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INDIRECT DETERMINATION OF O-ETHYL S-(2-DIISOPROPYLAMINOETHYL) METHYLPHOSPHONOTHIOATE IN AIR AT LOW CONCENTRATIONS

WILLIAM K. FOWLER* and JOSIAH E. SMITH, Jr.
Southern Research Institute, P.O. Box 55305, Birmingham, AL 35255-5305 (U.S.A.)
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SUMMARY

This paper describes an indirect method for the quantification of the toxic military agent O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate (VX) in the vapor state in air or other similar gases at ng/m^3 levels. The method begins with the passage of a gaseous sample through a filter impregnated with silver fluoride to convert the VX vapor to ethyl methylphosphonofluoridate. The latter compound is then trapped on a bed of Chromosorb 106, transferred to a smaller bed of the same sorbent, and desorbed thermally into a gas chromatograph equipped with a flame-photometric detector. The method is comparable in sensitivity to the principal alternative method, which is based on cholinesterase inhibition, and it is less subject to interference from common organic solvents and other cholinesterase inhibitors.

The detection limit was found to be limited by, and therefore dependent on, the nature and extent of any background substances that produced a significant chromatographic signal or response at the retention time of the analyte. In the absence of such substances, the instrument provided a response to 0.19 ng of VX that was thirty times larger than the peak-to-peak noise amplitude on the chromatographic base line. Moreover, the method bias (*i.e.*, 100% minus the percent VX recovery) was found to depend on VX concentration, with estimates of agent recovery ranging from 83% at a VX concentration of 0.67 ng/m^3 to 104% at a concentration of 0.084 ng/m^3. The relative standard deviation varied with VX concentration and with the nature of the test that was performed to estimate it. It ranged from 2.1% in one VX vapor-challenge test to 17% in an experiment involving spiked sampling tubes, and it was generally lower at the higher VX test concentrations.

INTRODUCTION

The very high toxicity of O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate, also known as VX, mandates the requirement for an analytical method that can determine it at exceedingly low concentrations in air. Defense-related research over the past two or three decades has produced numerous analytical methods

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for determining VX in a variety of matrices[1,2]. But methods based on the Schoenemann reaction[1-3], on enzyme inhibition [1,2,4], and on gas chromatography (GC) with a variety of detectors [1,5-8] are by far the most commonly used approaches to trace-level determinations of VX in complex matrices.

Because of its very high sensitivity, the enzymatic technique (in conjunction with sampling into a glass impinger or bubbler filled with a liquid absorbing medium) has been evaluated for determining very low concentrations of VX vapor. But this technique responds rather indiscriminately to any substance that can inhibit or destroy the activity of the enzyme (cholinesterase); such inhibitors include, *e.g.*, many common organic solvents[1].

For this reason, a sensitive, yet specific method based on GC was sought. However, VX vapor exhibits a troublesome tendency to adsorb strongly (often irreversibly) on any surface[9], a phenomenon that places extreme demands on the sampling device and on the gas chromatographic system with respect to inertness. Indeed, we have found that this adsorption problem leads to excessive inaccuracy and imprecision even in the enzymatic method, where the VX presumably adheres to the inner surface of the impinger sampler.

In the method reported here, the adsorption problem is effectively circumvented by first converting the VX to ethyl methylphosphonofluoridate, which is much less strongly adsorbed on most surfaces. This compound is frequently referred to as the G-analogue of VX because of its structural similarity to the G-type chemical agents. The V-to-G conversion reaction involves the use of solid silver fluoride as the reagent and is depicted in Fig. 1. Although this reaction has been widely used for facilitating determinations of VX[1], its original use occurred in classified military studies that were conducted over two decades ago, and the authors have not located the names of the original investigators. Nevertheless, it appears probable that V-to-G conversion has not been used previously in conjunction with solid-sorbent sampling, gas chromatographic determinations, or ppt[a] concentrations of VX vapor in air.

