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ENVIRONMENTAL FATE OF NITROGUANIDINE, DIETHYLENEGLYCOL  
DINITRATE, AND HEXACHLOROETHANE SMOKE

Final Report, Phase I

AD-A185 050

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19. ranging from 27 to 35 days in natural and pure waters. Biotransformation also occurs in water, with half-lives of 5 to 40 days, depending on the amount of organic nutrients present. DEGDN is expected to migrate readily through soil ( $K_p = 2$ ). In sediment, DEGDN was found to bind strongly by processes that are not yet understood. Biotransformation will also occur in sediment under anaerobic conditions, whereas aerobic biotransformation appears to be slow.

The fate of hexachloroethane (HCE) will be controlled by volatilization (half-life of 70 hr in a 180-cm-deep lake) and biotransformation (half-life 5-7 days) in water. In soil, biotransformation occurs under both aerobic and anaerobic conditions (half-lives ranging from 7 to 3 days). Once transported to the atmosphere HCE is projected to be quite stable.

HCE smoke results from the combustion of a mixture of HCE, grained aluminum, and zinc oxide. We have identified products of the combustion to be carbon tetrachloride, tetrachloroethylene, hexachlorobutadiene, hexachlorobenzene, hexachloroethane, hydrochloric acid, phosgene, carbon, aluminum chloride, zinc chloride, carbon monoxide, chlorine, and carbon dioxide.

The physical transport properties of the organic components of HCE smoke suggest that all components will rapidly volatilize from water (except for hexachlorobenzene) and that the rates will be approximately the same ( $t_{1/2} = 70$  hr,  $k_v = 0.01$  hr<sup>-1</sup>). Hexachlorobenzene will also volatilize with a half-life of approximately 150 hr. The  $K_{ow}$  values suggest that hexachlorobutadiene and hexachlorobenzene will partition to lipophilic materials, and they also will partition strongly to soil. These properties suggest that these compounds will remain at the surface of soil and transform by photolytic or hydroxyl radical processes. Carbon tetrachloride, tetrachloroethylene and hexachloroethane will readily volatilize back to the atmosphere after deposition.

The chemical transformation reactions of concern are those associated with the air phase. Tetrachloroethylene and hexachlorobutadiene will undergo reactions with hydroxyl radical, with projected half-lives of 10 and 5 days, respectively. Hexachlorobenzene undergoes slow photolysis, with a half-life of approximately 90 days.

Hydrolytic reactions of aluminum trichloride ( $AlCl_3$ ) and zinc chloride ( $ZnCl_2$ ) have been estimated from high-temperature hydrolysis studies to have half-lives of 30 and 17,000 min, respectively, in the aerosol state. These studies need to be re-evaluated to environmental temperatures and as a function of relative humidity.

The transformations of phosgene and chlorine are rapid and are projected not to be necessary to study in detail. The remaining compounds are projected to be stable to atmospheric chemistry.

The majority of the chemicals from hexachloroethane smoke are expected to remain or transform in the vapor or aerosol state and their persistence in soil or water is expected to be short.

The report includes SRI's recommendations for detailed studies to better define the fate of the subject chemicals.

## EXECUTIVE SUMMARY

This study was conducted to identify dominant loss and movement processes for nitroguanidine (NG), diethyleneglycol dinitrate (DEGDN), and hexachloroethane smoke products in air, soil, and water environments.

The loss of NG in the aqueous environment will be dominated by photolysis, with half-lives ranging from 1.6 days in the summer to 3.9 days in the winter. Biotransformation occurred under both aerobic and anaerobic conditions, with half-lives of 4 and 6 days, respectively, in the presence of extra organic nutrients. NG is projected to move readily through soils ( $K_p < 0.1$ ); however, anaerobic biotransformation occurs readily in soil, with an estimated half-life of 4 days.

DEGDN was found to undergo photolytic transformation, with half-lives ranging from 27 to 35 days in natural and pure waters. Biotransformation also occurs in water, with half-lives of 5 to 40 days, depending on the amount of organic nutrients present. DEGDN is expected to migrate readily through soil ( $K_p = 2$ ). In sediment, DEGDN was found to bind strongly by processes that are not yet understood. Biotransformation will also occur in sediment under anaerobic conditions, whereas aerobic biotransformation appears to be slow.

The fate of hexachloroethane (HCE) will be controlled by volatilization (half-life of 70 hr in a 180-cm-deep lake) and biotransformation (half-life 5-7 days) in water. In soil, biotransformation occurs under both aerobic and anaerobic conditions (half-lives ranging from 7 to 3 days). Once transported to the atmosphere HCE is projected to be quite stable.

HCE smoke results from the combustion of a mixture of HCE, grained aluminum, and zinc oxide. We have identified products of the combustion to be carbon tetrachloride, tetrachloroethylene, hexachlorobutadiene, hexachlorobenzene, hexachloroethane, hydrochloric acid, phosgene, carbon, aluminum chloride, zinc chloride, carbon monoxide, chlorine, and carbon dioxide.

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The report includes SRI's recommendations for detailed studies to better define the fate of the subject chemicals.



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## I. INTRODUCTION

The U.S. Army is in the process of defining the human health and environmental effects of nitroguanidine (NG), diethyleneglycol dinitrate (DEGDN), and hexachloroethane (HCE) smoke. Part of this definition is understanding the loss and movement of these chemicals when discharged in air, water, or soil environments. The objective of this research was to identify environmental fate processes that dominate the transport and transformation of the above chemicals. This objective was reached in Phase I by (1) reviewing the available literature on the environmental chemistry of the subject compounds, and (2) performing screening studies on selected processes for which the literature data were incomplete or not available.

Results of the studies reported herein provide a basis for recommending detailed kinetic rate studies to be performed in Phase II, a continuation of the environmental studies being considered by the government. The Phase II studies would generate rate constants for the dominant processes, which would then be incorporated into a computer model designed to simulate selected environments, thus permitting overall environmental assessments to be made.

## II. METHODS

### A. Literature Review

A literature review was conducted using computer searches of scientific literature data bases, including Chemical Abstracts, Biological Abstracts, Toxline, National Technical Information Service (NTIS), and files from the Defense Technical Information (DTIC) Center. These data were collected to understand the basic chemistry, physical properties, and abilities of the subject compounds to undergo environmental transport and transformation processes. Data were evaluated with the ultimate objective of defining the magnitude of rate and equilibrium constants that were relevant to environmental conditions. The literature review was centered primarily on nitroguanidine (CAS No. 556-88-7), diethyleneglycol dinitrate (CAS No. 693-21-0), and hexachloroethane (CAS No. 67-92-1). The fate of many of the hexachloroethane smoke products has been reviewed in EPA Document EPA-440/4-79-029b, entitled "Water-Related Environmental Fate of 129 Priority Pollutants, Volume II," by M. A. Callahan et al. (1979).

### B. Screening Studies

The test protocols used to evaluate the transport and transformation of the subject chemicals have been described by Mill et al. (1982). These protocols, developed in our laboratory, include hydrolysis, photolysis, and chemical oxidation in water; atmospheric chemistry; aquatic biotransformation; volatilization; and sorption of organics in sediment and soil. The protocols for those processes that affect the persistence of the subject chemicals are briefly described in the Results section.

### C. Analytical Methods

Analytical methods were developed for each chemical to follow its loss or partitioning in each screening study. The methods are described below.

#### 1. Nitroguanidine

Nitroguanidine was analyzed directly from aqueous solutions by high-performance liquid chromatography (HPLC) under the following conditions:

Instrument: Spectra-Physics 3500B liquid chromatograph

Column: 250 mm x 4.6 mm Zorbax C-8 (DuPont)

Solvent: Milli-Q water (100%)

Flow Rate: 0.8 ml/min

Detector: UV @ 254 nm

Retention Time: 5.31 min

Quantitation was achieved by the external standard method, using a standard calibration plot of nanograms of nitroguanidine versus peak area obtained from a Hewlett-Packard 3390A digital integrator.

2. Diethyleneglycol Dinitrate:

DEGDN was analyzed by HPLC under the following conditions:

Instrument: Spectra-Physics 3500B liquid chromatograph

Column: 4.6 mm x 250 mm Ultrasphere ODS-C<sub>18</sub>, 5 μm

Solvent: Methanol/water (50/50)

Flow Rate: 0.8 ml/min

Detector: UV @ 215 nm

Retention Time: 10.9 min, DEGDN; 12.7 min, 3,5-Dinitrotoluene

Quantitation was achieved by the internal standard method, using 3,5-dinitrotoluene as the internal standard and peak area responses recorded by a Spectra-Physics Minigrator digital integrator.

3. Hexachloroethane

Hexachloroethane was analyzed by gas chromatography (GC) of diethylether-sample extracts under the following conditions:

Instrument: Hewlett-Packard 5730 gas chromatograph.

Column: 1.8 m x 2 mm glass column packed with 10% DC-200 on 80/100 mesh Chromosorb W-HP

Flow Rate: 37 ml/min of 5% methane in argon

Temperature: 110°C isothermal

Detector: Electron capture -  $^{63}\text{Ni}$

Retention Time: 4.35 min, hexachloroethane;  
6.20 min, *o*-nitrotoluene (internal standard)

Quantitation was achieved by the internal standard method, using *o*-nitrotoluene as the internal standard and a Hewlett-Packard 3380A digital integrator.

#### 4. Hexachloroethane Smoke Products

Hexachloroethane smoke products were analyzed by employing several analytical techniques. One technique involved trapping the smoke aerosol in hexane and analyzing the hexane by capillary gas chromatography, using electron capture and mass spectrometer detectors. The following conditions were employed:

Instrument: Hewlett-Packard 5840 gas chromatograph.

Column: 30M Durabond DB-5 fused silica (J & W Scientific)

Column Temperature: 125°C

Carrier Gas:  $\text{H}_2$  @ 1.0 ml/min

Makeup Gas: Argon/methane (95/5) @ 40 ml/min

Detectors:  $^{63}\text{Ni}$  or Ribermag R-10-10 mass spectrometer

Smoke aerosols were also trapped in a 7.94-cm infrared cell and evaluated by infrared spectroscopy using a Perkin Elmer 137 infrared spectrometer.

#### D. Field Sampling

Field samples of natural waters, well waters, soil, and sediments were collected from Sunflower Army Ammunition Plant (SAAP) located in Desoto, Kansas, and from Radford Army Ammunition Plant (RAAP) located in Radford, Virginia. Samples were collected in glass bottles, cooled in styrofoam containers containing "Blue Ice", and air shipped to SRI. The samples were placed into refrigerator at 5°C within 24 hours of collection. The purpose of these samplings was to evaluate plant discharges and to collect microbial populations potentially acclimated to the subject chemicals. Nitroguanidine is manufactured at SAAP and diethyleneglycol dinitrate is manufactured at RAAP.

### III. RESULTS

#### A. Nitroguanidine (NG)

##### 1. Physical Transport

##### a. Sediment Sorption of NG

The sediment sorption properties of NG were tested with two of four EPA-characterized sediments. The properties of the sediments are given in Table 1.

Table 1

PROPERTIES OF EPA-CHARACTERIZED SEDIMENTS

<u>I.D.</u>	<u>pH</u> <u>(1:1)</u>	<u>CEC*</u> <u>(ml/100g)</u>	<u>Total N</u> <u>(%)</u>	<u>Organic</u> <u>Carbon</u> <u>(%)</u>	<u>Sand</u> <u>(%)</u>	<u>Clay</u> <u>(%)</u>	<u>Silt</u> <u>(%)</u>
EPA-5	7.44	19.00	0.192	2.28	33.6	31.0	35.4
EPA-6	7.83	33.01	0.097	0.72	0.2	68.6	31.2
EPA-12	7.63	13.53	0.214	2.33	0.0	35.4	64.6
EPA-18	7.76	15.43	0.062	0.66	34.6	39.5	25.8

---

\*Cation exchange capacity.

The two sediments that were chosen for evaluation with NG were EPA-6 and EPA-12. Both of these sediments have high surface areas (no sand fraction) and vary primarily in their cation exchange capacities.

Aliquots of dry sediment (0.1-0.5gm) were weighed into 25-ml Corex centrifuge tubes. Each sample was hydrated for 5 days by the addition of 5 ml of 0.01 M CaCl<sub>2</sub> solution. Following hydration, aqueous solutions of NG (20 ml) in 0.01 M CaCl<sub>2</sub> were added to each tube and the tubes were rotated for 24 hr.

After equilibration, each tube was centrifuged at 10,000 rpm for 20 min to separate the sediment and solution fractions. The supernatant was analyzed by HPLC for changes in NG concentration compared with similar control samples containing no soil. The results are shown in Table 2.

Table 2  
SEDIMENT ADSORPTION OF NG

<u>Tube No.</u>	<u>EPA Sediment No.</u>	<u>Initial Concentration (ppm)</u>	<u>Final</u>
1	6	0	0
2	6	1.6	1.6
3	6	3.2	3.2
4	6	6.4	6.4
5	12	0	0
6	12	1.6	1.6
7	12	3.2	3.2
8	12	6.4	6.1

The above results indicate that NG is not readily adsorbed by the two sediments. The sorption partition coefficient,  $K_p$ , was calculated to be  $<0.1$  in these sediments.

b. Octanol-Water Partition Coefficient ( $K_{ow}$ )

The  $K_{ow}$  for NG was determined by equilibrating 10 ml of water solutions of NG with various amounts of octanol and analyzing both phases for NG by HPLC. The results are summarized in Table 3.

Table 3  
RESULTS FOR THE DETERMINATION OF THE  $K_{ow}$  FOR NG

<u>Sample No</u>	<u>NG Solution (ml)</u>	<u>Octanol (ml)</u>	<u>ppm NG</u>		<u>% NG Recovered</u>	<u><math>K_{ow}</math></u>
			<u>H<sub>2</sub>O</u>	<u>Octanol</u>		
1	10.0	--	2014	--	--	--
2	10.0	2.0	1934	288	98.9	0.149
3	10.0	5.0	1873	276	99.8	0.148
4	10.0	10.0	1751	259	99.8	0.148

The average  $K_{ow}$  was  $0.148 \pm 0.001$ . This low  $K_{ow}$  reflects the charged character of NG at neutral pH (See Chemical Transformation section). Although the soil sorption partition coefficient,  $K_p$ , can be projected from this  $K_{ow}$  value through generally accepted correlation equations, we feel that they may not be applicable in this case, in which factors such as the cation exchange capacity of the soil or sediment may predominate.

c. Solubility of NG in Water

The aqueous solubility of NG was determined by suspending a 0.1-g sample of NG in each of three centrifuge tubes containing 25 ml of water. The tubes were rotated for four days in a closed chamber at 25°C and then centrifuged at 10,000 rpm for 60 min. Direct measurement of the aqueous fraction by HPLC at 25°C indicated that the aqueous solubility of NG was 2,600 ppm.

d. Summary of Physical Properties

Because of the charged nature of NG in water and its high aqueous solubility, Henry's Constant and vapor pressure are expected to be low and were not determined. The measured properties are summarized in Table 4.

Table 4

PHYSICAL PROPERTIES OF NG

$S_w$  - Aqueous solubility at 25°C = 2600 ppm

$K_{ow}$  - Octanol/water partition coefficient = 0.148

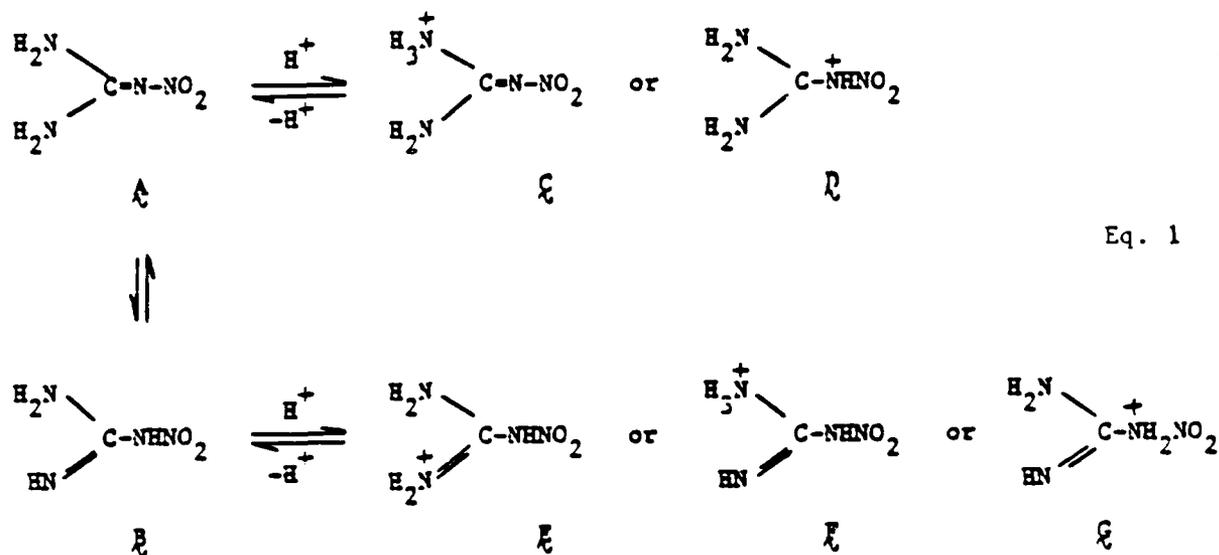
$K_p$  - Sediment sorption partition coefficient = <0.1

2. Chemical Transformation

a. Stability of NG in Water as a Function of pH

The principal tautomeric forms of NG are shown below (Equation 1), with structures C-G predominating under most environmental conditions. This is based on the reported pKa of 12.1 (DeVries and Gantz, 1954); therefore, NG will be unprotonated in water at pH values <12.

The hydrolysis of NG at different pH values was studied at 25° and 50°C. After 73 days, NG hydrolysis was insignificant at 25°C and pH 6.98 and gave 15% conversion at pH 9.56. The reaction was too slow to measure in 21 days at pH  $8.2 \pm 0.1$  at either 25° or 50°C.



Eq. 1

Experiments at pH 9.2 and 11.3 at 50°C gave consistent values of  $k(h)$  of  $5.5 \times 10^{-2} \text{M}^{-1} \text{s}^{-1}$ , which corresponds to half-life ( $t_{1/2}$ ) of 1500 days at pH 7 and 50°C; if the rate is five times slower at 25°C, half-life will be 20 years at pH 7 and 25°C. Table 5 summarizes the data.

In the hydrolytic studies at high pH, a product was observed to grow steadily with time. Product analysis studies were performed with a  $1.38 \times 10^{-2} \text{M}$  NG solution at 25°C (pH 12.0) and a  $1.05 \times 10^{-2} \text{M}$  NG solution at 50°C (pH 11.4). The hydrolysis rate constants for these two reactions were found to be  $3.49 \times 10^{-6} \text{s}^{-1}$  ( $t_{1/2} = 55 \text{ hr}$ ) and  $2.72 \times 10^{-5} \text{s}^{-1}$  ( $t_{1/2} = 7 \text{ hr}$ ), respectively. In both cases, the same hydrolysis product was observed. The UV spectrum of this product is very similar to that of NG except that the whole spectrum is shifted toward lower wavelengths, with a  $\lambda_{\text{max}}$  of 258 nm.

Table 5  
 HYDROLYSIS OF NG<sup>a</sup> AT DIFFERENT pHs AT 25°C AND 50°C

Temp (°C)	pH	Reaction Period	% Reaction	t <sub>1/2</sub>	10 <sup>8</sup> K <sub>h</sub> s <sup>-1</sup>
25	6.98	73 d	<3	> 8 yr	--
25	8.21 <sup>b</sup>	21 d	<3	>200 d	--
25	9.56 <sup>b</sup>	73 d	15	350 d	2.27 ± 0.56
25	10.52 <sup>b</sup>	12 d	55	13 d	60.7 ± 0.24
25	12.0 <sup>b,c</sup>	24 h	55	55 h	349
25	12.99 <sup>b</sup>	21 h	86	7.6 h	2520 ± 20
25	12.99 <sup>b</sup>	22 h	87	7.5 h	2500
50	6.97	42 h	8	360 d	11.3
50	8.30 <sup>b</sup>	21 d	<4	400 d	11.0
50	9.24	29 d	93	7.5 d	106 ± 4
50	11.35 <sup>b</sup>	6 h	90	2 hr	9770 ± 30
50	11.40 <sup>d</sup>	24 h	90	7 hr	2720

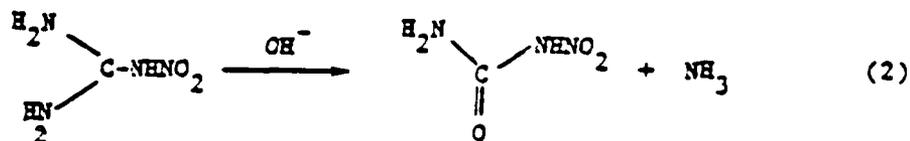
<sup>a</sup>[NG] = 1.24 x 10<sup>-5</sup> M.

<sup>b</sup>pH adjusted with NaOH solution only.

<sup>c</sup>[NG] = 1.38 x 10<sup>-2</sup> M.

<sup>d</sup>[NG] = 1.05 x 10<sup>-2</sup> M.

This product was identified as nitrourea (Eq. 2) based on comparative chromatography with an authentic standard and identical UV absorption spectrum.



We conclude from these data that the hydrolysis of NG will be extremely slow under most environmental conditions.

#### b. Photolysis of NG

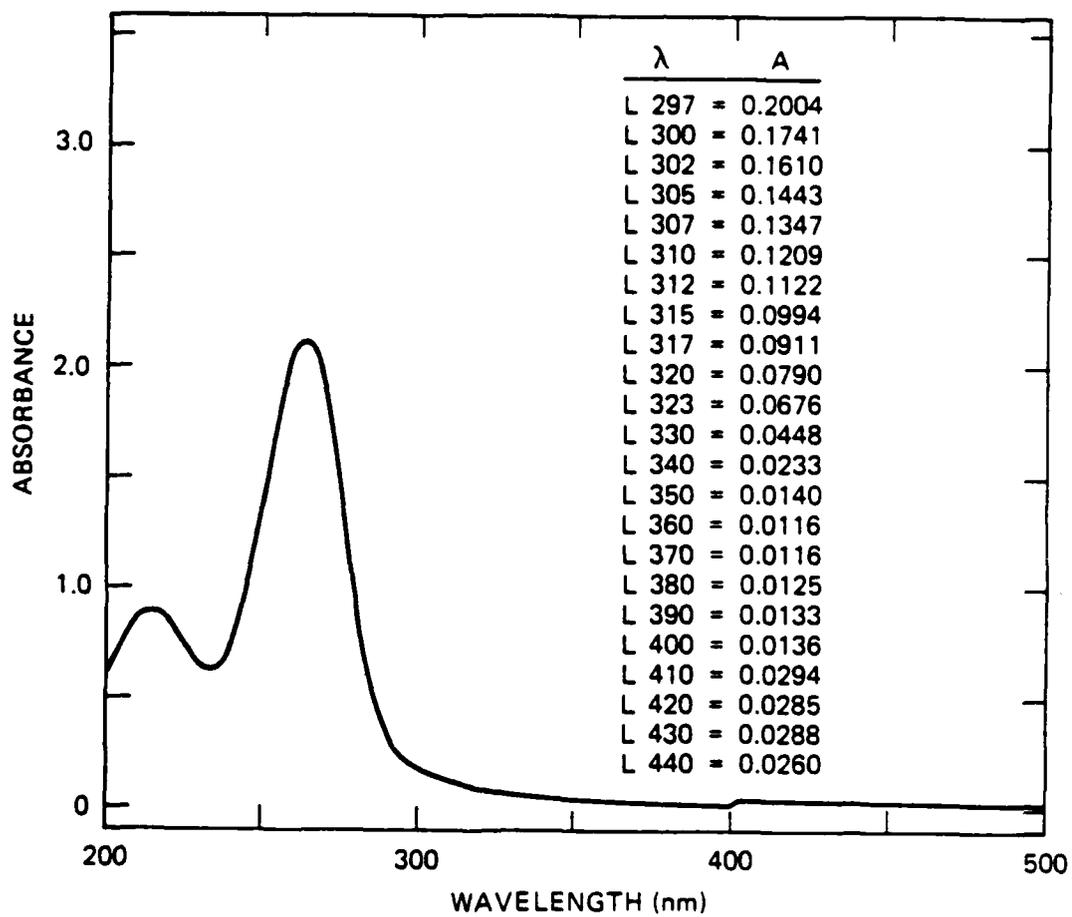
The UV spectrum of NG shown in Figure 1 has a long-wavelength tail, extending out to 350 nm. Extinction coefficients at 300, 310, 320, and 340 nm are 1130, 785, 513, and 150, respectively.

NG has been reported to photolyze in sunlight with a half-life of about 20 hr (Dennis, 1982). To confirm this, the sunlight photolysis of NG in several natural waters and in pure water was investigated. The natural waters were Kansas River water, Searsville Lake water, and Synthetic Natural water (water containing commercial humic acids). All of the natural waters were adjusted to have absorbance of about 0.03 a.u. at 313 nm and pH 8.

The UV spectra of the natural waters were weak and quite similar above 300 nm, although Kansas River water showed significantly stronger absorption below 240 nm (see Figure 2). The absorption of light by NG in the latter water was about 10% that of the natural waters. However, little light attenuation occurred because of the weak absorbance.

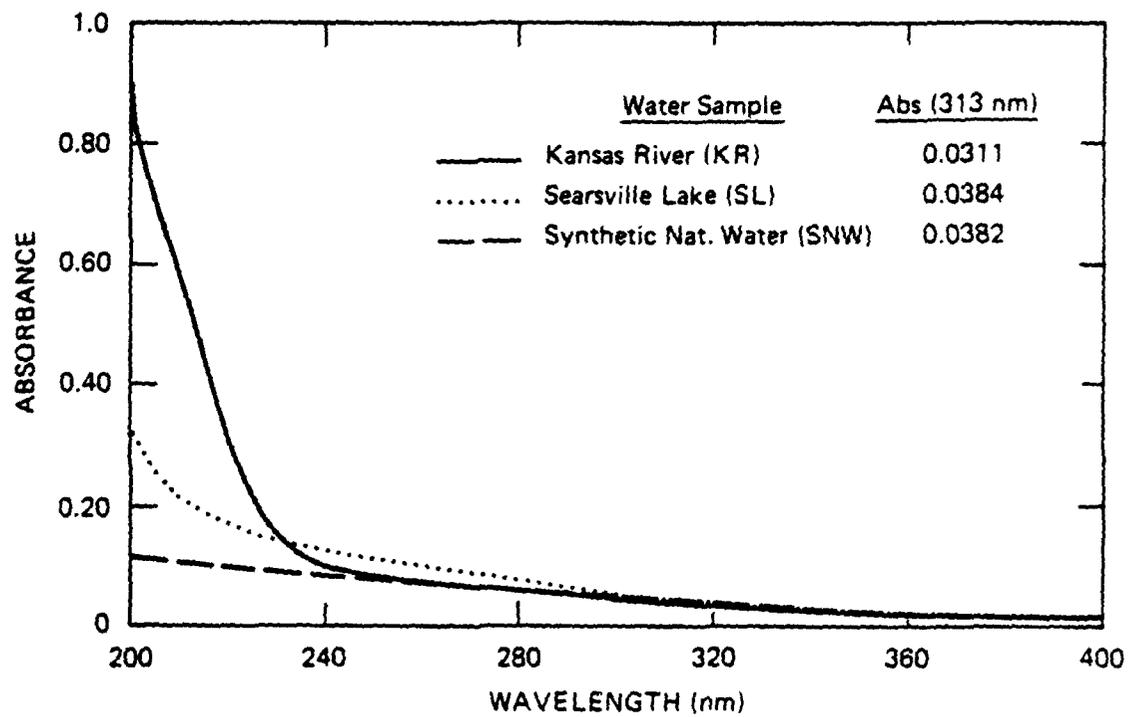
Photolysis experiments were performed with two different concentrations of NG in water. Solutions were exposed to March sunlight for varying periods of time and then analyzed at one time for the remaining NG. One product eluting before NG on the HPLC was noted in the solutions and grew with time. The light flux during each photolysis set was measured with the *p*-nitroacetophenone (PNAP) actinometer (Dulin and Mill, 1982).

With NG at a concentration of  $3.86 \times 10^{-6}$  M (absorbance at 313 nm = 0.003), we observed that NG photolyzed at very similar rates ( $k_p$ ) in all the waters, with half-lives of about 50 hr. The data are summarized in Tables 6 and 7.



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FIGURE 1 UV SPECTRUM OF  $1.54 \times 10^{-4}$  M NG IN WATER



LA-7706-9

FIGURE 2 UV SPECTRA OF NATURAL WATERS

Table 6

SUNLIGHT PHOTOLYSIS OF NG IN DIFFERENT WATERS<sup>a</sup>

Water <sup>b</sup>	pH	Abs (313)	$10^6 \times k_p \text{ s}^{-1c}$	Half-life (hr)
W	6.97	0.0016	4.31	45
KR	8.26	0.0311	3.39	57
SNW	8.64	0.0382	3.70	52
SL	8.33	0.0384	3.39	57

<sup>a</sup>[NG] =  $3.86 \times 10^{-6}$  M; abs (313) = 0.003 a.u.

<sup>a</sup>W = pure water; KR = Kansas river water; SNW = synthetic natural water; SL = Searsville Lake water.

<sup>c</sup> $k(\text{PNAP}) = 3.08 \times 10^{-6} \text{ s}^{-1}$ .

Table 7

SUNLIGHT PHOTOLYSIS OF HIGH CONCENTRATION OF NG IN DIFFERENT NATURAL WATERS<sup>a</sup>

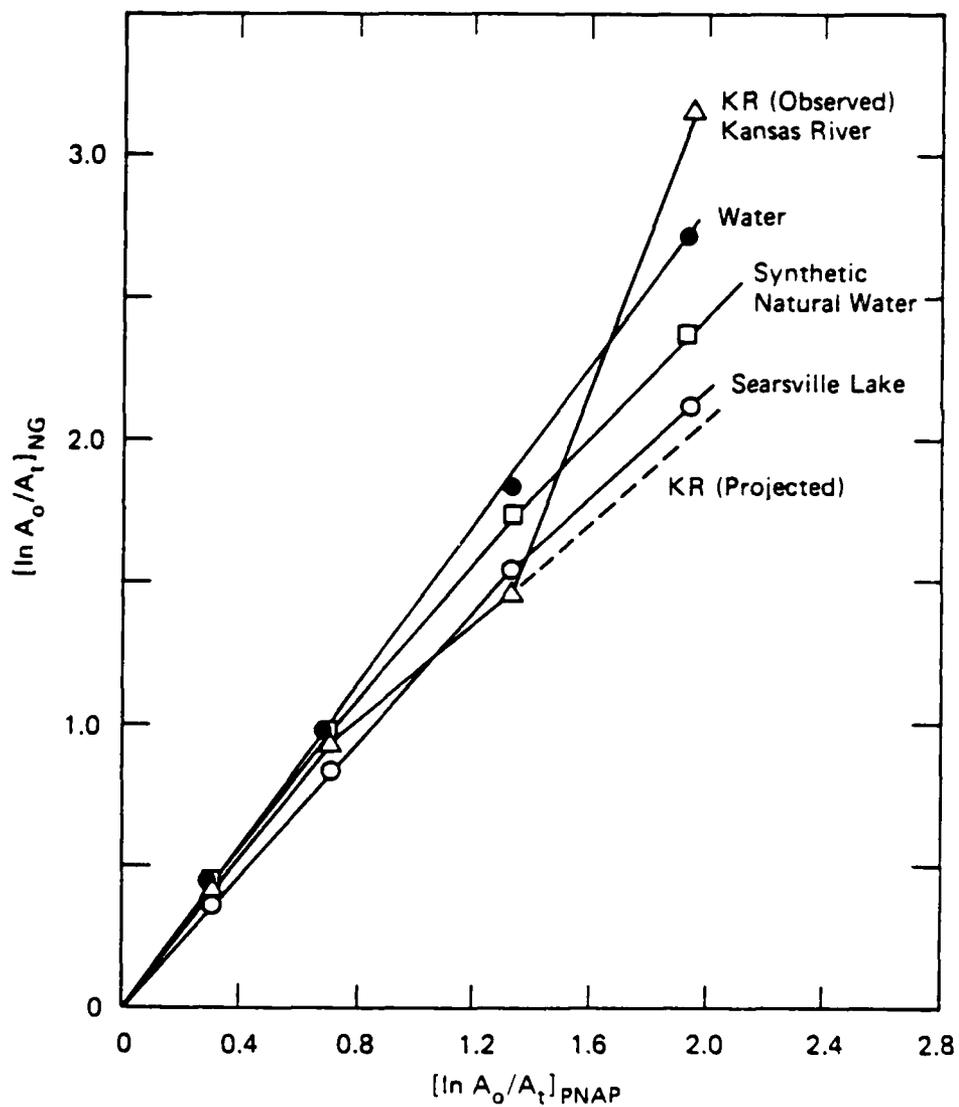
Waters <sup>b</sup>	$10^6 \times k_p \text{ s}^{-1c}$	Half-life (hr)
W	3.86	50
KR	4.73	41
SNW	4.46	43
SL	4.06	47

<sup>a</sup>[NG] =  $3.09 \times 10^{-5}$  M; abs. (313) = 0.022 a.u.

<sup>b</sup>See Table 6.

<sup>c</sup> $k(\text{PNAP}) = 3.33 \times 10^{-6} \text{ s}^{-1}$ .

Figure 3 shows a plot of log [NG] versus log [PNAP] that corrects for diurnal variations in sunlight; the dramatic upturn of the last segment of the KR experiment suggests that there is an error in the last point measured. Our results are in agreement with the early Army report (Dennis, 1982). Because Dennis' experiments were performed in August, when the summer light intensity is about twice that in the winter, our half-life values are consistent.



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FIGURE 3 SUNLIGHT PHOTOLYSIS OF NG IN FOUR WATERS AT  $3.86 \times 10^{-6}$  M

c. NG Quantum Yield

With optically thin solutions ( $A < 0.03$ ) of chemical C and actinometer A, the quantum yield can be expressed in terms of the day-averaged sunlight flux,  $L_\lambda$ , in the wavelength interval  $\lambda$ , the measured sunlight photolysis rate constants  $k_{pC}$ , and the extinction coefficients at wavelengths  $\lambda$ ,  $\epsilon_{\lambda C}$  and  $\epsilon_{\lambda A}$  (Equation 3):

$$\phi_c = \frac{k_{pC}}{k_{pA}} \times \frac{\sum L_\lambda \epsilon_{\lambda A}}{\sum L_\lambda \epsilon_{\lambda C}} \times \phi_A \quad (3)$$

Values of  $L_\lambda$  are available from Table 8.

Table 8

SPECTRAL DATA FOR NG AND PNAP AND SOLAR IRRADIANCE VALUES

$\lambda_{nm}$	$\epsilon_{\lambda A}^a$	$\epsilon_{\lambda C}^b$	Winter $L_{\lambda C}$	$L_\lambda \epsilon_{\lambda A}$	$L_\lambda \epsilon_{\lambda C}$
299	3100	1200	5.38 (-6)	1.67 (-2)	6.45 (-3)
304	2600	970	1.56 (-4)	4.05 (-1)	1.51 (-1)
309	2200	810	1.02 (-3)	2.24	8.26 (-1)
314	2000	673	3.19 (-3)	7.58	2.55
319	1500	540	7.53 (-3)	11.3	4.07
323	1100	440	8.10 (-3)	8.91	3.56
340	470	150	7.52 (-2)	35.3	11.3
370	71	75	1.47 (-1)	10.4	11.0
400	0	0	3.38 (-1)	0	0
$\sum L_\lambda \epsilon_\lambda$				76.1	33.5

<sup>a</sup>Calculated from spectrum of  $6.6 \times 10^{-5}$  M PNAP in 10% acetonitrile in 1-cm cell.

<sup>b</sup>Calculated from spectrum of  $1.54 \times 10^{-4}$  M NG in water in 1-cm cell.

<sup>c</sup>Values of  $L_\lambda$  taken from Mill et al. (1981).

<sup>d</sup>Values in parenthesis represent experimental factors (i.e.  $\times 10^{-6}$ ).

The quantum yield for this PNAP actinometer is  $1.68 \times 10^{-3}$ . The quantum yield for NG is  $5.3 \times 10^{-3}$ . Using Equation 3 and solar intensity values,  $L_\lambda$  (Mill et al., 1982), we calculated photolysis half-lives of NG in winter, spring, summer, and fall to be 3.9, 2.0, 1.0, and 1.6 days, respectively. The data and calculations are summarized in Table 9.

Table 9  
SOLAR ABSORPTION DATA ON NG AT DIFFERENT SEASONS

$\lambda_{nm}$	$\epsilon_\lambda$	$L_\lambda$ at $40^\circ N^a$			
		Winter	Spring	Summer	Fall
299	1200	5.38 (-6) <sup>c</sup>	1.09 (-4)	2.49 (-4)	1.09 (-4)
304	970	1.56 (-4)	1.37 (-3)	2.32 (-3)	1.37 (-3)
309	810	1.02 (-3)	2.96 (-3)	7.93 (-3)	5.35 (-3)
314	673	3.79 (-3)	7.99 (-3)	1.81 (-2)	1.38 (-2)
319	540	7.53 (-3)	1.38 (-2)	2.91 (-2)	2.31 (-2)
323	440	8.10 (-3)	1.42 (-2)	2.97 (-2)	2.39 (-2)
340	150	7.52 (-2)	1.78 (-1)	3.54 (-1)	1.08 (-1)
370	75	1.47 (-1)	2.30 (-1)	4.58 (-1)	3.84 (-1)
400	0	3.38 (-1)	5.26 (-1)	9.71 (-1)	7.31 (-1)
$\Sigma L_\lambda \epsilon_\lambda$ (NG)		33.5	66.9	137	83.1
$\Sigma L_\lambda \epsilon_\lambda$ (PNAP)		76.2	162.7	336	182.2
$t_{1/2}(d)^b$		3.9	2.0	1.0	1.6

<sup>a</sup>North Latitude

<sup>b</sup>Half-life defined in days

<sup>c</sup>Numbers in parenthesis represent experimental factors (i.e.,  $\times 10^{-6}$ )

#### d. Photolysis Products

The photolysis products of NG are reported to depend on pH. At pH between 3 and 10, guanidine and nitrate ion were reported to be the products (Noss and Chyrek, 1984). None of these products has been confirmed.

In our photolysis solution of NG, we observed a single dominant product peak eluting at 0.96 min before NG (Figure 4). The product has not been identified and, on the basis of comparative chromatography with an authentic standard is not nitrosoguanidine.

#### e. Chemical Oxidation/Reduction

In the hydrolytic and photolytic chemistry studies, NG was found to be stable in aerated and nonaerated aqueous solutions. This suggests that NG does not readily undergo chemical oxidation under environmental conditions. Also, the stability of NG in sterile aerobic and anaerobic soils (see Biotransformation section) suggests that chemical reduction processes are not significant relative to photolytic transformation. Therefore, further investigations into these processes were not conducted.

### 3. Biotransformation of NG

#### a. Aqueous Biotransformation

The screening for biotransformation of NG in samples of local pond water (Searsville Pond, Woodside, CA) with 1 g/liter phosphate buffer was conducted under aerobic and anaerobic conditions in the presence or absence of extra organic nutrients (50 ppm glucose and 10 ppm yeast extract) or 1% (dry weight) bottom sediment.

Aerobic waters (21) were prepared in 4-liter bottles and anaerobic waters (11) were prepared in Teflon-lined, screw-capped, 1-liter bottles. Autoclaved waters were used as the sterile control. Aerobic bottles were incubated in the dark at 25°C statically, with periodic shaking. Anaerobic bottles were incubated in a Forma Scientific Anaerobic System chamber, Model 1024, at 25°C. Periodically, samples were withdrawn from the bottles and analyzed by HPLC directly. At 5 ppm NG, no biotransformation was observed after 63 days of incubation under any of the above conditions.

Because Kaplan et al (1982) reported that NG biodegradation microorganisms were obtained from sewage sludge supplemented with nutrient broth under anaerobic conditions, the NG biotransformation test was also conducted with local sewage sludge. Samples of local sewage water (the South-Bayside System, Redwood City, CA) were collected and the organisms from the aerobic and anaerobic sludge were incubated in 4 g/liter Difco Nutrient Broth plus 15 ppm NG or basal salts medium (BSM) plus 100 ppm glucose, 20 ppm yeast extract, and 15 ppm NG. The aerobic media were incubated at 25°C, the anaerobic media at 35°C. In the aerobic media, no decrease in the NG concentration was observed during 24 days of incubation. In the anaerobic studies, the NG concentration did not decrease in the BSM plus glucose and yeast extract. However, a 25% decrease of NG was observed in the Nutrient Broth after 24 days and a 50% decrease after 49 days, which includes a 10-day lag period. These results are consistent with those reported by Kaplan et al. (1982).

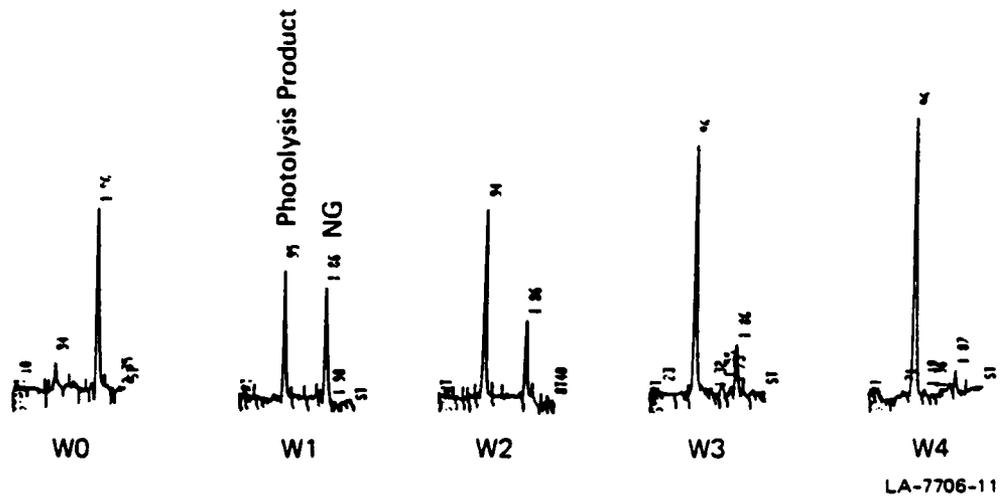


FIGURE 4 HPLC SIGNALS OF NG PHOTOLYSIS SOLUTION MIXTURE IN PURE WATER  
 NG elutes at 1.86 min; W0 (0 hr), W1 (24 hrs), W2 (48 hrs), W3 (76 hrs), and W4 (147 hrs).

The biotransformation of NG was investigated in waters collected at SAAP. The waters investigated were the Pond B lagoon water, which had received NG from past pilot-plant operations, and Hansen Creek water, which was collected 30 ft from the outfall of lagoon 002. NG (15 ppm) was added to each water alone, water plus 100 ppm glucose and 100 ppm yeast extract (Glucose + YE), or water plus 2 g/liter Difco Nutrient Broth. Each water was incubated aerobically and anaerobically. The results of NG analysis from these waters are shown in Figure 5.

Under aerobic conditions, 95% of the NG was lost in both Pond B and Hansen Creek waters after 6 days of incubation with Nutrient Broth, and NG was not detected after 9 days. In Pond B water with glucose and yeast extract, 53% of NG was lost after 6 days and 93% was lost after 9 days. In Hansen Creek water with added glucose and yeast extract, the loss of NG was 29% and 76% after 6 and 9 days of incubation, respectively. No significant decrease in NG concentration was observed in the waters incubated without extra nutrients.

The reason that NG biotransformation was faster in Nutrient Broth-containing water than in glucose and yeast extract-added water may be that the amount used was greater for Nutrient Broth than for the glucose-yeast extract, rather than being due to the quality of the organic nutrient.

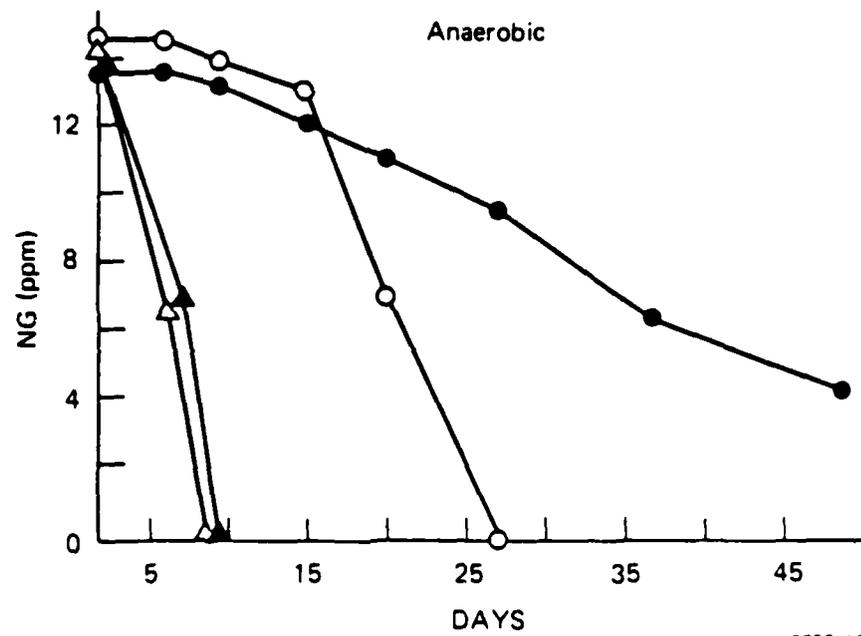
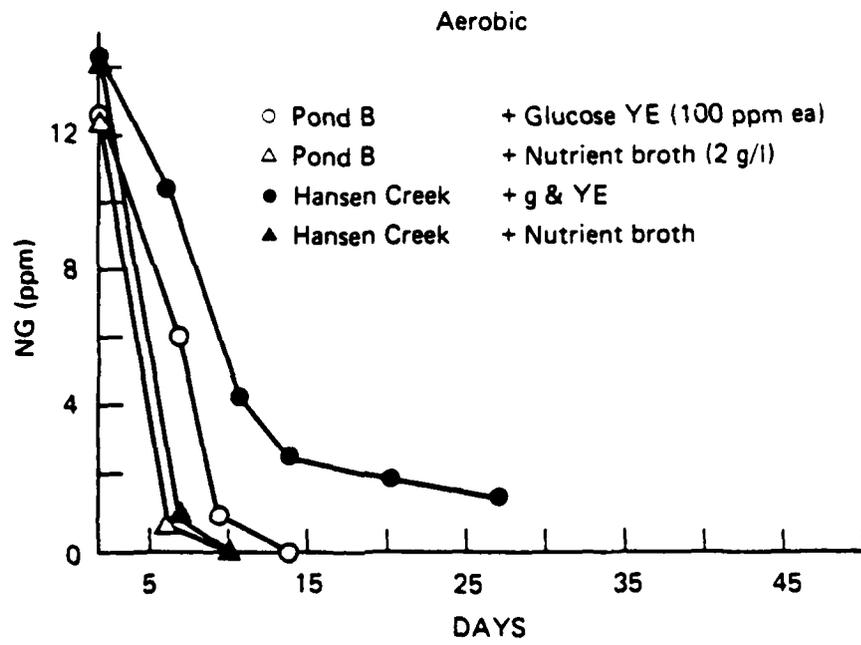
Under anaerobic conditions, 50% of the NG was lost after 6 days with Nutrient Broth, and NG was nondetectable after 13 days in both Pond B and Hansen Creek waters. With glucose and yeast extract, NG was transformed slowly for the first 13 days and was nondetectable after 27 days in Pond B water. NG was transformed to the extent of 80% after 47 days in Hansen Creek water with glucose and yeast extract. NG was not significantly transformed in the waters with no added organic nutrient.

When microorganisms in these NG-degraded waters were inoculated in BSM medium plus glucose and yeast extract (100 ppm each) or the original water plus glucose and yeast extract, biotransformation was observed again, confirming that the transformation was biological. However, the biotransformation was significantly slower in BSM ( $t_{1/2} = 2$  weeks) than in the nutrient-fortified natural water ( $t_{1/2} = 2$  days) under aerobic conditions.

Our previous laboratory studies have shown NG to be biodegradable only under anaerobic conditions, which is in agreement with the results of Kaplan et al. (1982). The SAAP environment apparently contains NG-adapted microorganisms that require only a small amount of organic nutrients to transform NG under aerobic conditions. This transformation proceeds at a much faster rate than we have observed under the anaerobic conditions. Thus, with both photochemical and microbial transformations, NG is not expected to persist in the aquatic environment.

#### b. Soil Biotransformation

An initial soil NG biotransformation study of NG was conducted with the local SRI soil. For a study of aerobic biotransformation in soil, 4 ml of water was added to each of a series of 20-g (dry weight) samples of soil to achieve a 70% moisture saturation. NG from an aqueous solution was added to yield 20 ppm NG/dry weight soil and the soils were incubated under aerobic conditions.



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FIGURE 5 AEROBIC AND ANAEROBIC BIOTRANSFORMATION OF NG IN SAAP WATERS

For the anaerobic study, 13 ml of water was added to a series of 20-g dry weight soils to create a flooded condition in 25 mm x 20 cm screw-capped test tubes. NG was added to achieve a concentration of 20 ppm NG/soil (dry weight). The test tubes were incubated in an anaerobic chamber.

The sterile control used as a baseline was local SRI soil that was autoclaved 1 hour on each of 3 successive days. Periodically, soil samples were removed and extracted with 2 x 40 ml of 0.1N HCl solution and analyzed for NG by HPLC.

The results showed that about 50% of NG was reduced in SRI soil under aerobic conditions after 6 weeks of incubation; however, the sterile control showed about the same loss, indicating that the loss was not biological. The nature of the loss was not clear. Under anaerobic conditions, the NG decrease was about 84% of original concentration; the control decrease was 30% of original concentration after 6 weeks of incubation, indicating that NG may have biotransformed in the flooded anaerobic soil.

The biotransformation of NG in sediment (anaerobic, upper lagoon 123) and soil (aerobic, bank of Pond A) from SAAP was also investigated. Water was added to 10 g of aerobic soil in a 125-ml flask to yield approximately 70% saturation. The anaerobic sediment was placed in a screw-capped, 20 cm x 25-mm screw-capped test tube and flooded with water to 2 cm above the sediment. Autoclaved sediment and soil (1 hr each day for 3 days) served as the sterile control. NG was added at 14 ppm to the soil and sediment (dry weight) and was extracted with 0.1N HCl for analysis by HPLC. The results are shown in Figure 6.

In the anaerobic sediment, NG was reduced to 4.9 and 1.0 ppm after 3 and 6 days of incubation and was nondetectable after 13 days. The sediment was black and appeared to be rich in organic matter from plant decay around the pond. These conditions appear ideal for anaerobic bacteria and demonstrate that NG can be rapidly biotransformed in the lagoon sediment.

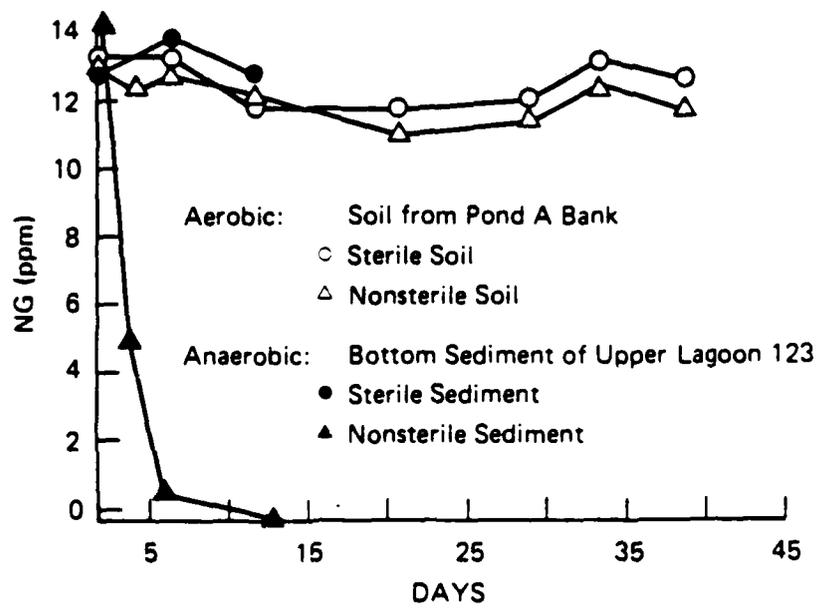
In soil collected near the Pond A pilot plant, NG was not significantly transformed under aerobic conditions after 41 days of incubation, although aerobic biotransformation was observed in Pond B and Hansen Creek water in the presence of 100 ppm of glucose and yeast extract. The failure to observe aerobic biotransformation in the soil may be because it lacked nutrients or because acclimated NG microorganisms were not present at the site where the sample was collected.

#### 4. Biouptake of NG and DEGDN by Aquatic Plants

The preliminary test for the uptake of NG and DEGDN by aquatic organisms was studied using a blue-green algae (*Anabena flow-aquae*) grown in Gorheims medium\* for one week in shaker flasks under 300 foot-candle

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\*Gorheim's Medium = NaNO<sub>3</sub>, 496 mg; K<sub>2</sub>HPO<sub>4</sub>, 39 mg; MgSO<sub>4</sub> · 7H<sub>2</sub>O, 75 mg; CaCl<sub>2</sub> · 2H<sub>2</sub>O, 36 mg; ferrrous citrate, 26 mg; Na<sub>2</sub>SiO<sub>3</sub> · 9H<sub>2</sub>O, 58 mg; Na<sub>2</sub>CO<sub>3</sub>, 20 mg; citric acid, 6 mg; EDTA, 1 mg; water, w<sup>2</sup> liter.



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FIGURE 6 NG BIOTRANSFORMATION IN SOILS FROM SAAP

fluorescent lighting at 24°C. The NG or DEGDN was added at 10 ppm; 24 hr later, the cultures were centrifuged and cell pellets were extracted with acetonitrile and analyzed, along with the supernatant, by HPLC. The average dry weight of the cell pellets indicated an algal concentration of 0.26 mg/ml. The  $K_B$  value for NG ( $\mu\text{g NG/g dry cells}/\mu\text{g NG/g water}$ ) was 130. No DEGDN was detected in the cell extract; therefore, the  $K_B$  for DEGDN was less than 100.

The study was repeated using  $^{14}\text{C}$ -NG, A. flos-aquae, and a green algae Selenastrum capricornutum. Nonlabeled and labeled NG were added to one-week-old algae solutions to yield 10 ppm and 80,000 dpm/ml. After 4 days of incubation on a shaker, the suspensions were centrifuged and the cell pellet was washed with phosphate buffer (pH 7), centrifuged again, and oxidized to  $^{14}\text{CO}_2$  by a Packard 306 oxidizer. Then radioactivity was counted. The  $K_B$  for  $^{14}\text{C}$ -NG in Anabena flos-aque was 89 using an algal concentration of 0.19 mg/ml cell dry weight and 146 in S. capricornutum with a cell dry weight of 0.45 mg/ml. The amount of NG in the algae appears to be no more than the amount sorbed on the algal surface, suggesting that NG does not partition strongly to algae.

To determine the uptake of DEGDN by aquatic plants, the algae S. capricornutum and A. flos-aquae were grown for 1 week in shaker flasks containing 50 ml of medium in 250 ml of water. To triplicate flasks of each organism was added enough DEGDN to yield 10 ppm, and then the solutions were incubated for 4 days. A suspension aliquot (30 ml) from each flask was centrifuged and the cell pellet was extracted with acetonitrile (2 x 5 ml) and analyzed, along with the supernatant, by HPLC. The remainder of the suspension was centrifuged and used for cell dry weight determinations.

The average cell dry weights were 0.36 and 0.25 mg/ml for S. capricornutum and A. flos-aquae, respectively. The average DEGDN concentrations in the supernatant were 9.6 and 8.1 ppm, respectively. DEGDN in the cell extract was not detectable, indicating that the aquatic plant uptake is low. The partition coefficient,  $K_B$ , is less than 10.

## B. Diethyleneglycol Dinitrate (DEGDN)

### 1. Physical Properties

#### a. Solubility of DEGDN

The solubility of DEGDN in water was determined by placing 0.3 ml of the DEGDN stock solution (containing 15% acetone) in each of four screw-capped 30-ml centrifuge tubes containing 1 ml of dichloromethane. The volatile solvents were removed by evaporation using a gentle flow of nitrogen gas, leaving >99% neat DEGDN. Milli-Q water was then added--7 ml to two of the tubes and 5 ml to the other two.

The water and DEGDN were equilibrated by rotation for 72 hr. The two phases were separated by centrifugation at 10,000 rpm for 60 min. The water phase was then analyzed for DEGDN by HPLC using reverse-phase techniques and ultraviolet detection at 215 nm.

The average water concentration of triplicate analyses was  $3.90 \text{ g L}^{-1}$  or 3,900 ppm.

b. Octanol-Water Partition Coefficient ( $K_{OW}$ ) for DEGDN

The  $K_{OW}$  for DEGDN was measured at four different octanol/water equilibration ratios. Neat DEGDN was prepared as stated above for the solubility determination. The DEGDN was solubilized in octanol-saturated Milli-Q water and diluted to 500 ml. Aliquots of this solution were mixed with water-saturated octanol in the following ratios: 5/20, 10/15, 15/10, and 20/5. These solutions were equilibrated by rotation for 24 hr, and then the phases were separated by centrifugation at 10,000 rpm for 30 min.

The initial solution and the aqueous phase from the four equilibrated samples were analyzed in triplicate by HPLC. The results (Table 10) indicated an average  $K_{OW}$  of 9.6.

Table 10

THE PARTITIONING OF DEGDN INTO OCTANOL/WATER  
AND THE MEASURED  $K_{OW}$

<u>Sample No.</u>	<u>Octanol/Water Ratio</u>	<u><math>K_{OW}</math></u>
1	5/20	9.9
2	10/15	9.2
3	15/10	9.8
4	20/5	9.6

c. Henry's Law Constant for DEGDN

An aqueous solution containing 17 ppm of DEGDN was prepared by the method described for the water-solubility determination. The solution (450 ml) was added to a Henry's Law apparatus, which consists of a 50-cm-long vessel containing an extra fine gas dispersion glass frit at the end of a glass tube extending to the bottom of the vessel. The vessel was placed in a constant temperature bath at  $25^{\circ}\text{C}$ . Humidified nitrogen gas was bubbled through the solution at a constant rate, which was periodically measured with a film bubble meter. Water samples were removed from the vessel by syringe through a septum port located at the center of the vessel. The samples were analyzed for DEGDN by HPLC.

Analysis of samples taken over a 10-day period at a nitrogen flow rate of  $57 \text{ ml min}^{-1}$  showed no significant change in the DEGDN concentration. We estimate the  $H_c$  value to be  $<0.01$ . The calculated  $H_c$  value from Equation 4 using a vapor pressure of 0.0036 torr and the water solubility value is shown below.

$$H_c = P/S = 0.0036 \text{ torr}/19.9 \times 10^{-3} \text{ M} = 0.018 \text{ torr M}^{-1} \quad (4)$$

d. Batch Sorption of DEGDN to Sediment

The  $K_p$  value of DEGDN was measured using EPA sediments #5 and #18. Samples (1g) of each sediment were equilibrated with aqueous solutions (20 ml) of DEGDN over a range of concentrations up to 17 ppm. The equilibration time was calculated from the concentration change in the solution phase.

The results of these equilibrations are shown in Table 11. The  $K_p$  values were low for both sediments. The  $K_p$  was somewhat higher for EPA #5 than for EPA #18 because the organic carbon content was higher for EPA #5 (2.3% versus 0.66%). The  $K_{oc}$  values were calculated to be 100 and 118.

Table 11

BATCH SORPTION OF DEGN ONTO EPA SEDIMENTS #5 AND #18

	Sample Number	Wt. of Sample (g)	DEGN in Water (ug/ml)	DEGN in Sediment (ug/g)	$K_p$
<u>Sed. #5:</u>	1	0.9939	0	0	
	2	0.9923	3.57	14.54	4.07
	3	1.0063	1.72	4.77	2.77
	4	1.0065	8.37	1.25	0.15
	5	0.9964	16.10	33.57	2.09
					Avg = 2.3
<u>Sed. #18:</u>	6	1.0091	0	0	
	7	1.0019	3.94	5.04	1.28
	8	1.0058	1.89	0.50	0.26
	9	0.9968	8.37	1.27	0.15
	10	1.0060	16.49	23.33	1.41
					Avg = 0.78
Controls	11			0	
	12			4.14	
	13			1.91	
	14			8.42	
	15			17.42	

## 2. Chemical Transformation

### a. Hydrolysis of DEGDN.

The hydrolysis of DEGDN was observed to be quite slow ( $\tau_{1/2} > 800$  days) at pH 7.00. The hydrolysis of DEGDN was then studied at pH 9, 10, and 12.8 over 31 days and our data (Table 12) show that the reactions were indeed very slow. These results were somewhat surprising in view of the much faster rates of hydrolysis of nitrate esters reported by Capellos et al. (1982, 1984a, 1984b). In our studies less than 5% of DEGDN was lost at pH 9 and 10. Only 15% loss was observed in pH 12.8 solution. The half-lives calculated from these data at these three pH values are 440, 530, and 130 days. These results are summarized in Table 12.

Fraser (1968) reported that DEGDN hydrolyzes in 90% EtOH, with a base hydrolysis constant,  $k_B = 340 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  at 60°C. Extrapolation of that value to 25°C gives  $k_B = 15 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ , which corresponds to a half-life of 5.3 days with 0.01 M NaOH. We measured the rate constant for hydrolysis of DEGDN at 25°C with 0.010 M NaOH in 90% EtOH and found a calculated half-life of 330 hr, which is equivalent to  $k_B = 5.8 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ . In view of the assumptions made in extrapolating the 60°C data to 25°C, the two values are in reasonable agreement. Moreover, they serve to give more confidence in our measurements in water and confirm the very slow hydrolysis observed.

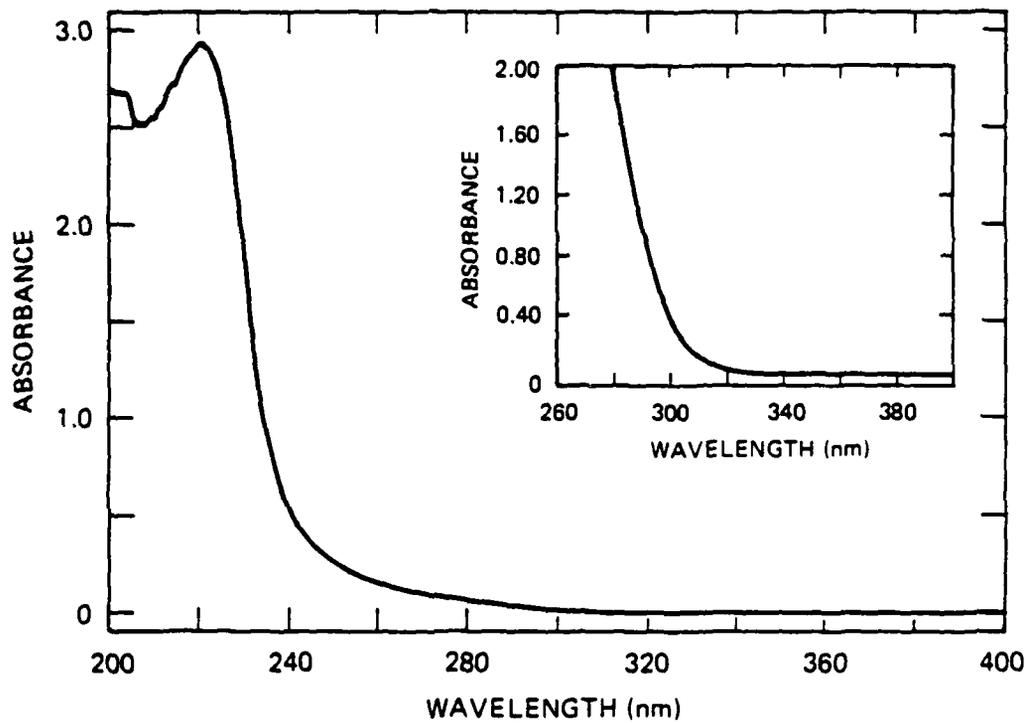
Table 12

#### HYDROLYSIS OF DEGDN IN WATER AT 25°C

pH	DEGDN Concentration	$k_H \times 10^3 \text{ d}^{-1}$	Half-life (days)	$k_B \text{ M}^{-1} \text{ s}^{-1}$
9.00	$8.32 \times 10^{-5} \text{ M}$	$1.57 \pm 0.35$	440	$1.82 \times 10^{-3}$
10.02	$1.04 \times 10^{-4} \text{ M}$	$1.31 \pm 0.53$	530	$1.44 \times 10^{-4}$
12.84	$2.78 \times 10^{-5} \text{ M}$	$5.43 \pm 0.48$	130	$9.08 \times 10^{-7}$

### b. Photolysis

DEGDN has strong UV absorbance at 230 nm, but its absorbance drops off sharply and is extremely weak in the solar region ( $> 300$  nm). A UV spectrum of a  $2.32 \times 10^{-3} \text{ M}$  solution of DEGDN (Figure 7 insert) shows that there is little absorption in the visible region. Extinction coefficients at 300 and 310 nm are 6 and  $2 \text{ M}^{-1} \text{ cm}^{-1}$ . Photolysis studies of DEGDN in pure water and in natural waters were conducted as described for NG. The optical absorbances of the natural waters were adjusted to 0.030 a.u. at 313 nm. After 23 days of exposure to sunlight, about 30 to 46% of  $3 \times 10^{-5} \text{ M}$  DEGDN was lost in most of the water solutions. The photolysis was fastest in Kansas River water and was



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FIGURE 7 UV SPECTRA OF DEGDN AT  $2.32 \times 10^{-3}$  M AND  $8.92 \times 10^{-2}$  M

slowest in standard humic acid water. The rate in pure water falls between the rates of those two waters, with a half-life of about 35 days in spring. These data show that the addition of humic substances to the water induced little enhancement of the photolysis rate of DEGDN. The results are summarized in Table 13.

