Design and Interpretation of Microcosm Studies for Chlorinated Compounds

Barbara H. Wilson and John T. Wilson
U.S. Environmental Protection Agency, National Risk Management Research Laboratory,
Ada, Oklahoma

Darryl Luce
U.S. Environmental Protection Agency, Region 1, Boston, Massachusetts

Introduction

Three lines of evidence are used to support natural attenuation as a remedy for chlorinated solvent contamination in ground water: documented loss of contaminant at field scale, geochemical analytical data, and direct microbiological evidence. The first line of evidence (documented loss) involves using statistically significant historical trends in contaminant concentration in conjunction with aquifer hydrogeological parameters (such as seepage velocity and dilution) to show that a reduction in the total mass of contaminants is occurring at the site. The second line of evidence (geochemical data) involves the use of chemical analytical data in mass balance calculations to show that decreases in contaminant concentrations can be directly correlated to increases in metabolic byproduct concentrations. This evidence can be used to show that concentrations of electron donors or acceptors in ground water are sufficient to facilitate degradation of the dissolved contaminants (i.e., there is sufficient capacity). Solute fate and transport models can be used to aid the mass balance calculations and to collate information on degradation.

Microcosm studies are often used to provide a third line of evidence. The potential for biodegradation of the contaminants of interest can be confirmed using of microcosms through comparison of removals in the living treatments with removals in the controls. Microcosm studies also permit an absolute mass balance determination based on biodegradation of the contaminants of interest. Further, the appearance of daughter products in the microcosms can be used to confirm biodegradation of the parent compound.

When To Use Microcosms

Microcosms have two fundamentally different applications. First, they are frequently used in a qualitative way to illustrate the important processes that control the fate of organic contaminants. Second, they are used to estimate rate constants for biotransformation of contaminants that can be used in a site-specific transport-and-fate model of a contaminated ground-water plume. This paper discusses the second application.

Microcosms should be used when there is no other way to obtain a rate constant for attenuation of contaminants, particularly when estimating the rate of attenuation from monitoring well data in the plume of concern is impossible. In some situations, there are legal or physical impediments to the comparison of concentrations in monitoring wells along a flow path. In many landscapes, the direction of ground-water flow (and water-table elevations in monitoring wells) can vary over short periods due to tidal influences or changes in barometric pressure. Changes in the stage of a nearby river or pumping wells in the vicinity can also affect the direction of ground-water flow. These changes in ground-water flow direction do not allow simple "snapshot" comparisons of concentrations in monitoring wells because of uncertainties in identifying the flow path. Rate constants from microcosms can be used with average flow conditions to estimate attenuation at some point of discharge or point of compliance.

Application of Microcosms

The primary objective of microcosm studies is to obtain rate constants applicable to average flow conditions. These average conditions can be determined by continuous monitoring of water-table elevations in the aquifer being evaluated. The product of the microcosm study, and the continuous monitoring of water-table elevations, will be a yearly or seasonal estimate of the extent of attenuation along average flow paths. Removals seen at field scale can be attributed to biological

activity. If removals in the microcosms duplicate removal at field scale, the rate constant can be used for risk assessment purposes.

Selecting Material for Study

Prior to choosing material for microcosm studies, the location of major conduits of ground-water flow should be identified, and the geochemical regions along the flow path should be determined. The important geochemical regions for natural attenuation of chlorinated aliphatic hydrocarbons are regions that are actively methanogenic, exhibit sulfate reduction and iron reduction concomitantly, or exhibit iron reduction alone. The pattern of chlorinated solvent biodegradation varies in different regions. Vinyl chloride tends to accumulate during reductive dechlorination of trichloroethylene (TCE) or tetrachloroethylene (PCE) in methanogenic regions (1, 2); it does not accumulate to the same extent in regions exhibiting iron reduction and sulfate reduction (3). In regions showing iron reduction alone, vinyl chloride is consumed but dechlorination of PCE, TCE, or dichloroethylene (DCE) may not occur (4). Core material must be acquired from each geochemical region in major flow paths represented by the plume, and the hydraulic conductivity of each depth at which core material is acquired must be measured. If possible, the microcosms should be constructed with the most transmissive material in the flow path.