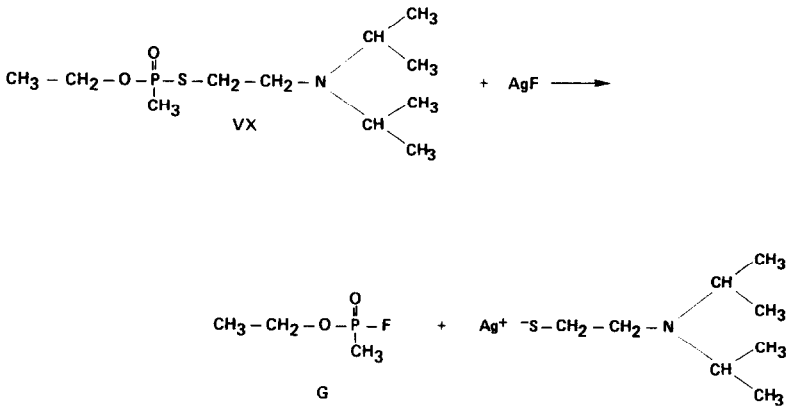

Fig. 1. Conversion of VX to its G-analogue (G).

[a] Throughout this article, the American trillion (10^{12}) is meant.

The method described in this publication entails the pumping of an air sample through a felt pad impregnated with silver fluoride, the collection of the G-analogue of VX in a solid-sorbent (Chromosorb 106) sampling tube, the transfer (by thermal desorption) of the collected G-analogue from the sampling tube to a much smaller transfer tube containing the same sorbent, and the thermal desorption of the G-analogue from the transfer tube into a gas chromatograph equipped with a flame-photometric detector in the phosphorus-specific mode. The only other method with comparable sensitivity is the impinger/enzymatic method, which is much more susceptible to interferences from common organic solvents and from other organophosphorus nerve agents than the method given here.

EXPERIMENTAL

The sampling tubes and the transfer tubes were constructed and conditioned essentially as described previously[10]. However, the glass blank for the sampling tube was 90 mm × 8 mm O.D. × 6 mm I.D., and the transfer-tube blank was 175 mm × 3 mm O.D. × 1.7 mm I.D. Moreover, the tubes were packed with unweighed portions of 60–80-mesh Chromosorb 106 (Alltech, Deerfield, IL, U.S.A.) to form either a 2-cm-long bed (*ca.* 180 mg) or a 5-cm-long bed (*ca.* 450 mg) in the sampling tube and a 1.5-cm-long bed (*ca.* 10 mg) in the transfer tube. Sampling rates up to about 4 l/min could be attained with the 8-mm-O.D. sampling tube that was packed with 2 cm of sorbent, whereas the 5-cm sorbent bed in this tube permitted sampling rates only up to about 1.5 l/min.

The V-to-G conversion filters were fabricated in three steps: (1) preparation of a solution of silver fluoride, (2) deposition of solid silver fluoride onto a felt pad, and (3) assembly of the filter unit at the inlet end of the sampling tube.

The silver fluoride solution was prepared by dissolving 37.5 g of $AgNO_3$ (Alfa Products, Danvers, MA, U.S.A.; purity = 99.9+ %) in 40.0 ml of deionized water. In a separate container, 12.5 g of $KF \cdot 2H_2O$ (Alfa Products; purity not specified) was dissolved in 44.0 ml of deionized water. The silver nitrate solution was then slowly added to the potassium fluoride solution as the latter was stirred. The resulting mixture was turbid and was therefore filtered through Whatman No. 42 filter paper before proceeding. A 12.5-ml aliquot of absolute ethanol was added to the filtrate with stirring; this treatment produced a brown precipitate, which was left as a suspension in the mixture. The above steps were carried out entirely with the use of polyethylene vessels. Moreover, the mixture, which was sufficient for the production of about 240 conversion filters, was invariably used immediately after its preparation and was thus never stored prior to use.

