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Journal of Chromatography A, 1003 (2003) 143–155
JOURNAL OF CHROMATOGRAPHY A
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Gas chromatographic–mass spectrometric characterisation of amiton and the recovery of amiton from concrete, paint, rubber and soil matrices

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Received 28 December 2001; received in revised form 24 April 2003; accepted 29 April 2003

Abstract

Amiton {*O*,*O*-diethyl *S*-[2-(diethylamino)ethyl] phosphorothiolate}, is an organophosphorus chemical included in Schedule 2 of the Chemical Weapons Convention (CWC). Verification provisions under the CWC rely on the existence of a database of analytical information for scheduled chemicals and related compounds. Little analytical information is available for amiton. In this study, gas chromatography–mass spectrometry (GC–MS) characterisation of amiton and its typical impurities (including by-products and degradation products), supported by selective GC detection and ^{31}P NMR data, was undertaken. Twenty-one compounds, including a by-product unique to amiton from an industrial source, were identified. Involatile degradation products of amiton were derivatised to enable their identification by GC–MS. The recovery of amiton from matrices that may be expected in an inspection scenario (i.e. concrete, paint, rubber and soil) was also examined. Paint and concrete matrices were the most useful matrices for the detection of amiton, and its by-products and degradation products. Amiton was readily detected in these matrices after 28 days.
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Keywords: Warfare agents; Concrete; Paint; Rubber; Soil; Amiton

1. Introduction

The United Nations Chemical Weapons Convention (CWC) [1], which was opened for signature in Paris in January 1993, prohibits the development, production, stockpiling and use of chemical weapons. Stockpiled chemical weapons are to be destroyed under the provisions of this treaty. The Convention, which became operational in April 1997, is administered by the Organisation for the Prohibition of Chemical Weapons (OPCW) based in The Hague.

A key feature of this Convention is its extensive verification provisions that serve the dual purposes of demonstrating compliance with, and deterring violation of, the Convention. These provisions allow for inspections of particular facilities, such as those used to store chemical weapons awaiting destruction, as well as those formerly used for production of chemical weapons. Relevant chemical industry

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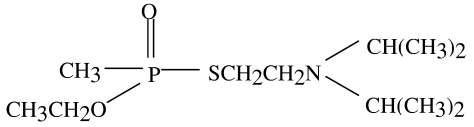
doi:10.1016/S0021-9673(03)00774-X

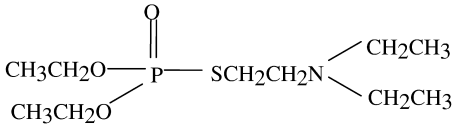
facilities will also be subject to verification provisions [2]. Inspections under the CWC of each type of facility may require collection and analysis of samples, as would an investigation of specific compliance concerns, such as alleged use of chemical weapons. Inspection procedures have been developed for on-site analysis (using equipment brought by an OPCW inspection team) and off-site analysis (at designated OPCW laboratories).

Routine verification of chemical industry is focused primarily on those chemicals which have been classified into three schedules, based on the risk they pose to the Convention through their potential applications either as chemical warfare (CW) agents or as precursors for the production of CW agents. For example, nerve agents which have been weaponised, such as VX {*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate} (Fig. 1) have been included in Schedule 1. Under the provisions of the CWC, such chemicals may only be produced in very limited quantities for research, medical and protective purposes.

A related chemical, amiton, *O*,*O*-diethyl *S*-[2-(diethylamino)ethyl] phosphorothiolate, also known as VG (Fig. 1), was included in Schedule 2 because of its potential application as a CW agent and interest in it amongst chemical weapon proliferators [3]. Amiton was originally developed during the early 1950s as an insecticide, but because of its toxicity has only limited applications, for example, as a systemic insecticide and miticide [4]. Under the CWC, production and use of this chemical for commercial purposes are permitted, but are subject to monitoring by data reporting and on-site inspections. The toxicity of compounds structurally similar to amiton were studied in the UK and USA. The inclusion of a P–C bond into the molecule produced a series of highly toxic compounds leading to the development of the chemical warfare agent, VX.



VX



AMITON

Fig. 1. Structure of the CW agent VX (*O*-ethyl-*S*-[2-(diethylamino)ethyl] methylphosphonothiolate) and amiton (*O*,*O*-diethyl-*S*-[2-(diethylamino)ethyl] phosphorothiolate).

There have been a number of studies on the extraction and chemical analysis from a variety of matrices for the presence of nerve agents that are known to have been weaponised. These have been reviewed by Black [5] and Kientz [6]. Early work on the analysis of CW agents includes studies on Sulphur mustard [7–11], and the nerve agents, sarin [8,10,11], soman [10], tabun [11] and VX [11]. In particular, studies undertaken during the development of the verification annex of the CWC have shown that GC–MS analysis can provide conclusive support for verification activities [12–14]. Rohrbaugh [15] demonstrated the utility of chemical ionisation GC–MS, using methanol as the CI reagent, to study the products of thermally degraded VX. Groenewold et al. [16] used secondary ion mass spectrometry for the detection of a range of chemical warfare related compounds and to detect VX [17] and some of its degradation products. Recent studies using atmospheric pressure ionisation mass spectrometry techniques of ionspray and electrospray [18–24] and LC–MS with atmospheric pressure chemical ionisation (APCI) [25–28] and electrospray ionisation (ESI) [6,18,29–31] have been shown to be suited to the direct analysis of organophosphorus compounds including their polar hydrolysis products without the need for prior derivatisation. LC–MS has been shown to be a useful method for the characterisation of VX degradation products [18]. Previously published work on the analysis of amiton is limited to an ion-selective electrode assay [32], ultraviolet colorimetric assay [33] and wastewater fluorescence assay [34]. These methods have detection limits at the ppb level, but are indirect non-specific assays. The mass spectrum and ^{31}P NMR data for amiton have been published [35] without discussion. However, there are no published studies on the analysis

of amiton in environmental samples, or its characterisation by GC–MS analysis.

The objective of this study has been to undertake the mass spectral characterisation of amiton, its typical impurities (including unreacted starting materials, by-products and degradation products), and to investigate the extraction efficiency of amiton (and some characteristic impurities) from concrete, paint, rubber and soil. These matrices might be expected in an OPCW inspection scenario.