Several characteristics of ground water from the same interval used to collect the core material should be determined, including temperature, redox potential, pH, and concentrations of oxygen, sulfate, sulfide, nitrate, ferrous iron, chloride, methane, ethane, ethene, total organic carbon, and alkalinity. The concentrations of compounds of regulatory concern and any breakdown products for each site must be determined. The ground water should be analyzed for methane to determine whether methanogenic conditions exist and for daughter products ethane and ethene. A comparison of the ground-water chemistry from the interval in which the cores were acquired with that in neighboring monitoring wells will demonstrate whether the collected cores are representative of that section of the contaminant plume.

Reductive dechlorination of chlorinated solvents requires an electron donor for the process to proceed. The electron donor could be soil organic matter, low molecular weight organic compounds (e.g., lactate, acetate, methanol, glucose), H_2, or a co-contaminant such as landfill leachate or petroleum compounds (5-7). In many instances, the actual electron donor(s) may not be identified.

Several characteristics of the core material should also be evaluated. The initial concentration of the contaminated material (in micrograms per kilogram) should be identified before constructing the microcosms. It is also necessary to determine whether the contamination is

present as a nonaqueous-phase liquid (NAPL) or in solution. A total petroleum hydrocarbon (TPH) analysis will reveal the presence of any hydrocarbon-based oily materials. The water-filled porosity, a parameter generally used to extrapolate rates to the field, can be calculated by comparing wet and dry weights of the aquifer material.

To ensure sample integrity and stability during acquisition, it is important to quickly transfer the aquifer material into a jar, exclude air by adding ground water, and seal the jar without headspace. The material should be cooled during transportation to the laboratory, then incubated at the ambient ground-water temperature in the dark before the construction of microcosms.

At least one microcosm study per geochemical region should be completed. If the plume is greater than 1 kilometer in length, several microcosm studies per geochemical region may need to be constructed.

Geochemical Characterization of the Site

The geochemistry of the subsurface affects the behavior of organic and inorganic contaminants, inorganic minerals, and microbial populations. Major geochemical parameters that characterize the subsurface include alkalinity, pH, redox potential, dissolved constituents (including electron acceptors), temperature, the physical and chemical characterization of the solids, and microbial processes. The most important of these in relation to biological processes are alkalinity, redox potential, the concentration of electron acceptors, and the chemical nature of the solids.

Alkalinity

Biologically active portions of a plume may be identified in the field by their increased alkalinity (compared with background wells), caused by the carbon dioxide resulting from biodegradation of the pollutants. Increases in both alkalinity and pH have been measured in portions of an aquifer contaminated by gasoline undergoing active utilization of the gasoline components (8). Alkalinity can be one of the parameters used to identify where to collect biologically active core material.

pH

Bacteria generally prefer a neutral or slightly alkaline pH level, with an optimum pH range for most microorganisms between 6.0 and 8.0; many microorganisms, however, can tolerate a pH range of 5.0 to 9.0. Most ground waters in uncontaminated aquifers are within these ranges. Natural pH values may be as low as 4.0 or 5.0 in aquifers with active oxidation of sulfides, and pH values as high as 9.0 may be found in carbonate-buffered systems (9). pH values as low as 3.0 have been measured for ground waters contaminated with municipal

waste leachates, however, which often contain elevated concentrations of organic acids (10). In ground waters contaminated with sludges from cement manufacturing, pH values as high as 11.0 have been measured (9).

Redox Potential

The oxidation/reduction (redox) potential of ground water is a measure of electron activity that indicates the relative ability of a solution to accept or transfer electrons. Most redox reactions in the subsurface are microbially catalyzed during metabolism of native organic matter or contaminants. The only elements that are predominant participants in aquatic redox processes are carbon, nitrogen, oxygen, sulfur, iron, and manganese (11). The principal oxidizing agents in ground water are oxygen, nitrate, sulfate, manganese(IV), and iron(III).

Biological reactions in the subsurface both influence and are affected by the redox potential and the available electron acceptors. The redox potential changes with the predominant electron acceptor, with reducing conditions increasing through the sequence oxygen, nitrate, iron, sulfate, and carbonate. The redox potential decreases in each sequence, with methanogenic (carbonate as the electron acceptor) conditions being most reducing. The interpretation of redox potentials in ground water is difficult (12). The potential obtained in ground water is a mixed potential that reflects the potential of many reactions and cannot be used for quantitative interpretation (11). The approximate location of the contaminant plume can be identified in the field by measuring the redox potential of the ground water.

To overcome the limitations imposed by traditional redox measurements, recent work has focused on measuring molecular hydrogen to accurately describe the predominant in situ redox reactions (13-15). The evidence suggests that concentrations of H_2 in ground water can be correlated with specific microbial processes, and these concentrations can be used to identify zones of methanogenesis, sulfate reduction, and iron reduction in the subsurface (3).

Electron Acceptors

Measuring the available electron acceptors is a critical step in identifying the predominant microbial and geochemical processes occurring in situ at the time of sample collection. Nitrate and sulfate are found naturally in most ground waters and will subsequently be used as electron acceptors once oxygen is consumed. Oxidized forms of iron and manganese can be used as electron acceptors before sulfate reduction commences. Iron and manganese minerals solubilize coincidently with sulfate reduction, and their reduced forms scavenge oxygen to the extent that strict anaerobes (some sulfate reducers and all methanogens) can develop. Sulfate is found in many depositional environments, and sulfate

reduction may be very common in many contaminated ground waters. In environments where sulfate is depleted, carbonate becomes the electron acceptor, with methane gas produced as an end product.

Temperature

The temperature at all monitoring wells should be measured to determine when the pumped water has stabilized and is ready for collection. Below approximately 30 feet, the temperature in the subsurface is fairly consistent on an annual basis. Microcosms should be stored at the average in situ temperature. Biological growth can occur over a wide range of temperatures, although most microorganisms are active primarily between 10°C and 35°C (50°F to 95°F).

Chloride

Reductive dechlorination results in the accumulation of inorganic chloride. In aquifers with a low background of inorganic chloride, the concentration of inorganic chloride should increase as the chlorinated solvents degrade. The sum of the inorganic chloride plus the contaminant being degraded should remain relatively consistent along the ground-water flow path.

Tables 1 and 2 list the geochemical parameters, contaminants, and daughter products that should be measured during site characterization for natural attenuation. The tables include the analyses that should be performed, the optimum range for natural attenuation of chlorinated solvents, and the interpretation of the value in relation to biological processes.

Table 1.   Geochemical Parameters

Analysis	Range	Interpretation
Redox potential	< 50 mV against Ag/AgCl	Reductive pathway possible
Sulfate	< 20 mg/L	Competes at higher concentrations with reductive pathway
Nitrate	< 1 mg/L	Competes at higher concentrations with reductive pathway
Oxygen	< 0.5 mg/L	Tolerated; toxic to reductive pathway at higher concentrations
Oxygen	> 1 mg/L	Vinyl chloride oxidized
Iron(II)	> 1 mg/L	Reductive pathway possible
Sulfide	> 1 mg/L	Reductive pathway possible
Hydrogen	> 1 nM	Reductive pathway possible; vinyl chloride may accumulate
Hydrogen	< 1 nM	Vinyl chloride oxidized
pH	5 < pH < 9	Tolerated range

Table 2. Contaminants and Daughter Products

Analysis	Interpretation
PCE	Material spilled
TCE	Material spilled or daughter product of perchloroethylene
1,1,1-Trichloroethane	Material spilled
cis-DCE	Daughter product of trichloroethylene
trans-DCE	Daughter product of trichloroethylene
Vinyl chloride	Daughter product of dichloroethylene
Ethene	Daughter product of vinyl chloride
Ethane	Daughter product of ethene
Methane	Ultimate reductive daughter product
Chloride	Daughter product of organic chlorine
Carbon dioxide	Ultimate oxidative daughter product
Alkalinity	Results from interaction of carbon dioxide with aquifer minerals

Microcosm Construction

During construction of the microcosms, manipulations should take place in an anaerobic glovebox. These gloveboxes exclude oxygen and provide an environment in which the integrity of the core material may be maintained, since many strict anaerobic bacteria are sensitive to oxygen. Stringent aseptic precautions are not necessary for microcosm construction; maintaining the anaerobic conditions of the aquifer material and solutions added to the microcosm bottles is more important.

The microcosms should have approximately the same ratio of solids to water as the in situ aquifer material, with minimal or negligible headspace. Most bacteria in the subsurface are attached to the aquifer solids. If a microcosm has too much water and the contaminant is primarily in the dissolved phase, the bacteria must consume or transform a great deal more contaminant to produce the same relative change in the contaminant concentration. As a result, the kinetics of removal at field scale will be underestimated in the microcosms.

A minimum of three replicate microcosms for both living and control treatments should be constructed for each sampling event. Microcosms sacrificed at each sampling interval are preferable to microcosms that are repetitively sampled. The compounds of regulatory interest should be added at concentrations representative of the higher concentrations found in the geochemical region of the plume being evaluated, and should be added as concentrated aqueous solutions. If an aqueous solution is not feasible, dioxane or acetonitrile may be used as solvents. Carriers that can be metabolized anaerobically should be avoided, particularly alcohols. If possible, ground water from the site should be used to prepare

dosing solutions and to restore water lost from the core barrel during sample collection.

Although no method is perfect, autoclaving is the preferred sterilization method for long-term microcosm studies, and mercuric chloride is excellent for short-term studies (weeks or months). Mercuric chloride complexes to clays, however, and control may be lost as it is sorbed over time. Sodium azide is effective in repressing metabolism of bacteria that have cytochromes but is not effective on strict anaerobes.

The microcosms should be incubated in the dark at the ambient temperature of the aquifer. Preferably, the microcosms should be inverted in an anaerobic glovebox as they incubate; anaerobic jars are also available that maintain an oxygen-free environment. Dry redox indicator strips can be placed in the jars to ensure that anoxic conditions are maintained. If no anaerobic storage is available, the inverted microcosms can be immersed in approximately 2 inches of water during incubation. Teflon-lined butyl rubber septa are excellent for excluding oxygen and should be used if the microcosms must be stored outside an anaerobic environment.

The studies should last from 12 to 18 months. The residence time of a plume may be several years to tens of years at field scale. Rates of transformation that are slow in terms of laboratory experimentation may have a considerable environmental significance, and a microcosm study lasting only a few weeks to months may not have the resolution to detect slow changes that are of environmental significance. Additionally, microcosm studies often distinguish a pattern of sequential biodegradation of the contaminants of interest and their daughter products.

Microcosm Interpretation

As a practical matter, batch microcosms with an optimal solids/water ratio that are sampled every 2 months in triplicate for up to 18 months, can resolve biodegradation from abiotic losses with a detection limit of 0.001 to 0.0005 per day. Rates determined from replicated batch microcosms are found to more accurately duplicate field rates of natural attenuation than column studies. Many plumes show significant attenuation of contamination at field-calibrated rates that are slower than the detection limit of microcosms constructed with that aquifer material. Although rate constants for modeling purposes are more appropriately acquired from field-scale studies, agreement between rates in the field and rates in the laboratory is reassuring.

The rates measured in the microcosm study may be faster than the estimated field rate. This may not be due to an error in the laboratory study, particularly if estimation of the field-scale rate of attenuation did not account for regions of preferential flow in the aquifer. The regions

of preferential flow may be determined using a down-hole flow meter or a geoprobe method for determining hydraulic conductivity in 1- to 2-foot sections of the aquifer.

