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HANDLING OF SAMPLES CONTAMINATED WITH CHEMICAL WARFARE AGENTS – PART 5

JOHNSEN Bjørn A, TØRNES John Aa,
AXELSSON Torill, KARLSEN Per J

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FORSVARETS FORSKNINGSINSTITUTT
Norwegian Defence Research Establishment
P O Box 25, N-2007 Kjeller, Norway

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JOHNSEN Bjørn A, TØRNES John Aa, AXELSSON Torill, KARLSEN Per J

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In order to improve the possibilities for verification of alleged use of chemical warfare agents different procedures of sample handling have been elaborated. Adsorption of chemical warfare agents to the porous polymer XAD-2 was tested both in laboratory and field exercises. This method decreases the size of the samples, prevents decomposition of agents present in the samples and is easy to perform even for untrained personnel. Another type of polymer tested was a C-18 cartridge which proved to be very efficient in adsorbing chemical warfare agents from aqueous solutions.

Several factors which may influence the recovries of chemical warfare agents when these methods are used in the field were tested. These factors were the amount of polymer, the flow rate during adsorption, the equilibration time during desorption, storage time and temperature during storage. The experiments were performed mainly by application of soman as a test substance, but different nerve agents (tabun, sarin and soman), mustard gas and the tear gas CS were used to test the procedures for sample handling.

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SAMPLE HANDLING OF CHEMICAL WARFARE AGENTS - PART V

Summary

In order to improve the possibilities for verification of alleged use of chemical warfare agents different procedures of sample handling have been elaborated. Adsorption of chemical warfare agents to the porous polymer XAD-2 was tested both in laboratory and field exercises. This method decreases the size of the samples, prevents decomposition of agents present in the samples and is easy to perform even for untrained personnel. Another type of polymer tested was a C-18 cartridge which proved to be very efficient in adsorbing chemical warfare agents from aqueous solutions.

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The field exercises during the winter 1985/86 were carried out in March with old and dirty snow. The procedures were in addition to snow samples successfully applied to samples from muddy water and water extracts of sand, soil and vegetation. They were shown to be of general use on an all-year basis. The methods were applicable both for concentrating samples and also for clean-up of samples of different origins.

1 INTRODUCTION

A complete procedure for verification of alleged use of chemical weapons should include procedures for sampling, sample handling and analysis of different types of samples. Positive verification of chemical warfare agents depends on the reliability of each of these procedures. In addition, knowledge about the persistence of the chemical agents under various conditions will increase the applicability of a verification procedure.

During the winter 1981/82 (1), 1982/83 (2), 1983/84 (3) and 1984/85 (4) the work was primarily concentrated on elaborating the persistency of the different chemical warfare agents under winter conditions. This includes the development and establishment of analytical methods for the chemical warfare agents. The research on methods for sample handling started during the winter of 1983/84. This work was concentrated on elaborating different ways of transportation of snow samples from the field to the laboratory. The most successful method was to extract the samples with an organic solvent in the field and transport the solvent to the laboratory for analysis.

The main objective of developing methods for sample handling is to prevent decomposition during transportation to the laboratory. It is important that the methods are easy to perform, are robust, make use of readily available equipment and have a wide applicability.

The methods tested during the winter 1985/86 were based on the adsorption of chemical warfare agents to polymers in the field. The two polymers elaborated are both commercially available and known to have excellent adsorption efficiency for non-polar organic compounds. The trade names of the polymers are Amberlite XAD-2 and Sep-Pak C-18.

It is noticed with satisfaction that different aspects of the problem of sample handling have been studied by other nations. The research has therefore taken account of the research described in the Canadian publication: Handbook for the investigation of allegation of the use of chemical or biological weapons (Document CD/677 (5)). It has also taken account of the Dutch Working Papers concerning the verification of the presence of nerve agents, their decomposition products or starting materials downstream of chemical production plants (Documents CD/306 (6) and CD/307 (7)).