Table 13  
SUNLIGHT PHOTOLYSIS OF DEGDN IN NATURAL WATERS<sup>a,b</sup>

<u>Waters<sup>c</sup></u>	<u><math>k_p \times 10^2 \text{ day}^{-1}</math></u>	<u>Half-life (days)</u>
Pure water	1.98 ± 0.08	35
Kansas River	2.54 ± 0.16	27
Searsville Lake	2.01 ± 0.14	34
Synthetic nat. water	2.35 ± 0.33	29
Std. Humic Acid	1.73 ± 0.19	40
Std. Fulvic Acid	1.87 ± 0.24	37

<sup>a</sup>Photolysis period of 23 days;  $k_p(\text{PNAP}) = 5.56 \times 10^{-1} \text{ d}^{-1}$ .

<sup>b</sup> $[\text{DEGDN}]_0 = 3.13 \times 10^{-5} \text{ M}$ .

<sup>c</sup>Absorbance of water at 313 nm = 0.03, pH = 7 ± 1, filtered through 0.20 mm paper.

c. Quantum Yield for DEGDN

Using the photolysis rate constant of DEGDN in pure water from Table 13, Equation 5 below, a quantum yield of the actinometer of  $\phi_{\text{PNAP}} = 1.68 \times 10^{-3}$ , and  $\sum L_{\lambda} \epsilon_{\lambda} (\text{DEGDN}) = 0.25$ , taken from Spring Solar Absorption Values (Table 14), we calculated the photolysis quantum yield of DEGDN to be 0.037.

$$\phi = \frac{\phi_a k_{pc} \sum \epsilon_{\lambda} L_{\lambda a}}{k_{pa} \sum \epsilon_{\lambda} L_{\lambda c}} \quad (5)$$

The symbol a denotes PNAP and c denotes DEGDN.

Table 14

## SPRING SOLAR ABSORPTION OF DEGDN AT 40°N

$\lambda_{nm}$	$\epsilon_{\lambda}$	$L_{\lambda} \epsilon_{\lambda}^a$
299	6.0	6.5(-4)
304	3.5	4.8(-3)
309	1.9	5.6(-3)
314	1.0	8.0(-3)
319	0.53	7.3(-3)
323	0.33	4.7(-3)
340	0.13	2.3(-2)
370	0.08	1.8(-2)
400	0.063	3.3(-2)
430	0.055	3.7(-2)
460	0.040	3.5(-2)
494	0.03	3.4(-2)
538	0.02	3.4(-2)

$$^a \Sigma L_{\lambda} \epsilon_{\lambda} = 0.25 \text{ d}^{-1}; t_{1/2} = 3 \text{ d.}$$

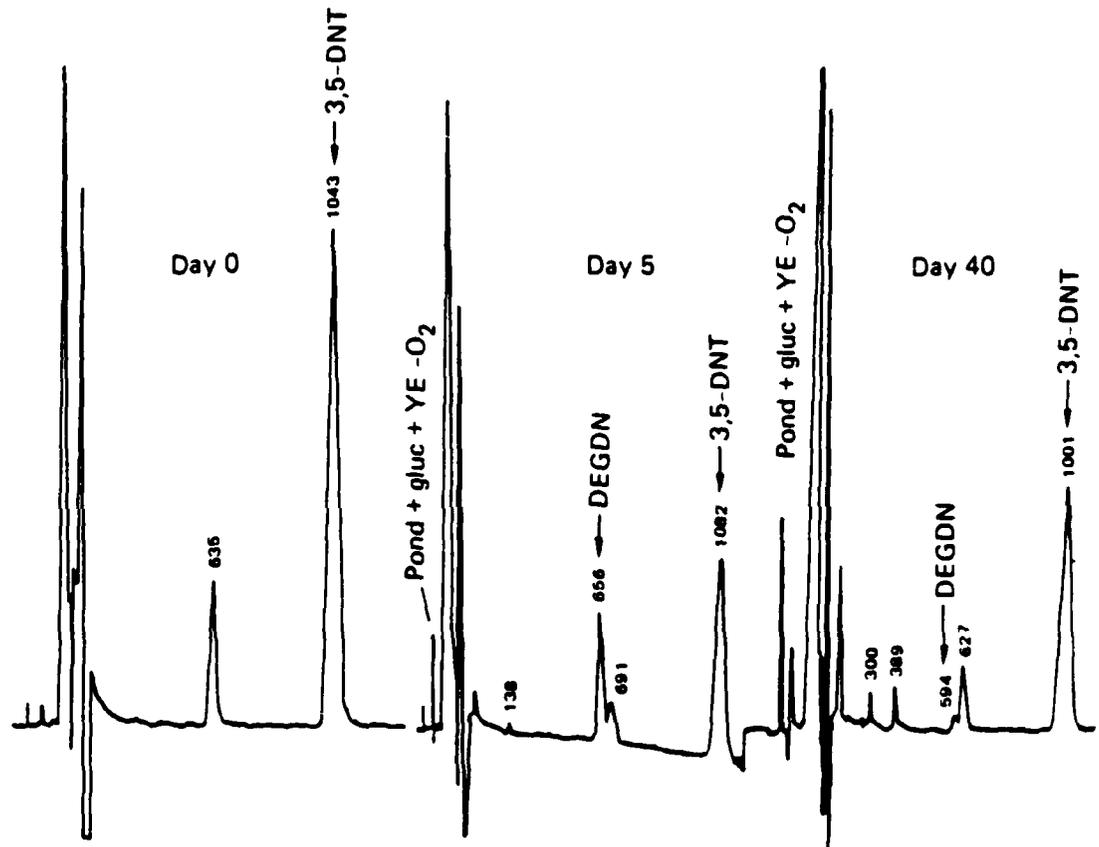
<sup>b</sup>Numbers in parenthesis represent exponential factors (i.e.  $\times 10^{-4}$ )

### 3. Biotransformation of DEGDN

#### a. Aqueous Biotransformation

The screening test for the biotransformation of DEGDN was conducted using a local water from Searsville Pond. DEGDN was added to the water alone, water plus 100 ppm of glucose and 100 ppm of yeast extract, or water plus 18 ppm diethyleneglycol. All waters were incubated under aerobic and anaerobic conditions. Autoclaved water served as a sterile control. The preparation and incubation conditions were the same as described for the NG biotransformation study.

Most of the waters did not show a significant decrease in DEGDN concentration during the 40 days of incubation. One exception was the water with added glucose and yeast extract, under anaerobic conditions. Analysis of this water by HPLC showed a 50% loss of DEGDN after 19 days of incubation, 94% loss after 40 days. This result suggests that DEGDN may be biotransformed with extra organic nutrients. To confirm that the transformation was biological, the water above was inoculated into BSM or pond water with added glucose and yeast extract. In both cases, 60% of the DEGDN was lost after 2 weeks of incubation and more than 80% was lost after 4 weeks, indicating that the degradation was caused by the transferred microorganisms. Three potential metabolites were observed in HPLC analysis (Figure 8).



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FIGURE 8 HPLC PROFILE OF DEGDN METABOLITES GENERATED AT DAYS 0, 5, AND 40

The biotransformation of DEGDN was investigated with waters obtained from the Radford Army Ammunition Plant (RAAP), including the biotreatment plant's aeration lagoon water, rotating biological contactor (RBC) effluent, anaerobic sludge, and New River water. Lagoon water, RBC water, and New River water were used for anaerobic study. In the aerobic study, ethanol was also used as supplemental organic nutrient because ethanol is the solvent used in the RAAP operation and is the major organic compound in the waste water.

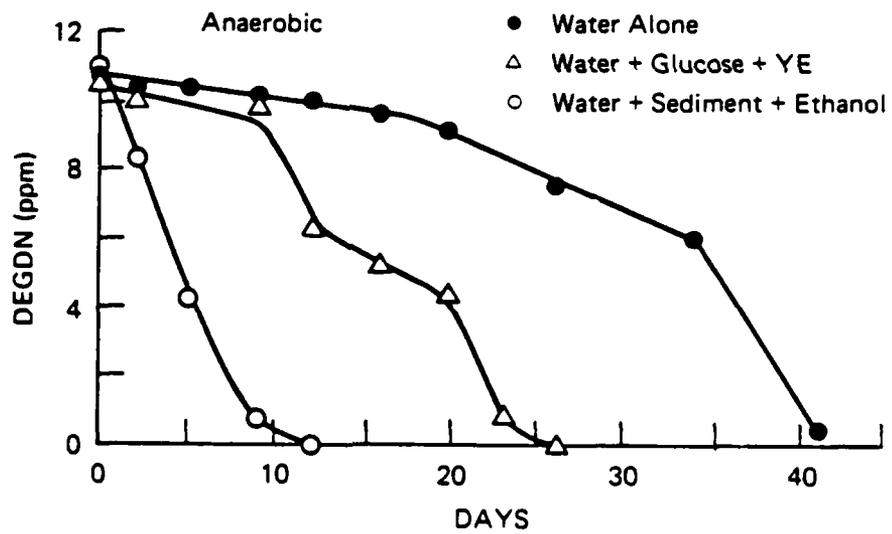
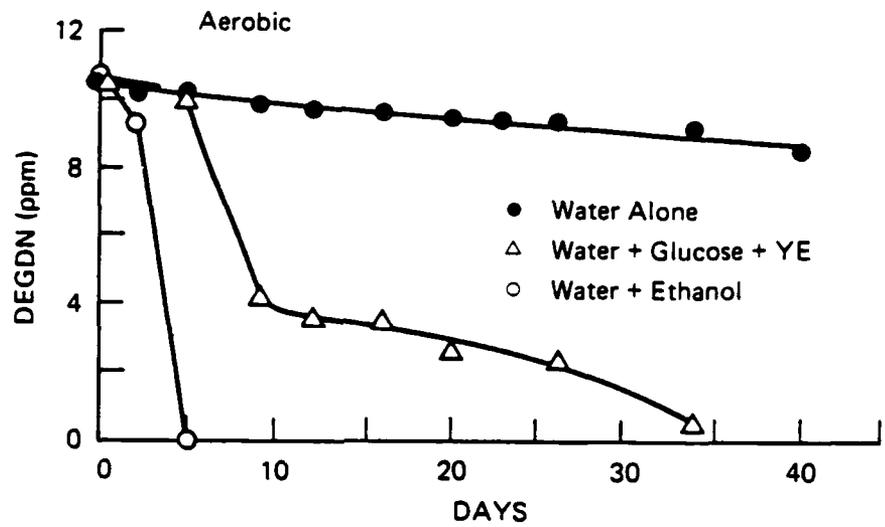
Under aerobic conditions, 10 ppm of DEGDN was reduced to <0.45 ppm in 5 days in aerated lagoon water and RBC effluent with or without added organic nutrient. In the New River water alone, loss was only 14% after 50 days; however, in the presence of 180 ppm ethanol (solvent used at RAAP), transformation was complete in 5 days. With added glucose and yeast extract (100 ppm each), there was 70% loss in 20 days and 90% loss after 34 days. These results indicate the presence of well-developed biotransformation organisms in the RAAP biological treatment plant. Aerobic biotransformation organisms are also present in the New River; however, they are organic nutrient-dependent, and ethanol serves as a better metabolic substrate than glucose plus yeast extract. The lagoon water and RBC effluent may have enough organic chemicals to be used by microorganisms as supplemental nutrients.

When the bioactive organisms from the biotreatment plant water were transferred into BSM with 10 ppm of DEGDN, no significant transformation was observed in 32 days. With the addition of glucose and yeast extract, only a 20% transformation was observed in 32 days, which suggests that other organic substrates, such as ethanol, are important for the biotransformation. When the organisms were inoculated into BSM containing 200 ppm of ethanol and 10 ppm of DEGDN, the DEGDN was completely metabolized in 6 days, indicating that ethanol is a preferred cometabolic substrate.

Under anaerobic conditions, DEGDN was reduced to an undetectable level (<0.45ppm) in 2% anaerobic sludge within 9 days in the presence of 1 g/liter Nutrient Broth, within 16 days in the presence of added glucose and yeast extract (100 ppm each), and within 20 days in the water alone. In New River water plus 1% bottom sediment and 180 ppm ethanol, DEGDN was completely transformed in 12 days. It took 16 days to transform 50% of the DEGDN in the presence of glucose and yeast extract, and DEGDN was non-detectable after 26 days. DEGDN loss in the New River water alone was 15% after 16 days, 30% after 26 days, 44% after 34 days, and 95% after 41 days. The loss of DEGDN in New River water under both aerobic and anaerobic conditions is shown in Figure 9.

These results show that even though DEGDN had not been manufactured recently, the RAAP waste water treatment plant still had well-developed aerobic and anaerobic biotransformation microbes, and that local waters contain anaerobic biodegraders. The biotransformation appears to occur by a cometabolic process, requiring extra organic nutrients. Ethanol seems to be a very efficient cometabolic substrate for the RAAP organisms, and is the major organic chemical in the RAAP wastewater.

Thus, biotransformation will be a major fate process of DEGDN when it is introduced into the waters with other waste substrates.



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FIGURE 9 AEROBIC AND ANAEROBIC DEGDN BIOTRANSFORMATION IN NEW RIVER WATER

### b. Soil Biotransformation

A study of DEGDN biotransformation in soil was conducted using soils and sediments from RAAP. Soil was collected from the land near the bio-reactor plant and sediment was collected from the New River approximately 500 meters below the treatment plant outflow. The procedures were similar to the methods described for NG soil biotransformation.

For the aerobic studies, 20 g of dry soil in each of a series of flasks was mixed with 3 ml of water, and DEGDN was added to give 20 ppm DEGDN per gram of soil. Soil autoclaved for one hour on each of three successive days was used as a sterile control. All soils were incubated at 20-25°C in the dark. Duplicate samples were periodically extracted with 2 x 40 ml of acetonitrile: methanol (1:1) containing an internal standard. After 5 weeks, the loss of DEGDN in the nonsterile soil was only 24%, whereas a 16% loss was observed in the sterile soil, suggesting that this transformation is very slow.

In anaerobic sediment taken from the New River incubated in test tubes and flooded with water, the loss of DEGDN in one day was 80% in sterile soil, >90% in the nonsterile sediment. These losses are puzzling because of DEGDN's high water-solubility, low soil sorption coefficient, and the fact that no metabolites were observed (by UV detection) in the HPLC profile. Repetition of this experiment gave similar results. To study this phenomenon further, 50 g of sediment was added to 250 ml of water and DEGDN was added to yield 10 ppm (based on sediment loading). The mixture was incubated anaerobically and the DEGDN concentration was monitored in the aqueous phase over an 8-day period. The results of this experiment are shown in Table 15.

Table 15

DEGDN CONCENTRATION (ppm) IN ANAEROBIC NEW RIVER  
SEDIMENT-WATER MIXTURE AS A FUNCTION OF TIME

Sample	DAY			
	0	2	5	8
Sterile	10.9	3.4	0.5	<0.4
Nonsterile	8.8	2.2	<0.4	---

After eight days, the sediment was filtered and extracted with acetonitrile. No DEGDN could be detected in the sediment extract by UV detection.

The above experiment was repeated under aerobic conditions; the results are shown in Table 16.

Table 16

DEGDN CONCENTRATION (ppm) IN AEROBIC NEW RIVER  
SEDIMENT-WATER MIXTURE AS A FUNCTION OF TIME

Sample	DAY						
	0	2	5	8	12	16	21
Sterile	10.7	9.7	4.1	3.0	3.6	2.4	1.9
Nonsterile	10.6	6.6	1.3	0.5	<0.4	---	---

Again, losses were observed in both sterile sediment and nonsterile samples. The loss was slightly faster in nonsterile than in sterile soil, indicating that biotransformation may also be occurring. The same experiment was conducted again, but with the addition of soil collected near the bioreactor of the Radford Army Ammunition Plant (RAAP). The study on the soil-water mixture was performed under aerobic and anaerobic conditions; the results are shown in Table 17.

Table 17

DEGDN CONCENTRATION (ppm) IN AEROBIC AND ANAEROBIC RAAP  
SOIL-WATER MIXTURES AS A FUNCTION OF TIME

Sample	DAY					
	0	5	9	12	16	21
Sterile-Aerobic	11.1	10.4	9.5	8.6	8.6	9.0
Nonsterile-Aerobic	10.5	10.1	9.3	8.3	7.8	7.3
Sterile-Anaerobic	10.5	10.0	9.9	8.8	8.6	8.3
Nonsterile-Anaerobic	10.7	8.1	6.3	4.3	1.9	<0.4

No transformation was observed in the sterile samples or in the aerobic-nonsterile sample, which is consistent with the results of our previous aerobic soil biotransformation study. DEGDN was totally transformed after 21 days under nonsterile anaerobic conditions. The results of these tests indicate that (1) DEGDN is not as readily transformed in soil-water mixtures as in sediment-water mixtures, (2) if aerobic biotransformation does occur, it is very slow, and (3) anaerobic biotransformation is occurring under the test condition.

The results of the above studies suggest that DEGDN is nonbiologically lost in New River sediment. No transformation products were observed in the HPLC profiles of sediment extracts using UV detection. Thus, if chemical transformation is occurring, the products are not UV-absorbing. Physical processes in which the DEGDN is tightly bound could also be occurring. The low  $K_{oc}$  and  $K_{ow}$  values for DEGDN suggest that a chemisorption process may be occurring rather than a lipophilic partitioning

process. We hope to obtain a radiolabeled tracer to evaluate this phenomenon further in Phase II studies.

To determine whether the loss of DEGDN in New River sediment-water mixtures was unique to New River sediment, we collected water and sediment from our local Searsville Pond and added DEGDN. The mixture was incubated under aerobic and anaerobic conditions. The results of this study (Table 18) indicate that the loss of DEGDN in sediment-water mixtures is not unique to the New River and that the losses occur more readily under anaerobic than under aerobic conditions. It is interesting that the non-sterile-aerobic study follows a first-order kinetic loss pattern whereas the sterile-aerobic loss does not. Thus, multiple loss processes could be occurring or sterilization of the sediment might be inducing changes in the sediment that affect the DEGDN loss rate under aerobic conditions. Under anaerobic conditions, the loss rates are nearly identical for sterile and nonsterile conditions. It appears from these studies that other nonbiological processes are competing with biotransformation in controlling the loss of DEGDN in sediment.

Table 18

DEGDN CONCENTRATION (ppm) IN AEROBIC AND ANAEROBIC SEARSVILLE POND SEDIMENT-WATER MIXTURE AS A FUNCTION OF TIME

Sample	DAY				
	0	3	5	7	10
Sterile-Aerobic	9.7	5.4	5.4	4.3	4.0
Nonsterile-Aerobic	9.3	4.8	3.1	2.0	1.1
Sterile-Anaerobic	9.8	2.9	1.0	<0.4	---
Nonsterile-Anaerobic	9.4	2.8	0.6	<0.4	---

C. Hexachloroethane Smoke

1. Hexachloroethane (HCE) Smoke Mixture

The HCE smoke mixture is reportedly composed of 46.7% hexachloroethane, 46.6% zinc oxide, and 6.7% aluminum metal (Katz et al., 1980).

The smoke mixture was evaluated by the percent of acetonitrile extractables, thermogravimetric analysis (TGA), metals determinations by emission spectral analysis and atomic adsorption, differential scanning calorimetry (DSC), and carbon and chlorine elemental analysis.

The percent of acetonitrile extractables was determined to be  $46.50 \pm 2.27$  (n = 4), which is consistent with the selective extraction of HCE from the smoke mixture. TGA showed a 49% weight loss on heating up to 250°C, with inflections at 100°C, 150°C, and 200°C, suggesting that impurities or thermally formed intermediates are being generated during the heating cycle.

Semiquantative emission spectral analysis showed the major metals present in HCE smoke mixtures to be zinc (46%), aluminum (14%), and trace amounts of silicon (0.25%), calcium (0.12%), magnesium (0.06%), and iron (0.04%). Atomic absorption spectroscopy showed 43.13% zinc, 11.30% aluminum. The high analysis for aluminum by emission and atomic absorption suggest that the samples are heterogeneous with respect to the metals.

DSC analysis showed four endotherms (Figure 10). We project that the observed transitions are involved with the hexachloroethane fraction (or organic impurities) in the HCE smoke mixture. No extensive exotherms were involved even when the mixture was heated to 650°C, which suggests that the initiation of combustion of the HCE smoke mixture is a high-temperature process.

Elemental analyses for carbon and chlorine in the HCE smoke mixture gave values that were far from the expected theoretical values (% C = 2.60 observed, 4.73 theoretical; % Cl = 33.35 observed, 41.93 theoretical). Poor correlations were also observed for hexachloroethane (% C = 7.75 observed, 10.15 theoretical; % Cl = 86.04 observed, 89.85 theoretical), probably resulting from the ability of hexachloroethane to sublime prior to oxidative transformation. The percent of HCE determined by gas chromatography (GC) was  $49.81 \pm 2.7\%$ .

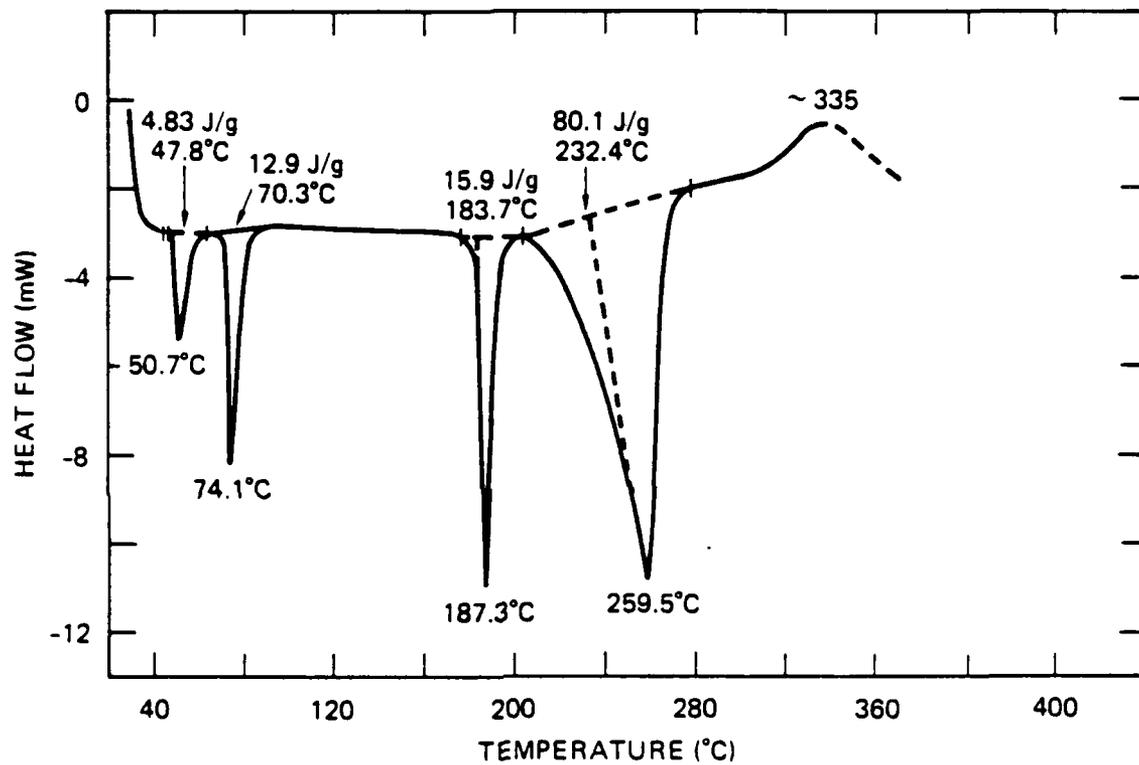
## 2. Products of HCE Smoke Mixture Combustion

We began preliminary investigations into the identification of HCE smoke combustion products. Samples of HCE smoke mixture were placed in a 492-cc Monel reactor and ignited with a Nichrom fuse. The gases were transferred to a 7.94-cm infrared cell and evaluated by infrared spectroscopy. Smoke components identified by this technique were HCl ( $2800\text{ cm}^{-1}$ ),  $\text{CO}_2$  ( $2330\text{ cm}^{-1}$ ), CO ( $2080\text{ cm}^{-1}$ ), tetrachloroethylene ( $914\text{ cm}^{-1}$ ), and phosgene ( $1825$  and  $850\text{ cm}^{-1}$ ). Gas chromatographic analysis of the gases on Porapak P (1/8" x 6') using a thermal conductivity detector confirmed the presence of the above gases (except for phosgene, due to the lack of a standard) and indicated the presence of chlorine in relatively high amounts.

We performed open-air burns of the HCE smoke mixture because of our observation of high amounts of carbon soot being generated in our Monel reactor, perhaps due to limited oxygen during the burn. In the open-air in a crucible, high amounts of carbon soot were also generated, yielding a semi-grey smoke. Product analyses were similar to those reported above except that the levels of phosgene, tetrachloroethylene, and hexachloroethane were apparently higher than previous values.

A 20-g sample of HCE was ignited with a Nichrom wire and the smoke effluent was bubbled through a hexane trap. A capillary GC profile of the hexane mixture using an ECD detector is shown in Figure 11.

Major components of the profile are hexachlorobenzene (55.38 min), hexachlorobutadiene (46.48 min), hexachloroethane (24.11 min), tetrachloro-



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FIGURE 10 DSC ENDOTHERM FOR HCE SMOKE MIXTURE

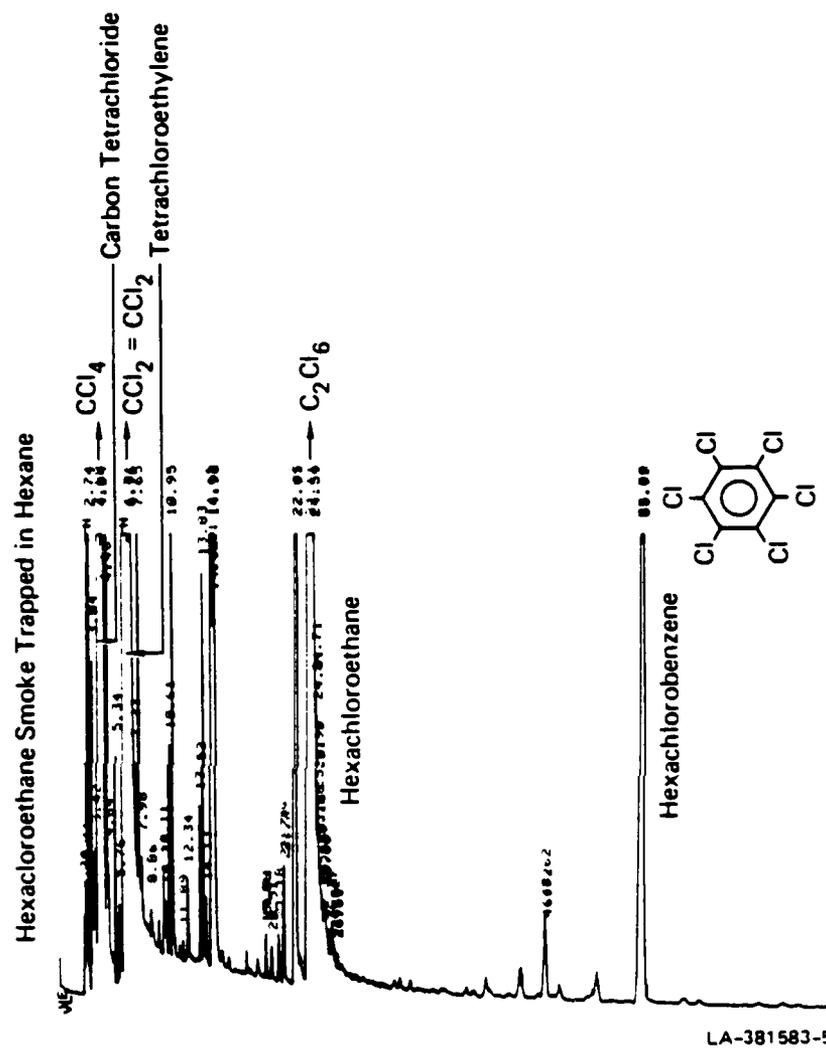


FIGURE 11 CAPILLARY GAS CHROMATOGRAM OF HC SMOKE AEROSOL TRAPPED IN HEXANE

ethylene (6.10 min), and carbon tetrachloride (3.68 min), which were identified by chromatographic comparison with authentic standards. Confirmation of these components was achieved by mass spectrometry.

The burn residue was extracted with acetone and chromatographed as above. Major components again were identified as hexachloroethane and tetrachloroethylene, with two new components appearing at 11.28 and 11.92 min (Figure 12) that could not be identified.

These results are consistent with those reported by Katz et al. (1980) who also report the metal fraction of the smoke to consist of aluminum and zinc chlorides.

### 3. Fate of the Combustion Products of HCE Smoke

HCE smoke results from the combustion of a mixture of HCE, grained aluminum, and zinc oxide. We have identified products of the combustion to be carbon tetrachloride, tetrachloroethylene, hexachlorobutadiene, hexachlorobenzene, hexachloroethane, hydrochloric acid, phosgene, carbon, carbon monoxide, chlorine, and carbon dioxide. These chemicals were identified from laboratory burns of 1- to 5-g samples of smoke materials. The identifications need to be confirmed with actual smoke devices.

Of the organic chemicals formed in HCE smoke, hexachloroethane was a major product. Because hexachloroethane is also a major starting material, the fate of this chemical was studied in detail.

#### a. Hexachloroethane

##### (1) Solubility in Water

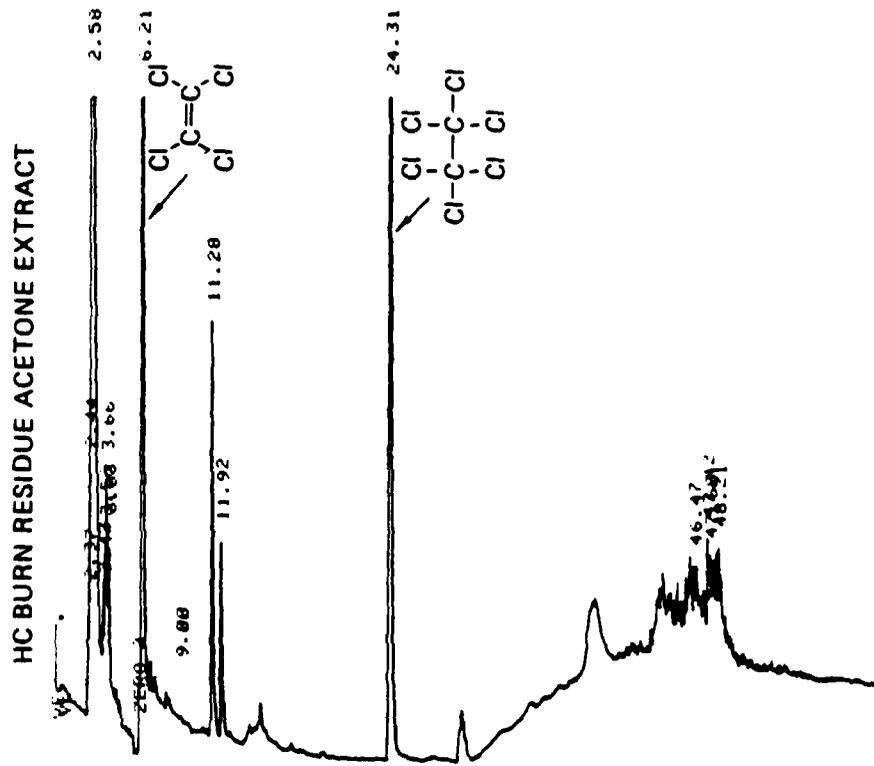
The aqueous solubility of HCE was determined by suspending a 1.0-g sample of HCE in each of three centrifuge tubes containing 25 ml of water. The tubes were rotated for four days in a closed chamber at 25°C and then centrifuged at 10,000 rpm for 60 min. Aliquots of the water were then removed, extracted with hexane, and analyzed by GC-ECD.

The measured solubility of HCE in water at 25°C was 14 ppm. This value compares to 8 ppm at 25°C reported by Dilling et al. (1975) and 50 ppm at 11°C reported by Verschueren (1977). Using the correlation equation developed by Yalkowsky and Valvani (1980), which relates  $K_{ow}$  to water solubility we calculated, a solubility value of 16 ppm, which is in good agreement with the measured value.

##### (2) Vapor Pressure of Hexachloroethane

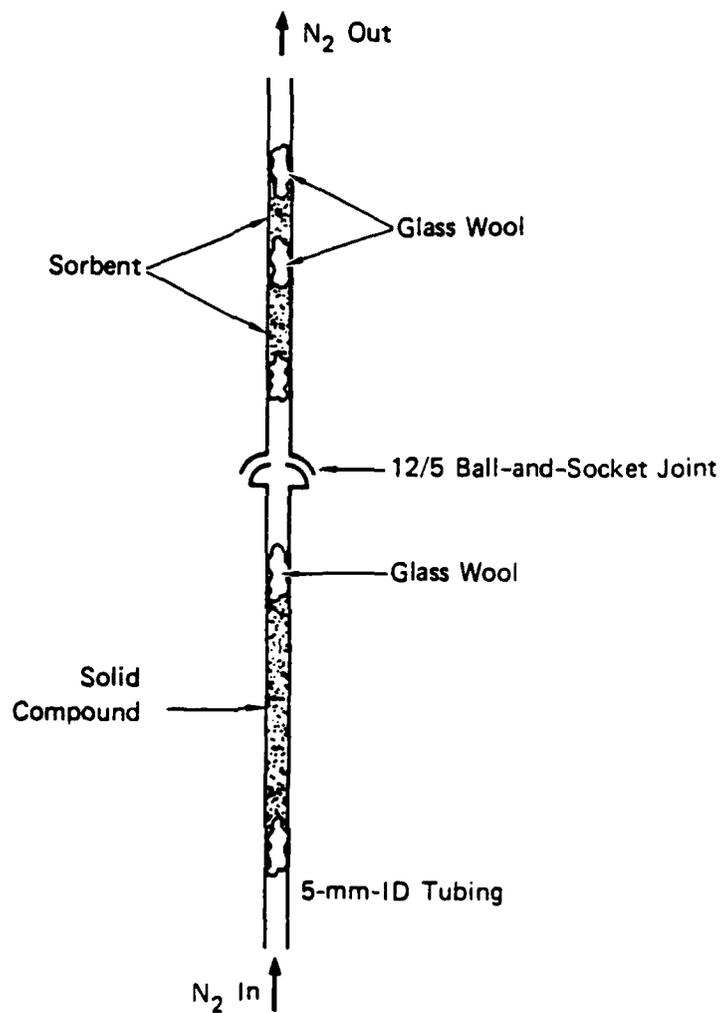
The vapor pressure of HCE was determined by the vapor saturation method developed by SRI for the EPA and described in the Federal Register [45FR227] (77345-77350).

Pure HCE was loaded into glass tubing (5 mm ID) between glass wool plugs and connected to a similar tube packed with Tenax-GC sorbent (Figure 13). Nitrogen gas was passed through the tubes and the HCE vapors were collected on the Tenax sorbent. After vapor collection, the front and back sorbent



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FIGURE 12 CAPILLARY GAS CHROMATOGRAM OF THE HEXANE EXTRACT OF HC SMOKE BURN RESIDUE



SA-8366-1

FIGURE 13 VAPOR SATURATION APPARATUS

sections were extracted separately with hexane and analyzed by GC-ECD.

Desorption efficiency was measured by injecting known quantities of HCE onto the Tenax sorbent followed by hexane extraction and GC-ECD analysis.

Four experiments were performed at 25°C for 30 min-periods using nitrogen flow rates ranging from 1.02 to 2.98 ml min<sup>-1</sup>. Vapor pressure was calculated from Equation 6.

$$P = \frac{nRT}{v} \quad (6)$$

where P = vapor pressure

n = moles HCE

R = gas constant (0.082 1-atm mole<sup>-1</sup> deg<sup>-1</sup>)

v = volume of purge gas

T = temperature (°K)

The results are shown in Table 19. The breakthrough of HCE was determined to be 9.6% in sample 1, 5.3% in sample 2, 0% in sample 3, and 12.2% in sample 4. We conclude, therefore, that the vapor pressure of HCE is 0.34 torr at 25°C.

Table 19

VAPOR PRESSURE OF HCE

<u>Sample No.</u>	<u>HCE Collected (moles x 10<sup>6</sup>)</u>	<u>Purge Volume (liter)</u>	<u>Vapor Pressure (torr)</u>
1	1.05	0.0660	0.29
2	0.60	0.0368	0.30
3	0.57	0.0308	0.34
4	1.34	0.0892	0.28

(3)  $K_{ow}$  for HCE

The octanol/water partition coefficient ( $K_{ow}$ ) for HCE was determined by mixing four different volumes of octanol-saturated water (5-20 ml) with four different volumes of water-saturated octanol (20-5 ml) containing HCE. The mixtures were rotated in sealed centrifuge tubes for 24 hr at 25°C. After equilibration, the octanol layer was removed. Aliquots of the aqueous fraction were extracted with hexane and analyzed for HCE by GC-ECD. The results, shown in Table 20, indicate a  $K_{ow}$  of  $8.9 \pm 0.1 \times 10^3$  for HCE at 25°C

Table 20

EXPERIMENTAL DATA USED TO CALCULATE  $K_{ow}$

<u>Sample Mixture (ml)</u>		<u>HCE in Octanol (ug/ml)</u>	<u>HCE in Water: (ug/ml)</u>	<u><math>K_{ow}</math></u>
<u>Octanol</u>	<u>Water</u>			
5	20	$1.02 \times 10^3$	0.116	$8.8 \times 10^3$
5	20	"	0.118	$8.6 \times 10^3$
10	15	"	0.113	$9.0 \times 10^3$
10	15	"	0.115	$8.9 \times 10^3$
15	10	"	0.114	$8.9 \times 10^3$
15	10	"	0.115	$8.9 \times 10^3$
20	5	"	0.113	$9.0 \times 10^3$
20	5	"	0.113	$9.0 \times 10^3$

(4) Henry's Constant for HCE

The Henry's Constant for HCE was measured by purging an aqueous solution of HCE with  $N_2$  gas and monitoring the HCE concentration as a function of purge volume in a Henry's apparatus controlled at 25°C. The measured Henry's Constant was  $6100 \text{ L-torr mole}^{-1}$ . This value compares to an estimated value of  $5800 \text{ L-torr mole}^{-1}$  calculated from the ratio of vapor pressure to water solubility ( $H_c = P/S_w$ ). According to these values, the volatilization of HCE will depend on mass transport resistance in the liquid phase, and volatilization from shallow turbulent water bodies will be rapid.

(5) Volatilization Rate for HCE

The volatilization of a chemical from a liquid to a gas phase can be controlled by transfer across a liquid-phase boundary layer, a gas-phase boundary layer, or both. The volatilization rate constant,  $k_v$ , is expressed

in terms of the mass transfer rates of the substance across liquid- and gas-phase boundary layers. The general expression for  $k_v$  is:

$$k_v = \left[ \frac{1}{L} \frac{1}{k_l} + \frac{RT}{H_c k_g} \right]^{-1} \quad (7)$$

where:

$k_v$  = Volatilization rate constant ( $h^{-1}$ )

$L$  = Depth (cm), which equals the liquid volume,  $F$ , divided by the interfacial area,  $A$

$k_l$  = Liquid film mass-transfer coefficient ( $cm\ h^{-1}$ )

$R$  = Gas constant (liter torr  $K^{-1}$  mole $^{-1}$ )

$T$  = Temperature (K)

$H_c$  = Henry's law constant (torr liter mole $^{-1}$ )

$k_g$  = Gas film mass-transfer coefficient ( $cm\ h^{-1}$ )

The relative magnitude of the two terms in Equation 7 determined whether liquid- or gas-phase mass transfer controls the volatilization.

If  $H_c > 3500$  torr  $M^{-1}$ , then mass transfer in the liquid phase controls about 95% of the volatilization rate constant. Since the Henry's constant for HCE is 6100 L-torr mole $^{-1}$ , Equation 7 reduces to Equation 8

$$k_v = \frac{1}{L} \left( \frac{1}{k_l} \right)^{-1} = \frac{k_l}{L} \quad (8)$$

For lakes and ponds,  $k_d$  is of the order of  $2 \text{ cm hr}^{-1}$ . Thus, for a water body 180 cm deep, the volatilization rate constant for HCE is  $0.01 \text{ hr}^{-1}$  and the estimated half-life is 70 hr.

(6) Sorption Partition Coefficient for HCE

Batch sorption isotherms for HCE were constructed using EPA sediments #5 and #18 according to the methods reported for NG except that the HCE concentrations were varied while the sediment concentration was held constant. The results are shown in Table 21 and plotted in Figure 14. Both sediments show a high degree of linearity over the concentration range tested. From the slope of the curves for EPA sediments #5 and #18,  $K_p$  values of  $12.9 \pm 0.4$  and  $4.4 \pm 0.2$ , respectively, were calculated. The isotherm for sediment #18 (lowest organic content) appears to have a declining slope with increasing HCE concentration. This may be due to saturation of the organic fraction active sites.

The  $K_{oc}$  values of 560 for #5 and 670 for #18 were calculated. It is surprising that the  $K_{oc}$  values do not fit the correlation equation of Karickhoff et al. (1979) with respect to  $K_{oc}$ , as shown below.

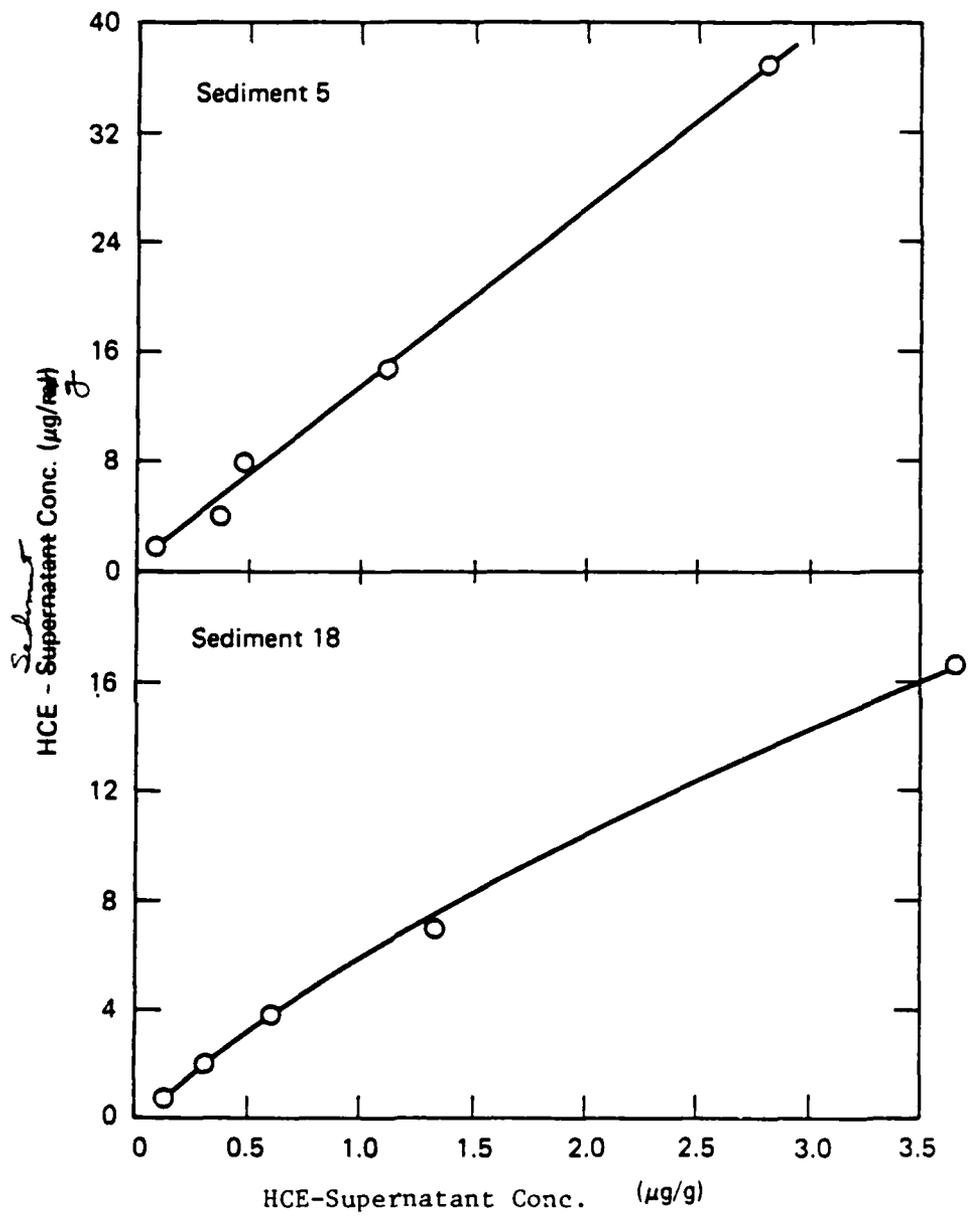
$$\log K_{oc} = 1.00 \log K_{ow} - 0.21 \quad (9)$$

The above equation predicts a  $K_{ow}$  of 970, but we found the measured  $K_{ow}$  to be 8,900 or one order of magnitude larger. Because the  $K_{ow}$  correlated well with the measured solubility of HCE using the correlation equation of Yalkowsky and Valvani (1980), we believe that the  $K_{ow}$  value measured in our laboratory is correct and that the Karickhoff correlation equation is not a good approximation in this case.

Table 21

BATCH SORPTION OF HCE ON EPA SEDIMENTS #5 AND #18

	Sample Number	Weight of Sediment (mg)	HCE in Water ( $\mu\text{g/g}$ )	HCE in Sediment ( $\mu\text{g/g}$ )	$K_p$
Sed. #5	1	1.0074	2.81	36.73	13.0
	2	1.0103	1.11	14.55	13.1
	3	1.0058	0.49	7.88	16.2
	4	1.0010	0.37	4.04	10.9
	5	1.0092	0.083	1.84	22.1
Sed. #18	6	1.0148	3.66	16.68	4.6
	7	1.0001	1.32	6.97	5.3
	8	1.0014	0.61	3.79	6.2
	9	1.0067	0.32	1.98	6.1
	10	1.0003	0.13	0.81	6.4

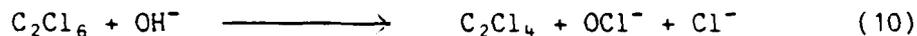


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FIGURE 14 SORPTION ISOTHERMS OF HCE ON EPA SEDIMENT 5 AND 18

### (7) Chemical Transformation

A Review of the literature on HCE revealed only a few papers containing useful data on chemical stability. Hydrolysis by  $\text{OH}^-$  was investigated by Taylor and Ward (1934), who reported that at  $25^\circ\text{C}$ , HCE hydrolyzes very slowly, even with 0.02 M NaOH in ethanol:water (90:10) to give a complex resinous mixture. They interpreted this finding as resulting from initial formation of tetrachloroethylene and NaOCl followed by oxidation and condensation of the solvent (Equation 10).



The rate constant for this reaction is reported to be  $6.3 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$  at pH 7, equivalent to a half-life of 350 centuries.

Photolysis of HCE in sunlight appears to be unimportant, judging from the UV spectrum of a 0.001 M solution in acetonitrile, where no absorption was detected above 260 nm. There is only a slight possibility that indirect photolysis will occur; if it does, it could not compete with volatilization. Apparently, volatilization of HCE will be the dominant loss process from water.

In the atmosphere, neither reaction with OH radical (highly chlorinated organics are generally refractory to OH radical) nor direct photolysis appears too important and we conclude that HCE is a conservative chemical in the environment in terms of chemical transformation.

### (8) Aqueous Biotransformation of HCE

Aqueous biotransformation of HCE was studied with waters collected from Searsville Pond (Woodside, CA) and Coyote Creek (San Jose, CA). For the aerobic study, HCE (in DMSO stock solution, 50 mg/ml) was added to 2 liters of Searsville pond water and 1 g/liter phosphate buffer at 5 ppm with or without bottom sediment (1% dry weight) or yeast extract (10 ppm) plus glucose (50 ppm). The 4-liter bottles containing the solutions were closed with a cotton plug and covered with aluminum foil in this initial experiment. Autoclaved pond water was used as a sterile control. The solutions were incubated in dark at  $25^\circ\text{C}$  without shaking. Periodically, a water sample was withdrawn, extracted twice with an equal volume of ether containing an internal standard (2-nitrotoluene), and analyzed by GC.

For the anaerobic study, 1 liter of water was prepared in Teflon-lined, screw-capped 1-liter bottles and incubated in an anaerobic chamber.

The HCE added to the nonsealed bottles of aerated water was lost rapidly in both sterile and nonsterile pond water (80-95% lost in 6 days), suggesting that volatilization may be a competitive process. However, in pond water with sediment, the GC profile showed the presence of an additional peak at 6 days, indicating that biotransformation may be occurring.

Under anaerobic conditions, the HCE concentration in autoclaved pond water remained at 4 ppm, but in nonsterile water with sediment or glucose plus yeast

extract added, 90% of the HCE was lost in 18 days. Figure 15 shows the profile of metabolites, which were tentatively identified as pentachloroethane, tetrachloroethylene, and trichloroethylene. These components began to increase after 3 days of incubation and were not observed in the sterile control.

The organisms (from Searsville Pond water that suggested microbial transformation of HCE) were transferred to BSM containing glucose, yeast extract, and 10 ppm of HCE. The media were inoculated in screw-capped flasks under aerobic and anaerobic conditions. Under both conditions, metabolites were observed in the GC profiles, with a concurrent decrease in HCE concentration. These results confirm that biotransformation of HCE is taking place in media containing pond water microbes.

The aqueous biotransformation of HCE was tested again in Coyote Creek water obtained in San Jose, CA. Anaerobic waters were incubated in 500-ml screw-capped bottles with no headspace. Aerobic waters (500 ml) were incubated in glass-stoppered, 1000-ml bottles with periodic shaking.

The results of the anaerobic and aerobic studies are shown in Figure 16. In the anaerobic waters with 100 ppm of glucose and yeast extract, the amount of HCE lost was 43%, 62%, 84%, and 92% after 4, 7, 12, and 14 days of incubation. No loss of HCE was observed in the sterile control. In the aerobic waters, the loss of HCE with glucose plus yeast extract was 60% after 14 days. A loss of 38% in the sterile control indicates that volatilization losses were still significant in this glass-stoppered bottle.

From these HCE aquatic biotransformation studies it was concluded that:

- 1) HCE biotransformation is nutrient-dependent.
- 2) HCE biotransformation proceeds at a faster rate under anaerobic than under aerobic conditions.
- 3) Under aerobic conditions, volatilization occurs faster than biotransformation .

Further evidence for the ability of microorganisms to transform HCE was obtained in our preliminary HCE biosorption study, in which a mixture of the four test organisms (Azotobacter, Bacillus, E. Coli, and Serratia) showed the presence of HCE metabolites in the GC profile of the cell suspension extract. No metabolites were observed in a heat-killed cell suspension extract. This result indicates that one or more of these test organisms can biotransform HCE and that the enzyme is constitutive because no acclimation to HCE was performed. This suggests that organisms capable of HCE biotransformation may be widespread in fresh water.

#### (9) Soil Biotransformation of HCE

The biotransformation of HCE in soil was studied with SRI soil. For the aerobic study, HCE in an ethanol stock solution (1 mg/ml) was added to a series of 125-ml Erlenmeyer flasks containing 20 g of autoclaved and nonsterile soils to yield a final HCE concentration of 5 ppm (per dry weight soil). Both soils were pretreated with 4 ml of water to ~70% moisture saturation. In the initial study, flasks were closed with aluminum foil-wrapped rubber stoppers.

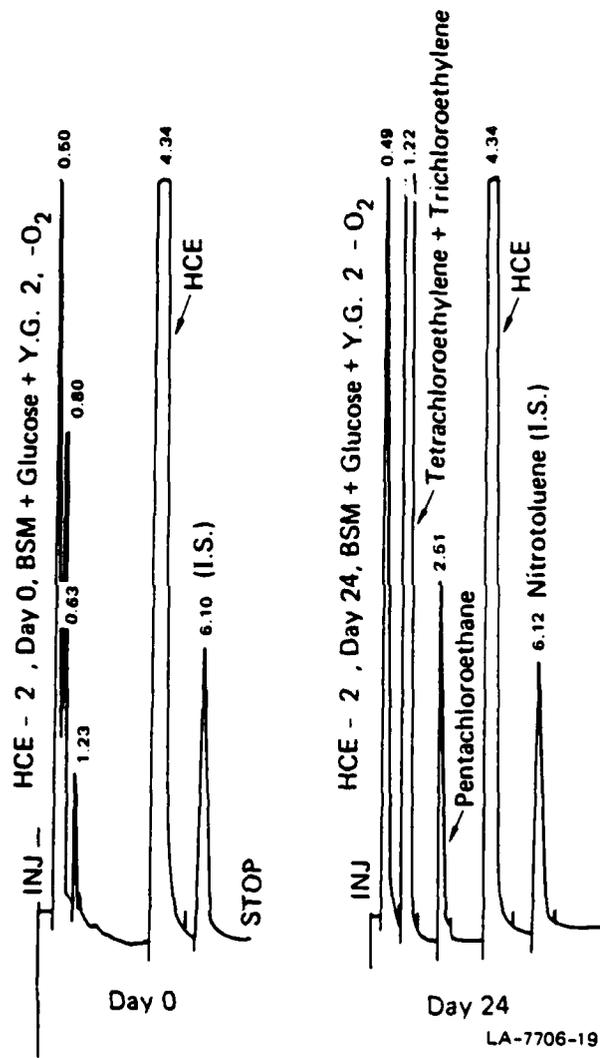
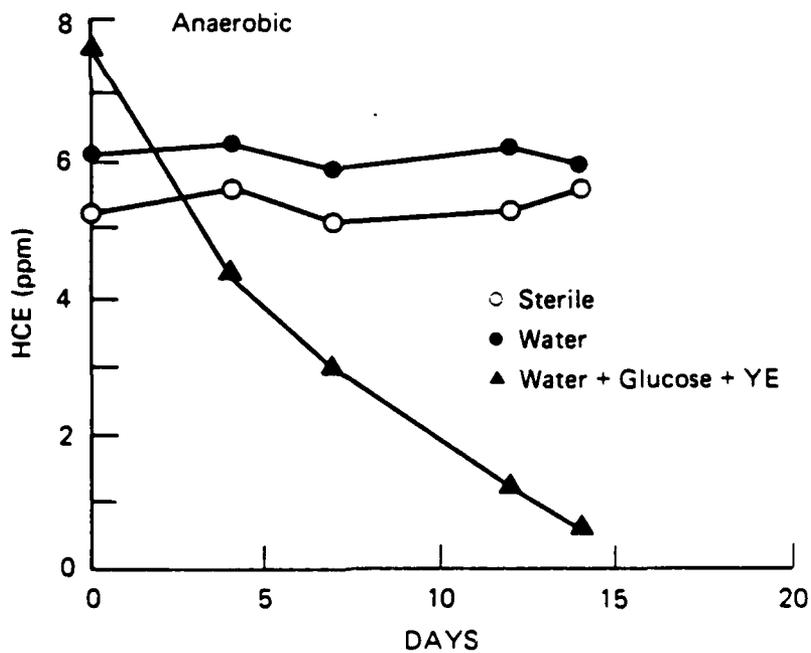
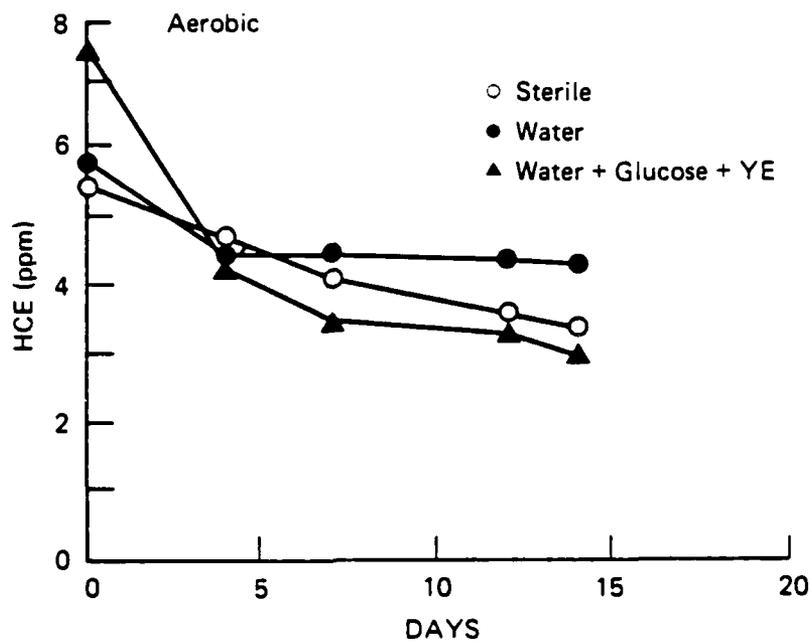


FIGURE 15 GAS CHROMATOGRAPHIC PROFILES OF HCE METABOLITES



LA-7706-20

FIGURE 16 HCE BIOTRANSFORMATION IN COYOTE CREEK WATER

For the anaerobic study, HCE was added to 25 mm x 20 cm Teflon-lined, screw-capped test tubes, each containing 20 g of soil and 14 ml of water to flood the soil. They were incubated in an anaerobic chamber.

The HCE was extracted from the soil with 40 ml of ether/acetone (1:1) followed by a second extraction with 40 ml of ether. The extracts were combined, carefully concentrated by  $N_2$  evaporation, and analyzed by GC.

After 7 days, 64% of the HCE was lost from the sterile soil and 80% was lost from the nonsterile soil under aerobic conditions. Under anaerobic conditions, 34% was lost from the sterile control and 99% was lost from the nonsterile soil. The GC profiles for Day 0 and Day 7 (Figure 17) show one major metabolite. These data suggest that HCE will be biotransformed in soil.

The soil biotransformation of HCE under aerobic and anaerobic conditions was repeated using shorter sampling periods to monitor HCE loss. The aerobic and anaerobic flasks and tubes were set up as described before. HCE was added to each flask to yield 5 ppm of the soil (dry weight). This time the aerobic soils were incubated in glass-stoppered Erlenmeyer flasks to reduce the evaporation loss. These results are plotted in Figure 18. In the anaerobic tubes, HCE concentration decreased to 2.6 (48% loss), 0.80 (84% loss), and 0.05 (99% loss) ppm after 1, 2, and 4 days of incubation. The sterile control showed minimal loss of HCE under the test conditions. The loss of HCE in aerobic soil that was incubated in glass-stoppered Erlenmeyer flasks was 66%, 94%, and 99% at the end of 1, 2, 3, and 4 weeks, respectively. The loss in sterile aerobic soil was 17% after 4 weeks of incubation.

The mineralization of HCE in soil was tested using  $^{14}C$ -HCE. To 50 g of soil was added 1.25  $\mu Ci$  of  $^{14}C$ -HCE and 5 ppm HCE in a glass-stoppered, 250-ml flask equipped with 5 ml of 0.5N KOH solution to trap evolved  $CO_2$ .  $^{14}C$ -HCE was obtained from Amersham International Ltd. with a specific activity of 18.2 mCi/mole.

Periodically (3 times a week), the KOH solution was withdrawn and replaced with fresh alkaline solution.  $^{14}CO_2$  in the KOH trap was precipitated with  $BaCl_2$ , centrifuged, retrapped into KOH solution after acidification in a sealed Warburg flask, and counted in a scintillation counter. The sterile soil with  $^{14}C$ -HCE was used as a blank.

After 27 days of incubation, less than 1% of the total activity appeared as  $^{14}CO_2$ . Because the radiolabeled starting material was 98% pure, the trapped  $^{14}CO_2$  could arise from HCE impurities. These initial investigations suggest that the total mineralization of HCE is slow or that HCE is cometabolically transformed to less chlorinated compounds, but not to  $CO_2$ .

The results showed that HCE can be rapidly biotransformed in soils under anaerobic conditions and moderately fast under aerobic conditions. The transformation does not require nutrients other than those present in the soil.

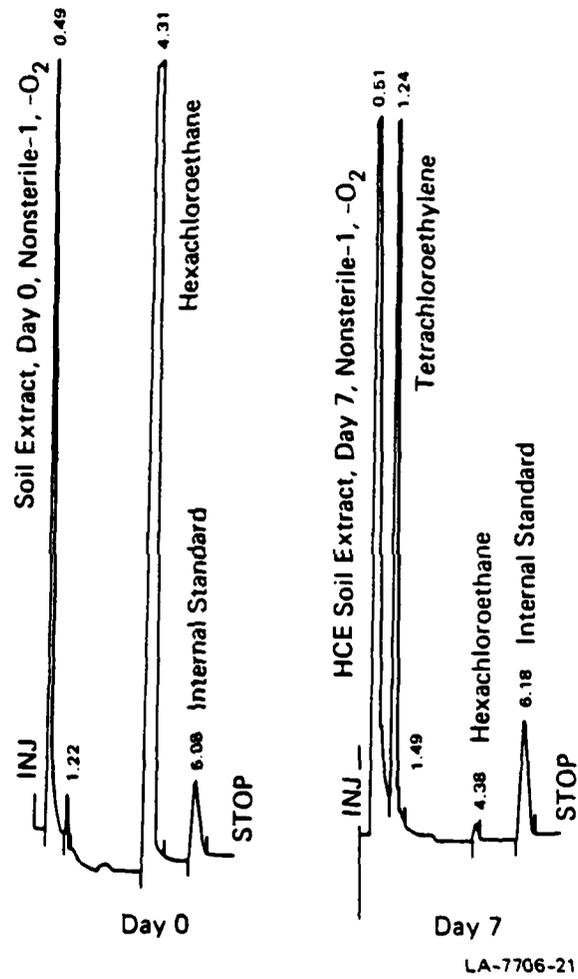
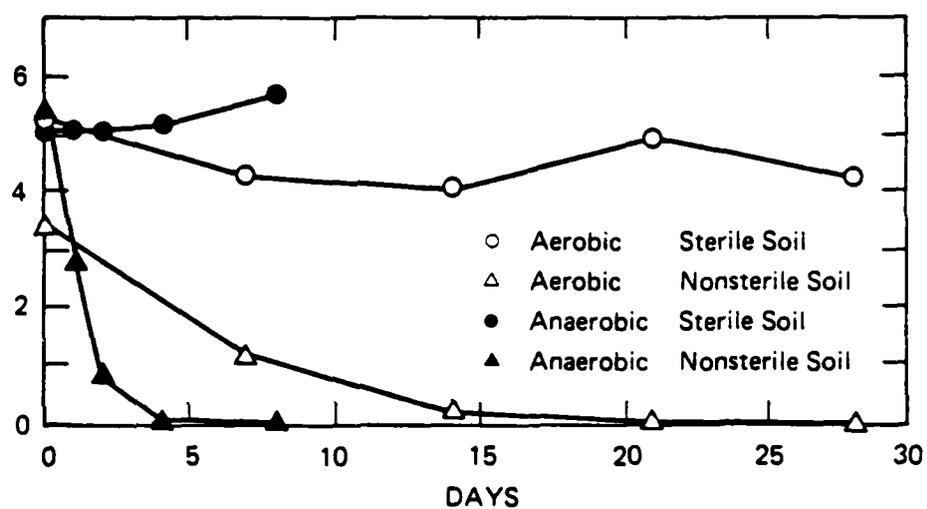


FIGURE 17 GAS CHROMATOGRAPHIC PROFILE OF HCE SOIL EXTRACT AT DAY 0 AND DAY 7



LA-7706-22

FIGURE 18 HCE BIOTRANSFORMATION IN SRI SOIL

Thus, it appears that biotransformation will be the major transformation process and volatilization will be the major transport process for HCE from soil and water. Once in the air environment, HCE appears to be stable until precipitative events recycle it back to soil and water.

HCE and its smoke-reaction products, tetrachloroethylene (TCE) and tetrachloromethane (TCM), have been shown to be biodegraded by settled domestic wastewater microorganisms in glass-stoppered reagent bottles under aerobic conditions (Tabak et al., 1981) and degraded in ground water (McCarty, 1985). TCE and TCM were also biodegraded by organisms under anaerobic conditions (Bouwer and McCarty, 1983a, 1983b; Vogel and McCarty, 1985).

Our results showed that HCE can be biotransformed by aquatic microorganisms and confirmed the results of Tabak et al. (1981), and McCarty (1985).

#### (10) Biosorption of HCE

The biosorption of HCE was studied using mixed-cell populations of Azotobacter beijerincki (ATCC 19360), Bacillus cereus (ATCC 1778), Escherichia coli (ATCC 9637), and Serratia marcescens (ATCC 13880).

