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# A SYSTEMATIC APPROACH TO IN SITU BIOREMEDIATION OF CARBON TETRACHLORIDE IN GROUNDWATER

<sup>1</sup>B. Faris and <sup>2</sup>ITRC-In Situ Bioremediation Team

<sup>1</sup>New Mexico Environment Department, Ground Water Quality Bureau, 4131 Montgomery NE, Albuquerque, NM, 87109; Phone: (505) 841-9466; Fax: (505) 884-9254.

<sup>2</sup>Interstate Technology Regulatory Council, In Situ Bioremediation Team, ECOS/ITRC 444 North Capitol Street, NW, Suite 445, Washington, DC, 20001; Phone: (202) 624-3660.

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## ABSTRACT

The historical use of carbon tetrachloride (CT) as a fumigant at grain silos has caused groundwater contamination at numerous sites. The Interstate Technology Regulatory Council (ITRC) – *In Situ* Bioremediation (ISB) Team has recently completed a guidance document that describes a systematic approach to ISB for CT in groundwater (ITRC, 2002). Contaminant reduction of CT through ISB typically occurs through a reductive process whereby an electron donor is introduced into the subsurface. Primary reductive pathways for CT have been documented to occur primarily through direct reductive dechlorination, cometabolic reductive dechlorination, and cometabolic denitrification. CT found at silos may be in conjunction with nitrate contamination that serves as an electron acceptor during ISB of CT. ITRC's ISB team guidance document describes regulatory concerns, provides a description of treatability tests, and feasibility; and defines the contaminant's pervasiveness, risks, sources, and site parameter criteria important to the evaluation of ISB for CT.

ITRC is a state-led coalition of more than 40 states working together with industry and stakeholders to achieve regulatory acceptance of environmental technologies. ITRC brings together a diverse mix of environmental experts and stakeholders from both the public and private sectors to broaden and deepen technical knowledge and streamline the regulation of new environmental technologies.

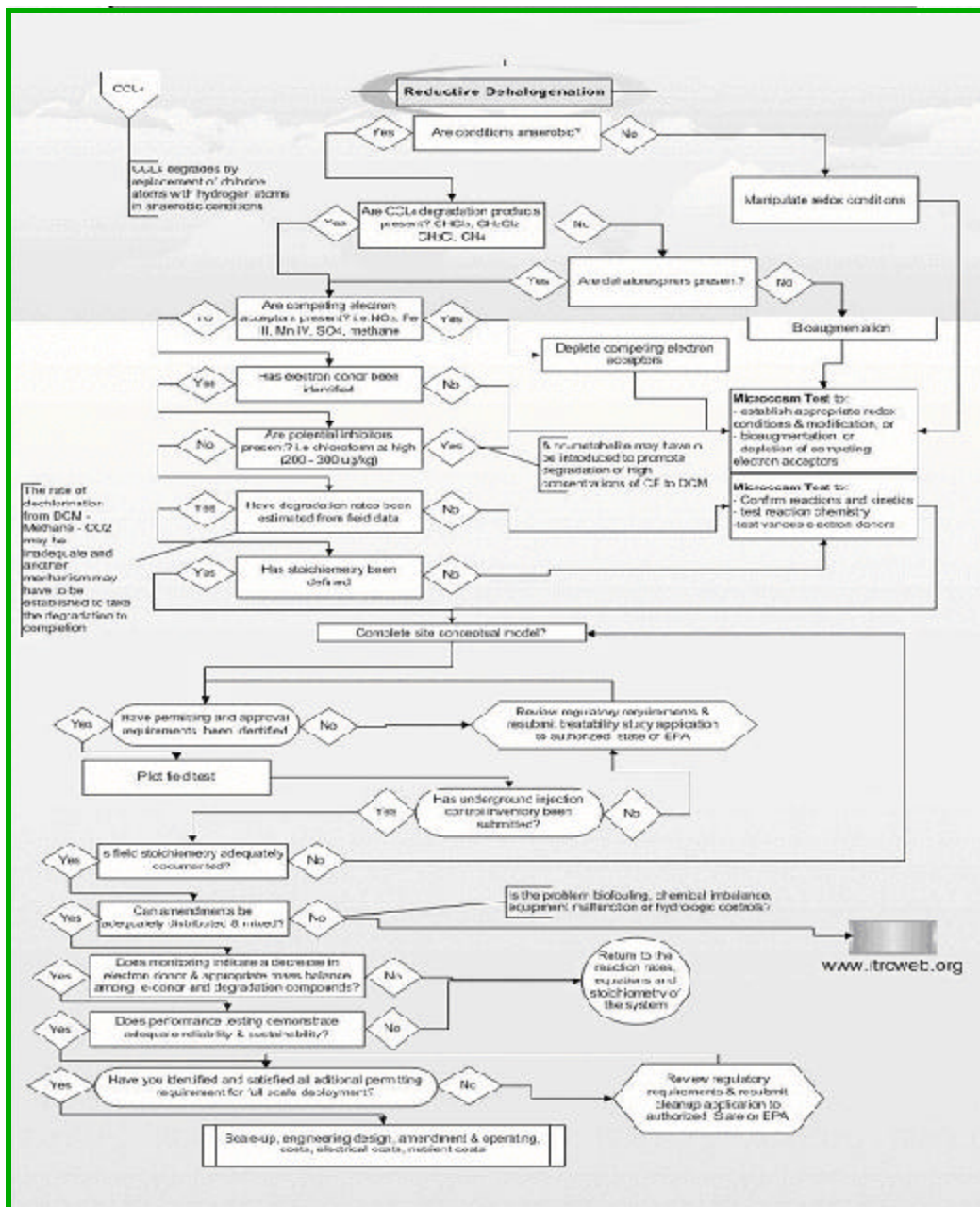
**Key words:** in situ bioremediation, dechlorination, cometabolic, electron donor and acceptor, denitrification

## INTRODUCTION

The purpose of the following sections is to apply the systematic approach for ISB of carbon tetrachloride. It defines the contaminant's pervasiveness, health and ecological risks, sources, and site parameters and criteria important to the evaluation of the application of ISB for carbon tetrachloride. Following the general systematic approach to ISB, this section further defines fate and transport of carbon tetrachloride, and provides decision-flow diagrams to follow the most effective pathways for ISB of carbon tetrachloride. It is important to note that ISB technologies for the remediation of carbon tetrachloride are in the developmental stage. Few field applications have been completed. This document is intended to represent the current state of ISB for carbon tetrachloride. New information will be added as ISB applications are completed.

