



Kinetics of the degradation of sulfur mustard on ambient and moist concrete

Carol A.S. Brevett^{a,*}, Kenneth B. Sumpter^b, Robert G. Nickol^a

^a SAIC, Gunpowder Branch, P.O. Box 68, APG, MD 21010-0068, United States

^b U.S. Army Edgewood Chemical Biological Center, 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424, United States

ARTICLE INFO

Article history:

Received 18 January 2008

Received in revised form 5 May 2008

Accepted 7 May 2008

Available online 15 May 2008

Keywords:

Sulfur mustard

H-2TG

Sulfonium ion

HD

¹³C SSMAS NMR

ABSTRACT

The rate of degradation of the chemical warfare agent sulfur mustard, bis(2-chloroethyl) sulfide, was measured on ambient and moist concrete using ¹³C Solid State Magic Angle Spinning Nuclear Magnetic Resonance (SSMAS NMR). Three samples of concrete made by the same formulation, but differing in age and alkalinity were used. The sulfur mustard eventually degraded to thiodiglycol and 1,4-oxathiane via the intermediate sulfonium ions CH-TG, H-TG, H-2TG and O(CH₂CH₂)₂S⁺CH₂CH₂OH on all of the concrete samples, and in addition formed 8–31% vinyl moieties on the newer, more alkaline concrete samples. This is the first observation of the formation of O(CH₂CH₂)₂S⁺CH₂CH₂OH on a solid substrate. The addition of 2-chloroethanol to concrete on which mustard had fully degraded to thiodiglycol and 1,4-oxathiane resulted in the formation of O(CH₂CH₂)₂S⁺CH₂CH₂OH, thus demonstrating the reversibility of sulfur mustard degradation pathways. The sulfur mustard degradation half-lives on ambient concrete at 22 °C ranged from 3.5 to 54 weeks. When the substrates were moistened, the degradation half-lives at 22 °C ranged from 75 to 350 h. The degradation of sulfur mustard occurred more quickly at elevated temperatures and with added water. The non-volatile toxic sulfonium ions persisted for months to years on concrete at 22 °C and weeks to months on concrete at 35 °C, before decomposing to the relatively non-toxic compounds thiodiglycol and 1,4-oxathiane.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

The Chemical Warfare Agent sulfur mustard, a potent vesicant, was used during World Wars I and II [1] and was used recently in the Iran–Iraq wars of the 1980s [2]. Poor disposal methods of unused sulfur mustard stockpiles and ordnance, especially ocean dumping, have led to the resurfacing and continuance of sulfur mustard in the environment [3–5]. In addition, there is the possibility that various terrorist groups may manufacture or procure chemical warfare agents (CWAs) to harm civilian populations, as happened when the group Aum Shinrikyo released Sarin (GB) in the Tokyo subway in March, 1995 [6].

In the event of contamination of a large area with sulfur mustard, knowledge of whether the sulfur mustard will degrade on a given surface, how quickly decomposition will occur, and the correct assessment of the identity, amount and toxicity of the products formed is crucial for making correct decisions about the need for additional decontamination.

Environmentally, sulfur mustard has been observed to persist for 4 years in soil [7]. Wagner and MacIver [8] used ¹³C SSMAS NMR to

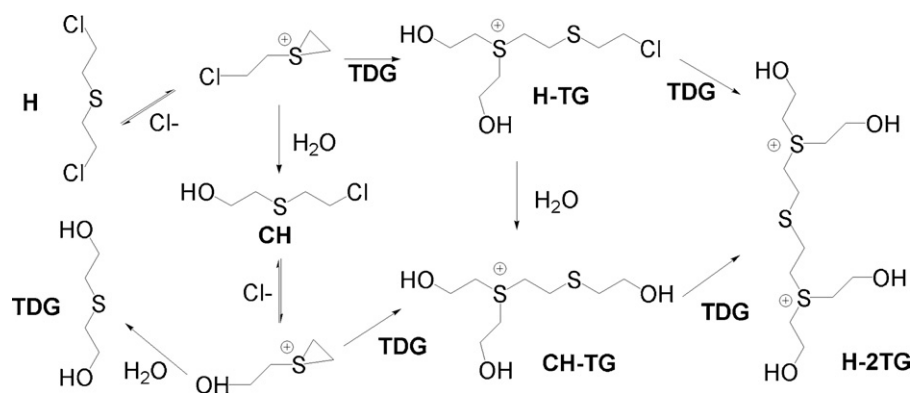
show that sulfur mustard persisted for several weeks on dry soil, but hydrolyzed and polymerized to form the toxic sulfonium ions CH-TG and H-2TG within 2 days when water was added.

Yang et al. [9] showed that although sulfur mustard has low solubility in water (1 mg/mL), forming droplets within it, reactions occurred at the water–sulfur mustard interface to form the hydrolysis products chlorohydrin (CH, ClCH₂CH₂SCH₂CH₂OH) and thiodiglycol (TDG, HOCH₂CH₂SCH₂CH₂OH), which subsequently formed the sulfonium ions H-2TG and CH-TG (Scheme 1) [10,11]. Tilley [12] measured the hydrolysis of 0.35 mM sulfur mustard in aqueous mixtures of ethanol, acetone and dimethyl sulfoxide at 25 °C, and calculated a hydrolysis half-life for mustard in pure water of 4 min. Bartlett and Swain [13] calculated a sulfur mustard hydrolysis half-life of 4.3 min. The half-life of 2 mM sulfur mustard in D₂O at 22 °C as measured by Logan and Sartori [14] was 7 min; thus showing a kinetic isotope effect of ~2.

Brevett et al. [15] showed that sulfur mustard on moist sand, limestone and asphalt degraded to form TDG via H-2TG and CH-TG [16]. Brevett et al. [17] demonstrated that sulfur mustard on ambient concrete would remain as mustard for 12 weeks, but after the passage of 2 weeks it was non-extractable.

Tang et al. [18] showed that sulfur mustard vapor diffused into cement, and droplets of sulfur mustard migrated through a layer of cement at the rate of 1.3×10^{-4} cm/min. Loss of sulfur mustard and formation of products were detected by extraction with

* Corresponding author. Tel.: +1 410 436 1761; fax: +1 410 436 7203.
E-mail address: carol.brevett@us.army.mil (C.A.S. Brevett).



Scheme 1.

acetonitrile followed by GC/MS. The rate for sulfur mustard loss was calculated to be $4.8 \times 10^{-5} \text{ min}^{-1}$, which corresponded to a first-order half-life of 10 days. Products observed in the GC/MS in were 2-chloroethyl vinyl sulfide (CEVS), 1,4-oxathiane, Q (sesquimustard, $\text{ClCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{Cl}$), and sulfur mustard disulfide [$(\text{ClCH}_2\text{CH}_2\text{S})_2$].

Wagner et al. showed that when sulfur mustard was placed on MgO [19] or CaO [20], the products TDG, CEVS and divinyl sulfide (DVS) were the major products. Sulfur mustard reacted on the surface of ambient alumina to give mostly TDG with minor amounts of CEVS and DVS. When excess water was added, the sulfonium ions H-2TG and CH-TG were formed, and $\text{Al}(\text{H}_2\text{O})_6^{3+}$ was liberated from the surface [21]. Vinyl products have also been observed in alkaline aqueous solutions [22].

Karwacki et al. [23] showed that sulfur mustard degradation on dry coconut shell carbon (CSC) at 50°C produced the elimination product, CEVS, and the cyclic ether 1,4-oxathiane. Wagner et al. [24] demonstrated that on wet CSC at 30°C the products CH and TDG were produced in addition to the products that were formed on the dry CSC. The first-order half-life was 25 days. Prasad and Singh [25] impregnated high surface area carbon with a variety of oxidizing agents to form the products, CH, TDG, 1,4-oxathiane, 1,4-thiazane and divinyl sulfone, depending upon the oxidant used [26]. Sharma et al. showed that carbon impregnated with a vanadium/ruthenium mixture would oxidize sulfur mustard to the sulfoxide [27].

Wagner et al. [28] studied the thermal degradation of sulfur mustard with and without water, finding that the “dry degradation” products were 1,2-dichloroethane, polysulfides Q ($\text{ClCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{Cl}$), and 1,4-dithiane, whereas the “moist degradation” products were 1,4-oxathiane, 2-chloroethanol, and numerous sulfonium ions, including the major product $\text{S}(\text{CH}_2\text{CH}_2)_2\text{S}^+\text{CH}_2\text{CH}_2\text{Cl}$ and the minor product $\text{O}(\text{CH}_2\text{CH}_2)_2\text{S}^+\text{CH}_2\text{CH}_2\text{OH}$.

The formation of sulfonium ions from sulfur mustard is important since they have been determined to be a cause of the toxicity of sulfur mustard [29,30], although the sulfonium ions are often less toxic than sulfur mustard itself [31].