The organisms were grown overnight in tryptic soy broth, centrifuged, washed with phosphate buffer (pH 7, 0.5 g/liter), and resuspended. Equal optical density suspensions of the four organisms were mixed and the cell suspension was heated in a boiling water bath for 10 min and centrifuged. The cells were heat-killed because preliminary tests showed that viable cells transform HCE. Aliquots of the mixed-cell suspension were placed into glass-stoppered centrifuge tubes, followed by phosphate buffer and HCE to give a total volume of 10 ml. The stoppered tubes were intermittently mixed over 1 hr and centrifuged at 4000 x g for 15 min. The supernatants and cell pellets were extracted with diethyl ether containing an internal standard (o-nitrotoluene) and the extract was analyzed by GC. The results (Table 22) showed an average  $K_B$  value of  $814 \pm 190$ . A relationship between  $K_B$  and  $K_{ow}$  has been described by Baughman and Paris (1981) as shown in Equation 11.

$$\log K_B = 0.907 \log K_{ow} - 0.361 \quad (11)$$

Using our measured  $K_{ow}$  value of 8900, we calculated  $K_B$  as 1700, which is twice the measured value. On the basis of the soil sorption partition coefficient ( $K_p$ ) of 4 to 13, we would have projected a smaller  $K_B$  value than that which was measured. We feel, however, that the measured value properly represents the partitioning behavior to cells and that the correlation equation of Baughman and Paris will give a closer value than the value expected from the soil sorption partition coefficient.

#### (11) Biouptake of HCE By Land Plants

Biouptake of HCE was studied using one monocot species and one dicot plant species. After several species of agronomic plants (corn, barley, bean, peas) were grown and their growth was observed, corn and pea plants were selected for the study.

Table 22

BIOSORPTION PARTITION COEFFICIENT ( $K_B$ ) FOR HCE

<u>Sample Number</u>	<u>Wt. of Cells (mg/ml)</u>	<u>Supernatant (<math>\mu\text{g/g}</math>)</u>	<u>Cell Pellet (<math>\mu\text{g/g}</math>)</u>	<u><math>K_B</math></u>
1	3.40	1.72	1047	609
2	3.40	1.98	1200	606
3	3.40	1.55	1081	706
4	1.89	2.31	1741	754
5	1.89	2.18	1857	852
6	1.89	2.23	1767	792
7	3.40	0.51	579	1135
8	3.40	0.64	497	777
9	3.40	0.35	359	1025
10	1.89	1.50	995	663
11	1.89	0.99	704	711
12	1.89	1.04	1196	1149

Average  $K_B = 814 \pm 190$

To successfully measure plant biouptake as distinct from soil partitioning or microbial accumulation, plant seedlings are germinated and grown without the presence of soil. After germination in aerated water, the plants were grown in pots containing Perlite for physical support. Mineral nutrients were supplied by regular watering with dilute Hoaglands solution. The plants were grown at ambient room temperature and given 12 hours of illumination each day with wide-spectrum fluorescent lamps at 200 foot-candle light.

There are distinct advantages to using young seedlings (5 to 10 days after germination), particularly for species with long primary roots such as corn, pea, and bean. Uptake is more vigorous in the apical portion of the root. Therefore, uptake on a per-gram-root basis should be greatest when young root tissue is used. Little uptake can be expected from older, lignified portions of long roots. In addition, one-week-old seedlings of corn, pea, and bean have minimum secondary root growth. As the seedling gets older, proliferation of secondary root growth makes it increasingly difficult to harvest the plant from the Perlite medium without root breakage.

Corn seeds (Zen mays var. Golden Bantam) were surface-sterilized for 5 min with a solution of 1% sodium hypochlorite (20% Chlorox) containing one drop of liquid detergent as a surfactant. The seeds were then given 20 rinses with deionized (D.I.) water to remove the oxidant and soap. The seeds were germinated in a 1-liter flask of deionized water in the dark at ambient room temperature. The water was changed twice during a 24-hr period.

After 24 hr, the germinating seeds were planted (4 seeds per pot) in pots containing Perlite. The pots were lined with 4-mil plastic bags to prevent the plant roots from growing out of the holes in the bottom of the pots.

Each pot was watered regularly with a complete nutrient solution. Excess solution was drained off by inverting the pot carefully. For the first 48 hr after planting, the pots were placed in a flat fitted with a clear plastic cover to reduce moisture loss. Droplets of "sweat" on the inside of the lid soon after it was put in place indicated that the relative humidity inside was high.

Eight days after germination was initiated, the plants were harvested for the uptake experiment. Each plant was removed carefully from the pot to avoid root breakage. Perlite granules were removed from the roots with a stream of deionized water. Each plant was then transferred to the uptake solution, which was contained in 1000-ml beakers filled to just under the brim. The top of each beaker was covered with aluminum foil with holes punched in it for the seedling roots. Each plant was lowered so that the root was submerged in the uptake solution. The seedling was held in place by the seed resting on the foil. Each uptake container held three plants to be tested after a specific exposure duration (3, 6, and 24 hr). Hence, there was a total of nine plants--three samples consisting of three plants each.

As soon as all nine plants were positioned in the uptake solution, solution samples were removed for analysis of HCE concentration by GC and  $^{14}\text{C}$  label-counting by liquid scintillation; these were the 0-hr samples. Solution samples were also taken to check HCE and  $^{14}\text{C}$  concentrations at the time-point intervals. Stirring bars provided constant circulation of the uptake solutions during exposure. All solutions used for the uptake experiment contained the same mineral composition and concentration as the watering solution so that the plant roots would not experience a change in ion equilibrium.

The uptake solution was prepared in the following manner. HCE crystals were added to 4 liters of deionized water. The mixture was sonicated for 30 min to solubilize the HCE close to saturation. Excess crystals were then removed from the solution by vacuum filtration through a 0.45- $\mu\text{m}$  membrane filter. Nutrient mineral ions were then added to the solution. The  $^{14}\text{C}$ -HCE spike was added in a small volume of ethanol (final ethanol concentration = 100 ppm). The uptake solution was mixed for 5 min with a stirring bar and was then divided into three 1-liter batches of uptake solution.

Following each uptake period, the plants from that uptake solution were transferred to a beaker containing cold (10°C) nutrient solution. Each plant was rinsed by hand agitation, then transferred to a 1-liter beaker filled with cold (10°C) nutrient solution. The roots were submerged in this solution while the seeds and shoots remained above an aluminum foil cover. The plants were kept in this solution for 15 min, with solution circulation being provided with a stir bar. The purpose of this rinse step is to allow the outward release or exchange of HCE out of the root-free space, which does not occur with short-term rinsing. Because the solution is chilled, metabolism is suspended and actively absorbed HCE is not actively desorbed.

Following the desorption step, the plants were removed, given one last rinse in deionized water, and blotted dry with paper towels. The roots, shoots, and seeds of the three plants in each sample set were separated with a razor blade and the subsets of roots, shoots, and seeds were weighed separately to the nearest milligram. After being weighed, each sample subset was placed in an ashless paper cup for sample oxidation.

The corn plants were divided into three sections consisting of the roots, seeds, and shoots. Each part of three plants was weighed and oxidized in a Packard 306 oxidizer, the  $^{14}\text{CO}_2$  was collected, and the radioactivity was counted. The distribution of activity as a function of time in each section is shown in Table 23.

The radioactivity was found mainly in the roots section, which contained two to three times the level in the nutrient solution (10,600 dpm). Much lower levels were found in the seeds and very little was found in the shoots. The change in activity between 3 and 24 hours is small.

When the roots were placed in fresh nutrient solution, HCE did not readily desorb back into the water phase. This finding suggests a rapid binding of  $^{14}\text{C}$ -HCE to the plant roots but little transport to the shoots over a 24-hr period. The loss of HCE from the solution was quite rapid, with 50%, 26%, and 3% remaining in the solution at 3, 6, and 24 hr, respectively. We conclude that plant biouptake of HCE will not be a major loss process.

The plant uptake study was also conducted with pea plants in a similar manner as described for corn plants. The distribution of activity found in the roots, seeds, and shoots of the pea plant is shown in Table 24.

Table 23

AVERAGE DISTRIBUTION (3 PLANTS) OF RADIOACTIVITY IN  
CORN PLANT SECTIONS AS A FUNCTION OF TIME

<u>Plant Sections</u>	<u>Time (hr)</u>	<u>Weight (g)</u>	<u>Activity (dpm/g)</u>
Roots	3	0.884 ± 0.024	21,924 ± 697
	6	0.853 ± 0.087	23,413 ± 2460
	24	0.894 ± 0.047	29,748 ± 4562
Seeds	3	1.025 ± 0.137	4,594 ± 334
	6	1.032 ± 0.042	4,026 ± 1326
	24	0.870 ± 0.036	5,627 ± 411
Shoots	3	1.304 ± 0.127	519 ± 192
	6	1.480 ± 0.067	439 ± 35
	24	1.480 ± 0.109	525 ± 27

Table 24

AVERAGE DISTRIBUTION (2 PLANTS) OF RADIOACTIVITY IN  
PEA PLANT SECTIONS AS A FUNCTION OF TIME

<u>Plant Sections</u>	<u>Time (hr)</u>	<u>Weight (g)</u>	<u>Activity (dpm/g)</u>
Roots	3	0.821 ± 0.080	23,846 ± 986
	6	0.641 ± 0.129	25,842 ± 2,946
	24	0.848 ± 0.060	18,214 ± 3,156
Seeds	3	1,194 ± 0.185	247 ± 96
	.6	1.25 ± 0.155	557 ± 232
	24	1.397 ± 0.118	363 ± 80
Shoots	3	603 ± 0.064	246 ± 95
	6	0.5013 ± 0.106	561 ± 220
	24	1.391 ± 0.118	619 ± 43

These results are very similar to those observed in corn plants. A two fold uptake (activity/g root per activity/ml nutrient solution) of activity was observed in the roots, with little activity being distributed to the seeds or shoots over a 24-hour period. The change in activity between 3 and 24 hours is small and suggests that plant biouptake of HCE will not be a major loss process. The loss of activity from the plant's nutrient solution (Table 25) indicates that most of the HCE is lost within 24 hours due to volatilization.

TABLE 25

<sup>14</sup>C-ACTIVITY (dpm/g) OF HCE IN PLANT NUTRIENT SOLUTIONS AS A FUNCTION OF TIME

Beaker	Time (hr)			
	0	3	6	24
1 <sup>a</sup>	10,258	5,841 (51%)	--	--
2 <sup>b</sup>	9,921	6,451 (65%)	4,039 (41%)	--
3 <sup>c</sup>	10,151	3,385 (33%)	278 (3%)	
4 <sup>d</sup>	10,568		5,350 (51%)	951 (9%)

( ) Percent of 0-hour counts

- a Beaker 1 was used for a 3-hr exposure.
- b Beaker 2 was used for a 6-hr exposure.
- c Beaker 3 was used for a 24-hr exposure.
- d Beaker 4 was used for a 24-hr exposure and did not contain plants.

b. Other HCE Smoke Products

(1) Physical Transport

The parameters useful for estimating transport properties of HCE smoke products measured in the laboratory were taken from Callahan et al. (1979). These properties are listed in Table 26.

Table 26

PARTITION COEFFICIENTS, SOLUBILITY, HENRY'S CONSTANT, AND  
VAPOR PRESSURE OF HCE SMOKE COMPONENTS at 25°C

<u>Chemical</u>	<u>K<sub>ow</sub></u>	<u>S<sub>w</sub></u> (mg/L)	<u>K<sub>oc</sub></u>	<u>H<sub>c</sub></u> (L-torr/mole)	<u>P</u> (torr)
Carbon tetrachloride	437	785	270	7,650	90
Tetrachloroethylene <sup>a</sup>	759	200	470	11,570	14
Hexachlorobutadiene <sup>b</sup>	5,500	2	3.400	524	0.15
Hexachlorobenzene <sup>b</sup>	1.51x10 <sup>6</sup>	0.006	9.3x10 <sup>5</sup>	20	1.1x10 <sup>5</sup>
Hexachloroethane <sup>c</sup>	8.9x10 <sup>3</sup>	14	~700	6100	0.34

<sup>a</sup>Photooxidation  $t_{1/2}$  ~ 10 days.

<sup>b</sup>No rate constants reported.

<sup>c</sup>All values measured at SRI.

The large Henry's Constants (except for hexachlorobenzene) suggest that all components will rapidly volatilize from water and that the rates will be approximately the same ( $t_{1/2} = 70$  hr,  $k_v = 0.01$  hr<sup>-1</sup>). Hexachlorobenzene will also volatilize with a half-life of approximately 150 hr. The  $K_{ow}$  values suggest that hexachlorobutadiene and hexachlorobenzene will partition to lipophilic materials and will also partition strongly to soil. These properties suggest that these two compounds will remain at the surface of soil and not migrate readily. Carbon tetrachloride, tetrachloroethylene, and hexachloroethane will readily volatilize back to the atmosphere after deposition.

Soil-partitioning parameters and the soil partitioning coefficient (soil/air),  $K_H$ , appear in Table 27 and suggest that the majority of the compounds will bind to soil if they are allowed to penetrate significantly below the soil surface.

Table 27

## CALCULATED SOIL PARTITIONING PARAMETERS

<u>Compound</u>	<u><math>\lambda_a</math></u>	<u><math>\lambda_w</math></u>	<u><math>\lambda_s</math></u>	<u><math>K_H</math></u>
Tetrachloroethylene	0.06	0.01	0.93	8
Hexachlorobutadiene	0.002	0.0003	0.998	32
Hexachlorobenzene	$1 \times 10^{-6}$	$1 \times 10^{-5}$	1.000	$3 \times 10^6$
Carbon tetrachloride	0.07	0.03	0.90	7
Hexachloroethane	0.03	0.02	0.95	18

$\lambda_a$  = Fraction of chemical in the soil-air phase

$\lambda_w$  = Fraction of chemical in the soil-water phase

$\lambda_s$  = Fraction of chemical bound to soil

(2) Chemical Transformation Photolysis of Hexachlorobenzene (HCB)

We measured the rate constants for photolysis of HCB in 20% acetonitrile in water, where it is quite volatile (initial concentration  $10^{-7}$  M) and in hexane (initial concentration  $10^{-5}$  M). HCB was sublimed at 15 torr before use. Solutions were photolyzed in sealed quartz tubes in late spring sunlight for periods up to 27 days. Analysis was by reverse-phase HPLC using UV detection at 216 nm; and dark controls were reanalyzed at each new time point. An actinometer solution of p-nitroacetophenone and pyridine in water was used to monitor the sunlight intensity and to estimate the quantum yields for photolysis of HCB (Dulin and Mill, 1982). The results are summarized in Table 28. Photolysis appears to be somewhat

Table 28

SUNLIGHT PHOTOLYSIS OF HCB<sup>a</sup>

<u>Solvent</u>	<u><math>[HCB]_0, M</math></u>	<u><math>10^7 k_p, s^{-1}{}^b</math></u>	<u><math>t_{1/2}, \text{days}</math></u>
Hexane	$1 \times 10^{-5}$	0.7	110
4:1 Water:acetonitrile	$1 \times 10^{-7}$	1.1	70

<sup>a</sup>Photolysed from 17 May to 13 June 1985 at Menlo Park, CA (37.5°N). The actinometer had a quantum yield of  $1.2 \times 10^{-4}$  and a half-life of 12 days.

<sup>b</sup>Based on replicate analyses of four time points plus a zero-time point.

faster in water than in hexane, but this could be an artifact owing to some volatilization from water that could not be eliminated.

In both solvents, photolysis of HCB is slow, with half-lives close to 90 days, which corresponds to a half-life of about 180 days in a flat water body that does not receive scattered light from all sides. In summer this half-life would decrease by about 30% and in winter it would increase by at least a factor of five owing to the large decrease in sunlight intensity in the region of spectral overlap with the HCB absorption.

The earlier data of Plimmer and Klingebiel (1976), which yielded an HCB half-life of 10 days in methanol, appear to be in error. From the data in Table 29 and spectral data for HCB and the actinometer, we can estimate the quantum yield for photolysis of HCB in sunlight. The ultraviolet spectrum of HCB in a 1:1 mixture of water and acetonitrile shows a weak tail extending to about 315 nm (see Figure 19). Quantum yields were calculated using  $k_p$  values in Table 28,  $\epsilon$  values in Table 29, and  $L_\lambda$  (day-averaged sunlight intensity) values for 40°N and summer as described in Dulin and Mill (1982). It is important to note that the rates in hexane and water are nearly the same within the error of the measurements, and the calculation gives an average value of  $\phi$  of  $0.0067 \pm 0.0013$ .

Table 29

UV SPECTRAL LISTINGS FOR HCB IN 1:1 ACETONITRILE/WATER<sup>a</sup>

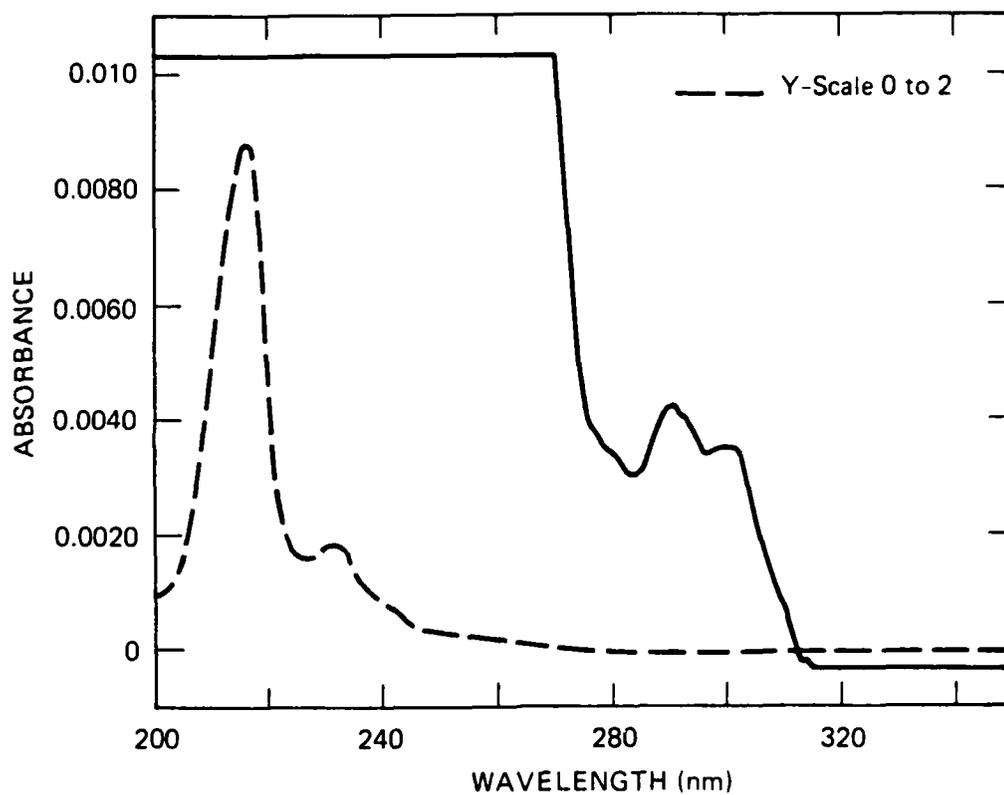
$\lambda$ , nm	$\epsilon_\lambda$ , M <sup>-1</sup> cm <sup>-1</sup>	$L_\lambda$ , M·cm d <sup>-1</sup>	$\epsilon L_\lambda$ , d <sup>-1</sup>
299	240	$3.3 \times 10^{-4}$	0.080
304	180	$2.8 \times 10^{-3}$	0.50
309	74	$9.9 \times 10^{-3}$	0.73
314	20	$2.1 \times 10^{-2}$	<u>0.43</u>

$$\Sigma \epsilon L_\lambda = 1.74 \text{ d}^{-1}$$

---


$$^a[\text{HCB}] = 2.0 \times 10^{-5} \text{ M.}$$

We have been unable to detect products from HCB photolyzed in water, but analogy to other systems suggests that pentachlorophenol is formed transiently. In hexane we observe one new product eluting before HCB and assume it is pentachlorobenzene.



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FIGURE 19  $20 \times 10^{-6}$  M HEXACHLOROBENZENE IN ACETONITRILE/WATER (1/1),  
1 CM CELL

Because HCB is volatile and unreactive in water and soils, its ultimate fate is probably photolysis in the atmosphere, where UV light intensity is slightly higher than under water. Using a  $\phi$  of 0.0067, we estimate a half-life of about 45 days in the lower atmosphere under equinox sunlight at 40° N.

The Henry's Constants for carbon tetrachloride and tetrachloroethylene are quite high. Because these components enter the environment from the vapor state, they are projected to remain there until precipitation events transport them to soil and water environments. Tetrachloroethylene has been reported to photooxidize with a half-life of ~10 days in the atmosphere (Dilling et al., 1975). Carbon tetrachloride is projected to be very stable in the environment because it does not readily transform by interaction with any environmental agents.

Although not much information has been reported for hexachloro-1,3-butadiene, its behavior in the atmosphere is believed to parallel that of tetrachloroethylene. Thus, we expect reactions with hydroxyl radical to predominate and a half-life of less than 10 days is projected. For material that deposits in soil or water, the sorption properties of hexachloro-1,3-butadiene are projected to be quite strong (based on  $K_{oc}$  value).

### (3) Biotransformation

Until recently, no definitive biotransformation studies on any of the above components have been reported. In this study, we have shown the biodegradability of hexachloroethane, and recently Vogel and McCarthy (1985) have reported on the biotransformation of tetrachloroethylene. However, the predominant environment for these chemicals to remain in is the atmosphere.

### (4) Smoke Metal Halides

The metal halides produced in hexachloroethane smoke are  $AlCl_3$  and  $ZnCl_2$ . The primary chemical reaction determining the fate of these metal halides is hydrolysis. Therefore, estimates of the rate constants for hydrolysis in the smoke, on soil, and in water are necessary to determine environmental half-lives.