2 EXPERIMENTAL

The field experiments during the winter 1985/86 were carried out to describe and develop methods for sample handling. The experiments can be divided into three categories.

The first includes experiments carried out to define the requirements for effective adsorption and desorption of chemical warfare agents on XAD-2 and C-18 columns. All experiments were carried out with snow samples which had a volume of 50-150 ml water when melted.

The second part was a field exercise to test the complete verification procedure. This exercise was performed to test the methods for sample handling under winter conditions to decide whether they were applicable in the field or not. The amount of water used as eluant in the experiments was 100 ml.

The third part comprise of experiments to test if the adsorption methods were of general use. They were therefore applied to samples obtained from muddy water and water extracts of sand, soil and vegetation.

2.1 XAD-2 method

XAD-2 is porous polymers of polystyrene and divinylbenzene. It is a good hydrophobic adsorbent with a large surface area. XAD-2 was used in a standard column with an inner diameter of 1cm and a height of 20 cm. The polymer was prewashed with water and methanol (Figure 2.1).

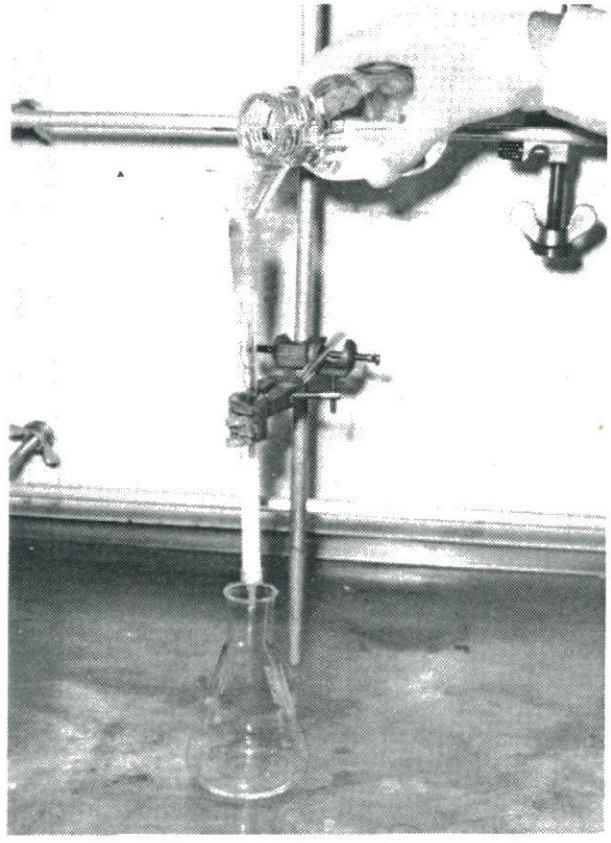

Figure 2.1 Standard column of XAD-2

2.1.1 Adsorption

There are several factors which may influence the amount of chemical compounds adsorbed to the polymer. These factors are water temperature, temperature of the adsorbent, flowrate and amount of XAD-2.

2.1.1.1 Sample temperature

Experiments were carried out using a standard column filled with 1 g XAD-2. Water samples of 0°C and 20°C together with a melted snow sample (0°C) were included in the experiments. The samples were added to the columns 15 minutes after the addition of a chemical warfare agent and passed through the columns in 5 minutes. After 2 minutes of equilibration with 5ml chloroform the columns were eluated. The recoveries were estimated by gas chromatographic analyses . The agents used in this experiment were tabun, sarin, soman and mustard.

2.1.1.2 Environmental temperature

To compare the adsorption efficiency at different environmental temperatures the standard XAD-2 columns were stored at -20°C for 3 hours and compared with columns stored at room temperature. The conditions of the experiments were the same as above. These experiments were performed with soman as a test agent.