In the current study, ^{13}C SSMA NMR techniques were employed to study the rate of and identify the products for sulfur mustard degradation on the environmentally and operationally relevant

substrates, ambient and moist concrete, mortar and limestone as a function of the temperature of the sample and the age of the concrete.

2. Experimental

2.1. Concrete and mortar substrates

The substrates used were three samples of concrete, mortar and limestone. The concrete samples had the same nominal formulation: 16.5% Portland cement, 34.2% natural river siliceous sand as fine aggregate, 49.3% Caldera Alabama limestone coarse aggregate, and a 0.45 water-to-cement ratio. Two samples, C03 and C04 were approximately 1 year old when used; they differed in that C03 was made in a small lab batch and C04 was made using industrial equipment. The third concrete sample, C90, had been cored from a used runway, and was estimated to be 10–20 years old. The mortar, M03, was C03 that lacked the coarse aggregate, limestone, which was studied separately. All samples were thoroughly ground and sieved through a ~ 2 mm sieve before use, in order to ensure good spinning in the NMR rotor, which held ~ 200 mg concrete. The surface areas of the crushed concrete were measured using nitrogen gas adsorption with a 5-point BET analysis [32], and were typically ~ 2 m²/g (Table 1). The pH values were measured using pH strips in a slurry of 200 mg concrete and 2 mL water; the pH values did not change during the duration of the experiments (Table 1). The ambient labile water content of the samples, measured by heating at 100°C for 24 h, was 3.5%.

2.2. Agent

Sulfur mustard, {bis(2-chloroethyl) sulfide} (ClCH_2CH_2)₂S, is commonly abbreviated H for munitions grade and HD for distilled; distillation removes the impurities that are commonly present in munitions grade mustard: Q (sesquimustard, bis(2-chloroethylthio) ethane, $\text{ClCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{Cl}$, 10.1%) the cyclic ether 1,4-dithiane, $(\text{S}(\text{CH}_2\text{CH}_2)_2\text{S}$, 3.2%), 1,2-dichloroethane (2.6%) and 4-chlorobutyl 2-chloroethyl sulfide (1.0%) and 16 other analytes (totaling 3.9%). The sulfur mustard used was 50% ^{13}C labeled at each carbon position, such that two ^{13}C would not be

Table 1
Physical properties of concrete and limestone samples

	C04	C90	C03	M03	Limestone
Surface area, m ² /g, crushed/monolith	2.2/3.1	1.9/1.4	2.0/1.3	2.8/na	2.2/na
Crushed pH, 1/24 h	12.5/12.5	8.0/8.5	12.0/12.0	12.0/12.0	6.0/7.0
Monolith pH, 1/24 h	10.5/12.0	6.5/7.5	8.5/9.0	na/na	7.5/7.5
Age of sample when pH tested	6 months	Est. 10–20 years	1 year	3 years	na

adjacent, and was 99.5% pure by GC/MSD. This allowed for good detection of the ^{13}C signal, but avoided the ^{13}C – ^{13}C couplings that would be present if 100% labeling were used. The notation for the sulfur mustard used was HD*; H for sulfur mustard, D denoting that it had been distilled, and the (*) denoting ^{13}C -labeling. Chemical shifts for sulfur mustard on concrete were 44.5 ppm for ClCH_2 and 35.2 ppm for the CH_2S . *Caution: Sulfur mustard is a potent vesicant, and care must be taken to prevent exposure to liquid or vapor. It should only be manipulated by trained personnel employing appropriate engineering controls and personal protective equipment.*

The rotor (silicon nitride, Doty Scientific, Columbia, SC) was packed with 200 mg concrete and 5 μL agent was added to the concrete using an Eppendorf pipette or a microliter syringe. Water, if used, was added second; a 13–20-fold molar excess was employed. The rotor was sealed with double-O-ring Kel-F end caps, and stored in a glass screw-cap vial. The 35 and 50 °C sample vials were placed in a heater block (Boekel, Feasterville, PA) that maintained the temperature within 0.1 °C of the set point.

2.3. NMR instrumentation

^{13}C SSMAS spectra for the 22 °C samples were collected at 9.4 T using a Varian Inova NMR spectrometer (Palo Alto, CA) equipped with a Doty Scientific 7 mm standard series VT-MAS (variable temperature magic angle spinning) probe using direct polarization at spinning rates of ~ 2000 Hz and a 90° pulse width of 7.5 μs . The spectra for the 35 and 50 °C samples were collected at room temperature at 9.4 T using a Varian Inova NMR spectrometer equipped with a Doty Scientific 7 mm supersonic VT-MAS probe using direct polarization at spinning rates of ~ 3500 Hz, and a 90° pulse width of 3.5 μs . Delay times between pulses were at least 5 times the measured T_1 , and spectra were referenced to external tetramethylsilane. When there was a great degree of overlap between peaks, and for the vinyl resonances, whose chemical shift overlaid the broad background resonance, the deconvolution software supplied by Varian Inc., VNMR version 6.1c was employed to determine relative peak areas.

2.4. Gas chromatography with mass spectrometry detection

GC/MSD analysis of the organic liquid extracts was performed on an Agilent Technologies (Santa Clara, CA) 6890N GC/5973 MSD equipped with a 30 m \times 0.25 mm HP-5 capillary column (Agilent Technologies, Santa Clara, CA). The oven temperature profile was ramped from 45 °C (5 min) to 265 °C at 10 °C/min. The injection temperature was 250 °C; MSD transfer line 280 °C; MSD quad 150 °C; and MSD source at 230 °C. The sample extracts were analyzed in the electron impact (EI) mode scanning from 40 to 350 amu. One microliter was injected on a split/splitless inlet with a purge time 0.5 min; purge flow rate 25 mL/min; with a constant helium column flow rate of 1 mL/min (average linear velocity of 36 cm/s). GC/MS of the extracts detected 1,4-oxathiane, 2-hydroxyethyl vinyl sulfide (HOEVS), CEVS, and TDG.

3. Results

3.1. Kinetics on ambient concrete

The first half-lives for the degradation of sulfur mustard at 22 °C on ambient concrete showed a great degree of variability, ranging from 3.5 to 54 weeks.

The degradation of sulfur mustard on concrete C03 exhibited very clean first-order kinetics (Figs. 1 and 2), and the half-life was 25 days. The degradation of sulfur mustard on concrete M03 exhibited zero-order kinetics, with a half-life of 54 weeks (Fig. 3).

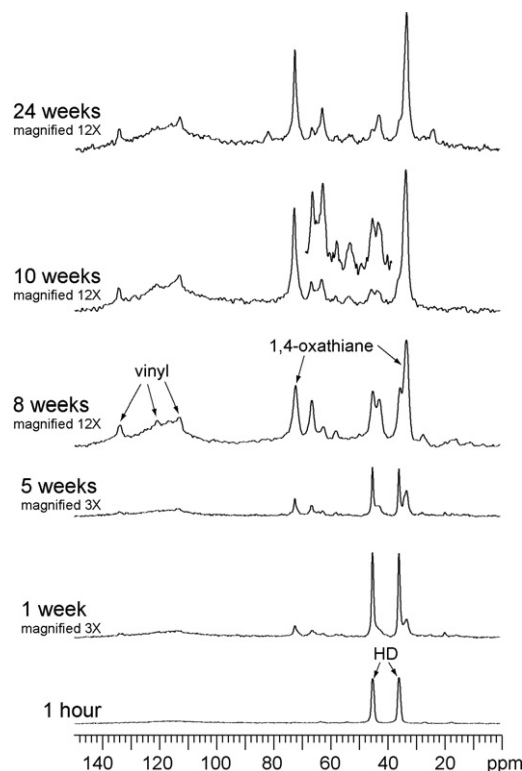


Fig. 1. ^{13}C SSMAS NMR spectra of sulfur mustard degradation on ambient concrete C03.

Sample C90 gave no reaction for several months, and then the degradation occurred (Fig. 4). Sample C90 had numerous spinning side bands and the peaks widths increased from 85 to 400 Hz as the degradation proceeded; hence the signal partially merged into the baseline, and the final total integrated peak area was only one-

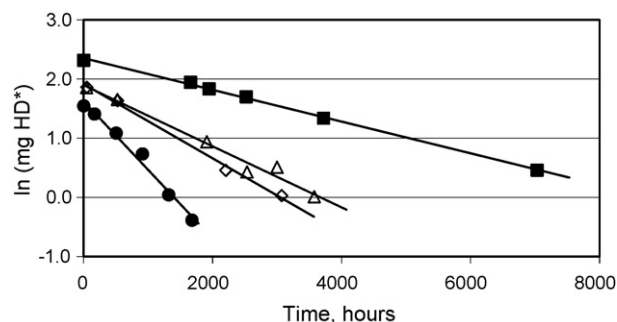


Fig. 2. First-order kinetic plots for sulfur mustard loss on ambient C04a at 22 °C (■), C04 at 35 °C (△, ◇), and C03 at 22 °C (●) concrete samples.

