Prepared in cooperation with the
TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION,
DIVISION OF SUPERFUND

Biodegradation of Chlorinated Ethenes at a Karst Site in Middle Tennessee

Water-Resources Investigations Report 99-4285

U.S. Department of the Interior
U.S. Geological Survey

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By Tom D. Byl *and* Shannon D. Williams

U.S. GEOLOGICAL SURVEY

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Nashville, Tennessee
2000

U.S. DEPARTMENT OF THE INTERIOR
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CONVERSION FACTORS, VERTICAL DATUM, WATER-QUALITY UNITS, AND ACRONYMS

Multiply	By	To obtain
micrometer (µm)	0.00003937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
cubic meter (m^3)	35.31	cubic foot
kilometer (km)	0.6214	mile
square meter (m^2)	10.6	square foot
liter (L)	33.82	liquid ounce
microliter (µL)	0.00003381	liquid ounce
milliliter (ml)	0.03381	liquid ounce
hectare (ha)	2.471	acre
kilogram (kg)	2.205	pound avoirdupois
liters per minute (L/min)	0.2642	gallons per minute

Temperature in degrees Celsius (°C) can be converted to degrees Fahrenheit (°F) as follows: °F = 1.8 x °C + 32

Concentrations in micromoles per liter (µM/L) and moles per liter (M/L) can be converted to micrograms per liter (µg/L) and grams per liter (g/L), respectively, by multiplying by the atomic or formula weight of the constituent.

Sea level: In this report “sea level” refers to the National Geodetic Vertical Datum of 1929—a geodetic datum derived from a general adjustment of the first-order level nets of the United States and Canada, formerly called Sea Level Datum of 1929.

WATER-QUALITY UNITS

CFU/100 mL	colony forming units per 100 milliliters of sample
mg/L	milligrams per liter
mV	millivolts
µg/L	micrograms per liter
m/h	meters per hour
cells/mL	cells per milliliter
µm	micrometer

ACRONYMS

AMO	Ammonia monooxygenase
DCA	1,2-Dichloroethane
DCE	Dichloroethylene
*c*DCE	*cis*-1,2-Dichloroethylene
*t*DCE	*trans*-1,2-Dichloroethylene
DO	Dissolved oxygen
DNAPL	Dense nonaqueous phase liquid
MMO	Methane monooxygenase
NADH	Nicotinamide adenine dinucleotide
ORP	Oxidation-reduction potential
PB	Phosphate buffer
PCE	Tetrachloroethylene
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
TCE	Trichloroethylene
TDEC-DSF	Tennessee Department of Environment and Conservation, Division of Superfund
USGS	U.S. Geological Survey
VC	Vinyl chloride

Biodegradation of Chlorinated Ethenes at a Karst Site in Middle Tennessee

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Abstract

This report presents results of field and laboratory investigations examining the biodegradation of chlorinated ethenes in a karst aquifer contaminated with trichloroethylene (TCE). The study site, located in Middle Tennessee, was selected because of the presence of TCE degradation byproducts in the karst aquifer and available site hydrologic and chlorinated-ethene information. Additional chemical, biological, and hydrologic data were gathered to evaluate whether the occurrence of TCE degradation byproducts in the karst aquifer was the result of biodegradation within the aquifer or simply transport into the aquifer. Geochemical analysis established that sulfate-reducing conditions, essential for reductive dechlorination of chlorinated solvents, existed in parts of the contaminated karst aquifer. Other areas of the aquifer fluctuated between anaerobic and aerobic conditions and contained compounds associated with cometabolism, such as ethane, methane, ammonia, and dissolved oxygen. A large, diverse bacteria population inhabits the contaminated aquifer. Bacteria known to biodegrade TCE and other chlorinated solvents, such as sulfate-reducers, methanotrophs, and ammonia-oxidizers, were identified from karst-aquifer water using the RNA-hybridization technique. Results from microcosms using raw karst-aquifer water found that aerobic cometabolism and anaerobic reductive-dechlorination degradation processes were possible when appropriate conditions were established in the microcosms. These chemical and biological results provide circumstantial evidence that several biodegradation processes are active in the aquifer. Additional site hydrologic information was developed to determine if appropriate conditions persist long enough in the karst aquifer for these biodegradation processes to be significant. Continuous monitoring devices placed in four wells during the spring of 1998 indicated that pH, specific conductance, dissolved oxygen, and oxidation-reduction potentials changed very little in areas isolated from active ground-water flow paths. These stable areas in the karst aquifer had geochemical conditions and bacteria conducive to reductive dechlorination of chlorinated ethenes. Other areas of the karst aquifer were associated with active ground-water flow paths and fluctuated between anaerobic and aerobic conditions in response to rain events. Associated with this dynamic environment were bacteria and geochemical conditions conducive to cometabolism. In summary, multiple lines of evidence developed from chemical, biological, and hydrologic data demonstrate that a variety of biodegradation processes are active in this karst aquifer.

INTRODUCTION

The biodegradation of chlorinated solvents such as trichloroethylene (TCE) in aquifers consisting of unconsolidated materials has been well documented, and several guidelines have been developed from those studies (Remediation Technologies Development Forum, 1997; U.S. Environmental Protection Agency, Region 4, 1997; and Wiedemeier and others,

1998). The guidelines for evaluating sites contaminated with chlorinated solvents incorporate hydrogeology, microbiology, and geochemistry, and groundwater modeling. A large component of those guidelines is for evaluating if significant biodegradation is taking place. One environment where chlorinated-solvent biodegradation has not been adequately investigated is karst aquifers because of the complex hydrology associated with these aquifers. Although chlorinated-solvent biodegradation products such as *cis*-1,2-dichloroethylene (*c*DCE) have been detected in karst aquifers, little research has been done in examining if biodegradation occurred in the karst aquifers or if the biodegradation products originated elsewhere.

Approximately 40 percent of the United States east of the Mississippi River is underlain by various types of karst aquifers (Quinlan, 1989), and more than two-thirds of the State of Tennessee is underlain by carbonate rocks and can be classified as karst (Wolfe and others, 1997). Potential industrial sources of ground-water contamination are common in karst regions; however, the fate and transport of contaminants such as chlorinated solvents in karst areas are poorly understood because of the distinctive hydraulic characteristics of karst aquifers (Field, 1993). Groundwater models that predict the fate and transport of contaminants in sandy aquifers have limited application to karst aquifers. Most natural attenuation and bioremediation guidelines specify that ground-water models are not applicable in fractured rock or karst aquifers (U.S. Environmental Protection Agency, 1997).

The U.S. Geological Survey (USGS), in cooperation with the Tennessee Department of Environment and Conservation, Division of Superfund (TDEC-DSF), is conducting a study of the occurrence, fate, and transport of chlorinated solvents in karst regions of Tennessee. One objective of this study was to examine the role of biodegradation in the fate and transport of chlorinated solvents in karst aquifers. This report presents results from field and laboratory data collected to examine chlorinated-ethene biodegradation at a TCE-contaminated karst site in Middle Tennessee. The study site was selected because of the presence of chlorinated-solvent degradation products in ground water and because of the availability of hydrologic and chlorinated-ethene data in TDEC-DSF files. The objectives of this report are to:

1. Summarize the current understanding of:
    a. chlorinated-ethene biodegradation processes and
    b. biodegradation of organic compounds in bedrock aquifers.
2. Characterize the microorganisms and geochemical conditions present in ground water at the study site.
3. Evaluate which chlorinated-ethene degradation processes may be occurring in ground water at the study site.
4. Document lessons learned from this study that may assist future investigations of chlorinated-solvent biodegradation in karst areas.

The body of the report is divided into six main sections. The first section is a general overview of chlorinated-ethene degradation processes. The second section reviews previous studies relevant to the effects of hydrology, bacteria, and geochemical conditions on the biodegradation of organic compounds in bedrock aquifers. The third section describes the study site with emphasis on hydrogeology and monitoring and remedial activities. The fourth section describes methods and procedures used to collect and interpret geochemical, chlorinated ethene and degradation product, and microbiological data. The fifth section presents results and interpretation of the multiple lines of evidence used to examine chlorinated-ethene degradation at the study site. Finally, the sixth section describes lessons learned during this study.

CHLORINATED-ETHENE BIODEGRADATION PROCESSES

Microbial organisms use a wide variety of metabolic processes to generate energy and maintain cellular growth. These processes involve the transfer of electrons from an electron donor (food source) to an electron acceptor. Three categories of metabolic processes are involved in the biological degradation of chlorinated ethenes. Reductive dechlorination is an anaerobic process in which chlorinated ethenes are used as electron acceptors (McCarty, 1994; Montgomery and others, 1994). Cometabolism is an aerobic process in which chlorinated ethenes are degraded as a result of fortuitous biochemical interactions which yield no benefit to the bacteria (Alvarez-Cohen and McCarty, 1991; Hanson and Brusseau, 1994; Ely, Williamson, and others, 1995). Direct oxidation is an aerobic or mildly anaerobic (iron reducing) process in

which sparsely chlorinated ethenes are used as electron donors (McCarty and Semprini, 1994; Bradley and Chapelle, 1996). One or all of these processes could be occurring at a given site, depending on environmental conditions.

Reductive Dechlorination

Generally, organic molecules with abundant carbon-hydrogen bonds are good food sources (electron donors) because they contain high-energy electrons. Highly chlorinated solvents such as tetrachloroethylene (PCE) and TCE, however, are electron poor because they have chlorine-carbon bonds. These compounds are likely to be used by bacteria as final electron acceptors instead of electron donors (Wiedemeier and others, 1998). During reductive dechlorination, chlorine atoms are replaced by electrons coupled to hydrogen atoms, resulting in sequential dechlorination from PCE to TCE to dichloroethylene (DCE) to vinyl chloride (VC) to ethene (fig. 1). During reductive dechlorination, *cis*-1,2-dichloroethylene (*c*DCE) is the most commonly formed isomer of DCE.

Soil and ground-water bacteria use a variety of natural electron acceptors. The use of these final electron acceptors is not arbitrary but is based on energy-transfer efficiency and availability (Montgomery and others, 1994). The most common inorganic electron acceptor in ground water is oxygen. Once the oxygen has been depleted, bacteria will preferentially use the next most efficient electron acceptor; usually this is nitrate (NO_3^-) or insoluble manganese (Mn^{4+}). After NO_3^- and Mn^{4+} have been depleted, the bacteria will use insoluble ferric iron (Fe^{3+}), followed by sulfate (SO_4^{2-}), and carbon dioxide (CO_2), respectively. The byproducts resulting from transfer of electrons to various electron acceptors are shown in table 1.

Dechlorination of PCE and TCE to DCE can occur under mildly reducing conditions such as NO_3^- or Fe^{3+} reduction (fig. 2); however, the dechlorination of DCE to VC, and VC to ethene requires the stronger reducing conditions of SO_4^{2-}-reduction or methanogensis (Vogel and others, 1987). The effectiveness of chlorinated solvents to serve as electron acceptors is proportional to the number of chlorine molecules attached (Vogel and McCarty, 1985). For example, PCE is more likely to serve as an electron acceptor and lose a chlorine atom through reductive dechlorination than is TCE, DCE, or VC.

Aerobic Cometabolism

Wilson and Wilson (1985) first reported that TCE was degraded under aerobic conditions by methanotrophic bacteria in a soil enriched with CH_4 and

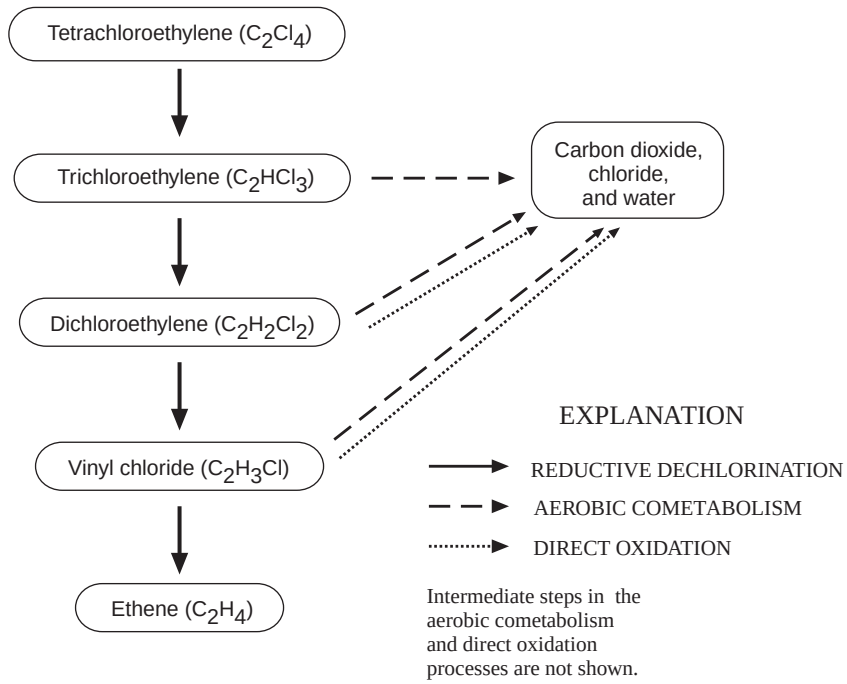

Figure 1. Common biological degradation processes for chlorinated ethenes.

Table 1. Terminal electron acceptors and byproducts involved in microbial processes

Electron acceptor[1]	Byproduct	Microbial process
Oxygen (O_2)	CO_2	Aerobic respiration
Nitrate (NO_3^-)	Ammonia (NH_3)	NO_3^- reduction
Manganese (Mn^{4+})	Manganese (Mn^{2+})	Mn^{4+} reduction
Iron (Fe^{3+})	Iron (Fe^{2+})	Fe^{3+} reduction
Sulfate (SO_4^{2-})	Sulfide (S^{2-})	SO_4^{2-} reduction
Carbon dioxide (CO_2)	Methane (CH_4)	Methanogenesis

[1]The electron acceptors are arranged from most energy efficient at the top to least efficient.

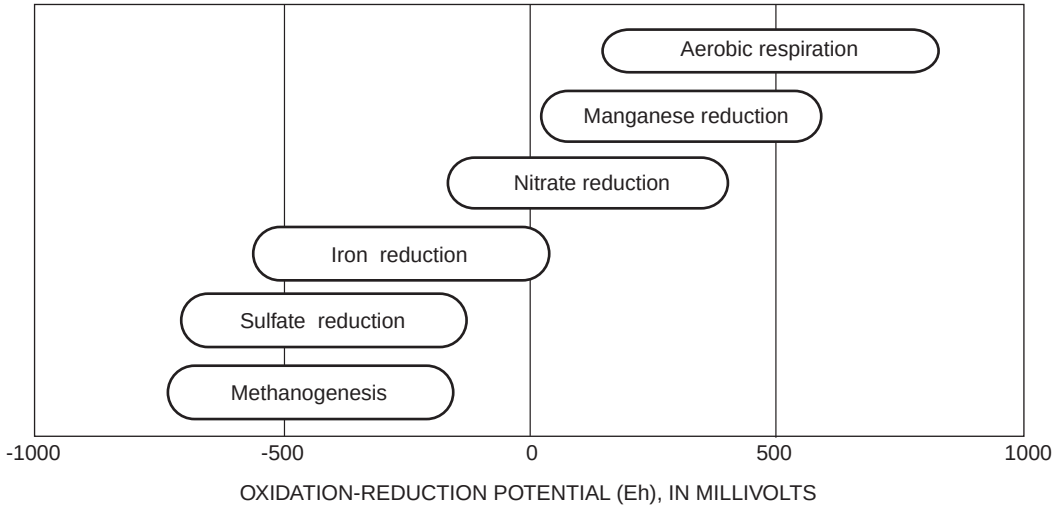

Figure 2. Oxidation-reduction potentials for selected microbial processes. (Modified from Stumm and Morgan, 1981.)

oxygen (O_2). Further studies revealed that the methane monooxygenase (MMO) enzyme was responsible for catalyzing the oxidation of TCE (Alvarez-Cohen and McCarty, 1991; Henry and Grbić-Galić, 1994). Other oxygenase enzymes such as ammonia monooxygenase (AMO) (Arciero and others, 1989; Rasche and others, 1991) and toluene dioxygenase (Nelson and others, 1988; Hopkins and others, 1993; Heald and Jenkins, 1994) also have been shown to oxidize certain chlorinated solvents. This oxidation reaction is called cometabolism because the reaction uses metabolic enzymes, but does not contribute any energy in return.

These oxidative enzymes catalyze a reaction that incorporates O_2 into the target substrates (fig. 3). This oxidation reaction requires an energy molecule such as nicotinamide adenine dinucleotide (NADH) to incorporate the O_2. The enzymes lack the ability to efficiently distinguish CH_4, NH_3, or toluene from certain chlorinated solvents. This lack of substrate specificity results in a chemical reaction in which oxygen is incorporated into the solvent molecule forming an unstable molecule such as TCE epoxide during MMO cometabolism (Alvarez-Cohen and McCarty, 1991). The unstable molecule will spontaneously degrade to one of several chloroacetic acids, such as dichloroacetic acid. These chloroacetic acids are soluble in water and will slowly degrade to CO_2, chloride, and water (fig. 3).

Cometabolism is limited to chlorinated solvents that have at least one hydrogen atom attached to the carbon (fig. 1). No studies have found a cometabolic pathway capable of degrading PCE (Henry and Grbić-Galić, 1994). Cometabolism tends to be an unsustainable process under stagnant conditions because of substrate competition and enzyme inhibition and inactivation (Ely and others, 1997). Competition occurs between the natural substrates, such as CH_4, NH_3, or toluene, and chlorinated solvents for binding

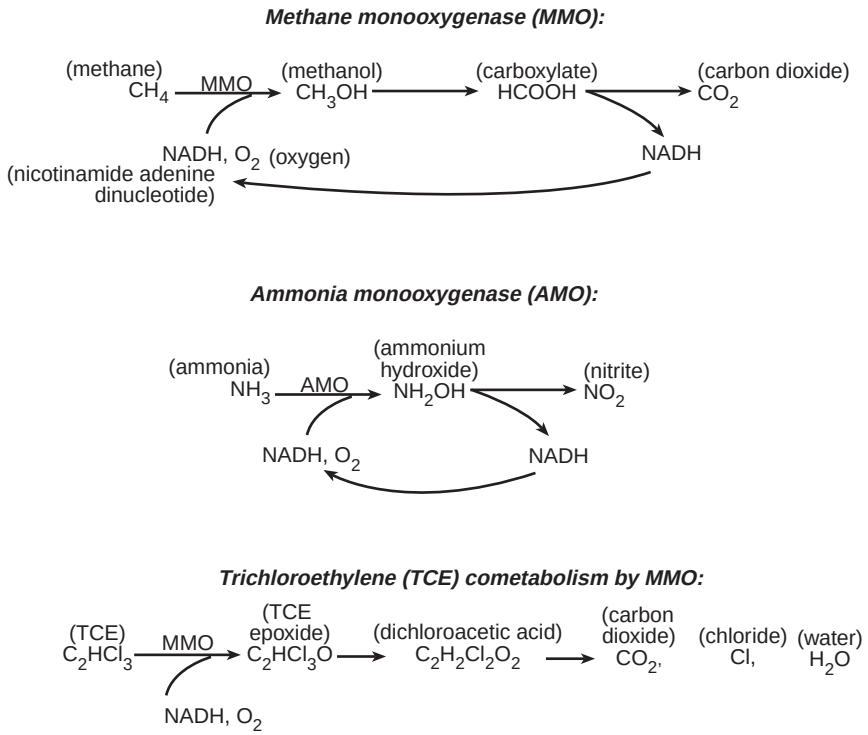

Figure 3. Examples of cometabolic processes that degrade chlorinated ethenes. (Information from Arciero and others, 1989; Hanson and Brusseau, 1994; Henry and Grbić-Galić, 1994; Ely, Williamson, and others, 1995.)

on the active site of the nonspecific oxygenase enzyme (Semprini and others, 1991; Broholm and others, 1992). Competitive inhibition occurs when enzymes cometabolize chlorinated solvents to the exclusion of natural substrates, ultimately depleting the bacteria of energy molecules (Chang and Alvarez-Cohen, 1995) (NADH in fig. 3). The TCE oxidation byproducts such as TCE epoxide may result in the inactivation of the oxygenase activity caused by damage to the enzymes (Ely, Hyman, and others, 1995). Inhibition and inactivation may be overcome by additional natural substrates (Alvarez-Cohen and McCarty, 1991; Ely and others, 1997).

Direct Oxidation

Recent studies have reported that chlorinated solvents with only one or two chlorine atoms (the least oxidized compounds) can serve as electron donors by bacteria. Several studies have shown that VC and 1,2-dichloroethane (DCA) can serve as food under aerobic conditions (McCarty and Semprini, 1994). Iron-reducing bacteria also can mineralize VC (Bradley and Chapelle, 1996) and DCE (Bradley and Chapelle, 1997) as a food source under aerobic conditions. Direct oxidation is limited to degrading lightly chlorinated solvents such as DCA, DCE, and VC; however, direct oxidation may serve a vital role in the sequential steps of chlorinated-solvent biodegradation. Aerobic or iron-reducing zones are commonly found downgradient of methanogenic or sulfate-reducing zones. Thus, partially dechlorinated byproducts (DCE and VC) produced by reductive dechlorination in the methanogenic or sulfate-reducing zones may be consumed in the more oxidized zones downgradient.

PREVIOUS STUDIES RELEVANT TO BIODEGRADATION IN BEDROCK AQUIFERS

Previous studies examining the biodegradation of organic compounds in fractured rock or karst settings generally have focused on biodegradation in the overburden or regolith above bedrock (Christensen and others, 1997). Wiedemeier and others (1998) described a case study in New Hampshire, where biodegradation of benzene, toluene, and TCE in overburden was documented by using microcosms and field

data. Few studies have examined biodegradation in bedrock aquifers. Major and others (1995) determined that microorganisms in a fractured bedrock aquifer in New York dechlorinated TCE to ethene. Acetone and methanol, which were also present in the aquifer, served as electron donors, and SO_4^{2-} and CO_2 were the predominant electron acceptors. Chapelle and others (1995) examined the microbial degradation of organic carbon in the Floridan aquifer, which consists of Tertiary limestone characterized by locally extensive conduits and karst topography. These researchers experienced difficulty in using patterns of electron acceptor consumption as a means to identify discrete oxidation-reduction potential zones in the aquifer; however, they did identify sulfate-reducing and methanogenic conditions in the aquifer.

The lack of studies examining biodegradation in karst aquifers may be due to the widespread perception that contaminants are rapidly flushed out of karst aquifers. In highly developed and well-connected conduit systems, the rate of contaminant migration is expected to be much faster than the rate of biodegradation. Field (1993) states that remediation techniques such as ground-water extraction or bioremediation are impractical in karst aquifers dominated by conduit flow; however, he also states that the belief that contaminants are rapidly flushed out of karst aquifers is a popular misconception. Large volumes of water may be trapped in fractures along bedding planes and other features isolated from active ground-water flow paths in karst aquifers (Wolfe and others, 1997). In areas isolated from the major ground-water flow paths, contaminant migration may possibly be slow enough that biodegradation could reduce contaminant mass if favorable microorganisms, food sources, and geochemical conditions are present.

Some researchers have implied that natural bioremediation in karst or fractured rock is unlikely to occur because of the microbiological characteristics of karst aquifers. Many microbes attach to the aquifer matrix and are not motile (swimmers); therefore, contact between those microbes and dissolved contaminants would be limited by the lack of surface area in fractures and conduits. However, adequate information does not exist to accept or reject the assumption that nonmotile bacteria dominate bacteria communities in karst conduits. Other researchers have implied that biodegradation in bedrock aquifers is limited by a lack of appropriate microorganisms. Vogel (1994), for example, states that hydrologic and geologic characteristics that would not benefit the natural bioremediation of chlorinated solvents include fractured rock systems where small microbial populations exist.

A few reports in the literature dispute that statement. Typical microbial numbers in material from unconsolidated aquifers have been reported to range from 1×10^4 to 1×10^7 cells/mL (Ghiorse and Wilson, 1988; Bedient and others, 1994). Studies have shown that water from bedrock (granite and karst) aquifers also may contain microbial populations within this range. For example, total microbial populations of approximately 1×10^5 cells/mL were detected in ground-water samples from a deep granite formation [800 meters (m) below land surface] in Sweden (Pedersen and Ekendahl, 1990). Total microbial populations of 9.7×10^5 to 8.5×10^6 cells/mL and heterotrophic bacteria populations of 3.5×10^3 to 5.0×10^5 cells/mL were detected in ground-water samples from a gasoline-contaminated karst aquifer in Missouri (O'Connor and Brazos, 1991).

Ground-water studies have detected significant bacteria concentrations in water samples from karst aquifers in Tennessee. For example, fecal coliform and fecal streptococci bacteria were detected and cultured in ground-water samples from wells completed as deep as 90 m below land surface in Williamson County in Middle Tennessee (Hanchar, 1991). Fecal coliform and fecal streptococci bacteria also have been detected in several wells in Bedford and Coffee Counties in Middle Tennessee (Roman-Mas and others, 1991) and in Grainger County in East Tennessee (Weaver and others, 1994). The detection of viable fecal bacteria suggests that a wide variety of other types of bacteria also may be present in karst aquifers in Tennessee.

Microorganisms responsible for chlorinated-ethene degradation have been detected in water samples from bedrock aquifers in other regions. Denitrifying and sulfate-reducing bacteria were identified in nutrient-poor, anoxic ground water from granite formations (240 m below land surface) in Manitoba, Canada, and lab studies indicated that the bacteria were able to mineralize a variety of organic substances (Jain and others, 1997).

Aerobic and anaerobic bacteria have several characteristics that may improve their ability to degrade chlorinated ethenes in karst aquifers. These characteristics include diverse metabolisms and the ability to withstand fluctuating anaerobic and aerobic conditions. These bacteria can derive energy from a

wide variety of foods. Research has shown that methanotrophs are capable of using ammonia instead of methane (Bedard and Knowles, 1989; Dalton, 1977) and that ammonia-oxidizing bacteria are capable of using methane (Arciero and others, 1989; Bedard and Knowles, 1989). Research also indicates that ammonia-oxidizing bacteria and methanotrophs have slow death rates and can shift into a dormant stage for extended periods when growth substrates are absent. Methanotrophs also function at low oxygen concentrations and are not inhibited until oxygen is completely consumed (Henry and Grbić-Galić, 1994).

Many bacteria, particularly those adapted for aqueous environments, are capable of moving through the use of flagella. For example, the soil bacteria order Pseudomonales have flagella at one or both ends (Chapelle, 1993) allowing them to swim upgradient or downgradient. Many chemolithotrophic bacteria such as ammonia- and sulfur-oxidizers develop flagella to swim towards or away from chemical stimuli as the need arises. At other times they become nonmotile to conserve energy or metabolize material that flows past them (Atlas, 1987).

Further evidence that bacteria are capable of degrading solvents in a conduit was revealed in a laboratory experiment that examined the aerobic degradation of TCE by methanotrophs in a 30-m by 5.0-cm stainless-steel pipe. TCE contaminated water (approximately 2 mg/L) was pumped into the pipe at a rate of 1 liter per minute, and methanotrophs (32 milligrams dry weight per liter) were added to the pipe water at a rate of 0.1 liter per minute. Results indicated that the TCE was reduced by 88 percent as the contaminated water traveled the length of the pipe at a velocity of 110 meters per hour (m/h) (residence time of 0.91 hour) (U.S. Environmental Protection Agency, 1993).

The ground-water velocity used in the experiment was within the range reported for karst conduits (10 to 500 m/h) by Quinlan (1989). The initial TCE concentration in the flow reactor was also within ranges reported for chlorinated solvent contaminated aquifers; however, the concentration of methanotrophs in the flow reactor may have been higher than concentrations typically found in karst aquifers. Assuming that cell mass is equal to 10^{-12} grams/cell (Chapelle, 1993), the concentration of methanotrophs in the flow reactor would be approximately 3×10^3 cell/mL. This concentration is within the range normally reported for total bacteria concentrations in water from karst aquifers, but the concentration does not account for species diversity.

STUDY SITE

The site used in this study is a manufacturing facility located on 65 ha in Marshall County, in Middle Tennessee (fig. 4). The manufacturing facility has been in operation since 1937 and is currently used to assemble air conditioning and heating equipment. A degreaser was installed in 1973 near the south end of the main manufacturing building. The chlorinated solvent TCE was piped underground from an above-ground storage tank to the degreaser. In 1980, the underground pipe connecting the storage tank to the degreaser ruptured and released an estimated 13,000 liters of TCE into a piping trench, allowing TCE to migrate into sanitary sewer pipes and the soil. The highest concentration of TCE (950,000 mg/L) was detected in 1987, when a 61-cm column of dense non-aqueous phase liquid (DNAPL) was measured in a shallow well screened near the top of the bedrock (Wolfe and others, 1997). In 1997, TCE was detected in a shallow well at a concentration (171 mg/L) indicating that DNAPL is still present beneath the manufacturing building.

Approximately 50 ground-water monitoring wells have been completed at the site (fig. 4). Shallow wells are screened near the top of bedrock (as much as 6 m into the bedrock) in a shallow water-bearing zone. Deeper wells range in depth from 18 to 67 m below land surface and are screened in a karst aquifer that consists of several water-bearing zones in the bedrock. Additional well completion data are given in table 2 (at end of the report). Remedial activities at the site include construction of two ground-water collection trenches on the top of the bedrock in 1989, excavation and thermal treatment of 1,150 m^3 of contaminated soil near the above-ground storage tanks in 1995, installation of several ground-water pump-and-treat wells, and construction of an aeration weir in Snell Branch, a small stream that flows through the study site (fig. 4). Air strippers are used to remove chlorinated solvents from water collected from the recovery trenches and the pump-and-treat wells.

