headache
- drowsiness
- slowness of recall and confusion

In the cases of nonfatal exposures, many of these effects are reversible upon recovery. More severe central nervous system effects resulting from exposure to nerve agents have been studied in man. Accidental exposure to lethal amounts of organophosphate pesticides (which also inhibit acetylcholinesterases) leads progressively to the following symptoms:

- ataxia (lack of muscle control)
- slurred speech
- coma
- areflexia (loss of reflexes)
- Cheyne-Stokes respiration (alternating periods of rapid breathing and not breathing)
- generalized convulsions
- finally, cessation of breathing and death

The toxicology of and treatment for exposure to mustard have also been reviewed in T.C. Marrs, R. L. Maynard, F. R. Sidell, *Chemical Warfare Agents: Toxicology and Treatment*. Interested readers are directed to Marrs, Maynard, and Sidell, and to the other works cited in their book for a full assessment of mustard toxicity.

The toxicity of Chemical Warfare Agents should not be judged simply on the basis of lethal doses or exposures. Mustard provides a case in point; during World War I, it was a very effective incapacitating agent, despite producing only 1 percent fatalities among its casualties.

Chemical weapons disperse mustard as an aerosol, which then evaporates to contaminate via inhalation. Exposure to aerosol droplets of mustard or mustard vapor causes no immediate effect. Itching, burning, and inflammation of areas where it contacts the skin generally begin about 4 hours after exposure (the exact length of time depends on the amount of agent involved), followed by swelling of the tissue. Twenty to 24 hours after exposure, small blisters form around the periphery of the affected area. Finally, fully developed blisters fill with a colorless to yellow liquid. Severe tissue degeneration occurs within the blisters, which are vulnerable to infection; the wound may take several months to heal. Inhalation of mustard vapor in high enough quantities leads to similar lesions in the lung and pulmonary edema.

The hydrolysis products of HD are also reported to exhibit toxicity. However, thiodiglycol, (2,2'-thiobis[ethanol]), the major breakdown product, exhibits low toxicity. Therefore, it is likely that most of the toxic effects attributed to the hydrolysis products are due to unreacted mustard and bis(chloroethyl)polysulfides present in the original material.

EXPERIMENTS

The compounds listed in Table 1 were obtained from commercial sources, except for the four highlighted in yellow. The four highlighted compounds are produced directly from the chemical warfare agents, and negotiations with the US Army Edgewood Chemical and Biological Center (ECBC) in Maryland are in progress to obtain these compounds to continue this project. The other compounds were purchased from Aldrich or Cerilliant as noted in Table 1, and used as received; no further purification was necessary.

The acidic organophosphorus (OP) degradation products from the nerve agents require derivatization before GC/MS analysis. A suggested method for derivatization is to use a silinizing agent to create the silyl ester of the organophosphate degradation product. The silinizing agent that has been successfully utilized is *N*-Methyl-*N*-[tert-butyldimethyl-silyl]trifluoroacetimide (MTBSTFA) with 1% tert-butyldimethylchloro silane (TBDMCS) which will convert hydroxyls, carboxyls, thiols and primary and secondary amines to TBDMS (*tert*-butyldimethylsilyl) derivatives (Figure 9). When this derivitizing agent is used on the degradation compounds, the following fragment data (Table 2) will be observed with GC/MS using Electron Impact (EI) ionization. The MTBSTFA/1%TBDCMS was purchased from Fisher Scientific, catalog number NC9306408, in 1mL sealed vials.

The nerve agent degradation compounds were mixed with the MTBSTFA/1%TBDCMS solution in a 5:1 ratio of MTBSTFA to degradation compound. This mixture was placed in a test tube heater and heated at 60°C ± 2°C for 3 hours. This produced the silyl ester of the respective organophosphate acids (generalized reaction scheme shown in Figure 9).