2.1.1.3 Flow rate

In practical field work it may be desirable to pass the sample through the columns at high speed in order to reduce the time necessary for sample preparation. To elaborate the effect of variation of the flow rate, melted snow samples (50 ml) were passed through columns of 1g XAD-2 in 0.5, 5 and 10 minutes. With a flow rate of 0.5 minutes the water had to be forced through by an

external air pressure (Figure 2.2). The experiments were performed with the nerve agents tabun, sarin and soman.

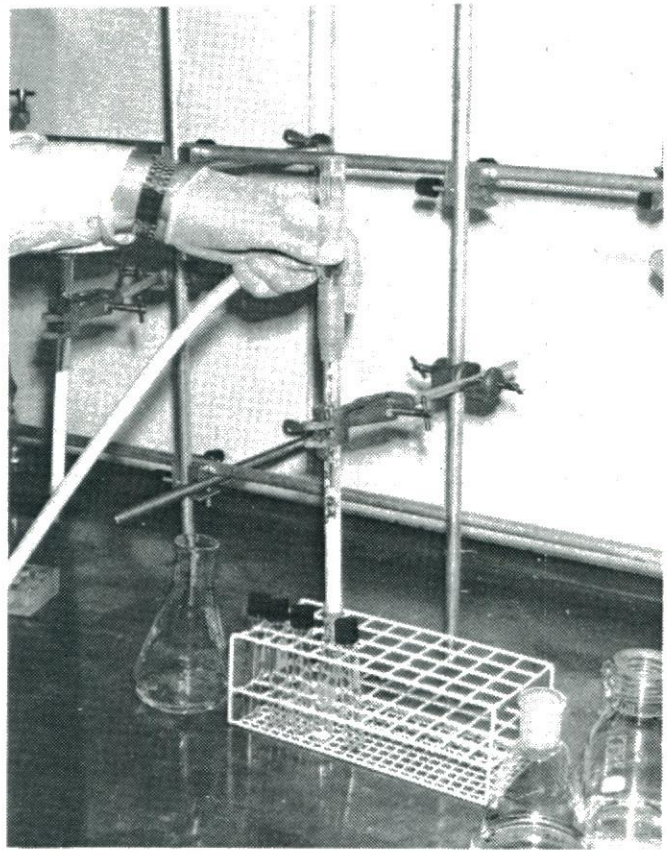

Figure 2.2 External air pressure was used to increase the flow rate

2.1.1.4 Amount of XAD-2

In order to test the effect of varying amounts of XAD-2 adsorption of chemical warfare agents was carried out with 1, 2, 3 and 5 g XAD-2. The experiments were performed with soman and mustard.

2.1.2 Transportation and storage

It is important that the chemical warfare agents are stored and transported under optimal conditions. Experiments were therefore carried out to study if storage time had any influence on the amount of agent recovered.

2.1.2.1 Storage time

To indicate whether there were any decomposition during storage XAD-2 columns were stored at 5°C up to 7 days. The experiments in the field were based on the assumption that samples shall be brought to a laboratory for analysis within 24 hours. This may, however, be difficult to achieve under all circumstances and long term experiments were therefore carried out to elaborate the effect of 7 days of storage. In these experiments soman was used as a test substance.

2.1.3 Desorption

Desorption of chemical warfare agents were performed by using organic solvents. The effect on the recovery was examined by varying the amount of eluant and the time of equilibration between eluant and adsorbent before elution.

2.1.3.1 Amount of eluant

Chloroform was used to eluate chemical warfare agents from the columns. Different volumes of chloroform were applied in order to define the effect on the recovery.

2.1.3.2 Equilibration time

Time of contact between adsorbent and eluant may play an important role for the recovery of chemical warfare agents from the columns . The effect was elaborated by measuring the desorbed amount of chemical warfare agent after varying the time of contact. The test substance was soman and the time of contact was 2, 5 and 10 minutes.

2.2 C-18 method

C-18 cartridges are commercially available columns of hydrocarbon polymers effectively used to isolate hydrophobic compounds from aqueous solutions. C-18 columns may therefore be important in cleanup, concentration and handling of aqueous samples. The cartridge is small and easy to handle (Figure 2.3).