The site is underlain by the Ordovician-age Lebanon, Ridley, and Pierce Limestones (Crawford, 1992; Crawford and Ulmer, 1994; Farmer and Hollyday, 1999) (table 3). The Ridley Limestone is susceptible to dissolution, especially where exposed at land surface. Sinkholes are common, and streams flowing off the Lebanon Limestone lose water and sink into the Ridley Limestone. Caves and cave streams primarily develop at contacts between the aquifer and confining

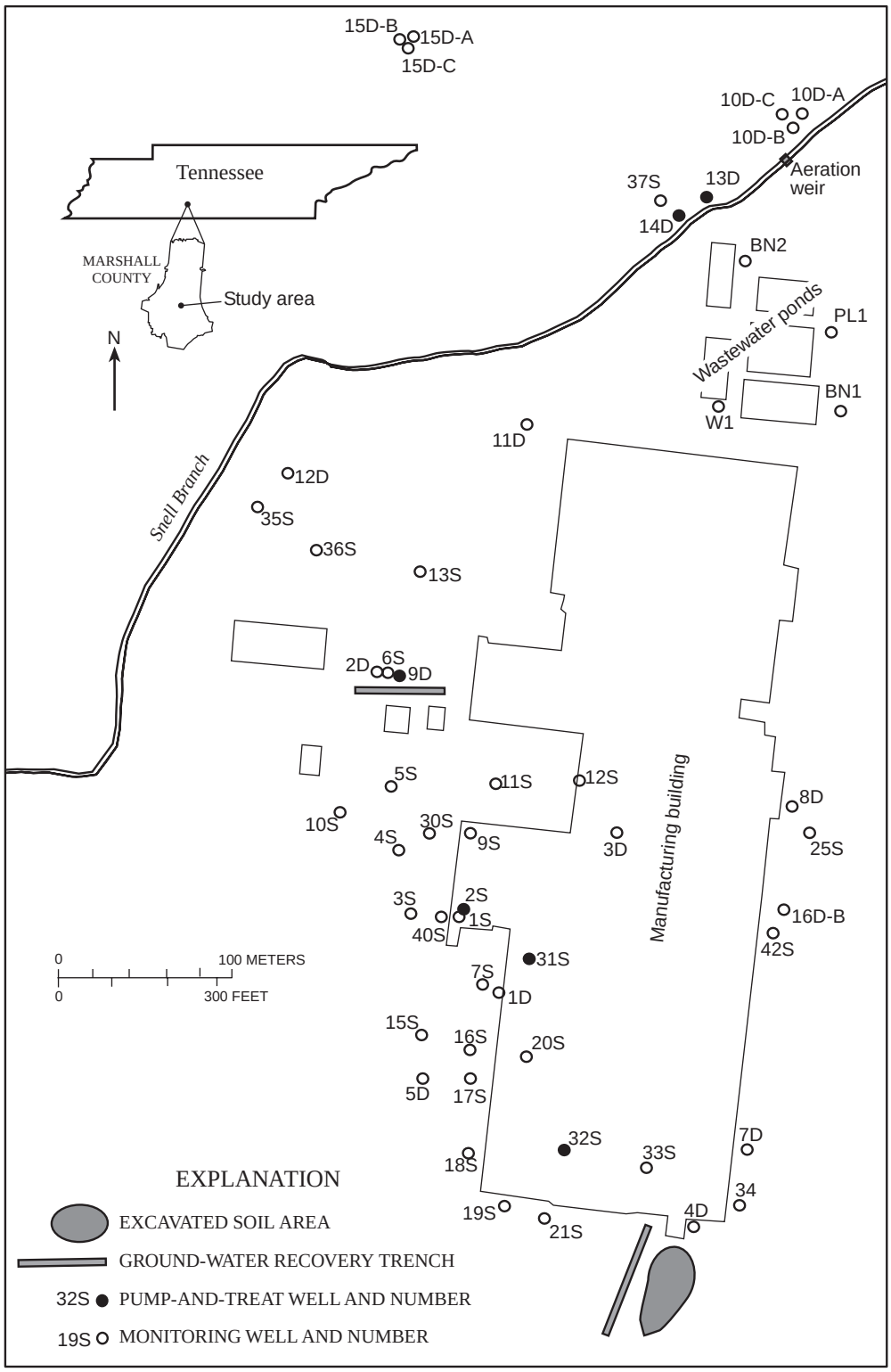

Figure 4. Location of ground-water monitoring and remediation activities at the study site in Marshall County, Tennessee.

Table 3. Geologic and hydrologic units at the study site

Stratigraphic unit	Lithology[1]	Thickness at study site[2] (meters)	Hydrogeologic characteristics at study site[2]
Regolith	Low permeability clay	1 to 6	Shallow water-bearing zone near top of bedrock.
Lebanon Limestone	Relatively pure limestone with beds averaging 5.1 to 7.6 cm in thickness. Limestone beds are separated by partings of calcareous shale ranging from 0 to 1.3 cm in thickness.	6 to 24	Leaky confining unit
Upper Ridley Limestone	Relatively pure, massively bedded limestone with little insoluble material. Beds ranging from 10 to 120 cm in thickness.	31	Karst aquifer
Thin-Bedded Member	Relatively pure limestone with beds 1.3 to 10 cm in thickness. Limestone beds are separated by thin partings of calcareous shale.	9	Confining unit
Lower Ridley Limestone	Similar to upper part of the Ridley Limestone	6	No water-bearing zones
Pierce Limestone	Thinly bedded limestone. Beds average 5.1 cm in thickness. One or more units of massively bedded limestone may be present. Limestone beds are separated by thin partings of calcareous shale as much as 1.3 cm in thickness.	9	Confining unit

[1] From Wilson, 1949.
[2] From J.J. Farmer and E.F. Hollyday, U.S. Geological Survey, written commun., 1999.

units (Crawford, 1992). The Ridley Limestone is exposed just north of the study site (Wilson and Luther, 1963), and Snell Branch sinks into the formation and flows to a spring approximately 2.7 km northeast of the site (Crawford, 1992). Water yields are low unless a well intersects a well-developed conduit or cave stream in the Ridley Limestone (Crawford and Ulmer, 1994).

The general flow direction of the shallow ground water is north-northwest toward Snell Branch creek as indicated by water levels in shallow wells (fig. 5). Water levels in the shallow water-bearing zone do not appear to be affected by the pump-and-treat wells. Along the western side of the manufacturing building, shallow ground water flows toward a trough in the bedrock surface (fig. 6). This trough is downgradient of the original TCE spill and appears to be the main route for horizontal transport of chlorinated ethenes in the shallow water-bearing zone (fig. 7).

Well-driller logs and well-pressure test data from unpublished TDEC-DSF files indicate that the karst aquifer at the site consists of at least three distinctive water-bearing zones located along bedding planes in the upper part of the Ridley Limestone (fig. 8). The first water-bearing zone is located at an altitude of approximately 208 m above sea level and was detected during the construction of wells 3D and 12D and the 10D wells. The second water-bearing zone is located at an altitude of approximately 200 m above sea level. The third zone is located just above the thin-bedded member of the Ridley Limestone at an altitude of 190 m above sea level. The second and third zones were intersected during the construction of several of the deep wells. Water-bearing zones were not detected in the lower part of the Ridley or Pierce Limestones.

Well-driller logs for wells 12D, 10D-A, 10D-B, and 10D-C, which are located near Snell Branch, indicate the presence of several highly fractured zones between the altitudes of 190 and 217 m above sea level. Driller logs for the three 10D wells (fig. 4) describe a highly fractured zone at an altitude of 216 m above sea level that is hydraulically connected to Snell Branch as well as several zones of fractures between the altitudes of 190 and 208 m above sea

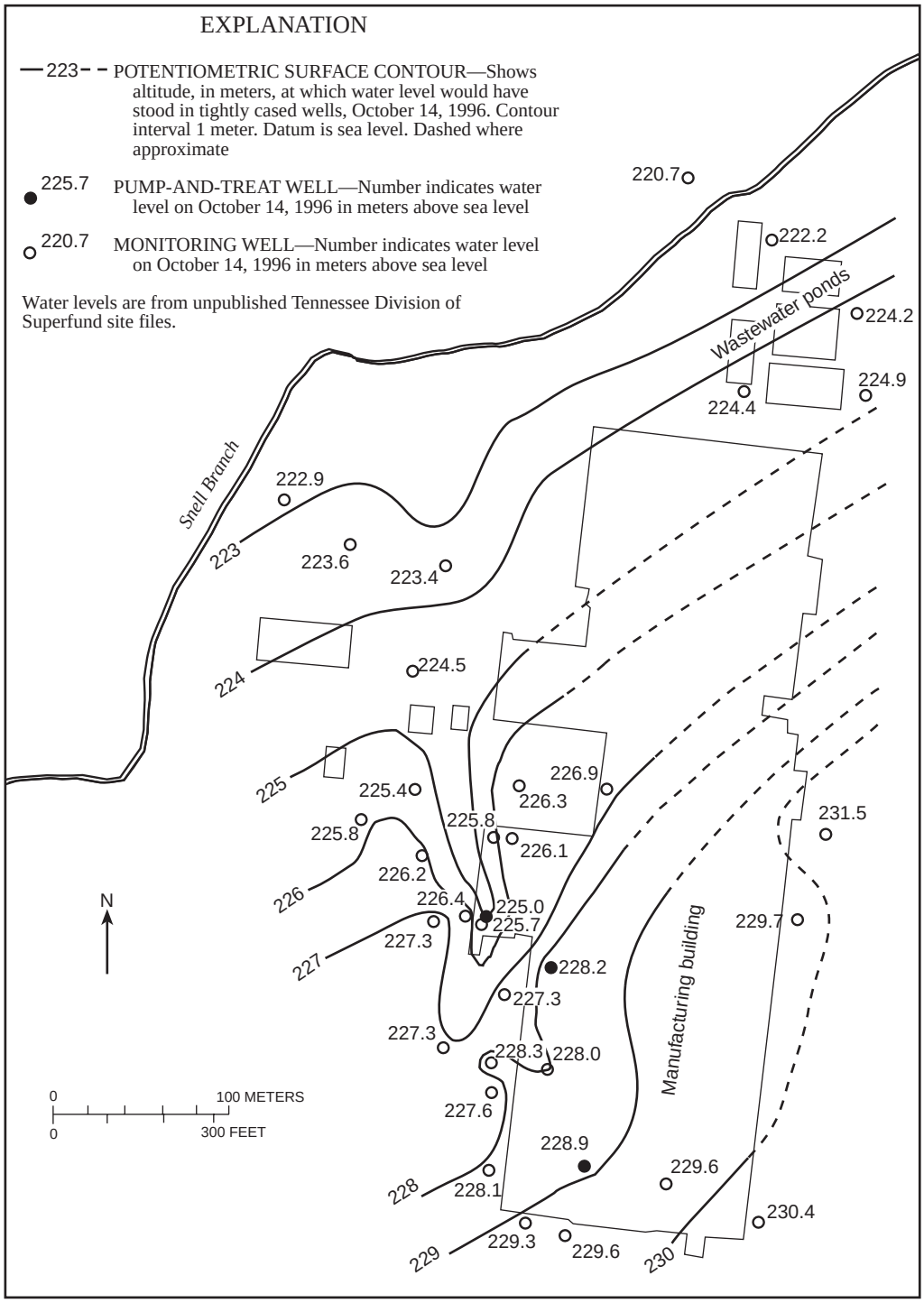

Figure 5. Typical potentiometric surface conditions for the shallow water-bearing zone.

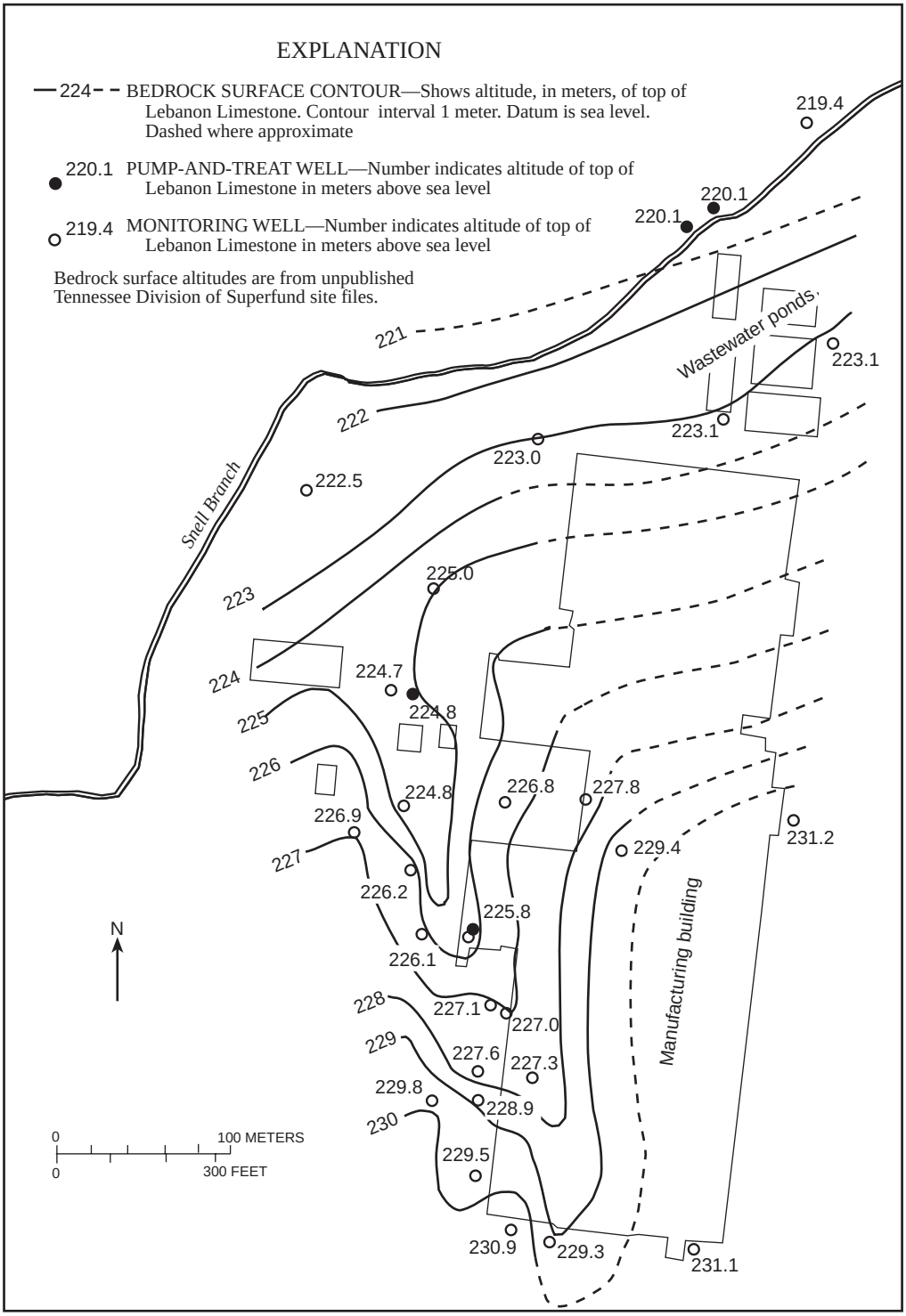

Figure 6. Bedrock surface at the study site.

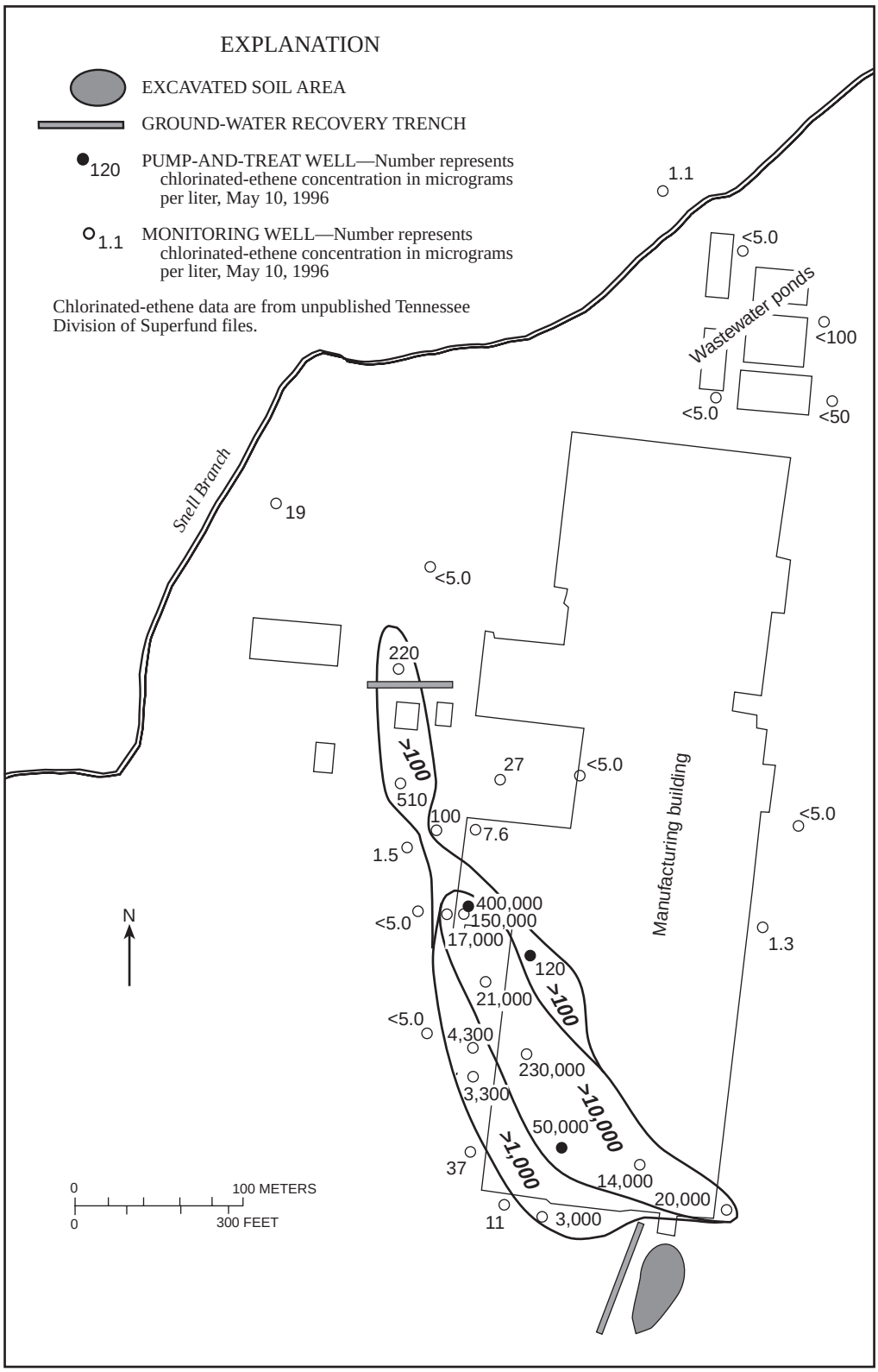

Figure 7. Spatial distribution of chlorinated ethenes in the shallow water-bearing zone.

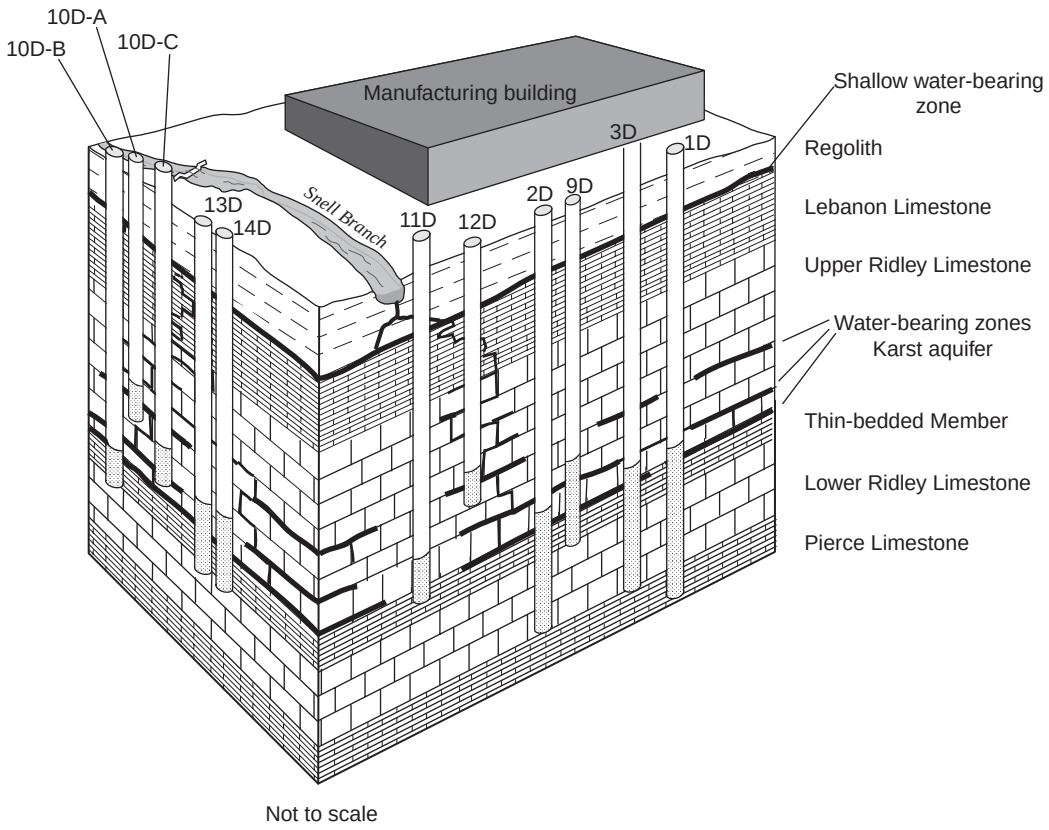

Figure 8. Generalized hydrogeology of the Middle Tennessee study site.

level that are hydraulically connected. During the construction of well 12D, an uncompleted well boring intersected a mud-filled cavity 1.2 m in height at an altitude of 208 m above sea level that was hydraulically connected to Snell Branch. The extensive vertical fracturing beneath Snell Branch, which runs approximately north 40° east through the study site, may be a result of jointing. Geologic investigations described in unpublished Tennessee Division of Superfund site files indicate that the local joint orientation is north 40° east and north 50° west.

Well-completion data indicate that the water-producing zones are not continuous beneath the site, and in some areas fractures appear to be isolated from the major zones of ground-water flow. For example, well 11D intersects the three water-producing zones in the karst aquifer, yet the well produced less than 3 L/min. Other wells intersected water-bearing zones and produced abundant water.

Because of the slight differences in water levels (fig. 9) when the pump-and-treat wells are operating and because the wells are screened in different water-bearing zones, determining the general direction of ground-water flow in the upper part of the Ridley Limestone is difficult. Since well 11D is screened in fractures isolated from the major zones of ground-water flow, changes in the water level of this well lag behind changes in other wells, and the water levels in 11D may be significantly higher or lower than water levels in other deep wells (fig. 9).

Pump-and-treat wells completed in the upper part of the Ridley Limestone (wells 9D, 13D, and 14D) draw down water levels in many of the deep wells and affect local ground-water flow (fig. 9). The pumping of well 9D (approximately 100 L/min) affects water levels in all of the deep wells located south of Snell Branch. The pumping of wells 13D and 14D (approximately 20 L/min at alternating intervals) affects a much smaller area north of Snell Branch (fig. 9). The pump-and-treat operations in the karst aquifer help to remove chlorinated solvent and contain the contaminant on site.

Ground-water tracing studies documented in unpublished TDEC-DSF site files verify that conduits between wells 3D and 9D and between wells 12D and 9D are hydraulically connected. Sodium chloride was

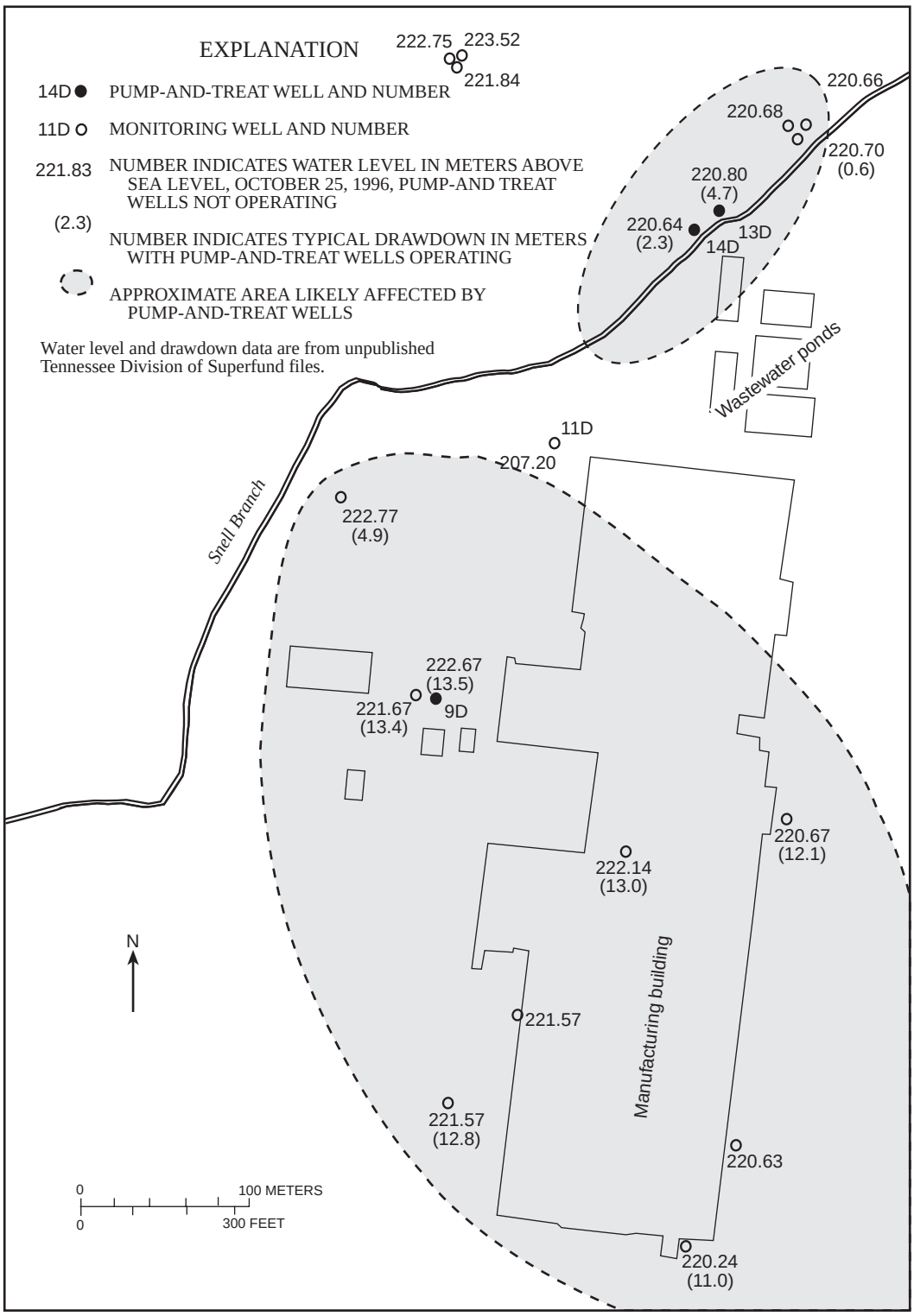

Figure 9. Effect of pump-and-treat wells on water levels in deep wells.

transported from well 3D to well 9D in 18 hours (average velocity 8 m/h) and rhodamine WT was transported from well 12D to well 9D in 28 hours (5 m/h). During these ground-water tracing studies, the pumping rate of well 9D was approximately 64 L/min. The reports were unclear on how much water was used to flush the tracers into the conduits. These tracer results indicate that ground water could flow relatively fast in the karst aquifer at this site but do not address the issue of ground-water storage.

Results from additional ground-water tracing studies demonstrate that some water-bearing zones are isolated from major zones of ground-water flow. These water-bearing zones may store water and contaminants for relatively long periods. Fluorescein (2.3 kg) was injected into well 8D on November 15, 1991, and the well was flushed with water (750 L/min) for 26.5 hours. During the fluorescein study, the pump-and-treat wells were turned off and nearby springs were monitored for fluorescein. Fluorescein was detected in some nearby springs; however, the results were inconclusive because of the lack of background samples from the springs. The deep wells at the study site were not sampled for fluorescein until 1996, 5 years after the dye injection. During those 5 years, pump-and-treat wells and other remediation efforts were active at the site. Unpublished TDEC-DSF files report that fluorescein was still present in water samples collected from deep wells during two separate sampling events in 1996. The dye was detected at significant concentrations in water samples from wells 3D (11 to 18 µg/L), 4D (0.54 to 1.2 µg/L), 5D (0.07 to 0.22 µg/L), and 8D (23 to 37 µg/L).

METHODS AND PROCEDURES

Multiple lines of evidence are often needed to demonstrate biodegradation processes at contaminated sites (National Research Council, 1993; Wiedemeier and others, 1998). The lines of evidence used to examine biodegradation of chlorinated solvents include (1) geochemical data that indicate depletion of electron donors and acceptors and increasing concentrations of metabolic byproducts, (2) chemical data that indicate decreasing concentrations of chlorinated solvents and increasing concentrations of degradation byproducts, and (3) laboratory or field microbiological data that indicate the bacteria present at a site can degrade contaminants (Chapelle and others, 1996; U.S. Environmental Protection Agency, 1997a). This study used field and laboratory techniques to develop data and address the three lines of evidence. Geochemical evidence was collected from selected wells for over 2 years during this study; and organic chemical data collected during a 10-year period was compiled, tabulated, and reviewed for spatial and temporal trends. Microbiological evidence was developed using microcosms and identification techniques.

Water-Quality Data Collection

Much of the water-quality data was already available from TDEC-DSF files (site 59-502), including chlorinated-ethene, ethene, and ethane data. Chlorinated-ethene data (TCE and byproducts of TCE degradation) were available for water samples collected quarterly between 1985 and 1998 from shallow and deep monitoring wells. Ethene and ethane (byproducts of VC degradation) data were available for water samples collected from selected shallow and deep wells beginning in 1997. These data included analytical printouts, information on analytical methods, quality control, and quality assurance data. The data were tabulated and examined, and changes in the analytical laboratory methods used were noted.

Additional data-collection activities conducted by the USGS included periodic water-quality sampling of selected shallow and deep monitoring wells, collection of water samples from selected deep wells for bacteria identification and enumeration, and continuous monitoring of water quality and water levels for selected deep wells. Water-quality samples were collected by the USGS during seven sampling events: November 1996; February, August, and November 1997; and February, April, and May 1998. Field measurements of temperature, pH, specific conductance, alkalinity, and dissolved oxygen (DO) were conducted using methods described by Wood (1981) and Hach Company (1992). Nutrient, major anion and cation, and total organic carbon analyses were conducted at the USGS regional laboratory in Ocala, Fla., using methods described by Fishman and Friedman (1989). Nutrient and major anion and cation analyses for samples collected during 1998 were conducted using spectrophotometric methods described by Hach Company (1992).