2. Experimental

2.1. Chemicals

Amiton was synthesised from diethyl phosphorochloridothionate and 2-diethylaminoethanol by the method of Ghosh and Newman [36], and purified by distillation (b.p. 74–82 °C at 0.005 mmHg; 1 mmHg=133.322 Pa). The distillation product from the synthesis of amiton was characterised using GC–MS and ^{31}P-NMR analysis. A sample of the distilled amiton was also stored for 7 years in a stoppered flask at ambient temperature. **Caution: amiton is a potent anticholinesterase and extreme care must be taken when working with it. In particular, the primary hazard of amiton is by absorption through the skin and proper protective gloves should be worn.**

Diethyl *N*,*N*-diethylaminophosphorothionate was synthesised from diethyl phosphorochloridothionate and diethylamine [37]. Triethyl phosphorothionate was synthesised by refluxing 170 mg of triethylphosphite with 60 mg of sulphur in 15 ml dichloromethane for 1 h [38]. Triethyl phosphorothiolate was synthesised by refluxing 10 mg of diethyl phosphorochloridothionate with 0.5 ml of 2 *M* aqueous sodium hydroxide in 10 ml ethanol for 1 h. The product was passed through a column of anhydrous sodium sulphate and ethyl iodide was added [39]. 2-Diethylaminoethanol, triethyl phosphate and all of the above starting materials were purchased from Aldrich (Sydney, Australia). GC–MS and ^{31}P NMR were used to confirm the identity of the starting materials and reaction products.

Diethyl phosphorothioic acid was prepared by adding 4.0 ml distilled water to 200 mg diethyl phosphorochloridothionate and heating to 75 °C for 1 h (until the solution became clear). Water was removed with a rotary evaporator, the residue dissolved in dichloromethane, filtered and the solvent removed under reduced pressure. The purity of the product was checked by ^{31}P NMR (purity 95%). Then, 10 mg of this product was derivatised using *N* - (*tert*. - butyldimethylsilyl) - *N* - methyltrifluoroacetamide (as described in Section 2.4 below). This solution was serially diluted to allow quantitation. Derivatisation with diazomethane [40] was performed to confirm the structural identity of diethyl phosphorothioic acid by electron impact ionisation (EI) GC–MS.

2.2. Instrumental

2.2.1. GC–MS

Positive ion EI and ammonia positive chemical ionisation (CI) GC–MS analyses were performed on a TRIO-1S quadrupole mass spectrometer (Fisons Instruments, Australia) interfaced to a Hewlett-Packard HP5890 Series II GC equipped with a HP7673 Autoinjector and a 25 m×0.32 mm BP5 capillary column (SGE, Australia). The GC injector and the GC–MS interface temperatures were 260 and 250 °C, respectively. A temperature program from 50 to 250 °C at 10 °C/min, then isothermal for 10 min was used. The ultra-high-purity helium (CIG, Australia) carrier gas linear velocity was 30 cm/s. Data were acquired and processed with an Intel 80386SX-based IBM computer using the LAB-BASE software package.

EI mass spectra were obtained using full scanning from 35 to 450 u at a rate of 1 scan/s with 70 eV electron energy, 150 μA emission current and 200 °C source temperature. Ammonia CI mass spectra were obtained using full scanning from 60 to 450 u at a rate of 1 scan/s with 70 eV electron energy, 350 μA emission current and 130 °C source temperature. Ammonia CI (99.99%, Matheson, USA) was optimised by maximising the ion intensity at *m*/*z* 183 for triethylphosphate introduced into the source via the reference gas reservoir inlet.

High-resolution measurements were acquired on a Bruker BioAPEX 47e system. The system was calibrated from 69 to 200 u using perfluorotributylamine (Aldrich) to give an error of less than 3 ppm.

The sample was introduced using a solids probe (without additional heating), into the EI source at an ionisation potential of 70 eV, and source temperature of 200 °C.

2.3. Gas chromatography (selective detectors)

A Varian 3700 GC system equipped with both a flame photometric detection (FPD–phosphorus or sulphur system) and a flame ionisation detection (FID) system was used for all capillary column analyses. Column effluent was split equally (1:1) between the two detectors. GC analyses were performed on a 25 m×0.22 mm BP1 capillary column (SGE Australia). Ultra-high-purity helium (velocity 22 cm/s) was used as carrier gas. The injector and detectors were maintained at 260 and 250 °C, respectively. A temperature program run from 50 to 250 °C at 10 °C/min was used. The ultra-high-purity helium (CIG) carrier gas linear velocity was 22 cm/s. Data were acquired on an NEC 80286 PC using the Delta chromatography data acquisition package (SGE Australia).

2.4. NMR

^{31}P NMR spectra were acquired at 300 MHz with proton decoupling using a Bruker AM300 Fourier transform (FT) NMR spectrometer. External tetrahydroxyphosphonium perchlorate served as the reference.

2.5. Spiking of matrices

Standardised sandy loam soil samples were obtained from the Victorian Ministry of Agriculture, consisting of 52% coarse sand, 37% fine sand, 8% clay and organic carbon content of 0.7%. Olive green alkyd painted steel panels (35×25×1 mm thick), commercial neoprene rubber samples (35×25×4 mm thick) and concrete samples chipped from typical footpath panels with thickness of 8–12 mm (irregular in shape) and weighing approximately 10 g each were also used.

A 10 g sample of each matrix was spiked with 10 mg of distilled amiton and sealed. Samples were stored in a fume hood at room temperature for periods of 1 h, 1 day, 7 days, 14 days, 21 days or 28 days prior to extraction and analysis. Duplicates were prepared for each matrix and time period.

2.6. Extraction and derivatization

Each spiked matrix sample underwent an organic extraction procedure, then an aqueous extraction and derivatisation procedure, as follows:

(a) Each sample was sonicated with two aliquots of 20 ml dichloromethane for 20 min. The combined dichloromethane extracts were filtered and concentrated to 1.0 ml.
(b) Each sample was then sonicated with 20 ml water–methanol (1:1) for 20 min. Each sample was filtered, acidified with 2 *M* aqueous hydrochloric acid to pH 1 and evaporated to dryness. Then, 50 mg of *N*-(*tert*.-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (Aldrich) in 2 ml acetonitrile was added to the dried extract and the mixture was heated at 60 °C for 1 h [41].

All sample extracts were analysed immediately using GC–MS (EI and CI), GC–FPD and GC–FID. Supporting evidence for structural assignments was obtained by ^{31}P NMR, as required.

3. Results and discussion

3.1. Characterisation of the distilled and stored amiton

The EI total ion chromatogram (TIC) of the distilled amiton, used for spiking the matrices, is shown in Fig. 2. This sample was determined to be 83% pure, with five major impurities identified as 2-diethylaminoethanethiol (**1**), triethylphosphorothiolate (**8**), diethyl-*N*,*N*-diethylaminophosphorothionate (**9**), *O*,*O*-diethyl-*O*-[2-(diethylamino) ethyl] phosphate (**15**) and *O*,*O*-diethyl *O*-[2-(diethylamino)ethyl] phosphorothionate (**16**) (see Table 1). Trace residues of 12 other impurities including the *tert*.-butyldimethylsilyl (TBDMS) derivatives of 2-diethylaminoethanol (**7**), diethyl phosphoric acid (**12**) and diethyl phosphorothioic acid (**13**) were also detected.

In order to examine the long-term stability of the distillation product, the amiton sample that had been stored for 7 years was also characterised by GC–MS

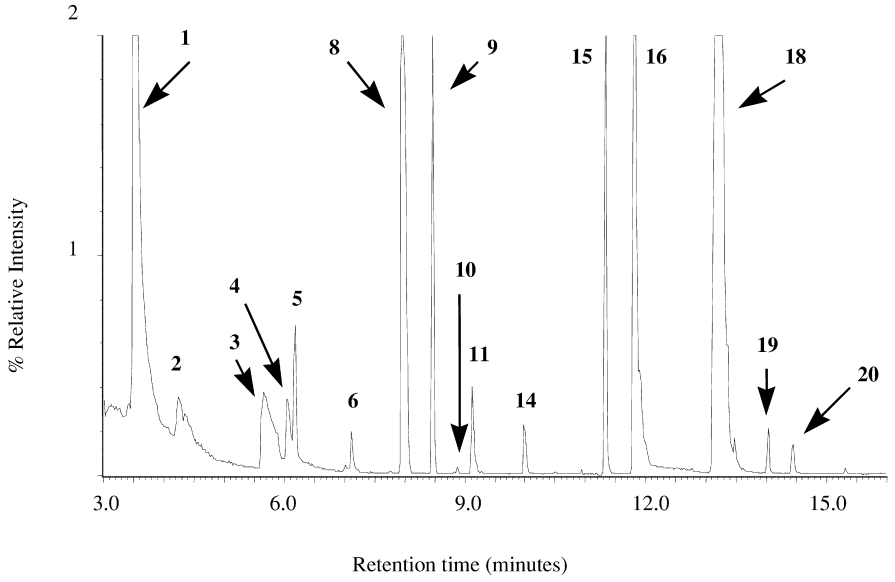


Fig. 2. GC–MS TIC of the distilled amiton (10 mg/ml in dichloromethane, 1 μl injection split 1:20) magnified (×50) to show the impurities. Note that the TBDMS derivatives of **7**, **12**, **13** and **17** are not present in this chromatogram.

and compared with the distilled amiton. The GC–MS TIC of stored amiton is shown in Fig. 3b. The amount of amiton (**18**) present in the stored sample had decreased significantly when compared with the distilled sample. There was also loss of some of the impurities detected in the distilled sample (Fig. 3a). The stored sample contained predominately **8**, **9** and **18** (Table 2), with traces of 4-ethylthiomorpholine (**2**), triethyl phosphate (**3**), triethyl phosphorothionate (**5**), 2-diethylaminoethyl chloride (**21**, not shown) and 4-methylthiomorpholine (**22**) also present. TBDMS derivatisation of the stored sample revealed a large number of products amongst which the TBDMS derivatives of **7**, **12**, **13**, ethyl phosphoric acid (**23**) and ethyl phosphorothioic acid (**24**) were detected.

The majority of the impurities observed in the distilled sample are stable by-products or degradation products of amiton, its thiono-isomer (**16**) and the initial starting materials. Compounds **1**, **7**, **12** and **13** are formed by base-catalysed degradation of amiton and its thiono-isomer (**16**) [42], and compounds **3**, **5** and **8** are formed by thermal degradation of amiton [43]. A degraded sample of a related commercial pesticide diazinon (*O*,*O*-diethyl *O*-[2-isopropyl-4-methyl-6-pyrimidinyl] phosphorothioate), was found to contain the impurities **3**, **5**, **8**, **12**, **13** and the structural analogues of **1**, *N*,*N*-diethyl-2-(ethylthio)ethanamine (**4**), **7**, **15**, **16** and 2,2′-dithiobis-(*N*,*N*-diethyl)ethanamine (**20**) [44]. These studies show that the presence of phosphorothionates or phosphorothiolates (other than amiton) in a sample could produce many of the phosphorus containing impurities detected in the distilled and stored samples, these impurities being indicative but not specific markers for amiton.

Of the impurities detected in our work, the diethylaminoethyl related impurities [i.e. **1**, **2**, **4**, **7**, *N*,*N*-diethyl-2-(ethyldithio)ethanamine (**11**), **15**, **16**, *O*,*O*-diethyl *S*-[2-(diethylamino)ethyl] phosphorodithionate (**19**), **20** and **22**] are more specific markers for amiton in a degraded sample (where no amiton remained) as they are unlikely to be present following the degradation of other organophosphorus pesticides. However, the majority of the impurities observed in the stored sample are related to the phosphorus part of the amiton molecule. In particular, **3**, **5**, **8** and **9** are stable indicators of amiton related material in a sample. Traces of **2**, **7**, **21** and **22** were the only diethylaminoethyl related impurities detected, suggesting that the amine impurities present in the distilled amiton sample are not

Table 1
Structure of chemicals identified in the distilled amiton and a sample of Amiton that had been stored for 7 years, and their retention times and methods of characterisation

No.	Structure	Chemical name	M_r	Distilled Amiton Rt. (min)[c], Characterisation[d]	Stored Amiton (7 years) Rt. (min)[c], Characterisation[d]
1	$(CH_3CH_2)_2NCH_2CH_2SH$	2-Diethylaminoethanethiol	133	3.55, CLS	nd
2	$S(CH_2CH_2)_2Net$	4-Ethylthiomorpholine[a]	131	4.30, CE	4.25, CE
3	$(CH_3CH_2O)_3P(O)$	Triethyl phosphate	182	5.63, ACR	5.71, ACR
4	$(CH_3CH_2)_2NCH_2CH_2SCH_2CH_3$	*N*,*N*-diethyl-2-(ethylthio)ethanamine	161	5.98, CE	nd
5	$(CH_3CH_2O)_2P(S)OCH_2CH_3$	Triethyl phosphorothionate	198	6.18, ACR	6.18, ACR
6	$(CH_3CH_2O)_2P(O)SCH_3$	Diethyl-*S*-methyl phosphorothiolate	184	7.12, ACR	nd
7	$(CH_3CH_2)_2NCH_2CH_2OH$	2-Diethylaminoethanol	116	7.47, ACRT	7.46, ACRT
8	$(CH_3CH_2O)_2P(O)SCH_2CH_3$	Triethyl phosphorothiolate	198	8.02, ACPRS	8, ACR
9	$(CH_3CH_2O)_2P(S)N(CH_2CH_3)_2$	Diethyl *N*,*N*-diethylamino phosphorothionate	225	8.48, ACRPS	8.46, ACR
10	$(CH_3CH_2O)_2P(S)SCH_2CH_3$	Triethyl phosphorodithionate	214	8.88, CL	nd
11	$(CH_3CH_2)_2NCH_2CH_2SSCH_2CH_3$	*N*,*N*-diethyl-2-(ethyldithio)ethanamine	193	9.12, CE	nd
12	$(CH_3CH_2O)_2P(O)OH$	Diethyl phosphoric acid	154	9.33, ACRT[b]	9.29, ACRT[b]
13	$(CH_3CH_2O)_2P(S)OH$	Diethyl phosphorothioic acid	170	9.87, ACERPST	9.83, ACRT
14	Unknown 1	–	209	9.97, CE	nd
15	$(CH_3CH_2O)_2P(O)OCH_2CH_2N(CH_2CH_3)_2$	*O*,*O*-diethyl *O*-[2-(diethylamino)ethyl] phosphate)	253	11.33, CE	nd
16	$(CH_3CH_2O)_2P(S)OCH_2CH_2N(CH_2CH_3)_2$	*O*,*O*-diethyl *O*-[2-(diethylamino)ethyl] phosphorothionate	269	11.83, CE	nd
17	Unknown 2	–	258	12.77, CET	nd
18	$(CH_3CH_2O)_2P(O)SCH_2CH_2N(CH_2CH_3)_2$	*O*,*O*-diethyl *S*-[2-(diethylamino)ethyl] phosphorothiolate (amiton)	269	13.27, CLNPS	13.19, ACR
19	$(CH_3CH_2O)_2P(S)SCH_2CH_2N(CH_2CH_3)_2$	*O*,*O*-diethyl *S*-[2-(diethylamino)ethyl] phosphorodithionate	285	14.05, CE	nd
20	$((CH_3CH_2)_2NCH_2CH_2S–)_2$	2,2′-Dithiobis-(*N*,*N*-diethyl)ethanamine	264	14.43, CL	nd
21	$(CH_3CH_2)_2NCH_2CH_2Cl$	2-Diethylaminoethyl chloride	135	nd	2.77, CL
22	$S(CH_2CH_2)_2NCH_3$	4-Methylthiomorpholine[a]	117	nd	3.94, CE
23	$CH_3CH_2OP(O)(OH)_2$	Ethylphosphoric acid	126	nd	nd
24	$CH_3CH_2OP(S)(OH)_2$	Ethylphosphorothioic acid	142	nd	nd

[a] Tentative assignment.
[b] Identified as an impurity in synthesised diethylphosphorothioic acid.
[c] nd, not detected.
[d] A, authentic chemical EI match; C, CI spectral data; E, EI spectra interpretation; L, EI reference spectrum library match; N, ^{31}P-NMR spectral data; P, phosphorus FPD data; R, GC column retention match with authentic chemical; S, sulphur FPD data; T, TBDMS derivative synthesised.

particularly stable and may gradually degrade to the piperazinium cation [45] and other polar or polymeric material.

The presence of **9** in the distilled and stored samples, while not initially expected, is characteristic of the amiton synthesis method used. Compound **9** has been registered as both a commercial pesticide [46] and a plant growth promoter [47], but it is apparently not widely used. Therefore, under certain circumstances, **9** would be a potentially useful marker for amiton and not other related phosphonothiolates or phosphonothionates.

3.2. Mass spectra of amiton and its impurities

The low resolution EI mass spectrum of amiton is presented (Fig. 4r) and accurate mass measurements of major ions are given in Table 3. The mass spectrum has a base peak at m/z 86 $[C_5H_{12}N]^+$, due to α-cleavage (observed with primary, secondary and tertiary *n*-alkyl amines [48]) of the diethylaminoethyl group $[(CH_3CH_2)_2NCH_2CH_2$-]. An analogous peak (at m/z 114) is observed in the mass spectrum of VX [49,50]. A prominent ion at m/z 99 associated with the amine $[C_6H_{13}N]^+$ was observed and, as

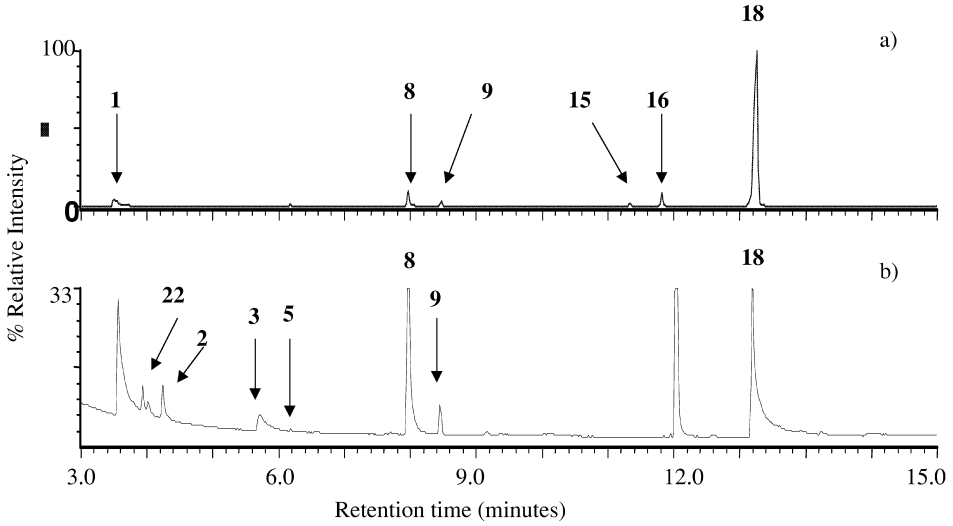


Fig. 3. GC–MS TIC of (a) the distilled amiton (10 mg/ml in dichloromethane, 1 μl injection split 1:20) and (b) the same sample stored for 7 years (8 mg/ml in dichloromethane, 1 μl injection split 1:20) magnified (×3). Note that compounds **1**, **15** and **16** were not detected in the stored sample and **21** are not shown. A diethyl phthalate peak was detected in the stored sample at 12.03 min and the peak at 3.57 min was not identified.

with many other organophosphorus compounds, the molecular ion is absent from the EI mass spectrum of amiton. Low intensity high mass ions are observed at *m/z* 197 ($[C_6H_{14}O_3PS]^+$) resulting from loss of the diethylamino group, and the additional losses of C_2H_4 to give *m/z* 169 ($[C_4H_{10}O_3PS]^+$) and 141 ($[C_2H_6O_3PS]^+$) respectively. The CI mass spectrum displayed a base peak $[M+H]^+$ ion at *m/z* 270. A ^{31}P NMR peak at 28.4 ppm is consistent with literature data for amiton [35].

The EI mass spectra of the impurities contained in the distilled amiton (Fig. 2) can be classed in two families depending on the presence of the diethylaminoethyl group. The impurities containing this group (**1**, **4**, **11**, **15**, **16**, **19**, **20** and the TBDMS derivative of **7**) feature mass spectra with a base peak at *m/z* 86 $[C_5H_{12}N]^+$, and no molecular ion (with the exception of **1**) as with amiton. The mass spectra of **15**, **16** and **19,** which are structurally similar to amiton, also feature a prominent peak at *m/z* 99 $[C_6H_{13}N]^+$. Low intensity characteristic high mass ions may assist in differentiating these chemicals.

A feature of the other family of impurities (i.e. not containing the diethylaminoethyl group) is their distinctive mass spectra. The EI mass spectrum of **9** displayed a molecular ion peak at *m/z* 225, a prominent *m/z* 192 ion arising from loss of HS, an intense *m/z* 121 ($[C_4H_{10}O_2P]^+$) ion arising from loss of $[C_4H_9N]$, a base peak at *m/z* 72 $[C_4H_{10}N]^+$, and a prominent ion at *m/z* 93 $[C_2H_6O_2P]^+$. The EI mass spectrum of **8** is characterised by a prominent molecular ion at *m/z* 198, and intense ions at *m/z* 170, 138, 111, and 109 are probably due to $[M–(CH_2CH_2)]^+$, $[M–(SCH_2CH_2)]^+$, $[HP(OH)_2(OCH_2CH_3)]^+$, and $[(OCH_2CH_3)P(O)OH]^+$, respectively. A peak at *m/z*

Table 2
Partial accurate mass spectrum of amiton showing peak assignments

Elemental composition	Calculated mass (u)	Measured mass (u)	Error (μ)
C_4H_9N	71.0735	71.0732	0.3
$C_5H_{12}N$	86.0970	86.0966	0.4
$C_6H_{13}N$	99.1048	99.1044	0.4
$C_2H_6O_3P$	109.0054	109.0051	0.3
$C_2H_6O_3PS$	140.9775	140.9769	0.6
$C_4H_{10}O_3PS$	169.0088	169.0078	1.0
$C_6H_{14}O_3PS$	197.0401	197.0389	1.2