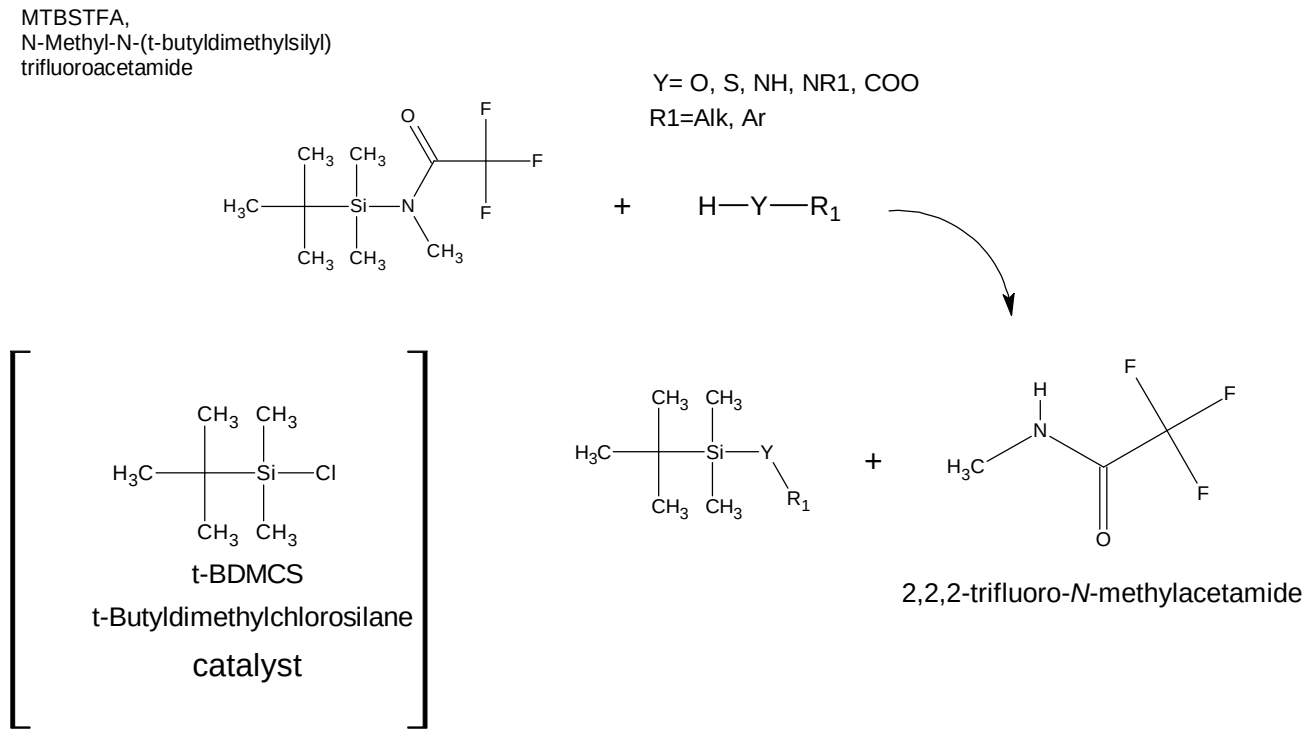


Figure 9. MTBSTFA/1%TBDCMS Reaction Scheme

The HD agent degradation compounds and the nonacidic organophosphorus (OP, nerve agents) compounds did not require any additional derivatization for GC/MS analysis.

The following conditions were used for all runs with the Agilent GC/MS (6890/5975 inert) in Electron Impact (EI) ionization mode.

- Injector: 300°C
- Oven: 40°C (4 min), 10°C/min to 270°C (8 min)
- Column: HP-5MS 30m x 250µm with 0.25µm film
- Flow: 1.0mL/min
- He: 7.07psi, 36cm/sec
- Mass range: 35-500 amu

With the conditions listed above, the following MS fragments were observed for the compounds (see Table 2).

Table 2. CWA Degradation Compound Fragments, OP Acids Derivatized with MTBSTFA/1%TBDCMS

Degradation/Hydrolysis Products	Parent	Predominant Masses in order of most to least abundant									
Diisopropyl methylphosphonate	180	97	123	79	43	45	41	27	80	139	121
***Isopropyl methylphosphonic Acid (IMPA)**	252	153	75	41	154	39	73	121	27	195	155
1,4-Dithiane	120	120	61	46	45	60	92	64	59	73	27
Thiodiglycol	122	61	45	104	91	47	60	31	46	27	44
1,4-Thioxane	104	46	28	61	104	45	27	26	74	76	47
***Ethyl methylphosphonate**	238	153	181	75	41	39	56	27	29	154	182
***MethylPhosphonic Acid**	324	267	73	268	135	195	133	153	269	75	212
***Pinacolyl methyl phosphonate**	294	153	154	237	41	75	211	73	43	69	121
***=MTBSTFA derivative**											

The actual chemical warfare agents have not been analyzed with these conditions, but the expected MS fragments are listed in Table 3.

Table 3. CWA Compounds

Agent	Parent Mass	Fragment Mass (% Relative Abundance)								
Sarin-GB	140	125(36%)	99(100%)	43(16%)	42(12%)	41(13%)	39(11%)	81(11)		
Soman-GD	182	126(100%)	99(78%)	84(12%)	83(10%)	69(48%)	57(16%)	41(35%)	82(38%)	81(7%)
Tabun-GA	162(34%)	133(47%)	106(23%)	70(92%)	117(19%)	44(71%)	43(100%)	42(60%)		
VX	267	167(7%)	107(7%)	79(13%)	114(100%)	72(30%)	30(27%)	127(23%)		
Mustard, Yperite -H, HD	158(23%)	109(100%)	73(10%)	96(8%)	61(7%)	60(9%)	59(13%)	63(31%)	27(22%)	
Lewisite-L	206(79%)	171(61%)	180(42%)	61(42%)	145(100%)	110(84%)				

