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## The Fate of the Chemical Warfare Agent During DNA Extraction\*

**ABSTRACT:** Forensic laboratories do not have the infrastructure to process or store contaminated DNA samples that have been recovered from a crime scene contaminated with chemical or biological warfare agents. Previous research has shown that DNA profiles can be recovered from blood exposed to several chemical warfare agents after the agent has been removed. The fate of four toxic agents, sulfur mustard, sodium 2-fluoroacetate, sarin, and diazinon, in a lysis buffer used in Promega DNA IQ™ extraction protocol was studied to determine if extraction would render the samples safe. Two independent analytical methods were used per agent, selected from GC-MS, <sup>1</sup>H NMR, <sup>19</sup>F NMR, <sup>31</sup>P NMR, or LC-ES MS. The methods were validated before use. Determinations were carried out in a semi-quantitative way, by direct comparison to standards. Agent levels in the elution buffer were found to be below the detectable limits for mustard, sarin, sodium 2-fluoroacetate or low (<0.02 mg/mL) for diazinon. Therefore, once extracted these DNA samples could be safely processed in a forensic laboratory.

**KEYWORDS:** forensic science, DNA, DNA extraction, chemical warfare agents, analytical chemistry, mass spectrometry, nuclear magnetic resonance

Since the distribution of anthrax-contaminated letters through the U.S. postal system in 2001, many government agencies have created chemical, biologic, radiological and nuclear (CBRN) response teams as part of a CBRN event management strategy. These response teams are multidisciplinary, comprised numerous individuals with varied and specialized knowledge, who are responsible for mitigating and investigating such an event. Peace Officers such as explosive disposal specialists and forensic identification specialists (FIS) would typically be members in a CBRN response team, tasked with explosive mitigation and crime scene examination respectively. The mandate for the Royal Canadian Mounted Police (RCMP) is clearly outlined in the statement below quoted from section 5.3(a) of the Memorandum of Understanding (MOU) signed by the Solicitor General of Canada, the Department of National Defence and the RCMP in 1994.

“Provide the expertise and equipment required to render safe or otherwise mitigate the dispersal effects of an Improvised Biological/Chemical Dispersal Device (B/CDD). As well, where necessary, manage a crime scene/collect and forensically examine evidence. In addition take on whatever roles may be required as a Peace Officer at such incidents;”

To fulfill such a mandate one obvious requirement for forensic identification was the creation of specialized courses designed to provide FIS with the knowledge and training to work in hazardous chemical, biological and radiological environments. Another need was to understand how the presence of chemical and biological warfare (CBW) agents, as well as the decontamination agents

used to neutralize them, will affect the performance of chemicals used to enhance friction ridge and footwear impression evidence. Several recent papers have described the effects of CW agents on friction ridge development (1), DNA recovery (2,3) and footwear enhancement (4). Other research has explored the effects of the decontamination agents on our ability to recover physical evidence (1–5).

CBRN-FISs have the requisite knowledge and training to process friction ridge and footwear impression evidence within the contaminated crime scene transmitting the images of chemically enhanced physical evidence out of the scene. The recovery of DNA evidence from a chemically or biologically hazardous crime scene involves removal of contaminated exhibits since the equipment required for DNA amplification and profiling is immobile.

As our previous research showed that blood exposed to several CW agents can still yield DNA profiles, further research into the effect of the DNA extraction process on the chemical stability of these CW agents was pursued. If the RCMP DNA extraction process was sufficient to neutralize the CW agents, then DNA samples could be safely handled in a forensic laboratory after only some preliminary processing in a level 3 environment. Level 3 isolators for BW agents are already commercially available and could be easily adapted for on-site DNA extraction. For CW agents, level 3 isolators are under development and close to being commercialized (6). Alternately, the DNA extraction could be performed inside the dirty zone.

The purpose of these experiments is to observe the chemical stability of mustard, sodium 2-fluoroacetate, sarin and diazinon to the Promega DNA IQ™ System-Small Casework Protocols. Hydrogen cyanide was also previously shown to not inhibit DNA recovery but it is not included in this study as it is a volatile agent that poses no health risks to DNA technicians because it evaporates so quickly.

To assess the chemical stability of the four selected agents, their persistence was monitored semi-quantitatively by two independent chemical analysis methods, during propagation through the DNA recovery protocol. Intact sulfur mustard, sarin and diazinon were

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traced by direct NMR analysis and by extraction and subsequent GC-MS, whereas sodium 2-fluoroacetate was monitored by direct LC-ESI MS and NMR. Other workers have shown that these methods can adequately be employed for the analysis of chemical warfare agents and pesticides. GC-MS and NMR analysis of sulfur mustard and sarin was elaborated in many studies, of which some recent examples are given (7–10). Diazinon is commonly analyzed at trace levels by GC-MS (11,12). Although, NMR has not been reported for diazinon, to our knowledge,  $^{31}\text{P}$  NMR will be applicable by analogy to sarin. Sodium 2-fluoroacetate is a strongly ionic compound of which the anionic 2-fluoroacetate is commonly analyzed by ion chromatography (13). To our knowledge, there are no reports available on LC-ESI MS analysis of 2-fluoroacetate, but its ionic character should make the compound amenable to LC-ESI MS analysis. In addition,  $^{19}\text{F}$  NMR has been reported suitable for the detection of the chemical in plant and tissue material (14,15). This brief review of the literature shows that these methods can be straightforwardly applied for monitoring of the four intact agents in DNA extraction liquids.

## Materials and Methods

### Materials

A Promega DNA IQ™ System kit was purchased from Promega Benelux B.V. (Leiden, The Netherlands, kit lot #181446; lysis buffer lot #16812710, elution buffer lot #17128605). *O*-isopropyl methylphosphonofluoridate (sarin, >98.5% pure) and bis-(2-chloroethyl)sulfide (sulfur mustard, 98.0% pure) were from TNO stock. Sodium 2-fluoroacetate (“analytical standard,” 99.7% pure) and diazinon (“analytical standard,” 98.4%) were purchased from Sigma-Aldrich (Seelze, Germany). DMSO-*d*<sub>6</sub>, sodium fluoride (NaF), trimethylphosphate (TMP), and deuteriated water (D<sub>2</sub>O) were purchased from Aldrich (Weinheim, Germany). Chloroform (CHCl<sub>3</sub>; for organic trace analysis) and formic acid (p.A.) were purchased from Merck (Darmstadt, Germany). All water used was deionized water (“milliQ”), obtained from a local Millipore unit (Millipore, Milford, MA).

### Agent Presence During the DNA Extraction Protocol

The first step in the DNA extraction protocol involves incubation of the DNA sample in lysis buffer at 95°C. These samples are referred to as Lysis Buffer Samples. The remainder of the DNA extraction protocol involves the following steps: addition of beads; incubation; separation of the beads from the lysis buffer; a first wash with lysis buffer; a second wash with wash solution; air-drying and addition of elution buffer. The presence of CW agent at the various stages was established by analysis of the following liquids, respectively: supernatant lysis buffer after addition of beads; separate or pooled wash liquids, and elution buffer.

These samples are referred to as Postlysis Buffer Samples and their preparation and analysis are described below.

### NMR Analysis

All spectra were recorded on a MercuryPlus 400 MHz NMR spectrometer (Varian Inc., Palo Alto, CA), equipped with the appropriate element specific probe.

**Preparation of Lysis Buffer Samples**—Each CW agent was processed in the following manner using the amounts and the internal standards shown in Table 1.

TABLE 1—Quantities of agents and internal standards for NMR.

Agent	Amount (mg)	Internal standard	NMR
Sulfur mustard	13.4	DMSO- <i>d</i> <sub>6</sub>	$^1\text{H}$ , $^{13}\text{C}$
Diazinon	10.7	TMP in DMSO- <i>d</i> <sub>6</sub>	$^{31}\text{P}$
Sarin	11.1	TMP in DMSO- <i>d</i> <sub>6</sub>	$^{31}\text{P}$
Sodium 2-fluoroacetate	10.2	NaF in D <sub>2</sub> O	$^{19}\text{F}$

The quantity of agent shown in Table 1 was weighed into an NMR tube. 1 mL of the lysis buffer and the appropriate internal standard were added; the sample was thoroughly mixed by vortexing. The internal standards were: a capillary with TMP in DMSO-*d*<sub>6</sub>, for  $^{31}\text{P}$  NMR, a capillary with NaF in D<sub>2</sub>O, for  $^{19}\text{F}$  NMR, a capillary with DMSO-*d*<sub>6</sub>, for  $^{13}\text{C}$  NMR, and the H<sub>2</sub>O signal in  $^1\text{H}$  NMR (for all  $^1\text{H}$  NMR, the  $^1\text{H}$  resonance was saturated by irradiation at 4.8 ppm). The sample was kept at room temperature (22°C). An NMR spectrum was recorded directly (0 h), after 24 and 48 h. Subsequently, the solution was heated to 95°C for 30 min and left to cool down to room temperature. Another NMR spectrum was then recorded. The spectra were compared to similarly obtained spectra of 1 mL of blank lysis buffer with the relevant internal standard.