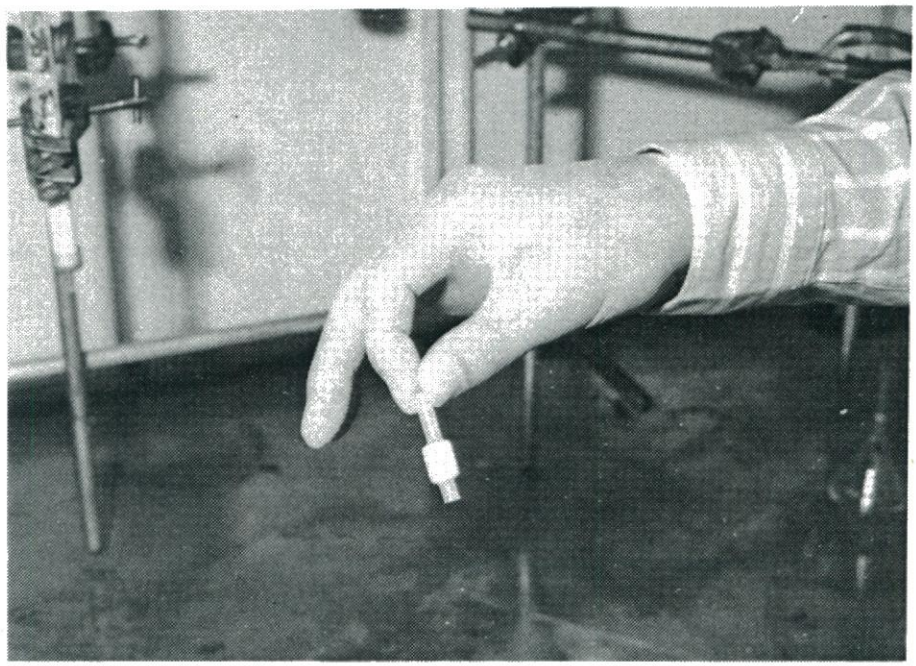

Figure 2.3 The Sep-Pak C-18 cartridge

2.2.1 Adsorption

Samples of melted snow (50 - 100 ml) were passed through the cartridges in 2-3 minutes. Due to large resistance it was difficult to increase the flow rate through the C-18 cartridge. A flow rate of 25 ml/min was therefore used as a standard procedure for C-18 cartridges in sample handling (Figure 2.4).

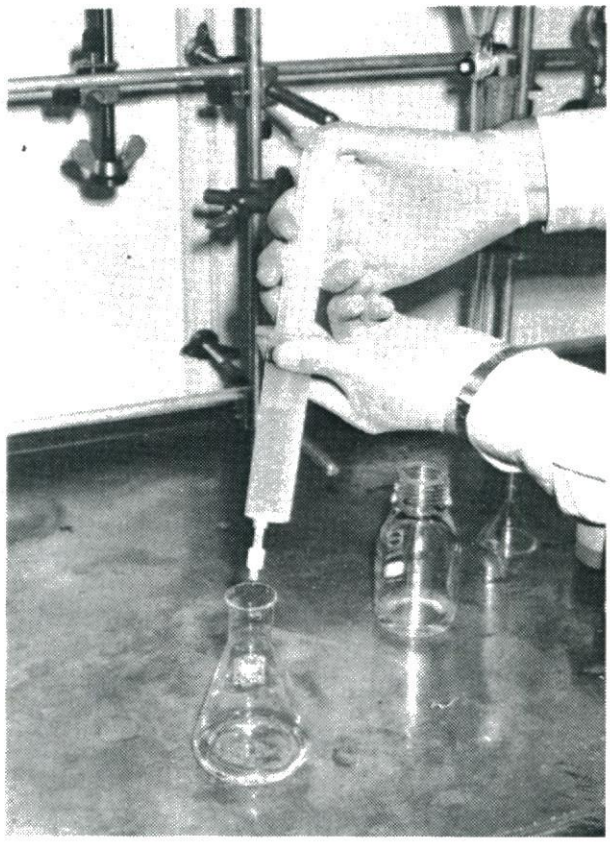

Figure 2.4 Sample passage through the C-18 cartridge

2.2.2 Transportation and storage

During transportation and storage the presence of water may cause hydrolysis of chemical warfare agents adsorbed on the column. In addition, temperature and storage time may be important for the recovery of chemical compounds from these columns.