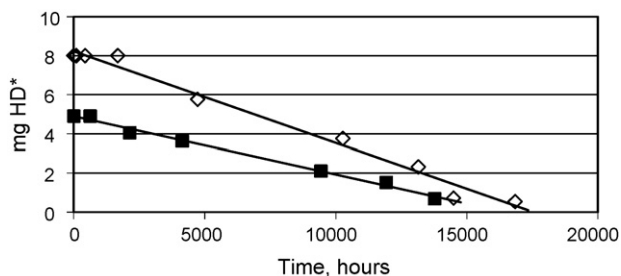


Fig. 3. Loss of sulfur mustard on ambient M03 at 22 °C (◇) and C04b at 22 °C (■).

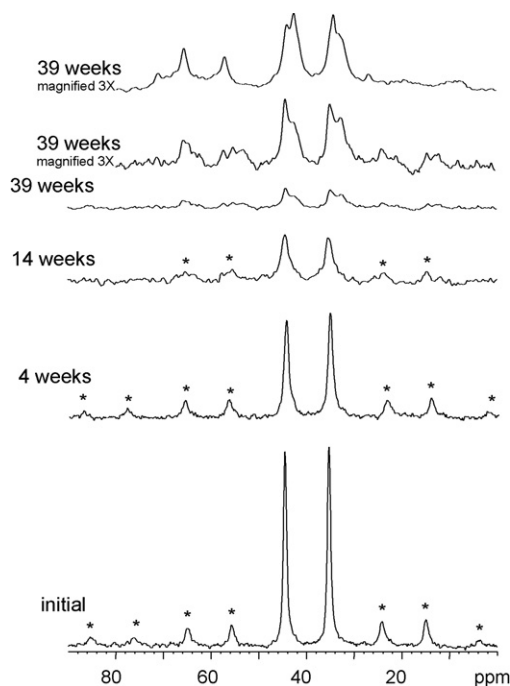


Fig. 4. ^{13}C SSMAS NMR spectra of sulfur mustard degradation on ambient concrete C90 at 22 °C; five bottom spectra were acquired using a 15-s recycle time; the top using a 0.3 s recycle time. The top two spectra are magnified. The (*) denote spinning side bands.

third of the original. In order to enhance the product resonances relative to the reactant, the 39-week spectrum was acquired with a shorter, 0.3 s recycle time, compared to the typical 15-s recycle time (Fig. 4, top spectrum). Thus, the product resonances at 65 and 57 ppm were more clearly seen.

Two samples of sulfur mustard on ambient concrete C04 at 22 °C were made within 4 months of each other. Sample C04a had an initial 10 mg dose of sulfur mustard, and degraded according to first-order kinetics with a half-life of 15 weeks (Fig. 2). Sample C04b had an initial 5 mg dose of sulfur mustard and degraded according to zero-order kinetics with a first half-life of 48 weeks (Fig. 3). The mass balance of sample C04b also decreased gradually during the course of the experiment, but due to the lengthy 82-week period of study, the calculated half-life was the same with and without the mass balance correction.

No degradation of sulfur mustard on the ambient limestone was seen for 1 year.

The degradation of mustard on ambient C04 at 35 °C was measured using duplicate samples that were made at the same time; the kinetics were first order and the half-lives were 46 and 56 days (Fig. 2).

3.2. Kinetics on moist concrete

Sulfur mustard on moist C03 at 22 °C exhibited degradation at 6 days, by 11 days it was difficult to distinguish between the sulfur mustard and the products (Fig. 5). Sulfur mustard degradation on moist M03, C90 and C04 at 22 °C gave similar results. First-order kinetic plots were used to calculate the rate constants for samples C03, M03 and C04; the half-lives were 92, 75 and 350 h, respectively. Due to the paucity of data the first-order half-life for sulfur mustard on C90 was estimated from one data point; it was ~107 h, based upon the loss of the 35 ppm HD* resonance (Fig. 6).

The degradation of sulfur mustard on moist C04 at 35 °C was measured using duplicate samples that were made at the same

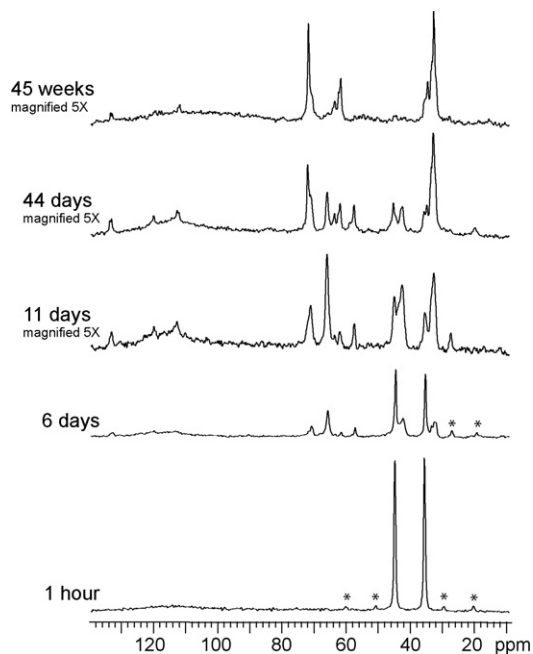


Fig. 5. ^{13}C SSMAS NMR spectra of sulfur mustard degradation on moist concrete C03 at 22 °C. The (*) denote spinning side bands.

time; the kinetics were first order and the half-lives were 23 and 24 h. The kinetics for the degradation of the sample at 50 °C were too fast to measure accurately, but were estimated to be less than 5 h.

Sulfur mustard on moist limestone at 22 °C exhibited degradation at 6 days and was complete in approximately 7 weeks; mass balance was maintained throughout the course of the reaction. The half-life was estimated to be 260 h.

3.3. Product identification

The products were identified based upon their relative integrated peak areas and chemical shifts, which, due to medium effects, were within 3 ppm of those observed in solution and on other matrices. Vinyl species and sulfonium ions formed immedi-

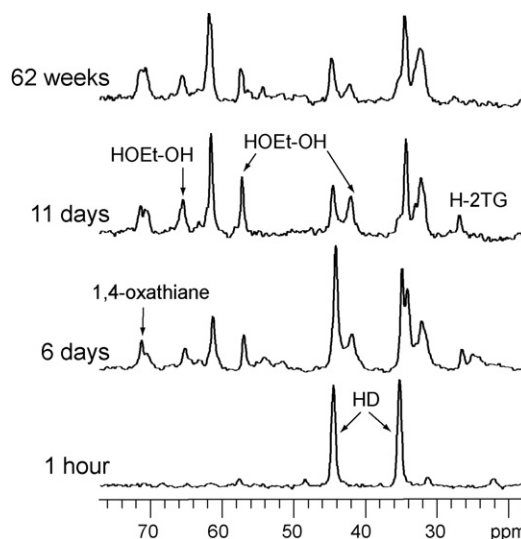
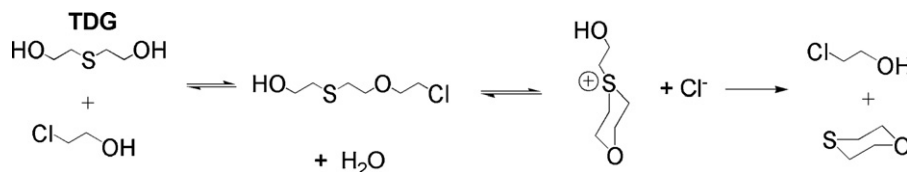


Fig. 6. ^{13}C SSMAS spectra of sulfur mustard degradation on moist C90 at 22 °C.



ately. The elimination product 2-chloroethyl vinyl sulfide (CEVS) had distinct vinyl resonances at 112 and 132 ppm, but the aliphatic CEVS resonances (34 and 43 ppm) were concurrent with the sulfur mustard resonances. In a few samples additional vinyl resonances were observed, possibly corresponding to 2-hydroxyethyl vinyl sulfide (HOEVS, 33, 60, 109, and 138 ppm) or divinyl sulfide (DVS, 114 and 129 ppm). Given the proximity of the chemical shifts and their propensity to shift in different media, it was difficult to definitively distinguish between the different vinyl species solely based upon the chemical shift.