Continuous monitoring of water quality and water levels was conducted from March through May 1998. Data were collected at 15-minute intervals from four wells (1D, 2D, 3D, and 12D). The water-quality monitors located in the screened intervals of wells

near water-producing zones measured conductivity, temperature, pH, ORP, and DO. The various sensors in the monitors were calibrated prior to placement in the wells and included rapid-pulse DO sensors that do not require stirring of the water in a well. Water-quality and water-level monitors were checked and calibrated every 2 to 3 weeks. Precipitation data (15-minute intervals) were obtained from a USGS gaging station approximately 1.5-km southeast of the study site. Precipitation data (daily totals) collected by employees at the site were obtained to verify that the data collected from the USGS gaging station were consistent with data from the site.

Geochemical Indicators

Geochemical data were used as indicators of terminal electron acceptor processes occurring in ground water at the site (Bouwer, 1994; Chapelle and others, 1995; Wiedemeier and others, 1998). The geochemical data used included DO, NO_3^-, NH_3, Mn^{2+}, Fe^{2+}, SO_4^{2-}, and S^{2-} concentrations (table 4). Calcium carbonate saturation indexes were calculated using methods described by Eaton and others (1995). Field measurements such as pH, alkalinity, specific conductance, temperature, and ORP were used to provide additional information concerning biodegradation processes and changes in water chemistry associated with the site hydrology.

Due to the complex ground-water flow paths in karst, geochemical isopleth maps may be difficult to construct in karst aquifers and the identification of discrete oxidation-reduction zones along a contaminant-plume transect is impractical (Chapelle and others, 1995). Thus, the geochemical data used to identify oxidation-reduction trends must be presented some other way. Radial diagrams can be used to identify spatial and temporal trends for geochemical indicators of biodegradation (Carey, 1998). Radial diagrams were used to illustrate oxidation-reduction conditions by arranging the axes of the diagrams in the sequential order of preferred electron acceptors (DO, NO_3^-, Mn, Fe, and SO_4^{2-}). Axis ranges were based on concentrations considered indicative of specific geochemical conditions (Wiedemeier and others, 1998). The axes for Mn^{2+} and Fe^{2+} were oriented with concentrations increasing toward the origin since these compounds are byproducts of Mn^{4+} and Fe^{3+} reduction (fig. 10).

Chlorinated-Ethene and Degradation Product Data

The chlorinated ethene released at this site was TCE. Degradation byproducts of TCE include DCE, VC, ethene, and ethane. Decreases in parent compounds and detection of chlorinated-ethene degradation byproducts may provide evidence that biodegradation has occurred (table 5). During reductive

Table 4. Geochemical indicators of terminal electron acceptor processes measured during this study

Constituent	Remarks concerning interpretation
Dissolved oxygen (DO)	If DO is greater than 1 mg/L (aerobic conditions), reductive dechlorination is unlikely; however, cometabolism and direct oxidation of partially dechlorinated solvents are possible.
Nitrate (NO_3^-)	Reductive dechlorination of tetrachloroethylene (PCE) and trichloroethylene (TCE) to *cis*-1,2-dichloroethylene (*c*DCE) can occur under these mildly reducing conditions. Direct oxidation of partially dechlorinated solvents also may occur under NO_3^--reducing conditions.
Ammonia (NH_3)	Elevated NH_3 concentrations may indicate NO_3^--reduction if DO is less than 1 mg/L. If NH_3 is present at 0.5 mg/L or greater and DO levels are greater than 1.0 mg/L, then partially dechlorinated solvents may be cometabolized through the NH_3-monooxygenase pathway.
Soluble manganese (Mn^{2+}) and ferrous iron (Fe^{2+}).	Increased concentrations of Mn^{2+} and Fe^{2+}, coinciding with low DO concentrations, are indicators that insoluble manganese and iron (Mn^{4+} and Fe^{3+}) are serving as electron acceptors. Reductive dechlorination of PCE and TCE to *c*DCE can occur under mildly reducing conditions. Mn^{4+}- and Fe^{3+}-reducing bacteria are capable of consuming vinyl chloride (VC) and *c*DCE.
Sulfate (SO_4^{2-})	Reductive dechlorination of TCE to *c*DCE, then to VC and ethene can occur under SO_4^{2-}-reducing conditions. The chlorinated solvents compete directly with SO_4^{2-} to serve as electron acceptors.
Sulfide (S^{2-})	Elevated S^{2-} concentrations may indicate SO_4^{2-}-reduction and conditions favorable for reductive dechlorination of chlorinated solvents.

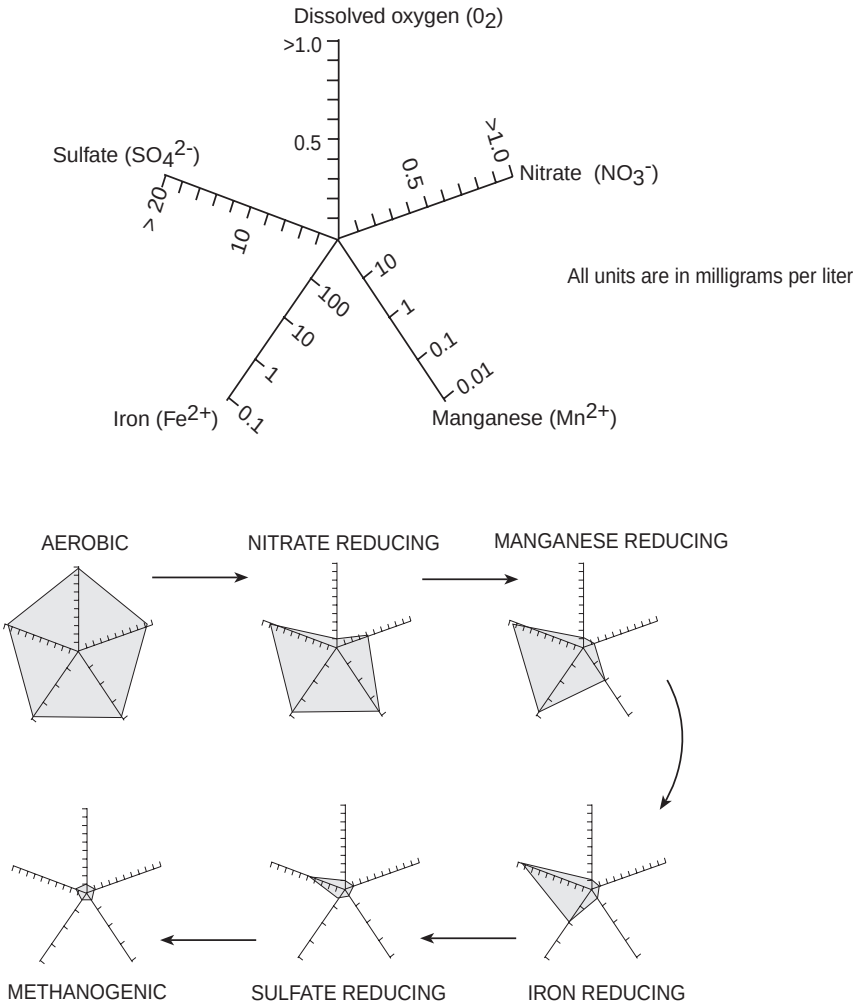

Figure 10. Radial diagrams used to illustrate electron acceptor processes. (Modified from Carey, 1988.)

dechlorination, all three isomers of DCE can be produced; however, *c*DCE is the most commonly produced isomer and 1,1-DCE is the least commonly produced. Chlorinated-ethene, ethene, and ethane data for water samples from shallow and deep wells were converted to micromolar concentrations and examined for spatial and temporal trends that could indicate the occurrence of reductive dechlorination. Aerobic degradation of chlorinated ethenes often results in intermediate byproducts that spontaneously degrade into a variety of compounds. Intermediate byproducts of aerobic degradation, such as TCE-epoxide and chloroacetic acids, were not examined during this study.

Microcosm Experiments

In addition to the field evidence, laboratory microcosms were used to determine if microorganisms at the site had the potential to degrade contaminants. A standard microcosm method does not exist; however, a variety of methods have been reported. In many of the studies, solid and liquid aquifer materials were placed in the microcosms (Hopkins and others, 1993; Chapelle and others, 1996; Wilson and others, 1996). Other studies have used only ground water from contaminated sites in the microcosms (Nelson and others, 1988; Krumme and others, 1993; Moran and Hickey, 1997).

Batch-type microcosms containing only water were used during this study because they may be more representative of conditions in karst aquifers. Since preliminary field data indicated the occurrence of aerobic and anaerobic conditions at the site, microcosms were set up under aerobic conditions to monitor for the occurrence of cometabolic degradation pathways. After depletion of DO, the microcosms were monitored for anaerobic degradation (reductive

Table 5. Organic constituents frequently measured at chlorinated-solvent contaminated sites

[Degradation byproducts are often indicative of biodegradation at the site]

Organic constituent	Remarks concerning interpretation
Tetrachloroethylene (PCE)	A commonly used chlorinated solvent that can be biodegraded only by reductive dechlorination. PCE was not used at this site and has not been detected in ground-water samples from the study site.
Trichloroethylene (TCE)	A commonly used chlorinated solvent. The most efficient biodegradation mechanisms include reductive dechlorination and methane cometabolism. TCE was the chlorinated solvent spilled at the study site and has been detected in ground-water samples from the study site.
Dichloroethylene (DCE)	
cis-1,2-DCE (*c*DCE)	The most common byproduct of reductive dechlorination of TCE. If the concentration of *c*DCE is greater than 80 percent of total DCE concentration, then reductive dechlorination is likely to have occurred. Significant concentrations of *c*DCE have been detected in ground-water samples from the study site.
trans-1,2-DCE (*t*DCE)	*Trans*-1,2-DCE is not produced significantly through any biologically mediated process. If *t*DCE is 50 percent or more of the total DCE, a good possibility exists that *t*DCE was released. Significant concentrations of tDCE have not been detected in ground-water samples from the study site.
1,1-DCE	This is the least common byproduct of reductive dechlorination of TCE. 1,1-DCE is also produced when 1,1,1-trichloroethane (TCA) undergoes abiotic chloride elimination. Significant concentrations of 1,1-DCE have not been detected in ground-water samples from the study site.
Vinyl chloride (VC)	VC is a byproduct of *c*DCE reductive dechlorination. VC has been detected in ground-water samples from the study site.
Ethane and ethene	These are strong indicators of complete reductive dechlorination. They have been detected in ground-water samples from the study site.

dechlorination). Water samples collected from wells 1D, 2D, 3D, and 12D on February 17, 1998 and May 21, 1998 were used to construct microcosms during two experiments. Four replicates were established for each treatment, and control procedures included sterilization of selected microcosms (table 6). During experiment 1, bacteria counts indicated that the sterilization procedure used for controls (experiment 1, treatment 9) were not successful. The control microcosms were cross-contaminated with bacteria from the other microcosms during the addition of TCE. The second microcosm experiment was set up to establish new sterile controls and define the loss of TCE due to abiotic factors.

Microcosms were set up in 40-mL glass vials equipped with Teflon-lined septum caps then stored inverted in the dark until the selected sampling dates. On the sampling dates, samples from the microcosms were analyzed for chlorinated ethenes using gas chromatography (GC) with hexane extractions and an electron capture detector. Additional chlorinated ethene analyses were performed using GC with headspace analysis and a photoionization detector.

Bacteria Identification and Enumeration

The geochemical information described earlier in this report helped to determine if conditions were suitable for biodegradation of chlorinated ethenes and to tentatively identify microbial processes in the karst aquifer. Other lines of evidence such as laboratory microcosm data and bacteria identification and enumeration strengthen the interpretation of the geochemical information. Bacteria in water samples collected on August 20 and 21, 1998, from selected deep wells were identified by the use of the RNA-oligonucleotide hybridization method. Facultative and aerobic heterotrophic bacteria were enumerated from microcosm samples that contained water from various wells using tryptic soy agar plate counts. This information was used to provide further evidence that sufficient bacteria capable of solvent biodegradation were present in the karst aquifer.

The RNA-oligonucleotide hybridization method is a technique that takes advantage of unique nucleotide sequences in the ribosomal RNA (rRNA) to identify the bacteria (Amann and others, 1995). This method can be used to identify groups of bacteria such as sulfate-reducing bacteria or specific genera such as

Table 6. Description of microcosms used to examine biodegradation of chlorinated ethenes

[TCE, trichloroethylene; mg/L, milligrams per liter; --, none; four replicates created for each treatment]

		Source of water		Microcosm treatments	
Experiment	Treatment	Wells(s)	Date	TCE (mg/L)	Additional
1	1	1D	Feb. 17, 1998	0.17	--
1	2	1D	Feb. 17, 1998	0.92	--
1	3	2D	Feb. 17, 1998	0.57	--
1	4	2D	Feb. 17, 1998	1.19	--
1	5	3D	Feb. 17, 1998	0.30	--
1	6	3D	Feb. 17, 1998	0.73	--
1	7	12D	Feb. 17, 1998	0.08	--
1	8	12D	Feb. 17, 1998	0.68	--
1	9	Multiple	Feb. 17, 1998	0.94	Sterilized[1]
2	1	1D	May 21, 1998	1.61	--
2	2	2D	May 21, 1998	1.53	--
2	3	3D	May 21, 1998	1.19	--
2	4	12D	May 21, 1998	2.50	--
2	5	2D/3D	May 21, 1998	0.73	Sterilized

[1]Bacteria data indicated sterilization not successful.

Nitrosomonas sp. The identification level used in this study was general bacteria groups such as iron-oxidizers, methanotrophs, ammonia-oxidizers, and sulfate-reducers (Byl and others, 1997; Farmer and others, 1998).

Bacteria samples were collected with sterile disposable bailers. Each water sample was poured into a sterile 1-liter bottle, placed on ice, and brought back to the laboratory. Sterile polycarbonate filters with 0.22-µm pores were used to concentrate the bacteria in the water samples. Bacteria were washed off the polycarbonate filters using a few drops of sterile phosphate buffer (PB) solution. The bacteria were preserved using a 4-percent paraformaldehyde-PB solution. The samples were stored in solution at 4 °C until they were subjected to the hybridization tests (Braun-Howland and others, 1992).

The sequences for the RNA-oligonucleotide probes of the various bacteria groups were selected from previously published sequences (Tsien and others, 1990; Kane and others, 1993; Amann and others, 1995). Bacteria have long been recognized to play an important role in oxidizing Fe^{2+} to Fe^{3+}. The reverse process, bacteria mediated reduction of Fe^{3+} to Fe^{2+}, has been studied as well (Chapelle, 1993). Field microbial investigations are indicating that bacteria-catalyzed Fe^{3+} reduction and Fe^{2+} oxidation are occurring within close proximity of each other (Lovley and Phillips, 1988; Lovley and others, 1990), which implies a synergistic relation between the organisms. This potential synergistic relation is one reason why these bacteria have been difficult to isolate and culture in the laboratory. To avoid this problem, Siering and Ghiorse (1997) have developed a rRNA-targeted hybridization probe to identify iron- and manganese-oxidizing bacteria in environmental samples. The RNA-hybridization probe was used to identify these potential synergistic bacteria in ground-water samples from this site.

The oligonucleotide probes were tagged with rhodamine, fluorescein, or acridine orange dye. The probes were stored at –20 °C until used. Bacteria were prepared for hybridization by taking a 100-µL sample from the preserved cells and washing the preservative out by using a series of washes and centrifugation steps (Brawn-Howland and others, 1992) and replacing the preservative with a hybridization buffer. The bacteria cells were incubated for 30 minutes at 90 °C to relax and open the RNA coils for hybridization with the oligonucleotide probes. The fluorescent-tagged probes were added to the hybridization mixture and incubated at 37 °C for 2 hours on a gentle shaker. Probes hybridized to complimentary sequences in the bacteria. The cells were spun down in a centrifuge and resuspended in clean PB solution. The un-hybridized probes were washed away in a series of PB-solution centrifugation washes. After two washes, the cells

were resuspended and filtered through a 0.2-µm, clear polycarbonate filter. The filter with the cells was placed directly on a slide and examined using an epifluorescent microscope (X 1,000 magnification) equipped with appropriate excitation and emission filters for the targeted dye.

The tryptic soy agar and sterile dilution buffer used to count facultative anaerobic and aerobic heterotrophic bacteria were prepared as described by Eaton and others (1995). Autoclaved glass filtration devices were used to hold 0.45-µm pore-size sterile filters. A 0.01- or 1-mL aliquot of shaken well water was transferred to 20 mL of sterile dilution buffer and drawn onto the filter as described in the membrane-filtration method (Britton and Greeson, 1989). The filters were placed on the agar plates by using sterile forceps and placed in an incubator at 35 °C. Bacteria-colony forming units were counted after 48 hours. Results were reported as colony forming units per 100 mL of water.

RESULTS AND INTERPRETATION

TCE and TCE-degradation byproducts are present in both the shallow water-bearing zone near the top of bedrock and the deeper karst aquifer at the study site (fig. 8, table 8). The monitoring data was unclear whether the byproducts of TCE degradation were produced in the shallow water-bearing zone alone, or if conditions were suitable for additional biodegradation to occur in the karst aquifer. This investigation attempted to address the biodegradation issue by reviewing hydrogeologic and organic chemistry records, running geochemical analyses, identifying microbes, establishing microcosms, and integrating the results into multiple lines of evidence. The results for the shallow water-bearing zone are presented first, followed by results for the karst aquifer.

Biodegradation of Chlorinated Ethenes in the Shallow Water-Bearing Zone

Ground water in the shallow water-bearing zone flows along a trough in the bedrock surface, which serves as the main route for horizontal transport of chlorinated ethenes in the shallow zone. The shallow ground-water contamination plume moved laterally from well 33S to well 7S to well 2S to well 5S (fig. 11). Because the general direction of ground-water flow and the extent of the chlorinated-ethene plume in the shallow water-bearing zone were known, identifying discrete oxidation-reduction zones along the flow path in the shallow zone was possible. The delineation of oxidation-reduction zones, supplemented with organic chemistry data, was used to infer which chlorinated-ethene degradation processes prevailed along the plume.

Geochemical data for water samples collected from well 33S indicate that anaerobic conditions were present in shallow ground water beneath the manufacturing building (fig. 11). Compared to water samples from other shallow wells, samples collected from well 33S on August 22, 1997, contained low DO and NO_3^- concentrations (<0.1 and <0.06 mg/L, respectively) and high NH_3, Mn^{2+}, and Fe^{2+} concentrations (1.6, 2.6, and 18 mg/L, respectively). Anaerobic conditions were present in water samples from well 33S (table 7), and concentrations of electron acceptors were within ranges normally associated with iron or possibly sulfate-reducing conditions. Those oxidation-reduction conditions are associated with reductive-dechlorination processes.

Chlorinated-ethene and degradation-product data from shallow wells under the building also support the inference that reductive dechlorination was occurring in shallow ground water beneath the manufacturing building [table 8 (at end of the report)]. Water samples from well 33S contained lower molar concentrations of TCE than molar concentrations of *c*DCE and VC (reductive-dechlorination byproducts) and *c*DCE and VC concentrations generally increased in water samples collected between 1994 and 1997 (fig. 12). Ethene and ethane (degradation products of VC) also were detected in water samples from shallow wells screened beneath the manufacturing building. Water samples collected from well 33S contained 60 to 93 µM/L of ethene and 28 to 34 µM/L of ethane (table 7). Water samples from well 32S, also screened beneath the manufacturing building, contained 278 to 379 µM of ethene. A low molar ratio of parent material to byproducts and increasing concentrations of byproducts are evidence of biologically mediated reductive dechlorination of TCE.

As the shallow ground water moved downgradient from the building, direct recharge resulted in higher DO concentrations in the ground water. Water samples from shallow wells not screened beneath the manufacturing building commonly contained DO concentrations greater than 1.0 mg/L (fig. 11). Conditions in wells away from the manufacturing building, near

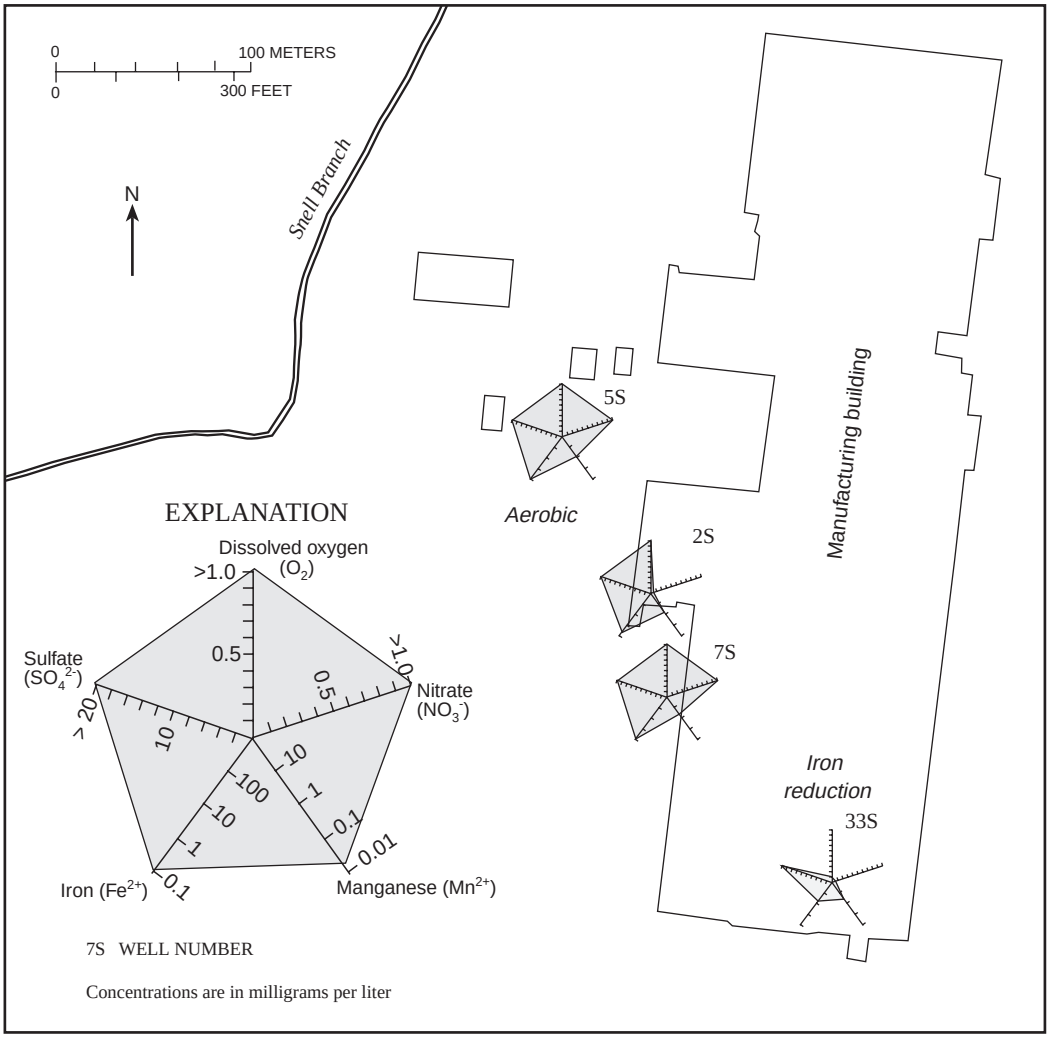

Figure 11. Geochemical conditions of water samples collected from selected shallow wells, August 21, 1997.

well 5S for example, were consistently aerobic (table 7), which would preclude reductive dechlorination from occurring. Aerobic degradation (cometabolism) of chlorinated ethenes is possible if sufficient food and oxygen are available. Ethene and ethane, which were found in shallow ground water under the building, are suitable food substrates for inducing cometabolism. However, concentrations in water samples from wells to the west of the manufacturing building (5S, 6S, and 35S) were usually below detection limits.

A more consistent food supply would be required to sustain cometabolism in the aerobic zone. Ammonia is an alternative food substrate of the AMO cometabolic pathway. The anaerobic conditions underneath the manufacturing building resulted in elevated concentrations of NH_3 (approximately 0.5 to 2.5 mg/L) and low NO_3^- concentrations in water samples from wells near the manufacturing building (table 7). Farther downgradient, where conditions were consistently aerobic (near well 5S), NH_3 concentrations were lower (less than 0.10 mg/L) and NO_3^- concentrations increased (0.59 to 1.8 mg/L) indicating that NH_3 was oxidized to NO_3^- (fig. 13). Concurrent decreases in NH_3 concentrations and increases in NO_3^- concentrations along the delineated flow path are consistent with the AMO pathway and the cometabolic degradation of TCE, *c*DCE, and VC. These geochemical data indicate that cometabolism by the AMO pathway may have been occurring in shallow ground water to the west of the manufacturing building. Direct oxidation of VC and *c*DCE is another potential biodegradation pathway in the aerobic part of the shallow water-bearing zone, but this was not confirmed in this study.

Geochemical conditions of water samples from wells located near the western side of the manufacturing building (7S and 2S) changed in response to rain

Table 7. Water-quality data for samples collected from selected shallow wells

[µS/cm, microsiemens per centimeter; $CaCO_3$, calcium carbonate; mg/L, milligrams per liter; µM/L, micromoles per liter; <, less than; --, no data; [a], sample possibly aerated during collection; *, see table 8 for date samples were collected for trichloroethylene, *cis*-1,2,-dichloroethylene, and vinyl chloride]

Well name	Date	pH (standard units)	Specific conductance (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Dissolved oxygen (mg/L)	Nitrogen nitrate, dissolved (mg/L as N)	Nitrogen ammonia, dissolved (mg/L as N)	Manganese, dissolved (mg/L as Mn)	Iron, dissolved (mg/L as Fe)	Sulfate, dissolved (mg/L as SO_4)
Well 33S	11/12/96*	6.2	1,276	413	<0.1	0.21	1.40	1.70	5.70	61
	02/11/97	6.7	1,250	386	<0.1	0.05	1.40	2.00	6.00	75
	08/22/97	6.8	1,585	480	<0.1	0.06	1.60	2.60	18.10	170
	5/98	--	--	--	--	--	--	--	--	--
Well 7S	11/12/96*	6.4	1,118	434	2.4[a]	0.63	0.84	0.03	0.01	37
	02/11/97	6.7	1,024	441	1.1[a]	0.22	2.50	4.40	<0.01	32
	08/21/97*	7.1	1,080	387	3.5	1.90	0.98	1.90	<0.01	110
	11/05/97	6.7	1,311	350	5.1	0.20	1.47	--	0.01	78
	5/98	--	--	--	--	--	--	--	--	--
Well 2S	11/12/96*	6.4	908	335	2.5[a]	0.25	0.48	2.20	0.30	72
	02/11/97	7.0	886	359	0.8[a]	<0.02	0.48	1.90	0.24	69
	08/22/97	6.8	918	363	2.1	<0.02	0.47	2.10	0.28	67
	5/98	--	--	--	--	--	--	--	--	--
Well 5S	11/12/96*	7.4	678	329	--	0.91	0.02	<0.01	<0.01	75
	02/11/97	7.6	645	265	7.5[a]	0.59	0.02	<0.01	<0.01	75
	08/22/97	--	581	340	5.6	1.80	0.10	1.40	<0.01	78
	11/05/97	7.5	578	255	5.4	1.10	0.05	--	0.01	122
	5/98	--	--	--	--	--	--	--	--	--

events. DO and NO_3^- concentrations decreased and NH_3 and Mn^{2+} concentrations increased significantly between November 12, 1996 and February 11, 1997 in water samples from well 7S (fig. 14), indicating a change from aerobic conditions to manganese-reducing conditions. The geochemical sampling technique used during the November 12, 1996 and February 11, 1997 sampling events may have resulted in some aeration of samples and actual DO concentrations were probably lower than the measured concentrations. The sampling technique was modified after the February 11, 1997 sampling event to minimize aeration of samples. The geochemical data indicate that at times the zone of anaerobic reductive dechlorination may have extended beyond the edge of the building; however, whether significant reductive dechlorination occurred in these fluctuating zones of transition is not clear.

Geochemical conditions in this fluctuating transition zone were probably affected by both infiltration of water from the surface after rainfall events (fig. 15) and transport of shallow ground water from the upgradient anaerobic zone beneath the manufacturing building. As the shallow ground water moves laterally out from under the building, recharge from rain would supply DO and dilute the contaminated water. Thus, the anaerobic water containing ammonia, small aliphatic hydrocarbons, and chlorinated ethenes coming from under the building mixes with aerobic waters, making the conditions suitable for cometabolism. Methanotrophic and ammonia-oxidizing bacteria would consume methane and ammonia as well as oxygen in this transition zone. Cometabolic destruction of chlorinated ethenes would also occur in this transition zone. Undoubtedly some of the water carrying bacteria, contaminants, electron donors, and electron acceptors in the shallow water-bearing zone also migrates down into the karst aquifer. Evidence that aerobic and anaerobic biodegradation pathways remained active in the karst aquifer is examined in the next section.