**Preparation of Postlysis Buffer Samples**—To allow direct NMR measurement of the agents in wash liquid and elution buffer, the procedure was scaled up, proportionally to the indications in the manufacturer's protocol. Samples of the separate agents in lysis buffer, 2100 µL at 10 mg/mL, were prepared (in lysis buffer with 1% 1 M DTT). Solutions were heated at 95°C for 30 min. Approximately, 98 µL of the Fe bead suspension was added to the 2100 µL solution and the mixture was incubated for 5 min. The supernatant was removed and 1400 µL of lysis buffer (no DTT) was added. After shaking, the supernatant was removed and 1400 µL of wash buffer was added (wash buffer, 1:1 diluted with 50% ethanol/50% isopropanol). After shaking, the supernatant was removed and the beads were dried under a gentle flow of air. 700 µL of elution buffer was then added and the beads were incubated at 65°C for 5 min. The supernatants removed (lysis buffer with DTT, lysis buffer, wash buffer) were combined for each separate agent. NMR experiments were carried out with 700 µL of the pooled wash solutions. After separation of the beads from the elution buffer, 700 µL of elution buffer was used for NMR.

### GC-MS Analysis

**Preparation of Lysis Buffer Samples**—A specific amount of CW agent was weighed into a teflon capped vial and the corresponding volume of DNA lysis buffer was added to obtain a solution with a concentration of *c.* 10 mg/mL. Prior to every analysis, the mixture was shaken by hand for 1–2 min and 100 µL (or 50 µL) of the mixture was then pipetted into another teflon capped vial and 2.0 mL of CHCl<sub>3</sub> was added (or 1.0 mL with 50 µL subsample). The buffer/chloroform mixture was shaken by hand, for 2 min, and left for about 1 min to separate in two layers. Part of the CHCl<sub>3</sub> layer was removed and diluted 10-fold with CHCl<sub>3</sub>. Of this diluted extract, 1 µL was used for GC-MS analysis. Samples were taken at 24-h intervals, from 0 to 168 h (1 week).

For semi-quantitative comparison, a solution of CW agent in CHCl<sub>3</sub>, 50 µg/mL, was prepared as a standard; 50 µg/mL corresponds to the extract CW agent concentration without decomposition and with complete extraction recovery. Of this 50 µg/mL solution, 1 µL was used for comparative analysis.

Attempts at extraction of 2-fluoroacetic acid from acidified solutions failed, due to irreproducible recovery. The extracts, apparently acidic, damaged the GC column. Therefore, GC-MS analysis of 2-fluoroacetate was abandoned and LC-MS was performed.

**Preparation of Postlysis Buffer Samples**—As sulfur mustard did not carry on through the lysis buffer sequence of the DNA extraction process and as sodium 2-fluoroacetate was not extractable, postlysis buffer samples were only studied by GC-MS for diazinon and sarin.

After each subsequent step in the protocol, 100  $\mu$ L of the corresponding solution was removed for agent extraction. This 100  $\mu$ L was vigorously shaken with 2.0 mL of chloroform. For the prewash liquids, 100  $\mu$ L was removed and diluted to 1.0 mL with another 900  $\mu$ L of chloroform; 1  $\mu$ L of this final dilution was used for GC-MS analysis.

For the wash steps, 100  $\mu$ L wash liquid was extracted with 2 mL of chloroform and 1  $\mu$ L of the chloroform layer was directly analyzed by GC-MS. Of the elution buffer, 50  $\mu$ L was extracted with 1.0 mL of chloroform; 1  $\mu$ L of the chloroform layer was directly analyzed by GC-MS.

**Analysis**—All GC-MS analyses were carried out with a 6890 Series II gas chromatograph, equipped with a type 5973N mass selective detector (MSD; Agilent, Amstelveen, The Netherlands). The gas chromatograph was equipped with the standard split/splitless injector (Agilent), used in splitless mode (1  $\mu$ L injection, injector at 220°C). Gas chromatography (GC) was carried out with a CpSil 8 CB column (50 m  $\times$  0.32 mm i.d., 0.25  $\mu$ m film thickness; Chrompack, Middelburg, The Netherlands), with helium as the carrier gas, at a constant flow of 1.0 mL/min. An oven temperature program was used, as follows: 40°C (1 min), 10°C/min to 280°C, and 280°C maintained for 5 min. The GCMS interface temperature was kept at 280°C. The MSD was operated under electron ionization (EI, 70 eV ionization energy), at a source temperature of 200°C and scanning from 25 to 500 Da at three scans per second.

#### LC-MS Analysis

LC-MS analysis was used exclusively for sodium 2-fluoroacetate.

**Preparation of Samples**—A 10 mg/mL solution of sodium 2-fluoroacetate in lysis buffer (with 1% 1 M DTT) was prepared, with a total volume of *c.* 20 mL. Aliquots of 10  $\mu$ L were removed for analysis, at the appropriate time intervals between 0 and 48 h. Following the DNA extraction kit protocol, the solution was then heated to 95°C for 30 min and a 10  $\mu$ L aliquot was removed for analysis. The supernatant was then cooled to room temperature, an appropriate amount of the Fe-beads suspension was added and left standing for incubation (5 min). The supernatant was removed and a 10  $\mu$ L was taken for analysis.

The washing steps were executed and of the pooled wash liquids 10  $\mu$ L was taken for analysis.

All analyses were carried out directly after removal of an aliquot from the DNA extraction process. Prior to LC-MS analysis, samples were diluted with eluent, 0.1% formic acid in milliQ water, at ratios between 1:1000 and 1:200,000, appropriate for the Q-TOF sensitivity range.

**Analysis**—All analyses were carried out with a Q-TOF (Micro-mass, Altrincham, U.K.) mass spectrometer, operated in negative ion electrospray mode (3.0 kV spray voltage, 25 V cone voltage,

desolvation gas N<sub>2</sub> at 400 L/min, nebulizer gas N<sub>2</sub> at 20 L/min). For separation, the mass spectrometer was equipped with an LC system consisting of a PepMap C18 column (15 cm  $\times$  1 mm ID, 3  $\mu$ m particles; LC Packings, Amsterdam, the Netherlands), and an Alliance Liquid Chromatograph (type 2690; Waters, Milford, MA, U.S.A.). A pump flow of 0.6 mL/min was generated by the liquid chromatograph; the flow was split, precolumn, to give a flow of *c.* 40  $\mu$ L/min through the column.

The analyses were run isocratically, using 0.1% formic acid in milliQ water. Injection of 10  $\mu$ L of dilute sample was achieved by a sixport switching valve (Valco, Schenkon, Switzerland) with a 10  $\mu$ L loop mounted. The Q-TOF mass spectrometer was calibrated according to a common procedure; the negative ion low mass scale was verified with methylphosphonic acid (95 Da, for [M-H]<sup>−</sup>).

## Results

### Blank DNA Lysis Buffer

Spectra of the blank lysis buffer were obtained to establish a background for the CWA spiked material. The DNA lysis buffer did not produce any signals in <sup>31</sup>P and <sup>19</sup>F NMR spectra. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the lysis buffer showed complicated patterns, with 30 <sup>1</sup>H signals, and over 50 <sup>13</sup>C signals. The resonances were not individually assigned to the individual constituent compounds of the DNA lysis buffer, as identification of those compounds was not relevant to the study. The proton chemical shifts mainly lie in the saturated alkyl region (0.9–4.5 ppm), and they derive from the alkyl protons of Tris, EDTA, and SDS. As the blank <sup>1</sup>H and <sup>13</sup>C spectra were only used for detailed comparison to corresponding spectra of the sulfur mustard spiked lysis buffer, the number of <sup>1</sup>H and <sup>13</sup>C signals in the blank slightly hampered analysis of sulfur mustard in lysis buffer.

In GC-MS analysis, the chloroform extract of blank lysis buffer did not show any significant signals to interfere with the agent analysis. Some minor peaks were observed in the total ion current (TIC) chromatogram of the extract, but inspection of the corresponding spectra showed that these signals belonged to minor impurities that typically occur in buffers and solvents, for instance plasticizer (diisooctylphthalate) and tetrachloroethene (from the chloroform).

### Sulfur Mustard in Lysis Buffer

**<sup>1</sup>H and <sup>13</sup>C NMR**—The initial <sup>1</sup>H NMR spectrum, at 0 h, did not show any evidence for the presence of sulfur mustard (see Fig. 1). In addition to the blank lysis buffer signals, the spectrum displayed distinct signals for the three hydroxyethyl protons, <sup>A</sup>HO-<sup>B</sup>H<sub>2</sub>C-<sup>C</sup>H<sub>2</sub>C, typical of hydrolysis products thiodiglycol and 2(2-chloroethyl)thioethanol (hemimustard). The <sup>13</sup>C NMR spectrum shows resonances for carbon probably adjacent to sulfoxide (-CH<sub>2</sub>-SOCH<sub>2</sub>-) or sulfone (-CH<sub>2</sub>-SO<sub>2</sub>-CH<sub>2</sub>-). From these observations it is concluded that the bulk (>99%) of sulfur mustard is rendered harmless in DNA lysis buffer within *c.* 30 min. The reaction product is most probably thiodiglycol sulfoxide or -sulfone. In addition, the spectra obtained at later stages of buffer exposure and after heating at 95°C for 30 min look identical to the 0 h spectrum.

**GC-MS**—Sulfur mustard eluted at 12.30 min under the GC-MS conditions used. A typical chromatogram and spectrum, from sulfur mustard in lysis buffer after 24 h, are shown in Fig. 2. The GC peak area was used in semi-quantitative determinations. The

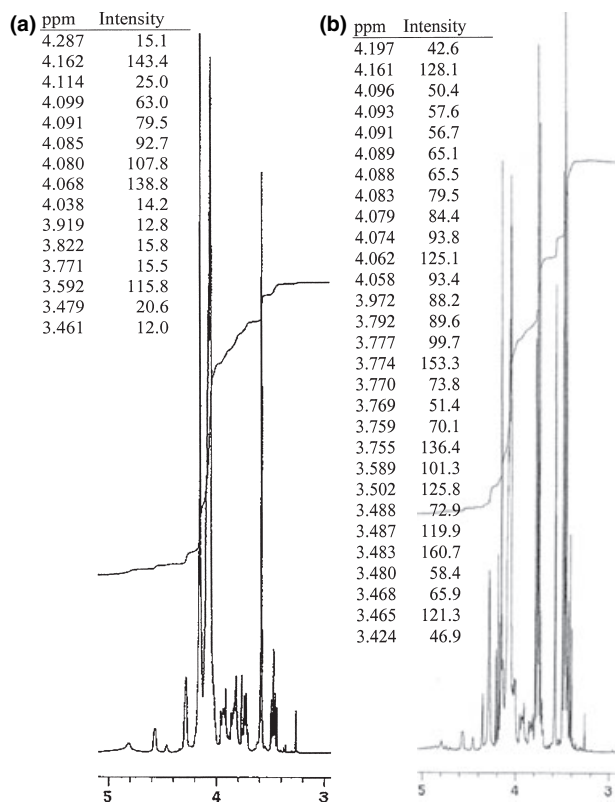


FIG. 1—Partial  $^1\text{H}$  NMR spectra of lysis buffer blank (a) and lysis buffer spiked with sulfur mustard (b), at time 0 h.

TABLE 2—Summary of GC-MS results on degradation of sulfur mustard, diazinon, and sarin in lysis buffer.

Time (h)	Sulfur mustard		Diazinon		Sarin	
	% to 0 h	% to standard*	% to 0 h	% to standard*	% to 0 h	% to standard*
0	100	37	100	90	100	95
24	1.2	0.4	50	45	76	73
48	1	0.4	46	42	55	52
72	0.9	0.3	28	25	43	41
96	0.8	0.3	32	28	24	23
120	0.3	0.1	15	14	14	13
144	0.3	0.1	19	18	4	4
168	0.1	0.04	20	18	0.7	0.7

\*By comparison to a solution of 50  $\mu\text{g/mL}$  agent in  $\text{CHCl}_3$ .

response in the TIC chromatogram of the sample taken at 0 h was compared to that in the TIC chromatogram of a 50  $\mu\text{g/mL}$  sulfur mustard solution in  $\text{CHCl}_3$ , which analysis corresponds to conditions of no decomposition and full extraction recovery. Only 37% of sulfur mustard was intact at 0 h. This is at some variance with the 0-h observation in NMR, because the time between mixing and spectrum recording was shorter for GC-MS and extraction than for NMR. The GC-MS results are summarized in Table 2.

Table 2 shows that degradation of sulfur mustard in lysis buffer proceeded relatively fast. After a few minutes (effectively during sample preparation, mixing and shaking), about two-thirds of the original quantity was converted. After 2 days, the sulfur mustard concentration was down to less than a percent, 0.1  $\text{mg/mL}$ , of the original contamination level, 10  $\text{mg/mL}$ . After a week, the sulfur

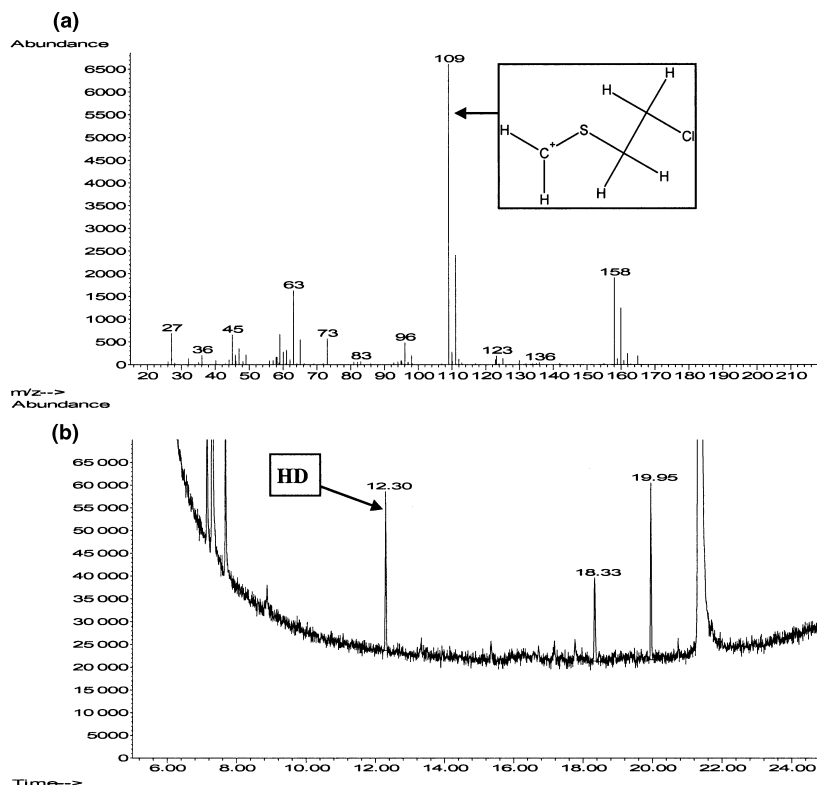


FIG. 2—Total ion current (TIC) chromatogram of the dichloromethane extract of sulfur mustard spiked lysis buffer, at 24 h (b; “HD” indicates the sulfur mustard peak), and mass spectrum of sulfur mustard (a).

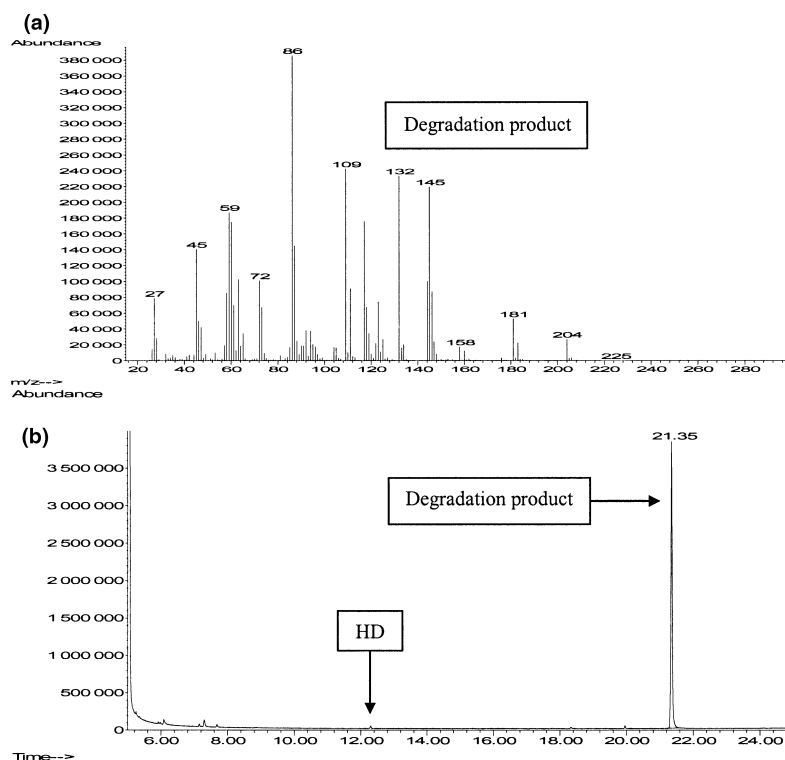


FIG. 3—Total ion current chromatogram, at 24 h (from the same analysis as in Fig. 2; (b), and mass spectrum of an apparent 2-(2-chloroethyl)thioethyl-derivative formed by sulfur mustard in lysis buffer, at retention time 21.35 min (a).

mustard concentration had dropped below one part per thousand ( $\text{‰}$ ), 0.01 mg/mL, of the original spiking concentration.

Attempts were made to assess whether alkylating 2-(2-chloroethyl)thioethyl containing degradation products, for instance hemimustard, are formed from sulfur mustard in the lysis buffer. Most characteristic for such derivatives is the  $m/z$  109 fragment, representative of the alkylating structure  $+\text{CH}_2\text{SCH}_2\text{CH}_2^{35}\text{Cl}$  and abundant in the sulfur mustard mass spectrum. Only one such product was observed to an appreciable amount in any of the GC-MS analyses. The spectrum of this compound, eluting at 21.35 min, is given in Fig. 3a. The spectrum clearly displays the above mentioned  $m/z$  109 signal and  $m/z$  111 for the corresponding  $^{37}\text{Cl}$  isotope. In addition,  $m/z$  123 and 125 confirm the presence of a  $\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{Cl}$  moiety. The other signals present, for example  $m/z$  204, 132 and 86, correspond to the remainder of the sulfur mustard adduct structure. However, they do not match fragments of the known buffer constituents, sodium decylsulfate, EDTA, or tris, present at mol/L concentrations. Hence, the compound corresponding to the Fig. 3a spectrum was not identified beyond the presence of the alkylating 2-chloroethylthioethyl moiety. The dominant product observed by NMR is either bis(2-hydroxyethyl)sulfoxide or -sulfone. However, these hydrolysis products are generally not extractable with chloroform and, therefore, not observed in GC-MS analysis. There was no indication in any of the NMR spectra that 2-chloroethyl containing compounds were present. However, signals from low levels of such a compound may be masked by the signals of the main degradation product. In that respect, it is noted that NMR did not yield any signals of the  $c.$  0.5% sulfur mustard that survived after 24 h. This effectively puts the upper limit for  $\text{C}_{10}\text{H}_{21}\text{-O-SO}_2\text{-O-C}_2\text{H}_4\text{-S-C}_2\text{H}_4\text{-Cl}$  at a percent of the sulfur mustard concentration. Hence, at no time are any 2-(2-chloroethyl)thioethyl compounds present at a level above 1% of the original sulfur mustard concentration. In addition, the primary adduct

between sulfur mustard and decyl sulfate degrades further over time: the peak area for that compound decreases after prolonged standing (followed up to 96-h exposure of sulfur mustard to lysis buffer).

The toxicity of the particular adduct of sulfur mustard and decyl sulfate,  $\text{C}_{10}\text{H}_{21}\text{-O-SO}_2\text{-O-C}_2\text{H}_4\text{-S-C}_2\text{H}_4\text{-Cl}$ , is not known. Because the other degradation products do not retain the alkylating capability, represented by the 2-chloroethyl group, these degradation products qualify as “nontoxic,” as compared to sulfur mustard.

#### Sarin in Lysis Buffer

$^{31}\text{P}$  NMR—The initial  $^{31}\text{P}$  NMR spectrum of sarin in lysis buffer, at 0 h, shows two intense  $^{31}\text{P}$  signals (29.88 and 36.33 ppm) for sarin, due to  $^{31}\text{P}$ – $^{19}\text{F}$  coupling, a third signal (25.5 ppm) for the first degradation product and a minor signal (31.81 ppm) for a P-containing contaminant. Table 3 gives a summary of relative quantities from  $^{31}\text{P}$  NMR results over time. The lysis buffer itself, as a blank, did not yield any  $^{31}\text{P}$  signals.

TABLE 3—Summary of  $^{31}\text{P}$  NMR results for sarin in DNA lysis buffer.

Compound	Resonance (ppm)	Relative quantity (%)			
		0 h	24 h	48 h	48 h+*
Sarin	29.88 + 36.33 <sup>†</sup>	99	96	94	45
1st degradation product	25.55	0.8	4	6	21
Contaminant	31.81	0.4	0	0	0
2nd degradation product	31.20	0	0	0	23
3rd degradation product	27.97	0	0	0	12

\*Forty-eight hours and 30 min of heating at 95°C and cooling down to room temperature.

<sup>†</sup>Two signals, due to  $^{31}\text{P}$ – $^{19}\text{F}$  coupling.

After 24 h, over 95% of the original sarin is still intact, whereas after 48 h over 94% is still intact. Even subsequent heating at 95°C for 30 min, and cooling to room temperature, leaves 45% of the original sarin intact. Heating generates another two degradation products, probably through phosphorylation of buffer components.

**GC-MS**—Sarin eluted at 5.93 min under the GC-MS conditions used. A typical chromatogram and spectrum are shown in Fig. 4. The GC peak area was used in semi-quantitative determinations. The response in the TIC chromatogram of the sample taken at 0 h was compared to that in the TIC chromatogram of a 50 µg/mL sarin solution in CHCl<sub>3</sub> (corresponding to the conditions of no decomposition and full extraction recovery); all sarin was intact at 0 h. The results are summarized in Table 4.

At 0 h, the GC peak area from sarin in lysis buffer is slightly larger than that of the 10 mg/mL equivalent standard; that results in an apparent quantity of over a 100% (relative to standard). At 24 h, the quantity of sarin appears to have increased to a 120%. However, this is due to some variability in the response of the GC-MS equipment and the semi-quantitative determination. At best, the 24-h value indicates that no degradation took place. Degradation is obvious at 48 h and after additional heating.

#### Diazinon in Lysis Buffer

**<sup>31</sup>P NMR**—The initial <sup>31</sup>P NMR spectrum of diazinon in lysis buffer, at 0 h, showed the single diazinon signal at 59.56 ppm (relative to TMP, at 2.71 ppm). The <sup>31</sup>P NMR spectrum of blank lysis buffer did not show any signals. After 24 h, a second signal

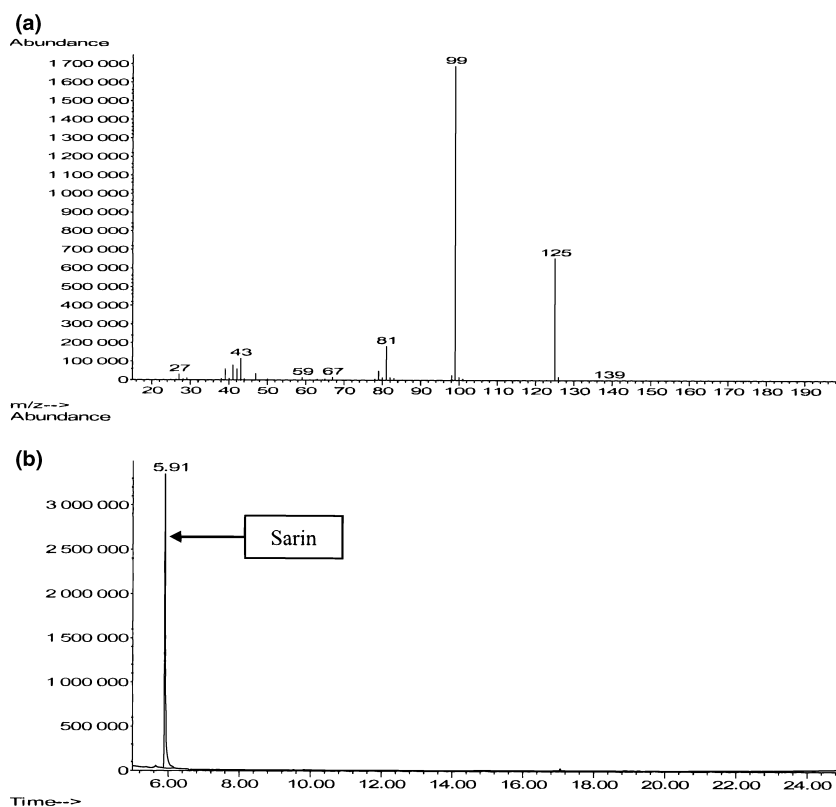


FIG. 4—Total ion current chromatogram of the dichloromethane extract of sarin spiked lysis buffer, at 0 h and scaled to the sarin peak (b), and mass spectrum of sarin (a).

TABLE 4—Summary of GC-MS results for sarin and diazinon and LC-MS results for 2-fluoroacetate on degradation of agents in lysis buffer.

Time (h)	Sarin (GC-MS)		Diazinon (GC-MS)		2-Fluoroacetate (LC-MS)*	
	% to 0 h <sup>†</sup>	% to standard <sup>†,‡</sup>	% to 0 h <sup>†</sup>	% to standard <sup>†,‡</sup>	% to 0 h <sup>†</sup>	% to standard <sup>†,§</sup>
0	100	110	100	98	100	100
24	120	120	110	110	100	100
48	91	97	70	69	100**	100**
48+ <sup>¶</sup>	8	8	7	7	100 <sup>††</sup>	100 <sup>††</sup>

\*Determined in duplo.

<sup>†</sup>% intact agent recovered.

<sup>‡</sup>By comparison to a solution of 50 µg/mL agent in CHCl<sub>3</sub>.

<sup>§</sup>By comparison to a solution of 50 µg/mL agent in milliQ water.

<sup>¶</sup>Forty-eight hours and 30 min of heating at 95°C with cooling down to room temperature.

\*\*Determined after 94 h (see text).

<sup>††</sup>Ninety-four hours and 30 min of heating at 95°C with cooling down to room temperature.

TABLE 5—Summary of  $^{31}\text{P}$  NMR results for diazinon in DNA lysis buffer.

Compound	Resonance (ppm)	Relative quantity (%)			
		0 h	24 h	48 h	48 h+*
Diazinon	59.56	100	91	85	34
1st degradation product	49.51	0	9	15	52
2nd degradation product	41.68	0	0	0	14

\*Forty-eight hours and 30 min of heating at 95°C and cooling down to room temperature.

showed up, at 49.51 ppm (relative to TMP, at 2.71 ppm). That signal resulted from a P-containing degradation product, probably hydrolyzed diazinon. After 48 h, the degradation product signal was more intense, but diazinon still persisted. After subsequent heating to 95°C, a second P-containing degradation product showed up, at 41.68 ppm (relative to TMP, at 2.71 ppm), but diazinon still persisted. The second degradation product is probably doubly hydrolyzed diazinon. Table 5 summarizes the relative quantities of diazinon and its P-containing degradation products.

Table 5 shows that degradation of diazinon had proceeded to 9%, after 24 h, and to 15%, in 48 h. Even after 48 h at room temperature and heating at 95°C, 34% of the original quantity of diazinon persisted in the lysis buffer.

**GC-MS**—Diazinon eluted at 19.96 min under the GC-MS conditions used. A typical chromatogram and spectrum are shown in Fig. 5. The GC peak area was used in semi-quantitative determinations. The response in the TIC chromatogram of the sample taken at 0 h was compared to that in the TIC chromatogram of a 50 µg/mL diazinon solution in  $\text{CHCl}_3$  (corresponding to conditions of no decomposition and full extraction recovery); over 90% of diazinon was recovered intact at 0 h. The results are summarized

in Table 2. That table shows that more than 40% of the original quantity of diazinon “survives” 48 h in the lysis buffer. Almost 20% of the original quantity “survives” a week of buffer exposure.

Table 4 gives a summary of results on degradation of diazinon when the protocol was strictly followed. At 24 h, the quantity of diazinon appears to have increased to 110%. However, this is due to some variability in the response of the GC-MS equipment and the semi-quantitative determination. In this second series of experiments, diazinon seems to have degraded less (*c.* 30%) than in the first series (*c.* 60%; Table 2). For degradation of diazinon, heating is more efficient than prolonged storage: <10% of the original quantity persists after 2 days in lysis buffer and subsequent heating.

#### Sodium 2-Fluoroacetate in Lysis Buffer

**$^{19}\text{F}$  NMR**—The initial  $^{19}\text{F}$  NMR spectrum, at 0 h, shows a triplet  $^{19}\text{F}$  signal (−212.7, −212.8, and −213.0 ppm in a 1:2:1 intensity ratio, relative to NaF standard, at −120.00 ppm) for sodium 2-fluoroacetate, due to  $^{19}\text{F}$ – $^1\text{H}$  coupling. The  $^{19}\text{F}$  NMR spectrum of blank lysis buffer did not show any signals. The  $^{19}\text{F}$  spectrum after 24 h is identical to that at 0 h, whereas a minor degradation product signal, at −106.6 ppm, appears after 48 h. These results are summarized in Table 6. From these experiments, it is clear that almost all 2-fluoroacetate survives 48 h of buffer solution, even after 30 min heating at 95°C.

**GC-MS**—Attempts with extraction of 2-fluoroacetic acid from acidified solutions, pH *c.* 1, were unsuccessful. Some of the acid was extracted, with dichloromethane, but results were irreproducible. Moreover, the strongly acidic extract damaged the GC column. For these reasons, it was decided to use liquid chromatography with negative ion electrospray mass spectrometry detection (LC-MS).

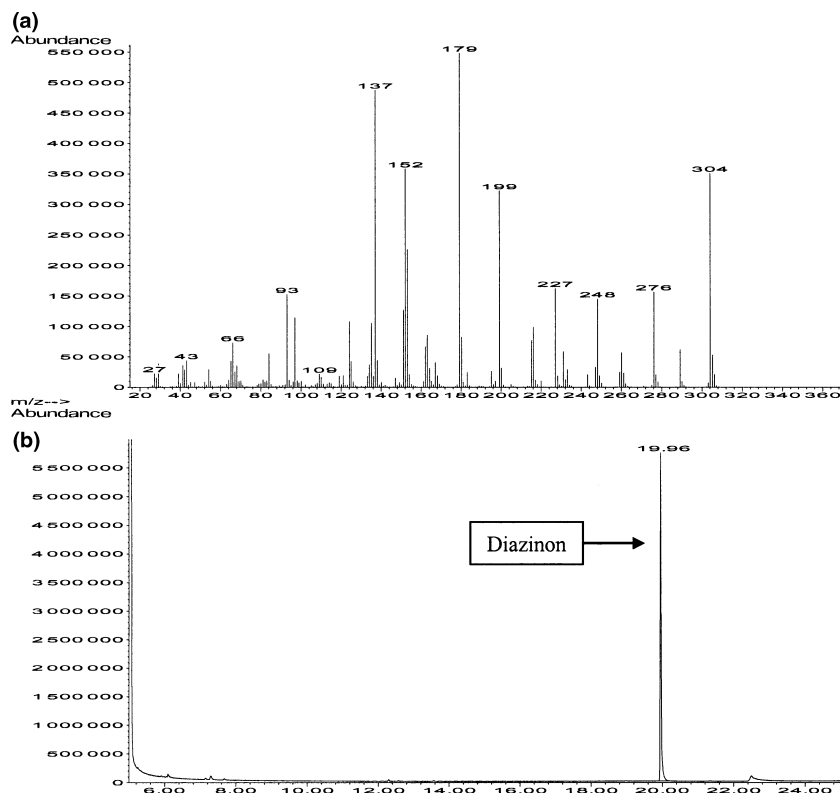


FIG. 5—Total ion current chromatogram of the dichloromethane extract of diazinon spiked lysis buffer, at 0 h and scaled to the diazinon peak (b), and mass spectrum of diazinon (a).

TABLE 6—Summary of  $^{19}\text{F}$  NMR results for sodium 2-fluoroacetate in DNA lysis buffer.

Compound	Resonance (ppm)	Relative quantity (%)			
		0 h	24 h	48 h	48 h+*
2-Fluoroacetate	-212.7, -212.8, -213.0	100	100	99	98
1st degradation product	-106.6	0	0	0.8	2

\*Forty-eight hours and 30 min of heating at 95°C and cooling down to room temperature.

**LC-MS**—The LC negative ion electrospray mass spectrum of sodium 2-fluoroacetate typically displays a signal of the compound at 77 Da,  $[\text{FH}_2\text{C-COO}]^-$  (see Fig. 6). The spectrum, a background spectrum, and an example chromatogram are given in Fig. 6. The blank spectrum (Fig. 6b) shows that negative ion signals other than  $m/z$  77 in the fluoroacetate spectrum (Fig. 6a) belong to unidentified background components present in the blank buffer or in the eluent. A semi-quantitative determination was carried out *in duplo* against a freshly prepared solution of sodium 2-fluoroacetate in milliQ water. The 2-fluoroacetate content was quantified by the ion chromatograms of  $[\text{FH}_2\text{C-COO}]^-$ ,  $m/z$  77. Samples were taken after 94 h, instead of after 48 h. As no degradation was observed at 94 h, this point in time is considered equivalent to the situation at 48 h. Results are summarized in Table 4.

#### Agent Presence During the Remainder of the DNA Extraction Protocol

**Sarin**—The pooled wash liquid  $^{31}\text{P}$  NMR spectra displayed intense signals of sarin (29.63 and 36.28 ppm), along with two signals for three degradation products (25.13, 26.19, and 31.09 ppm).

Detail of the  $^{31}\text{P}$  NMR spectrum, demonstrating the  $^{31}\text{P}$ - $^1\text{H}$  spin coupling in sarin and the degradation products and the  $^{31}\text{P}$ - $^{19}\text{F}$  coupling of sarin, is shown in Fig. 7. The concentration of sarin in the pooled liquids is *c.* 0.7 mg/mL. That implies that degradation and washing are the main pathways for loss of sarin in the DNA extraction procedure. In the elution buffer extract, no  $^{31}\text{P}$  response of sarin was observed. Therefore, sarin was not present in the blank elution buffer.

GC-MS analysis was performed to assess the sarin content of the various liquids in the extraction process. Results are summarized in Table 7. In addition, Fig. 8 gives a summary of ion chromatograms for sarin from chloroform extracts of the various liquids. The decrease in GC peak areas, in going from lysis buffer heating to elution buffer, shows that the process liquids carry away the original sarin load.

The measurements also show that sarin does not appreciably bind to the beads, as there is no difference in recovery before and after addition of the beads. Note that sarin could not have been recovered from the elution buffer, because air-drying of the beads would have evaporated any remaining sarin.

**Diazinon**—The chloroform extract of the pooled wash liquids displayed intense  $^{31}\text{P}$  NMR signal of diazinon (59.66 ppm), along with signals of phosphorus containing degradation products (0.73, 42.25, 49.70, and 54.10 ppm). Detail of the  $^{31}\text{P}$  NMR spectrum, displaying the  $^{31}\text{P}$ - $^1\text{H}$  spin coupling, is shown in Fig. 9. The concentration of diazinon in the pooled liquids is *c.* 1.51 mg/mL, which corresponds to *c.* 15% of the original concentration. In the elution buffer extract, no  $^{31}\text{P}$  response of diazinon was observed. Therefore, diazinon was not present in the elution buffer at any appreciable amount.

GC-MS analysis was performed to assess the diazinon content of the various liquids in the extraction process. Results are

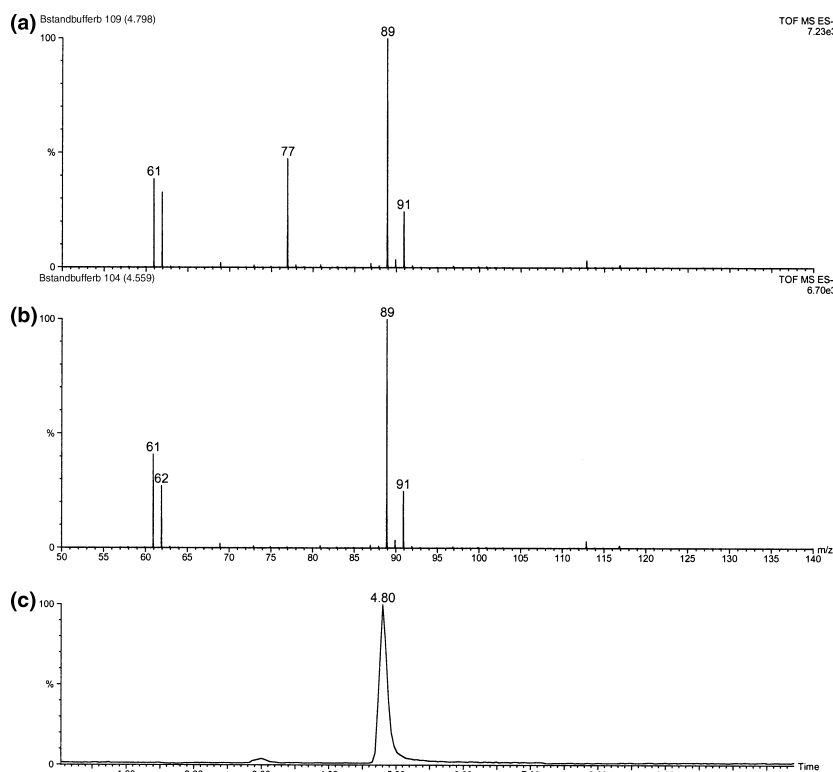


FIG. 6—Ion chromatogram,  $m/z$  77 of  $\text{FH}_2\text{C-C(O)O}^-$  (c), and negative ion electrospray mass spectra from the negative ion LC-ESI MS analysis of sodium 2-fluoroacetate in lysis buffer, at 0 h:  $\text{FH}_2\text{C-C(O)O}^-$  at 4.80 min LC peak (a) and eluent background spectrum (b).



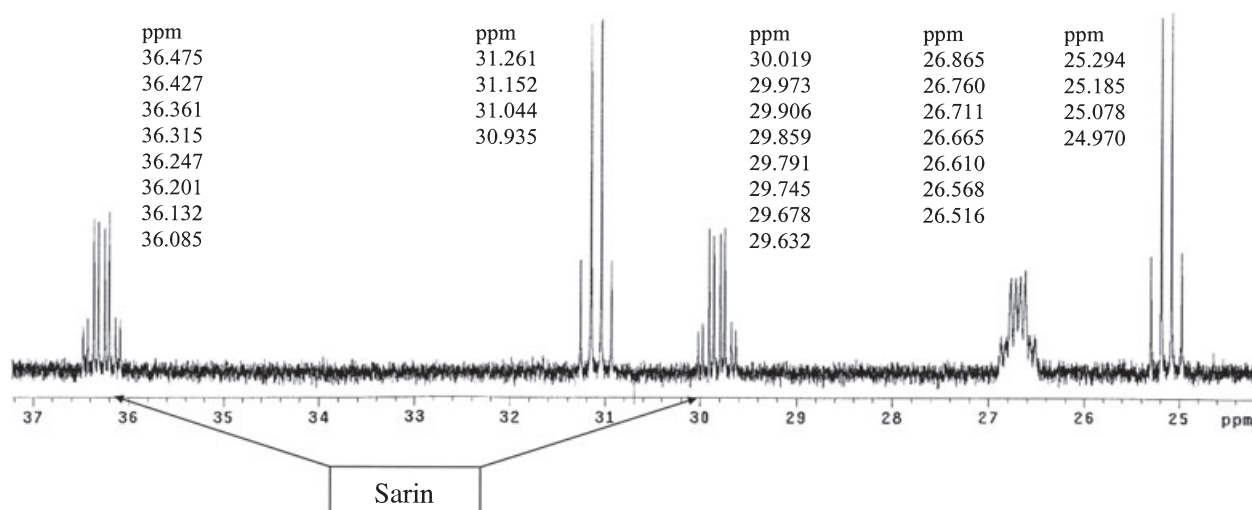


FIG. 7—Partial  $^{31}\text{P}$  NMR spectrum of pooled wash liquid during processing of a sample contaminated with sarin, showing  $^{31}\text{P}$ – $^1\text{H}$  and  $^{31}\text{P}$ – $^{19}\text{F}$  spin coupling.

TABLE 7—Summary of GC-MS results for sarin and diazinon and LC-MS results for 2-fluoroacetate in the postlysis stage of the DNA extraction process.

Stage	Sarin (GC-MS)		Diazinon (GC-MS)		2-Fluoroacetate (LC-MS)*	
	% to 0 h <sup>†</sup>	% to standard <sup>†,‡</sup>	% to 0 h <sup>†</sup>	% to standard <sup>†,‡</sup>	% to 0 h <sup>†</sup>	% to standard <sup>†,§</sup>
After heating 95°C, 30 min	8	8	7	7	100	100
Supernatant after adding beads	8	8	0.6	0.6	100	100
Lysis buffer wash	0.07	0.08	0.03	0.03	3	3
Wash solution	0.004	0.004	0.004	0.004	0.3	0.3
Elution buffer	0	0	0.01	0.01	<0.1	<0.1

\*Determined *in duplo*.

<sup>†</sup>% intact agent recovered.

<sup>‡</sup>By comparison to a solution of 50 µg/mL agent in  $\text{CHCl}_3$ .

<sup>§</sup>By comparison to a solution of 50 µg/mL agent in milliQ water.