The presence of the most prevalent sulfonium ion, $O(CH_2CH_2)_2S^+CH_2CH_2OH$, 4-(2-hydroxyethyl)-1,4-oxathian-4-ium (HOEt-OT, 33, 46, 57, and 65 ppm, Scheme 2) was monitored by the characteristic 65 ppm resonance. The presence of a 27 ppm resonance suggested the H-2TG (27, 44, 46, and 57 ppm), CH-TG (27, 35, 43, 45, 58 and 62 ppm) and H-TG (29, 33, 43, 44, 46 and 59 ppm) sulfonium ions (Scheme 1). The number of overlapping peaks in the spectra and the low intensity of the 27 ppm peak made it difficult to distinguish between these three sulfonium ions. Thus, any future references to or assignments of H-2TG include the possibility that the species may be CH-TG or H-TG. The H-2TG, CH-TG and H-TG have characteristic resonances at 27–29 and 57–59 ppm; the resonances at 33–35, 43–46 and 62 ppm were concurrent with resonances for other compounds, and thus were not diagnostic. The HOEt-OT had characteristic resonances at 57 and 66 ppm, in addition to the 46 and 32 ppm resonances. Hence, whenever a 57 ppm resonance was present, the 27 and 65 ppm resonances were used to identify which sulfonium ion was present. The expected 32 ppm resonance was concurrent with the thiodiglycol (TDG), 1,4-oxathiane and 1,4-dithiane resonances, and thus was not diagnostic.

After the completion of the degradation of the sulfur mustard and sulfonium ions, TDG (32, 61 ppm) and 1,4-oxathiane (32, 72 ppm) were identified by the relative integrated peak areas of their two resonances. A 32 ppm resonance that was larger than that due to the sum of TDG and 1,4-oxathiane was taken as evidence of 1,4-dithiane. These assignments were confirmed by spiking the rotors with the neat compounds at the end of the degradation.

Chlorohydrin (CH, 33, 35, 45, 61 ppm), an intermediate in the formation of TDG directly from sulfur mustard (Scheme 1) was only observed on moist limestone, and was identified by additional peaks near the 34 ppm TDG resonance.

3.3.1. Products on ambient concrete

Degradation of sulfur mustard on ambient concrete C03 at 22 °C, yielded the intermediates H-2TG, HOEt-OT, and the products CEVS, TDG and 1,4-oxathiane (Fig. 1). The vinyl species at 112 and 133 ppm grew to a plateau (10%) and persisted. A plot of the 57 and 66 ppm sulfonium ion peaks with time showed that they grew and then decreased in intensity (Fig. 7). The 32, 62 and 72 ppm peaks for TDG and 1,4-oxathiane grew consistently for 4000 h.

Ambient M03, which was C03 without the limestone filler, had the products CEVS (112 and 133 ppm), 1,4-oxathiane (32 and 72 ppm), and 1,4-dithiane (32 ppm), coexistent with the mustard (Fig. 8).

Little degradation of sulfur mustard on ambient C90 occurred over a period of 11 months; the only additional species observed was HOEt-OT (Fig. 4).

The difference between the two ambient C04 samples was quite marked. Sample C04a had a first-order half-life of 15 weeks, and yielded CEVS (112 and 131 ppm, 14%), the two sulfonium ions (HOEt-OT 65, 57 ppm and H-2TG, 57, 27 ppm), 1,4-oxathiane (33 and 72 ppm), and 1,4-dithiane (33 ppm) (Fig. 8) with 100% mass balance. By contrast, sample C04b had a zero-order half-life of 45 weeks, a 50% mass balance after 80 weeks, and yielded products

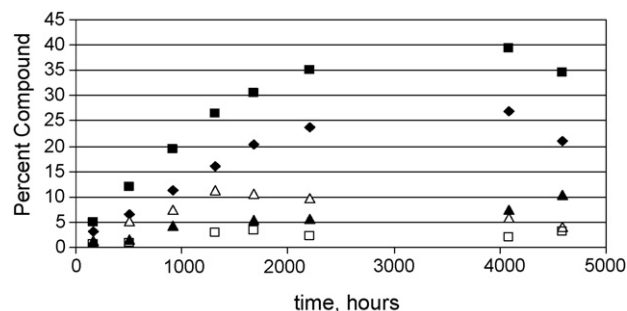


Fig. 7. Evolution of peaks in ^{13}C SSMSMAS NMR spectra for sulfur mustard degradation on ambient C03 concrete at 22 °C: 72 (◆), 33 (■), 61 (▲), 66 (△), and 57 (□) ppm.

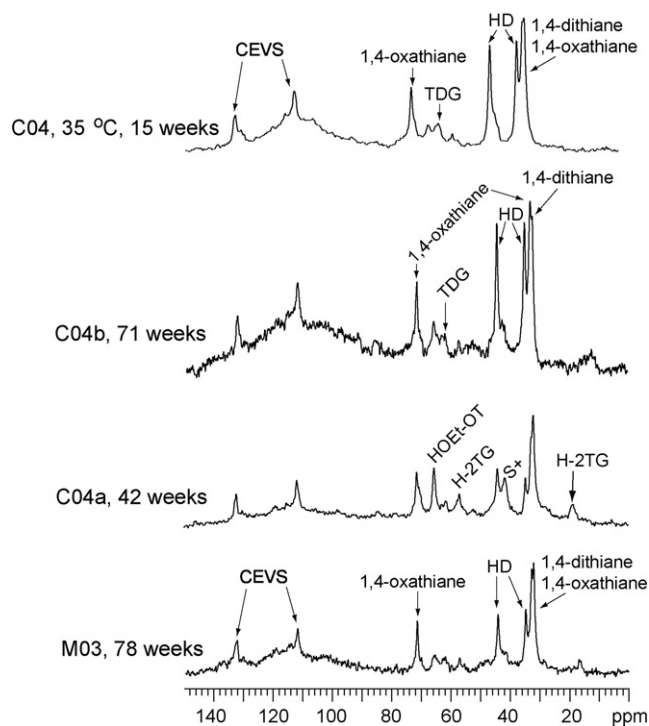


Fig. 8. ^{13}C SSMSMAS spectra of the intermediates formed during sulfur mustard degradation on ambient substrates.

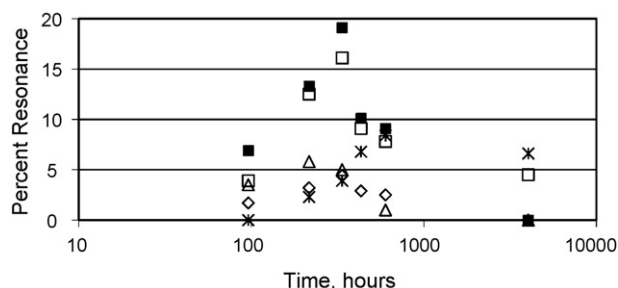


Fig. 9. Evolution of peaks in ^{13}C SSMAS NMR spectra for sulfur mustard degradation on moist M03 at 22 °C: 57 (◇), 66 (□), 43 (■), vinyl (*) and 27 (△) ppm.

CEVS (13%), 1,4-oxathiane, TDG and 1,4-dithiane; sulfur mustard was still present in the sample (Fig. 8).

Sulfur mustard degradation on ambient C04 at 35 °C yielded CEVS (18–19%), 1,4-oxathiane, 1,4-dithiane and TDG (Fig. 8); and yielded CEVS, 1,4-oxathiane, and 1,4-dithiane at 50 °C.

3.3.2. Products on moist concrete

Sulfur mustard degradation on moist C90 at 22 °C formed a trace of H-2TG, HOEt-OT, TDG and 1,4-oxathiane immediately; no CEVS was formed (Fig. 6). After a period of 62 weeks as much HOEt-OT was present as had been at 11 days and had not decomposed, in contrast to the other concretes studied.

The sulfur mustard on moist C03 at 22 °C initially formed H-2TG (27 and 57 ppm), CEVS (133 ppm, 10%) and prominent 65 and 43 ppm resonances (Fig. 5). The final spectrum showed 1,4-oxathiane and the minor products TDG and vinyl species. The degradation of sulfur mustard on moist M03 at 22 °C was similar to C03 at 22 °C. A plot of the 27, 43, 57 and 66 ppm sulfonium ion peaks with time showed that they grew and then decreased in intensity (Fig. 9), whereas the vinyl species at 112 and 133 ppm grew to a plateau of 8%, and persisted. The 32, 62 and 72 ppm peaks for TDG, 1,4-oxathiane and 1,4-dithiane grew consistently, and persisted (Fig. 10). The intermediates and products for the decomposition of sulfur mustard on moist C04 at 22 °C were similar to moist C03 and M03 at 22 °C (Fig. 11).

When sulfur mustard degradation on moist C04 was studied at 35 °C, intermediate peaks at 27, 57 and 66 ppm were seen immediately, corresponding to H-2TG and HOEt-OT. The 65 and 57 ppm peaks reached a maximum intensity in 72 h, and totally degraded over 1000 h. TDG and 1,4-oxathiane were the final products; the final spectrum had additional peaks at 34 and 63 ppm, both downfield of TDG (Fig. 11). There was also a gradual downfield shifting of the chemical shifts of the products during the reaction. The downfield shift was similar to shifts seen previously for sulfonium ions from sulfur mustard degradation on sand [16], due to the formation of ions in the medium. The vinyl moieties observed accounted for 11–12% of the total final resonances. The sample was

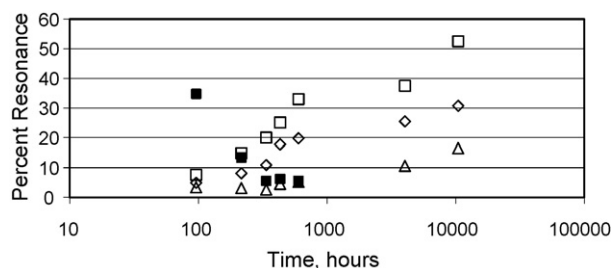


Fig. 10. Evolution of peaks in ^{13}C SSMAS NMR spectra for sulfur mustard degradation on moist M03 at 22 °C: 71 (◇), 32 (□), 35.4 (■), and 62 (△) ppm.

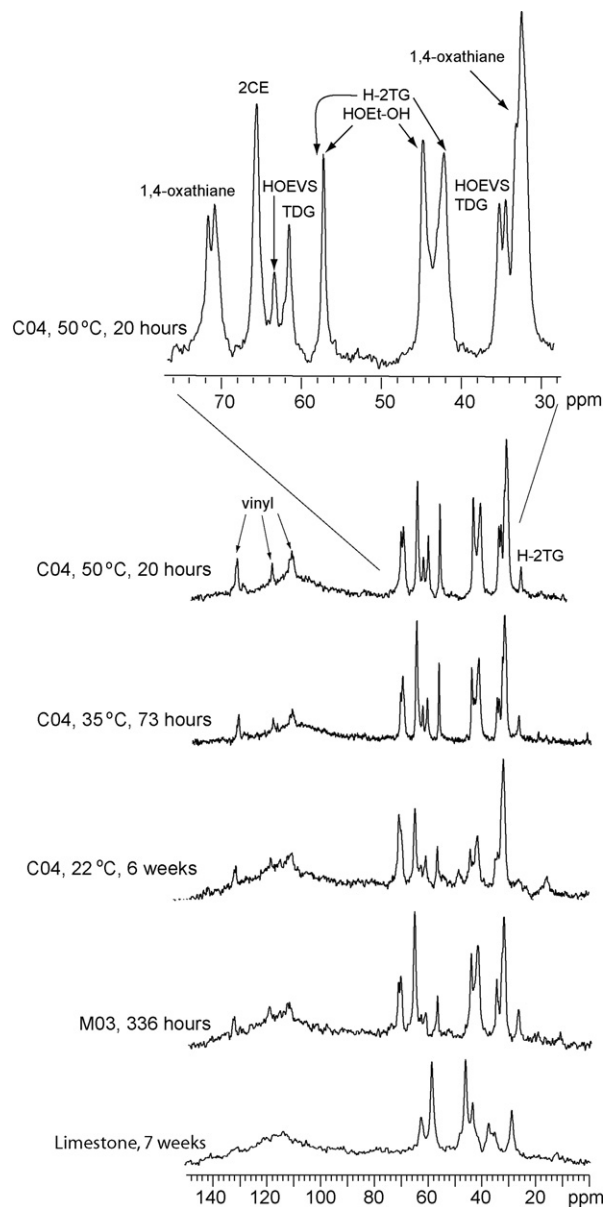


Fig. 11. ^{13}C SSMAS spectra of the intermediates formed during sulfur mustard degradation on moist substrates.

extracted with acetonitrile after the final NMR spectrum; GC/MS of the extract detected 1,4-oxathiane, 2-hydroxyethyl vinyl sulfide (HOEVS), CEVS, and TDG; overall only 2% of the original sulfur mustard spike was recovered, and no unreacted sulfur mustard was observed. In the NMR, the HOEVS would have vinyl peaks coincident with CEVS and aliphatic carbon peaks similar to TDG, hence the additional resonances observed at 63 and 34 ppm may be due to HOEVS.

The degradation of sulfur mustard on moist C04 at 50 °C ultimately yielded 13% vinyl moieties, TDG, and 1,4-oxathiane; the vinyl species may be present as HOEVS since there was a peak at 64 ppm in addition to the 62 ppm TDG peak, and there was a peak at 34 ppm in addition to the 32 ppm peak for TDG, similar to moist C04 at 35 °C. The intermediate sulfonium ions H-2TG and HOEt-OT were also detected (Fig. 11).

In all of the samples of sulfur mustard degradation on moist concrete, the 65 ppm peak was initially very prominent, and then disappeared entirely. Since it was more than twice the size of the

sulfonium ion 57 ppm peak, additional species may be present at this chemical shift. One possible assignment for the 65 ppm transient peak is reactive 2-chloroethanol (63, 47 ppm); ethylene glycol (64 ppm) is not a candidate since it is expected to be a stable product.

Sulfur mustard degradation on moist limestone produced 72% H-2TG sulfonium ion, 15% chlorohydrin and 13% thiodiglycol (Fig. 11).

3.4. Rate of sulfonium ion decomposition

The characteristic 65 ppm peak for the sulfonium ion HOEt-OT that was observed on the concrete samples generally reached a maximum, and then decomposed. The time for the formation of the maximum HOEt-OT peak on ambient concrete at 22 °C spanned a range of 10–77 weeks. The HOEt-OT persisted for at least 30 weeks; in one case it was still present after a 95-week period of observation (Table 2). The duplicate samples that were made at 35 °C exhibited a peak maximum at 13 and 15 weeks, one sample had no HOEt-OT remaining after 30 weeks, but the other sample still had the HOEt-OT present after 38 weeks (Table 2). Thus, the variability for the HOEt-OT formation and degradation was more extensive than the variability for the sulfur mustard degradation on the same samples.

The time for the formation of the maximum HOEt-OT peak on moist concrete at 22 °C spanned a range of 260–530 h, persisted for at least 22 weeks, and was detected in one sample after 60 weeks (Table 3). The duplicate samples that were made at 35 °C both exhibited a peak maximum at 45 h, and no HOEt-OT was detected after 800 and 1000 h (5 and 6 weeks). The first half-life for loss of the 66 ppm peak showed great variability—24 h for one sample, and 172 h for the other (Table 3). Thus, although the time to the maximum HOEt-OT concentration was weeks on the ambient concrete and hours on the moist concrete, the time for the HOEt-OT degradation was weeks to months on both moist and ambient concrete.

3.5. Formation of HOEt-OT from added 2-chloroethanol to TDG and 1,4-oxathiane on moist concrete

The formation of HOEt-OT was demonstrated by the addition of 18 μ L of neat, natural abundance 2-chloroethanol (63, 47 ppm) to the 62-week-old C03 sample, which contained the products

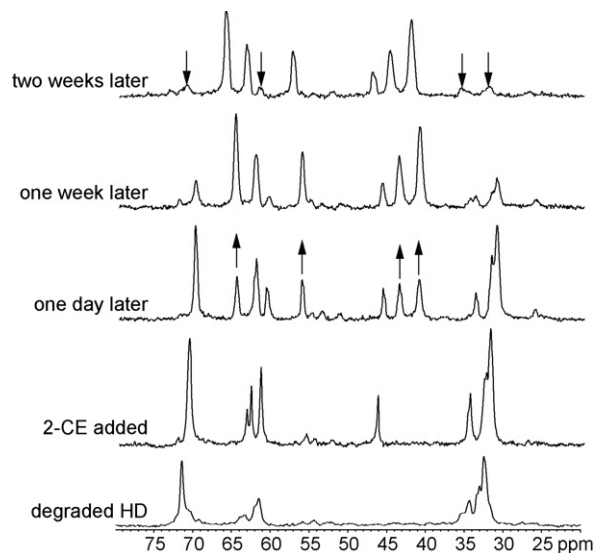


Fig. 12. ^{13}C SSMA NMR spectra of sulfur mustard degradation on moist concrete C03 and after the addition of 2-chloroethanol. The arrows show the gain of HOEt-OT and the loss of TDG and 1,4-oxathiane.

TDG and 1,4-oxathiane. After 24 h the sulfonium ion HOEt-OT was observed; over a period of 2 weeks it grew at the expense of the TDG and 1,4-oxathiane (Fig. 12). The integrated peak area of the 2-chloroethanol peaks remained relatively unchanged, since the reaction was catalytic in 2-chloroethanol.