2.2.2.1 Temperature

Experiments were carried out at 5°C and 20°C to see whether any variation in temperature would influence the recovery of agents from the columns. The effect was registered 24 hours after application on the columns. Tabun, sarin, soman, mustard and CS were used as test agents.

2.2.2.2 Water removal

In order to prevent hydrolysis an air stream was passed through the columns to remove any residual water after adsorption of the agents. The experiments were performed at 5°C and 20°C and analysis was carried out 24 hours after application on the columns.

2.2.2.3 Storage

The effect of long term storage of samples adsorbed to C-18 columns was tested to determine the degradation of chemical warfare agents. The columns were stored in the refrigerator. The agents tested were tabun, sarin, soman, mustard and CS.

2.2.3 Desorption

The chemical warfare agents were desorbed from the columns by using chloroform. The amount of desorbed agent may vary with the volume of eluant used. The experiments carried out with

the C-18 columns all showed high recoveries when using 5 ml chloroform for desorption of the chemical warefare agents.

2.2.3.1 Volume of eluant

Experiments with different volumes of chloroform for elution from the columns were carried out to study the effects of using larger volumes f or desorption. The volumes were 5, 10 and 15 ml and test substances were mustard gas and CS.

2.3 Analytical methods

All samples were analysed by gas chromatography. The qualitative analyses were based on relative retention times and multiple ion detection (MID), while the quantitative analyses were performed by integration of peak areas after adding an internal standard.

3 SAMPLE HANDLING

The procedures for sample handling of aqueous solutions were tested in field experiments on snow samples. In addition, a field test was carried out with aqueous extractions of samples from soil, sand and vegetation . A sample from muddy water was also included in the experimental work.

3.1 Snow samples

Snow samples were contaminated with 1 mg of the chemical warfare agents tabun, sarin, soman, CS and mustard. The samples were exposed to the prevailing weather conditions over night. Sample handling was elaborated with C-18 cartridges, XAD-2 columns and direct chloroform extraction.

3.1.1 Procedures

Snow samples contaminated with 1mg of a chemical warfare agent were collected and melted on a water bath to give 50-100ml water. This was carried out with all samples, while further treatment and procedures varied for the different methods.

3.1.1.1 XAD-2

The sample was passed through columns containing 2g XAD-2 in 30 seconds by using an external air pressure. The columns were sealed and brought to the laboratory for analysis after 24 hours without any further precaut ions. The columns were eluted with 10 ml CHCl3 after 10 minutes of equilibration between the adsorbent and eluant. An internal standard was added before analysis by gas chromatography.

3.1.1.2 C-18

The samples were passed through the cartridge in 3 minutes and transported to the laboratory for analysis after 24 hours. The columns were eluted with 10 ml chloroform. An internal standard was added before analysis by gas chromatography.

3.1.1.3 Direct extraction

The snow samples were melted and afterwards extracted by 10 ml chloroform. The chloroform samples were brought to the laboratory for analysis after 24 hours. Before analysis by gas chromatography an internal standard was added to the chloroform samples.

3.2 Soil, sand, vegetation and water samples

Samples of soil, sand vegetation and muddy water were contaminated with 1 mg of the chemical warfare agents tabun, sarin and soman. The samples of soil sand and vegetation were extracted with 50 ml water and isolated by using the three methods of sample handling described in chapter 3.1.1. The methods were applied directly to the samples of muddy water.