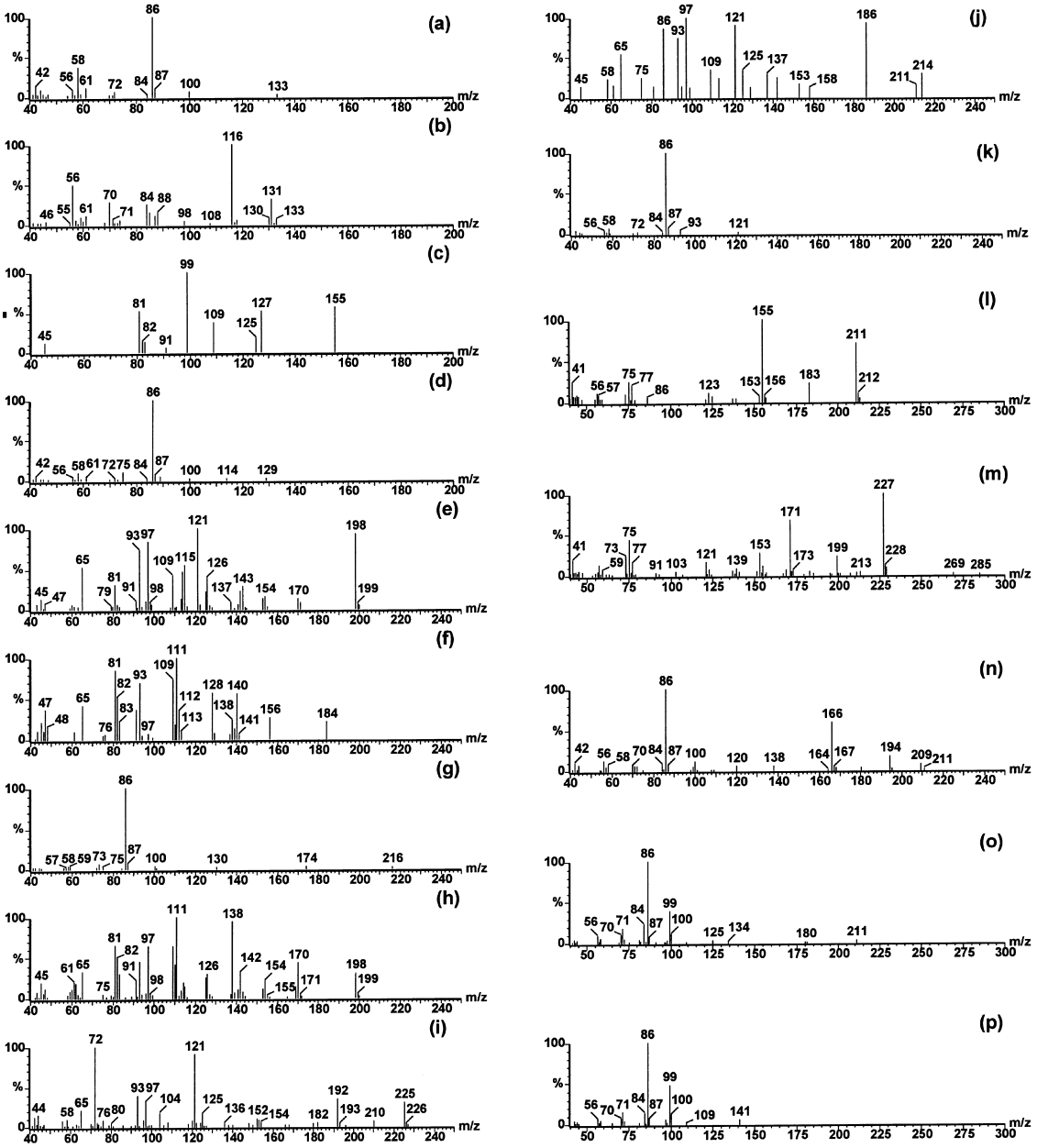


Fig. 4. EI mass spectra of amiton and impurities (a) 2-diethylaminoethanethiol, **1**;(b) 4-ethylthiomorpholine, **2**;(c) triethylphosphate, **3**;(d) *N*,*N*-diethyl-2-(ethylthio)ethanamine, **4**;(e) triethylphosphorothionate, **5**;(f) diethyl-*S*-methylphosphorothiolate, **6**;(g) 2-diethylaminoethanol TBDMS derivative, **7**;(h) triethylphosphorothiolate, **8**;(i) diethyl-*N*,*N*-diethylaminophosphorothionate, **9**;(j) triethylphosphorodithionate, **10**;(k) N,N-diethyl-2-(ethyldithio)ethanamine, **11**;(l) diethylphosphoric acid TBDMS derivative, **12**;(m) diethylphosphorothioic acid TBDMS derivative, **13**;(n) unknown 1, **14**;(o) *O*,*O*-diethyl-*O*-[2-(diethylamino)ethyl]phosphate), **15**;(p) *O*,*O*-diethyl-*O*-[2-(diethylamino)ethyl]-phosphorothionate, **16**;(q) unknown 2, **17**;(r) amiton, **18;**(s) *O*,*O*-diethyl-*S*-[2-(diethylamino)ethyl]phosphorodithionate, **19**;(t) 2,2′-dithiobis-(*N*,*N*-diethyl)ethanamine),**20**;(u) 2-diethylaminoethyl chloride, **21**; (v) 4-methylthiomorpholine, **22**;(w) ethylphosphoric acid TBDMS derivative, **23**;(x) ethylphosphorothioic acid TBDMS derivative, **24**.

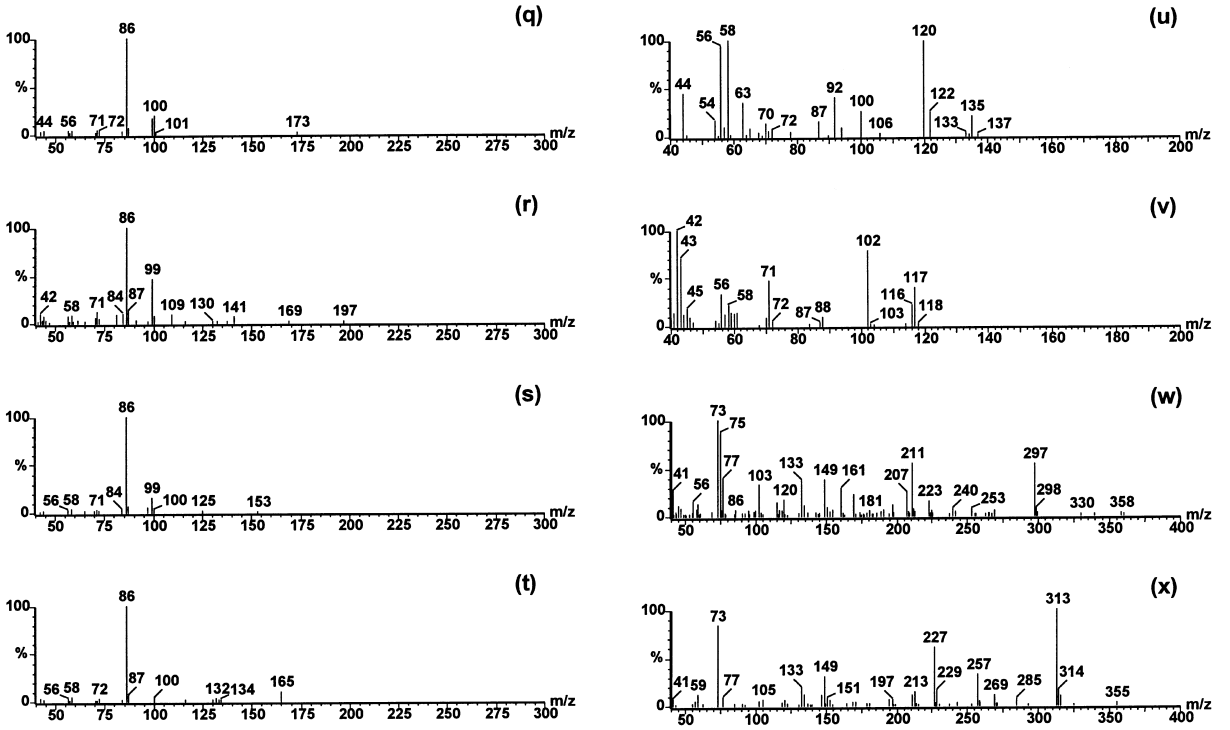


Fig. 4. (*continued*)

97 is probably due to $[(HO)_2PS]^+$, perhaps via a thiono-thiolo rearrangement in the source, and is also a significant peak in the mass spectra of *O,O*-diethylphosphorothionate esters. The absence of prominent ions at *m/z* 138 ($[C_4H_{11}O_3P]^+$) and 111 ($[C_2H_8O_3P]^+$) distinguish **8** from its thionate isomer triethyl phosphorothionate (**5**), likewise the base peak *m/z* 121 ($[C_4H_{10}O_2P]^+$) ion for **5** is not a significant peak for **8** [51].