Table 7. Water-quality data for samples collected from selected shallow wells—Continued

Well name	Date	Sulfide, dissolved (mg/L as S^{2-})	Chloride, dissolved (mg/L as Cl)	Calcite saturation index	Trichloroethylene (μM/L)	*cis*-1,2-Dichloroethylene (μM/L)	Vinyl chloride (μM/L)	Ethene (μM/L)	Ethane (μM/L)
Well 33S	11/12/96*	--	150	-0.52	<0.76	182.6	78.4	--	--
	02/11/97	--	140	-0.10	0.30	147.5	47.7	--	--
	08/22/97	0.056	190	0.14	<0.76	150.6	56.0	92.67	27.57
	5/98	--	--	--	<6.85	312.9	36.0	60.24	33.59
Well 7S	11/12/96*	--	80	-0.28	63.55	90.3	1.02	--	--
	02/11/97	--	70	-0.01	6.15	11.4	0.08	--	--
	08/21/97*	0.060	56	0.49	50.23	142.4	<0.02	<0.93	<0.86
	11/05/97	0.015	14	--	28.92	168.1	0.98	<0.93	<0.86
	5/98	--	--	--	59.25	280	<7.20	<0.93	<0.86
Well 2S	11/12/96*	--	45	-0.47	1,415	330.1	32.8	--	--
	02/11/97	--	47	0.15	610.4	466.2	29.0	--	--
	08/22/97	0.068	46	0.00	1,302	355.9	<8.00	7.77	3.72
	5/98	--	--	--	702.1	279.4	17.3	10.37	2.39
Well 5S	11/12/96*	--	15	0.42	0.74	0.47	0.06	--	--
	02/11/97	--	18	0.48	2.09	0.44	<0.02	--	--
	08/22/97	0.034	11	--	--	--	--	<0.93	<0.86
	11/05/97	0.005	137	--	3.73	3.77	<0.02	<0.93	<0.86
	5/98	--	--	--	10.96	5.20	<0.02	<0.93	<0.86

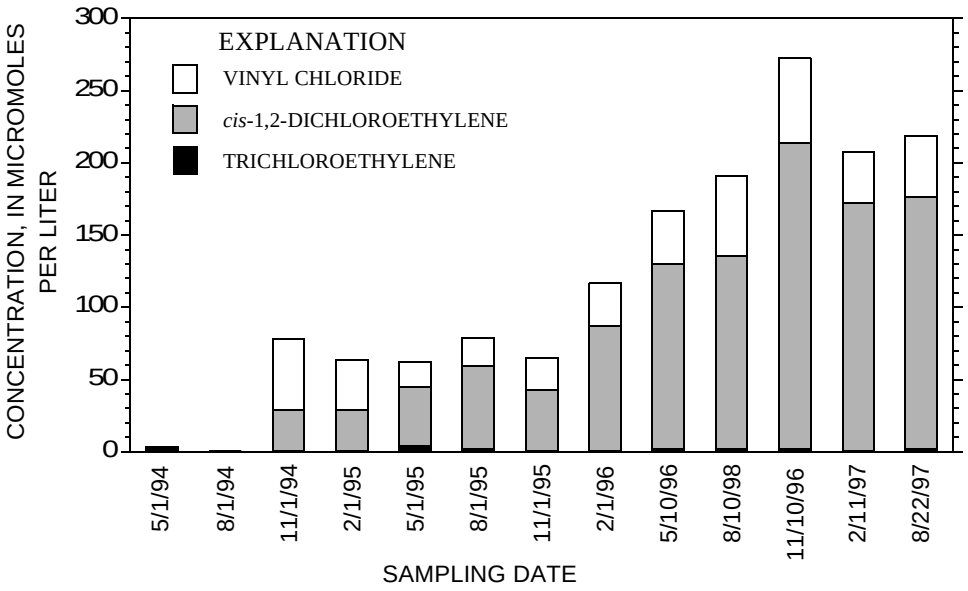

Figure 12. Chlorinated-ethene data for water samples collected from well 33S.

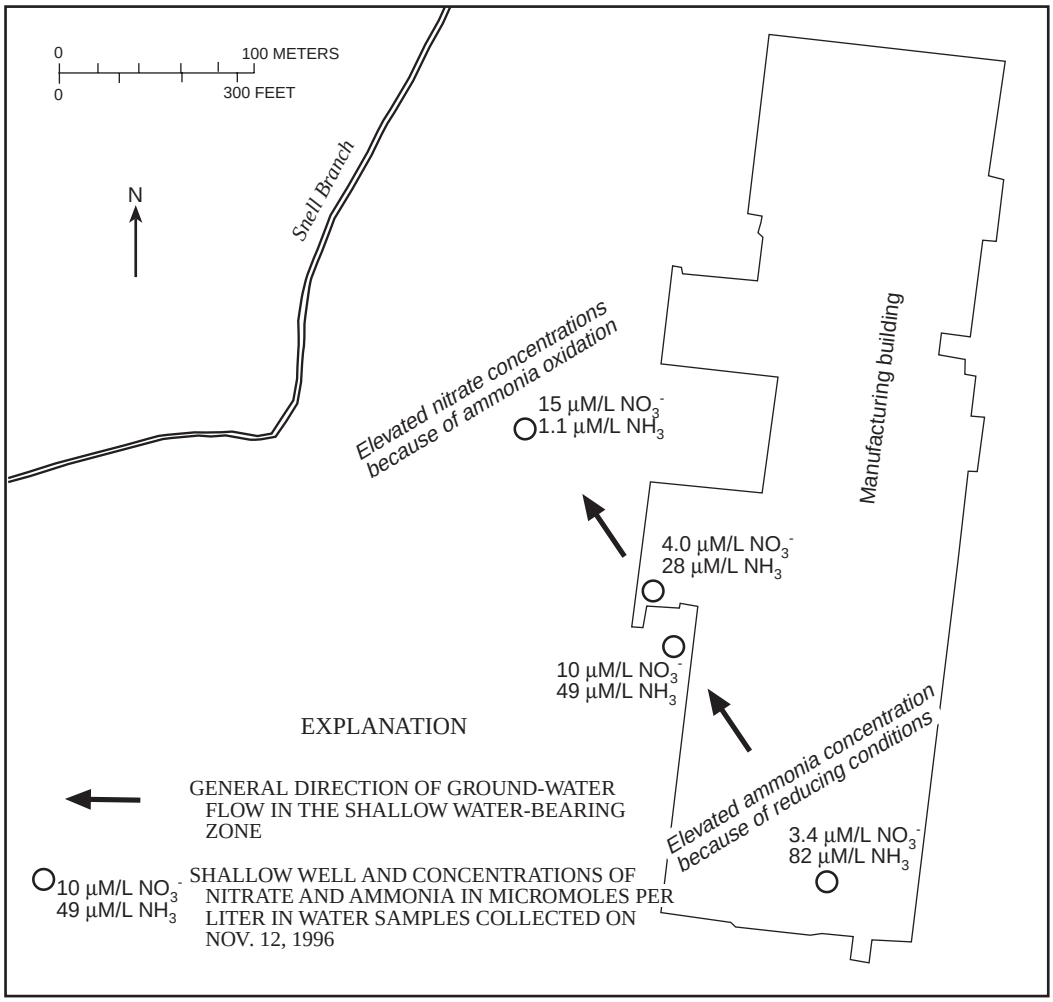

Figure 13. Nitrate and ammonia concentrations in samples from selected shallow wells.

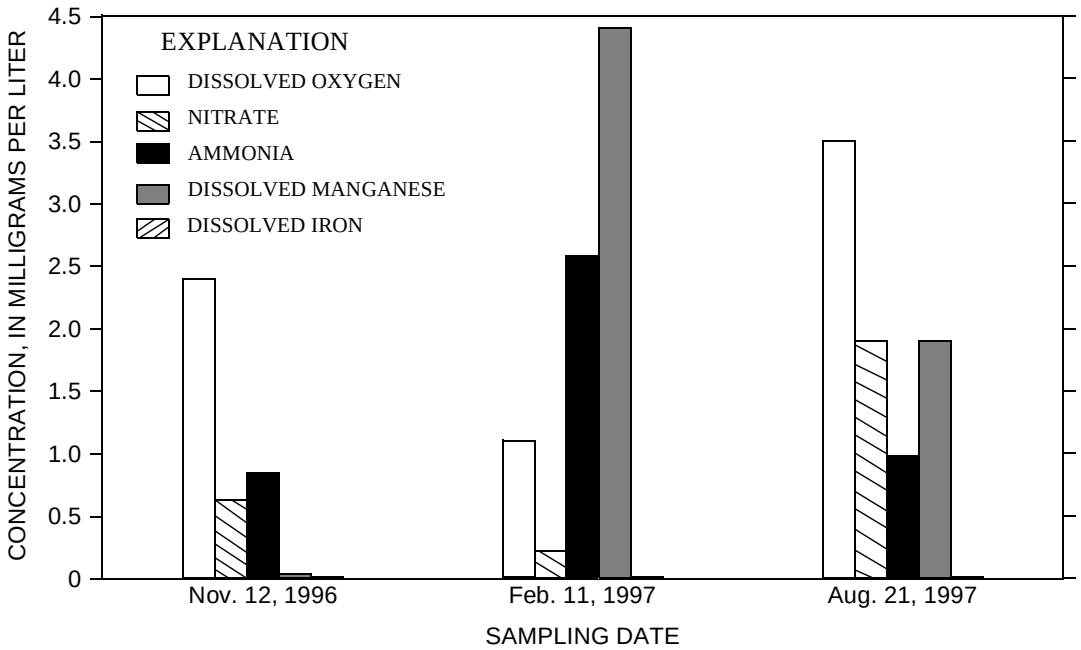

Figure 14. Geochemical data for water samples from well 7S.

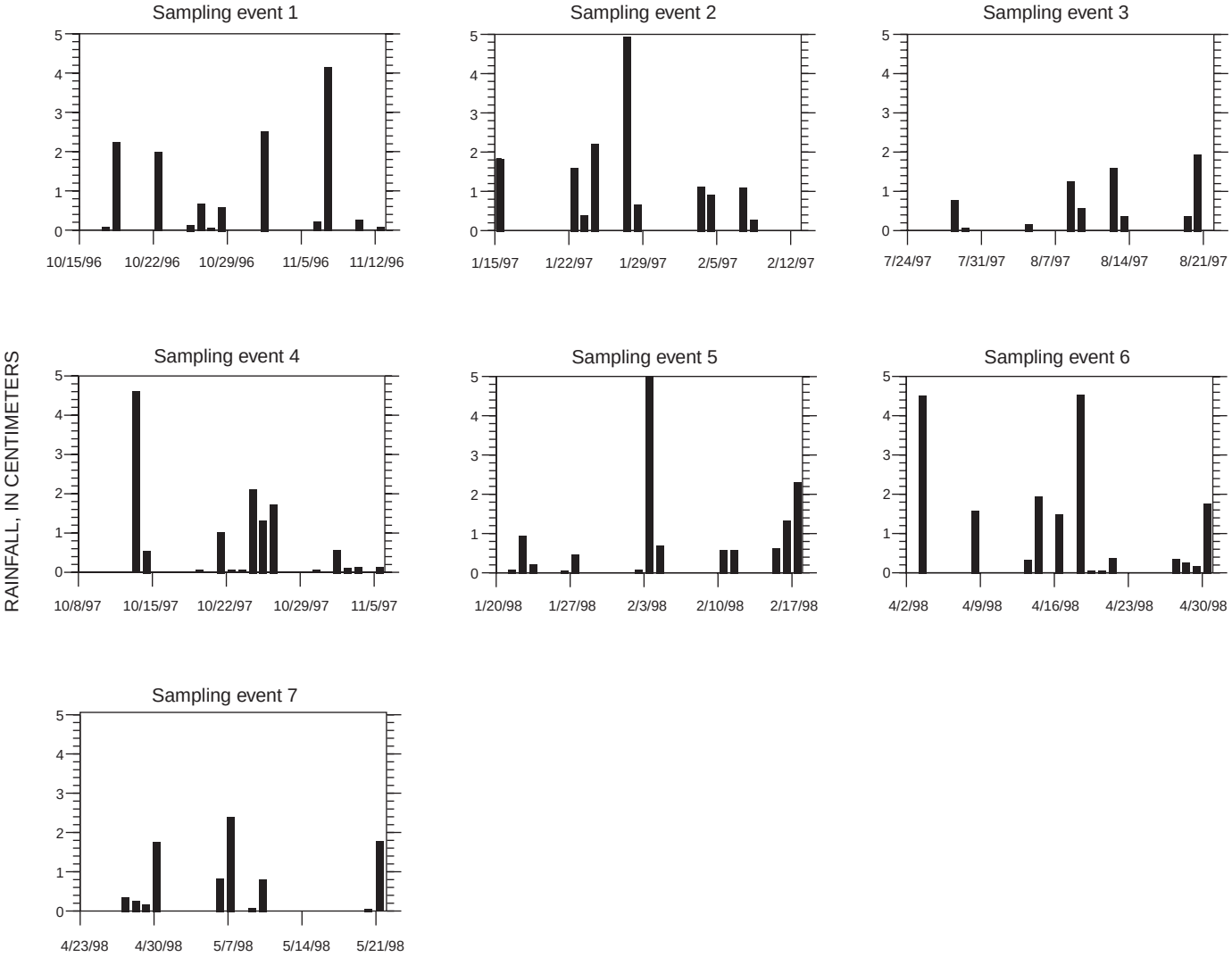

Figure 15. Daily rainfall totals for a 1-month period before each ground-water sampling event.

Biodegradation of Chlorinated Ethenes in the Karst Aquifer

Water-quality data collected during periodic sampling by the USGS indicate that conditions in water-producing zones of the upper part of the Ridley Limestone vary spatially and temporally at the study site. Conditions in some zones, such as those intersected by wells 1D and 3D, were consistently anaerobic, whereas conditions in other zones, such as those intersected by wells 2D and 12D, fluctuated between anaerobic and aerobic (table 9). When anaerobic conditions were present, Mn^{4+} or Fe^{3+} reduction may have been the dominant electron acceptor (fig. 16); however, sulfate reduction also may have been occurring. Sulfide concentrations ranging from 0.002 to 3.46 mg/L were detected in water samples from deep wells (table 9). The sulfate and sulfide concentrations were generally higher in samples from the karst aquifer compared to the shallow wells (tables 7 and 9). This is probably due in part to the availability of sulfate from the gypsum nodules of the karst bedrock. Bacteria can accelerate the remobilization of sulfate and convert the sulfate to sulfide.

Significant concentrations of chlorinated ethenes (TCE, *c*DCE, and VC) were detected in water samples from several deep wells (table 9). Those wells containing chlorinated ethenes, except for well 12D, had higher molar concentrations of reductive-dechlorination byproducts (*c*DCE and VC) than of TCE (fig. 17). The concentration of chlorinated ethenes varied with hydrologic conditions and weather events. Periodic increases in VC and *c*DCE concentrations in water samples were detected. For example, between May 10 and August 10, 1996, *c*DCE concentrations in water samples from wells 3D and 4D increased from

Table 9. Water-quality data for samples collected from selected deep wells

[µS/cm, microsiemens per centimeter; $CaCO_3$, calcium carbonate; mg/L, milligrams per liter; µM/L, micromoles per liter; --, no data; <, less than; *, see table 8 for date samples were collected for trichloroethylene, *cis*-1,2,-dichloroethylene, and vinyl chloride]

Well name	Date	Water level (meters above sea level)	pH (standard units)	Specific conductance (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Dissolved oxygen (mg/L)	Total organic carbon (mg/L)	Nitrogen nitrate, dissolved (mg/L as N)	Nitrogen ammonia, dissolved (mg/L as N)	Manganese, dissolved (mg/L as Mn)
Well 4D	02/12/97	215.01	7.4	476	180	0.2	--	<0.02	0.28	0.04
	08/21/97*	213.12	7.0	179	90	1.2	--	0.06	0.02	0.04
	5/98	--	--	--	--	--	--	--	--	--
Well 1D	11/12/96*	210.34	6.9	830	265	<0.1	--	0.42	0.04	0.07
	02/11/97	214.71	6.7	995	335	<0.1	--	0.03	0.30	0.11
	08/21/97*	213.36	7.6	818	259	<0.1	2.5	0.12	0.32	<0.01
	11/05/97	206.24	7.5	756	253	<0.1	1.3	<0.02	0.50	<0.01
	02/17/98		7.2	634	230	0.3	1.4	0.30	0.50	<0.20
	04/30/98	210.85	7.4	708	202	<0.1	7.5	0.30	0.09	<0.20
	05/21/98	207.80	6.2	736	220	0.2	0.7	0.20	0.13	<0.20
Well 3D	11/13/96*	210.30	7.1	725	251	--	--	0.14	0.47	0.01
	02/12/97	214.52	7.3	2,342	450	--	--	<0.02	3.40	<0.01
	08/21/97*	213.23	7.5	818	284	<0.1	--	0.04	0.43	0.05
	11/05/97	206.18	7.3	844	319	<0.1	--	<0.02	0.71	0.01
	02/17/98		7.1	711	294	<0.1	4.0	0.10	0.77	0.10
	04/30/98	210.08	7.3	781	240	0.2	8.4	0.20	0.85	0.90
	05/21/98	207.11	6.7	688	263	<0.1	1.3	0.20	0.78	0.20
Well 2D	02/11/97	214.27	7.0	598	--	<0.1	--	0.26	1.70	0.02
	08/20/97*	213.37	7.3	427	156	3.4	3.8	0.11	0.02	0.02
	11/04/97*	205.73	7.3	406	153	3.3	--	0.10	0.41	--
	02/17/98		6.9	340	135	2.0	4.0	0.60	0.14	<0.20
	04/30/98	210.57	7.3	509	138	<0.1	7.7	0.20	0.24	<0.20
	05/21/98	207.21	6.4	400	159	0.1	7.9	0.20	0.26	<0.20
Well 11D	11/13/96*	209.47	8.2	1,242	113	<0.1	--	0.03	0.47	<0.01
	02/11/97*	213.35	7.7	1,095	163	<0.1	--	0.02	0.30	0.02
	08/20/97*	216.23	8.2	776	137	<0.1	--	0.16	0.14	0.02
Well 12D	08/20/97*	222.65	7.1	507	105	5.5	6.3	0.72	0.05	0.05
	11/04/97	218.43	7.2	557	266	0.4	--	0.10	0.13	--
	02/17/98		6.9	389	135	5.8	4.6	0.20	0.02	<0.20
	04/30/98	220.32	7.4	369	82	5.3	5.9	0.30	0.10	<0.20
	05/21/98	217.22	6.3	590	210	0.2	2.4	0.40	0.32	<0.20
Well 14D	11/13/96*	220.54	6.8	738	333	--	--	0.04	0.06	0.14
	02/11/97*	219.30	7.2	750	330	--	--	<0.02	0.16	0.14
	11/04/97*	220.03	7.3	789	338	1.0	1.8	0.19	0.09	0.28
Well 15D-A	11/13/96	223.35	6.9	713	277	--	--	0.58	0.03	0.03
	02/12/97	223.60	7.2	830	230	--	--	0.53	0.02	0.03
Well 15D-C	11/13/96	221.67	9.4	329	128	--	--	0.05	0.06	<0.01
	02/12/97	221.88	9.6	346	155	<0.1	--	--	0.17	<0.01
Well 16D-B	08/22/97	--	9.6	627	140	1.8	38.0	0.15	2.30	0.02
	11/04/97	--	10.5	697	126	0.3	--	0.20	4.08	--
Well 10D-B	08/22/97	220.52	8.2	310	138	<0.1	2.7	0.06	0.05	0.04
	11/04/97	220.24	7.0	531	227	<0.1	--	<0.02	0.08	--

Table 9. Water-quality data for samples collected from selected deep wells—Continued

Well name	Date	Iron, dissolved (mg/L as Fe)	Sulfate, dissolved (mg/L as SO_4)	Sulfide, dissolved (mg/L as S^{2-})	Chloride, dissolved (mg/L as Cl)	Calcite saturation index	Trichloroethylene (µM/L)	*cis*-1,2-Dichloroethylene (µM/L)	Vinyl chloride (µM/L)	Ethene (µM/L)	Ethane (µM/L)
Well 4D	02/12/97	0.27	50	--	7	-0.15	0.01	0.06	<0.02	--	--
	08/21/97*	0.72	2	0.038	3	--	<0.01	<0.01	<0.02	--	--
	5/98	--	--	--	--	--	<0.01	<0.01	<0.02	<0.04	<0.03
Well 1D	11/12/96*	0.41	22	--	91	-0.39	0.42	0.13	0.74	--	--
	02/11/97	0.13	52	--	150	-0.44	<0.01	0.06	0.09	--	--
	08/21/97*	0.17	36	0.024	86	0.45	0.11	0.10	0.05	--	--
	11/05/97	0.01	34	0.002	66	0.25	0.02	0.36	1.90	3.39	67.18
	02/17/98	0.04	72	0.009	45	--	--	--	--	--	--
	04/30/98	0.05	50	0.003	47	--	--	--	--	--	--
	05/21/98	0.02	50	0.028	77	--	0.06	0.12	0.08	<0.04	<0.03
Well 3D	11/13/96*	0.06	93	--	23	-0.16	0.02	1.89	1.17	--	--
	02/12/97	<0.01	110	--	440	0.27	0.02	0.62	0.31	--	--
	08/21/97*	0.03	67	1.570	53	0.41	0.01	0.06	<0.02	--	--
	11/05/97	0.11	71	2.340	47	0.19	<0.01	0.20	<0.02	<0.93	126.71
	02/17/98	0.13	115	0.130	38	--	--	--	--	--	--
	04/30/98	0.08	88	0.013	12	--	--	--	--	--	--
	05/21/98	0.04	90	0.014	23	--	<0.01	2.37	2.24	<0.04	21.22
Well 2D	02/11/97	0.02	48	--	26	0.18	6.96	14.34	3.92		
	08/20/97*	0.01	35	0.028	10	-0.04	1.28	4.82	4.10		
	11/04/97*	0.01	46	0.011	8	--	6.70	58.80	12.16	6.10	40.48
	02/17/98	0.02	49	0.031	9	--	4.54	11.04	2.50	<0.07	4.42
	04/30/98	0.08	36	0.006	>10	--	--	--	--	--	--
	05/21/98	0.02	45	0.019	11	--	0.88	5.27	1.17	<0.04	<0.03
Well 11D	11/13/96*	0.01	590	--	9	0.71	0.52	1.76	<0.02	--	--
	02/11/97*	0.03	450	--	6	0.35	0.02	1.46	<0.02	--	--
	08/20/97*	<0.01	260	3.460	3	0.66	<0.01	0.73	<0.02	--	--
Well 12D	08/20/97*	0.03	100	0.030	8	-0.35	3.04	0.98	<0.02	--	--
	11/04/97	0.03	72	0.014	8	--	8.56	3.88	<0.02	<0.93	<0.87
	02/17/98	0.01	75	0.023	37	--	0.14	0.04	<0.03	<0.07	<0.07
	04/30/98	0.06	74	0.012	>10	--	--	--	--	--	--
	05/21/98	<0.01	90	0.010	18	--	1.29	0.78	0.06	<0.04	<0.03
Well 14D	11/13/96*	0.03	65	--	9	-0.24	2.21	2.39	0.10	--	--
	02/11/97*	0.23	63	--	10	0.16	1.51	3.94	0.44	--	--
	11/04/97*	0.01	120	0.015	11	0.36	2.32	5.21	0.93	<0.93	<0.87
Well 15D-A	11/13/96	0.02	130	--	<1	-0.21	<0.02	--	--	--	--
	02/12/97	0.13	130	--	5	--	<0.01	--	--	--	--
Well 15D-C	11/13/96	<0.01	26	--	6	0.52	--	--	--	--	--
	02/12/97	<0.01	26	--	4	--	--	--	--	--	--
Well 16D-B	08/22/97	<0.01	160	0.034	15	1.33	<0.01	0.05	<0.02	--	--
	11/04/97	<0.01	150	0.014	17	--	<0.01	0.03	<0.02	<0.93	4.19
Well 10D-B	08/22/97	0.01	19	0.504	3	0.43	<0.01	0.06	0.09	--	--
	11/04/97	<0.01	49	0.124	4	--	0.07	0.15	0.12	<0.93	<0.87

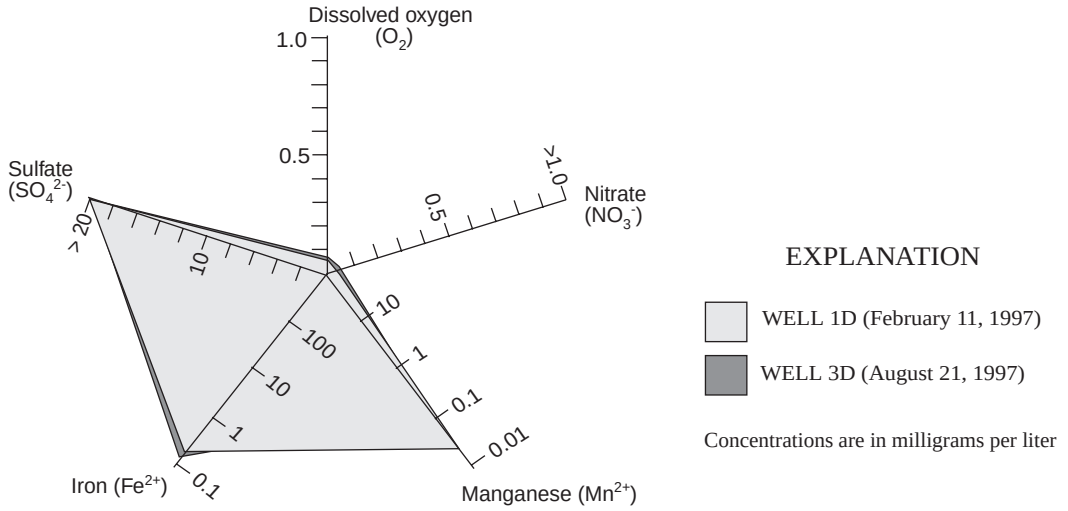

Figure 16. Typical geochemical conditions for anaerobic water samples collected from deep wells.

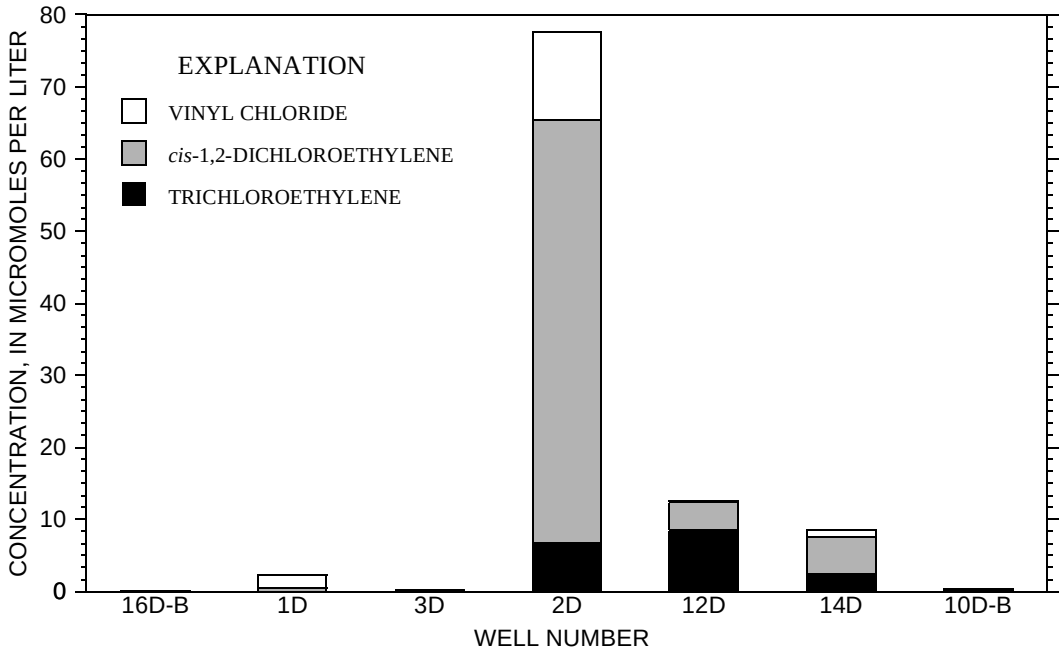

Figure 17. Concentrations of chlorinated ethenes in water samples collected from selected deep wells on November 4 and 5, 1997.

0.01 to 7.48 µM/L and from 0.04 to 5.41 µM/L, respectively (fig. 18). Ethene and ethane also were detected in water samples from wells 1D, 2D, and 3D (table 9). The ethene and ethane are final products of reductive dechlorination of VC and are readily consumed by bacteria.

The high ratio of degradation byproducts to TCE implies that reductive dechlorination is occurring at the site. The location of the reductive-dechlorination process is not clear from the chlorinated-ethene data. The degradation byproducts may have been formed in the shallow water-bearing zone and transported down into the upper part of the Ridley Limestone without any additional dechlorination occurring in the karst aquifer. Traditionally, sequential patterns of oxidation-reduction zones along a contaminant plume transect are used to identify where reductive dechlorination is active in the aquifer. A simple contaminant flow path and sequential oxidation-reduction zones could not be identified for the karst aquifer because of the complex hydrogeology at the study site. The geochemical and biological information derived from sampling karst-aquifer wells were considered individually instead of as a section of a continuum along a flow path. The data for an individual well represents geochemical and biological conditions in that part of the karst aquifer, whether the aquifer is in an active or less active flow zone. Multiple lines of biodegradation evidence are presented for karst wells 12D, 1D, 3D, and 2D in the following text.

Multiple Lines of Evidence from Well 12D

Water-quality conditions of samples from well 12D, located near Snell Branch and screened in the upper water-producing zone of the Ridley Limestone, changed significantly after rainfall events. Continuous data collected from well 12D indicated that water levels rose (sometimes to land surface), specific conductance decreased due to dilution by rainwater, and DO concentrations and ORP (expressed as Eh) increased (fig. 19). These changes typically occurred within hours of rainfall events (fig. 20) indicating that water was quickly transported from land surface to the upper water-producing zone of the Ridley Limestone intersected by well 12D. These changes in water chemistry are consistent with the detection of a mud-filled cavity that was hydraulically connected to Snell Branch during the construction of well 12D. After rainfall events, the DO would start to decrease and anaerobic conditions would develop until the next rainfall (fig. 19).

During dry periods such as late May 1998, anaerobic conditions persisted and oxidation-reduction potentials decreased (fig. 19D) to levels associated with iron-reducing conditions (fig. 2). However, during the 3 months of continuous monitoring, anaerobic conditions did not persist long enough for reductive dechlorination to occur. The lack of VC (fig. 21), ethene, and ethane (table 9) in water samples from well 12D also suggests that little reductive dechlorination had occurred. Some periodic increases in *c*DCE concentrations were detected in water samples collected from well 12D; however, these increases were often accompanied by increases in TCE (fig. 21) and are most likely the result of transport processes, not reductive dechlorination in the water-producing zone intersected by well 12D.

These results indicate that natural attenuation of chlorinated ethenes in the part of the karst aquifer intersected by well 12D is probably limited to aerobic degradation or mechanisms such as dilution. Ammonia-oxidizing bacteria and methanotrophs (table 10) were detected in water samples from well 12D; however, microcosms containing water from well 12D (experiment 2, treatment 4) did not exhibit significant aerobic degradation of TCE during a 17-week period (fig. 22). Microcosm data are given in table 11 (at end of the report). The water samples used to construct these microcosms were collected during a dry period (May 21, 1998) and contained unusually low DO concentrations (table 9) and ORP (fig. 19D), which limited aerobic degradation. Between weeks 17 and 23, some reduction in TCE concentrations occurred in excess of the reductions in control samples (fig. 22) which indicate that reductive dechlorination was beginning to occur.