4 RESULTS

4.1 Results of experiments with XAD-2

All experiments with XAD-2 were performed by using columns with an internal diameter of 10 mm. As shown in Table 4.1 the amount of XAD-2 may be a limiting factor for adsorbing the chemical warfare agents. This was shown for soman by the larger recovery when increasing the amount of XAD-2 from 1 to 5 g. On the other hand adsorption of mustard gas seemed to be independent of the amount of XAD-2.

Amount of XAD-2 (g)	Per cent recovery of applied amount of agent	
	HD	GD
1	31	28
2	47	35
3	29	41
5	33	72

Table 4.1 Results of adsorption experiments with different amounts of XAD-2

It was examined whether the adsorption efficiency of XAD-2 could be influenced by the temperature of the aqueous solution. The results of the experiments carried out with temperatures varying from 0°C to 20°C showed no such effect (Table 4.2). Mustard, tabun and soman were slightly better adsorbed at 0°C than at 20°C, while sarin showed the opposite effect. This shows that samples may be directly applied to the columns without taking into account the temperature of the sample.

The adsorption efficiency might also be influenced by the temperature of the adsorbent. The effect of decreased temperature of XAD-2 in adsorption of soman was positive which indicated that no preheating of adsorbents was necessary when used under winter conditions. The results are shown in Table 4.3.

Temperature (°C)	Per cent recovery of applied amount of agent			
	HD	GA	GB	GD
0	14	25	55	81
5	-	-	-	69
10	-	-	-	76
20	12	20	70	71

Table 4.2 *Results of adsorption experiments with different temperatures of the solution*

Temperature (°C)	Per cent recovery of applied amount of agent
	GD
20	38
0	50

Table 4.3 *Results of experiments with XAD-2 stored at different temperatures*

The adsorption of different agents was dependent on the flow rate which is a measure of the contact time between agent and adsorbent (Table 4.4). The adsorbed amounts of tabun and soman were relatively constant at flow rates below 10 ml per minute, but the amount was decreased when the flow rate was increased to 100 ml per minute. Sarin seemed to be more difficult to adsorb and had the highest recovery at flow rate 5 ml/min. It was, however, noticed that at high flow rate the recovery was at least half that at low flow rate.

Flow rate (ml/min)	Per cent recovery of applied amount of agent		
	GA	GB	GD
100	17	30	42
10	22	32	90
5	25	55	81

Table 4.4 *Results of experiments with adsorption at different flow rates*

The time for transportation of samples from a battlefield to a laboratory and for storage in the laboratory before the analysis may vary. It is therefore important that the chemical warfare agents are stable when adsorbed on the XAD-2. Table 4.5 shows that no decomposition of soman was found after storage for 1 week at +5°C.

Days	0	1	2	7
Per cent recovery of applied amount of soman	39	29	33	36

Table 4.5 Results of experiments with storage on XAD-2 columns

The chemical warfare agents were desorbed from XAD-2 by elution with chloroform. The results of the experiments with varying volumes of chloroform are shown in Table 4.6. The experiments showed that 10 ml and 15 ml eluant enhanced the recovery of both soman and mustard compared to 5 ml.

Volume of eluant (ml)	Per cent recovery of applied amount of agent	
	HD	GD
5	24	16
10	49	35
15	45	39

Table 4.6 Results of experiments with different volumes of eluant for desorption

During desorption the time of equilibration with eluant on the column had a large effect on the recovery of soman. Increasing the equilibration time from 2 to 5 minutes showed an increase in recovery from 12 to 29 per cent, and the recovery was further increased to 44 per cent after 10 minutes of equilibration.

Equilibration time (min)	Per cent recovery of applied amount of agent
	GD
2	12
5	29
10	44

Table 4.7 Results of experiments with different equilibration times before elution

4.2 Results of experiments with the C-18 cartridge

The results in Table 4.8 show that the temperature during storage was important for positive identification of tabun and mustard gas, while no effect was found for the three other agents.