The reagent mixture was absorbed into the felt (Fiber-Taxis, Bellingham, MA, U.S.A., Type PE-9080 non-woven polyester felt) by pouring the mixture into each of two shallow polyethylene trays and immersing a 15 × 13 cm rectangle of felt in the solution of each tray. After a 30-s soak period, the felt pieces were removed from the reagent mixture, squeezed gently on a polyethylene surface to remove excess solution, placed in dry polyethylene trays, and dried in a forced-air oven at 50°C for 6 h. It was found that best results were achieved when the above manipulations leading to the drying step were performed as rapidly as possible, preferably within 5 min. After the drying step, circular pads of the impregnated felt were punched from the material

with a 5/16-in.-diameter arch punch, and the pads were sealed in a polyethylene jar and stored in the dark until ready for use in air sampling. All handling of the reagent or impregnated felt, either before or after drying, was performed with the use of long forceps and/or protective rubber gloves to avoid skin contact with the reagent.

Just prior to the collection of a sample, a conversion pad was installed in a 5/16-in. polypropylene union (Cole-Parmer, Chicago, IL, U.S.A.; Part No. N-06381-20) as shown in Fig. 2. A back-up filter pad of unimpregnated felt was used just

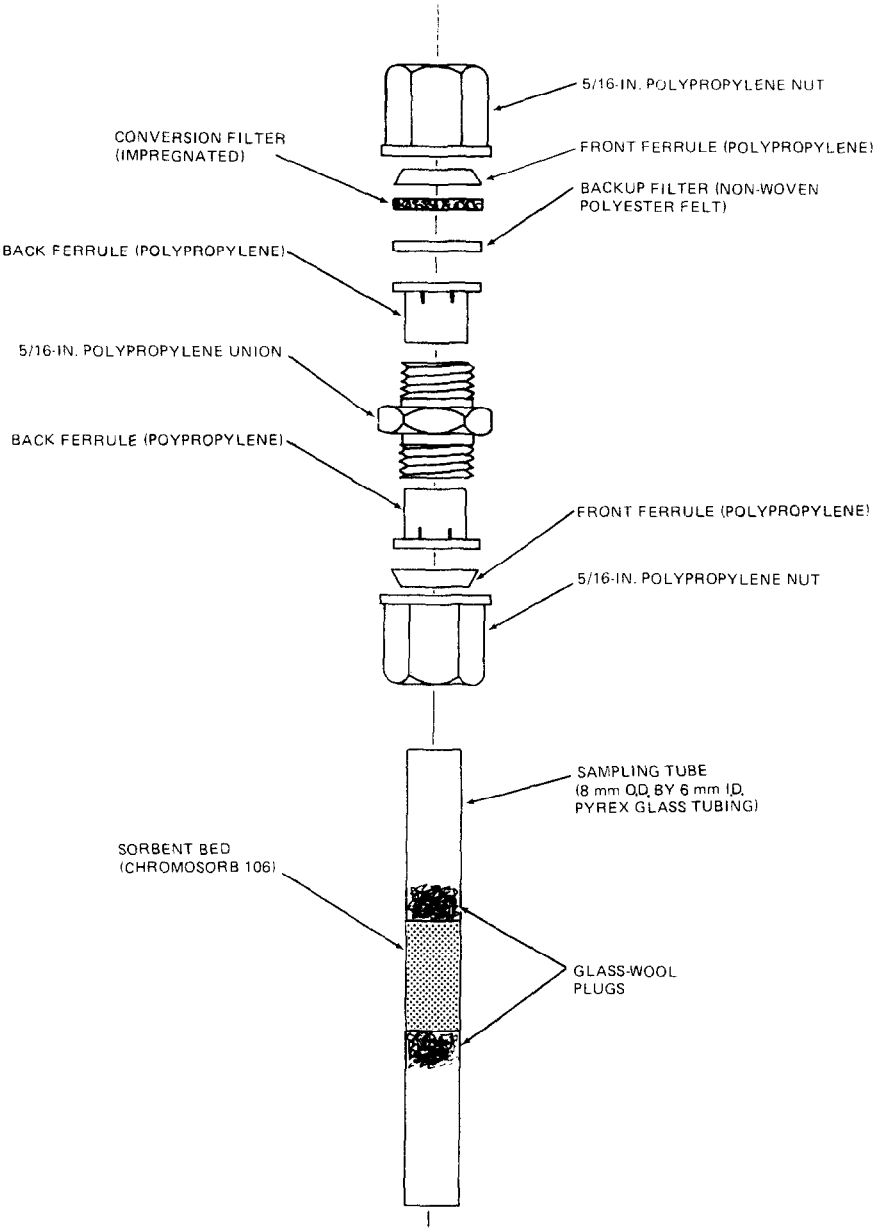

Fig. 2. Construction of the V-to-G conversion filter and attachment of the filter to the sampling tube.

beneath the impregnated pad to prevent loose particles of the solid silver fluoride reagent from being swept into the sampling tube. The union, with conversion pad installed, was then fitted onto the inlet end of a sampling tube (Fig. 2). In the apparatus of Fig. 2, the recess between the top of the uppermost polypropylene ferrule and the upper surface of the conversion pad (*ca.* 3–4 mm) was intended to be sufficient to prevent significant back-diffusion of spiked VX or G-analogue out of the device but not deep enough to allow substantial wall contact with incoming VX vapor during sampling.

The sampling tubes, the transfer tubes, and the V-to-G conversion filters are reusable, but their useful life depends on the conditions under which they are used. For example, chemically harsh gases (such as nitrogen dioxide, chlorine and ozone) and excessively high desorption temperatures will promote the deterioration of the Chromosorb 106 sorbent, and strong light will degrade the V-to-G conversion reagent. Although the vendor of the Chromosorb 106 recommends an upper temperature limit of 250°C, we have found that sorbent deterioration is rapid in this application at desorption temperatures above about 220°C.