Data from microcosm experiment 1 indicate the occurrence of reductive dechlorination in microcosms containing water from well 12D. During microcosm experiment 1, the discovery was made that control microcosms (experiment 1, treatment 9) were not sterile. Plate counts for facultative and aerobic heterotrophic bacteria indicated greater than 2×10^4 bacteria colony-forming units per 100 milliliters (CFU/100mL) of water sample in the control microcosms after a 1-week incubation period. Bacteria enumeration data are given in table 12 (at end of the report).

Without adequate controls, determining to what degree the TCE decreases in the microcosms was caused by biodegradation was impossible. Significant

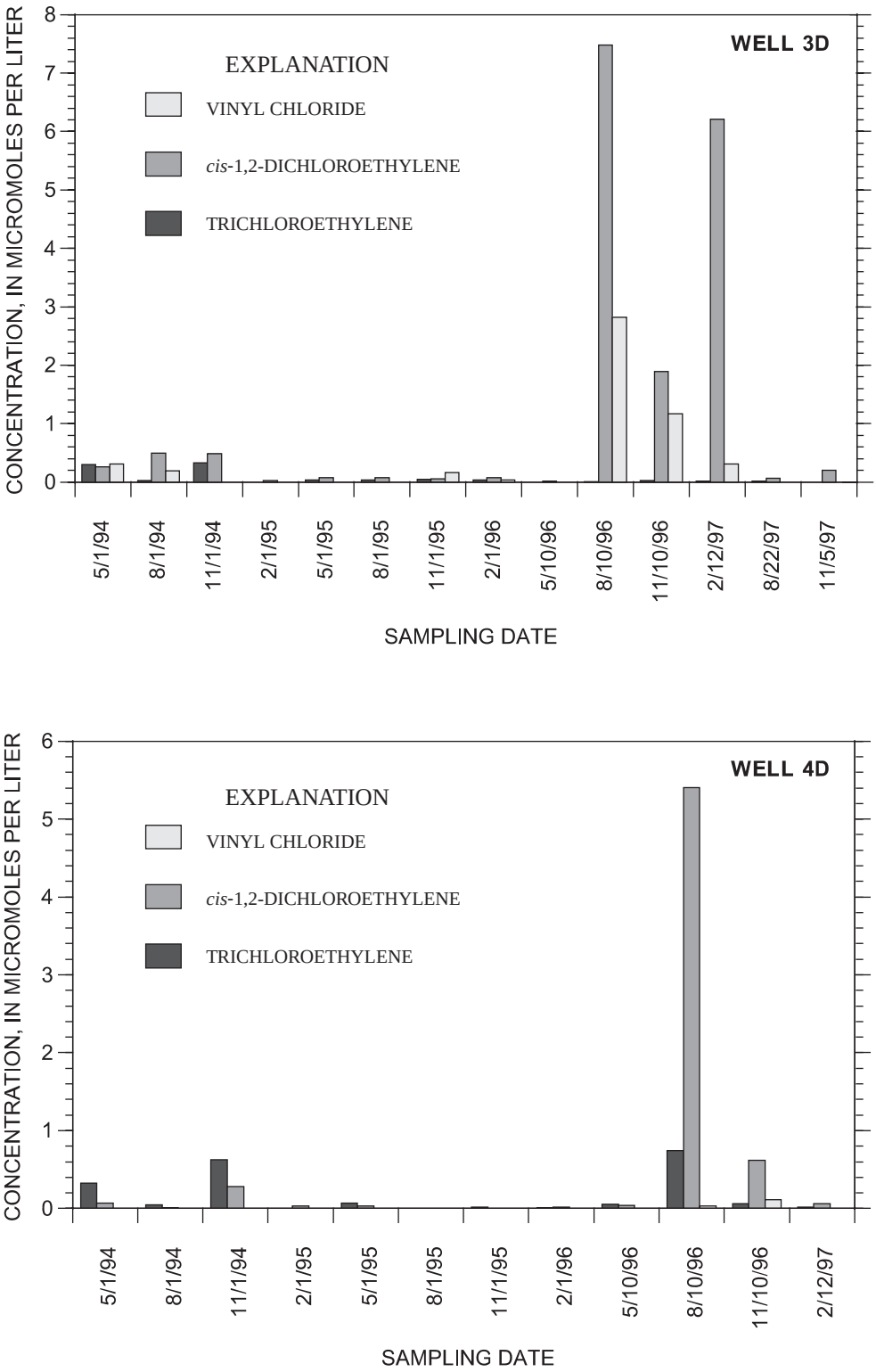

Figure 18. Temporal changes in chlorinated-ethene concentrations in water samples from wells 3D and 4D.

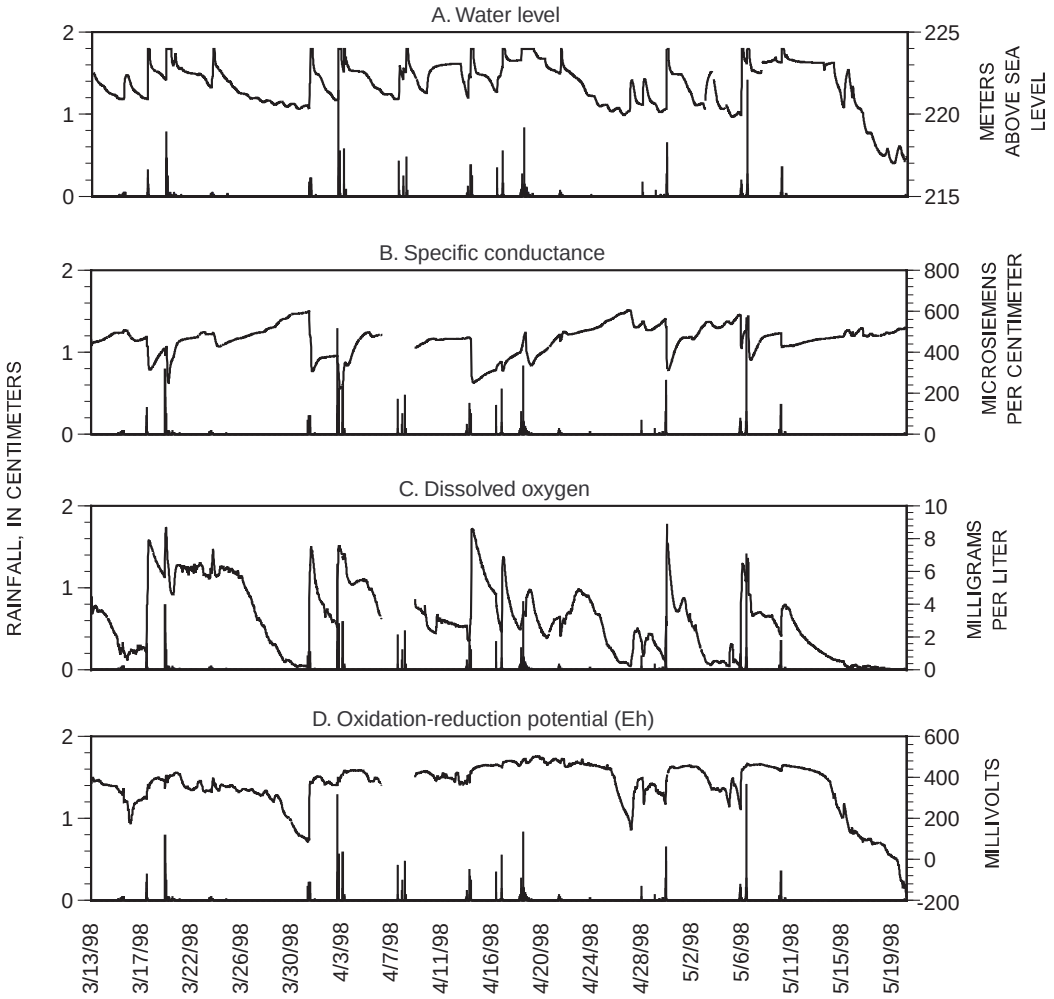

Figure 19. Continuous ground-water monitoring data collected from well 12D for (A) water level, (B) specific conductance, (C) dissolved oxygen, and (D) oxidation-reduction potential, March 13 through May 19, 1998. (Line gaps indicate missing data.)

*c*DCE and VC concentrations, however, were detected in several of the treatments after a 10-month incubation period, indicating that some reductive dechlorination had occurred in the microcosms. VC and *c*DCE were not present in the microcosms when they were first constructed, and the detection of the compounds could only be due to reductive dechlorination of TCE.

After a 10-month incubation period, *c*DCE represented 98 and 67 percent of the total chlorinated-ethene concentration remaining in microcosm treatments containing water from well 12D (experiment 1, replicates 7 and 8, respectively) (fig. 23). Microorganisms responsible for reductive dechlorination (sulfate reducers) were detected in water samples from well 12D (table 10), and the microcosms from experiment 1 indicate that the microorganisms were able to dechlorinate TCE after oxygen and other electron acceptors were depleted during the 10-month incubation period. In spite of microcosm evidence that reductive dechlorination can occur in water from 12D, it is unlikely that reductive dechlorination is a significant biological process in that part of the karst aquifer. Field geochemical conditions indicate that the reducing conditions in 12D are disrupted with each rainfall event.

Multiple Lines of Evidence from Well 1D

Well 1D is screened in the lower water-producing zone of the karst aquifer and is located close to the manufacturing building. Water-quality conditions in the part of the karst aquifer intersected by well 1D exhibited little change in response to storms during the 2 months (March 1998 to May 1998) of continuous

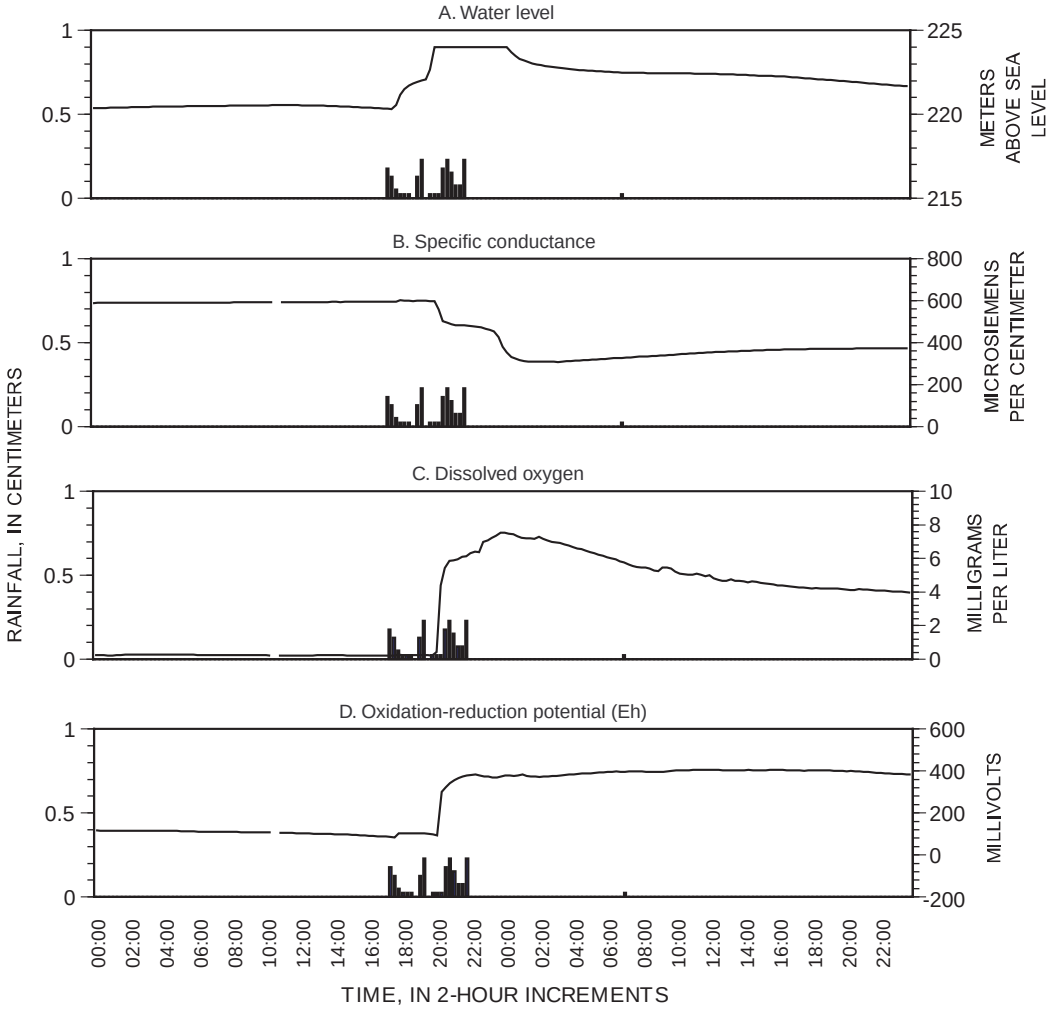

Figure 20. Continuous ground-water monitoring data collected from well 12D showing rapid changes in (A) water level, (B) specific conductance, (C) dissolved oxygen, and (D) oxidation-reduction potential, March 31 through April 1, 1998. (Line gaps indicate missing data.)

monitoring. Anaerobic conditions (less than 0.3 mg/L DO) were consistently detected in water samples collected from well 1D during quarterly sampling events (table 9), and geochemical data indicate manganese-reducing conditions (fig. 16). Continuous water-quality monitoring indicated that although water levels increased after precipitation events, specific conductance, DO, and ORP (expressed as Eh) changed little (fig. 24). Equilibrium of the ORP electrode took approximately 4 days to come to equilibrium with the aquifer water. Once equilibrium was reached, ORP measured during continuous monitoring was normally between –150 and –200 millivolts (mV), which is normally associated with nitrate-, iron-, and sulfate-reducing conditions (figs. 2 and 24D).

During a 23-week period, significant aerobic degradation of TCE was not detected in microcosms using water collected from well 1D on May 1, 1998 (experiment 2, treatment 1) (fig. 22). Ammonia oxidizers were not detected in water samples collected from well 1D; however, methanotrophs were detected (table 10). Based on the geochemical data and the microcosm results, biodegradation of chlorinated ethenes in the water-producing zone intersected by well 1D would be limited to reductive dechlorination. Between 17 and 23 weeks, TCE concentrations in the microcosms using well 1D water decreased faster than in the control samples (fig. 22) which could indicate that reductive dechlorination was beginning to occur. During the 10-month incubation period of microcosm experiment 1, TCE degradation byproducts (VC and *c*DCE) increased from zero to approximately 80 and 50 percent of the average total chlorinated-ethene concentration remaining in microcosms containing water from well 1D

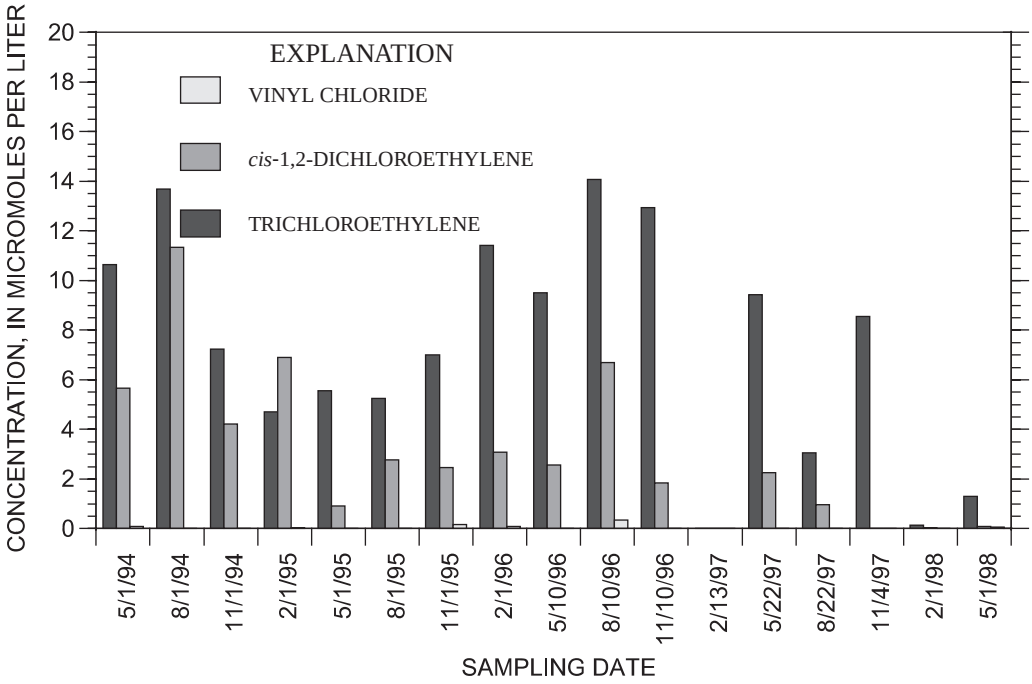

Figure 21. Chlorinated-ethene concentrations in water samples collected from well 12D.

Table 10. Results from bacteria identification for samples collected on November 4 and 5, 1997 from selected wells
[N, not detected; Y, detected; --, data not available]

	Wells					
Type of bacteria	**1D**	**2D**	**3D**	**12D**	**16D-B**	**10D-B**
Ammonia oxidizers	N	N	Y	Y	Y	Y
Methanotrophs	Y	Y	Y	Y	Y	Y
Manganese/iron oxidizers	--	Y	Y	Y	N	Y
Sulfate reducing	--	Y	Y	Y	Y	Y
E. coli	Y	--	Y	Y	Y	N

(experiment 1, treatments 1 and 2, respectively) (fig. 23). Results from microcosm experiment 1 demonstrate that reductive dechlorination of TCE is possible in the water from well 1D.

The lag time for reductive dechlorination to occur in the microcosms was probably due to aeration of the experimental water during set up. No lag time would be expected in the aquifer around well 1D since the conditions are consistently anaerobic. Further evidence of reductive dechlorination in well 1D is found in the chlorinated-ethene data (table 8). Byproducts of TCE reductive dechlorination, DCE and VC, were found in every sample reported. Thus, the multiple lines of evidence indicate reductive dechlorination occurs in the part of the karst aquifer intersected by well 1D.

Multiple Lines of Evidence from Well 3D

Well 3D is located near the center of the manufacturing building and is screened in the lower water-producing zone of the upper part of the Ridley Limestone. Continuous water-quality data indicate that rainfall events and changes in the pumping rate of well 9D affect water-quality conditions in the part of the karst aquifer intersected by 3D. When pump-and-treat well 9D was operating (March 19, 1998 to April 8, 1998) the water moving through the screened interval of well 3D appeared to have been stored in the karst aquifer longer than the water moving through the screened interval of well 12D. Evidence for this inference includes increased specific conductance after precipitation events (fig. 25B). The higher specific conductance is indicative of increased dissolved solids

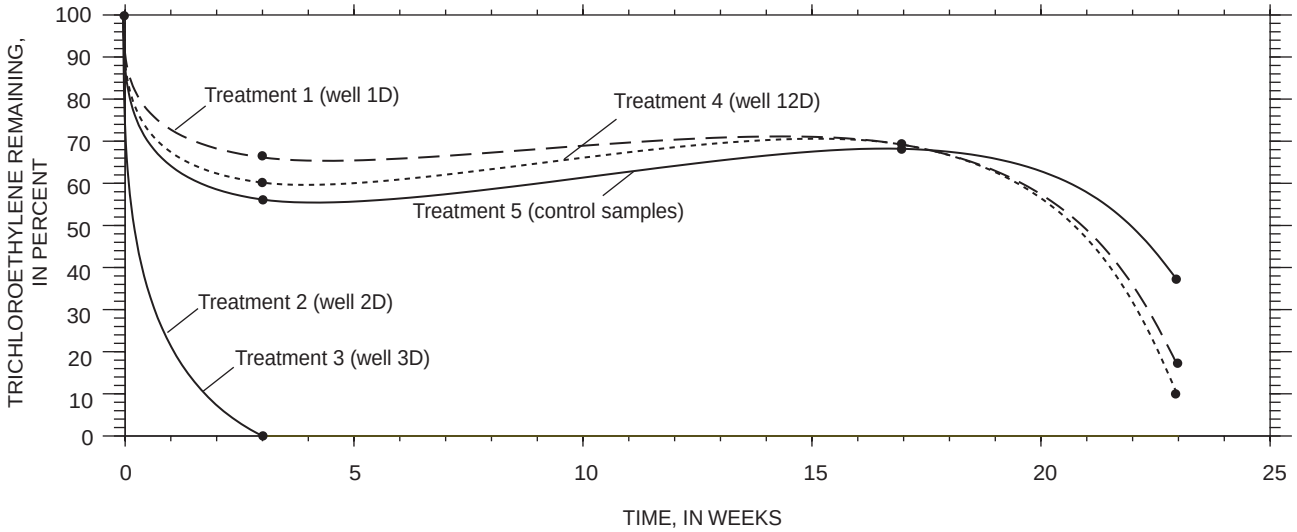

Figure 22. Trichloroethylene (TCE) concentrations during microcosm experiment 2.

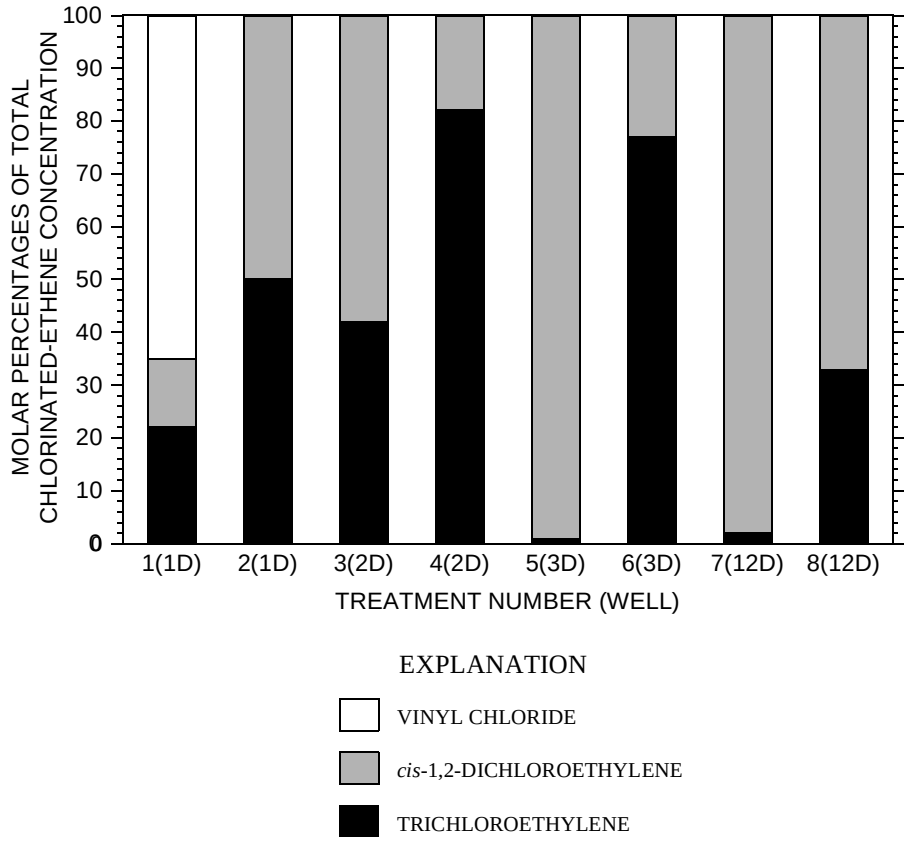

Figure 23. Relative amounts of trichloroethylene and reductive-dechlorination degradation products in experiment 1 microcosms after 10 months of incubation.

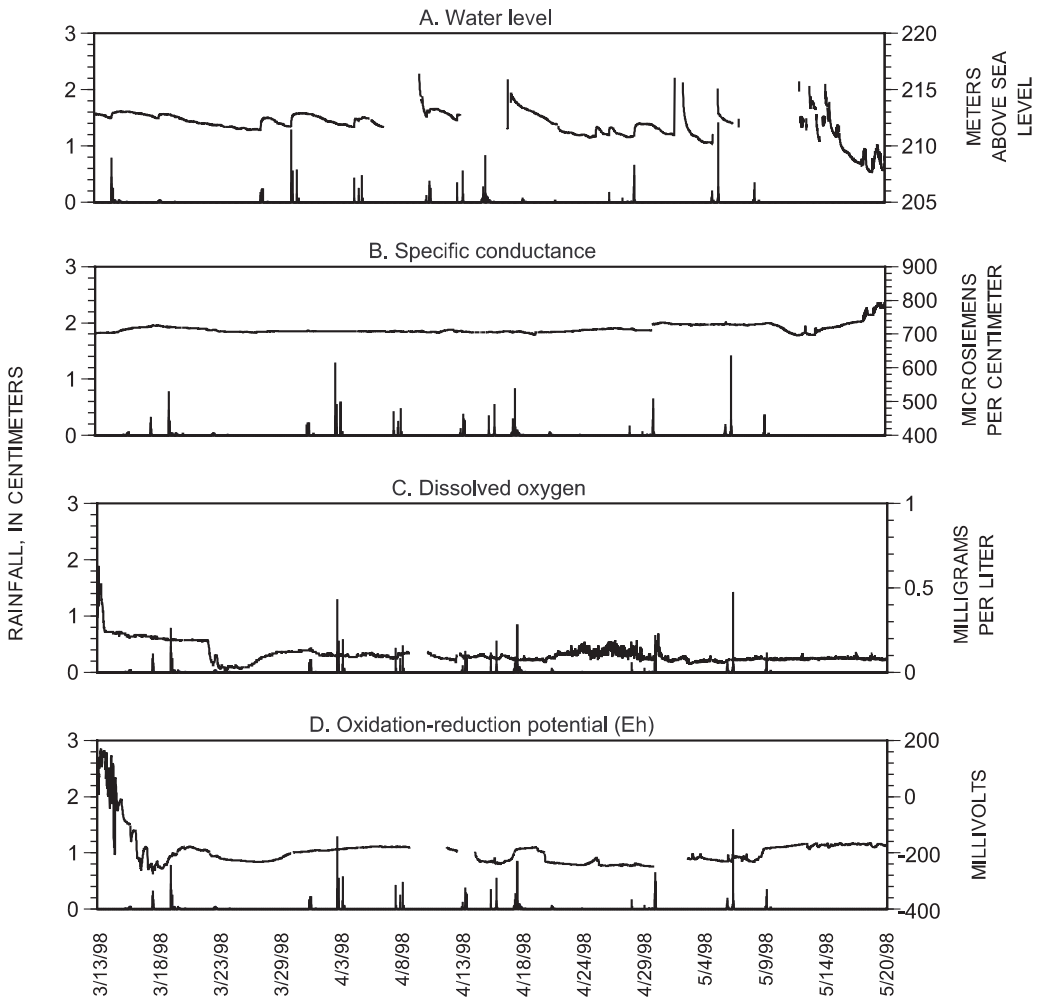

Figure 24. Continuous ground-water monitoring data collected from well 1D for (A) water level, (B) specific conductance, (C) dissolved oxygen, and (D) oxidation-reduction potential, March 18 through May 20, 1998. (Line gaps indicate missing data.)

normally associated with limestone dissolution over time. Also, dye was detected in water samples collected from well 3D in 1996, 5 years after the dye was injected into well 8D which was screened in the same lower water-producing zone of the karst aquifer. Water samples collected from well 3D during quarterly sampling normally had low DO concentrations (less than 0.2 mg/L) (table 9). During the continuous monitoring period, DO concentrations in the well water were less than 0.5 mg/L and ORP was less than –100 mV (fig. 25D) when the pump-and-treat well was operating.

The ORP (fig. 25D) was within ranges normally associated with nitrate-, iron-, and sulfate-reducing conditions (Stumm and Morgan, 1981). Sulfide concentrations detected in some water samples from well 3D (>1 mg/L) were significantly higher than sulfide concentrations detected in water samples from shallow wells (tables 7 and 9), which indicate sulfate-reduction occurred in the aquifer near well 3D. Sulfate-reducing bacteria were identified in water samples collected from well 3D (table 10). Results from microcosm experiment 1 indicate that bacteria from well 3D can reductively dechlorinate TCE. After a 10-month incubation period, *c*DCE represented 98 and 24 percent of the total chlorinated ethene remaining in microcosms using well 3D water (experiment 1, treatments 5 and 6, respectively) (fig. 23).

The specific conductance of water in well 3D decreased, and DO increased, when pump-and-treat well 9D was not operating (various times between April 8, 1998 and May 20, 1998) (fig. 25). The slight decrease in specific conductance and increased DO

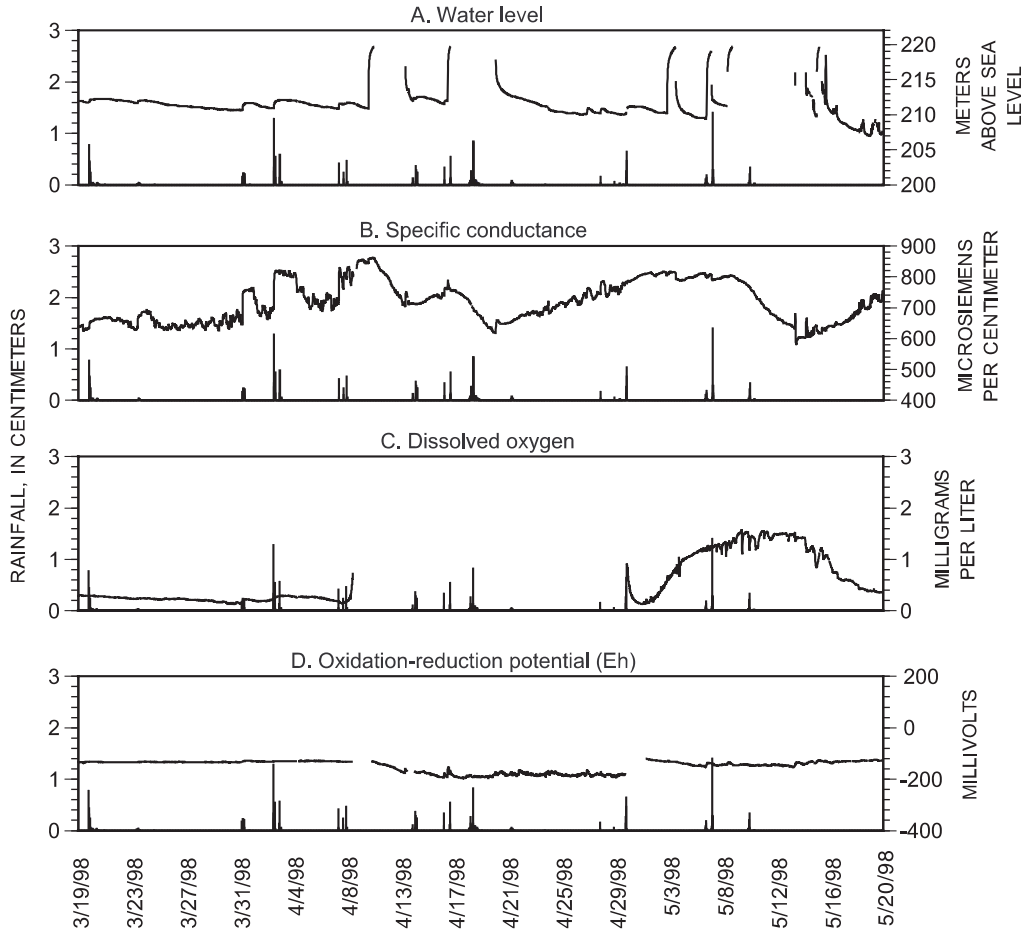

Figure 25. Continuous ground-water monitoring data collected from well 3D for (A) water level, (B) specific conductance, (C) dissolved oxygen, and (D) oxidation-reduction potential, March 19 through May 20, 1998. (Line gaps indicate missing data.)

associated with pump-and-treat downtime indicate recharge of the karst aquifer near well 3D by fresh rainwater. The resulting change would tend to benefit the aerobic bacteria, ammonia oxidizers and methanotrophs, detected in water samples from well 3D. Results from the second microcosm experiment using water collected on May 21, 1998, suggest that significant cometabolism occurred when aerobic conditions prevailed. During a 3-week incubation period, TCE in the microcosms containing well 3D water (experiment 2, treatment 3) was completely degraded (fig. 22).