The results in Table 4.8 show further that removal of water by passing an air stream through the column did not have a positive effect on the recovery. On the contrary, the recovery was considerably lower for all the agents. Desorption probably occurred during the air stream treatment.

Storage time and temperature	Amount of agent relative to immediate extraction by chloroform				
	GA	GB	GD	CS	HD
Immediate extraction	100	100	100	100	100
24 h 20°C water removal	0	36	74	24	0
24 h 20°C	8	58	100	27	0
24 h 5°C water removal	50	36	80	24	52
24 h 5°C	100	73	100	26	63

Table 4.8 *Results of experiments after storage for 1 day at 5 and 20°C, with and without water removal*

The results of storage at 5°C for different periods up to 1 week showed only a slight decrease in the recovery of all agents elaborated. The results show that the agents may be detected for a much longer period than 1 week of storage (Table 4.9).

Time	Percent agent recovered compared with immediate extraction				
	GA	GB	GD	CS	HD
Immediate extraction	100	100	100	100	100
" eluation	100	95	100	85	63
1 day	100	73	100	75	66
2 days	55	48	100	70	42
5 days	37	59	100	53	40
7 days	35	43	100	56	33

Table 4.9 *Results of long term storage of the C-18 cartridge*

As seen from Table 4.10 about 60 % of the agents were desorbed by 5 ml chloroform. Another 20% is desorbed by elution with 10 ml chloroform. The effect of increasing the volume to 15 ml is however small.

Volume of $CHCl_3$ (ml)	Percent recovered of applied amount	
	HD	CS
5	63	58
10	81	85
15	87	89

Table 4.10 Results of desorption by different volumes of chloroform

4.3 Results of sample handling experiments

The report describing the work undertaken during the winter 1983/84 (Document CD/509 (3)) shows that extraction carried out in the field by using chloroform is an excellent method to preserve any agent present in a snow sample. The results obtained by using this method was compared with the results obtained with the method of adsorption to polymers.

As shown in Table 4.11 XAD-2 gave relatively good recoveries for sample handling of all the agents even if the recoveries of tabun and sarin were reduced to 50% of the amount recovered by

Exercise/ method	Per cent recovered				
	GA	GB	GD	CS	HD
1) In chloroform solution	100 (121 µg)	100 (21 µg)	100 (121 µg)	100 (490 µg)	100 (170 µg)
XAD-2	54	47	100	100	100
2) In chloroform solution	100 (21 µg)	100 (37 µg)	100 (145 µg)	100 (290 µg)	100 (204 µg)
XAD-2	0	83	100	93	100

Table 4.11 Results of field experiments with XAD-2 columns

chloroform extraction. This exercise was carried out at temperatures just below 0°C. The snow was grainy, but dry. The second exercise was performed at temperatures between 0 and 3°C when the snow was wet and grainy. As seen in Table 4.11, this reduced the amount of tabun recovered by direct extraction and made it impossible to identify tabun from the XAD-2 column. This result is in conformity with the results from our earlier reports showing that tabun may be difficult to verify under winter conditions, probably due to hydrolysis.

Method	Per cent recovered				
	GA	GB	GD	CS	HD
In chloroform solution	100 (121 µg)	100 (119 µg)	100 (226 µg)	100 (630 µg)	100 (323 µg)
XAD-2	63	60	100	75	100
C-18	35	62	100	100	98

Table 4.12 Results of field experiments comparing three methods of sample handling

Since C-18 columns are efficient for clean-up of aqueous solution this was expected to be a promising tool also in sample handling. Concentration of the sample, clean-up and transformation to a medium easy to handle and transport could be carried out in one step.

Table 4.12 shows that the recovered amounts of the chemical warfare agents on a C-18 column were comparable with those of the XAD-2 method.