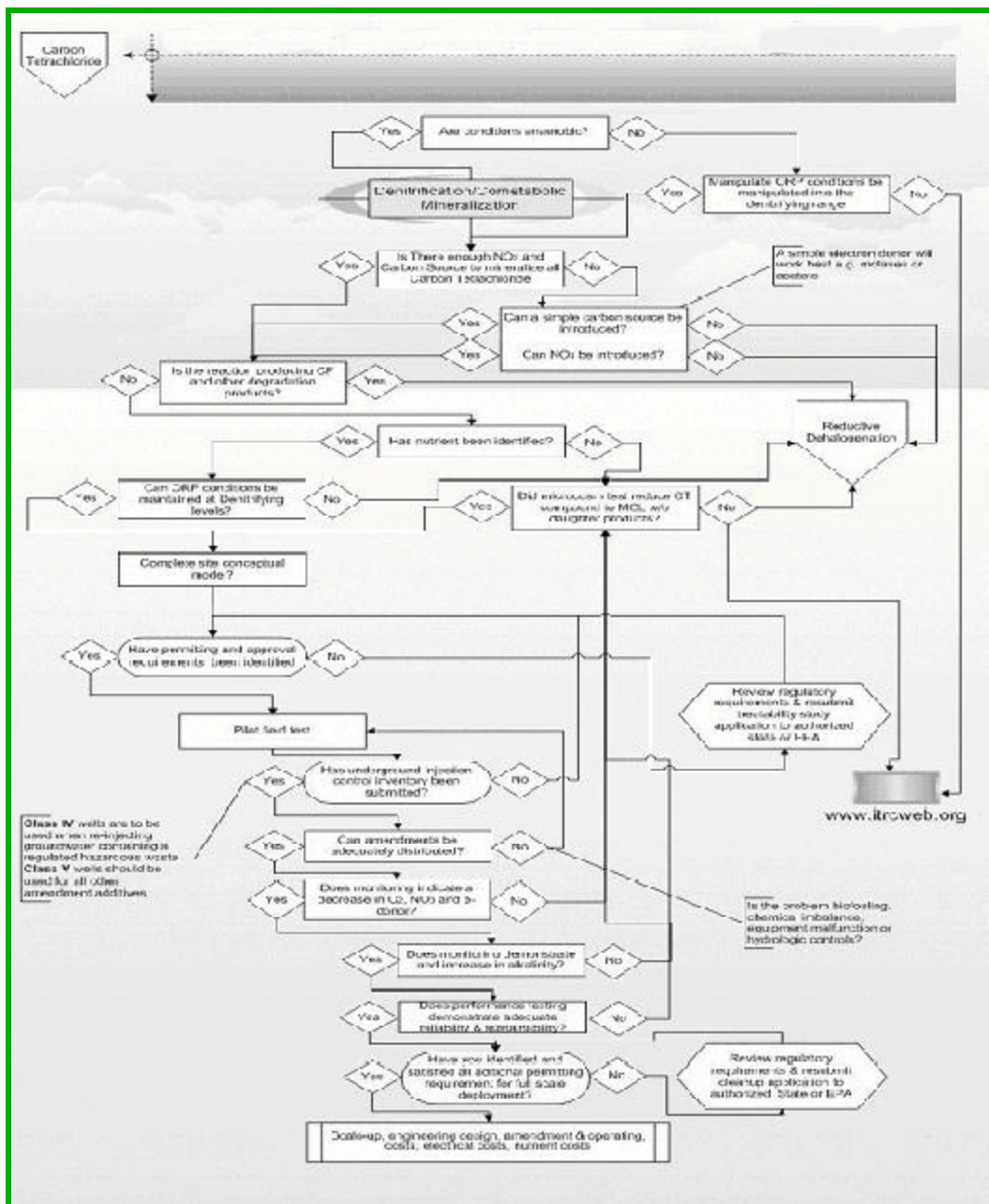
## DECISION TREE

The following decision-tree flow diagrams are supporting documentation for application of ISB toward remediation of carbon tetrachloride. Together they provide a decision pathway to characterize the



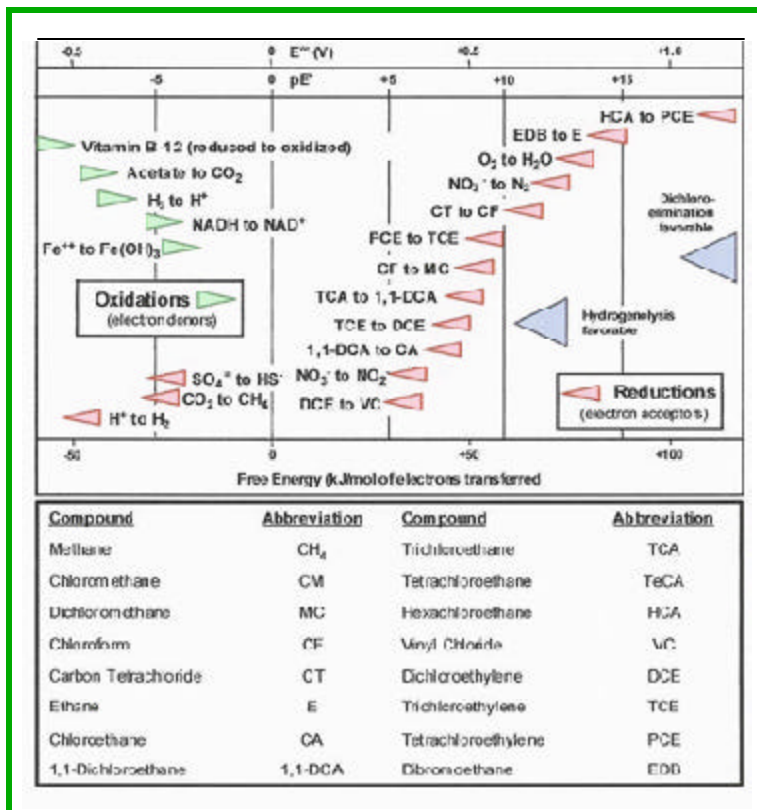
**Figure 1.** *In situ* bioremediation of carbon tetrachloride.

applicability of *in situ* bioremediation of carbon tetrachloride in groundwater. This document does not evaluate bioremediation in the unsaturated zone. The decision-tree flow diagrams and text will guide the reader to understand and evaluate aspects of decision making for deployment of ISB for carbon tetrachloride. The overall objective of these decision trees is to identify potential limiting factors of ISB, define when laboratory treatability tests are warranted, describe regulatory concerns or barriers, encourage implementation of a pilot field test, and ultimately determine if a full-scale system containing *in situ* bioremediation is practical. This ITRC team has identified major reductive pathways for ISB of carbon tetrachloride and



**Figure 2.** *In situ* bioremediation of carbon tetrachloride.

presents the two most viable anaerobic pathways for biodegradation. One pathway is reductive dechlorination and the other is cometabolic. The cometabolic pathway may occur either through reductive dechlorination or denitrification. These pathways are described in Sections Reductive Dechlorination and Reductive Denitrification/Cometabolic Pathway. The first decision tree describes reductive dechlorination through direct or cometabolic reduction, while the second decision tree describes a reductive denitrification/cometabolic pathway.



**Figure 3.** Half-reaction potentials for ORP of halogenated aliphatic compounds: potential electron acceptors and electron donors. Bases of arrows align with the potentials of the half reaction shown in volts (Cookson, 1995).

## REDUCTIVE DECHLORINATION

The key to this pathway is the presence of degradation products and the need to carry this reduction to completion so that neither contaminant nor its degradation products are above site closure criteria. Since it can only occur under reducing conditions, the first question is whether the current site conditions are anaerobic. If so, check for degradation products. If not, then a carbon source needs to be introduced to reduce oxidation-reduction potential (ORP) conditions. This can be determined through laboratory treatability tests. These tests can also determine if halorespirers are present. If degradation products are present, what are the ORP conditions, or better yet, what are the competing terminal electron acceptors? This can be determined through monitoring during the site characterization process.

It is important to understand the effect ORP conditions have on the presence or absence of degradation products, and under what conditions one might expect these degradation products to degrade. Please refer to Figure 3 to review under what ORP conditions the practitioner may expect these products to appear or degrade. Competing electron acceptors need to be depleted for successful dechlorination of

carbon tetrachloride. The laboratory treatability tests will establish this along with identifying the most suitable electron donor. Once these factors have been determined, the degradation rates should be established from site characterization data. Be aware that if no reductive dechlorination is evident from field data, the laboratory treatability test will provide expected degradation rates once the selected electron donor (carbon source) has been chosen.

From the field data or laboratory treatability test data, the site-specific stoichiometry can be defined. This is important to ascertain the concentration of electron donor and nutrients, if required, based on the laboratory treatability tests needed to introduce to the contaminant plume for complete reductive dechlorination of carbon tetrachloride and its degradation products. It should be pointed out that the first degradation product, chloroform (CF), may become an inhibitor to this process under certain methanogenic mixed cultures. Chloroform was shown to inhibit reductive dechlorination of cis-DCE and VC at concentrations as low as  $77\mu\text{ g/l}$  (Maymo-Gatell, 2001). Chloroform has been observed to inhibit its own degradation and acetate consumption at approximately  $330\mu\text{ g/l}$ , under perhaps methanogenic conditions, and the inhibition appears to be less under sulfate-reducing conditions at a concentration of approximately  $2\text{ mg/l}$  (Gupta et al., 1996). However, during another study, carbon tetrachloride degradation continued even when chloroform concentrations accumulated as high as  $10\text{ mg/L}$  (Freedman et al., 1995). Accumulated chloroform concentrations higher than  $2\text{ mg/l}$  did not seem to inhibit carbon tetrachloride degradation in pure cultures of a methanogen, a sulfate reducer, and a clostridium species (Egli et al., 1988). Bower and McCarty (1983) showed chloroform degradation occurred at  $40\mu\text{g/l}$ , even though they observed carbon tetrachloride degradation around the same concentration. In a further study by Bower and McCarty (1983) it was shown that no chloroform degradation occurred at  $60\mu\text{g/l}$ , even though they saw carbon tetrachloride degradation at the same concentration under denitrifying conditions. Hansen (1993) showed that chloroform accumulation does not seem to inhibit degradation at carbon tetrachloride concentrations of  $1\text{ mg/l}$ , if nitrate and acetate are not limiting.

With a completed site conceptual model, a pilot field test should be conducted. Final permitting issues need to be completed prior to deployment of the pilot field test. The decision tree helps identify regulatory requirements of the application. During the field test, the practitioner should answer at least two issues related to enhanced ISB: 1) Are the amendments properly distributed according to design?, and

2) Did biofouling occur? Corrections to the design are often made during this test. Results of the pilot field test should show if reductive dechlorination of carbon tetrachloride occurred to completion, if the electron donor was consumed, and if ISB of carbon tetrachloride will be sustainable. If so, the enhanced ISB system can be taken to full deployment.

### **REDUCTIVE DENITRIFICATION/COMETABOLIC PATHWAY**

There are two main reductive cometabolic pathways for carbon tetrachloride. The reductive dechlorination pathway yields degradation products through cometabolic processes and not by serving as electron acceptors. They are produced fortuitously when biologically produced enzymes or cofactors degraded carbon tetrachloride during the microbial consumption of an alternate carbon source. Please refer to Section Anaerobic Cometabolism for more detail.

What makes the reductive denitrification pathway attractive is that no or little degradation products are produced, specifically chloroform, which is a regulated contaminant of concern (COC). Furthermore, at many carbon tetrachloride-contaminated grain silo sites, nitrate is present in groundwater above standards. Therefore, this reductive pathway provides for destruction of both COCs, carbon tetrachloride, and nitrate. The difficulties of this pathway are to maintain the denitrifying conditions, and since there are few sites that have demonstrated this pathway, acceptance may be mixed. The practitioner should refer to Section Cometabolic Denitrification for a description of this degradation pathway. If the denitrifying conditions are not maintained and ORP conditions are lowered, carbon tetrachloride reduction will follow the reductive dechlorination pathway. With that stated, this pathway begins by asking if enough nitrate is present to complete the mineralization of carbon tetrachloride. This can be determined by a laboratory treatability test. If site conditions show that substantial amounts of chloroform and/or degradation products are present in the groundwater, then reductive dechlorination may be occurring and this pathway should be further investigated. However, if little to no chloroform is present, this reductive denitrification/cometabolic pathway may be preferred. An electron donor that maintains ORP conditions in the denitrification range should be evaluated and chosen. Typically this has been acetate. Again the laboratory treatability test will determine the electron donor, nutrient requirements, possible microbial augmentation, and whether conditions can be maintained at the denitrification rate. If these tests show that the site is conducive to the denitrification/cometabolic pathway, a site conceptual model can be developed. Following that, a pilot field test can be

designed and implemented after regulatory concerns are addressed. This flow chart describes those concerns. Once the pilot test is deployed, concerns on amendment mixing, biofouling, contaminant destruction, and maintaining denitrifying conditions are answered. Again, if results from this test show successful destruction of carbon tetrachloride through the denitrification/cometabolic pathway, a full-scale design can be prepared and deployed.

## **CONTAMINANT BACKGROUND**

### ***Pervasiveness of Carbon Tetrachloride Contamination***

Carbon tetrachloride has been found as a contaminant in soil and/or groundwater at approximately 22% of sites investigated under CERCLA (ECO-USA, 2001). Carbon tetrachloride in the soil above the saturated zone may be at concentrations high enough to act as a source for groundwater contamination, even though use of carbon tetrachloride as a product had been discontinued. This residual carbon tetrachloride in the soil should be assessed and dealt with as a source of contamination

### ***Site Operational History***

Determining and documenting the operational history of carbon tetrachloride contamination sites may be one of the most important steps in the bioremediation decision-making process. There are a wide variety of potential carbon tetrachloride contamination generators, and each may have additional and unique factors that will play an important role in the decision-making process. For instance, in an industry where the only contaminating substance is/was carbon tetrachloride, the issues are fairly straightforward. However, in an industry where a number of other substances with long persistence may also have been released, ISB of carbon tetrachloride may not be feasible. In some industries, carbon tetrachloride may be a small component of a waste stream and may thus not behave as the pure product would in the subsurface.

As an example, consider carbon tetrachloride used as a fumigant in grain storage facilities in the Midwest. Carbon tetrachloride was typically applied to these facilities as a mixed product generically referred to as “80-20,” composed of 80% carbon tetrachloride and 20% carbon disulfide or “70-30,” composed of 70% ethylene dichloride (1,2-dichloroethane [DCA]) and 30% carbon tetrachloride. Trade names for carbon tetrachloride products include Carbona, Benzinoform, Flukoids, Necatorina, Tetrafinol, Tetraform, Tetrasol, Univerm, and Vermoestroid. Operational history of the facility will likely show that releases to the environment were cyclic, based on annual grain storage patterns. Investigators may also find

that carbon tetrachloride was not the only fumigant (EDB and EDB/carbon tetrachloride mixtures, also known as Maxkill 10 have been documented) in use at the facility, or that the facility also had associated seed treatment operations that may have contributed mercury compounds to the subsurface. The time of the releases will also prove important in modeling the contaminant plume movement and remediation options. If the operational history is not carefully considered, very incorrect decisions may result.

**Table 1. Properties of carbon tetrachloride and its degradation products.**

Property	Carbon Tetrachloride (C-Cl <sub>4</sub> )	Chloroform (C-H-Cl <sub>3</sub> )	Dichloromethane (C-H <sub>2</sub> -Cl <sub>2</sub> )	Chloromethane (C-H <sub>3</sub> -Cl)
CAS #	56-23-5	67-66-3	75-09-2	74-87-03
Molecular Weight	153.82	119.38	84.93	50.49
Color/Form	Colorless, clear, heavy liquid	Clear, colorless liquid	Colorless liquid (a gas above 104 °F)	Clear, colorless liquid
Taste/Odor	Characteristic, ether-like odor	Pleasant, etheric, nonirritating odor	Odor similar to chloroform	Faint sweet odor (noticeable at toxic levels)
Density/Specific Gravity	1.5940 @ 20°C	1.4835 @ 20 °C	1.3255 @ 20 °C	0.92 @ 20 °C
Octanol/Water Partition Coefficient (log K <sub>ow</sub> )	2.83	1.97	1.25	0.91
Partition Coefficient (K <sub>oc</sub> )*	110 (71 EPA Web site)	31	21	6
Water Solubility	793 mg/L @ 25 °C	7,710 mg/L @ 25 °C	13,000 mg/L @ 25 °C	6,500 mg/L @ 25 °C
Henry's Law Constant	2.76 x 10 <sup>-2</sup> atm-cu meter/mole @ 25 °C	3.67 x 10 <sup>-3</sup> atm-cu meter/mole @ 24 °C	3.25 x 10 <sup>-3</sup> atm-cu meter/mole @ 25 °C	1.27 x 10 <sup>-2</sup> atm-cu meter/mole @ 25 °C
Boiling Point	76.8 °C	61.2 °C	39.75 °C	-24.2 °C
Melting Point	-23 °C	-63.2 °C	-95 °C	-97.6 °C
Vapor Density (Air=1)	5.32	4.12	2.93	1.8
Vapor Pressure (mmHg)	115 mm Hg @ 25 °C	197 mm Hg @ 25 °C	435 mm Hg @ 25 °C	2,103 mm Hg @ 25 °C
Evaporation Rate** (BuAc=1)	12.8	11.6	71	No available data

\*K<sub>oc</sub> values of compounds in the family of chloromethane vary widely depending on the analytical methodology and site (see Truex et al., May 2001).

\*\*Relative evaporation rate listed as compared to ether (100) rather than butyl acetate.



### ***Sources of Carbon Tetrachloride Contamination***

Historically carbon tetrachloride was produced in large quantities to make refrigerants, fluids, and propellants for aerosol cans. Carbon tetrachloride has been widely used as cleaning fluid in the home, as dry cleaning fluid, in the production of semiconductors, as a degreaser, as a gasoline additive in some formulations, and to recover tin from tin-plating waste. It was used as a catalyst in the manufacturing of soap perfumes and insecticides and because carbon tetrachloride is nonflammable, it was also used in fire extinguishers. The primary agricultural use for carbon tetrachloride was as a fumigant in grain silos.