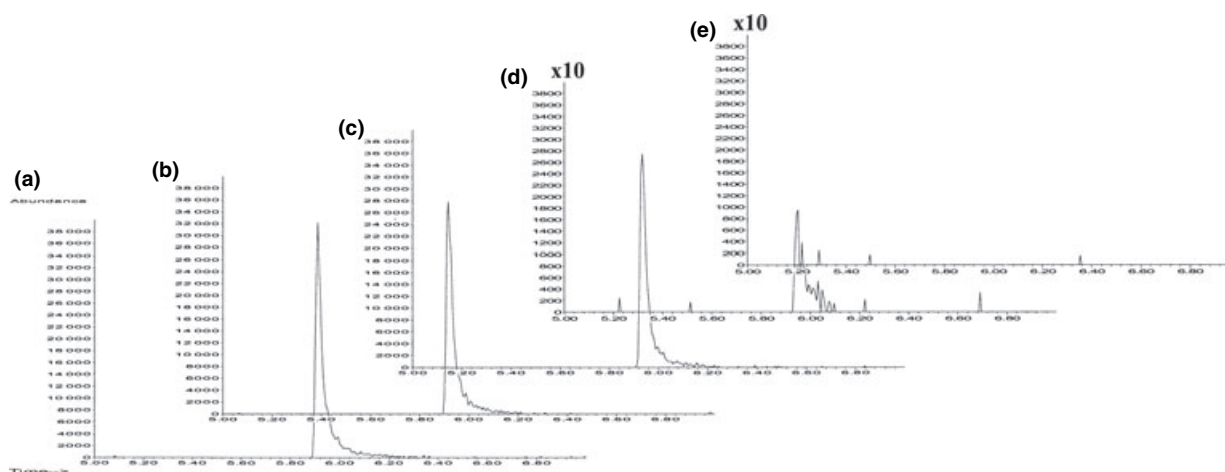


FIG. 8—Overview of ion chromatograms for sarin from chloroform extracts of the various postlysis buffer liquids; (a) through (e): from lysis buffer after heating (95°C, 30 min); after addition of beads; lysis buffer wash; wash solution; elution buffer.

summarized in Table 7. These measurements show that diazinon does bind to the beads, with trapping of *c.* 90% of the quantity present before the addition of beads. Washing with lysis buffer and washing liquid does not extract much of the diazinon from the beads. Subsequent washing with elution buffer still liberates diazinon from the beads. In contrast to sarin, diazinon is not sufficiently volatile to be removed by the air-drying process.

**Sodium 2-Fluoroacetate**—The  $^{19}\text{F}$  NMR spectrum of pooled wash liquids showed a clear triplet signal for fluoroacetate, at  $-213.04$  ppm, and no other signals of possible fluorine containing degradation products. The concentration of 2-fluoroacetate in the pooled wash liquid is *c.* 2.52 mg/mL, which corresponds to 25% of the original concentration in lysis buffer. No  $^{19}\text{F}$  signals were observed in the elution buffer  $^{19}\text{F}$  NMR spectrum. Therefore,

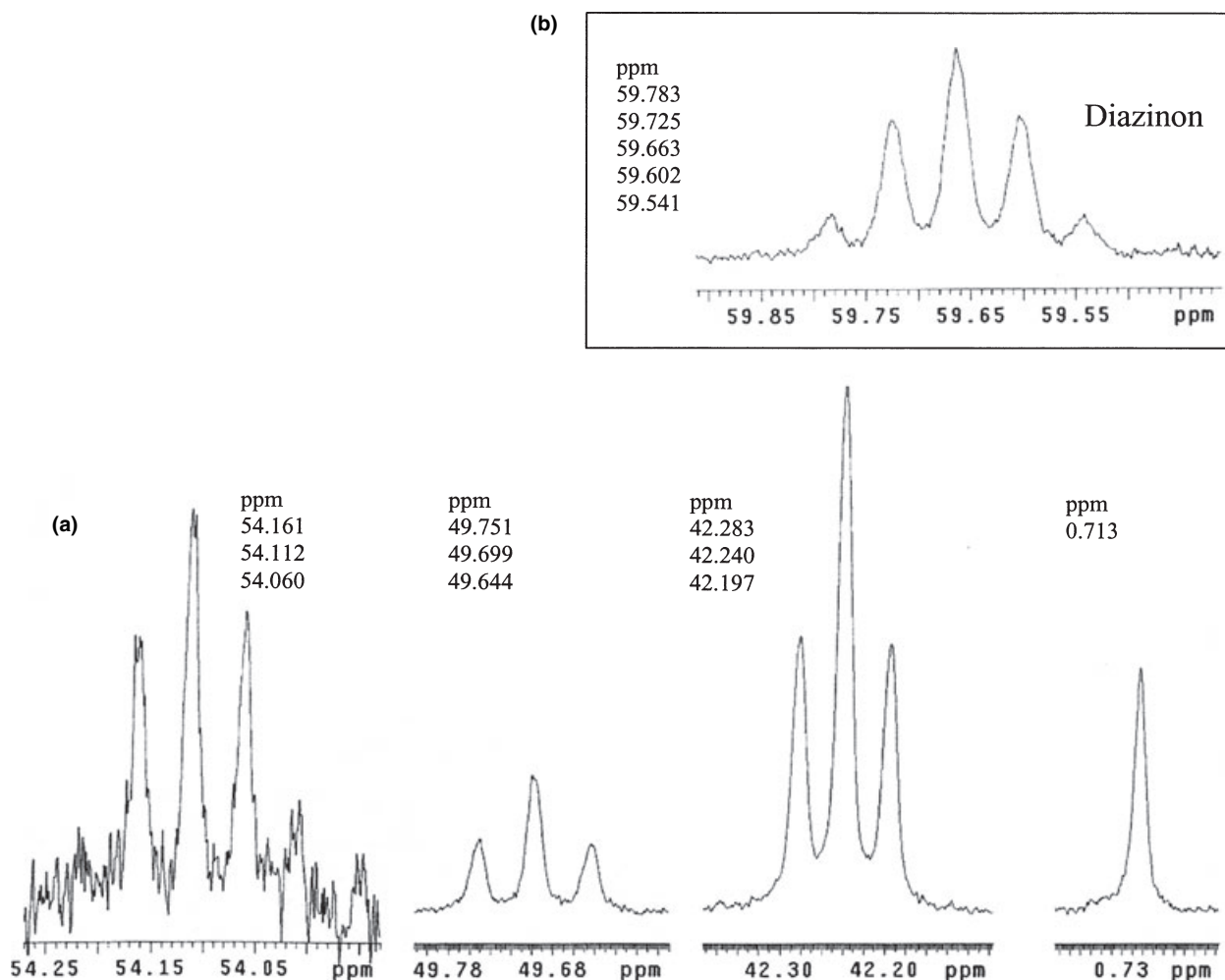


FIG. 9—Partial  $^{31}\text{P}$  NMR spectrum of pooled wash liquid during processing of a sample contaminated with diazinon, showing  $^{31}\text{P}$ – $^1\text{H}$  spin coupling (a); the diazinon signal is shown in the insert (b).

elution buffer no longer contains an appreciable amount of 2-fluoroacetate. As with lysis buffer, a semi-quantitative determination was carried out *in duplo* against a freshly prepared solution of sodium 2-fluoroacetate in milliQ water. The 2-fluoroacetate content was quantified by the ion chromatograms of  $[\text{FH}_2\text{C-COO}]^-$ ,  $m/z$  77. As an example, chromatograms of the elution buffer are given in Fig. 10, whereas the results are summarized in Table 7.

This shows that most 2-fluoroacetate, *c.* 97%, is discarded with the lysis buffer supernatant. Approximately 3% of the agent concentration is then recovered in the washing liquid of the first step washing. Note that the agent affinity for the beads material is apparently low. The next washing liquid contains a fraction of that, *c.* 0.3%. Finally, the elution buffer used to draw any adsorbed DNA from the beads, contains <0.1% of the original agent concentration of 10 mg/mL.

## Discussion

### Comparison of NMR and MS Results of CW Agents in Lysis Buffer

**Sulfur Mustard**—The GC-MS and NMR results for sulfur mustard agree well, given the characteristics of both spectrometry methods. Only the time 0 h differs considerably, with no

observable sulfur mustard in NMR and 37% recovery in GC-MS. This difference is due to a combination of relatively fast degradation and the differences in sample preparation time for the two techniques. Sample preparation for GC-MS typically required a few minutes, with degradation virtually stopping upon addition of  $\text{CHCl}_3$ , whereas that for NMR typically took 30 min or more. At the “0 h” time of the NMR experiment sulfur mustard degradation had proceeded further than at the “0 h” time of the GC-MS experiment. From 24 h on, NMR did not indicate any sulfur mustard, whereas GCMS detected small quantities. However, the relatively large quantity of the major sulfur mustard degradation product produced so much signal intensity that signals from the traces of sulfur mustard were likely masked in NMR. Therefore, the GC-MS and NMR results are consistent.