Statistical comparisons can determine whether removals of contaminants of concern in the living treatments are significantly different from zero or significantly different from any sorption that is occurring. Comparisons are made on the first-order rate of removal, that is, the slope of a linear regression of the natural logarithm of the concentration remaining against time of incubation for both the living and control microcosm. These slopes (removal rates) are compared to determine whether they are different and, if so, the extent of the difference that can be detected at a given level of confidence

The Tibbetts Road Case Study

The Tibbetts Road Superfund site in Barrington, New Hampshire, a former private home, was used to store drums of various chemicals from 1944 to 1984. The primary ground-water contaminants in the overburden and bedrock aquifers were TCE and benzene, with respective concentrations of 7,800 µg/L and 1,100 µg/L.

High concentrations of arsenic, chromium, nickel, and lead were also found.

Material collected at the site was used to construct a microcosm study evaluating the removal of benzene, toluene, and TCE. This material was acquired from the waste pile near the origin of Segment A (Figure 1), the most contaminated source at the site. Microcosms were incubated for 9 months. The aquifer material was added to 20-milliliter headspace vials; dosed with 1 milliliter of spiking solution; capped with a Teflon-lined, gray butyl rubber septa; and sealed with an aluminum crimp cap. Controls were prepared by autoclaving the material used to construct the microcosms overnight. Initial concentrations for benzene, toluene, and TCE were 380 µg/L, 450 µg/L, and 330 µg/L, respectively. The microcosms were thoroughly mixed by vortexing, then stored inverted in the dark at the ambient temperature of 10°C.

The results (Figures 2 through 4 and Table 3) show that significant biodegradation of both petroleum aromatic hydrocarbons and the chlorinated solvent had occurred. Significant removal in the control microcosms also occurred for all compounds. The data exhibited more variability
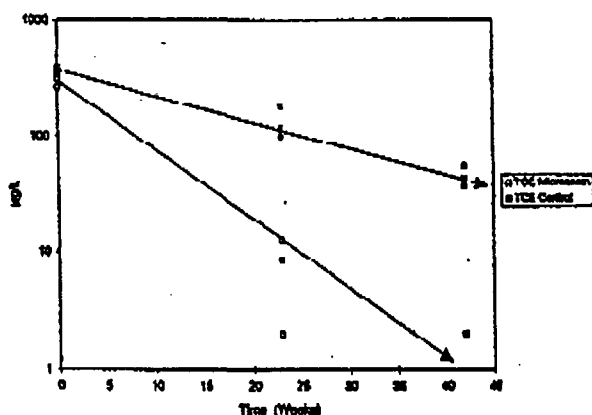

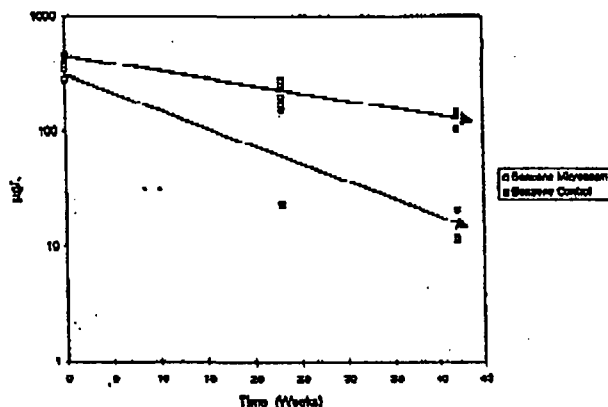Figure 1. TCE concentrations in the Tibbetts Road microcosm study.

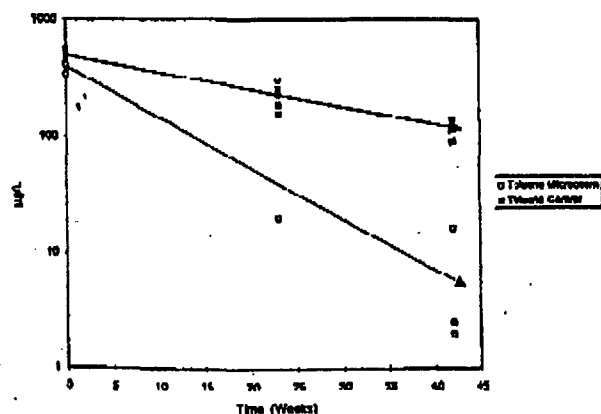Figure 2. Benzene concentrations in the Tibbetts Road microcosm study.