Prior to the analysis step, the collected vapor samples were passed from the sampling tubes to the smaller transfer tubes by thermal desorption. This step was performed by first attaching the transfer tube to the sampling tube (after removal of the conversion filter) by means of a 5/16-to-1/8-in. stainless-steel Swagelok reducing union (Crawford, Solon, OH, U.S.A.) equipped with PTFE ferrules. Next, the free end of the transfer tube was connected to a vacuum sampling pump, which was switched on and adjusted to pump room air at 300 ml/min into the sampling tube and out through the transfer tube. With the pump running, the free end of the sampling tube was inserted into a 9-mm-I.D. hole bored through a small aluminum block that was maintained at 200°C. The sampling tube was kept inside the heated block for 2 min to ensure a quantitative desorption and transfer of G-analogue from the sampling tube to the transfer tube.

The G-analogue that accumulated in the 3-mm-O.D. transfer tube was desorbed thermally inside the hot injection port of a gas chromatograph the injection port of which had been modified in a manner similar to Method A of the previous publication[10]. Also described in this reference is the desorption procedure except that, in the current work, the transfer tube resided in the injection port for 15 s prior to the initiation of carrier gas flow. All desorptions, whether in the transfer step or in the analysis step, were carried out in the backflush direction. The GC instrumental conditions are summarized in Table I.

The instrumental response was calibrated by the spiking of sampling tubes with known amounts of VX, the thermal desorption of spiked VX (as its G-analogue) into the gas chromatograph, and the linear regression of response *versus* VX amount. The previously reported tube-spiking procedure[10] was used here except that the solvent used in preparing the standard solutions was cyclohexane rather than chloroform, as a matter of convenience. Additionally, the VX solution was deposited directly onto the V-to-G conversion pad, rather than onto the inner wall of the sampling tube, during the spiking procedure. The VX concentration ranges employed in calibrations were chosen to bracket the expected sample concentrations. The linear range of the instrument extended up to about 100 ng of VX per desorption.