**Sarin**—NMR and GC-MS experiments agree in the observation that degradation of sarin in lysis buffer in the first 48 h is <10%. In contrast, there is a large discrepancy between the GC-MS and NMR results after heating at 95°C, for 30 min: *c.* 90% degradation (GC-MS) against *c.* 55% (NMR). Repeated experiments with sarin showed the same discrepancy between GC-MS and NMR results, while the time-resolved degradation observed by the separate methods remained the same between experiments. We have no physical or chemical explanation for the discrepancy. The degradation

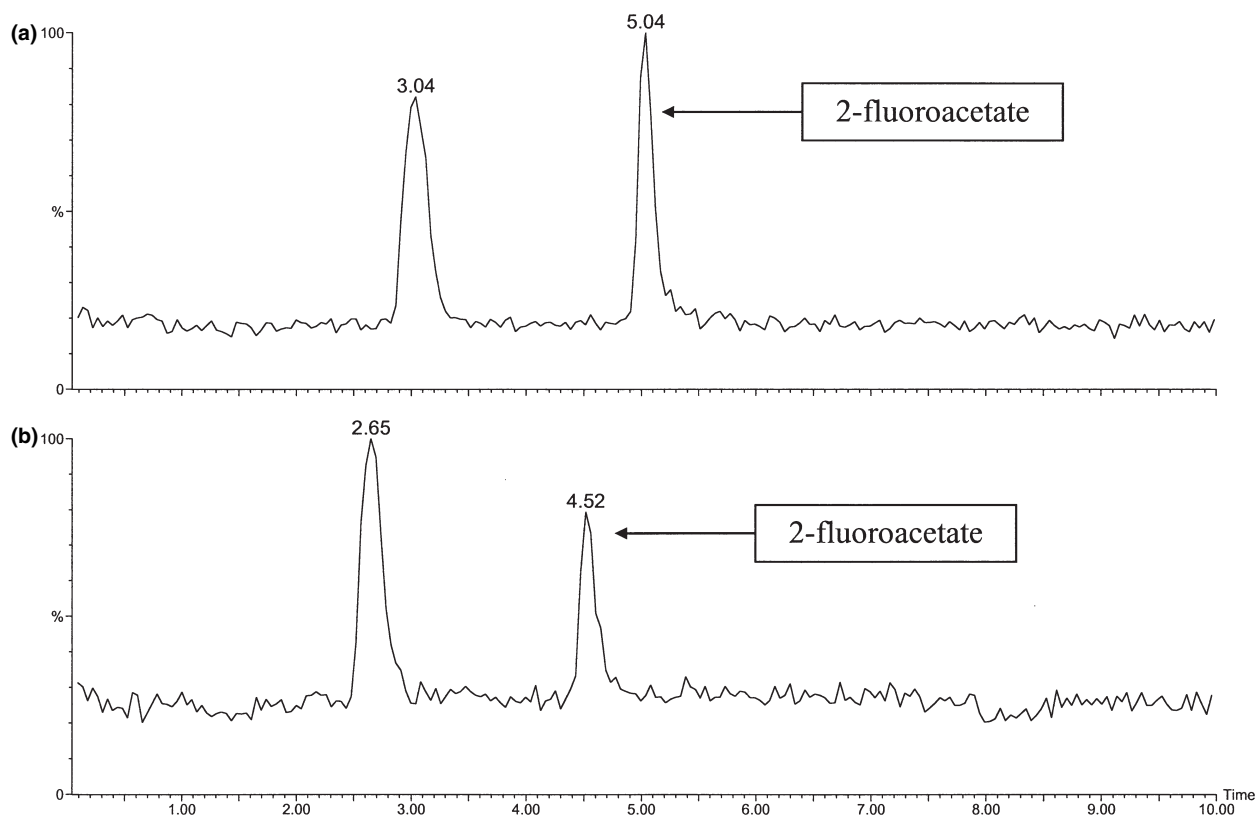


FIG. 10—Ion chromatograms,  $m/z$  77 [ $\text{FH}_2\text{C-COO}^-$ ], from LC negative ion electrospray analysis of a standard sodium 2-fluoroacetate in milliQ water (10 mg/mL, diluted 1:200,000; a) and of sodium 2-fluoroacetate in elution buffer (10 mg/mL, diluted 1:1000; b); retention time shifts are due to matrix effects.

comes down to a persistence of 1–4 mg/mL sarin in lysis buffer which at least justifies the conclusion that agent contamination levels are still not safe for benchtop or common fume hood processing.

**Diazinon**—Observations with NMR and GC-MS are at some variance. GC-MS indicates a much faster degradation of diazinon than NMR in the first 48 h. In a broader perspective, results largely agree. Degradation of diazinon in lysis buffer does not proceed fast. After 1 day, between 50% and 90% is still recovered. After 2 days, between 45% and 70% is still intact. Subsequent heating of diazinon in lysis buffer more than 10% of the original quantity of 10 mg/mL intact (7% by GC-MS, 34% by NMR).

**Sodium 2-Fluoroacetate**—The LC-MS results show no evidence of degradation within the measurement accuracy of  $\pm 5\%$ . Also, no degradation product signals were observed in the scan range accessible by the Q-TOF (50 Da and up). In the  $^{19}\text{F}$  NMR experiments, degradation occurs to the extent of  $c. 1\%$ , while the degradation product had a spectral signature. The minor degradation product is not fluoride,  $\text{F}^-$ , because the fluoride  $^{19}\text{F}$  NMR signal would coincide with the standard signal (at  $-120.0$  ppm). That degradation product is probably a low molecular weight ( $<50$  Da) compound, because it is not visible in LC mass spectra. These characteristics make it unlikely that the degradation product is a compound with a toxicity which exceeds that of fluoroacetate. Overall, both methods agree in the assessment that the degradation of 2-fluoroacetate in lysis buffer is negligible.

#### Comparison of NMR and MS Results for CW Agents Postlysis Buffer

**Sarin**—NMR and GC-MS results for the postlysis buffer stage agree. Sarin is removed by the wash liquid and the agent is no longer present at the processing stage of the elution buffer. Therefore, after processing of a sarin contaminated lysis buffer sample, elution buffer can be taken out of chemical agent containment for further handling.

**Diazinon**—GC-MS and  $^{31}\text{P}$  NMR agree that the wash liquid contains diazinon; according to GC-MS analysis, the wash liquid carries less than 1% of the original quantity of diazinon (10 mg/mL).  $^{31}\text{P}$  NMR shows that the pooled wash liquids contain much more. That implies that the original lysis buffer, from before the lysis buffer wash, carries away most of the diazinon present. GC-MS analysis shows that the elution buffer extract still contains diazinon, albeit at  $<0.02\%$  of the original quantity of 10 mg/mL; this quantity is below the  $^{31}\text{P}$  NMR limit of detection. Given the low quantity, diazinon contaminated elution buffer can be taken out of chemical agent containment for further handling, provided that direct contact is avoided.

**Sodium 2-Fluoroacetate**—Sodium fluoroacetate is mainly carried away by the first lysis buffer supernatant. The subsequent washing steps, with lysis buffer and wash buffer, carry another few percent. The remaining elution buffer contains  $<0.1\%$  of the original agent concentration of 10 mg/mL.

TABLE 8—Persistence (%) of CW agents to different stages of DNA extraction protocol.

CW agent	% Recovered			
	Lysis buffer (h)	Lysis buffer (48 h) + 95°C	Wash buffer	Elution buffer
Sulfur mustard	0.40 (24) 0.35 (48)	n.d.	n.a.	n.a.
Sarin	>90 (24) >75 (48)	>10	<0.04	n.d.
Diazinon	>50 (24) >50 (48)	>10	<0.1	<0.02
Sodium 2-fluoroacetate	100 (24) 100 (48)	>98	<0.3	<0.1

n.d., not detected; n.a., not analyzed.

The persistence of CW agents observed at the various steps in the Promega DNA IQ™ Protocol is summarized in Table 8.

It was established that sulfur mustard does not persist after prolonged standing in lysis buffer, whereas the other agents all survived prolonged lysis buffer contact and, to a significant amount, the application of heat.

For these three persistent agents, the remaining steps of the Promega DNA IQ™ Protocol appeared to be very effective at removing significant quantities. The liquids in the postlysis buffer stages of the process carried decreasingly less agent. Agent levels in the final liquid, the elution buffer were zero (sarin, 2-fluoroacetate) or low (<0.02 mg/mL for diazinon).

## Conclusions

Given these findings, DNA extraction from samples contaminated at relatively high levels of 10 mg/mL of sarin, diazinon, or sodium 2-fluoroacetate should preferably be conducted in chemical containment. The final product of the DNA extraction process, the elution buffer solution, can safely be taken out of that chemical containment. In contrast, samples contaminated with levels of 10 mg/mL of sulfur mustard are rendered almost harmless by <1 day of standing at room temperature. Hence, treatment of sulfur mustard contaminated samples does not require the chemical containment.

However, it should be noted, that these investigations were not carried out with real DNA samples, for example blood or tissue, but with neat buffer. Different CW agent behavior may occur in a real-world situation. It is known that salt content (ionic strength) and pH affect chemical agent degradation. As chemical containment would be required for the processing of samples contaminated at levels of 10 mg/mL of sarin, diazinon, or sodium 2-fluoroacetate, processing of samples similarly contaminated with sulfur mustard would be best also done in chemical containment to maintain a consistent protocol.

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