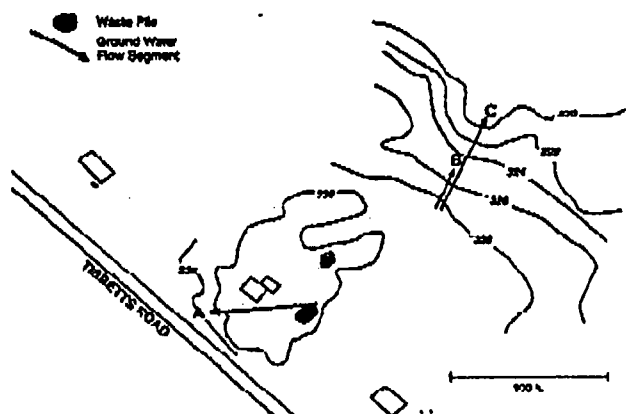Figure 3. Toluene concentrations in the Tibbetts Road microcosm study.

Figure 4. Location of waste piles and flow path segments at the Tibbetts Road Superfund site.

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Table 3. Concentrations of TCE, Benzene, and Toluene in the Tibbetts Road Microcosms

Compound	Time Zero Microcosms	Time Zero Controls	Week 23 Microcosms	Week 23 Controls	Week 42 Microcosms	Week 42 Controls
TCE	328	337	1	180	2	36.3
	261	394	12.5	116	2	54.5
	309	367	8.46	99.9	2	42.3
Mean ± standard deviation	299 ± 34.5	366 ± 28.5	7.32 ± 5.83	132 ± 42.4	2.0 ± 0.0	44.4 ± 9.27
Benzene	366	396	201	236	11.1	148
	280	482	276	180	20.5	105
	340	433	22.8	152	11.6	139
Mean ± standard deviation	329 ± 44.1	430 ± 33.1	167 ± 130	189 ± 42.8	14.4 ± 5.29	130 ± 21.9
Toluene	443	460	228	254	2	136
	342	557	304	185	2.5	92
	411	502	19.9	157	10.6	115
Mean ± standard deviation	399 ± 51.6	506 ± 48.6	184 + 147	199 + 49.9	7.03 ± 8.29	114 ± 22.0

in the living microcosms than in the control treatment, a pattern that has been observed in other microcosm studies. The removals observed in the controls are probably due to sorption; however, this study exhibited more sorption than typically seen.

The rate constants determined from the microcosm study for the three compounds are shown in Table 4. The appropriate rate constant to be used in a model or a risk assessment would be the first-order removal in the living treatment minus the first-order removal in the control. In other words, the removal that is in excess of the removal in the controls.

The first-order removal in the living and control microcosms was estimated as the linear regression of the natural logarithm of concentration remaining in each microcosm in each treatment against time of incubation. Student's t distribution with n - 2 degrees of freedom was used to estimate the 95 percent confidence interval. The standard error of the difference of the rates of removal in living and control microcosms was estimated as the

square root of the sum of the squares of the standard errors of the living and control microcosms, with n - 4 degrees of freedom (16).

Table 5 presents the concentrations of organic compounds and their metabolic products in monitoring wells used to define line segments in the aquifer for estimation of field-scale rate constants. Wells in this aquifer showed little accumulation of trans-DCE, 1,1-DCE, vinyl chloride, or ethene, although removals of TCE and cis-DCE were extensive. This can be explained by the observation that iron-reducing bacteria can rapidly oxidize vinyl chloride to carbon dioxide (4). Filterable iron accumulated in ground water in this aquifer.