Due to the toxicity of carbon tetrachloride, consumer and fumigant uses have been discontinued and only industrial uses remain. Use of the compound was curtailed in the 1960s due to concerns over the effects of exposure. In 1986, carbon tetrachloride was banned for use as a pesticide. The major current use of carbon tetrachloride is in the production of chlorofluorocarbons that are used primarily as refrigerants. Due to the effort to reduce ozone-depleting chemicals, carbon tetrachloride was phased out for non-feedstock uses in 1996. It is now used only in those feedstock operations in which it is totally consumed.

### ***Properties of Carbon Tetrachloride***

Carbon tetrachloride is a dense, nonaqueous-phase liquid (DNAPL) that does not occur naturally. The physical properties of carbon tetrachloride and its most common degradation products (chloroform, dichloromethane, and chloromethane) can be found at the TOXNET Web site and are summarized in Table 3. TOXNET was developed by the U.S. National Institute of Health and can be found at the following URL: <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/d?./temp/~AAAnFaqbV:0:BODY>.

### ***Contaminant Relationship***

Carbon tetrachloride contamination has been found in association with other contaminants, specifically VOCs and nitrates. During the course of many fuel-related (gasoline, diesel) investigations, carbon tetrachloride has been identified as a COC when a complete VOC suite has been run on the groundwater sample. When ISB is being evaluated for benzene, toluene, ethylbenzene, and total xylenes (BTEX), and polyaromatic hydrocarbons (PAH) contamination at a site and carbon tetrachloride is present, BTEX and PAHs may serve as electron donors, and the carbon tetrachloride may become the electron acceptor. When this occurs, a sequential dechlorination of carbon tetrachloride and the degradation products should be measured.

In many rural and agricultural regions, nitrate contamination is co-mingled with carbon tetrachloride plumes due to past use of carbon tetrachloride at grain silos. The relationship of these two contaminants is important to ISB systems. Even though carbon tetrachloride is an organic compound, it rarely serves as an electron donor in reductive ISB systems. Furthermore, if an electron donor is present (i.e., acetate) along with nitrate contamination serving as an electron acceptor, carbon tetrachloride is cometabolically mineralized without the appearance of reductive by-products such as chloroform. This process has been identified for the bacteria *Pseudomonas stutzeri* KC (Michigan State Univ., 2000).

Other fumigants may be present. A document from Foster (1984) entitled Preventing Insect Problems in Farm-Stored Corn from Purdue Cooperative Extension Service shows that the following pesticides have been used in grain storage: malathion, dichlorvos, methyl bromide, chloropicrin, 70-30 (1,2-DCA/carbon tetrachloride), 80-20 (carbon tetrachloride/carbon disulfide), and aluminum phosphide. Malathion (O,O-dimethyl-S-1,2-di(carboethoxy) ethyl phosphorodithioat) is an aliphatic organophosphate that was heavily used as a pesticide and its degradation products are of concern. Dichlorvos (O,O-dimethyl-O-2,2-dichloro-vinyl phosphate) is a volatile organophosphate and its degradation products (dimethyl phosphate and dichloroacetaldehyde) are of health concern. Methyl bromide is lethal to all plant and animal life, is classified as a sterilant (Ware, 1983), and has been phased out of usage in the United States. Chloropicrin (Cl<sub>3</sub>CNO<sub>2</sub>) has been used as an olfactory warning agent in grain fumigants but also serves as a fumigant itself (Ware, 1983) and is susceptible to anaerobic ISB with the degradation products being carbon dioxide, chloride, and possibly nitromethane. "70-30" was a liquid fumigant composed of 70% ethylene dichloride (1,2-DCA) and 30% carbon tetrachloride used at grain silos. Aluminum phosphide is a solid and had been used at grain silos. These compounds or their degradation products may be found as contaminants, along with carbon tetrachloride, and should be considered during site investigation activities to identify other contaminants of concern.

Technical-grade carbon tetrachloride product is typically reported as 99 to 100 percent carbon tetrachloride. Doherty (2000) reports that trace amounts of carbon disulfide, bromine, chloroform, and hydrochloric acid may be present. Additionally, Doherty (2000) reports that diphenylamine, ethyl acetate, and ethyl cyanides may be present as corrosion inhibitors in some commercial formulations.

Sometimes chloroform is present in analytical results from groundwater samples when site investigation activities have clearly identified carbon tetrachloride as the only contaminant discharged. This information can

be perplexing to the site manager if groundwater conditions are not anaerobic and site characterization data does not indicate any reductive conditions. Just because chloroform is present does not necessarily indicate that the site is undergoing complete biological reduction of carbon tetrachloride. Possible solutions to this observation are that chloroform was a trace constituent in the carbon tetrachloride discharged, or the vadose or saturated zone directly beneath the discharge point has been or is conducive to anaerobic reduction of carbon tetrachloride, thus forming chloroform as a degradation product. This anaerobic reduction zone directly beneath the discharge point may not be substantial enough for complete reduction of the carbon tetrachloride, allowing for the remaining carbon tetrachloride and the degradation product, chloroform, to persist.

Carbon tetrachloride as a solvent appears to predate some of the other ubiquitous solvents found at remediation sites on defense facilities, especially those that were in place by the 1940s. Newer facilities tend to have the TCE, PCE, and TCA solvents present as soil and/or groundwater contaminants. However, carbon tetrachloride contamination can be found on many relatively new military installations. The common thread on these military sites is carbon tetrachloride's use as a degreasing agent in association with vehicle maintenance and communications / electronic gear cleaning. Where these types of operations were conducted is where the contaminants are commonly detected. Landfills on these sites also can contain carbon tetrachloride contamination. Fire extinguishers that contained carbon tetrachloride also may have been present. Examples of Department of Defense (DOD) sites that have carbon tetrachloride contamination include ammunition plants; base realignment and closure (BRAC) sites including air bases, naval yards, and depots; military reservations; and active defense installations.

Historically, Hanford Department of Energy Reservation disposed of carbon tetrachloride to the soil at several sites adjacent to a plutonium refinishing plant during operation of the plant from 1955 to 1973. The carbon tetrachloride was used with mixtures of other organics to recover plutonium from the aqueous stream in the plant. The resultant organic liquids discharged to the disposal site consisted primarily of carbon tetrachloride mixed with tributyl phosphate, dibutyl butyl phosphate, and lard oil (Truex et al., 2001). DOE's Y-12 Plant in Oak Ridge, Tennessee, has a historical carbon tetrachloride plume with suspected DNAPL in fractured bedrock (TechCon Program, Oak Ridge). A pump-and-treat system is planned for plume containment. Oak Ridge is following a systematic approach to evaluate the effectiveness of ISB for carbon tetrachloride at the Y-12 Plant (DOE, Project Status) that has included site characterization for ISB, laboratory-scale treatability tests, and limited pilot-scale field demonstrations.

## Regulatory Standards and Guidance of Carbon Tetrachloride

Carbon tetrachloride is no longer registered under FIFRA Section 3 and is no longer used in pesticides. Carbon tetrachloride is designated as a hazardous air pollutant under Section 112 of the Clean Air Act [40 CFR 61.01 (7/1/99)]. The compound is also designated as a hazardous substance under Section 311(b)(2)(A) of the Federal Water Pollution Control Act, and is also regulated under the Clean Water Act

**Table 2. Selected state groundwater standards.**

State	Numeric Standard (µg/L)				State Regulation
New Mexico	CT-10 CF-100 DCM-100 CM-no numeric standard				New Mexico Water Quality Control Regulation 20.6.2.3103 NMAC
New Hampshire	CT-5 CF-6 DCM-5 CM-3				New Hampshire Groundwater Management and Groundwater Release Detection Permits Env-Wm 1403
Arizona	CT-5 CF-no numeric standard DCM-5 CM-no numeric standard				
Virginia					Uses Safe Drinking Water Act, part 141, title 40 CFR
Colorado	CT-0.27 CF-6 DCM-4.7 CM-no numeric standard				Water Quality Control Commission (5 CCR 1002-41)
Missouri	Scenario				RSMo §260.565-260.575 and administrative rule 10 CAR 25-15.010
	CT	A	B	C	
	CF	0.8	1	1	
	DCM	51	71	150	
	CM	No numeric standard			
Oklahoma	CT-4 CF-10 DCM-no numeric standard CM-2.7				Oklahoma Standard for Groundwater and Protection Action Subchapter 7, §785:45-7-2
North Dakota	CT-5 CF-100 MCL or HAL DCM-5 CM-3				Standards of Quality for Waters of the State, Chapter 33-16-02, North Dakota Administrative Code
Pennsylvania	Used Aquifers		Used Aquifers		Pennsylvania Land Recycling Program Regulations Subchapter C, §250.304 and §250.305
	TDS≤2,500		TDS>2,500		
	CT	5	500		
	CF	100	10,000		
	DCM	3	300		
CM	3	300			

CT=carbon tetrachloride; CF=chloroform; DCM=dichloromethane (methylene chloride); CM=chloromethane

Amendments of 1977 and 1978 [40 CFR 116.4 (7/1/99)]. The MCL established by the National Revised Primary Drinking Water Regulations for carbon tetrachloride in community and non-transient, non-community water systems is 5 micrograms per liter [40 CFR 141.61 (7/1/99)]. Certain states have promulgated numeric water standards for carbon tetrachloride and its degradation products, as shown in the following table for selected states. Due to differences in groundwater standards for carbon tetrachloride and its degradation products, the reader should be aware of differing cleanup standards among states.

## **SITE DESCRIPTION/CHARACTERIZATION**

Adequate site characterization is required at any carbon tetrachloride-contaminated site prior to remediation. The extent and magnitude of a carbon tetrachloride plume must be fully characterized. Persistence of carbon tetrachloride in the environment mandates characterization of the contamination, including identifying carbon tetrachloride sources in the vadose zone. Since carbon tetrachloride is a DNAPL, characterization of a site should identify and address DNAPL presence, if possible. Following site characterization and evaluation, ISB may be the chosen remediation technology; however, source control must be addressed prior to achievement of closure criteria. The following section discusses site characterization as it relates to enhanced ISB of carbon tetrachloride. This section discusses which hydrogeologic and geochemical parameters should be characterized and why for ISB of carbon tetrachloride.

### ***Hydrogeologic Environment***

All hydrogeologic conditions at the site should be defined [(i.e., vadose zone source, lithology, hydraulic conductivity ( $K$ ), octanol-water partitioning coefficient ( $K_{ow}$ ), organic carbon partitioning coefficient ( $K_{oc}$ ), partitioning coefficient ( $K_d$ ), or the retardation coefficient ( $Rf$ )] and measured to estimate the movement of fluids and solutes through the subsurface. Retardation is necessary to develop a representative conceptual characterization of the contaminant plume and unsaturated zone sources from residual compound. Carbon tetrachloride, in a relative view, has a higher retardation than does MTBE but lower than other DNAPLs such as TCE. Understanding retardation of the compound and external elements of the subsurface influencing retardation provides the chemical basis for describing the dimensions of the contaminated plume.

### ***Geochemistry***

Carbon tetrachloride reduction occurs during anaerobic respiration. In the absence of oxygen and the presence of a carbon source, bacteria may utilize the carbon tetrachloride as an electron acceptor (reductive dechlorination), or carbon tetrachloride may be transformed cometabolically, depending on the

ORP conditions, the bacteria, and available electron acceptors. ISB of carbon tetrachloride is a remediation technology that introduces a carbon source to a contaminated aquifer. Depending on the type of carbon source introduced and the ORP conditions achieved, carbon tetrachloride may either be dechlorinated to its degradation products, or mineralized into carbon dioxide and water with little or no formation of degradation products. Since many shallow aquifers are aerobic, the introduced carbon is initially consumed by the indigenous aerobic bacteria as a carbon source (electron donor). As the aquifer becomes depleted in dissolved oxygen, conditions become anaerobic. When carbon remains in excess, indigenous denitrifying bacteria proliferate and the reduction of carbon tetrachloride occurs. Figure 3 shows the expected sequence of selected subsurface ORP transformations, and may be useful for assessing whether degradation products may be expected to appear or disappear, based on site ORP conditions. The reader is cautioned that the figure is meant as a template, based on the thermodynamics of the reduction and oxidation reactions considered, and Eh values are referenced to a pH of 7. Accurate ORP measurements in the field are difficult to obtain and may not be representative of the heterogeneous subsurface conditions encountered. Nevertheless, the diagram shows the relative strength (or oxidizing power) of the relevant electron acceptors. Since low dissolved-oxygen conditions are necessary for ISB of carbon tetrachloride to occur, the following geochemical parameters should be measured: concentration of carbon tetrachloride and its degradation products, chloride, nitrate, alkalinity, DO, pH, ORP, and phosphorous; and possibly dissolved iron and manganese, total organic carbon, and methane. Tables 3 and 4 describe the importance of geochemical parameter identification for ISB of carbon tetrachloride.

### ***Contaminant Degradations/Microorganisms***

Carbon tetrachloride reduction may occur either through a biological sequential reduction or a direct mineralization process. Figure 4 shows possible carbon tetrachloride degradation pathways.

Carbon tetrachloride biological destruction and its degradation products have been observed under denitrifying, sulfate-reducing, acetogenic, fermentative, and methanogenic conditions by a variety of organisms. These reductive processes do not solely follow a sequential reduction whereby a chloride ion is removed during each transformation. Research studies suggest that carbon tetrachloride destruction pathways may be both reductive and substitutional. DOE has presented these research studies and transformation pathways

while evaluating carbon tetrachloride contamination at the Hanford site and the Y-2 Oak Ridge site and can be viewed at this site (Truex, 2001) under appendix C entitled, “Literature Review: Natural Attenuation Mechanisms and Rates for Chloromethane Subsurface Contamination at Hanford.” This document provides a comprehensive literature review of studies that identify and evaluate transformation pathways for carbon tetrachloride and its degradation products.

**Table 3. Analytes important to evaluating *in situ* bioremediation of carbon tetrachloride.**

Primary Analyte	Reason for Analysis
Carbon Tetrachloride	Decreases in concentration if ISB is occurring.
Chloroform	This COC is a degradation product of reductive dechlorination of carbon tetrachloride.
Dichloromethane	This COC is a degradation product of reductive dechlorination of carbon tetrachloride.
Methyl Chloride (chloromethane)	This COC is a degradation product of reductive dechlorination of carbon tetrachloride.
Chloride	An increase in chloride concentration from background may indicate a reductive dechlorination of carbon tetrachloride.
Nitrate/nitrite	This COC is expected to decrease in concentration if bioremediation is occurring. Also, if this electron acceptor becomes depleted, carbon tetrachloride may reductively dechlorinate, creating degradation products.
Dissolved Manganese and Iron	If dissolved manganese or iron is present, indicates ORP is too low and matrix Mn/Fe is serving as e <sup>-</sup> acceptor.
Sulfate	If sulfate concentrations are less than background and ORP is low, sulfate may be serving as an electron acceptor and reduction may be occurring.
Sulfide	If sulfide (H <sub>2</sub> S) concentrations are greater than background, sulfate may be serving as an electron acceptor producing sulfides.
Phosphorous (P)	For ISB of carbon tetrachloride to occur effectively, sufficient P needs to be available for microbial metabolism. (P may need to be added as an amendment.)
Total Organic Carbon	TOC analysis will indicate availability of naturally occurring carbon sources (e <sup>-</sup> donor).
Methane	This constituent may be present as the final degradation product of carbon tetrachloride dechlorination or may be present if ORP conditions are so low that methanogenesis is occurring.

The two most significant pathways for ISB systems are under anaerobic conditions and are described.