Konigsbuch et al. (1975) studied the kinetics of the hydrolysis of small metal halide particles in spray reactors, and their work is applicable to both the smoke and soil environments. This heterogeneous reaction was carried out in such a manner that the rate of hydrolysis was limited by the chemical reaction and not by diffusional processes. Under these conditions, the equation describing the rate constant was determined to be:

$$kt = [1 - A/A_0]^{1/3}, \quad (12)$$

where  $A$  and  $A_0$  are the concentrations of the metal halides expressed as activities of  $^{38}Cl$  hydrolysis of  $AlCl_3$  and  $ZnCl_2$  at time  $t$  and  $t_0$ , respectively. The results for the hydrolysis of  $AlCl_3$  and  $ZnCl_2$  particles, shown in Table 30, assume that the energy of activation ( $E_a$ ) and the con-

stant A from the Arrhenius equation are independent of temperature. A particle-size dependence, which was approximately linear over the range of 1.2 to 2.1 mm, was also observed but was not given explicitly.

Table 30

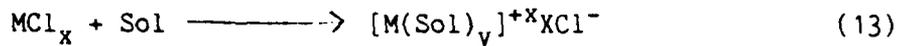
ESTIMATED RATE CONSTANTS AND HALF-LIVES FOR THE  
HYDROLYSIS OF METAL HALIDES AT 25°C<sup>a</sup>

Compound	$k_h$ (min <sup>-1</sup> )	$E_a$ (Kcal/mole)	$t_{1/2}$ (min)
AlCl <sub>3</sub>	$2.6 \times 10^{-2}$	4.6	30
ZnCl <sub>2</sub>	$4.8 \times 10^{-5}$	8.4	17,000

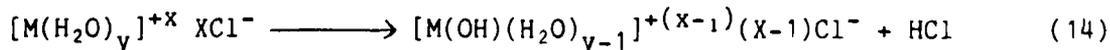
<sup>a</sup>Values of k calculated from values at >250°C and  $E_a$

These data have a number of potential problems. First, the reactions were conducted at high temperatures (230-800°C) and we have extrapolated them to room temperature using their values of the Arrhenius  $E_a$ . The reactions were also studied at 70 torr water pressure, and no water-pressure dependence was reported. In addition, the method employed measures the loss of chloride from the particles as HCl and may not accurately measure the rate of hydrolysis. Therefore, these studies need to be re-evaluated at environmental temperatures and as a function of relative humidity.

The literature contains a number of papers on the hydrolysis of metal halides in aqueous solutions (Levitskii and Maksimov, 1961; Schoersch, 1964; Frink and Peech, 1963; Biedermann, 1964; Takahashi, 1968; Movius and Matwiyoff, 1968; Spivakovskii and Makovskaya, 1968; Spivakovskii and Moisa, 1968). In each paper, the mechanism discussed involves a rapid displacement of the inner sphere chlorides by solvent molecules to give an outer sphere complex (Eq. 13).



When the solvent is water, the reaction is followed by a proton exchange as shown in Eq. 14.



All authors assume that the rate of reaction shown in Eq. 13 is very fast (reaction goes to completion within 1 hour). The second reaction (Eq. 12) depends on pH, ionic strength, etc. The cited references give equilibrium constants for Eq. 13 and no information on the rate. Hence, we conclude that metal halides in smoke aerosols depositing in aquatic environments will hy-

drate rapidly, according to Eq. 14. The release of HCl (Eq. 13) is expected to be as fast as or faster than the rates shown in Table 30.

#### 4. Bioconcentration Factors of HCE Smoke Products

The bioconcentration factors (BCF) of many of the HCE smoke products were adopted from those found in Lyman et al. (1982) and are shown in Table 31.

Table 31

#### BIOCONCENTRATION FACTORS OF SELECTED HCE SMOKE PRODUCTS IN AQUATIC ORGANISMS

<u>Compound</u>	<u>BCF</u>	<u>Aquatic Species</u>
Tetrachloroethylene	49	Bluegill sunfish
Hexachloroethane	139	Bluegill sunfish
Hexachlorobenzene	7,760	Rainbow trout
"	18,500	Fathead minnow
Carbon tetrachloride	30	Bluegill sunfish
" "	4	Rainbow trout
Hexachlorobutadiene	414 (est.)	

Several regression equations have been proposed based on  $K_{ow}$  and aqueous solubility to estimate BCFs when the aquatic studies are not available (Lyman, 1982). These equations are as follows:

$$\log BCF = 0.76 \log K_{ow} - 0.23 \quad (15)$$

$$\log BCF = 2.791 - 0.564 \log S_w \quad (16)$$

Using these equations, the BCF for hexachlorobutadiene was calculated to be 410 ( $K_{ow}$  of 5500) and 418 (aqueous solubility of 2 mg L<sup>-1</sup>) based on the  $K_{ow}$  and  $S_w$  values previously reported. The correlation from both equations is quite high; although these values are in close agreement, this agreement does not imply high accuracy; the 414 BCF in Table 31 is probably correct within an order of magnitude.

#### IV. CONCLUSIONS AND RECOMMENDATIONS

##### A. Nitroguanidine

The physical properties of NG suggest that it is nonvolatile, does not readily partition to lipophilic phases ( $K_{ow} = 0.156$ ), and does not partition to soils ( $k_p < 0.1$ ). The reason for these results is that NG is protonated under most environmental conditions and therefore is present as the nitroguanidium ion. This property should make NG very leachable from soil. However, in wells sampled at SAAP near NG-contaminated areas, no NG could be found ( $< 0.05$  ppm). This is probably the result of microbial activity in the soil.

Biotransformation occurred readily under both aerobic and anaerobic conditions in soils and waters obtained from SAAP. This transformation appears to be nutrient-dependent and half-lives of 4 and 6 days were found in water under anaerobic and aerobic conditions, respectively. In soils, anaerobic transformation dominated and a half-life of 4 days was estimated. In local soils and waters, anaerobic biotransformation proceeded slowly and no aerobic biotransformation was observed. These results indicate that NG-adaptive microorganisms exist in NG-contaminated areas and readily transform NG. Thus, biotransformation will be an important factor in controlling the fate of NG.

Photolysis was also found as a dominant pathway for NG transformation in the environment. Photolysis half-lives were calculated to range from 3.9 days in the winter to 1.6 days in the summer. The photolysis occurs by a direct photolytic mechanism and is not affected by naturally occurring organics. Other chemical transformation processes are projected to be extremely slow compared to photolysis.

According to the above findings, the fate of NG in the environment will be controlled by photolysis and biotransformation in water and by biotransformation in soil. We recommend that the following NG studies be done in Phase II of this project:

1. Detailed studies to refine the first-order photolysis rate constant.
2. Identification of photolysis products.
3. Establishment of pseudo-first-order and second-order aerobic biotransformation rate constants in water.
4. Identification of aerobic biotransformation products.
5. Establishment of pseudo-first-order and second-order anaerobic biotransformation rate constants in water and soil.
6. Identification of anaerobic biotransformation products.

## B. Diethyleneglycol Dinitrate

DEGDN was found to have a water solubility of 3,900 mg/liter. From this fact and its vapor pressure of 0.0036 torr, Henry's Constant was calculated to be  $0.018 \text{ torr M}^{-1}$ . The magnitude of Henry's Constant suggests that volatilization will be slow and dependent on the gas phase mass transport coefficient. The soil sorption partition coefficients for DEGDN yield  $K_{oc}$  values of 100 and 108 on EPA standardized soils. The magnitude of these values suggests that sorption will not be great. This fact was confirmed by the octanol-water partition coefficient,  $K_{ow}$ , which was measured to be 9.6. However, in biotransformation studies we did observe strong DEGDN binding to sediment and poor extraction recoveries. This process needs to be studied further.

DEGDN was found to undergo biotransformation under both aerobic and anaerobic conditions in waters collected from RAAP. In both cases, the biotransformation appears to be organic nutrient-dependent. This dependence needs to be studied further to extrapolate biotransformation rate constants to low-nutrient environmental conditions. Biotransformation half-lives ranged from 5 days in the presence of ethanol to greater than 40 days in New River water.

Biotransformation appeared to be very slow in soil under aerobic conditions. In sediment, an irreversible binding of DEGDN was observed and it was difficult to distinguish biotransformation processes from adsorptive properties. The observation that sterilized sediments bound DEGDN as readily as did non-sterile sediment suggests that the physical processes will dominate the loss and movement of DEGDN in sediment.

Photolysis was found to be the major chemical transformation loss process for DEGDN, and half-lives ranging from 35 days in pure water to 27 days in Kansas River water were observed. The estimated half-lives suggest that photolysis will compete with biotransformation in affecting the persistence of DEGDN in aqueous environments. Hydrolysis was found to be extremely slow under most environmental conditions.

Based on the above findings, the DEGDN studies recommended for Phase II are:

1. A detailed rate study on the photolysis of DEGDN.
2. Identification of major photolytic transformation products.
3. A detailed aerobic biotransformation study to determine the first and second biotransformation rate constants and the role of organic nutrients in regard to the transformation rate.
4. Identification of aerobic biotransformation products.
5. A detailed anaerobic biotransformation study as stated in item 3 above.
6. Identification of anaerobic biotransformation products.
7. Study of the processes binding DEGDN to sediment.

### C. Hexachloroethane (HCE)

The fate of HCE was considered apart from the other HCE smoke products because this chemical is a major component in uncombusted smoke devices and also appears in the smoke aerosol. Consequently, air, soil, and water environments could become contaminated more significantly with HCE than with other components in HCE smoke due to spillage, incomplete combustion, or disposal of smoke devices.

The solubility of HCE in water was measured at 14 ppm. The vapor pressure of HCE was measured at 0.34 torr. From these values we calculated a Henry's Constant of 5800 1-torr mole<sup>-1</sup>. The measured Henry's Constant was 6100 1-torr mole<sup>-1</sup>. The magnitude of Henry's Constant indicates that volatilization will be rapid in shallow, turbulent water bodies and will be dependent on mass transport resistance in the liquid phase only. For a water body 2 m-deep, the half-life for volatilization, based on a two-film model, is approximately 70 hr and the first-order volatilization rate constant is 0.01 hr<sup>-1</sup>.

The  $K_{ow}$  value for HCE was measured to be  $8.9 \times 10^3$  ( $\log K_{ow} = 3.9$ ), which suggests that partitioning to lipophilic materials will be strong. The soil sorption partition coefficient,  $K_{oc}$ , was measured to be 600, approximately eight-fold smaller than that projected from  $K_{ow}/K_{oc}$  correlation equations. The  $K_{oc}$  values were estimated from the linear portions of sorption isotherms derived from two standardized soils. On the basis of the relatively low  $K_{oc}$  and high water-solubility values, this result suggests that HCE should migrate moderately fast in soil.

HCE undergoes rapid biotransformation in anaerobic soil and moderate biotransformation in aerobic soil. The loss of HCE in aerobic soil that was inoculated into glass-stoppered Erlenmeyer flasks was 66%, 94%, 98% and 99% at the end of 1, 2, 3, and 4 weeks, respectively. The loss in sterile aerobic soil was 17% after 4 weeks of incubation.

In the anaerobic waters with 100 ppm of glucose and yeast extract, the amount of HCE lost was 43%, 62%, 84%, and 92% after 4, 7, 12, and 14 days of incubation. No loss of HCE was observed in the sterile control.

In the aerobic waters, the loss of HCE with glucose plus yeast extract was 60% after 14 days. A loss of 38% in the sterile control indicates that volatilization losses are significant.

The above results suggest that biotransformation will be a major loss process for HCE in aerobic and anaerobic soils. In water, biotransformation will compete with volatilization (see above) under anaerobic conditions, especially in deep, quiescent water bodies.

No chemical transformation processes were identified that dominate the transformation of HCE in soil, water, or air.

Based on the above findings, the recommendation for Phase II studies on HCE are:

1. Detailed biotransformation studies of HCE in soil under aerobic and anaerobic conditions.
2. Detailed biotransformation studies of HCE in water under anaerobic conditions.
3. Detailed soil leachability studies to verify soil-migration rates. This will also resolve the issue between the high  $K_{ow}$  and low  $K_{oc}$  values.
4. Identification of soil and water aerobic and anaerobic biotransformation products.

#### D. Hexachloroethane Smoke

HCE smoke results from the combustion of a mixture of HCE, grained aluminum, and zinc oxide. We have identified products of the combustion to be carbon tetrachloride, tetrachloroethylene, hexachlorobutadiene, hexachlorobenzene, hexachloroethane, hydrochloric acid, phosgene, carbon, aluminum chloride, zinc chloride, carbon monoxide, chlorine, and carbon dioxide. These chemicals were identified from laboratory burns of 1- to 5-g samples of smoke materials. The identifications need to be confirmed with actual smoke devices. The confirmation will be achieved in Phase II during the scale-model deployment studies using actual smoke grenades.

Hexachloroethane has been discussed in the previous section and recommendations have been made.

The physical transport properties of the organic components of HCE smoke suggest that all components will rapidly volatilize from water (except for hexachlorobenzene) and that the rates will be approximately the same ( $t_{1/2} = 70$  hr,  $k_v = 0.01$  hr<sup>-1</sup>). Hexachlorobenzene will also volatilize, with a half-life of approximately 150 hr. The  $K_{ow}$  values suggest that hexachlorobutadiene and hexachlorobenzene will partition to lipophilic materials and also will partition strongly to soil. These properties suggest that these compounds will remain at the surface of soil and transform by photolytic or hydroxyl radical processes. Carbon tetrachloride, tetrachloroethylene, and hexachloroethane will readily volatilize back to the atmosphere after deposition.

The chemical transformation reactions of concern are those associated with the air phase. Tetrachloroethylene and hexachlorobutadiene will undergo reactions with hydroxyl radical, with projected half-lives of 10 and 5 days, respectively. Hexachlorobenzene undergoes slow photolysis, with a half-life of approximately 90 days.

Hydrolytic reactions of aluminum trichloride ( $AlCl_3$ ) and zinc chloride ( $ZnCl_2$ ) have been estimated from high-temperature hydrolysis studies to have half-lives of 30 and 17,000 min, respectively, in the aerosol state. These studies need to be repeated at environmental temperatures and as a function of relative humidity.

The transformations of phosgene and chlorine are rapid and are projected not to be necessary to study in detail. The remaining compounds are projected to be stable to atmospheric chemistry.

The majority of the chemicals from hexachloroethane smoke are expected to remain or transform in the vapor or aerosol state, and their presence in soil or water is expected to be short. Therefore, no biotransformation studies on HCE smoke are recommended for Phase II.

The studies on HCE smoke recommended for Phase II are as follows:

1. Detailed chemical hydrolysis studies of  $ZnCl_2$  and  $AlCl_3$  in the aerosol state as a function of relative humidity.
2. A study of the hexachlorobutadiene reaction with hydroxyl radical in the vapor state. Estimates for the reaction half-life are based on the reported reaction rate of tetrachloroethylene. We need to confirm that this reaction proceeds along similar pathways and establish the rate constant.
3. Identification of hexachlorobutadiene-hydroxyl radical reaction products.

The studies recommended for NG, DEGDN, HCE, and HCE Smoke products should provide the necessary information to perform computer simulations of environments receiving chemical wastes. These environments could include evaporation lagoons, ponds, creeks, the Kansas River in the vicinity of SAAP, and the New River in the vicinity of RAAP. The scale-model deployment studies will provide the data to construct an aerosol model to project the deposition and fate of HCE smoke components.

## REFERENCES

- Baughman, G., and D. Paris. Microbial bioconcentration of organic pollutants from aquatic systems--A critical review. *CRC Crit. Rev. Microbiol.* 8, 205 (1981)
- Biedermann, G. Some recent results on the hydrolysis equilibria of metal ions. *Proc. 8th Int. Conf. Coord. Chem.* Sept 7-11, 1964, pp. 434-437.
- Bouwer, E. J., and P. L. McCarty. Transformation of halogenated organic compounds under denitrification conditions. *Appl. Environ. Microbiol.* 45:1295 (1983b). *Appl. Environ. Microbiol.* 45, 1286 (1983a).
- Callahan, M. A., M. W. Slimak, N. G. Gabel, I. P. May, C. F. Fowler, J. R. Freed, P. Jennings, R. L. Durfee, F. C. Whitmore, B. Maestri, W. R. Mabey, B. R. Holt, and C. Gould. Water-Related Environmental Fate of 129 Priority Pollutants, Volume II. EPA Report, EPA-440/4-79-0296, December 1979.
- Capellos, C., W. J. Fisco, C. R. Ribanco, V. D. Hogan, J. Campisi, F. X. Murphy, T. C. Castorina, and D. H. Rosenblatt. Basic hydrolysis of glyceryl nitrate esters. I. 1-Glyceryl and 2-glyceryl nitrate esters. *Int. J. Chem. Kinet.* 14, 903-917 (1982).
- Capellos, C., W. J. Fisco, C. Ribando, V. D. Hogan, J. Campisi, F. X. Murphy, T. C. Castorina, and D. H. Rosenblatt (1984a). Basic hydrolysis of glyceryl nitrate esters. *Int. J. Chem. Kinet.* 16, 1009-1026 (1984a)
- Capellos, C., W. J. Fisco, C. Ribando, V. D. Hogan, J. Campisi, F. X. Murphy, T. C. Castorina, and D. H. Rosenblatt. Basic hydrolysis of nitrate esters. III. Trinitroglycerin. *Int. J. Chem. Kinet.*, 16, 1027-1051 (1984b)
- Dennis, W. H., Jr.. A memorandum report to W. D. Burrows on photolysis of nitroguanidine. SGRD-UBG-R, Sept. 29, 1982.
- DeVries, J. E., and E. St. Clair Gantz. Spectrophotometric studies of dissociation constants of nitroguanidines, triazoles, and tetrazoles. *J. Am. Chem. Soc.* 76, 1008-1010 (1954).
- Dilling, W. L., N. B. Terfertiller, and G. J. Kallos. Evaporation rates of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene and other chlorinated compounds in dilute aqueous solution. *Environ. Sci. Technol.* 9, 833-838 (1975)

- Dulin, D., and Mill, T. (1982). Development and evaluation of sunlight actinometers. *Environ. Sci. Technol.* 16, 815-821 (1982).
- Frink, C, and M. Peech. Hydrolysis of the aluminum ion in dilute aqueous solutions. *Inorg. Chem.* 2, 473-478 (1963).
- Kaplan, D. L., J. H. Cornell, and A. M. Kaplan. Decomposition of nitroguanidine. *Environ. Sci. Technol.* 16, 488-492 (1982)
- Karickhoff, S. W., D. S. Brown, and T. A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res.* 13, 241.
- Katz, S., Karickhoff A., Snelson, R. Farlow, R. Welker, and S. Mainer, Physical and chemical characterization of fog oil and hexachloroethane smoke. IIT Research Institute, Chicago, IL Report to the U.S. Army Medical Research and Development Command Contract DAMD17-78-C-8085, 1980.
- Konigsbuch, M., E. Foa, Y. Schachter, and V. Schild. Kinetics of solid-gas reactions in spray reactors. *Isr. J. Technol.* 13, 371-376 (1975).
- Levitskii, E. and V. Maksimov. The composition of hydrolysis products of aluminum chloride solutions. *Dokl. Akad. Nauk. SSR*, 141, 865-868 (1961).
- Lyman, W. J., W. F. Reehl, and D. H. Rosenblatt (1982). Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Company, New York.
- McCarthy, P. L. (1985). A Biodegradation of trace organics in groundwater Presented at Am. Soc. Microbiol. annual meeting, 1985.
- Mill, T., W. R. Mabey, D. C. Bomberger, T. W. Chou, D. G. Hendry, and J. H. Smith. Laboratory Protocols for Evaluating the Fate of Organic Chemicals in Air and Water. SRI Final Report on EPA Contract 68-03-2227, September 1982, p. 60.
- Movius, W., and N. Matwiyoff. Magnetic resonance studies of outer-sphere ion-pair formation in N,N-dimethylformamide solutions of aluminum (III) halides. *J. Phys. Chem.* 72, 3063-3066 (1968).
- Noss, C. I., and Chyrek, R. H. Technical report 8309, U.S. Army Medical Bioengineering R&D Lab., Fort Detrick, Jan. 1984.
- Plimmer, J. R. and U. I. Klingebiel. Photolysis of hexachlorobenzene. *J. Agr. Food Chem.* 24, 721-723 (1976).
- Schoersch, G. Hydrolysis of the Zn<sup>2+</sup> ion at 25° in 2 M KCl and 2 M NaCl. *Bull. Soc. Chim. France*, 1449-1455 (1964).
- Spivakovskii, V. and G. Makovskaya. Fine hydroxide and hydroxide chlorides precipitated from aqueous ethanol solutions. The method of five variables. *Russ. J. Inorg. Chem.* 13, 1423-1427 (1968).
- Spivakovskii, V., and L. Moisa. Basic chlorides of fire precipitated from aqueous ethanol solutions. The method of five variables. *Russ. J. Inorg. Chem.* 13, 1423-1427 (1968)

Tabak, H. H., S. A. Quave, C. I. Mashni, and E. F. Barth. Biodegradability studies with organic priority pollutant compounds. *J. Water Pollut. Control Fed.* 531503 (1981).

Takahashi, A. NMR studies on proton transfer between hydration and solvent water molecules in aqueous solution of  $AlCl_3$ . *J. Phys. Soc. Japan* 24, 657-658 (1968)

Taylor, W., and A. M. Ward. The kinetics of the interactions of sodium hydroxide with penta- and hexachloro- and bromo-ethanes in aqueous ethyl-alcoholic solutions. *J. Chem. Soc.* 2003-2011 (1934).

Verschueren, K. Handbook of environmental data on organic chemicals. Van Nostr and Reinhold Press, New York, 1977, 659 p.

Vogel, T. M., and P. L. McCarthy. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* 49, 1080-1083 (1985).

Yalkowsky, S. H., and S. C. Valvani. Solubility and partitioning. I: Solubility of nonelectrolytes in water. *J. Pharm. Sci.* 69, 912 (1980).

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