4. Discussion

4.1. Effect of concrete properties on the product distribution

The concrete samples were studied under ambient conditions and with added water. Ambient conditions, meaning that the concrete was in equilibrium with room temperature and humidity, with no prior drying in an oven, were chosen since drying in an oven would cure the concrete and change the structure from the ambient [33]. The amount of atmospheric water that equilibrated into concrete C04 was 3.5%; this corresponded to 7 mg of water, which would be roughly an 8-fold excess of water to sulfur mustard on

Table 2
Formation and decay of the sulfonium ion HOEt-OT (66 ppm Peak) during sulfur mustard degradation on ambient concrete

Concrete	Temperature (°C)	Time to maximum (weeks)	Time to 50% loss (weeks)	Time of no detection (weeks)
C03	22	10	~14	>30 ^a
M03	22	77	>95	≥95 ^a
C90	22	n/a	n/a	n/a
C04a	22	42	>60	≥60 ^a
C04b	22	72	~12	>83 ^a
C04a	35	15	~6	~30
C04b	35	13	~8	>38 ^a

^a The decomposition of the HOEt-OT was incomplete when the last spectrum was taken.

Table 3
Formation and decay of the sulfonium ion HOEt-OT (66 ppm peak) during sulfur mustard degradation on moist concrete

Concrete	Temperature (°C)	Time to maximum (h)	Time to 50% loss (h)	Time of no detection (weeks)
C03	22	260	~800	~54
M03	22	340	~260	~42
C90	22	264	>10000	≥60 ^a
C04a	22	530	~500	>22 ^a
C04a	35	45	~24	~5
C04b	35	45	~172	~6

^a The decomposition of the HOEt-OT was incomplete when the last spectrum was taken.

the surface of the concrete. This was enough water to react with the sulfur mustard, however, the production of the “dry degradation” product 1,4-dithiane, and the absence of H-2TG under ambient conditions (M03 and C04b) implied that the bulk of the water remained bound to the concrete and did not react with the sulfur mustard.

The surface areas of the concrete samples did not vary greatly from one sample to another (Table 1). The pH of the C90 sample was much lower than that of the newer C04, C03 and M03 samples, due to carbonation occurring on the concrete with time [34]. This lower pH in turn affected the product mixture—no vinyl products, which are generally associated with alkaline surfaces [19,20] were detected on the low pH concrete samples.

Assuming a molecular surface area of 38 \AA^2 [10], 5 mg of sulfur mustard, the amount typically used in these experiments, would require 7 m^2 for monolayer coverage of the concrete. However, the available surface area was only $\sim 0.4 \text{ m}^2$, thus approximately 6% of the sulfur mustard would form the first monolayer. The typical sample formed $\sim 10\%$ vinyl species based on the integrated peak area of the vinyl chemical shifts, suggesting that some of the vinyl species migrated from the surface, and allowed another molecule of sulfur mustard to react. In addition, the surface area of the product molecules is smaller than that of the sulfur mustard, which would also allow for additional molecules on the surface.

The largest percentage vinyl species observed (31%) was on the ambient M03 at 22°C sample (Fig. 8). The only other observed products in this sample over the period of a year were 1,4-oxathiane and 1,4-dithiane, and sulfur mustard was still present. The high percentage vinyl species may be due to the lack of other reaction pathways—that is, insufficient of ambient water to form sulfonium ions. The ambient C04 at 35°C samples both formed $\sim 18\%$ vinyl moieties (Fig. 9). The higher percentage of elimination products on C04 at 35°C than 22°C may be due to a higher thermal mobility of the reactants and products, and hence more reactant is able to reach the concrete surface. Thus, the major contributor to the percentage of vinyl species formed was the surface alkalinity, but the absence of other reaction pathways, especially those involving water, also contributed to a higher percentage of vinyl species. In general, more vinyl species were produced on the ambient concrete than the moist counterpart.

4.2. Sulfur mustard degradation

The half-lives observed for the hydrolysis of sulfur mustard on concrete were 1500 times longer than those observed in dilute solution; 100 h compared to 4 min, respectively. The much slower hydrolysis rate on concrete compared to aqueous solution, plus the formation of elimination and sulfonium ion products, showed that once formed, the 1-(2-chloroethyl) thiiranium ion, $\text{ClCH}_2\text{CH}_2\text{S}^+(\text{CH}_2)_2$, reacted preferentially with sulfur mustard rather than with H_2O . This was not surprising, since sulfur mustard was highly solvated in dilute aqueous solution, whereas on the concrete the amount of sulfur mustard and water was enough to moisten the surface, but no free liquid was observed. Visual observations of droplets of sulfur mustard on concrete indicated that the wetting of the concrete by the sulfur mustard was complete within 30 min, and spinning the sample in the rotor aided this wetting process.

The slightly narrower sulfur mustard peak widths obtained upon the addition of water indicated that the water absorbed on the hydrophilic concrete surface, and the sulfur mustard formed droplets between the concrete particles. However, as the reaction proceeded, the products formed may have migrated between the sulfur mustard-rich and the water-rich portions of the mixture.

Since the NMR data were collected under quantitative relaxation time conditions, and the samples were stored in sealed rotors, the

mass balance should have been preserved throughout the course of the reaction. This was true for 8 of the 14 reactions studied. Two samples, ambient C90 and C04b, lost mass balance gradually during the course of the reaction, possibly due to the adsorption of the products and reactants into the concrete. Four samples, ambient C03, ambient M03, moist C03 and moist C90 lost $\sim 30\%$ of the mass within the first few days of monitoring, possibly due to the formation of multiple minor products whose chemical shifts were too small to distinguish from the baseline. The inhomogeneity of the concrete substrate may also influence the mass balance, that is, some components of the concrete may have been more proficient at forming and absorbing minor products.

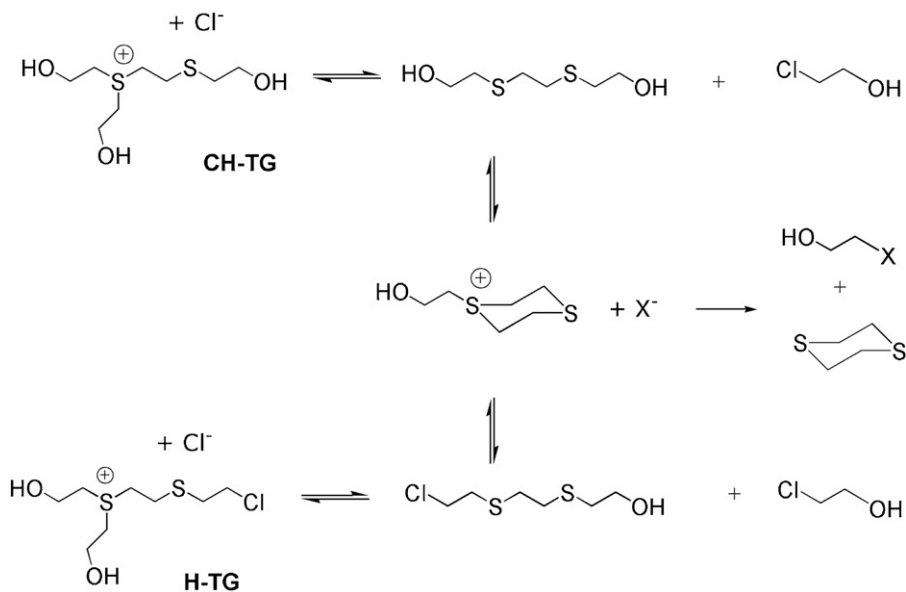
An examination of the order of the degradation reaction showed that nine of the reactions examined exhibited first-order kinetics based upon a plot of $\{\ln(\text{mg HD}^*)\}$ versus time. Two ambient samples, M03 and C04b, which exhibited zero-order kinetics based upon a plot of $\{\text{mg HD}^*\}$ versus time produced 1,4-dithiane, 1,4-oxathiane and a trace of TDG, but no sulfonium ions. This suggested that in the presence of water, ambient or added, first-order kinetics occurred, forming sulfonium ions, but when water was lacking, zero-order kinetics were observed. Zero-order kinetics are known to occur when a surface or enzyme catalyst is present, and are observed when the reactant saturates the catalyst surface [34], which would be possible in the absence of water.

The reason for the apparent dearth of water is not well understood, but may be related to the inhomogeneity of the concrete, which in turn affected the amount of entrained water, which in turn affected the products and order of the reactions. Although great care was taken to thoroughly grind and mix the concrete samples before use, the greatly different degradation rates and products observed on samples C04a and C04b at 22°C suggested that the samples were not totally homogeneous. Comparison of the sulfonium ion degradation rates on moist C04 at 35°C also suggested that these duplicate samples were not totally homogeneous.