Both VX and the G-analogue of VX in neat liquid form were supplied in several

TABLE I

GC INSTRUMENTAL CONDITIONS FOR DETERMINATIONS OF VX IN AIR

Instrument	Hewlett-Packard Model 5880A
Detector	Flame-photometric detector in the phosphorus-specific mode
Column	15 m × 0.53 mm I.D., DB-210 fused-silica capillary column with a 1.0-μm thick coating of the stationary phase
Chart speed	1.0 cm/min
Gas flows	
Carrier gas (He)	20 ml/min
Air	44 ml/min
Oxygen	20 ml/min
Hydrogen	80 ml/min
Temperatures	
Oven	60°C
Injection port	200°C
Detector	200°C

lots or batches by the U.S. Army. All batches were found to be in excess of 90% pure, and most were better than 95% pure, when assayed by GC with flame-ionization detection. Each working standard solution was prepared by serial dilution from a stock standard that had been prepared gravimetrically in a small volumetric flask. All solution concentrations were corrected for the less-than-100% purity of the starting materials. All reagents and solvent were of reagent grade except as otherwise noted.

Test atmospheres containing VX vapor were generated in an all-PTFE, diffusion-tube generator (constructed in-house) the output stream of which could be adjusted to any relative humidity and any temperature between room temperature and 50°C. In addition, NO_2 and SO_2 concentrations of (nominally) 0.01 ppm could be established when desired by bleeding in the respective gases from pressurized cylinders. (These gases were tested as potential sources of interference because they are known to be chemically aggressive substances.) The diffusion tube was about 14 cm long and 0.64 cm I.D.; its temperature was adjusted between 25 and 50°C to obtain VX vapor concentrations ranging from 3.9 to 51 ng/m^3.

RESULTS AND DISCUSSION

Fig. 3 displays a chromatogram that resulted from spiking a sampling tube, through its attached V-to-G conversion filter, with 0.19 ng of VX and sampling outdoor air at 2 l/min for 50 min. Because the total air volume in this case was 100 l, this corresponded to the detection of VX in air at a concentration of 1.9 ng/m^3 or about 0.18 ppt. Furthermore, as is evident from Fig. 3, even lower levels of VX could have been detected in this instance. The practical lower limit of detection may be expected to depend on the extent of interference from background constituents (atmospheric contaminants and sorbent degradation products) in most applications of the proposed method. Because the G-analogue response in Fig. 3 agreed closely with

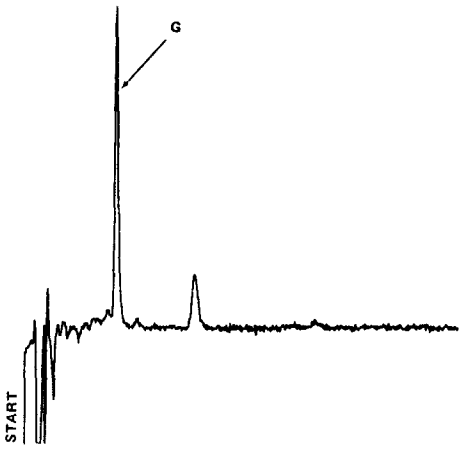

Fig. 3. Typical chromatogram obtained by spiking a sampling tube with 0.19 ng of VX, sampling air at 2 l/min for 50 min, and analyzing the tube for the G-analogue of VX. The retention time of the G-analogue (G) in this figure was 1.77 min.

the instrument-calibration responses for 0.19-ng VX spikes, a significant interference from coeluting background substances was unlikely.

The chromatogram of Fig. 3 reflects a loss of resolution that occurred as a result of the particular mode of thermal desorption that was employed in this study. Specifically, the G-analogue peak in this figure is approximately twice as wide as the G-analogue peak obtained by solution injection into the gas chromatograph under otherwise identical conditions. But this loss is still acceptable in many applications.

In a preliminary test of the proposed method, we sampled and determined VX vapor in the output stream from a VX vapor generator under various sets of conditions. In each test run under a given set of conditions, six replicate determinations of VX concentration were made. The first eleven test runs covered the VX concentration range from zero to 51 ng/m^3, as determined by the analytical method described in this paper. The last five runs were conducted at an essentially constant VX concentration (also as determined by the proposed method), which was about 12 ng/m^3. However, the generator output concentration appeared to be drifting, and it thus required an occasional minor adjustment between test runs. A gravimetric assay of the generator output concentration, based on measurement of the weight lost by the diffusion tube, was considered impractical because of the very small amount of weight lost by the tube during the course of these experiments. Because no suitable reference method was available for use in this work, no evaluation of method accuracy could be performed in this test. Except where noted otherwise below, sampling was conducted at 1.0 l/min for 2 h through sampling tubes containing 2 cm of sorbent.