The extent of attenuation from well to well (Table 5) and the travel time between wells in a segment (Figure 4) were used to calculate first-order rate constants for each segment (Table 6). Travel time between monitoring wells was calculated from site-specific estimates of hydraulic conductivity and from the hydraulic gradient. In the area sampled for the microcosm study, the estimated Darcy

Table 4. First-Order Rate Constants for Removal of TCE, Benzene, and Toluene in the Tibbetts Road Microcosms

Parameter	Living Microcosms	Autoclaved Controls	Removal Above Controls
	First-Order Rate of Removal (per year)		
TCE	6.31	2.62	3.69
95% confidence interval	± 2.50	± 0.50	± 2.31
Minimum rate significant at 95% confidence			1.38
Benzene	3.87	1.51	2.36
95% confidence interval	± 1.96	± 0.44	± 1.83
Minimum rate significant at 95% confidence			0.53
Toluene	5.49	1.86	3.63
95% confidence interval	± 2.87	± 0.45	± 2.64
Minimum rate significant at 95% confidence			0.99

Table 5. Concentration of Contaminants and Metabolic Byproducts in Monitoring Wells Along Segments In the Plume Used To Estimate Field-Scale Rate Constants

Parameter	Segment A		Segment B		Segment C	
Monitoring well	80S	79S	70S	52S	70S	53S
	Upgradient g/L	Downgradient g/L	Upgradient g/L	Downgradient g/L	Upgradient g/L	Downgradient g/L
TCE	200	13.7	710	67	710	3.1
cis-DCE	740	10.9	220	270	220	2.9
trans-DCE	0.41	< 1	0.8	0.3	0.8	< 1
1, 1-DCE	0.99	< 1	< 1	1.6	< 1	< 1
Vinyl Chloride	< 1	< 1	< 1	< 1	< 1	< 1
Ethene	< 4	< 4	7	< 4	7	< 4
Benzene	510	2.5	493	420	493	< 1
Toluene	10,000	< 1	3,850	800	3,850	< 1
o-Xylene	1,400	8.4	240	71	240	< 1
m-Xylene	2,600	< 1	360	59	360	< 1
p-Xylene	1,400	22	1,100	320	1,100	< 1
Ethylbenzene	1,300	0.7	760	310	760	< 1
Methane	353	77	8	3	8	< 2
Iron						27,000

Table 6. First-Order Rate Constants in Segments of the Tibbetts Road Plume

Flow Path Segments In Length and Time of Ground-Water Travel

Compound	Segment A 130 feet = 6.4 years	Segment B 80 feet = 2.4 years	Segment C 200 feet = 10 years
	First-Order Rate Constants in Segments (per year)		
TCE	0.41	0.59	0.54
cis-DCE	0.65	Produced	0.43
Benzene	0.82	0.04	> 0.62
Toluene	> 1.42	0.30	> 0.83
o-Xylene	0.79	0.30	> 0.55
m-Xylene	> 1.20	0.45	> 0.59
p-Xylene	0.64	0.31	> 0.70
Ethylbenzene	1.16	0.22	> 0.66

flow was 2.0 feet per year. With an estimated porosity in this particular glacial till of 0.1, this corresponds to a plume velocity of 20 feet per year.

Summary

Table 7 compares the first-order rate constants estimated from the microcosm studies with the rate constants estimated at field scale. The agreement between the independent estimates of rate is good, indicating that the rates can appropriately be used in a risk assessment. The rates of biodegradation documented in the microcosm study could easily account for the disappearance of TCE, trichloroethylene, benzene, and toluene observed at field scale. The rates estimated from the microcosm study are several-fold higher than the rates estimated at field scale, which may reflect an underestimation of the true rate in the field. The estimates of plume velocity assumed that the aquifer was homogeneous. No attempt was made in this study to correct the estimate of plume velocity for

Table 7. Comparison of First-Order Rate Constants in a Microcosm Study and in the Field at the Tibbetts Road NPL Site

Parameter	Microcosms Corrected for Controls		Field Scale		
	Average Rate	Minimum Rate Significant at 95% Confidence	Segment A	Segment B	Segment C
	First-Order Rate (per year)				
Trichloroethylene	3.69	1.38	0.41	0.59	0.54
Benzene	2.36	0.53	0.82	0.04	> 0.62
Toluene	3.63	0.99	> 1.42	0.36	> 0.83

the influence of preferential flow paths. Preferential flow paths with a higher hydraulic conductivity than average would result in a faster velocity of the plume, thus a lower residence time and faster rate of removal at field scale.

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