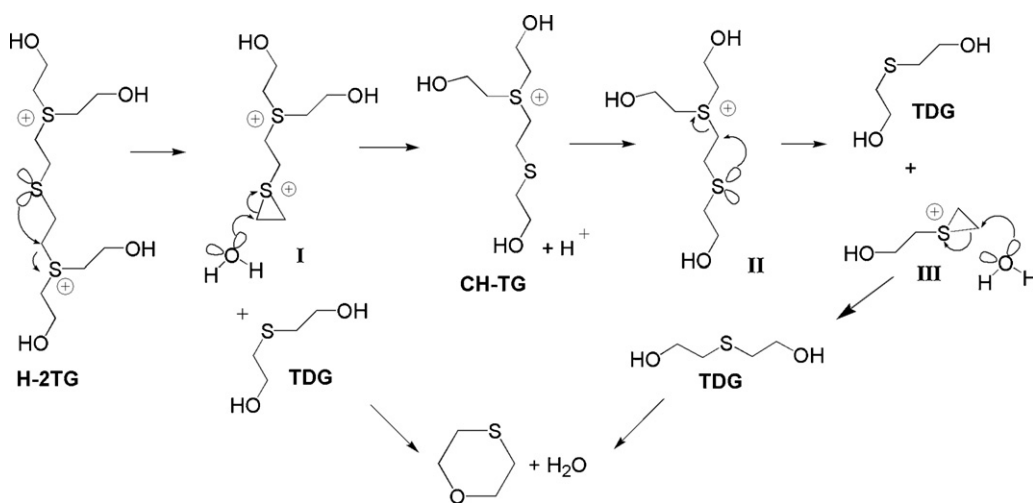
4.3. Sulfonium ion formation and decomposition

The formation and decomposition of HOEt-OT to 1,4-oxathiane (32 and 71 ppm, 1:1 ratio), was observed on several concrete samples. According to Scheme 2, HOEt-OT would form from TDG and 2-chloroethanol, and then degrade to form 1,4-oxathiane and 2-chloroethanol. Thus, the net effect was to form 1,4-oxathiane and water from TDG with 2-chloroethanol functioning as a catalyst. Initially, 2-chloroethanol would be formed from the decomposition of CH-TG and H-TG (Scheme 3), which in turn were formed according to Scheme 1. Eventually, all of the 2-chloroethanol formed from the sulfur mustard would have reacted, since no residual 2-chloroethanol resonances (63 and 47 ppm) were seen in the spectra when the reaction was followed to completion. One possible mechanism for the removal of 2-chloroethanol would be via the reversible formation of H-TG or CH-TG, which would then degrade independently to TDG (Schemes 4 and 5).

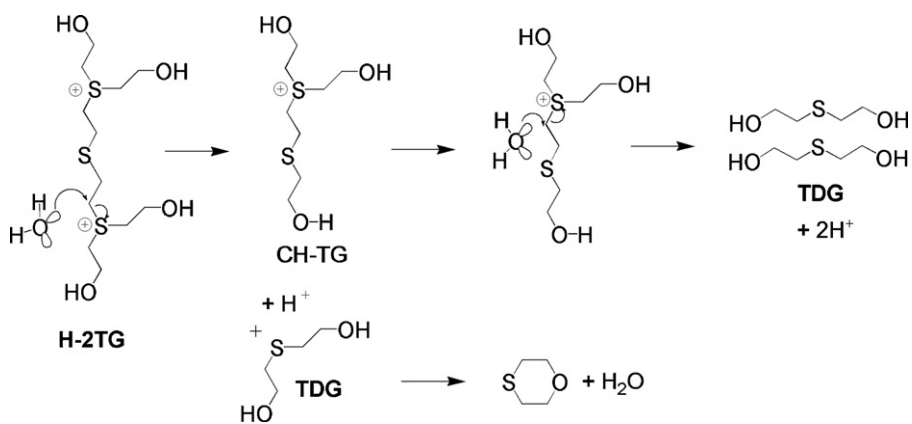
The product 1,4-dithiane may be formed by the decomposition of $\text{S}(\text{CH}_2\text{CH}_2)_2\text{S}^+\text{CH}_2\text{CH}_2\text{OH}$, 1-(2-hydroxyethyl)-1,4-dithianium, which has chemical shifts at 56, 43, 37 and 23 ppm (Scheme 3). The characteristic resonances at the 23 and 37 ppm chemical shifts were not seen, which suggested that any 1-(2-hydroxyethyl)-1,4-dithianium formed and reacted quickly. An additional mechanism for the formation of 1,4-dithiane from sulfur mustard is via the intermediates H-H (45, 44, 43, 38, 35, 28 ppm), Q (43, 34, 32 ppm), and $\text{S}(\text{CH}_2\text{CH}_2)_2\text{S}^+\text{CH}_2\text{CH}_2\text{Cl}$, 1-(2-chloroethyl)-1,4-dithianium (38, 37, 43, 23 ppm) (Scheme 6) [28]. The characteristic resonances for the $\text{S}(\text{CH}_2\text{CH}_2)_2\text{S}^+\text{CH}_2\text{CH}_2\text{Cl}$ at the 23 and 38 ppm chemical shifts were not seen, thus any formed would have reacted quickly. The resonances for H-H and Q would have had chemical



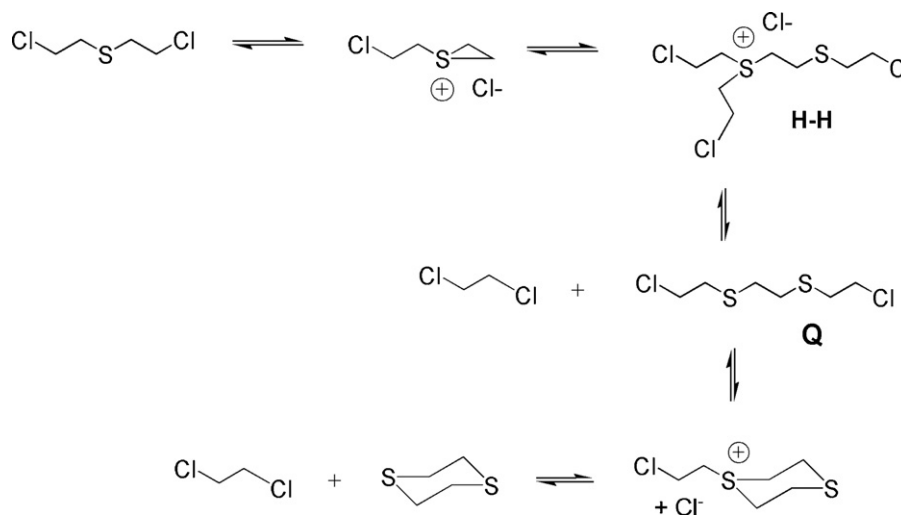
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

shifts that were similar to the other species observed, and would not have been unequivocally detected had they formed.

In prior studies, sulfur mustard degradation products were extracted from soils with hexane several years after contamination. CEVS, TDG, 1,4-dithiane and 1,4-oxathiane, and multiple other species, many of which were related to the impurities present in munitions grade sulfur mustard were detected by GC/MS [35]. The products observed in this study using SSMAS NMR were consistent with the prior GC/MS studies, although no sulfonium ions were detected in the prior studies. The passage of time would result in the degradation of the intermediate sulfonium ions to the products that were observed via GC/MS. In addition, any sulfonium ions present would not extract into hexane, nor would they be volatile on the GC, and hence would not have been detected by the GC/MS technique used.

5. Conclusions

The kinetics of the degradation of sulfur mustard on three samples of airport runway concrete were studied. The first half-lives for the degradation of sulfur mustard at 22 °C on moist concrete fell within a narrow range, between 75 and 350 h, whereas on ambient concrete the range was broad, ranging from 25 days to 54 weeks. The rate was slower on the older, less alkaline concrete. Increasing the temperature also resulted in a faster degradation rate. The wide range of decomposition rates on ambient concrete samples indicated that it would be difficult to accurately predict degradation rates in an environment where the age and degree of hydration of the concrete are unknown.

The degradation immediately produced the minor product 2-chloroethyl vinyl sulfide on the younger, more alkaline concrete samples, but not on the aged sample. The intermediate sulfonium ions 4-(2-hydroxyethyl)-1,4-oxathian-4-ium, and toxic H-2TG formed immediately; the sulfonium ions decomposed to form 1,4-oxathiane and thiodiglycol on the moist samples. On ambient concrete 1,4-oxathiane and 1,4-dithiane were observed; the intermediate sulfonium ions were present in low quantities or not at all, apparently depending upon the amount of ambient water that had been entrained on the concrete. The formation of only non-toxic products would take years on some samples at 22 °C. The products observed were consistent with those observed on alkaline metal oxides, aqueous hydrolysis of mustard, and during the thermal degradation of mustard.

In the event of contamination of concrete with sulfur mustard, the production of toxic intermediates would lead to a condition in which air monitors would not detect any mustard, but vesicant materials would still be present in the concrete. Thus, the concrete would need to be tested for the presence of both sulfur mustard and toxic sulfonium ions.