Sample	Method	Per cent recovered	
		HD	GD
Muddy water	Chloroform	100 (480 µg)	100 (59 µg)
	XAD-2	16	33
	C-18	100	100
Soil	Chloroform	100 (345 µg)	100 (270 µg)
	XAD-2	12	22
	C-18	71	67
Sand	Chloroform	100 (418 µg)	100 (240 µg)
	XAD-2	20	33
	C-18	100	58
Vegetation	Chloroform	100 (96 µg)	100 (240 µg)
	XAD-2	100	17
	C-18	91	66

Table 4.13 *Results of experiments to test three extraction methods on various aqueous samples*

Even if the methods of extraction from aqueous solutions both with chloroform and adsorbents were developed for sample handling of snow samples these methods are applicable for other aqueous samples as well. The results of handling aqueous extracts of soil, sand and vegetation and samples of muddy water are shown in Table 4.13. The recoveries vary, but show that the methods may be useful for all kinds of aqueous solutions.

5 CONCLUSIONS

The ultimate objective of the research programme is to develop comprehensive procedures for selection, handling, transportation and analysis of samples collected in the field, on which the verification of alleged use of chemical weapons may be based.

This report has focused on sample handling and transportation of samples from the field to the laboratory for unambiguous analysis. It is important that the samples are not degraded or contaminated during this process. In this connection it may often be necessary with a clean-up and concentration step of the samples in the field both to reduce volume and prevent further degradation. Samples from soil and vegetation may easily be submitted to bacterial growth and degradation. Samples from water and snow may require a large reduction in sample volume before transportation. The needs and type of equipment a fact-finding team will need for sample-handling have been identified.

The experiments carried out to define suitable conditions for application of XAD-2 columns in sample handling were performed with columns with an internal diameter of 1 cm. This column was chosen as a standard column to give a defined bed height of the adsorbent. This column size prevented too high resistance during liquid passage. The experiments showed further that 2

grams of XAD-2 was a sufficient amount to adsorb a relatively high per cent of the chemical warfare agents present in an aqueous sample. Larger amounts of XAD-2 gave higher adsorption of the chemical warfare agents, but required larger volume of eluant for desorption. The samples would then be more diluted and would probably need to be concentrated. The experiments showed that the temperature of the samples did not influence the adsorption of chemical warfare agents to XAD-2. The chemical warfare agents showed a high stability during storage on XAD-2 columns for 1week, which indicated that this was not a limiting time for storage. The recovery of the XAD-2 method was dependent on the equilibration time of eluant and XAD-2 before desorption. A much higher yield was achieved when the equilibration time was increased from 2 to 10 minutes.

As an alternative, the C-18 cartridge was also ideal for field experiments. This cartridge is small, easy to handle and had a high adsorption efficiency for chemical warfare agents in aqueous solutions. The experiments indicated that chemical warfare agents were relatively easily desorbed by an air stream. Removal of residual water by blowing air through the cartridge should therefore be avoided. The recovery of chemical warfare agents showed a slow decrease during storage for 1week but the recovered amount was still more than 30% of the applied amount.

On the basis of these field experiments and research, procedures have been developed for two different methods of sample handling. Sample handling in the field may be carried out both by using chloroform extraction or one of the two adsorption methods using columns with porous polymers .

The method of organic solvent extraction necessitates use of glassware and organic solvents in the field. This method, which is simple to perform, gives a high recovery of all known chemical warfare agents and may easily be applied under field conditions.

The alternative method is based on the adsorption of chemical warfare agents to columns containing porous polymers (the method of porous polymer adsorption). This method is slightly less efficient than the extraction with the organic solvent. The columns are, however, easy to use and to transport and have also excellent storage properties. In addition, the amount of field equipment necessary is reduced to a minimum when using this method.

The methods have successfully been used with samples from snow, muddy water and aqueous extracts of soil, sand and vegetation. These methods are both of general use on an all year basis and may be used in the field for verification of alleged use of chemical weapons. The two methods may supplement each other and application of these methods require only simple equipment.

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