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Interaction of abiotic and microbial processes in hexachloroethane reduction in groundwater

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Abstract

In order to gain insight into mechanisms of hexachloroethane reduction, hexa- and pentachloroethane transformation rates were measured in anaerobic groundwater samples. For samples spiked with pentachloroethane, disappearance of pentachloroethane was accompanied by tetrachloroethylene production. Transformation rates were similar in unpoisoned and in $HgCl_2$-poisoned samples, and rates were within ±20% of predictions based on measured pH and second-order dehydrochlorination rate constants determined in clean laboratory systems, indicating that the fate of pentachloroethane in this system is dominated by abiotic reactions. No hexachloroethane transformation was observed in $HgCl_2$-poisoned samples, whereas in unpoisoned samples, hexachloroethane disappearance was accompanied by production of tetrachloroethylene as well as traces of pentachloroethane. Although only minor amounts of pentachloroethane accumulated, as much as 30% of the hexachloroethane transformation pathway proceeds via a pentachloroethane intermediate. This suggests that the microbial reduction of hexachloroethane proceeds at least in part through a free-radical mechanism. To the extent that hexachloroethane reduction to tetrachloroethylene occurs through a pentachloroethane intermediate, the first step in the sequence, the microbially-mediated step, is the slow step; the subsequent abiotic dehydrohalogenation step occurs much more rapidly.

1. Introduction

A great deal of recent research has focused on the reductive dehalogenation of organic compounds in aquatic environments. Many such studies have emphasized or included the reduction of hexachloroethane to tetrachloroethylene as a model

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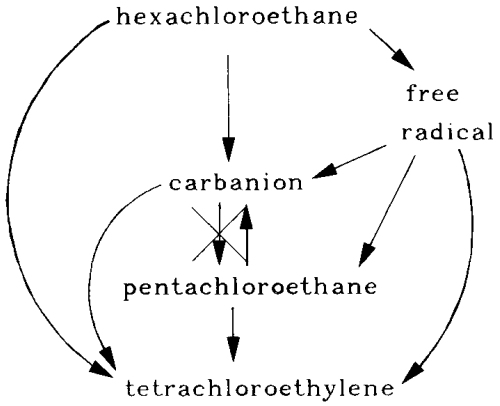

Fig. 1. Potential pathways for hexachloroethane reduction, indicating significance of pentachloroethane. *Crossed-out* pathway reflects results of previous study (Roberts and Gschwend, 1991).

reaction (e.g., Criddle et al., 1986; Jafvert and Wolfe, 1987; Bouwer and Wright, 1988; Reinhard et al., 1988, 1990; Curtis and Reinhard, 1989; Peijnenburg et al., 1989; Schanke and Wackett, 1992), although to date little progress has been made in elucidating the actual reaction pathway(s) in environmental samples. In principle, reductive dehalogenation could occur either through a two-electron (nucleophilic reduction) pathway or via two sequential one-electron transfer steps (free-radical pathway), as reviewed by Baciocchi (1983). Being able to determine which pathway is adopted may well prove critical to developing valid models relating structure to reactivity, through which it may ultimately be possible to predict the environmental fate of new compounds.

Investigations of hexachloroethane reduction by Fe(II)-porphyrins (Mansuy and Fontecave, 1982) and rat liver microsomal preparations (Nastainczyk et al., 1982) have noted pentachloroethane as a minor product. Pentachloroethane could conceivably be generated either via hydrogen atom abstraction by a pentachloroethyl radical (formed by a free-radical reduction step) or via protonation of a pentachloroethyl carbanion (resulting either from a single two-electron transfer step or from two consecutive one-electron transfer steps). Pentachloroethane formed from hexachloroethane reduction can itself react to tetrachloroethylene, albeit via dehydrochlorination, a reaction that does not involve a change in oxidation state. In a recent study (Roberts and Gschwend, 1991), we studied the dehydrochlorination reaction of pentachloroethane in order to assess the probable stability of a pentachloroethyl carbanion. Our results indicated that pentachloroethane formed during hexachloroethane reduction is unlikely to result from protonation of a carbanion; this is illustrated schematically in Fig. 1. Rather, production of pentachloroethane may be diagnostic of a free-radical reduction pathway. Because pentachloroethane dehydrochlorination to tetrachloroethylene proceeds relatively rapidly, with a half-life of ~2.9 days at pH 7, 25°C, even minor accumulation of pentachloroethane may signify a role as a reaction intermediate rather than a side product.

The purpose of the present study was to investigate the transformation of hexa- and pentachloroethane in environmental samples. We chose to investigate transfor-

mations that proceed in anaerobic groundwater obtained from a site on Cape Cod, Massachusetts, U.S.A., because preliminary studies had indicated that minor amounts of pentachloroethane were formed during the course of hexachloroethane reduction. If confirmed by the present study, this would help to identify whether reductive dehalogenation takes place via a two-electron or a free-radical mechanism. Moreover, if reaction rate constants could be obtained for both hexa- and pentachloroethane, we could address the question of whether pentachloroethane represents an intermediate or a side product. The difference between rates observed in unaltered groundwater samples and rates observed in poisoned samples would reveal whether transformations were directly mediated by microorganisms, or alternatively resulted from the interaction of chemical species with alkyl halides. Finally, most studies of dehydrohalogenation kinetics have been conducted in relatively simple, clean chemical systems, whereas environmental samples may contain a host of other reagents (which might include microorganisms or bases other than OH^- or H_2O; Roberts et al., 1993) potentially capable of interacting with the halogenated organic compounds of interest. A comparison of rates of pentachloroethane transformation in anoxic groundwater samples with rates predicted on the basis of our prior laboratory studies would permit an assessment of the magnitudes of errors that may result from extrapolating the results of laboratory studies to environmental conditions.