Acknowledgements

The authors thank Drs. James Savage, H. Dupont Durst and Mark Brickhouse for programmatic support, Dr. George Wagner for stimulating discussions, and Mr. David Sorrick for the supply of ¹³C-labeled sulfur mustard (U.S. Army Research, Development and Engineering Command, Aberdeen Proving Ground, MD). The authors acknowledge Ms. Christine Franklin (SAIC) for administrative support, Mr. Carroll Cook (SAIC) for assistance with the agent operations, Mr. Dennis Bevilacqua (SAIC) for instrumentation support, Mr. Richard Smith (U.S. Air Force Civil Engineering Support Agency, Tyndall, FL) for the aged concrete sample; and Dr. C. Weiss (U.S. Army Engineering R&D Center, Vicksburg, MS) for the concrete, mortar and limestone samples. The work described in this report was performed under SAIC Contract No DAAD13-03-D-0017.

References

- [1] L. Szinicz, History of chemical and biological warfare agents, *Toxicology* 214 (2005) 167–181.
- [2] S. Budiansky, United Nations accuses Iraq of military use, *Nature* 308 (1984) 483.
- [3] K. Korzeniewski, War gases in the southern Baltic Sea, *Studia I Materialy Oceanologiczne* 67 (1994) 91–101.
- [4] M. Billington, Army to destroy mustard gas shell, *The News Journal*, New Castle, Delaware, USA. Saturday February 11, 2006, B2.
- [5] T. Sanginiti, Clamshell cleanup cost nears \$6 million, *The News Journal*, New Castle, Delaware, USA. Tuesday July 19, 2005, B1–B2.
- [6] P. Zurer, Japanese Cult Used VX to Slay Member, *C&EN*, 76 (1998) August 31, 7.
- [7] R.M. Black, R.J. Clark, R.W. Read, M.T.J. Reid, Identification of nerve agent and sulphur mustard residues in soil samples collected four years after a chemical attack; in Proceedings of the ERDEC Scientific Conference on Chemical Defense Research held at Aberdeen Proving Ground, Maryland on November 16–19, 1993 ERDEC-SP-024; J.D. Williams, D.A. Berg, P.J. Reeves (Compilers), U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1994, pp. 227–233; UNCLASSIFIED Report (AD-A286 742).
- [8] G.W. Wagner, B.K. MacIver, Degradation and fate of mustard in soil as determined by ¹³C MAS NMR, *Langmuir* 14 (1998) 6930–6934.
- [9] Y.-C. Yang, L.L. Szafraniec, W.T. Beaudry, J.R. Ward, Direct NMR measurements of sulfonium chlorides produced from the hydrolysis of 2-chloroethyl sulfides, *J. Org. Chem.* 52 (1987) 1637–1638.

- [10] Y.-C. Yang, L.L. Szafraniec, W.T. Beaudry, J.R. Ward, Kinetics and mechanism of the hydrolysis of 2-chloroethyl sulfides, *J. Org. Chem.* 53 (1988) 3293–3297.
- [11] Y.-C. Yang, J.A. Baker, J.R. Ward, Decontamination of chemical warfare agents, *Chem. Rev.* 92 (1992) 1729–1743.
- [12] R.I. Tilley, The hydrolysis of bis(2-chloroethyl) sulfide (sulfur mustard) in aqueous mixtures of ethanol, acetone and dimethyl sulfoxide, *Aust. J. Chem.* 46 (1993) 293–300.
- [13] P.C. Bartlett, C.G. Swain, Kinetics of hydrolysis and displacement reactions of β,β' -dichlorodiethyl sulfide (mustard gas) and of β -chloro- β' -hydroxydiethyl sulfide (mustard chlorohydrin), *J. Am. Chem. Soc.* 71 (1949) 1406–1415.
- [14] T.P. Logan, D.A. Sartori, Nuclear magnetic resonance analysis of the solution and solvolysis of sulfur mustard in deuterium oxide, *Toxicol. Mech. Methods* 13 (2003) 235–240.
- [15] C.A.S. Brevett, K.B. Sumpter, G.W. Wagner, C.L. Cook, Degradation of mustard on moist sand, asphalt and limestone using ^{13}C SSMAS NMR, Technical Report, ECBC-TR-523 (2006) Document AD-A460 354.
- [16] C.A.S. Brevett, K.B. Sumpter, G.W. Wagner, Degradation of sulfur mustard on moist sand as determined by ^{13}C SSMAS NMR, *Spectrosc. Lett.* 41 (2008) 29–39.
- [17] C.A.S. Brevett, K.B. Sumpter, G.W. Wagner, J.S. Rice, Degradation of the blister agent sulfur mustard, bis(2-chloroethyl) sulfide on concrete, *J. Hazard. Mater.* 140 (2007) 353–360, <http://dx.doi.org/10.1016/j.jhazmat.2006.09.067>.
- [18] H. Tang, Z. Cheng, M. Xu, S. Huang, L. Zhou, A preliminary study on sorption, diffusion and degradation of mustard (HD) in cement, *J. Hazard. Mater.* 128 (2006) 227–232.
- [19] G.W. Wagner, P.W. Bartram, O. Koper, K.J. Klabunde, Reactions of VX, GD and HD with nanosize MgO, *J. Phys. Chem. B.* 103 (1999) 3225–3228.
- [20] G.W. Wagner, O. Koper, E. Lucas, S. Decker, K.J. Klabunde, Reactions of VX, GD and HD with Nanosize CaO. Autocatalytic dehydrogenation of HD, *J. Phys. Chem. B* 104 (2000) 5118–5123.
- [21] G.W. Wagner, L.R. Procell, R.J. O'Connor, S. Munavalli, C.L. Carnes, P.N. Kapoor, K.J. Klabunde, Reactions of VX, GD and HD with Nanosize Al_2O_3 . Formation of aluminophosphonates, *J. Am. Chem. Soc.* 123 (2001) 1636–1644.
- [22] K.E. Jackson, β,β' -Dichloroethyl sulfide (mustard gas), *Chem. Rev.* 15 (1934) 425–462.
- [23] C.J. Karwacki, J.H. Buchanan, J.J. Mahle, L.C. Buettner, G.W. Wagner, Effect of temperature on the desorption and decomposition of mustard from activated carbon, *Langmuir* 15 (1999) 8645–8650.
- [24] G.W. Wagner, B.K. MacIver, Y.-C. Yang, Magic angle spinning NMR study of adsorbate reactions on activated charcoal, *Langmuir* 11 (1995) 1439–1442.
- [25] G.K. Prasad, B. Singh, Reactions of sulphur mustard on impregnated carbons, *J. Hazard. Mater.* B116 (2004) 213–217.
- [26] G.K. Prasad, B. Singh, M.V.S. Suryanarayana, B.S. Batra, Kinetics of degradation of sulphur mustard on impregnated carbons, *J. Hazard. Mater.* B121 (2005) 159–165.
- [27] A. Sharma, A. Saxena, B. Singh, M. Sharma, M.V.S. Suryanarayana, R.P. Semwal, K. Ganeshan, K. Sekhar, In-situ degradation of sulphur mustard and its simulants on the surface of impregnated carbons, *J. Hazard. Mater.* B133 (2006) 106–112.
- [28] G.W. Wagner, B.K. MacIver, D.K. Rohrbaugh, Y.-C. Yang, Thermal degradation of bis(2-chloroethyl) sulfide (mustard gas), *Phosphorus, Sulfur, Silicon* 152 (1999) 65–76.
- [29] W.H. Stein, S. Moore, M. Bergmann, Chemical reactions of mustard gas and related compounds. I. The transformations of mustard gas in water. Formation and properties of sulfonium salts derived from mustard gas, *J. Org. Chem.* 11 (1946) 664–674.
- [30] J. Borak, F.R. Sidell, Agents of chemical warfare: sulfur mustard, *Ann. Emerg. Med.* 21 (1992) 303–307.
- [31] N.B. Munro, S.S. Talmage, G.D. Griffin, L.C. Waters, A.P. Watson, J.F. King, V. Hauschild, The sources, fate and toxicity of chemical warfare agent degradation products, *Env. Health Perspect.* 107 (1999) 933–974.
- [32] Surface areas were measured by Micromeritics Inc., Norcross, GA.
- [33] J.J. Beaudoin, J. Marchand, Pore structure, in: V.S. Ramachandran, J.J. Beaudoin (Eds.), *Handbook of Analytical Techniques in Concrete Science and Technology*, Noyes Publications, Park Ridge, New Jersey, 1999, pp. 597–600.
- [34] I.N. Levine, *Physical Chemistry*, McGraw-Hill, New York, 1978, pp. 518–524.
- [35] P.A. D'Agostino, L.R. Provost, A.S. Hansen, G.A. Luoma, Identification of mustard related compounds in aqueous samples by gas chromatography/mass spectrometry, *Biomed. Environ. Mass. Spectrom.* 11 (1989) 484–491.