Of the minor impurities in this family, the EI mass spectrum of diethyl *S*-methyl phosphorothiolate (**6**) displayed a peak for the molecular ion at *m/z* 184 and intense ions at *m/z*, 156, 140, 128, 111, and 109, for $[M–(CH_2CH_2)]^+$, $[(C_2H_5O)P(OH)SCH_3]^+$, $[(OH)_2P(O)SCH_3)]^+$, $[(OH)P(O)(SCH_3)]^+$ and $[(OH)P(O)(OC_2H_5)]^+$ [33]. The absence of prominent ions at *m/z* 79, 95 and 107 for $[(OH)POCH_3]^+$, $[CH_3OPSH]^+$ and $[(C_2H_5O)POCH_3]^+$, respectively, distinguish **6** from its thionate isomer [51,52]. The mass spectrum of triethyl phosphorodithionate (**10**) displayed a prominent molecular ion at *m/z* 214 and intense ions at *m/z* 186, 121, 97, for $[M–(CH_2CH_2)]^+$, $[C_4H_{10}O_2P]^+$, and $[(HO)_2PS]^+$, respectively. The mass spectrum of **3** has been well characterised [52] and is dominated by successive losses of alkyl groups to give ions at *m/z* 155, 127, and 99 for $[(C_2H_5O)_2P(OH)_2]^+$, $[(C_2H_5O)P(OH)_3]^+$, and $[P(OH)_4]^+$, respectively and at *m/z* 109 $[(OH)P(O)(OC_2H_5)]^+$ and *m/z* 81 $[P(O)(OH)_2]^+$. The *tert*.-butyldimethylsilyl (TBDMS) derivative of both **12** and **13** feature a low intensity $[M–CH_3]^+$ ion, and prominent $[M–C_4H_9]^+$, $[M–C_4H_9Si]^+$, $[M–C_6H_{13}Si]^+$, $[C_2H_7OSi]^+$ and $[C_3H_9Si]^+$ ions as with other phosphonic acids [41]. The mass spectrum of **13** also features prominent ions at *m/z* 153 ($[C_4H_{10}PO_2S]^+$) and 121 ($[C_4H_{10}PO_2]^+$). The mass spectrum of both **2** and **22** feature a prominent molecular ion (*m/z* 131 and 117, respectively), and a base peak at *m/z* 116 ion for **2** and a prominent *m/z* 102 ion for **22** arising from loss of CH_3. These are probably cyclic alkylthiomorpholine related chemicals.

The observed mass spectra and retention behaviour for **3**, **5**, **6**, **8**, **9** and the TBDMS derivatives of

Table 3
Comparison between chemicals identified in the distilled amiton and those present in an aged VX sample [55]

General formula (amiton)	Amiton	General formula (VX)	VX
$Et_2NCH_2CH_2X$	$(Et)_2NCH_2CH_2SH$[a]	$iPr_2NCH_2CH_2X$	$(iPr)_2NCH_2CH_2SH$
	$(Et)_2NCH_2CH_2SEt$		$(iPr)_2NCH_2CH_2SEt$
	$(Et)_2NCH_2CH_2OH$		–
	$(Et)_2NCH_2CH_2SSEt$		–
	$(Et)_2NCH_2CH_2SSCH_2CH_2N(Et)_2$		$(iPr)_2NCH_2CH_2SSCH_2CH_2N(iPr)_2$[a]
	–		$(iPr)_2NCH_2CH_3$
	–		$CH_3SCH_2CH_2N(iPr)_2$
	–		$(iPr)SCH_2CH_2N(iPr)_2$
	–		$(iPr)_2NCH_2CH_2SCH_2CH_2N(iPr)_2$[a]
	–		$(iPr)_2NCH_2CH_2SCH_2CH_2SCH_2CH_2N(iPr)_2$
$(EtO)_2P(O)X$	$(EtO)_3P(O)$	$(EtO)CH_3P(O)X$	$(EtO)CH_3P(O)OEt$
	$(EtO)_2P(O)SEt$[a]		$(EtO)CH_3P(O)SEt$
	$(EtO)_2P(O)OH$		–
	$(EtO)_2P(O)OCH_2CH_2N(Et)_2$[a]		$(EtO)CH_3P(O)OCH_2CH_2N(iPr)_2$
	$(EtO)_2P(O)SCH_2CH_2N(Et)_2$		$(EtO)CH_3P(O)SCH_2CH_2N(iPr)_2$
	–		(EtO) CH_3 P(O) O P(O) CH_3 (OEt)
$(EtO)_2P(S)X$	$(EtO)_2P(S)OEt$	$(EtO)CH_3P(S)X$	$(EtO)CH_3P(S)OEt$
	$(EtO)_2P(S)OCH_3$		–
	$(EtO)_2P(S)N(Et)_2$[a]		–
	$(EtO)_2P(S)SEt$		–
	$(EtO)_2P(S)OH$		–
	$(EtO)_2P(S)OCH_2CH_2N(Et)_2$[a]		–
	$(EtO)_2P(S)SCH_2CH_2N(Et)_2$		–
	–	$(EtS)CH_3P(O)X$	$(EtS)CH_3P(O)SCH_2CH_2N(iPr)_2$

[a] The major impurities in each sample.

7, **12** and **13** match those of synthesised authentic materials. The CI mass spectra for **1**, **2**, **3**, **5**, **6**, **8**, **9**, **10**, **12**, **13**, **14**, **21**, **22** and **24** displayed a base peak $[M+H]^+$ ion, with the remainder displaying a prominent $[M+H]^+$ peak. ^{31}P NMR peaks obtained for **5**, **8** and **9** at 29.3, 67.5 and 75.2 ppm respectively is consistent with literature data [53].

The EI mass spectra of a **4**, **9**, **11**, **15**, **16**, **19**, and the TBDMS derivatives of **7**, **12**, **13**, **23** and **24** have not previously been reported.

3.3. Behaviour in matrices

Amiton has previously been shown to be relatively stable [45,54]. This was reflected in the dichloromethane extracts of all four matrices after 28 days where amiton was detected by GC–FID analysis (Fig. 5). The quantity of amiton recovered decreased with time in all four matrices, possibly due to a combination of adsorption and chemical degradation. Recovery was relatively high from the painted panels, and remained significant over the 28 days. This is consistent with previous work in which painted metallic bomb fragments proved to be a useful matrix for recovery of undegraded sarin four years after a Kurdish village was attacked with CW agents [13]. In soil, the recovery was high initially, but in concrete and rubber extracts the amiton recovery was low initially and became more difficult to recover with time.