The multiple lines of evidence combined indicate that aerobic and anaerobic degradation occurred in the water-producing zone of the karst aquifer intersected by well 3D. Anaerobic conditions were present with the pump-and-treat well operating (the normal condition during this study) and reductive dechlorination was likely. When pump-and-treat well 9D was turned off, aerobic conditions suitable for aerobic cometabolic degradation of chlorinated ethenes prevailed. Bacteria suitable for both anaerobic and aerobic degradation were present in well 3D, and evidence of both degradation pathways was found in the microcosm studies.

Multiple Lines of Evidence from Well 2D

Well 2D is located to the west of the manufacturing building near pump-and-treat well 9D and is screened in the lower water-producing zone of the karst aquifer, as is well 9D. Because well 2D is located very close to pump-and-treat well 9D, water passing through the screened interval of well 2D probably flows from several parts of the karst aquifer in response to the gradient imposed by pumping well 9D. Water in well 2D represents a composite of waters

from parts of the karst aquifer affected by the pumping. The ORP and DO conditions in well 2D tended to be moderate whereas the bacteria composition ranged from aerobic to anaerobic bacteria types.

Water-quality conditions in the part of the karst aquifer intersected by well 2D changed in response to rainfall events, although not as quickly as conditions in well 12D. Specific conductance decreased after rainfall events due to dilution by rainwater (fig. 26B). DO concentrations initially increased after rainfall events then rapidly decreased as anaerobic aquifer water with a high chemical oxygen demand mixed with the rainwater (fig. 26C). In a pattern similar to the DO concentrations, ORP decreased after rainfall events (fig. 26D). These data indicate that freshwater was initially transported toward the pump-and-treat well after rainfall events (fig. 26). However, this fresh rainwater was soon followed by a mixture of anaerobic aquifer water and rainwater as indicated by the lower specific conductance, DO, and ORP concentrations. Presumably, old aquifer water displaced by the rainwater contained chemicals such as sulfide and reduced-metals (Fe^{2+} and Mn^{2+}) that would scavenge DO carried in by the rainwater, also driving the ORP down. When the rain ceased, the pre-rain event flow pattern would return to the karst aquifer within days. The implication is that the hydrology in the karst aquifer was stable during dry periods punctuated by recharge events that carried in water, DO, food, and other compounds. Bacteria as diverse as those found in the karst aquifer would take advantage of these changing environments, switching between anaerobic and aerobic conditions.

The quarterly geochemical data (table 9) and continuous ORP monitoring (approximately +200 mV) (fig. 27D) indicate that baseline conditions ranged from slightly aerobic to Mn^{4+} and NO_3^- reducing. Aerobic bacteria and NO_3^--reducing bacteria were identified in these water samples. During the anaerobic periods after a rain event, ORP commonly decreased to sulfate-reducing conditions (fig. 27D), and

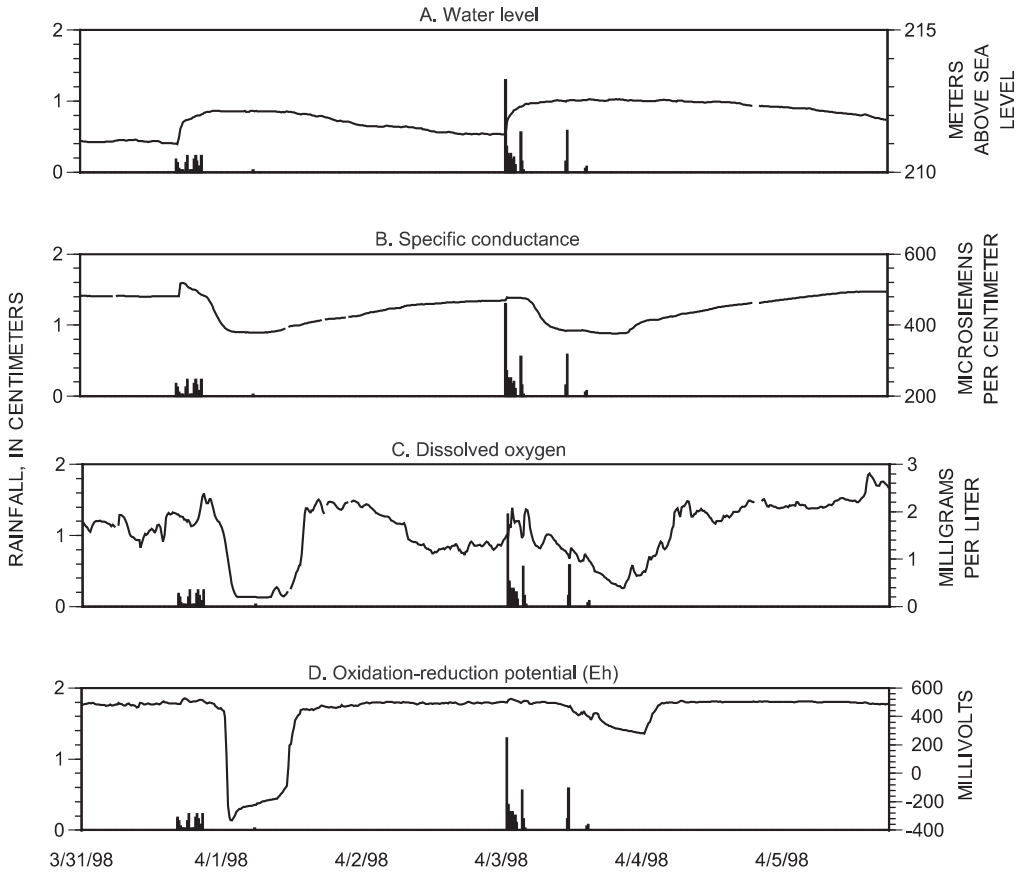

Figure 26. Continuous ground-water monitoring data collected from well 2D for (A) water level, (B) specific conductance, (C) dissolved oxygen, and (D) oxidation-reduction potential, March 31 through April 5, 1998. (Line gaps indicate missing data.)

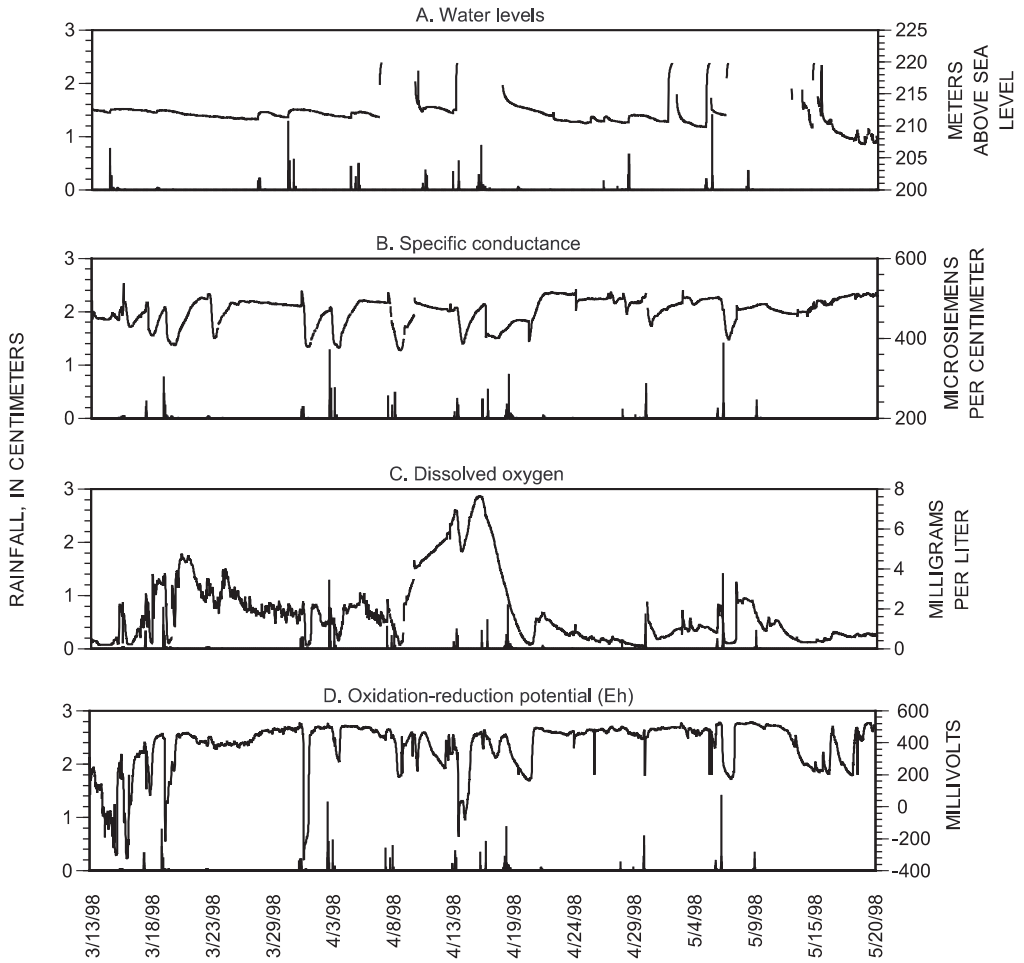

Figure 27. Continuous ground-water monitoring data collected from well 2D for (A) water level, (B) specific conductance, (C) dissolved oxygen, and (D) oxidation-reduction potential, March 13 through May 20, 1998. (Line gaps indicate missing data.)

SO_4-reducing bacteria were detected in water samples from well 2D (table 10). However, the sulfate-reducing conditions lasted only a few days in well 2D, which would be insufficient time for significant reductive dechlorination to take place. The ORP conditions and bacteria types found in well 2D represent the wide range of conditions present in the karst aquifer. The pattern of ORP and DO with regard to baseline and post-rain conditions provides insight into the displacement of older ground water by recharge from rainwater.

Cometabolism appeared to be a more significant biological degradation process than reductive dechlorination in microcosms set up with water from well 2D. Experiment 2 microcosms using water from well 2D exhibited significant aerobic degradation. Within 3 weeks, TCE in the microcosms was completely degraded (fig. 22, treatment 2). Methanotrophs were identified in well 2D water samples; however, ammonia oxidizers were not detected. Bacteria identification and microcosm results in conjunction with geochemical monitoring indicate that environmental conditions suitable for cometabolism occurred more consistently in well 2D than did conditions for reductive dechlorination. However, results from microcosms in experiment 1 indicate that native bacteria from well 2D were capable of reductively dechlorinating TCE. Evidence of reductive dechlorination was found in microcosms after a 10-month incubation period. The TCE degradation byproduct *c*DCE was present in microcosms as a result of reductive dechlorination (experiment 1, treatments 3 and 4) (fig. 23).

Data Collected from Other Deep Wells

Geochemical data from other deep wells at the site indicated a similar pattern of anaerobic zones in the aquifer or zones fluctuating between aerobic and anaerobic conditions. The DO concentration in water samples from well 4D ranged from 0.2 to 1.2 mg/L and the DO concentration in water samples from well 16D-B ranged from 0.3 to 1.8 mg/L (table 9). Both aerobic (ammonia oxidizers and methanotrophs) and anaerobic (sulfate reducers) bacteria were detected in water samples from well 16D-B (table 10).

Water samples from monitoring wells 10D-B and 11D contained less than 0.1 mg/L of DO in three or fewer sampling events. Well 11D is screened in a bedding plane that appeared to be isolated from the active ground-water-flow system. Water from well 11D had such high sulfide concentrations that it had a strong odor and would start to form a black precipitate within minutes after being brought to the surface. These observations suggest that DO concentrations in well 11D were consistently less than 0.1 mg/L. Unlike well 11D, well 10D-B was screened in a highly fractured area of the karst aquifer. Limited geochemical data (table 9) indicate anaerobic conditions occurred in well 10D-B; however, both aerobic and anaerobic bacteria were present in water samples (table 10), implying a close association with aerobic conditions. More data are required to determine whether conditions in 10D-B are consistently anaerobic.

Geochemical data from 11 deep wells indicate that geochemical conditions varied greatly throughout the karst aquifer at the site (table 9). For example, during the August 20-22, 1997 sampling event, DO ranged from less than 0.1 to 5.5 mg/L, ammonia ranged from 0.02 to 2.30 mg/L, and sulfide ranged from 0.02 to 3.46 mg/L.

A large diverse microbial population was also present throughout the karst aquifer (table 10). The concentration of viable bacteria present in water samples from the deep wells was higher than expected. For example, aerobic heterotrophic bacteria concentrations in water samples from the karst aquifer were greater than 6×10^4 colony forming units per 100 mL of sample (table 12). This range of bacteria does not account for strict anaerobic bacteria or others that would not grow on the tryptic soy agar plates. Still, these unenriched bacteria concentrations occurring in the karst aquifer were equivalent to culture concentrations used in laboratory biodegradation studies (Dalton, 1977; Nelson and others, 1988). This suggests enough bacteria are present in the karst aquifer to support TCE biodegradation.

The hydrologic, biological, and chemical data demonstrate the large range of hydrologic conditions in the karst aquifer and provide insight into its complexity. Some areas in the karst aquifer responded rapidly to rainfall events whereas other areas were more stable and changed little regardless of rainfall, probably reflecting longer ground-water residence times. This range of hydrologic conditions provides an opportunity for bacteria to enter, reproduce, and spread throughout the aquifer system. The bacteria appear to have flourished wherever conditions were right. As the bacteria multiplied, they influenced the geochemistry of the system and degraded chlorinated solvents. Despite the complexity of the karst system, the same biological degradation processes are active in this karst system as in any unconsolidated aquifer. The hydrologic, biological, and chemical data provide multiple lines of evidence, supported by laboratory microcosm results, that a variety of TCE biodegradation pathways are active in the karst aquifer (table 13). The central issue for bioremediation is not whether biodegradation is occurring in the karst aquifer, but whether water is retained long enough in the karst aquifer to degrade a meaningful mass of contaminants. The observation of dyes in various deep wells 5 years after injection and the stable anaerobic conditions in parts of the aquifer indicate long local residence times. This study, however, does not determine what volume of the aquifer is represented by such areas and how much contamination they contain.

LESSONS LEARNED

This project examined the potential for biological degradation of TCE in karst aquifers. Biodegradation is often presumed to be an irrelevant attenuation process in karst aquifers because of short ground-water residence times, lack of bacteria, and unsuitable environmental conditions. To address these issues, hydrologic, biological, and geochemical information was gathered. The greatest challenge of this investigation was interpreting the results within the framework of the complex karst hydrology. Examining biodegradation in the shallow water-bearing zone near the top of the limestone bedrock was relatively straightforward. Identification was possible of a chlorinated-solvent plume, a general ground-water flow path, and a sequential pattern of oxidation-reduction zones in the

Table 13. Summary and interpretation of multiple lines of evidence using data collected from deep wells

Well	Geochemical indicators (quarterly data)	Geochemical indicators (continuous monitoring data)	Chlorinated-ethene data
1D	Anaerobic, nitrate-reducing conditions.	Anaerobic, sulfate-reducing or methanogenic conditions.	*cis*-1,2-Dichloroethylene, vinyl chloride, ethene, and ethane detected.
3D	Anaerobic, nitrate-reducing conditions. Possible sulfate-reducing conditions at times (high sulfide concentrations detected).	Anaerobic, sulfate-reducing or methanogenic conditions. Aerobic conditions when the 9D pump-and-treat well is not operating.	*cis*-1,2-Dichloroethylene, vinyl chloride, and ethane detected.
2D	Fluctuates between aerobic and anaerobic conditions. Possible ammonia oxidization and nitrate reduction during aerobic and anaerobic conditions, respectively.	Rapid fluctuations between aerobic and anaerobic conditions. Oxidation-reduction potentials indicate manganese or iron-reducing levels.	*cis*-1,2-Dichloroethylene, vinyl chloride, ethene, and ethane detected.
12D	Fluctuates between aerobic and anaerobic conditions. Possible ammonia oxidization and nitrate reduction during aerobic and anaerobic conditions, respectively.	Anaerobic conditions only present for a few days after rainfall events. During dry periods, reducing conditions may be present for a few weeks.	*cis*-1,2-Dichloroethylene, vinyl chloride, ethene, and ethane detected. Higher molar concentrations of trichloroethylene than degradation products detected.
Other wells.	Aerobic and anaerobic conditions	Data not available	Reductive-dechlorination degradation products detected.

shallow water-bearing zone. Two lines of evidence, geochemical and chlorinated-ethene data, sufficed to demonstrate the occurrence of TCE biodegradation in the shallow anaerobic zone (fig. 28). Since the aim of this study was to determine if biological degradation of chlorinated solvents was occurring in the karst aquifer, further examination of the shallow water-bearing zone was not done.

Byproducts of TCE reductive dechlorination were detected in water samples from the deeper karst aquifer; however, additional data was required to determine if the degradation byproducts were simply transported from the shallow water-bearing zone or if they were the result of biodegradation processes within the karst aquifer. Also, the complexity of the karst aquifer system made computerized fate-and-transport models useless for this site. In the karst aquifer, ground-water zones in close proximity to each other often varied in hydrology, biology, and geochemistry.

Geochemical parameters and chlorinated solvents measured quarterly in deep wells provided some understanding of spatial and temporal patterns in the karst aquifer. These data demonstrated that conditions were favorable for reductive dechlorination or cometabolic degradation pathways in various parts of the karst aquifer. After four sampling events, evidence showed that temporal patterns were not adequately characterized by the quarterly sampling schedule. Continuous monitoring devices were placed in four karst aquifer wells to gather information on temperature, pH, DO, ORP, specific conductance, and water levels to monitor aquifer stability. These continuous monitoring devices provided some of the most important information for identifying areas capable of sustaining biological degradation processes. The continuous monitoring devices confirmed water storage areas and active flow zones in the karst aquifer by linking specific conductance, ORP, and DO with hydrogeologic and weather information. This continuous monitoring information was used to interpret the bacteria and microcosms results (fig. 29).

Biological information similar to that described in other studies (Wilson and others, 1996) was gathered during the investigation. Additional biological data were needed because of the widespread perception that karst aquifers are not able to sustain large microbial populations. Determining if a sufficiently large and diverse microbial population capable of biodegrading the chlorinated solvents existed in the karst aquifer was critical for this investigation (fig. 30). This line of evidence was established by identifying bacteria known to degrade chlorinated solvents using molecular methods and traditional plate counts. Microcosms prepared from non-enriched well water demonstrated cometabolism or reductive dechlorination of TCE. Data resulting from these activities provided biological evidence that sufficient microbes were present in the karst aquifer and were capable of degrading TCE under expected site conditions.

Table 13. Summary and interpretation of multiple lines of evidence using data collected from deep wells—Continued

Well	Bacteria-identification data	Microcosms	Conclusions
1D	Ammonia oxidizers not detected. Methanotrophs detected. Data for other types of bacteria not available.	No significant aerobic degradation. Reductive-dechlorination degradation products present after a 10-month incubation.	Reductive dechlorination possible. No aerobic degradation because of the continuously anaerobic conditions.
3D	Ammonia oxidizers, methanotrophs, manganese and iron oxidizers, and sulfate reducers detected.	Significant aerobic degradation during a 3-week incubation. Reductive-dechlorination degradation products present after a 10-month incubation.	Reductive dechlorination possible. Aerobic degradation may occur when the 9D pump-and-treat well is not operating.
2D	Methanotrophs, manganese and iron oxidizers, and sulfate reducers detected.	Significant aerobic degradation during a 3-week incubation. Reductive-dechlorination degradation products present after a 10-month incubation.	Rapid fluctuations between aerobic and anaerobic conditions may prevent reductive dechlorination from occurring. Some aerobic degradation is possible.
12D	Ammonia oxidizers, methanotrophs, manganese and iron oxidizers, and sulfate reducers detected.	No significant aerobic degradation. Reductive-dechlorination degradation products present after a 10-month incubation.	Geochemical conditions would limit reductive dechlorination. Aerobic bacteria were present, but no aerobic degradation in microcosms.
Other wells.	Aerobic and anaerobic bacteria detected in samples from 10D-B and 16D-B.	Microcosm data not available	Potential aerobic and anaerobic degradation. Additional data needed.

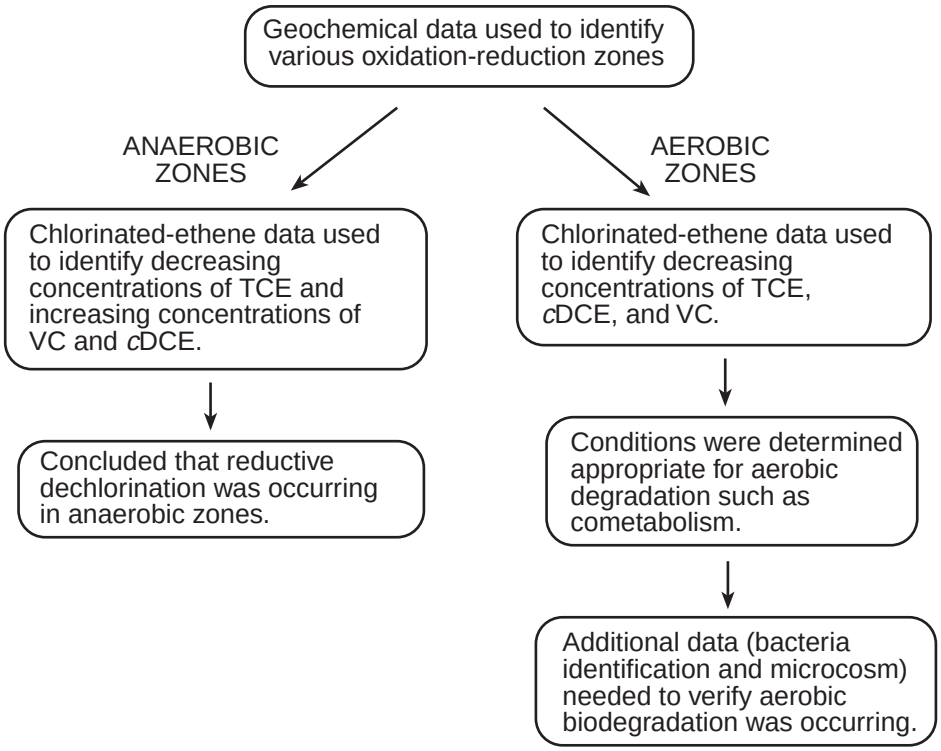

Figure 28. Lines of evidence needed to evaluate biodegradation of chlorinated solvents in the shallow water-bearing zone.

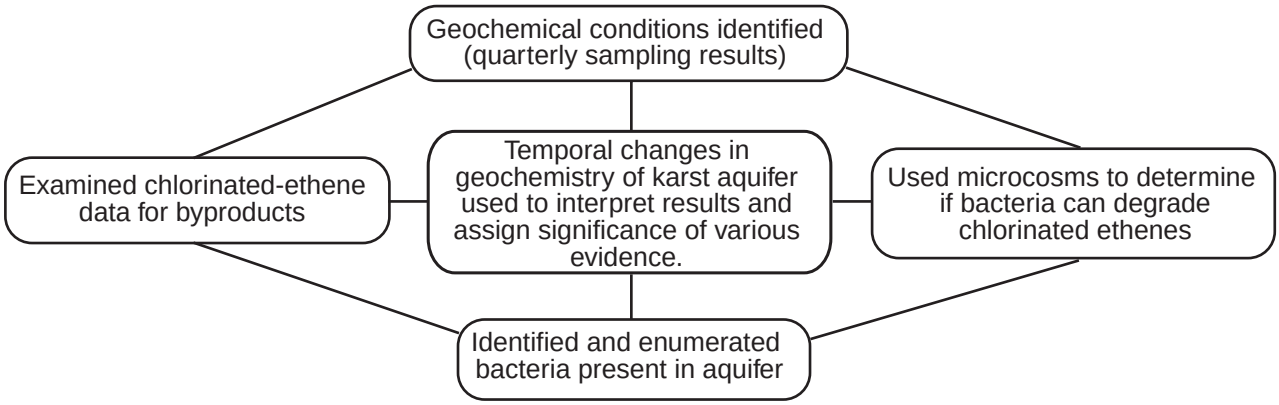

Figure 29. Lines of evidence needed to evaluate biodegradation of chlorinated ethenes in the karst aquifer.

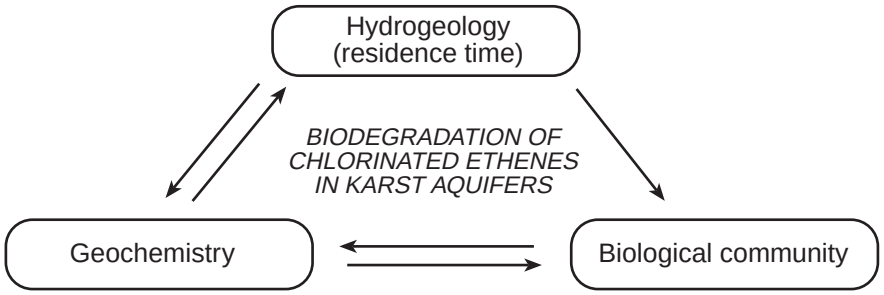

Figure 30. Hydrogeology determines residence time of ground water, which influences the biological community, the geochemistry, and the biodegradation of chlorinated ethenes in karst aquifers.

Hydrogeology was the principal factor influencing the biological and geochemical conditions in the karst aquifers (fig. 30). Therefore, the chemical and biological evidence had to be considered in the context of the hydrology. For example, temporal and spatial continuity of the aquifer geochemistry and ORP had to be taken into account before applying the results from the microcosm studies to the karst aquifer. Results from the microcosm studies indicated that anaerobic reductive dechlorination was possible in each well given strong anaerobic conditions. However, geochemical conditions in some parts of the aquifer fluctuate with the weather, preventing reductive dechlorination from occurring to the same extent as in the microcosms.

Although hydrology had the principal influence on the karst system, the appropriate geochemical conditions and biological assemblages also had to be present for TCE biodegradation to occur. Individually, hydrologic, biological, or chemical observations were insufficient to prove that TCE biodegradation was occurring in the karst aquifer. Together they provided convincing evidence that anaerobic and aerobic biological degradation processes were active in distinct areas of the karst aquifer.

SUMMARY AND CONCLUSIONS

The biodegradation of chlorinated ethenes in aquifers consisting of unconsolidated material has been well documented; however, chlorinated-ethene biodegradation has not been adequately investigated in karst areas even though chlorinated-solvent biodegradation products have been detected in karst aquifers. Factors that affect the biodegradation of chlorinated

solvents include hydrology, microbiology, and geochemistry.

Three categories of metabolic processes are involved in the biological degradation of chlorinated ethenes. Reductive dechlorination is an anaerobic process in which chlorinated ethenes are used as electron acceptors and results in a sequential dechlorination from PCE to TCE to DCE to VC to ethene. Cometabolism is an aerobic process in which chlorinated ethenes are degraded as a result of oxygenase enzymes, such as methane and ammonia monooxygenase, inserting oxygen into TCE, DCE, or VC molecules. Cometabolism needs additional substrates like methane or ammonia, as well as dissolved oxygen, to sustain the process. Direct oxidation is an aerobic or mildly anaerobic process in which lightly chlorinated ethenes (DCE and VC) are used as electron donors. At a given site, one or all of these processes could be occurring depending on environmental conditions.

Most studies of contaminant biodegradation in fractured rock or karst settings examined biodegradation in the overburden or saprolite above bedrock. The lack of studies examining biodegradation in karst aquifers may be due to the widespread perception that the hydrologic and microbiologic characteristics of karst aquifers prevent biodegradation of contaminants in karst aquifers. Previous research, however, has indicated that large volumes of water may be isolated from active ground-water flow paths in karst aquifers. Other studies have shown that water from bedrock aquifers may contain large and diverse microbial populations, which include bacteria responsible for the degradation of chlorinated ethenes.

The biodegradation of chlorinated ethenes was examined at a TCE contaminated karst site in Middle Tennessee. A shallow water-bearing zone is present at the site and chlorinated-ethenes are transported along a trough in the bedrock surface. Some deep wells intersect fractures that are part of the active ground-water flow system of the karst aquifer, whereas other deep wells intersect fractures isolated from the active ground-water flow system. Pump-and-treat wells completed in the upper part of the Ridley Limestone draw down water levels in many of the deep wells and affect local ground-water flow.