The results of the test are given in Table II, where it can be seen that the relative standard deviations (R.S.D. values) of replicate determinations were less than 10% in all test runs. Moreover, no obvious effect due to relative humidity, temperature, NO_2, or SO_2 could be discerned.

As a further test of the method at lower effective VX concentrations, we spiked a set of five sampling tubes with different amounts of VX on each of four separate

TABLE II

RESULTS OF VX DETERMINATIONS IN SAMPLES COLLECTED FROM A VX-VAPOR GENERATOR

Run No.	*Relative humidity (%)*	*Vapor temperature (°C)*	*Average found VX concentration (ng/m³)*	*R.S.D. (%)*
1	<20	25	0	0
2	<20	25	3.9	3.8
3	<20	25	5.8	5.2
4	<20	25	9.2	4.0
5	<20	25	12	5.0
6	<20	25	13	4.6
7	<20	25	17	6.1
8	<20	25	23	2.1
9	<20	25	34	5.2
10	<20	25	51	5.7
11[a]	<20	25	10	8.4
12	<20	50	11	4.8
13	>80	25	14	6.7
14	>80	50	11	6.1
15[b]	<20	25	13	4.1
16[c]	<20	25	11	3.7

[a] In run No. 11, sampling was conducted at 0.20 l/min for 10 h.
[b] In run No. 15, the sampled VX vapor contained 0.009 ppm SO_2.
[c] In run No. 16, the sampled VX vapor contained 0.016 ppm NO_2.

days. The sampling tube used in this particular test contained Chromosorb 106 beds that were 5 cm, rather than 2 cm, in length. Because it was desired to test the capabilities of the method in the absence of atmospheric contaminants, each spiked sampling tube was fitted with a small charcoal filter at the inlet of the V-to-G conversion filter just prior to the initiation of air sampling to exclude airborne contaminants. Each day, the spiked tubes were allowed to sample outdoor air at 1.0 l/min for 24 h and were then promptly analyzed for VX. The VX spikes ranged in mass from zero (solvent only) to 0.96 ng each day, corresponding to VX concentrations ranging from zero to 0.67 ng/m^3.

Table III displays the results of this test. In this table, the VX spike levels are expressed as the equivalent 'target concentrations', and the amounts of VX found by analysis of the various sampling tubes are similarly expressed as 'found concentrations'. The average found concentrations, the R.S.D. values of the found concentrations, and the average spike recoveries (expressed as percentages of the target concentrations) for the entire four-day period are also shown in Table III.

The R.S.D. values of Table III were clearly higher than those from the previous experiment (Table II), but even at these very low concentrations, they were still acceptable for applications not requiring high accuracy. Of course, the variability in spiking may have contributed significantly to the R.S.D. values of Table III but not to those of Table II. Note also that the found concentrations appeared to be positively biased at the low end of the concentration range and negatively biased elsewhere. But we observed this same behavior in similar tests involving other analytes and other

TABLE III

RESULTS OF THE DETERMINATION OF VX FROM SPIKED SAMPLING TUBES AFTER THE SAMPLING OF OUTDOOR AIR

Target VX concentration (ng/m^3)	*Found VX concentration (ng/m^3)*						*Percent of target concentration*
	Day 1	*Day 2*	*Day 3*	*Day 4*	*Average*	*R.S.D. (%)*	
0	0	0	0	0	0	0	100
0.084	0.11	0.096	0.075	0.075	0.087	17	104
0.17	0.16	0.16	0.12	0.14	0.14	13	85
0.33	0.29	0.32	0.25	0.29	0.29	10	88
0.67	0.50	0.63	0.52	0.58	0.56	11	83

solid sorbents. Although the source of the bias is currently unknown, its magnitude is acceptably small for many potential applications at these very low concentration levels, and its nature suggests that it was an experimental artifact rather than an inherent, unavoidable characteristic of the method.