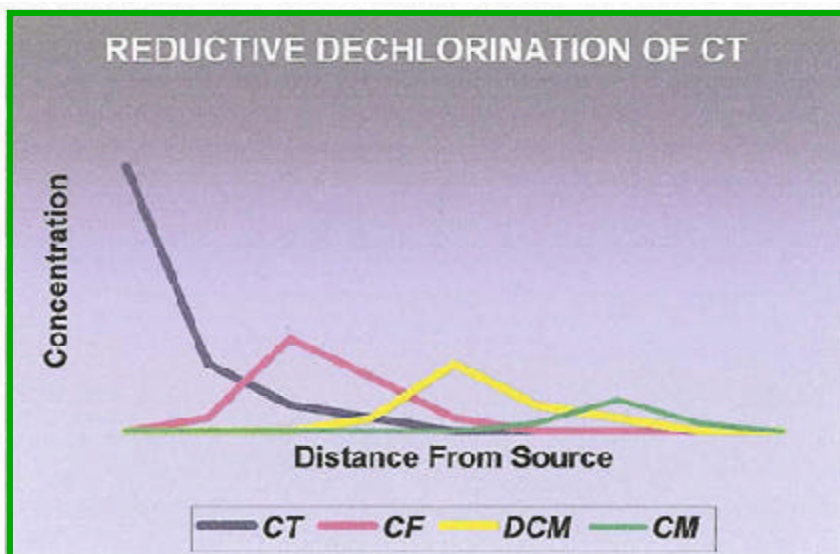
### 1. Reductive Dechlorination

Reductive dechlorination is the process whereby bacteria halorespire the chlorinated compound which serves as an electron acceptor. A carbon source (electron donor) is necessary for this process to occur. Carbon tetrachloride undergoes a series of reductions where the chlorine ion is substituted with a hydrogen ion. During each of these degradations, carbon tetrachloride releases a chlorine ion and gains a hydrogen ion. Two electrons are transferred at each step during this process, potentially providing a source of energy for the microbe. Therefore, a reduction of carbon tetrachloride would occur along with an initial increase in chloroform, with a subsequent decrease followed by an increase in dichloromethane (methylene chloride) with its subsequent decrease, and the formation of chloromethane (methyl chloride) with its decrease, until methane becomes the final product. Consequently, the occurrence of reductive dechlorination can be measured by observing the presence of degradation products such as chloroform, dichloromethane, chloromethane, and methane. This direct reductive-dechlorination pathway, whereby carbon tetrachloride and its degradation products serve as electron acceptors, has, until recently, not been identified. Due to the multiple biological-degradation pathways possible for carbon tetrachloride, this reductive-dechlorination pathway may not be easily identified. Figure 5, is a hypothetical graph of concentrations of carbon tetrachloride with its degradation products as a function of distance from the source.

**Table 4.** Field parameters important to evaluating *in situ* bioremediation of carbon tetrachloride.

Primary Analyte	Reason for Analysis
Alkanlinity	Due to microbial respiration production of CO <sub>2</sub> , you can expect an increase in alkalinity from background.
Dissolved Oxygen (DO)	For ISB of carbon tetrachloride to occur, DO concentrations must be depleted (<2mg/l).
pH	ISB of carbon tetrachloride occurs effectively in wide pH ranges (5.5-9.5).
ORP	The ORP may be used in conjunction with electron acceptor concentrations as a qualitative indicator of ORP conditions and in identifying which electron acceptor(s) may be active.





**Figure 5.** Hypothetical reduction of carbon tetrachloride with degradation products (CT=carbon tetrachloride [CCL<sub>4</sub>], CF=chloroform [CCl<sub>3</sub>H], DCM=dechloromethane [CCl<sub>2</sub>H<sub>2</sub>], and CM=chloromethane [CClH<sub>3</sub>]).

Reductive dechlorination of carbon tetrachloride needs to be taken to completion, allowing total destruction of carbon tetrachloride and its degradation products. Since chloroform is a degradation product and a known carcinogen, any degradation pathway that does not produce chloroform is preferred.

## 2. Anaerobic Cometabolism

Carbon tetrachloride, like other chlorinated aliphatic compounds, degrades under reducing conditions. Carbon tetrachloride has been shown to degrade under reducing conditions via two major cometabolic pathways. “During cometabolic reactions, the chlorinated aliphatic compound is caused by an enzyme or cofactor produced during microbial metabolism of another compound. Chlorinated aliphatic compound degradation or oxidation does not yield any energy or growth benefit for the microorganism mediating the cometabolic reaction” (EPA, Engineered Approaches to *In Situ* Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications). The following discussion describes the two major reductive-cometabolic pathways for carbon tetrachloride.

### a. Cometabolic Reductive Dechlorination

During this reductive pathway, carbon tetrachloride is fortuitously degraded by an enzyme or cofactor during the microbial consumption of an alternate carbon source. There appears to be no benefit to the microorganisms during this process. These enzymes or cofactors reduce carbon tetrachloride, systematically producing degradation products as would be seen during direct anaerobic-reductive dechlorination of

carbon tetrachloride. “Carbon tetrachloride is degraded to chloroform, dichloromethane, chloromethane, and ultimately methane by hydrogenolytic dechlorinations. Many anaerobic bacteria can catalyze the first two reactions of this pathway using the cofactors heme, factor F430, and corrinoids, such as aquocobalamin and methylcobalamin” [(Carbon Tetrachloride Pathway Map (Anaerobic)) (Tripp, 2000)]. It is difficult in the pilot field test or full-scale deployment to distinguish which is the preferred pathway. It may not be necessary for the site manager to determine which pathway is occurring during field deployment.

#### *b. Cometabolic Denitrification*

The denitrification/cometabolism degradation of carbon tetrachloride results in little to no production of chloroform. However, mechanisms of this degradation process are not as well understood as reductive dechlorination and contain numerous limiting factors. Basically carbon tetrachloride is cometabolized to eventually produce carbon dioxide and possibly formate (Lasatoskie, 1999). This process works as follows. A carbon source is introduced into the contaminant zone to create denitrifying conditions. That is, the ORP is slightly reduced and all available oxygen has already been consumed as an electron acceptor. If nitrate is in the aquifer, it serves as an electron acceptor and is denitrified (Dybas, 2000), thus removing another contaminant from the aquifer. Various denitrifying bacteria produce oxygenase enzymes that fortuitously metabolize carbon tetrachloride and the end result is carbon dioxide without the production of chloroform.

An important discovery by Criddle (1990) identified the bacteria *Pseudomonas stutzeri* KC as being able to reduce carbon tetrachloride without production of CF. This specific bacteria secretes the molecule PDTC [pyridine-2,6-bis(thiocarboxylate)], believed to be an iron chelator which alone may transform carbon tetrachloride into carbon dioxide, chloride ions, formate, and other nonvolatile products. This molecule is thought to be the cometabolic factor for destruction of carbon tetrachloride. Criddle and colleagues discovered that *Pseudomonas stutzeri* KC must be grown in an anaerobic, alkaline environment (pH 8) that lowers dissolved iron (Mayotte et al., 1996). Dissolved iron limits the capability of this bacteria to degrade carbon tetrachloride. Criddle’s work, along with others, resulted in the field deployment of a biocurtain to treat carbon tetrachloride at a site in Schoolcraft, Michigan (Criddle, 1990).

A fed batch experiment was conducted by Sherwood and Peterson (Sherwood, 1996) to test the effects of electron donors and acceptors on the production of chloroform. This study showed that under nitrate- (electron acceptor) limiting conditions there was chloroform production, while under acetate-(electron

donor) limiting conditions, there was little chloroform production. What this suggests is that if an abundant carbon source (electron donor) is available and nitrate (electron acceptor) is limiting, the bacteria will search out an alternate electron acceptor. Typically, for an alternate electron acceptor to be available to the bacteria, the ORP will be lower. This will occur with an abundant carbon source. When the ORP is lowered, carbon tetrachloride will undergo reductive dechlorination and the denitrifying bacteria that can convert carbon tetrachloride directly to carbon dioxide are out competed. When the electron donor is limited and nitrate is abundant, the denitrifiers remain predominant producing more oxygenase enzymes, causing minimal production of chloroform and mineralization of carbon tetrachloride to carbon dioxide. Therefore, if an ISB system can be optimized so that denitrification conditions are consistently met (ORP 500-700 mv) and nutrient requirements are supplied, carbon tetrachloride should be reduced without production of chloroform, provided the bacteria that produce these cometabolic factors are present.

This denitrification/cometabolism of carbon tetrachloride has been observed in the field and has been deployed. McQuillan et al. (1998) showed through monitored, natural attenuation at a co-mingled gasoline and carbon tetrachloride plume that reductive dechlorination and denitrification/cometabolism of carbon tetrachloride occurred. In the source zone where electron donors (benzene, toluene, ethylbenzene, and xylene) were abundant, ORP was suppressed (sulfate-reducing conditions), and nitrate had been depleted, reductive dechlorination of carbon tetrachloride occurred and degradation products were present. However, where electron donors were limiting, the ORP was slightly suppressed, background nitrate was abundant, and direct mineralization of carbon tetrachloride was observed at pH ranging between 7.2 and 7.4. A field demonstration of ISB of carbon tetrachloride and nitrate at the DOE Hanford site showed that nitrate was reduced and carbon tetrachloride was cometabolically mineralized with minor production of chloroform (Truex et al., 1996).

This denitrification/cometabolic reduction pathway of carbon tetrachloride may be preferred since little to no chloroform is produced; but as mentioned above, numerous limiting factors or conditions may exist. First, for this reductive pathway to occur, a fine balance between nitrate addition and electron donor amendment must be maintained. This task may be difficult to achieve in field conditions. Secondly, if direct mineralization of carbon tetrachloride is not occurring in spite of achieving optimal conditions (i.e., appropriate electron donor and acceptor amendment), bio-augmentation of suitable bacteria may be necessary, as in

the Schoolcraft case. Finally, this reductive pathway for carbon tetrachloride has not been deployed at numerous sites and could be considered an emerging application that may impede its acceptance.

### ***Adverse Human Health Effects***

Carbon tetrachloride and some of its degradation products are considered carcinogens or suspected carcinogens, and are regulated substances and hazardous materials. Exposure to high concentrations of carbon tetrachloride may cause liver, kidney, and central nervous system damage (ATSDR, 1995). Long-term exposure to carbon tetrachloride has the potential to cause liver damage and/or cancer (EPA-Office of Water, 2001). Studies in certain animals have shown an increase in liver tumors to some species when ingested. The Department of Health and Human Services has determined that carbon tetrachloride may reasonably be anticipated to be a carcinogen. The International Agency for Research on Cancer has determined that carbon tetrachloride is possibly carcinogenic to humans, and the EPA has determined that carbon tetrachloride is probably a human carcinogen (ECO-USA, 2001).

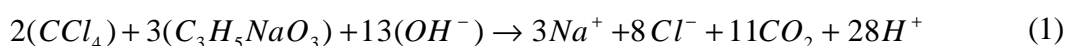
### ***Adverse Ecological Effects***

Due to carbon tetrachloride's relatively high evaporation rate when released to the environment, most moves quickly into the air. Carbon tetrachloride is stable in air (30-100 years) (ATSDR, 1995) and may react with other chemicals that have the potential to destroy upper atmosphere ozone. It is reported not to accumulate in animals, and it is unknown whether it accumulates in plant tissue.

## **FATE AND TRANSPORT**

### ***Stoichiometry and Kinetics***

In the case of organic compounds such as carbon tetrachloride, there is not a simple single stoichiometric equation because there are many competing reactions occurring simultaneously, and each is catalyzed by different bacteria. The rates for each reaction will vary from site to site and hence impact or alter the overall stoichiometry. Reductive dechlorination of carbon tetrachloride to methane is, however, straightforward. Each molecule of carbon tetrachloride requires 8 electrons from a carbon source (electron donor) to convert it to methane. The single-carbon atom in carbon tetrachloride changes valence from fully oxidized (+4) to fully reduced (-4), or a net change of 8 electrons. If sodium lactate ( $C_3H_5NaO_3$ ) is the supplied carbon donor, three molecules will ideally reduce two molecules of carbon tetrachloride. The three carbons in sodium lactate have a zero valence and will oxidize with a yield of 12 electrons per sodium lactate molecule. That is



Therefore, the amount of sodium lactate amendment necessary to reduce one gram of carbon tetrachloride would be approximately 2.2 grams. Please note that many ISB systems add an additional 10% to 20% of amendment as a safety factor.

### ***Modeling***

Fate and transport models are site specific and must be developed accordingly. These models are representations of field conditions. As previously stated, the conceptual model is a simplified version of the particular site's groundwater system, as inferred from the site characterization. Groundwater computer models are used to simulate and predict conditions regarding site-specific, sub-surface flow and transport. This information is important to assist in determining amendment mixing, hydraulic control, biofouling, first-order decay rates, and travel times and directions. This helps in identifying the number and locations of recovery and injection wells and flow rates to achieve ISB of carbon tetrachloride. Since this decision criteria module for a systematic approach to ISB is not site specific, generic models are not applicable.

Once the site is characterized to a degree and the various hydrogeological, chemical, and microbial relationships are adequately understood, a conceptual model of carbon tetrachloride behavior is developed. The fate and transport of carbon tetrachloride through the porous media is controlled by its density and the pressure resulting from its release into the subsurface. The magnitude of the pressure is proportional to the interfacial tension between the carbon tetrachloride and the water and is inversely proportional to the diameter of the pore openings.

After the conceptual model is developed, a mathematical model is formulated. Proper physical constants, variables, and boundary conditions are identified. There are numerous assumptions and simplifications that can be implemented at this stage, and sensitivity analysis could be performed.

### ***Movement of Carbon Tetrachloride Through the Saturated Zone***

In the saturated zone, interaction between carbon tetrachloride, which is a DNAPL, and the water phase is important. The carbon tetrachloride as a product migrates below the water table due to its density, which is 1.59 g/cc. The aqueous or dissolved phase of a carbon tetrachloride forms the plume, which travels in the direction of the groundwater flow. More permeable media and higher carbon tetrachloride saturation will cause a higher rate of flow of the aqueous phase. Nearly all movement of carbon tetrachloride as a product (DNAPL) takes place through the "connected pores" under the influence of gravity. In order to

displace the water in the pores, any DNAPL must have sufficient mass to overcome the capillary forces that hold the water in the pores.

### ***Applicability***

The first step to establish the suitability of a site for ISB of carbon tetrachloride is to determine site conditions as defined above. The most critical factor at a site is the presence or absence of an electron donor that will affect the ORP. A decreased ORP indicates reducing conditions required for carbon tetrachloride reduction, either through reductive dechlorination or denitrification/cometabolism. This condition can only occur if a carbon source is or has been present to reduce the ORP. However, the level of ORP is the determining factor for which a reductive process is dominant. If the ORP is systematically lowered, then reductive dechlorination of the carbon tetrachloride will be preferred. This can be achieved by electron donor selection. For instance, lactate typically can reduce the ORP to sulfate-reducing and possibly methanogenic conditions, thus favoring reductive dechlorination. On the other hand, if an electron donor such as acetate or ethanol is chosen, the ORP will not be reduced as much. If the ORP is maintained at denitrification conditions, then cometabolism of the carbon tetrachloride will occur with minor or no production of degradation products. A laboratory-scale treatability study or determination will assist the project manager in determining which pathway is preferred and what conditions can be manipulated.

During site characterization, if there are no electron donors present, then site conditions should indicate the following:

little or no decrease in carbon tetrachloride concentration attributed to biological activity;

little to no decrease in ORP, little to no decrease in nitrate concentrations if present;

little to no increase in dissolved iron or manganese or sulfides;

little to no decrease in sulfate concentrations; and

lack of carbon tetrachloride degradation products (i.e., CF, DCM, CM).

Realistically though, many carbon tetrachloride sites are discovered at sites suspected of other VOC releases such as petroleum hydrocarbons. If this is the case, then an electron donor will be present in groundwater as another contaminant of concern. If an electron donor is present, certain site conditions may be observed such as the presence of degradation products (i.e., CF, DCM, CM); a decrease in ORP, dissolved oxygen, nitrate and sulfate concentrations; and an increase in dissolved manganese and/or iron and sulfides.

With this information, first-order decay rates can be calculated for the site. Furthermore, the site data will help in determining what non-biological attenuation factors are occurring in the contaminant plume.

With this site characterization data, a project manager can decide which ISB reduction pathway is most suited for the site. That is, if conditions are not reductive or slightly reductive, than the addition of a specific electron donor to the saturated zone that favors denitrification/cometabolism of the carbon tetrachloride may be preferred and chosen as the ISB remediation process. Please note that in this case, nitrate addition may be necessary to maintain these conditions to prevent the production of chloroform and other degradation products. This can be determined through stoichiometry and mass balance equations. On the other hand, if conditions are already reductive and reductive dechlorination of the carbon tetrachloride is present at the site, then these conditions may be maintained by the addition of specific electron donors to keep the ORP suppressed. These conditions must be maintained so that degradation products do not remain but are completely reduced. This will prevent persistence of a degradation product (i.e., CF) in groundwater.