Multiple lines of evidence usually are needed to evaluate potential biodegradation processes. These lines of evidence normally include: (1) geochemical data that indicate depletion of electron donors and acceptors and increasing concentrations of metabolic byproducts, (2) chemical data that indicate decreasing concentrations of chlorinated solvents and increasing concentrations of degradation products, and (3) laboratory or field microbiological data that indicate the bacteria present at a site can degrade contaminants. Chlorinated-ethene, ethene, and ethane data for water samples from shallow and deep wells were obtained from TDEC-DSF files. Additional data-collection activities conducted by the USGS included periodic water-quality sampling of selected shallow and deep monitoring wells, collection of water samples from selected deep wells for bacteria identification and enumeration, continuous monitoring of water quality and water levels for selected deep wells, and microcosm studies.

Multiple lines of evidence indicate that reductive dechlorination was the dominant biodegradation process occurring in the anaerobic shallow water-bearing zone underneath the manufacturing building. Ground water in the shallow water-bearing zone was influenced by precipitation recharge as the ground water moved away from the manufacturing building. The recharge water supplied DO and diluted the contaminated water in this transition zone between the anaerobic zone upgradient and the aerobic zone downgradient. Geochemical data indicate aerobic bacteria capable of cometabolizing or directly oxidizing the chlorinated ethenes were active in the aerobic zone downgradient, further decreasing the concentration of chlorinated ethenes in the shallow water-bearing zone. Undoubtedly some of the water, carrying with it bacteria, contaminants, electron donors, and electron acceptors, in the shallow water-bearing zone also migrates down into the karst aquifer.

Water-quality conditions in the karst aquifer varied both spatially and temporally. Significant concentrations of chlorinated-ethene biodegradation products were detected in the karst aquifer; however, the biodegradation products could have been transported from the shallow water-bearing zone. Because of the complex hydrology at the site, it was not possible to identify a chlorinated-ethene plume or discrete oxidation-reduction zones. The deep wells were treated as individual areas instead of as a direct continuum along a flow path. For example, well 12D is thought to intersect an active flow conduit in the aquifer. Wells 1D and 3D intersect water-bearing zones in the aquifer with less active flow. The data indicated that samples from wells which intersect the active ground-water flow system of the karst aquifer

(well 12D) contained bacteria that dechlorinated TCE; however, the geochemical conditions present would limit the occurrence of reductive dechlorination. Samples from wells which intersected fractures isolated from the active ground-water flow system (well 1D) contained bacteria which could dechlorinate TCE. Anaerobic conditions persisted in these zones and the geochemical conditions in these fractures were suitable for reductive dechlorination.

A vast consortia of bacteria capable of degrading chlorinated ethenes were detected in the water samples from the karst aquifer. Bacteria capable of cometabolism and direct oxidation of chlorinated ethenes were identified in water samples from wells that fluctuated between aerobic and anaerobic conditions (wells 2D and 3D). During periods of anaerobic conditions, constituents essential to cometabolism such as methane, ethane, and ammonia could be produced. Microcosm results indicated that the aerobic bacteria in samples from the karst aquifer could quickly (within less than 3 weeks) degrade TCE.

The greatest challenge to this investigation was interpreting the results within the framework of the complex karst hydrology. Data such as continuous water-quality monitoring and microbiological data were necessary to compose sufficient evidence that significant biodegradation occurred in the karst aquifer. Continuous monitoring provided some of the most useful information about the geochemical conditions and variability in the karst aquifer. Together, the multiple lines of evidence helped to identify the relation between hydrology, geochemistry, and biology and the biodegradation of chlorinated ethenes in the karst aquifer.

Table 2. Well completion and hydrogeologic data for selected wells

[--, data not available; altitudes are in meters above sea level; data from Tennessee Division of Superfund files]

Well	Top of casing	Top of Lebanon Limestone	Screen interval	Bottom of benonite seal	Notes
1D	232.48	227.0	171-192	194	
2D	226.18	224.7	170-191	193	
3D	231.31	229.4	175-193	194	
4D	231.84	231.1	186-189	190	
5D	232.14	229.8	188-191	191	
7D	231.96	230.2	169-203	204	Well abandoned
8D	233.77	231.2	166-206	206	Well abandoned
9D	226.86	224.8	183-203	203	Pump-and-treat well
10D-A	221.58	219.3	203-206	207	
10D-B	221.55	--	185-188	188	
10D-C	221.67	--	197-200	201	
11D	227.17	223.0	184-187	191	
12D	223.81	222.5	203-206	206	
13D	222.49	220.1	183-186	204	Pump-and-treat well
14D	222.57	220.1	180-183	205	Pump-and-treat well
15D-A	230.36	--	209-212	210	
15D-B	230.12	--	186-189	190	
15D-C	230.50	--	168-174	176	
16D	--	--	--	--	
1S	231.65	225.8	223-	--	
2S	231.65	225.8	223-	--	Pump-and-treat well
3S	229.77	226.1	224-	--	
4S	228.40	226.1	224-	--	
5S	227.92	224.8	223-	--	
7S	230.64	227.1	225-	--	
9S	231.65	229.4	223-	--	
10S	227.71	226.9	223-	--	
11S	230.25	226.8	224-	--	
12S	231.86	227.8	226-	--	
13S	226.46	225.0	221-	--	
15S	230.93	228.9	226-	--	
16S	231.37	227.6	225-	--	
17S	231.36	228.9	226-	--	
18S	232.36	229.5	227-	--	
19S	232.36	230.9	228-	--	
20S	231.65	227.3	225-	--	
21S	231.49	229.3	226-	--	
25S	233.61	230.1	228-	--	
30S	231.33	--	229-	--	
31S	231.58	--	227-	--	Pump-and-treat well
32S	231.55	--	228-	--	Pump-and-treat well
33S	231.52	--	229-	--	
34S	230.75	--	230-	--	
35S	224.12	--	222-	--	
36S	224.70	--	223-	--	
37S	222.76	--	220-	--	
40S	230.83	--	226-	--	
42S	230.55	--	226-	--	
BN-1	227.09	--	223-	--	
BN-2	225.61	--	221-	--	
W-1	225.35	223.1	223-	--	
PL-1	225.52	223.1	223-	--	

Table 8. Chlorinated-ethene data for samples from selected wells

[Data from Tennessee Division of Superfund files; TCE, trichloroethylene; *c*DCE, *cis*-1,2-dichloroethylene; VC, vinyl chloride; µg/L, micrograms per liter; --, no data; <, less than]

Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)	Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)
2S	10/31/85	950,000	--	--	5S	08/10/96	137	89	<1
2S	11/05/85	750,000	--	--	5S	11/10/96	97	46	4
2S	11/12/85	1,080,000	--	--	5S	02/11/97	274	119	<1
2S	01/03/86	920,000	--	--	5S	08/22/97	744	43	<1
2S	01/09/87	520,000	--	5,300	5S	11/05/97	490	365	<1
2S	02/05/87	800,000	--	88,000	5S	5/21/98	1,440	504	<1
2S	03/13/87	36,000	--	<10	6S	10/31/85	270	--	--
2S	11/13/88	730,000	--	--	6S	11/05/85	490	--	--
2S	05/16/91	<10	--	<10	6S	11/12/85	720	--	--
2S	10/25/91	68,000	--	<5,000	6S	01/03/86	250	--	--
2S	02/10/92	80,000	--	4,500	6S	01/09/87	32	--	<10
2S	04/17/92	170,000	--	<5,000	6S	02/05/87	120	--	1,300
2S	07/31/92	1,800,000	--	<200,000	6S	03/13/87	<10	--	<10
2S	02/24/93	48,000	--	1,900	6S	11/13/88	62	--	--
2S	05/14/93	52,000	--	2,200	6S	05/16/91	2,100	--	<10
2S	05/01/94	300,000	100,000	3,000	6S	10/25/91	22	--	<10
2S	08/01/94	350,000	<20	2,900	6S	02/10/92	15	--	<10
2S	11/01/94	190,000	54,000	<500	6S	04/17/92	3	--	23
2S	02/01/95	140,000	53,000	3,400	6S	07/31/92	5	--	<10
2S	05/01/95	170,000	46,000	<500	6S	10/30/92	8	--	<10
2S	08/01/95	270,000	35,000	<5,000	6S	02/24/93	<5	--	<10
2S	11/01/95	129,600	36,900	2,160	6S	05/14/93	3	--	<10
2S	02/01/96	151,200	36,450	2,300	6S	10/22/93	<5	--	75
2S	05/10/96	352,000	48,000	3,300	6S	02/09/94	4	--	<10
2S	08/10/96	287,000	43,000	4,000	6S	05/01/94	95	1,300	<25
2S	11/10/96	186,000	32,000	2,050	6S	08/01/94	<25	340	<25
2S	02/11/97	80,200	45,200	1,810	6S	11/01/94	1	32	12
2S	08/22/97	171,000	34,500	<500	6S	02/01/95	<1	170	1
2S	5/21/98	92,250	27,090	1,080	6S	05/01/95	<1	86	21
5S	10/31/85	5,600	--	--	6S	08/01/95	<3	39	<3
5S	11/05/85	9,800	--	--	6S	11/01/95	<2	98	14
5S	11/12/85	14,800	--	--	6S	02/01/96	<1	136	40
5S	01/03/86	16,600	--	--	6S	05/10/96	<1	170	49
5S	01/09/87	110,000	--	900	6S	08/10/96	<1	516	106
5S	02/05/87	280,000	--	21,000	6S	11/10/96	12	81	14
5S	03/13/87	130,000	--	<10	6S	02/14/97	3	114	22
5S	11/13/88	68,100	--	--	6S	08/27/97	<1	80	42
5S	05/16/91	320,000	--	4,300	6S	5/21/98	<1	86	27
5S	10/25/91	3,000	--	<100	7S	10/31/85	48,000	--	--
5S	02/10/92	910	--	30	7S	11/05/85	31,000	--	--
5S	04/17/92	470	--	10	7S	11/12/85	71,000	--	--
5S	07/31/92	5,400	--	<500	7S	01/03/86	31,000	--	--
5S	10/30/92	4,400	--	<500	7S	01/09/87	430,000	--	56
5S	02/24/93	2,700	--	<250	7S	02/05/87	120,000	--	<10
5S	05/14/93	160,000	--	<500	7S	03/13/87	150,000	--	<10
5S	02/09/94	980	--	<100	7S	11/13/88	18,900	--	--
5S	05/01/94	650	1,700	<25	7S	05/16/91	95,000	--	400
5S	08/01/94	2,400	960	<25	7S	10/25/91	59,000	--	290
5S	11/01/94	1,300	170	<25	7S	02/10/92	75,000	--	<5,000
5S	02/01/95	1,100	300	<50	7S	04/17/92	200,000	--	<5,000
5S	05/01/95	160	170	<50	7S	07/31/92	160,000	--	<10,000
5S	08/01/95	200	110	<5	7S	10/30/92	92,000	--	<10,000
5S	11/01/95	148	108	12	7S	02/24/93	93,000	--	<5,000
5S	02/01/96	71	110	3	7S	05/14/93	140,000	--	<10,000
5S	05/10/96	221	285	<1	7S	10/22/93	82,000	--	<10,000

Table 8. Chlorinated-ethene data for samples from selected wells—Continued

Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)	Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)
7S	02/09/94	87	--	<10	20S	02/01/95	49,000	110,000	5,100
7S	05/01/94	140,000	120,000	<500	20S	05/01/95	52,000	64,000	2,400
7S	08/01/94	16,000	22,000	500	20S	08/01/95	120,000	79,000	4,800
7S	11/01/94	17,000	28,000	<500	20S	11/01/95	6,300	1,200	36
7S	02/01/95	6,400	23,000	<500	20S	02/01/96	60,750	28,350	4,100
7S	05/01/95	18,000	28,000	<500	20S	05/10/96	92,000	124,000	8,500
7S	08/01/95	47,000	52,000	<500	20S	08/10/96	80,000	115,000	10,000
7S	11/01/95	12,800	24,920	146	20S	11/10/96	78,000	102,000	8,500
7S	02/01/96	810	1,163	21	20S	02/19/97	31,200	99,200	10,200
7S	05/10/96	10,930	10,100	70	20S	08/27/97	40,000	77,000	3,000
7S	08/10/96	5,660	7,850	85	20S	5/21/98	18,000	76,950	9,180
7S	11/10/96	8,350	8,750	64	33S	10/22/93	<5	--	2,000
7S	02/11/97	808	1,110	5	33S	02/09/94	<100	--	480
7S	08/22/97	6,600	13,800	<1	33S	05/01/94	2	110	140
7S	11/05/97	3,800	16,300	61	33S	08/01/94	1	1	5
7S	5/21/98	7,785	27,140	<450	33S	11/01/94	1	2,400	4,100
11S	01/09/87	35	--	<10	33S	02/01/95	3	2,400	2,900
11S	02/05/87	<10	--	11	33S	05/01/95	300	3,400	1,500
11S	03/13/87	<10	--	<10	33S	08/01/95	140	4,800	1,600
11S	11/13/88	36	--	--	33S	11/01/95	12	3,500	1,900
11S	02/10/92	14	--	<10	33S	02/01/96	<1	7,200	2,500
11S	04/17/92	<5	--	<10	33S	05/10/96	<100	10,700	3,100
11S	07/31/92	13	--	<10	33S	08/10/96	<100	11,200	4,600
11S	10/30/92	33	--	<10	33S	11/10/96	<100	17,700	4,900
11S	02/24/93	17	--	<10	33S	02/11/97	39	14,300	2,980
11S	05/14/93	20	--	<10	33S	08/22/97	<100	14,600	3,500
11S	10/22/93	31	--	<10	33S	5/21/98	<900	30,330	2,250
11S	02/09/94	18	--	<10	34S	10/22/93	<500	--	770
11S	05/01/94	18	58	<1	34S	02/09/94	25,000	--	<5,000
11S	08/01/94	18	50	<1	34S	05/01/94	55	400	720
11S	11/01/94	15	41	<1	34S	08/01/94	140	1,800	2,400
11S	02/01/95	14	33	<1	34S	11/01/94	8,400	8,200	<100
11S	05/01/95	7	25	<1	34S	02/01/95	810	2,900	1,300
11S	08/01/95	11	26	<1	34S	05/01/95	300	2,500	130
11S	11/01/95	8	21	<1	34S	08/01/95	180	5,700	670
11S	02/01/96	9	33	<1	34S	11/01/95	675	5,490	900
11S	05/10/96	7	20	<1	34S	02/01/96	1,140	3,290	710
11S	08/10/96	6	38	<1	34S	05/10/96	460	15,620	3,940
11S	11/10/96	9	26	<1	34S	08/10/96	368	3,200	1,400
11S	02/18/97	7	40	<1	34S	11/10/96	115	1,850	709
11S	08/27/97	6	32	<1	34S	02/18/97	1,200	3,800	1,130
11S	5/21/98	<1	2	<1	34S	08/26/97	2	820	350
20S	01/09/87	270,000	--	<2,000	34S	5/21/98	212	1,449	338
20S	02/05/87	530,000	--	<10	35S	10/22/93	<5	--	<10
20S	03/13/87	500,000	--	<10	35S	02/09/94	6	--	<10
20S	11/13/88	79,000	--	--	35S	05/01/94	280	110	2
20S	05/16/91	61,000	--	<10	35S	08/01/94	2	11	1
20S	10/25/91	54,000	--	<5,000	35S	11/01/94	5	38	<1
20S	02/10/92	290,000	--	<10,000	35S	02/01/95	<3	16	<3
20S	04/17/92	13,000	--	11,000	35S	05/01/95	<1	7	<1
20S	10/30/92	460,000	--	<10,000	35S	08/01/95	3	27	<1
20S	02/24/93	360,000	--	<10,000	35S	11/01/95	6	38	8
20S	05/14/93	960,000	--	4,700	35S	02/01/96	4	24	<1
20S	10/22/93	380,000	--	<25,000	35S	05/10/96	3	16	<1
20S	02/09/94	558,000	--	<50,000	35S	08/10/96	<1	20	3
20S	05/01/94	49,000	36,000	1,700	35S	11/10/96	1	18	2
20S	08/01/94	13,000	150,000	3,000	35S	02/13/97	4	8	<1
20S	11/01/94	180,000	90,000	<500	35S	08/27/97	2	29	5

Table 8. Chlorinated-ethene data for samples from selected wells—Continued

Well	Date sampled	TCE (μg/L)	*c*DCE (μg/L)	VC (μg/L)	Well	Date sampled	TCE (μg/L)	*c*DCE (μg/L)	VC (μg/L)
35S	5/21/98	2	17	1	1D	11/05/97	3	35	119
37S	10/22/93	<5	--	<10	1D	5/21/98	8	12	5
37S	02/09/94	<5	--	<10	2D	02/14/89	<10	--	--
37S	05/01/94	<5	<5	<5	2D	04/20/89	<5	--	--
37S	08/01/94	<1	<5	3	2D	02/28/91	3,900	--	--
37S	11/01/94	<1	<1	<1	2D	04/11/91	5,200	--	16
37S	02/01/95	<1	<1	<1	2D	07/25/91	16,000	--	13
37S	05/01/95	<1	<1	<1	2D	10/18/91	90,000	--	440
37S	08/01/95	<1	<1	<1	2D	02/19/92	130,000	--	1,400
37S	11/01/95	<1	<1	<1	2D	04/15/92	68,000	--	<5,000
37S	02/01/96	<1	<1	<1	2D	07/31/92	59,000	--	<5,000
37S	05/10/96	<1	1	<1	2D	10/30/92	130,000	--	<10
37S	08/10/96	<1	<1	<1	2D	02/26/93	51,000	--	<10,000
37S	11/10/96	<1	<1	<1	2D	05/19/93	17,000	--	<1,000
37S	02/18/97	<1	<1	<1	2D	10/22/93	27,000	--	6,800
37S	08/27/97	<1	<1	<1	2D	02/10/94	8,000	--	1,000
37S	5/21/98	<1	<1	<1	2D	05/01/94	14,000	16,000	<25
42S	08/01/94	1	10	1	2D	08/01/94	11,000	3,900	<250
42S	11/01/94	250	<50	<50	2D	11/01/94	38,000	12,000	<500
42S	02/01/95	<1	<1	<1	2D	02/01/95	1,700	540	<1
42S	05/01/95	<1	<1	<1	2D	05/01/95	510	8,300	<500
42S	08/01/95	<1	<1	<1	2D	08/01/95	8,300	2,000	<100
42S	11/01/95	<1	<1	<1	2D	11/01/95	101,700	106,200	4,500
42S	02/01/96	<1	<1	<1	2D	02/01/96	2,990	6,400	1,005
42S	05/10/96	<1	1	<1	2D	05/10/96	1,650	5,100	1,165
42S	08/10/96	<1	<1	<1	2D	08/10/96	1,500	2,300	50
42S	11/10/96	<1	<1	<1	2D	11/10/96	1,590	2,680	<10
42S	08/18/97	<1	<1	<1	2D	02/11/97	915	1,390	245
42S	08/27/97	<1	<1	<1	2D	05/20/97	5,290	520	38
42S	5/21/98	<1	4	<1	2D	08/22/97	168	467	256
1D	02/14/89	3,998,000	--	--	2D	11/05/97	880	5,700	760
1D	04/20/89	260,000	--	--	2D	2/17/98	597	1,070	156
1D	02/28/91	180	--	--	2D	5/21/98	116	511	73
1D	04/11/91	180	--	160	3D	01/25/90	26,000	--	--
1D	07/25/91	<10	--	400	3D	02/28/91	2,900	--	--
1D	10/18/91	96	--	17	3D	04/11/91	2,200	--	120
1D	02/06/92	12	--	2,600	3D	07/25/91	240	--	13
1D	04/15/92	54	--	8	3D	10/18/91	<5	--	390
1D	07/31/92	48	--	<10	3D	02/06/92	130	--	170
1D	10/30/92	<5	--	<10	3D	04/15/92	34	--	<10
1D	02/26/93	32	--	450	3D	07/31/92	1,100	--	<50
1D	05/19/93	32	--	9	3D	10/30/92	30	--	<10
1D	10/22/93	18	--	1,000	3D	02/26/93	11	--	<10
1D	02/10/94	7	--	3,500	3D	05/19/93	14	--	<10
1D	05/01/94	29	14	19	3D	10/22/93	<5	--	<10
1D	08/01/94	140	24	<3	3D	02/10/94	12	--	16
1D	11/01/94	270	300	46	3D	05/01/94	39	25	19
1D	02/01/95	9	120	110	3D	08/01/94	3	48	12
1D	05/01/95	3	130	220	3D	11/01/94	43	47	<1
1D	08/01/95	5	18	25	3D	02/01/95	<1	2	<1
1D	11/01/95	28	47	65	3D	05/01/95	4	7	<1
1D	02/01/96	8	119	740	3D	08/01/95	5	7	<1
1D	05/10/96	10	81	940	3D	11/01/95	6	5	10
1D	08/10/96	89	20	24	3D	02/01/96	4	7	2
1D	11/10/96	55	13	46	3D	05/10/96	<1	1	<1
1D	02/11/97	<1	6	5	3D	08/10/96	1	725	176
1D	08/22/97	14	10	3	3D	11/10/96	3	183	73

Table 8. Chlorinated-ethene data for samples from selected wells—Continued

Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)	Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)
3D	02/12/97	2	60	19	10D-A	02/06/92	3,600	--	<100
3D	08/22/97	2	6	<1	10D-A	02/12/92	3,100	--	<100
3D	11/05/97	<1	19	<1	10D-A	04/15/92	1,800	--	<250
3D	5/21/98	<1	230	140	10D-A	07/31/92	340	--	<100
4D	01/25/90	2,300	--	--	10D-A	10/30/92	900	--	<10
4D	02/28/91	210	--	--	10D-A	02/26/93	190	--	<10
4D	04/11/91	180	--	150	10D-A	05/19/93	280	--	<100
4D	07/25/91	48	--	<10	10D-A	10/22/93	780	--	<100
4D	10/18/91	<5	--	<10	10D-A	02/10/94	160	--	<20
4D	02/06/92	31	--	12	10D-A	05/01/94	110	33	<5
4D	04/15/92	1,100	--	<10	10D-A	08/01/94	70	71	<1
4D	07/31/92	250	--	<100	10D-A	11/01/94	120	79	<1
4D	10/30/92	<5	--	<10	10D-A	02/01/95	5	34	<1
4D	02/26/93	2	--	<10	10D-A	05/01/95	73	42	<1
4D	05/19/93	190	--	<10	10D-A	08/01/95	29	15	<1
4D	10/22/93	<5	--	<10	10D-A	11/01/95	86	39	<2
4D	02/10/94	<5	--	<10	10D-A	02/01/96	46	24	<1
4D	05/01/94	43	7	<1	10D-A	05/10/96	39	15	<1
4D	08/01/94	6	1	<1	10D-A	08/10/96	10	8	<1
4D	11/01/94	82	27	<1	10D-A	11/10/96	30	14	<1
4D	02/01/95	<1	3	<1	10D-A	02/21/97	35	18	<1
4D	05/01/95	9	3	<1	10D-A	08/26/97	1	<1	<1
4D	08/01/95	<1	<1	<1	10D-A	5/21/98	7	16	4
4D	11/01/95	2	<1	<1	10D-B	02/06/92	280	--	<10
4D	02/01/96	1	2	<1	10D-B	02/19/92	150	--	<10
4D	05/10/96	7	4	<1	10D-B	04/15/92	160	--	<10
4D	08/10/96	98	524	2	10D-B	07/31/92	140	--	<10
4D	11/10/96	8	60	7	10D-B	10/30/92	120	--	<10
4D	02/12/97	2	6	<1	10D-B	02/26/93	120	--	15
4D	08/22/97	<1	<1	<1	10D-B	05/19/93	81	--	32
4D	5/21/98	<1	<1	<1	10D-B	10/22/93	150	--	<10
5D	01/25/90	7	--	--	10D-B	02/10/94	75	--	<4
5D	02/28/91	<5	--	--	10D-B	05/01/94	51	45	6
5D	04/11/91	<5	--	<5	10D-B	08/01/94	59	91	6
5D	07/25/91	<10	--	<10	10D-B	11/01/94	32	130	33
5D	10/18/91	93	--	<5	10D-B	02/01/95	19	30	3
5D	02/06/92	20	--	<10	10D-B	05/01/95	33	45	<1
5D	04/15/92	1,300	--	<10	10D-B	08/01/95	37	38	<1
5D	07/31/92	49	--	<10	10D-B	11/01/95	60	46	10
5D	10/30/92	360	--	<100	10D-B	02/01/96	35	37	4
5D	02/26/93	<5	--	<10	10D-B	05/10/96	50	47	1
5D	05/19/93	170	--	<10	10D-B	08/10/96	11	42	14
5D	10/22/93	45	--	<10	10D-B	11/10/96	50	44	<1
5D	02/10/94	<5	--	<10	10D-B	02/21/97	24	38	1
5D	05/01/94	15	25	<1	10D-B	05/22/97	46	35	1
5D	08/01/94	31	5	<1	10D-B	08/22/97	<1	6	6
5D	11/01/94	20	7	<1	10D-B	11/04/97	9	15	8
5D	02/01/95	6	11	<1	10D-B	2/17/98	<2	<2	<2
5D	05/01/95	<1	<1	<1	10D-B	5/21/98	<1	<1	<1
5D	08/01/95	4	7	<1	10D-C	02/01/96	86	45	<1
5D	11/01/95	8	1	<2	10D-C	05/10/96	76	38	1
5D	02/01/96	<1	<1	<1	10D-C	08/10/96	36	33	<1
5D	05/10/96	<1	<1	<1	10D-C	11/10/96	35	25	<1
5D	08/10/96	<1	2	4	10D-C	02/21/97	21	20	<1
5D	11/10/96	2	<1	2	10D-C	08/26/97	7	4	<1
5D	02/13/97	<1	<1	<1	10D-C	5/21/98	<1	<1	<1
5D	08/26/97	2	<1	<1	11D	02/06/92	5,900	--	<100
5D	5/21/98	<1	<1	<1	11D	02/19/92	700	--	13

Table 8. Chlorinated-ethene data for samples from selected wells—Continued

Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)	Well	Date sampled	TCE (µg/L)	*c*DCE (µg/L)	VC (µg/L)
11D	04/15/92	720	--	<50	12D	11/10/96	1,700	180	<10
11D	07/31/92	390	--	<100	12D	02/13/97	<1	<1	<1
11D	10/30/92	1,000	--	<50	12D	05/22/97	1,240	220	<10
11D	02/26/93	500	--	<50	12D	08/22/97	400	95	<1
11D	05/19/93	500	--	<100	12D	11/04/97	1,125	376	<1
11D	10/22/93	200	--	<100	12D	2/17/98	18	4	<2
11D	02/10/94	910	--	<50	12D	5/21/98	170	8	4
11D	05/01/94	1,400	1,400	10	13D	10/22/93	4,600	--	<10
11D	08/01/94	650	750	<25	13D	02/10/94	1,400	--	<100
11D	11/01/94	970	800	<50	13D	05/01/94	180	23	<5
11D	02/01/95	540	450	4	13D	08/26/97	255	203	3
11D	05/01/95	650	350	<25	13D	10/14/97	71	102	2
11D	08/01/95	540	420	<25	13D	11/05/97	62	72	<1
11D	11/01/95	420	335	9	14D	10/22/93	2,800	--	<10
11D	02/01/96	416	263	1	14D	02/10/94	610	--	17
11D	05/10/96	140	410	<5	14D	05/01/94	470	140	12
11D	08/10/96	42	274	1	14D	08/01/94	210	130	<3
11D	11/10/96	68	171	<1	14D	11/01/94	130	150	<5
11D	02/12/97	3	142	<1	14D	02/01/95	150	170	2
11D	08/22/97	<1	71	<1	14D	05/01/95	180	84	<5
11D	5/21/98	<1	27	4	14D	08/01/95	120	92	<1
12D	02/06/92	200,000	--	<480	14D	11/01/95	198	138	16
12D	02/12/92	230,000	--	<1,000	14D	02/01/96	252	234	21
12D	04/15/92	14,000	--	<1,000	14D	05/10/96	428	230	10
12D	07/31/92	10,000	--	<1,000	14D	08/10/96	320	310	8
12D	10/30/92	2,300	--	<10	14D	11/10/96	290	232	6
12D	02/26/93	170	--	15	14D	02/12/97	198	382	28
12D	05/19/93	2,100	--	7	14D	05/22/97	520	310	<10
12D	10/22/93	2,200	--	<20	14D	08/26/97	270	330	24
12D	02/10/94	7	--	<10	14D	11/05/97	305	505	58
12D	05/01/94	1,400	550	5	16D-A	05/28/97	<1	2	<1
12D	08/01/94	1,800	1,100	<50	16D-A	08/26/97	<1	3	<1
12D	11/01/94	950	410	<5	16D-A	11/04/97	<1	2	<1
12D	02/01/95	620	670	3	16D-A	2/17/98	<2	<2	<2
12D	05/01/95	730	90	<50	16D-A	5/21/98	<1	<1	<1
12D	08/01/95	690	270	<25	16D-B	05/28/97	<1	2	<1
12D	11/01/95	920	240	11	16D-B	08/22/97	<1	5	<1
12D	02/01/96	1,500	300	6	16D-B	11/04/97	<1	3	<1
12D	05/10/96	1,250	250	<5	16D-B	2/17/98	<2	7	<2
12D	08/10/96	1,850	650	21	16D-B	5/21/98	<1	<1	<1

Table 11. Microcosm data used to examine biodegradation of chlorinated ethenes

[Concentrations are in milligrams per liter; GC, gas chromatography; ECD, electron capture detector; PID, photoionization detector; *c*DCE, *cis*-1,2-dichloroethylene; ND, not detected; <, less than; --, no data]