2. Materials and methods

2.1. Groundwater sample collection

Groundwater samples were obtained from well FSW *300-50* at the U.S. Geological Survey Toxic Substances Hydrology Research Site on Cape Cod on August 24, 1989. An extensive plume of contamination has developed at this site as the result of the continuous disposal over more than 50 years of secondary treated sewage effluent into a shallow unconfined aquifer (LeBlanc, 1984). The fate and distribution of organic contaminants at this site, as well as the characteristics of the indigenous microflora, have been extensively investigated by others (Harvey et al., 1984; LeBlanc, 1984; Thurman et al., 1986; Harvey and George, 1987; Barber et al., 1988, 1992; Field et al., 1992; Harvey and Barber, 1992). The monitoring well from which the samples were obtained is located $\sim$ 500 m downgradient of the source of contamination, and is constructed of 5-cm polyvinylchloride (PVC) casing, with a 60-cm slotted PVC screen at a depth of 15 m below ground surface.

A sampling system was developed to allow collection of groundwater samples with a minimum of atmospheric or microbial contamination. This system, which represents a modification of the design described by Backhus et al. (1986), is illustrated in Fig. 2. Samples were collected with a positive-displacement gear-driven pump (model SP-*202*, Fultz Pumps Inc., Lewistown, Pennsylvania, U.S.A.) powered by an AC generator and an AC/DC converter. Samples were collected at a low flow rate (250 $mL\,min^{-1}$) to minimize turbulence in the sample flask that might cause sample degassing, altering the groundwater chemistry. An inflatable packer was used in an

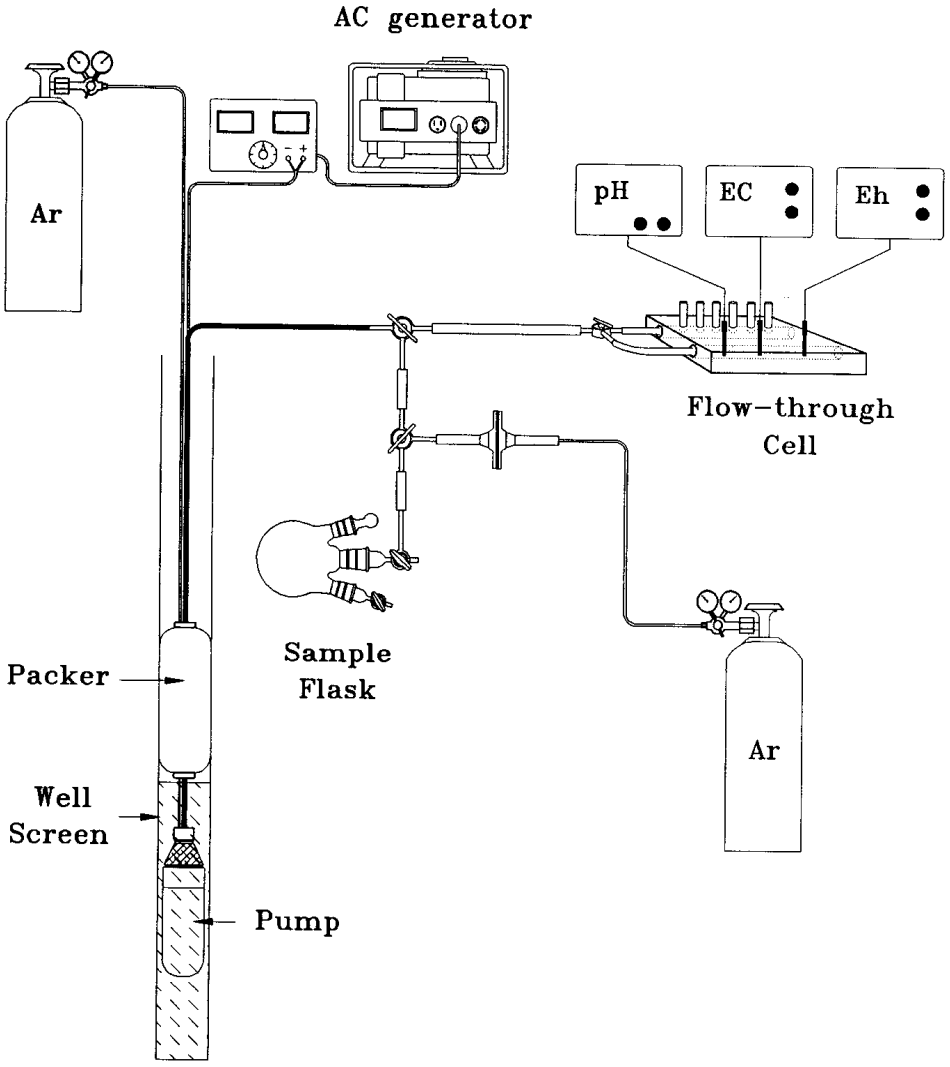

Fig. 2. Schematic of groundwater sampling setup.

attempt to provide a sample as representative as possible of the groundwater within the aquifer. The well was pumped for 1 h (25 well volumes) before beginning sample collection.

Sampling tubing (1-cm diam., polypropylene) was connected via Teflon® stopcocks and Teflon® tubing either to a flow-through cell for measuring chemical parameters such as pH, Eh and electrical conductivity (EC), or to sample collection flasks. Groundwater could be diverted into one of two sections of the flow-through cell; one section contained test tube inserts containing pH buffers or hydroquinone solutions to allow calibration of pH and Eh electrodes at groundwater temperature, and the other was designed to allow connection of electrodes to the flow-through cell for measurement of parameters without sample exposure to the atmosphere. Determinations of pH were performed with an Orion® Model SA *720* pH/ISE meter and

an Orion®–Ross® combination pH electrode. Electrical conductivity was measured with a Hanna Instruments® HI *8333* instrument, and Eh was determined with a Cole-Parmer® ORP electrode connected to a Horizon® Model *5996* meter. A tank of compressed argon was connected to the sampling setup via copper and Teflon® tubing and a 0.2-μm sterile filter so that sample flasks could be purged with argon prior to filling to minimize oxidation of groundwater samples. Sample flasks consisted of 250-mL (nominal volume; actual volume $\approx$ 300 mL) three-necked flasks equipped with a three-way and a two-way stopcock adapter and a ground-glass stopper. Each flask also contained a small Teflon® stir bar. The sample tubing, stopcock assembly and sample flasks were all autoclaved immediately prior to field work, and were transported to the field in sterile polypropylene bags.