A preliminary examination of the recovery of the five major impurities from all four matrices by full scanning GC–MS analysis showed that, in the absence of detectable levels of amiton, they could be used to confirm the prior existence of amiton in an environmental sample. Paint yielded the best recoveries for **8** and **9** over the 28 day period (data not shown). Recoveries of **8** and **9** in concrete, paint and rubber extracts followed a similar pattern to amiton, although their recovery from paint is reduced after 14 days. The quantity of **8** recovered from soil increased significantly up to day 14, possibly re-

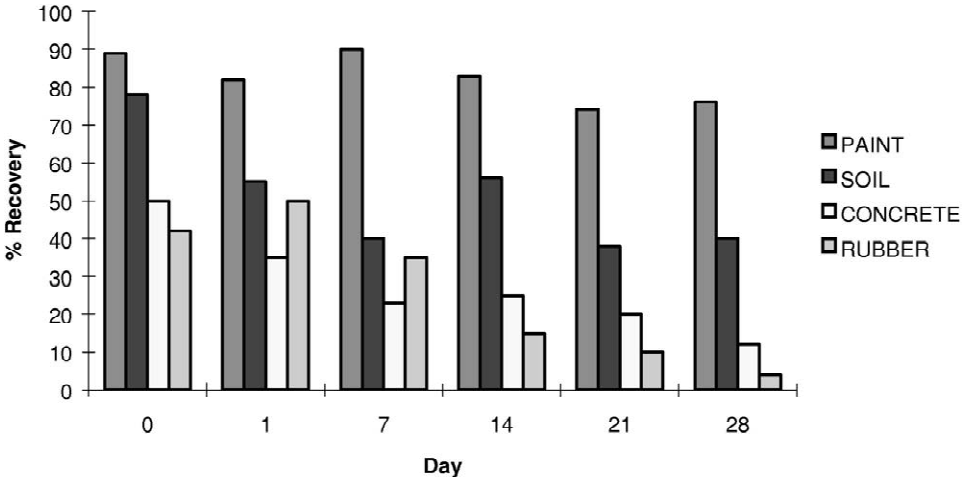


Fig. 5. Recovery of amiton by GC–FID analysis of four matrices (single sample of each) studied over the 28 day period.

sulting from degradation of amiton during this period. However, recovery of **9** from soil proved difficult using dichloromethane. Of the other major impurities, only paint and concrete extracts contained traces of **1**, with increasing levels detected in paint extracts after 14 days, suggesting that it may be formed by degradation of amiton or related chemicals in this matrix. Traces of **16** were detected in concrete and paint extracts after 14 days.

Traces of 13 additional amiton impurities were detected by GC–MS analysis of selected paint and concrete extracts (Fig. 6). While many of these could result from the degradation of related organophosphorus pesticides, the detection of the amiton impurities **2** and **22**, together with a selected ion monitoring (SIM) GC–MS screening procedure for the impurities **1**, **4**, **16**, **19** and **20** in these extracts would also assist in confirming the presence of amiton in a degraded environmental sample.

Amongst the amiton impurities detected, **8**, **9** and

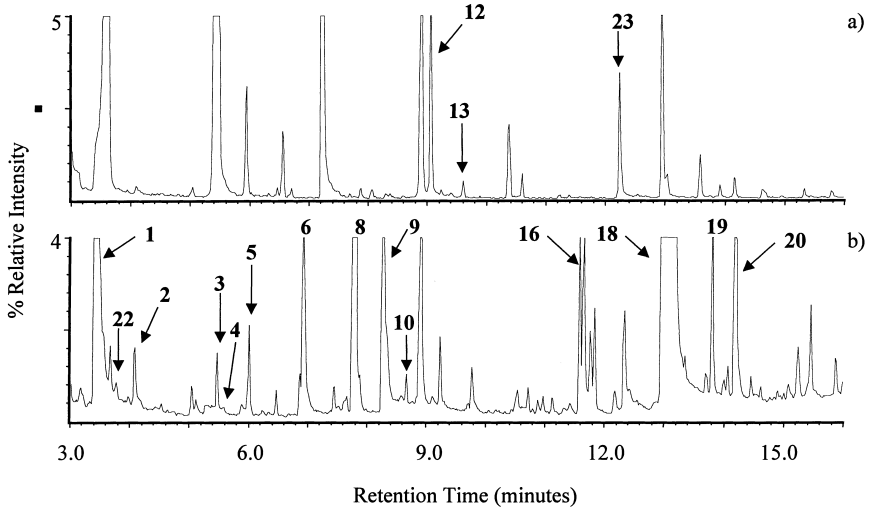


Fig. 6. GC–MS TIC of (a) the concrete day 21 methanol–water (1:1) derivatised extract magnified (×20) and (b) the paint day 21 dichloromethane extract magnified (×25).

the TBDMS derivative of **13** have both distinctive mass spectra, and were detected in all extracts up to 21 days after spiking samples with distilled amiton. SIM GC–MS analysis for characteristic ions of these impurities (i.e. *m*/*z* 111, 138, 198 for **8**, *m*/*z* 121, 192, 225 for **9** and *m*/*z* 171, 199, 227 for the TBDMS derivative of **13**) may be useful as part of a screening technique for establishing prior existence of amiton in an environmental sample.

3.4. Comparisons between amiton and VX impurities

Not suprisingly, a number of the impurities observed in the sample of amiton were found to be structurally similar to those previously identified following long-term storage (10–15 years) of the agent VX (Table 3) [55]. Table 3 shows the general formula of the impurities identified in the amiton and VX samples, and highlights the similarities between their structure e.g. $R_2NCH_2CH_2X$ ($R=CH_3CH_2-$ for amiton, $R=(CH_3)_2CH_2-$ for VX), where X represents a thiol, ethanethiol or dialkylaminoethyldisulphide, are common to both agents. Similarly, for $R_1R_2P(O)X$ ($R_1=R_2=CH_3CH_2O-$ for amiton, $R_1=CH_3CH_2O-$, $R_2=CH_3$ for VX), where X represents ethanol, ethanethiol or dialkylaminoethanol, are also common to both agents. However, for $R_1R_2P(S)X$ ($R_1=R_2=CH_3CH_2O-$ for amiton, $R_1=CH_3CH_2O-$, $R_2=CH_3$ for VX), only the ethyl ester is a common impurity and this is a degradation product of other phosphorothiolates and phosphonothiolates. The CWC [1] requires verification of amiton and related *O*-alkyl *S*-[2-(dialkylamino)ethyl] alkylphosphonothiolates (the VX family of chemicals). Detection of these impurities could prove useful as markers for amiton and individual members of the VX family of chemicals in a degraded sample.

4. Conclusion

The synthesis of amiton, using the method of Ghosh and Newman, followed by purification by distillation, resulted in an 83% pure sample containing five major impurities and trace levels of a large number of minor impurities. Seventeen impurities were detected and characterised by EI and CI GC–MS, with supporting evidence from ^{31}P-NMR and GC-FPD analyses. Many of these impurities were found to be structurally similar to those observed with long-term storage of VX. Eight of these impurities and four additional degradation products were also identified by GC–MS analysis of an amiton sample that had been stored for 7 years.

This study demonstrated that GC–MS analysis can provide evidence for the presence of amiton in concrete, paint, rubber and soil samples in support of the verification provisions of the CWC.

Acknowledgements

The authors would like to thank Sally Duck of Monash University, Clayton, Melbourne, for the high-resolution mass spectrometry measurements for amiton.

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