Microcosm identification					Trichloroethylene		*c*DCE	Vinyl chloride
Experiment number	Treatment number	Treatment description	Time (weeks)	Replicate number	GC/ECD	GC/PID	GC/PID	GC/PID
1	1	1D live	0	1	0.15	--	--	--
1	1	1D live	0	2	0.15	--	--	--
1	1	1D live	0	3	0.17	--	--	--
1	1	1D live	0	4	0.20	--	--	--
1	2	1D live	0	1	0.92	--	--	--
1	2	1D live	0	2	0.98	--	--	--
1	2	1D live	0	3	0.97	--	--	--
1	2	1D live	0	4	0.79	--	--	--
1	3	2D live	0	1	0.61	--	--	--
1	3	2D live	0	2	0.56	--	--	--
1	3	2D live	0	3	0.62	--	--	--
1	3	2D live	0	4	0.50	--	--	--
1	4	2D live	0	1	1.41	--	--	--
1	4	2D live	0	2	1.09	--	--	--
1	4	2D live	0	3	1.10	--	--	--
1	4	2D live	0	4	1.15	--	--	--
1	5	3D live	0	1	0.07	--	--	--
1	5	3D live	0	2	0.07	--	--	--
1	5	3D live	0	3	0.08	--	--	--
1	5	3D live	0	4	0.08	--	--	--
1	6	3D live	0	1	0.81	--	--	--
1	6	3D live	0	2	0.71	--	--	--
1	6	3D live	0	3	0.74	--	--	--
1	6	3D live	0	4	0.65	--	--	--
1	7	12D live	0	1	0.09	--	--	--
1	7	12D live	0	2	0.08	--	--	--
1	7	12D live	0	3	0.08	--	--	--
1	7	12D live	0	4	0.07	--	--	--
1	8	12D live	0	1	0.74	--	--	--
1	8	12D live	0	2	0.71	--	--	--
1	8	12D live	0	3	0.67	--	--	--
1	8	12D live	0	4	0.62	--	--	--
1	9	Unsterile control	0	1	--	--	--	--
1	9	Unsterile control	0	2	1.05	--	--	--
1	9	Unsterile control	0	3	0.87	--	--	--
1	9	Unsterile control	0	4	0.91	--	--	--
1	1	1D live	2	1	0.17	--	--	--
1	1	1D live	2	2	0.12	--	--	--
1	1	1D live	2	3	--	--	--	--
1	1	1D live	2	4	0.18	--	--	--
1	2	1D live	2	1	0.58	--	--	--
1	2	1D live	2	2	0.28	--	--	--
1	2	1D live	2	3	0.47	--	--	--
1	2	1D live	2	4	0.53	--	--	--
1	3	2D live	2	1	0.33	--	--	--
1	3	2D live	2	2	0.30	--	--	--
1	3	2D live	2	3	0.33	--	--	--
1	3	2D live	2	4	0.32	--	--	--
1	4	2D live	2	1	0.57	--	--	--

Table 11. Microcosm data used to examine biodegradation of chlorinated ethenes—Continued

Microcosm identification					Trichloroethylene		*c*DCE	Vinyl chloride
Experiment number	Treatment number	Treatment description	Time (weeks)	Replicate number	GC/ECD	GC/PID	GC/PID	GC/PID
1	4	2D live	2	2	0.63	--	--	--
1	4	2D live	2	3	0.78	--	--	--
1	4	2D live	2	4	0.73	--	--	--
1	5	3D live	2	1	0.05	--	--	--
1	5	3D live	2	2	0.05	--	--	--
1	5	3D live	2	3	0.04	--	--	--
1	5	3D live	2	4	0.03	--	--	--
1	6	3D live	2	1	0.34	--	--	--
1	6	3D live	2	2	0.32	--	--	--
1	6	3D live	2	3	0.31	--	--	--
1	6	3D live	2	4	0.30	--	--	--
1	7	12D live	2	1	0.07	--	--	--
1	7	12D live	2	2	0.06	--	--	--
1	7	12D live	2	3	0.05	--	--	--
1	7	12D live	2	4	0.07	--	--	--
1	8	12D live	2	1	0.30	--	--	--
1	8	12D live	2	2	0.24	--	--	--
1	8	12D live	2	3	0.21	--	--	--
1	8	12D live	2	4	0.28	--	--	--
1	9	Unsterile control	2	1	0.30	--	--	--
1	9	Unsterile control	2	2	0.17	--	--	--
1	9	Unsterile control	2	3	0.13	--	--	--
1	9	Unsterile control	2	4	0.18	--	--	--
1	1	1D live	39	1	0.08	0.018	0.008	0.001
1	1	1D live	39	2	0.10	0.035	0.004	0.013
1	1	1D live	39	3	0.10	0.037	0.005	0.016
1	1	1D live	39	4	0.12	0.031	0.040	0.141
1	2	1D live	39	1	0.37	1.038	0.037	<0.002
1	2	1D live	39	2	0.56	0.824	0.106	<0.001
1	2	1D live	39	3	0.59	1.282	0.035	<0.001
1	2	1D live	39	4	0.66	0.963	2.878	<0.001
1	3	2D live	39	1	0.34	0.312	0.291	<0.001
1	3	2D live	39	2	0.32	0.331	0.096	<0.001
1	3	2D live	39	3	0.32	0.243	0.918	<0.001
1	3	2D live	39	4	0.42	0.458	0.118	<0.001
1	4	2D live	39	1	0.59	0.963	0.291	<0.002
1	4	2D live	39	2	0.88	1.038	0.162	<0.002
1	4	2D live	39	3	0.57	1.116	0.162	<0.002
1	4	2D live	39	4	0.75	1.197	0.111	<0.002
1	5	3D live	39	1	0.04	0.009	0.013	<0.001
1	5	3D live	39	2	0.03	0.007	0.179	0.001
1	5	3D live	39	3	0.03	0.007	0.495	<0.001
1	5	3D live	39	4	0.04	0.007	0.683	<0.001
1	6	3D live	39	1	0.51	0.759	0.131	<0.002
1	6	3D live	39	2	0.50	0.531	0.110	<0.002
1	6	3D live	39	3	0.48	0.584	0.131	<0.002
1	6	3D live	39	4	0.45	0.435	0.162	<0.002
1	7	12D live	39	1	0.09	0.027	0.855	<0.001
1	7	12D live	39	2	0.09	0.027	1.375	<0.001
1	7	12D live	39	3	0.07	0.023	0.179	<0.001

Table 11. Microcosm data used to examine biodegradation of chlorinated ethenes—Continued

Microcosm identification					Trichloroethylene		*c*DCE	Vinyl chloride
Experiment number	Treatment number	Treatment description	Time (weeks)	Replicate number	GC/ECD	GC/PID	GC/PID	GC/PID
1	7	12D live	39	4	0.07	0.012	2.332	<0.001
1	8	12D live	39	1	0.46	0.391	1.056	<0.001
1	8	12D live	39	2	0.44	0.413	6.968	<0.001
1	8	12D live	39	3	0.43	0.331	0.320	<0.001
1	8	12D live	39	4	0.37	0.371	0.350	<0.001
1	9	Unsterile control	39	1	--	--	--	--
1	9	Unsterile control	39	2	--	--	--	--
1	9	Unsterile control	39	3	--	--	--	--
1	9	Unsterile control	39	4	--	--	--	--
2	1	1D live	0	1	1.74	--	--	--
2	1	1D live	0	2	1.90	--	--	--
2	1	1D live	0	3	1.69	--	--	--
2	1	1D live	0	4	1.11	--	--	--
2	2	2D live	0	1	1.60	--	--	--
2	2	2D live	0	2	1.38	--	--	--
2	2	2D live	0	3	1.75	--	--	--
2	2	2D live	0	4	1.37	--	--	--
2	3	3D live	0	1	1.03	--	--	--
2	3	3D live	0	2	1.25	--	--	--
2	3	3D live	0	3	1.19	--	--	--
2	3	3D live	0	4	1.27	--	--	--
2	4	12D live	0	1	2.14	--	--	--
2	4	12D live	0	2	3.10	--	--	--
2	4	12D live	0	3	2.19	--	--	--
2	4	12D live	0	4	2.57	--	--	--
2	5	2D/3D sterile	0	1	0.70	--	--	--
2	5	2D/3D sterile	0	2	0.83	--	--	--
2	5	2D/3D sterile	0	3	0.71	--	--	--
2	5	2D/3D sterile	0	4	0.66	--	--	--
2	1	1D live	3	1	0.70	--	--	--
2	1	1D live	3	2	1.05	--	--	--
2	1	1D live	3	3	0.92	--	--	--
2	1	1D live	3	4	1.59	--	--	--
2	2	2D live	3	1	ND	--	--	--
2	2	2D live	3	2	ND	--	--	--
2	2	2D live	3	3	<0.01	--	--	--
2	2	2D live	3	4	<0.01	--	--	--
2	3	3D live	3	1	<0.01	--	--	--
2	3	3D live	3	2	ND	--	--	--
2	3	3D live	3	3	ND	--	--	--
2	3	3D live	3	4	ND	--	--	--
2	4	12D live	3	1	1.36	--	--	--
2	4	12D live	3	2	1.68	--	--	--
2	4	12D live	3	3	1.46	--	--	--
2	4	12D live	3	4	--	--	--	--
2	5	2D/3D sterile	3	1	0.43	--	--	--
2	5	2D/3D sterile	3	2	0.47	--	--	--
2	5	2D/3D sterile	3	3	0.34	--	--	--
2	5	2D/3D sterile	3	4	0.39	--	--	--
2	1	1D live	17	1	1.46	--	--	--

Table 11. Microcosm data used to examine biodegradation of chlorinated ethenes—Continued

Microcosm identification					Trichloroethylene		*c*DCE	Vinyl chloride
Experiment number	Treatment number	Treatment description	Time (weeks)	Replicate number	GC/ECD	GC/PID	GC/PID	GC/PID
2	1	1D live	17	2	1.21	--	--	--
2	1	1D live	17	3	0.72	--	--	--
2	1	1D live	17	4	1.04	--	--	--
2	2	2D live	17	1	ND	--	--	--
2	2	2D live	17	2	ND	--	--	--
2	2	2D live	17	3	<0.01	--	--	--
2	2	2D live	17	4	<0.01	--	--	--
2	3	3D live	17	1	ND	--	--	--
2	3	3D live	17	2	<0.01	--	--	--
2	3	3D live	17	3	ND	--	--	--
2	3	3D live	17	4	ND	--	--	--
2	4	12D live	17	1	2.15	--	--	--
2	4	12D live	17	2	2.21	--	--	--
2	4	12D live	17	3	2.50	--	--	--
2	4	12D live	17	4	<0.01	--	--	--
2	5	2D/3D sterile	17	1	0.64	--	--	--
2	5	2D/3D sterile	17	2	0.51	--	--	--
2	5	2D/3D sterile	17	3	0.54	--	--	--
2	5	2D/3D sterile	17	4	0.27	--	--	--
2	1	1D live	22	1	0.39	--	--	--
2	1	1D live	22	2	0.44	--	--	--
2	1	1D live	22	3	0.26	--	--	--
2	1	1D live	22	4	<0.01	--	--	--
2	2	2D live	22	1	ND	--	--	--
2	2	2D live	22	2	<0.01	--	--	--
2	2	2D live	22	3	<0.01	--	--	--
2	2	2D live	22	4	<0.01	--	--	--
2	3	3D live	22	1	<0.01	--	--	--
2	3	3D live	22	2	<0.01	--	--	--
2	3	3D live	22	3	<0.01	--	--	--
2	3	3D live	22	4	<0.01	--	--	--
2	4	12D live	22	1	0.94	--	--	--
2	4	12D live	22	2	0.05	--	--	--
2	4	12D live	22	3	<0.01	--	--	--
2	4	12D live	22	4	<0.01	--	--	--
2	5	2D/3D sterile	22	1	0.26	--	--	--
2	5	2D/3D sterile	22	2	0.35	--	--	--
2	5	2D/3D sterile	22	3	--	--	--	--
2	5	2D/3D sterile	22	4	0.19	--	--	--

Table 12. Aerobic and facultative anaerobic heterotrophic bacteria enumeration data

[mL, milliliters; CFU, colony-forming units; CFU/100mL, colony-forming units per 100 milliliters; TNTC, too numerous to count; >, greater than]

Microcosm description	Replicate number	Incubation (weeks)	Source of water (date collected)	Enumeration results		
				mL of sample	CFU	CFU/100 mL
Experiment 1, treatment 9	1	1	2D/12D (2/17/98)	1.00	TNTC	$> 2 \times 10^4$
Experiment 1, treatment 9	2	1	2D/12D (2/17/98)	1.00	TNTC	$> 2 \times 10^4$
Experiment 1, treatment 9	3	1	2D/12D (2/17/98)	1.00	TNTC	$> 2 \times 10^4$
Experiment 1, treatment 9	4	1	2D/12D (2/17/98)	1.00	TNTC	$> 2 \times 10^4$
Experiment 2, treatment 1	2	3	1D (5/21/98)	0.01	6	6×10^4
Experiment 2, treatment 1	4	3	1D (5/21/98)	0.01	89	8.9×10^5
Experiment 2, treatment 2	3	3	2D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 2	4	3	2D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 3	3	3	3D (5/21/98)	0.01	134	1.3×10^6
Experiment 2, treatment 3	4	3	3D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 4	3	3	12D (5/21/98)	0.01	121	1.2×10^6
Experiment 2, treatment 4	4	3	12D (5/21/98)	0.01	86	8.6×10^5
Experiment 2, treatment 5 (control)	1	3	2D/3D (5/21/98)	1.00	0	0
Experiment 2, treatment 5 (control)	2	3	2D/3D (5/21/98)	1.00	0	0
Experiment 2, treatment 5 (control)	3	3	2D/3D (5/21/98)	1.00	0	0
Experiment 2, treatment 5 (control)	4	3	2D/3D (5/21/98)	1.00	0	0
Experiment 2, treatment 1	1	17	1D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 1	4	17	1D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 2	1	17	2D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 2	4	17	2D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 3	1	17	3D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 3	1	17	3D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 4	4	17	12D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 4	4	17	12D (5/21/98)	0.01	TNTC	$> 2 \times 10^6$
Experiment 2, treatment 5 (control)	1	17	2D/3D (5/21/98)	0.01	0	0
Experiment 2, treatment 5 (control)	2	17	2D/3D (5/21/98)	0.01	0	0
Experiment 2, treatment 5 (control)	3	17	2D/3D (5/21/98)	0.01	0	0
Experiment 2, treatment 5 (control)	4	17	2D/3D (5/21/98)	0.01	0	0

REFERENCES

Alvarez-Cohen, L., and McCarty, P.L., 1991, A cometabolic biotransformation model for halogenated compounds exhibiting product toxicity: Environmental Science and Technology, v. 25, p. 1381-1387.

Amann, R.I., Ludwig, W., and Schleifer, K.H., 1995, Phylogenetic identification and in situ detection of individual microbial cells without cultivation: Microbiological Reviews, v. 59, no. 1, p. 143-169.

Arciero, D., Vannelli, T., Logan, M., and Hooper, A.B., 1989, Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europeae*: Biochemical and Biophysical Research Communications, v. 159, no. 2, p. 640-643.

Atlas, R.M., 1987, Microbiology ecology: fundamentals and applications: New York, N.Y., MacMillan Publishing Company, 533 p.

Bedard, C., and Knowles, R., 1989, Physiology, biochemistry, and specific inhibitors of CH_4, NH_4^+, and CO oxidation by methanotrophs and nitrifiers: Microbiology Review, v. 53, no. 1, p. 68-84.

Bedient, P.B., Rifai, H.S., and Newell, C.J., 1994, Ground water contamination: transport and remediation: Englewood Cliffs, N.J., Prentice-Hall, Inc., 541 p.

Bouwer, E.J., 1994, Bioremediation of chlorinated solvents using alternate electron acceptors, *in* Norris, R.D., and Kerr, R.S., Handbook of bioremediation: Boca Raton, Fla., Lewis Publishers, p. 149-175.

Bradley, P.M., and Chapelle, F.H., 1996, Anaerobic mineralization of vinyl chloride in Fe(III)-reducing aquifer sediments: Environmental Science and Technology, v. 23, no. 6, p. 2084-2086.

———1997, Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions: Environmental Science and Technology, v. 31, no. 9, p. 2692-2696.

Braun-Howland, E.B., Danielsen, S.A., and Nierzwicki-Bauer, S.A., 1992, Development of a rapid method for detecting bacterial cells in situ using 16S rRNA-targeted probes: Biotechniques, v. 13, no. 6, p. 928-934.

Britton, L.J., and Greeson, P.E., eds., 1989, Methods for collection and analysis of aquatic biological and microbiological samples: Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, chapter A4, 363 p.

Broholm, K., Christensen, T.H., and Jensen, B.K., 1992, Modelling TCE degradation by a mixed culture of methane-oxidizing bacteria: Water Resources, v. 26, no. 9, p. 1177-1185.

Byl, T.D., Farmer, J.J., Williams, S.D., Wolfe, W.J., and Bailey, F.C., 1997, Identification of bacteria in contaminated ground water to determine the feasibility of intrinsic remediation [abs.], *in* Bridging the Global Environment-Technology, Communication, and Education, 18th, San Francisco, 1997: Society of Environmental Toxicology and Chemistry, p. 312.

Carey, G.R., 1998, SEQUENCE V1.1 for visualizing natural attenuation users' guide: Ottawa, Ontario, Environmental Software Solutions, 50 p.

Chang, H.L., and Alvarez-Cohen, L., 1995, Transformation capacities of chlorinated organics by mixed cultures enriched on methane, propane, toluene, or phenol: Biotechnology and Bioengineering, v. 45, no. 5, p. 440-449.

Chapelle, F.H., 1993, Ground-water microbiology and geochemistry: New York, N.Y., John Wiley and Sons, Inc., 424 p.

Chapelle, F.H., Bradley, P.M., Lovely, D.R., and Vroblesky, D.A., 1996, Measuring rates of biodegradation in a contaminated aquifer using field and laboratory methods: Ground Water, v. 34, no. 4, p. 691-698.

Chapelle, F.H., McMahon, P.B., Dubrovsky, N.M., Fujii, R.F., Oaksford, E.T., and Vroblesky, D.A., 1995, Deducing the distribution of terminal electron-accepting processes in hydrologically diverse groundwater systems: Water Resources Research, v. 31, no. 2, p. 359-371.

Christensen, T.H., Lyngkilde, J., Nielsen, P.E., Albrechtsen, H.J., and Heron, G., 1997, Natural aerobic biological attenuation: Integrative transition zones, *in* Ward, C.H., Cherry, J.A., Scalf, M.R., eds., Subsurface Restoration Handbook: Chelsea, Mich., Ann Arbor Press, p. 329-342.

Crawford, N.C., 1992, Groundwater flow in the vicinity of three water wells contaminated with trichloroethylene (TCE), near Lewisburg, Marshall County, Tennessee: Prepared for the U.S. Environmental Protection Agency, Region IV, and O.H.M. Corporation, 152 p.

Crawford, N.C., and Ulmer, C.S., 1994, Hydrogeologic investigations of contaminant movement in karst aquifers in the vicinity of a train derailment near Lewisburg, Tennessee: Environmental Geology, v. 23, p. 41-52.

Dalton, H., 1977, Ammonia oxidation by the methane oxidizing bacterium *Methylococcus capsulatus* strain bath: Archives of Microbiology, v. 114, p. 273-279.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., and Branson, M.A.H., eds., 1995, Standard methods for the examination of water and wastewater (19th ed.): Washington, D.C., American Public Health Association, 1268 p.

Ely, R.L., Hyman, M.R., Arp, D.J., Guenther, R.B., and Williamson, K.J., 1995, A cometabolic kinetics model incorporating enzyme inhibition, inactivation, and recovery: II. Trichloroethylene degradation experiments: Biotechnology and Bioengineering, v. 46, p. 232-245.

Ely, R.L., Williamson, K.J., Guenther, R.B., Hyman, M.R., and Arp, D.J., 1995, A cometabolic kinetics model incorporating enzyme inhibition, inactivation, and recovery: I. Model development, analysis, and testing: Biotechnology and Bioengineering, v. 46, p. 218-231.

Ely, R.L., Williamson, K.J., Hyman, M.R., and Arp, D.J., 1997, Cometabolism of chlorinated solvents by nitrifying bacteria: kinetics, substrate interactions, toxicity effects and bacterial response: Biotechnology and Bioengineering, v. 54, no. 6, p. 520-534.

Farmer, J.J., Byl, T.D., Williams, S.D., and Bailey, F.C., 1998, Identification of bacteria in contaminated ground water using the RNA-hybridization technique [abs.], *in* The Natural Connection-Environmental Integrity and Human Health, 19th, Charlotte, N.C.: Society of Environmental Toxicology and Chemistry, p. 3.

Farmer, J.J., and Hollyday, E.F., 1999, Subsurface correlation of the Pierce Limestone and adjacent confining units of Middle Tennessee, Annual Tennessee Water Resources Symposium, 9th, Nashville, Tennessee, 1999: Tennessee Section of the American Water Resources Association, p. 1B1-1B4.

Field, M.S., 1993, Karst hydrology and chemical contamination: Journal of Environmental Systems, v. 22, no. 1, p. 1-26.

Fishman, M.J., and Friedman, L.C., 1989, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A1, 545 p.

Ghiorse, W.C., and Wilson, J.T., 1988, Microbial ecology of the terrestrial subsurface: Advances in Applied Microbiology, v. 33, p. 107-172.

Hach Company, 1992, Hach water analysis handbook: Loveland, Colo., Hach Company, 831 p.

Hanchar, D.W., 1991, Effects of septic-tank effluent on ground-water quality in northern Williamson County and southern Davidson County, Tennessee: U.S. Geological Survey, Water-Resources Investigations Report 91-4011, 15 p.

Hanson, R.S., and Brusseau, G.A., 1994, Biodegradation of low-molecular-weight halogenated organic compounds by aerobic bacteria, *in* Chaundry, G.R., ed., Biological Degradation and Bioremediation of Toxic Chemicals: Portland, Ore., Dioscorides Press, p. 277-297.

Heald, S., and Jenkins, R.O., 1994, Trichloroethylene removal and oxidation toxicity mediated by toluene dioxygenase of *Pseudomonas putida*: Applied and Environmental Microbiology, v. 60, no. 12, p. 4634-4637.

Henry, S.M., and Grbić-Galić, D., 1994, Biodegradation of trichloroethylene in methanotrophic systems and implications for process applications, *in* Chaundry, G.R., ed., Biological Degradation and Bioremediation of Toxic Chemicals: Portland, Ore., Dioscorides Press, p. 314-344.

Hopkins, G.D., Semprini, L., and McCarty, P.L., 1993, Microcosm and in situ field studies of enhanced biotransformation of trichloroethylene by phenol-utilizing microorganisms: Applied and Environmental Microbiology, v. 59, p. 2277-2285.

Jain, D.K., Stroes-Gascoyne, S., and Cord, I., 1997, Characterization of microbial communities in deep groundwater from granitic rock: Canadian Journal of Microbiology, v. 43, no. 3, p. 272- 283.

Kane, M.D., Poulsen, L.K., and Stahl, D.A., 1993, Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide hybridization probes designed from environmentally derived 16S rRNA sequences: Applied and Environmental Microbiology, v. 59, no. 3, p. 682-686.

Krumme, M.L., Timmis, K.N., and Dwyer, D.F., 1993, Degradation of trichloroethylene by *Pseudomonas cepaci* G4 and the constitutive mutant strain G4 5223 PR1 in aquifer microcosms: Applied and Environmental Microbiology, v. 59, no. 8, p. 2746-2749.

Lovley, D.R., Chapelle, F.H., and Phillips, E.J., 1990, Fe(III)-reducing bacteria in deeply buried sediments of the Atlantic Coastal Plain: Geology, v. 18, no. 10, p. 954-957.

Lovley, D.R., and Phillips, E.J.P., 1988, Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese: Applied and Environmental Microbiology, v. 54, no. 6, p. 1472-1480.

Major, D.W., Cox, E.E., Edwards, E., and Hare, P.W., 1995, Intrinsic dechlorination of trichloroethene to ethene in a bedrock aquifer, *in* Hinchee, R.E., Wilson, J.T., and Downey, D.C., eds., Intrinsic Bioremediation, 3, v. 1: Columbus, Ohio, Batelle Press, p. 197-203.

McCarty, P.L., 1994, An overview of anaerobic transformation of chlorinated solvents *in* Symposium on intrinsic bioremediation of ground water: Washington, D.C., U.S. Environmental Protection Agency, EPA 540/R-94/515, p. 135-142.

McCarty, P.L., and Semprini, L., 1994, Ground-water treatment for chlorinated solvents, *in* Norris, R.D., and Matthew, J.E., Handbook of bioremediation: Boca Raton, Fla., Lewis Publishers, p. 87-116.

Montgomery, Larry, Assaf-Anid, Nada, Nies, Loring, Anid, P.J., and Vogel, T.M., 1994, Anaerobic biodegradation of chlorinated organic compounds, *in* Chaundry, G.R., ed., Biological degradation and bioremediation of toxic chemicals: Portland, Ore., Dioscorides Press, p. 256-276.

Moran, B.N., and Hickey, W.J., 1997, Trichloroethylene biodegradation by mesophilic and psycrophilic ammonia oxidixers and methanotrophs in groundwater microcosms: Applied and Environmental Microbiology, v. 63, no. 10, p. 3866-3871.

National Research Council, 1993, In situ bioremediation: when does it work?: Washington, D.C, National Academy Press, 207 p.

Nelson, M.J.K., Montgomery, S.O., and Pritchard, P.H., 1988, Trichloroethylene metabolism by microorganisms that degrade aromatic compounds: Applied and Environmental Microbiology, v. 54, no. 2, p. 604-606.

O'Connor, J.T., and Brazos, B.J., 1991, The response of natural groundwater bacteria to groundwater contamination by gasoline in a karst region, *in* Proceedings of the

Conference on Hazardous Waste Research: Manhattan, Kan., Kansas State University, p. 281-293.

Pedersen, K., and Ekendahl, S., 1990, Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden: Microbial Ecology, v. 20, no. 1, p. 37-52.

Quinlan, J.F., 1989, Ground-water monitoring in karst terranes: recommended protocols and implicit assumptions: Las Vegas, Nev., U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, EPA/600/X-89/050, 100 p.

Rasche, M.E., Hyman, M.R., and Arp, D.J., 1991, Factors limiting aliphatic chlorocarbon degradation by *Nitrosomonas auropaea*: co-metabolic inactivation by ammonia monoxygenase and substrate specificity: Applied and Environmental Microbiology, v. 57, p. 2986-2994.

Remediation Technologies Development Forum (RTDF), 1997, Natural attenuation of chlorinated solvents in groundwater: principles and practices: prepared by the Industrial Members of the Bioremediation of Chlorinated Solvents Consortium of the RTDF, Version 3.0, 63 p.

Roman-Mas, Angel, Bennett, M.W., and Hamilton, K.G., 1991, Reconnaissance of ground-water quality at selected sites in Bedford and Coffee Counties, Tennessee, June and July 1991: U.S. Geological Survey Open-File Report 91-510, 1 sheet, scale 1:180,000.

Semprini, L., Hopkins, G.D., Roberts, P.V., Grbić-Galić, D., and McCarty, P.L., 1991, A field evaluation of in-situ biodegradation of chlorinated ethenes: part 3, studies of competitive inhibition: Ground Water, v. 29, no. 2, p. 239-250.

Siering, P.L., and Ghiorse, W.C., 1997, Development and application of 16S rRNA-targeted probes for detection of iron- and manganese-oxidizing sheathed bacteria in environmental samples: Applied and Environmental Microbiology, v. 63, no. 2, p. 644-651.

Stumm, Werner, and Morgan, J.J., 1981, Aquatic Chemistry: an introduction emphasizing chemical equilibria in natural waters (2d ed.): New York, John Wiley and Sons, 780 p.

Tsien, H.C., Bratina, B.J., Tsuji, K., and Hanson, R.J., 1990, Use of oligodeoxynucleotide signature probes for identification of physiological groups of methylotrophic bacteria: Applied Environmental Microbiology, v. 56, no. 9, p. 2858-2865.

U.S. Environmental Protection Agency, 1993, Pilot-scale demonstration of a two-stage methantrophic bioreactor for biodegradation of trichloroethene in groundwater, Emerging Technology Summary: U.S. Environmental Protection Agency EPA/540/S-93/505, 5 p.

———1997a, Draft EPA Region 4 Suggested practices for evaluation of a site for natural attenuation (biological degradation) of chlorinated solvents, Version 3.0: Atlanta, Ga., U.S. Environmental Protection Agency, Region 4, 41 p.

———1997b, Use of monitored natural attenuation at Superfund, RCRA corrective action, and underground storage tank sites: Washington, D.C., U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response Directive 9200.4-17, 25 p.

Vogel, T.M., 1994, Natural bioremediation of chlorinated solvents, *in* Norris, R.D., and Matthew, J.E., eds., Handbook of bioremediation: Boca Raton, Fla., Lewis Publishers, p. 201-224.

Vogel, T.M., Criddle, C.S., and McCarty, P.L., 1987, Transformations of halogenated compounds: Environmental Science and Technology, v. 21, p. 722-735.

Vogel, T.M., and McCarty, P.L., 1985, Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions: Applied and Environmental Microbiology, v. 49, no. 5, p. 1080-1083.

Weaver, J.D., Patel, A.R., and Hickey, A.C., 1994, Groundwater quality for Grainger County, Tennessee: U.S. Geological Survey Open-File Report 93-365, 14 p.

Wiedemeier, T.H., Swanson, M.A., Moutoux, D.E., Gordon, E.K., Wilson, J.T., Wilson, B.H., Kampbell, D.H., Hass, P., Miller, R.N., Hansen, J.E., and Chapelle, F.H., 1998, Technical protocol for evaluating natural attenuation of chlorinated solvents in ground water: Cincinnati, Ohio, U.S. Environmental Protection Agency, National Risk Management Research Laboratory, Office of Research and Development, EPA/600/R-98/128, 248 p.

Wilson, B.H., Wilson, J.T., and Luce, Darryl, 1996, Design and interpretation of microcosm studies for chlorinated compounds, *in* Symposium on natural attenuation of chlorinated organics in ground water, Proceedings: Washington, D.C., U.S. Environmental Protection Agency EPA/540/R-96/509, p. 21-30.

Wilson, C.W., 1949, Pre-Chattanooga stratigraphy in central Tennessee: Nashville, Tenn., Tennessee Division of Geology Bulletin 56, 407 p.

Wilson, C.W., and Luther, E.T., comps., 1963, Geologic map of the Lewisburg Quadrangle, Tennessee: Nashville, Tenn., Tennessee Department of Conservation, scale 1:24,000.

Wilson, J.T., and Wilson, B.H., 1985, Biotransformation of trichloroethylene in soil: Applied and Environmental Microbiology, v. 49, no. 1, p. 242-243.

Wolfe, W.J., Haugh, C.J., Webbers, A., and Diehl, T.H., 1997, Preliminary conceptual models of the occurrence, fate, and transport of chlorinated solvents in karst aquifers of Tennessee: U.S. Geological Survey Water-Resources Investigations Report 97-4097, 80 p.

Wood, W.W., 1981, Guidelines for the collection and field analysis of ground-water samples for selected unstable constituents (2d ed.): Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 1, chapter D2, 24 p.