In any event, it should be understood that the VX-recovery data of Table III merely approximate the true total accuracy of the method since these data reflect an unnatural error component (*i.e.*, that due to the error in spiking) and do not reflect a component due to the volumetric error in sampling.

The efficiency with which the V-to-G conversion filter converts VX to the G-analogue was also studied. The theoretical yield from the conversion of 1 ng of VX should be 0.47 ng of G-analogue. The actual yield from several replicate determinations was about 80% of this value, suggesting a conversion efficiency of 80%. But the response of the method to VX has been observed to be virtually constant under widely varying conditions (*e.g.*, Table II), suggesting that the conversion efficiency is largely unaffected by environmentally significant factors such as temperature and humidity.

The breakthrough volume of the G-analogue on Chromosorb 106 was not measured at room temperature becasue the data of Table III indicated little or no loss of G-analogue even after sampling 1440 l of air through sampling tubes containing 5-cm-long beds (*ca.* 450 mg) of Chromosorb 106. This implies a breakthrough volume of not less than 3.2 liters per milligram of Chromosorb 106, which is well above the limit on sample volume likely to be imposed by sample background concomitants that appear as extraneous peaks in the chromatograms. But when the sampling tube was maintained at 50°C, about 7% of a 1.8-ng VX spike was found, after conversion to the G-analogue, to have broken through a 2-cm-long (ca. 180-mg) bed of Chromosorb 106 (as the G-analogue) following the sampling of 160 l of air. This implies a breakthrough volume, at the 7% breakthrough level, of 0.89 l/mg at 50°C.

In the use of this method, it has been observed that the vapors from either liquid or solid chlorine/hypochlorite bleach will interfere with the method by producing a background constituent that coelutes with the G-analogue of VX. This constituent was identified by combined GC–mass spectrometry as *p*-dichlorobenzene, which is thought to arise from a reaction between Chromosorb 106 (a cross-linked polystyrene) and chlorine gas. In addition, the pesticide malathion appeared to undergo a reaction in the V-to-G conversion filter, yielding a phosphorus-containing product that eluted just before the G-analogue peak with a peak-to-peak retention time difference of 0.35 min between them. At a malathion concentration approaching that of VX, the peak overlap was significant.

However, the method reported here was found to be insusceptible to interference from milligram amounts of common organic solvents, *i.e.*, chloroform, methanol, ethanol, 2-propanol, *n*-hexane, cyclohexane, dichloromethane, 1,1,2-trichloro-1,2,2-trifluoroethane, acetone, ethyl acetate, formamide, carbon tetrachloride, benzene and toluene. Moreover, the reported chromatographic conditions were observed to successfully resolve the G-analogue peak from three other organophosphorus nerve agents —GA or tabun (ethyl N,N-dimethylphosphoramidocyanidate), GB or sarin (isopropyl methylphosphonofluoridate) and GD or soman (1,2,2-trimethylpropyl methylphosphonofluoridate). These represent distinct advantages for the method given here relative to the enzymatic method.

Because of the similarity of the G-analogue to the chemical agent GB, it is reasonable to expect that this method may be useful for simultaneous determinations of both GB and VX. Recent data have suggested that this is true, as GB has been found to pass through the V-to-G conversion filter efficiently, and the GB chromatographic peak is baseline-resolved from the G-analogue peak.

CONCLUSION

It was concluded that VX vapor can be determined with high sensitivity by converting it to a simpler compound (*i.e.*, the G-analogue of VX) during the sampling step, by trapping the G-analogue vapor on a Chromosorb 106 sampling tube, and by thermally desorbing the G-analogue into a gas chromatograph equipped with a flame-photometric detector. The accuracy and precision of the method were found to be adequate for many likely applications.

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