Samples were also collected in 300-mL biological oxygen demand (BOD) bottles for analysis of chemical constituents. Additional samples were collected in 50-mL plastic syringes and were filter-sterilized (0.2-μm syringe filter) into sterile plastic bottles for analysis of dissolved nitrogen species.

2.2. Laboratory analysis of groundwater chemistry

Analyses were conducted for selected inorganic species in order to characterize the redox status of the groundwater. Nitrogen species (NO_3^-, NO_2^- and NH_4^+) were determined via flow injection analysis with a Lachat Instrument Co. autoanalyzer. Total manganese and iron were determined via graphite furnace atomic absorption analysis of samples that had been acidified to pH $\approx$ 1 with concentrated HNO_3. Dissolved oxygen was determined for samples collected in BOD bottles, and (after first removing samples for measurement of pH at 25°C) for sample flasks at the completion of experiments, via a modification of the Winkler titration method, using $2.5 \cdot 10^{-3}$ *N* thiosulfate as a titrant. Alkalinity was determined by titration of a 50-mL sample with $4.3 \cdot 10^{-3}$ *M* HCl, standardized by potentiometric titration against Na_2CO_3. End points were determined via the Gran function.

2.3. Kinetic experiments

To determine the kinetics of hexa- and pentachloroethane transformation, groundwater samples were spiked with either hexa- or pentachloroethane, or a combination of the two, and concentrations were monitored over the course of a month. Trichlorofluoromethane (CCl_3F) was included in all experiments to verify the integrity of our incubation flasks against volatilization losses. All timecourses were conducted using the 300-mL three-necked flasks in which the samples had been collected. The three-way stopcock adapters on the flasks allowed all sample manipulation steps, such as spiking with alkyl halides or removal of aliquots for analysis, to be conducted under a stream of argon gas, minimizing exposure to the atmosphere.

In order to inhibit microbial activity in selected samples, flasks were spiked with 0.5 mL of a deoxygenated (argon-purged) mercuric chloride solution to yield a final concentration of 10 mg L^{-1}. Poisoning was performed 3 days before spiking flasks with alkyl halides to allow adequate time for die-off.

Experiments were begun by spiking flasks (via a sterile glass syringe) with 300 μL of a methanol stock solution of alkyl halides to yield concentrations of 0.6 μM CCl_3F + 0.9 μM $Cl_3C–CHCl_2$, or 6 μM CCl_3F + 7 μM $Cl_3C–CCl_3$, or 6 μM CCl_3F + 0.9 μM $Cl_3C–CHCl_2$ + 7 μM $Cl_3C–CCl_3$. We were concerned that biotransformation of the methanol carrier might alter the biogeochemical conditions within the flasks, creating reduced species capable of effecting the abiotic transformation of hexachloroethane and thus hampering a comparison of rates obtained in poisoned vs. unaltered samples. To address this concern, selected samples were spiked with 300 μL methanol, allowed to incubate for 1 week, poisoned with mercuric chloride, and then (after 3 more days) spiked with alkyl halides. Reaction rates in flasks treated in this manner proved indistinguishable from rates in $HgCl_2$-poisoned flasks that were not pretreated with methanol.

After initial mixing, flasks were sampled by removing 100-μL aliquots via sterile glass syringes; sampling was repeated at 1- to 2-day intervals. Flasks were incubated in the dark in a water bath at 25°C. Immediately after sampling, aliquots (0.5 μL) were removed from the sterile glass syringes via a 10-μL GC syringe for analysis via cold on-column direct aqueous injection gas chromatography (GC) with electron capture detection (ECD), as previously described (Roberts and Gschwend, 1991). Samples from flasks spiked with hexachloroethane required a preliminary 10-fold dilution step to bring concentrations within the operating range of the electron capture detector. This was done in order to increase our chances of observing trace concentrations of reaction products. Samples from these flasks were analyzed with and without such a dilution step.

Mid-way through the timecourses, selected flasks were sacrificed for analysis via combined gas chromatography–mass spectrometry (GC–MS). These samples were transferred to 250-mL volumetric flasks and extracted 3× with 1 mL of pentane. The extracts were combined, and 1 mL of hexane containing 70 μM *1,2*-dibromoethane as an internal standard was added. The extracts were blown down with argon to a final volume of 1 mL, and were analyzed with a Hewlett-Packard® benchtop gas chromatograph–mass spectrometer [model *5995*B, modified as described by Jensen et al. (1982)] equipped with a Teknivent® Vector/One controller/data system. Separations were achieved with a 30-m, thin-film (0.25-μm) fused-silica capillary column with a nonpolar DB-*5* cross-linked phase (J & W Scientific, Folsom, California, U.S.A.). For quantifying alkyl halide concentrations, only the following ions were monitored to maximize sensitivity: *m/e* 59–60, 107, 117–119, 130–132, 165–169 and 201. Complete scans (*m/e* 59–250) were, however, also performed on each sample to confirm the identity of the compounds present.

2.4. Data analysis

For each experiment, monitoring $Cl_3C–CHCl_2$ or $Cl_3C–CCl_3$ disappearance and $Cl_2C{=}CCl_2$ or $Cl_3C–CHCl_2$ appearance permitted independent estimates of rate constants. These were determined by fitting the data from flasks spiked with pentachloroethane via a regression of the form:

$$\ln(Cl_3C–CHCl_2) = \ln(Cl_3C–CHCl_2)_o - k_{obs}t$$

A similar expression was used for determining the rate of $Cl_3C–CCl_3$ transformation. The groundwater proved to be contaminated with both tri- and tetrachloroethylene, requiring corrections to be made in order to account for the tetrachloroethylene initially present in determining rate constants from tetrachloroethylene data. The $Cl_2C{=}CCl_2$ data were fit to an equation of the form:

$$(Cl_2C{=}CCl_2) = (Cl_3C–CHCl_2)_o[1 - \exp(-k_{obs}t)] + (Cl_2C{=}CCl_2)_o$$

for flasks spiked with pentachloroethane, or

$$(Cl_2C{=}CCl_2) = (Cl_3C–CCl_3)_o[1 - \exp(-k_{obs}t)] + (Cl_2C{=}CCl_2)_o$$

for flasks spiked with hexachloroethane. Because the extent of hexachloroethane transformation over the course of the 1-month incubations was slight ($\leqslant 25\%$), it was difficult to assess how well the hexachloroethane concentrations obeyed a pseudo-first-order decay model. As a result, the tetrachloroethylene data for flasks spiked with hexachloroethane were also analyzed by assuming a pseudo-zero-order model, by fitting the data to an expression of the form:

$$(Cl_2C{=}CCl_2) = k_{obs}t + (Cl_2C{=}CCl_2)_o$$

3. Results

3.1. Groundwater chemistry

As shown by the results of chemical analyses summarized in Table 1, Winkler titrations indicated low levels of dissolved oxygen to be present in the groundwater samples. This may reflect limitations in our ability to measure low levels of dissolved oxygen by this technique. Results obtained for groundwater samples were not significantly different from results obtained with argon-purged Milli-Q® water, leading us to infer that the results for the groundwater samples are of questionable reliability. The remaining parameters suggest that biogeochemical conditions can best be described as intermediate between manganese- and iron-reducing: nitrate, nitrite and iron are absent or present at only low levels, while manganese is present at concentrations vastly exceeding the solubility of Mn(IV)-oxides [yet still below the solubility of phases such as $MnCO_{3(s)}$ and $Mn(OH)_{2(s)}$]. Together with the low Eh, this suggests the manganese is predominately present as relatively soluble Mn(II) species.

GC analyses indicated the groundwater to be contaminated with both tri- and tetrachloroethylene, as indicated in Table 1. These results were confirmed by GC–MS analysis. Analyses of samples collected from this well in the spring of 1988 indicated a total organic carbon (TOC) content of 2.3 mg L^{-1}, and a dissolved organic carbon (DOC) content of 2.0 mg L^{-1} (L.B. Barber, pers. commun., 1993).

3.2. Reaction of pentachloroethane to tetrachloroethylene

Pentachloroethane concentrations in an unpoisoned sample spiked with CCl_3F

Table 1
Groundwater chemistry

Parameter	Concentration
Dissolved oxygen	13 ± 16 μM
NO_3^-	0–4 μmol L^{-1} N
NO_2^-	0 μmol L^{-1} N
NH_4^+	120–130 μmol L^{-1} N
Total manganese	93 ± 4 μM
Total iron	0.41 ± 0.07 μM
pH (field)	6.32–6.51
pH (lab)	6.56–6.65
Alkalinity	$1.4 \cdot 10^{-3}$ eq L^{-1}
Eh (rel. to SHE)	+245 to +257 mV
Electrical conductivity	225–254 μS
Trichloroethylene	0.16 μM
Tetrachloroethylene	0.21 μM

SHE = standard hydrogen electrode.

plus pentachloroethane exhibited exponential decay, as shown in Fig. 3a. No lag period was observed that might be attributed to adaptation of microorganisms to new metabolic pathways. The increase in tetrachloroethylene concentration was only 47% of what would be expected if it were to represent the sole reaction product, as indicated by the discrepancy between the observed concentrations and the model predictions obtained from the pentachloroethane data. Gradual increases in trichloroethylene concentration were observed over time in this flask, potentially representing a reductive dehalogenation product either of pentachloroethane or of tetrachloroethylene. These increases (on the order of 0.13 μM) can only account for 10–15% of the pentachloroethane initially added. No increase in trichloroethylene concentration was observed in an unpoisoned flask spiked with a combination of CCl_3F, pentachloroethane and hexachloroethane; this may reflect a competitive inhibition of pentachloroethane or tetrachloroethylene reduction to trichloroethylene introduced by the presence of hexachloroethane. Competitive inhibition of this type might be anticipated if binding to the active sites of the enzyme(s) or prosthetic groups responsible were to occur through hydrophobic interactions.

Any biological transformation of pentachloroethane must proceed in addition to an abiotic component; thus, predictions of contaminant transformation based on known rate constants for abiotic reactions represent the minimum rate of parent compound disappearance. In both unpoisoned flasks, pentachloroethane disappearance rates were within ±20% of those predicted for abiotic dehydrochlorination from second-order rate constants determined in clean laboratory systems (Roberts and Gschwend, 1991) and measured pH. This close correspondence of observed rates to predicted abiotic transformation rates indicates that the fate of pentachloroethane is dominated by the abiotic dehydrohalogenation reaction to tetrachloroethylene.

The mass-balance discrepancy for pentachloroethane could be due either to some process acting on pentachloroethane in addition to its abiotic dehydrochlorination,

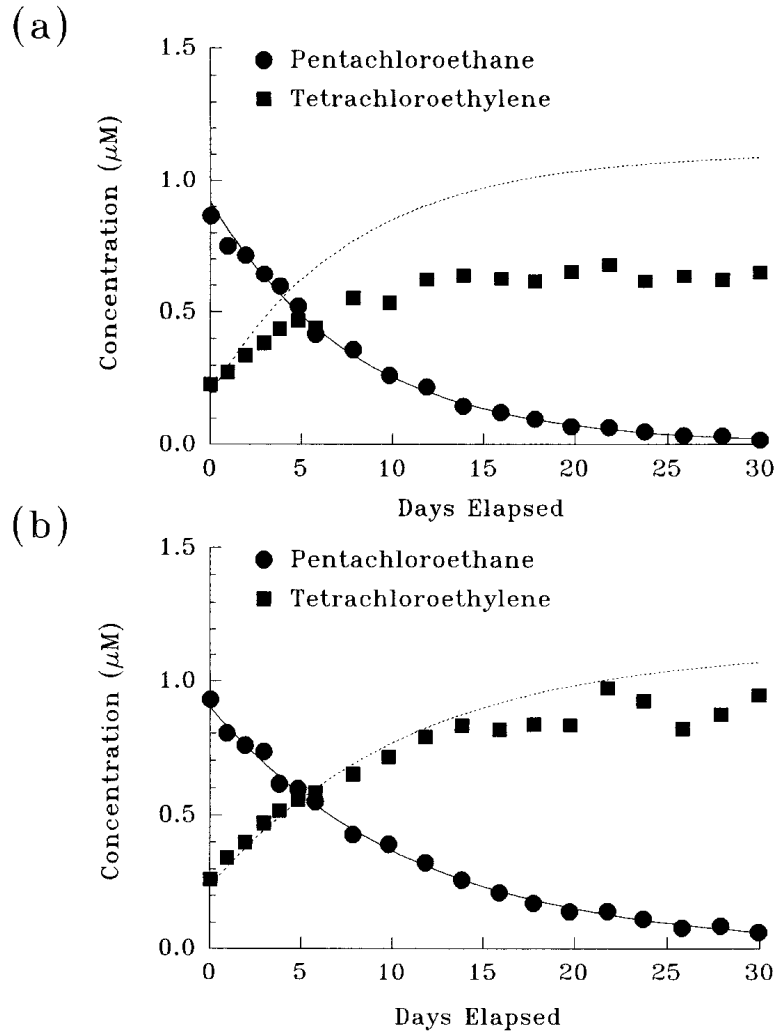

Fig. 3. Example results of pentachloroethane transformation in groundwater: (a) unpoisoned sample; and (b) $HgCl_2$-poisoned sample. *Solid lines* indicate model fits to pentachloroethane data; *dashed lines* represent predicted tetrachloroethylene concentrations, assuming no other products are formed.

or alternatively to transformation of the products tetra- or trichloroethylene. The similarity of observed pentachloroethane transformation rates to rates predicted for abiotic dehydrochlorination would favor the second explanation. On the other hand, the measured product concentrations appear to be levelling off with time, indicating tetra- and trichloroethylene transformations are negligible within the time frame of our experiments.

As indicated in Fig. 3b, pentachloroethane concentrations observed in poisoned samples also exhibited exponential decay. For these flasks, however, mass-balance calculations indicated that tetrachloroethylene accumulation was significantly greater than in the unpoisoned flask, being on the order of $74 \pm 6\%$ of predicted values.

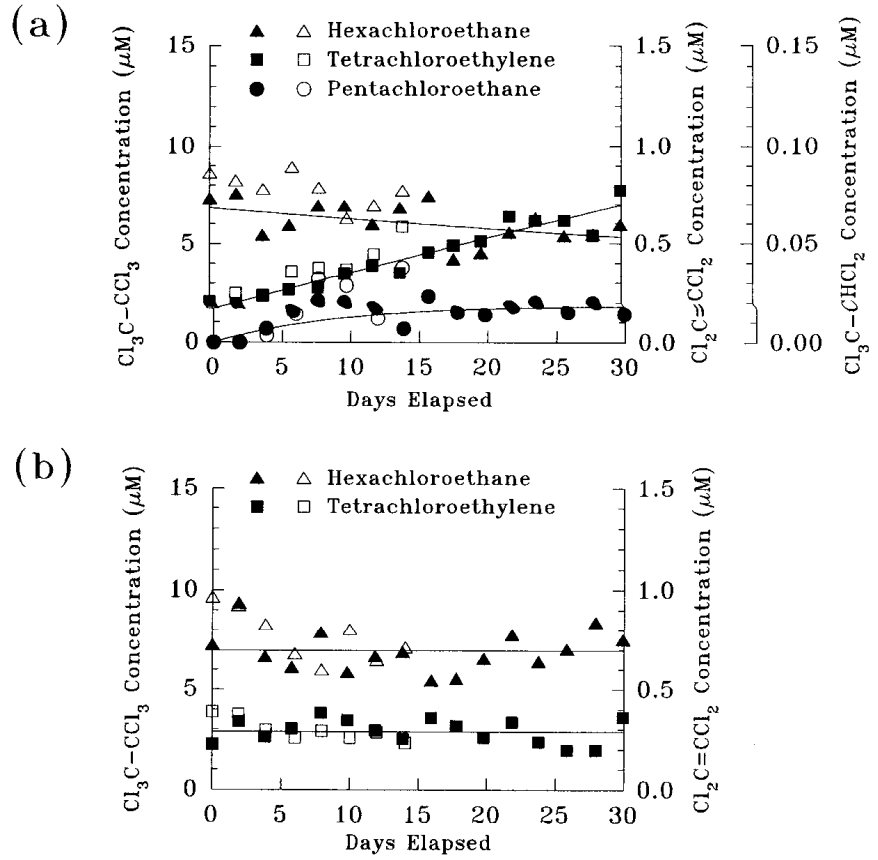

Fig. 4. Example results of hexachloroethane transformation in groundwater: (a) unpoisoned samples; and (b) $HgCl_2$-poisoned samples. *Solid symbols* indicate measurements in flasks incubated for a month; *open symbols* indicate measurements in flasks sacrificed after 14 days for solvent extraction. *Solid lines* indicate pseudo-first-order model fits to data represented by *solid symbols* or [for (b)] mean concentrations.

Pseudo-first-order rate constants obtained in poisoned flasks were within ±4% of values predicted from second-order dehydrochlorination rate constants and measured pH.

A possible explanation for differences observed in poisoned vs. unpoisoned flasks could be that the added Hg(II) is somehow catalyzing the abiotic dehydrohalogenation reaction. We do not believe this to be the case; although metal ions can catalyze the hydrolysis of esters and ester analogs, they do so by functioning as Lewis acids, facilitating the acid-catalyzed pathway. Such a role is implausible for dehydrohalogenation reactions, which lack an acid-catalyzed mechanism. Indeed, Cooper et al. (1989) have looked for, but failed to observe, any catalysis of *1,1,2,2*-tetrachloroethane dehydrochlorination by a variety of heavy metals, including Hg(II). Nor have we observed any effect of $HgCl_2$ (or its omission) on pentachloroethane dehydrochlorination rates in previous studies conducted in well-characterized chemical systems (Roberts and Gschwend, 1991).

3.3. Reaction of hexachloroethane to pentachloroethane and tetrachloroethylene

Example results for flasks spiked with hexachloroethane are indicated in Fig. 4. The relatively large scatter in the hexachloroethane data undoubtedly results from errors introduced in diluting samples to bring concentrations within the usable range of the electron capture detector. Despite this scatter, it is clear that hexachloroethane transformation only occurs in unpoisoned flasks (Fig. 4a): no decrease in hexachloroethane concentration was observed in poisoned flasks (Fig. 4b), and tetrachloroethylene production was only observed in unpoisoned flasks. Of greatest interest to the present study, trace amounts of pentachloroethane (absent in poisoned flasks and absent initially in unpoisoned flasks) gradually accumulated over time. Note that pentachloroethane and tetrachloroethylene accumulation in the flask that was sacrificed after 14 days for GC–MS analysis tended to parallel behavior in the replicate flask that was monitored for a month.

The absence of pentachloroethane and tetrachloroethylene production in poisoned flasks suggests that reductive dehalogenation of hexachloroethane is microbially mediated. Preliminary studies, in which inhibition was accomplished with sodium azide, yielded similar results. That two very different chemical species ($HgCl_2$ and N_3^-) were equally effective in inhibiting hexachloroethane reduction supports a direct involvement of microorganisms. Abiotic reducing agents, such as reactive colloids or perhaps dissolved reductants such as thiols or transition-metal ions, would be expected to interact very differently with Hg(II) species and with azide.

No lag period was observed before hexachloroethane reduction began. This might indicate enzymatic pathways required for reductive dehalogenation had already been induced by the presence of tetra- and trichloroethylene in the groundwater. Alternatively, the absence of a lag period could signify that reduction is occurring via a cometabolic process involving enzymes already active, or that reduction is accomplished through nonspecific enzymes or prosthetic groups that might function in a variety of electron-transport systems.

The production of pentachloroethane in unpoisoned samples was confirmed by GC–MS analyses by comparing retention times and mass spectrum with an authentic sample. This compound was only observed in unpoisoned groundwater samples spiked with hexachloroethane.

3.4. Data modeling

In principle, hexachloroethane could be reduced to pentachloroethane via hydrogenolysis or could be reduced directly to tetrachloroethylene via reductive elimination; pentachloroethane could itself undergo dehydrohalogenation to tetrachloroethylene. The overall reaction sequence can be given by:

$$Cl_3C{-}CCl_3 \xrightarrow{k_1} Cl_3C{-}CHCl_2 \quad (1)$$

$$Cl_3C{-}CCl_3 \xrightarrow{k_2} Cl_2C{=}CCl_2 \quad (2)$$

$$Cl_3C{-}CHCl_2 \xrightarrow{k_3} Cl_2C{=}CCl_2 \quad (3)$$

In these sequences, k_1, k_2 and k_3 refer to observed rate constants (pseudo-first-order or pseudo-zero-order) for hexa- and pentachloroethane reactions. Our results indicate that pentachloroethane transformation exhibited pseudo-first-order decay, as shown by the good agreement in Fig. 3 between observed concentrations and the fits obtained from an exponential regression. The extent of hexachloroethane transformation was too slight over the course of the incubations to assess whether these observations were better explained by a pseudo-first-order or a pseudo-zero-order model. Either sort of behavior could be anticipated in microbial transformations, depending on whether or not the hexachloroethane was present at concentrations that were saturating with respect to the enzyme system(s) involved. We suspect that a pseudo-zero-order model may in fact be more appropriate at the concentrations employed in the present study; in preliminary studies conducted with much lower initial hexachloroethane concentrations ($\sim 0.5\ \mu M$ vs. $7\ \mu M$), hexachloroethane was transformed to a greater extent over a comparable period of time ($\sim 50\%$ vs. $< 25\%$).

If we assume that hexachloroethane obeys a pseudo-first-order decay expression, then its transformation rate can be given by:

$$\frac{d(Cl_3C\text{–}CCl_3)}{dt} = -(k_1 + k_2)\cdot(Cl_3C\text{–}CCl_3) \tag{4}$$

for which the solution is:

$$(Cl_3C\text{–}CCl_3) = (Cl_3C\text{–}CCl_3)_o \cdot \exp[-(k_1 + k_2)t] \tag{5}$$

The corresponding governing equations and integrated rate expressions for pentachloroethane and tetrachloroethylene are:

$$\frac{d(Cl_3C\text{–}CHCl_2)}{dt} = k_1\cdot(Cl_3C\text{–}CCl_3) - k_3\cdot(Cl_3C\text{–}CHCl_2) \tag{6}$$

$$(Cl_3C\text{–}CHCl_2) = (Cl_3C\text{–}CCl_3)_o \cdot \frac{k_1}{k_3 - (k_1 + k_2)} \times [\exp -(k_1 + k_2)t - \exp(-k_3 t)] \tag{7}$$

$$\frac{d(Cl_2C{=}CCl_2)}{dt} = k_2\cdot(Cl_3C\text{–}CCl_3) + k_3\cdot(Cl_3C\text{–}CHCl_2) \tag{8}$$

$$(Cl_2C{=}CCl_2) = (Cl_3C\text{–}CCl_3)_o \cdot \left[1 - \frac{k_3 - k_2}{k_3 - (k_1 + k_2)}\exp[-(k_1 + k_2)t] + \frac{k_1}{k_3 - (k_1 + k_2)}\exp(-k_3 t)\right] + (Cl_2C{=}CCl_2)_o \tag{9}$$

If $k_3 \gg k_1 + k_2$, Eq. 9 can be approximated by:

$$(Cl_2C{=}CCl_2) \approx (Cl_3C\text{–}CCl_3)_o\cdot[1 - \exp\{-(k_1 + k_2)t\}] + (Cl_2C{=}CCl_2)_o \tag{10}$$

which at small t can be further simplified to:

$$(Cl_2C{=}CCl_2) \approx (Cl_3C\text{–}CCl_3)_o\cdot(k_1 + k_2)t + (Cl_2C{=}CCl_2)_o \tag{11}$$

Table 2
Estimates of $(k_1 + k_2)$ obtained from flasks spiked with hexachloroethane

Sample treatment	From $Cl_3C–CCl_3$ data	From $Cl_2C{=}CCl_2$ data	
	$10^3(k_1 + k_2)$ (day^{-1})	$10^3(k_1 + k_2)$ (day^{-1})	$10^3(k_1 + k_2)$ ($\mu M\ day^{-1}$)
Unpoisoned[a]	8.4 ± 4.2	2.7 ± 0.2	18 ± 1
Unpoisoned[b]	14 ± 8	2.9 ± 0.4	24 ± 3
Unpoisoned[c]	14 ± 5		
Poisoned[d]	0.1 ± 4.1	−0.2 ± 0.2	−1.3 ± 1.7
Poisoned[e]	6.6 ± 9.3	−0.8 ± 0.3	−5.6 ± 2.0
Poisoned[f]	24 ± 10	−1.2 ± 0.3	−9.6 ± 2.5

Numbers in parentheses represent one standard deviation. Values in units of (day^{-1}) correspond to pseudo-first-order rate constants; values in units of ($\mu M\ day^{-1}$) represent pseudo-zero-order rate constants.
[a]Unpoisoned sample monitored for 1 month.
[b]Sacrificed on day 14 for GC–MS analysis.
[c]Spiked with both penta- and hexachloroethane.
[d]Poisoned with 0.5 mL deoxygenated $HgCl_2$ solution 3 days before spiking with hexachloroethane.
[e]Spiked with 300 μL methanol, incubated 1 week at 25°C, poisoned with 0.5 mL deoxygenated $HgCl_2$ solution, and after 3 additional days spiked with hexachloroethane.
[f]Poisoned with 0.5 mL deoxygenated $HgCl_2$ solution 3 days before spiking with hexachloroethane; sacrificed on day 14 for GC–MS analysis.

Alternatively, if we assume that hexachloroethane transformation follows a pseudo-zero-order decay model, then the transformation rates and integrated rate expressions for hexachloroethane, pentachloroethane and tetrachloroethylene can be given as:

$$\frac{d(Cl_3C–CCl_3)}{dt} = -(k_1 + k_2) \tag{12}$$

$$(Cl_3C–CCl_3) = (Cl_3C–CCl_3)_o - (k_1 + k_2)t \tag{13}$$

$$\frac{d(Cl_3C–CHCl_2)}{dt} = k_1 - k_3 \cdot (Cl_3C–CHCl_2) \tag{14}$$

$$(Cl_3C–CHCl_2) = \frac{k_1}{k_3}[1 - \exp(-k_3 t)] \tag{15}$$

$$\frac{d(Cl_2C{=}CCl_2)}{dt} = k_2 + k_3 \cdot (Cl_3C–CHCl_2) \tag{16}$$

$$(Cl_2C{=}CCl_2) = (k_1 + k_2)t - \frac{k_1}{k_3}[1 - \exp(-k_3 t)] + (Cl_2C{=}CCl_2)_o \tag{17}$$

If $k_3 \gg k_1$, Eq. 17 simplifies to:

$$(Cl_2C{=}CCl_2) \approx (k_1 + k_2)t + (Cl_2C{=}CCl_2)_o \tag{18}$$

Rates of hexachloroethane transformation, given by $(k_1 + k_2)$ and summarized in Table 2, were determined by fitting these integrated rate expressions to the hexachloroethane and tetrachloroethylene data for flasks that were spiked with

Table 3
Estimates of k_3 obtained from flasks spiked with pentachloroethane, and comparison to predicted abiotic dehydrohalogenation rates

Sample treatment	k_3 (day^{-1})	pH	k_3/k_{pred}
Unpoisoned	0.127 ± 0.002	6.62	1.20
Unpoisoned[a]	0.093 ± 0.002	6.64	0.84
Poisoned[b]	0.089 ± 0.002	6.56	0.97
Poisoned[c]	0.094 ± 0.002	6.59	0.96

Numbers in parentheses represent one standard deviation.
[a]Spiked with both penta- and hexachloroethane.
[b]Poisoned with 0.5 mL deoxygenated $HgCl_2$ solution 3 days before spiking with pentachloroethane.
[c]Spiked with 300 μL methanol, incubated 1 week at 25°C, poisoned with 0.5 mL deoxygenated $HgCl_2$ solution, and after 3 additional days spiked with pentachloroethane.

hexachloroethane. For purposes of comparison, half-lives on the order of 150 days were observed in homogeneous systems by Criddle et al. (1986), 14–37 min in anaerobic sediment–water slurries by Jafvert and Wolfe (1987), and 3 to > 60 days in the presence of Borden (Ontario, Canada) aquifer sand (Criddle et al., 1986; Reinhard et al., 1990). As previously discussed, the results indicate hexachloroethane reduction only occurs in unpoisoned flasks. Because of scatter in the hexachloroethane data resulting from sample dilution, it is difficult to obtain a precise estimate of $(k_1 + k_2)$ from the hexachloroethane data. Much more precise estimates can be obtained from the tetrachloroethylene data, which were obtained from undiluted samples. Note that estimates of $(k_1 + k_2)$ obtained in this manner were essentially equivalent in the two unpoisoned flasks. The most reliable estimates of the rate of hexachloroethane reduction to pentachloroethane and tetrachloroethylene can be obtained from the unpoisoned flask that was monitored over the course of a month (solid symbols in Fig. 4a); fits to these tetrachloroethylene data yield estimates for $(k_1 + k_2)$ of $(2.7 \pm 0.2) \cdot 10^{-3}$ day^{-1} (pseudo-first-order model) or $(18 \pm 1) \cdot 10^{-3}$ μM day^{-1} (pseudo-zero-order model). These values can be compared to pentachloroethane transformation rates to assess what fraction of the hexachloroethane reduction pathway proceeds through a pentachloroethane intermediate.

Pentachloroethane transformation rates (k_3) determined from data for flasks spiked with pentachloroethane are indicated in Table 3, along with a comparison of measured rates to dehydrohalogenation rates predicted from measured pH and the second-order rate constant determined in a previous study (Roberts and Gschwend, 1991). In all cases, the observed values of k_3 compare reasonably well with the predicted values, indicating pentachloroethane transformation is dominated by the abiotic dehydrochlorination reaction. Note that our calculated k_3-values are significantly greater than our estimates of $(k_1 + k_2)$, validating the approximations used to derive Eqs. 11 and 18 and thus our determination of $(k_1 + k_2)$ from the rate of tetrachloroethylene accumulation. We should, however, point out that estimating $(k_1 + k_2)$ from tetrachloroethylene accumulation according to Eq. 11 or

Eq. 18 assumes that this compound represents the sole product of pentachloroethane transformation. Calculations suggest that the resulting error in k_1- and k_2-values is on the order of $\pm 10\%$, which we feel is within experimental error.

Estimates of $k_1/(k_1 + k_2)$, that is, the fraction of the hexachloroethane reduction pathway that proceeds via a pentachloroethane intermediate, can be obtained via several different methods. Observed concentrations of pentachloroethane in the unpoisoned groundwater sample spiked with hexachloroethane (solid symbols, Fig. 4a) can be fit as a function of time with k_1 as a fitting parameter via a nonlinear least-squares regression. The data can be fit either to Eq. 7 or Eq. 15, depending on whether a pseudo-first-order or a pseudo-zero-order model for hexachloroethane transformation is felt to be more appropriate. Observed pentachloroethane concentrations were fit to Eq. 7 by constraining the value of $(k_1 + k_2)$ to equal the value obtained from a fit to the tetrachloroethylene data via Eq. 11. For both the pseudo-first-order and the pseudo-zero-order models, k_3 was constrained to equal the rate of the abiotic dehydrohalogenation reaction predicted from the measured pH. If $k_1/(k_1 + k_2)$ is determined in this manner, the results indicate that $\sim 11\%$ of the hexachloroethane transformation proceeds through a pentachloroethane intermediate if a pseudo-first-order decay model is assumed for hexachloroethane, $\sim 12\%$ if a pseudo-zero-order model is assumed.

One problem with calculating the fraction of hexachloroethane reduction that proceeds through a pentachloroethane intermediate by fitting observed pentachloroethane concentrations as a function of time in this manner is that the pentachloroethane concentrations determined by direct aqueous injection GC were close to our detection limit, and thus may be particularly subject to error. An alternative method of assessing the fraction $k_1/(k_1 + k_2)$ is to use the data for the unpoisoned sample which was sacrificed for solvent extraction and GC–MS analysis (open symbols, Fig. 4a). The concentration of pentachloroethane in this solvent extract, as determined by GC–ECD, is subject to much less uncertainty than concentrations determined from direct aqueous injection GC, although data are available for only one point in time rather than for a complete time series. If we substitute this estimate of the pentachloroethane concentration at known t into Eq. 7 or Eq. 15, constraining $k_3 = 9.9 \cdot 10^{-2}$ day^{-1} and $(k_1 + k_2) = 2.7 \cdot 10^{-3}$ day^{-1} or $18 \cdot 10^{-3}$ μM day^{-1}, we can solve for k_1. Results of such calculations indicate that $\sim 21\%$ of the hexachloroethane transformation proceeds through a pentachloroethane intermediate assuming a pseudo-first-order decay model, $\sim 29\%$ if a pseudo-zero-order model is assumed.

4. Discussion

The absence of hexachloroethane transformation in $HgCl_2$-poisoned groundwater samples indicates that its reduction is microbially mediated under the conditions investigated, while the close correspondence of pentachloroethane disappearance rates to rates predicted on the basis of measured pH and laboratory-derived second-order rate constants implies that its transformation is dominated by abiotic

dehydrohalogenation to tetrachloroethylene. Although only trace quantities of pentachloroethane accumulated in unpoisoned flasks spiked with hexachloroethane, a comparison of the relative kinetics of hexachloroethane and pentachloroethane transformation indicates that at least 11–29% of the hexachloroethane reduction proceeds via a pentachloroethane intermediate. Note that these calculations assume a pentachloroethane transformation rate equal to the abiotic dehydrohalogenation rate; if pentachloroethane were to undergo transformation at a faster rate, these calculations would underestimate this fraction. For example, if we assume pentachloroethane reacts at a rate 20% faster than the abiotic rate (as observed in an unpoisoned sample spiked with pentachloroethane), we can estimate (according to a pseudo-zero-order model for hexachloroethane decay) that the fraction $k_1/(k_1 + k_2)$ increases from 29% to 35%. To the extent that hexachloroethane reduction does pass through a pentachloroethane intermediate, the slow step in the reaction sequence is the initial, microbially-mediated reduction step; the subsequent abiotic dehydrohalogenation reaction proceeds at a substantially faster rate. It is often assumed that microbial transformation processes involved in the fate of halogenated compounds will be much more rapid (and thus far more important) than abiotic processes; our results for pentachloroethane indicate that this is not necessarily the case.

The appearance of pentachloroethane in samples spiked with hexachloroethane indicates that at least part of the hexachloroethane reduction occurs via a free-radical mechanism. We cannot determine whether the remaining fraction proceeds via a two-electron (nucleophilic elimination) reduction process or whether it occurs via two sequential one-electron transfer steps. The rate of one-electron transfer to an enzyme-bound radical, thus yielding an enzyme-bound carbanion that undergoes an elimination step to form tetrachloroethylene, may merely be faster than the rate of dissociation of the enzyme-bound radical, followed by abstraction of a hydrogen atom to yield pentachloroethane.

Although we have observed a close correspondence of predicted vs. observed disappearance rates for pentachloroethane, we should note that this may not always be the case for other substrates subject to dehydrohalogenation reactions. Pentachloroethane, with a large Brønsted coefficient β, is expected to be much less sensitive to buffer catalysis than halogenated compounds with intermediate values of β. This issue was discussed in detail by Roberts and Gschwend (1991); Brønsted coefficients for other polyhalogenated alkanes are discussed in Roberts et al. (1993). Depending on the susceptibility of the particular halogenated alkane to buffer catalysis, dehydrohalogenation rates in the environment might be substantially greater than rates predicted on the basis of experiments conducted in simple aqueous solution.

These results should not be taken to imply that hexachloroethane reduction invariably proceeds via a free-radical mechanism, nor that its transformation is always directly mediated by microorganisms. Results of preliminary investigations conducted in sulfidic lake water (Roberts, 1991) suggest that in this environment, hexachloroethane reduction is dominated by abiotic reactions. Further, the absence of pentachloroethane production in filter-sterilized lake water samples indicates that reduction may be occurring by a nucleophilic elimination mechanism. Further studies

are required to better understand what factors may influence pathways adopted for reductive dehalogenation before valid models relating structure to reactivity can be derived.

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