#### ***Monitored Natural Attenuation (MNA)***

Dilution, dispersion, and sorption of a carbon tetrachloride plume will occur to some degree. These physical conditions should have been determined and quantified during the site characterization process. However, microbiological effects of MNA will not occur unless an electron donor is present in the subsurface and if conditions are reductive. If an electron donor is present, the degree of MNA occurrence should be identified and evaluated during site characterization. That is, analyses of the analytes described in Table 3 should have been performed, which reductive pathway is predominant should have been determined, and an evaluation to see if remediation goals can be achieved through MNA alone should have been conducted. Once MNA evidence has been gathered, it must be presented in a clear and logical sequence to secure acceptance from the responsible party and the regulators. Please refer to the ITRC document titled “Natural Attenuation of Chlorinated Solvents in Groundwater: Principles and Practices” [www.itrcweb.org](http://www.itrcweb.org) (link ISB-7) (ITRC, 1999).

#### ***Enhanced ISB Systems***

If following site characterization, evaluation of MNA, and the laboratory treatability test, all data indicates that an enhanced ISB system will reduce the carbon tetrachloride, then a pilot test system should be engineered to introduce amendments (i.e., electron donor, electron acceptor, nutrients, or microbes) to the subsurface. An enhanced ISB system should be tailored for a specific contaminant site and should be dictated by site-specific conditions. Please refer to ITRC’s Technical and Regulatory Requirements of

Chlorinated Solvents in Groundwater (link ISB-3) (ITRC, 1998) and EPA's Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications (link) for good descriptions of engineered systems with associated references.

### ***Hybrid Treatment Systems***

The site manager should determine if an enhanced ISB system for carbon tetrachloride reduction can stand alone to meet remediation goals based on the site characterization, laboratory treatability studies, and field pilot tests. If these remediation goals cannot be achieved solely through an enhanced ISB system, then a hybrid treatment system that includes an enhanced ISB system should be instituted. Since carbon tetrachloride is volatile, an ISB system may be used in conjunction with volatilization technologies such as air sparging, soil-vapor extraction, six-phase heating, *in situ* thermal destruction, and bioventing. Be aware that these volatilization technologies may aerate groundwater, thus changing the ORP conditions making the site unsuitable for ISB anaerobic reduction of carbon tetrachloride. Furthermore, if a site already contains remediation systems, then an enhanced ISB system may be suitable as a "polishing" technology in the final stages of achieving remediation goals or standards in a more timely fashion. ISB remediation technologies for carbon tetrachloride should be considered and deployed, where appropriate, as part of a treatment system to reach remediation goals.

### ***Laboratory-Scale Treatability Tests***

Laboratory treatability studies are necessary to determine if bioremediation of carbon tetrachloride can or will occur at the contaminant site, and to determine which reductive pathway may be most beneficial and productive. A laboratory treatability study, along with site characterization data, will identify if reductive dechlorination of carbon tetrachloride is occurring or has the potential to occur at the site. If it has been determined that reductive dechlorination is occurring at the site, the laboratory treatability study will provide information on how to maximize this reductive process. The study will show which electron donor is most suitable to reduce the ORP to low enough levels to assure that degradation products are completely reduced. The study may include an evaluation of different electron donors that may be suitable. If no reductive dechlorination is occurring at the site, the laboratory treatability study will determine if the site is capable of sustaining this reductive process. The study will also determine not only which electron donor is most appropriate but what nutrients, if any, are necessary; what will be the reductive rates for each degradation



product; and assist in designing the quantity of amendments needed to add to the subsurface. Furthermore, if reductive dechlorination is occurring at the site and the project manager decides to pursue the denitrification/cometabolism of carbon tetrachloride instead of reductive dechlorination, the laboratory treatability study will help determine how to adjust site conditions to induce the denitrification/cometabolism reductive pathway. For instance, the study can be tailored to increase the ORP to denitrification conditions that may eliminate degradation product production and stimulate the denitrification / cometabolism reductive pathway.

If the denitrification/cometabolism pathway is the preferred reductive pathway, the laboratory treatability study will provide the essential data of electron donor selection (one that does not reduce the ORP below denitrification levels), any nutrient requirements (i.e., phosphorous), and what is required to maintain these finely balanced conditions. Furthermore, the study will determine if bacteria that produce the cometabolic factor are present or if bioaugmentation of these bacteria is necessary (i.e., *Pseudomonas stutzeri* KC). The study will further determine the ORP values that must be maintained and whether nitrate (electron acceptor) must be added to maintain these conditions.

In general, these studies are recommended to provide specific, contaminant-degradation information, provide the information about the types of biodegradation that occur naturally at the site, and assist in determining the best amendments to be added. Also, these studies can evaluate different amendments and different levels of amendments to determine which is most effective.

### ***Pilot-Scale Field Demonstration***

Pilot studies provide needed information to determine the viability of the use of full-scale, in situ bioremediation technologies for a site's cleanup instead of traditional technologies. Sites like the Schoolcraft Project in Schoolcraft, Michigan; the DOE Hanford Site in Washington state; and the DOE Oak Ridge Y-12 site used pilot studies to prove that ISB would effectively remediate the carbon tetrachloride plumes from their respective aquifers. Additional large-scale field demonstrations are planned for the summer of 2002 at Oak Ridge and results are pending.

A plume of carbon tetrachloride and nitrates was found in the Schoolcraft aquifer. Since the contaminant plume was extensive, traditional technologies were determined to be very costly and slow. With discovery of the nonnative bacterium, *Pseudomonas stutzeri* KC, a novel technology was proposed using in situ bioaugmentation to remediate the carbon tetrachloride and nitrate plume. The *Pseudomonas stutzeri* KC

bacterium degraded the carbon tetrachloride with little or no production of chloroform. The pilot site was constructed. The aquifer's water was extracted and mixed with a base to pH 8.2. The buffered water was injected into the injection well and allowed to re-equilibrate. The pumping system was allowed to become fully operational. While site preparation occurred, the *Pseudomonas stutzeri* KC bacterium was grown in filtered, sterilized groundwater amended with phosphate, acetate (food), and base. The resultant inoculum was pumped into the test site through the injection well. The pilot test results determined that the in situ bioaugmentation was successful if the pH was maintained at levels above pH 8.0 and if the *Pseudomonas stutzeri* KC bacterium was kept viable with a proper acetate diet (<http://www.egr.msu.edu/schoolcraft>).

Over the long history of various DOE sites, many contaminants were discharged into the subsurface, migrating to and affecting their aquifers. Traditional treatment technologies were determined to be too costly and time consuming to remediate the sites. The 200 Area at Hanford was chosen for a pilot study for the DOE Hanford, Washington, site. This pilot study used indigenous microorganisms to remediate the carbon tetrachloride and nitrate plume under anaerobic conditions. Part of the pilot site contained a computer-based accelerated bioremediation design tool (ABDT), which proved effective in the design, monitoring, and operation of the pilot study site. The resultant pilot study determined that with the proper additions of nutrient (acetate) solution, as pulses alternated with the correct nitrate pulses, the indigenous anaerobic microorganisms reduced the nitrates to nitrogen gas, and the carbon tetrachloride to CO<sub>2</sub> and chlorine ions. Some chloroform was produced as a by-product. Use of the ABDT system allowed for controlled growth of the microorganisms without plugging the reinjection well. (CLU-IN.ORG "In Situ Bioremediation for the Hanford Carbon Tetrachloride Plume, Subsurface Contaminants Focus Area," U.S. Department of Energy, April 1999).

## **LIMITATIONS**

An enhanced ISB system for carbon tetrachloride has four major limitations. The first two are biofouling and amendment mixing issues. The third major limitation is not just determining the reductive pathway that is most suitable for the site but managing the balance of amendment injections to achieve the reductive pathway of carbon tetrachloride that has been chosen. The final limitation is that very few pilot-scale and full-scale field demonstrations have been deployed to date. What this indicates is that as more pilot-scale field demonstrations and full-scale deployment systems are installed, further information and data should help define and optimize ISB systems for carbon tetrachloride.

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