Table 4. CWA Degradation Products GC-MS Retention Time and TIC Signal-to-Noise (S/N) Ratios

Compound	Retention Time (min)	Concentration (ppm)	TIC S/N
1,4-Thioxane	7.68	53.1	970.5
1,4-Dithiane	11.30	4.4	425.0
Thiodiglycol	13.11	57.5	186.0
Pinacolyl Methyl Phosphonate	17.87	10.7	31477.8
Methylphosphonic acid	18.25	1.9	18134.1
Ethyl methyl phosphonic acid	14.64	7.2	45809.1
Isopropylmethylphosphonic acid	15.01	5.0	665.7

The detection limit used in this work was determined by making the confidence limit 1%. This corresponds to a signal-to-noise (S/N) ratio of about 5:1. The instrument noise level using blanks was determined and compared to the signal of the derivatized compounds (Table 4). The theoretical instrument detection limit (IDL) would be five times the standard deviation of the noise. Extrapolation of the TIC S/N to a 5:1 ratio would put the instrument detection limits for these compounds at the values noted in Table 5.

Table 5. Theoretical IDL

Compound	Concentration theoretically producing 5:1 S/N (ppb)
1,4-Thioxane	273.57
1,4-Dithiane	51.76
Thiodiglycol	1545.70
Pinacolyl Methyl Phosphonate	1.70
Methylphosphonic acid	0.52
Ethyl methyl phosphonic acid	0.79
Isopropylmethylphosphonic acid	37.55

The limits that the EPA will be looking at for action levels during any site remediation are shown in Table 6.

Table 6. Risk-Based Concentrations

Compound	CAS RN	Risk concentration	
		Water (µg/L)	Air (µg/m^3)
Cyclohexyl Sarin (GF)	329-99-7	140	
1,2-Diisopropyl methylphosphonate (DIMP)	1445-75-6	2800	
1,4-Dithiane	505-29-3	350	
O-ethyl methylphosphonic acid (EMPA)	1832-53-7	880	30
Isopropyl methylphosphonic acid (IMPA)	1832-54-8	3500	
Lewisite (L-1)	541-25-3	3.5	3
Methylphosphonic acid (MPA)	993-13-5	700	24
Mustard Distilled (HD)	505-60-2	0.25	0.02
S-[2-(diisopropylamino)ethyl] methylphosphonic acid EA2192	73207-98-4	0.021	0.0007
Sarin (GB)	107-44-8	0.7	0.001
Soman (GD)	96-64-0	0.14	0.001
Tabun (GA)	77-81-6	1.4	0.001
Thiodiglycol (TDG)	111-48-8	18000	
VX	50782-69-9	0.021	0.0006

The method was calibrated by preparing the silyl esters as described above of the acidic OP's, diluting the resulting solution to 30μg/mL, 20μg/mL, 10μg/mL, 5μg/mL, and 1μg/mL of the respective analyte to a volume of 1mL. 10μL of an internal standard (IS) solution of 1000μg/mL of each IS was added to produce an IS concentration of 40ng/μL. The internal standard compounds used for calibration are 1,4-dichlorobenzene-d_4, Naphthlene-d_8, and Acenaphthene-d_{10}. The internal standards are added so relative retention times can be calculated.

The acidic compounds, EMPA, IMPA, PMPA and MPA, will be extracted from the 50ml samples using SPE columns (500mg, quaternary amine) that have been attached to a vacuum manifold and left to dry. The water sample will be loaded into the SPE column, and with vacuum, a flow rate of 2.5 to 3 ml/min through the columns will be established. The sample reservoir is reloaded as needed until the entire 50ml sample has passed through the column. The SPE columns are rinsed with 1ml of methanol, and the columns are left on full vacuum (~500mm Hg) for 1 hour to ensure that they are dry. The analytes bound to the column will be eluted using 1.4ml of 0.3M ammonium hydroxide and collected directly into a vial.

All other compounds will be extracted with C18 SPE columns, and analytes bound to the column will be eluted with ethyl acetate, and analyzed with no derivatization.

CONCLUSIONS

Chemical Warfare Agents and their Hydrolysis Products from the US EPA Standardized Analytical Methods, collects quite a lot of data on the physical, chemical, and toxicological properties of the Chemical Warfare Agents discussed in this summary. This will provide the EPA with a more complete set of data to make decisions to protect health and welfare during site remediation after a terrorist event using chemical weapons. As analytical methods are developed and refined to cover the wide range of compounds produced from CWA degradation, this document will grow to encompass all of these developments.

DISCLAIMER

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency.