Technical Protocol for Evaluating the Natural Attenuation of MtBE

Regulatory and Scientific Affairs Department

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EXECUTIVE SUMMARY

Methyl tert-butyl ether (MtBE) has been produced commercially in the United States since 1979 and has been used as both an octane enhancer and oxygenate in gasoline. Over the last few years, MtBE-oxygenated gasoline has been phased-out of most US markets, though it continues to be used in various international markets. Releases of MtBE-containing gasoline have led to the detection of MtBE in soil, surface water, and groundwater. MtBE may be removed from environmental media by some of the active or passive strategies typically employed for gasoline remediation, including Monitored Natural Attenuation (MNA).

The objective of this protocol is to provide a framework for incorporating MtBE (and other oxygenates or degradation byproducts e.g., tert-butyl alcohol) MNA into an overall site remediation strategy at any site where these compounds have been released to the subsurface. The fundamental approaches for MNA were documented by EPA and ASTM beginning in the late-1990s. This protocol follows these approaches in principle and includes specific data needs and evaluation flowcharts for selecting and implementing an MNA strategy for MtBE.

This technical protocol addresses data collection, evaluation, and interpretation procedures that consider the physical, chemical and biological properties of MtBE and other oxygenates and degradation byproducts. A tiered approach is provided that can be used by stakeholders to interpret several lines of evidence to evaluate natural attenuation on a site-specific basis. Several resources are provided to support an MNA evaluation, including:

- a review of basic scientific principles relevant to the evaluation of MtBE natural attenuation, including biodegradation and physicochemical attenuation mechanisms;
- a discussion of data that can be used to assess MtBE (and other oxygenates or degradation byproducts) natural attenuation;
- technical references for relevant chemical properties, analytical methods, and field sampling techniques;
- guidance for data quality assurance and interpretation, including statistical analysis; and
- guidance on the presentation of natural attenuation data/information to facilitate regulatory and other stakeholder review and acceptance of MNA remedies.

1 INTRODUCTION

1.1 Purpose and Objectives of Document

Releases of MtBE-containing gasoline have led to the detection of MtBE in soil, surface water, and groundwater. Methyl tert-butyl ether may be removed from environmental media by some of the active or passive strategies typically employed for gasoline remediation, including Monitored Natural Attenuation (MNA). However, based upon the physical and chemical properties of MtBE, the data needs and evaluation procedures for selecting and implementing an MNA strategy for MtBE differ from those typically used for other gasoline constituents. This technical protocol addresses data collection, evaluation, and interpretation procedures that consider the properties of MtBE when evaluating natural attenuation.

Natural attenuation refers to all biological and abiotic processes that dilute, remove, degrade, or detoxify chemicals in the environment (USEPA, 1999 and 2001). Natural attenuation has become an accepted strategy for many classes of chemicals provided that risks can be managed effectively and the remedial timeframe is comparable to other practicable alternatives. Evaluation of the performance of natural attenuation strategies relies upon monitoring networks that can quantify changes in chemical concentration and/or mass and related geochemistry and hydrology that influence, or are products of, attenuation processes. This remedial approach is often referred to as MNA.

This protocol provides guidance to those interested in assessing MtBE natural attenuation, and those with the responsibility of reviewing such work. This manual is designed to:

- Present the basic scientific principles relevant to the evaluation of MtBE natural attenuation;
- Develop a framework for assessing the feasibility of incorporating MtBE natural attenuation into an overall site strategy;
- Identify those data that can be used to assess MtBE natural attenuation;
- Provide a concise technical reference for relevant chemical properties, analytical methods, and field sampling techniques;
- Provide protocols and guidance for data interpretation; and
- Provide guidance on the presentation of natural attenuation data/information to facilitate regulatory and other stakeholder review and acceptance of MNA remedies.

This protocol is not a prescriptive manual to be followed step by step for all sites with MtBE. Rather, the protocol helps stakeholders identify and quantify attenuation mechanisms and assess whether these mechanisms provide for sufficient MtBE natural

attenuation in a particular environment. This protocol may be used at any stage of a site evaluation, from site discovery to remedy re-evaluation.

The material presented in this protocol is intended to support work plan development, site investigation, data analysis, and decision making for regulators, site managers, and practitioners. The remainder of this document is organized as follows:

- The remainder of Section 1 summarizes the regulatory status of MNA for MtBE and discusses attenuation mechanisms specific to MtBE;
- Section 2 presents a tiered approach that can be used by stakeholders to interpret several lines of evidence to evaluate natural attenuation on a site-specific basis;
- Section 3 provides methods for field data collection and chemical analysis; and
- Section 4 describes data evaluation and presentation methods for the three tiers of data.

1.2 Regulatory Status of MNA of MtBE

Monitored Natural Attenuation as a tool for the remediation of petroleum hydrocarbons has reached wide-spread acceptance among State and Federal regulators since the late 1990s (ASTM, 1998; USEPA, 1998 and 2001; Wiedemeier and Chapelle, 2000). The stability of benzene, toluene, ethylbenzene, and xylene (BTEX) plumes at sites with a continuing primary source of contamination, or the rapid shrinkage of these plumes, especially following source removal (only secondary source [as defined in Section 2.1.2] remaining), has provided empirical evidence that MNA is "working" at many sites. State acceptance of MNA for MtBE, however, has lagged, largely because of the perception that MtBE is resistant to biodegradation as well as the relative scarcity of site data on the attenuation of MtBE in groundwater. However, as data showing biodegradation and effective attenuation of MtBE at multiple sites have been developed, many regulators have become willing to accept this strategy for MtBE on a site-by-site basis.

In the mid-1990s, numerous hydrocarbon plume studies contributed to our understanding of natural attenuation. Surveys of gasoline plumes by such groups as the Lawrence Livermore National Laboratory (Happel et al., 1998; Rice et al., 1995), the Texas Bureau of Geology (Mace et al., 1997), and Chevron Research and Technology Company (Buscheck and O'Reilly, 1995) showed that plumes of benzene from gasoline sites were generally small (less than 250 feet), with significant decreases in concentrations occurring over time even in the absence of active remediation. The development of guidance for implementation of MNA for BTEX prepared by government, industry, and others provided consensus and direction for development of MNA strategies for BTEX, as discussed in Section 2.1.1 (ASTM, 1998; Buscheck and O'Reilly, 1995, 1997; USEPA, 1998 and 1999; Wiedemeier and Chapelle, 2000; Wiedemeier et al., 1995).

Early surveys of MtBE plumes were hampered by limited historical data. Methyl tert-butyl ether was rarely included as an analyte at petroleum sites prior to the late 1990s, when several studies were published regarding the behavior of MtBE plumes (Happel et al., 1998; IST, 1999; USEPA, 1998 and 1999). Since that time, evidence of natural attenuation and biodegradation of MtBE has been observed at multiple field sites (Wilson et al., 2000) and guidance and directives issued by the Environmental Protection Agency (EPA) have broadened the regulatory definition of MNA (USEPA, 1999). Researchers in academia, government, and industry have provided proof of MtBE biodegradation by many naturally occurring aerobic and anaerobic organisms and have elucidated the aerobic metabolic pathways, as discussed in Appendix A. These data provide scientific evidence that is also helping to promote regulatory acceptance of MNA strategies for MtBE.

The next step in the process of regulatory acceptance is developing MNA protocols, such as this one, specifically to address the unique physical, chemical, and biological properties of MtBE that differentiate this compound from BTEX and other chemicals.

1.3 Anticipating and Addressing Stakeholder Concerns

A large body of scientific evidence supports the occurrence of natural attenuation as a protective remedial alternative for many contaminants at many sites. Monitored Natural Attenuation has been applied to thousands, if not tens of thousands of sites, across the country and around the world. Nevertheless, at many sites where the community is aware of a contaminant plume, considerable skepticism is voiced, even though natural attenuation is clearly working to remediate the plume and is protective of human health and the environment. In many cases, the public may perceive natural attenuation as an alternative that allows responsible parties to save money while exposing the public to continued risk or a reduction in property values. Because of the skepticism often exhibited by the public, more extensive outreach efforts are usually required when evaluating and, where appropriate, implementing MNA. Thus, efforts should be made to educate the public to the protectiveness of MNA where it is appropriate. Based on the experience of the authors, stakeholders often indicate a greater willingness to accept MNA if it can be shown that contaminants are being transformed to innocuous byproducts and not simply being diluted or transferred to another environmental medium. Recent laboratory and field data show that MtBE biodegradation occurs under aerobic and a range of anaerobic conditions. Thus, biodegradation could be the dominant mechanism working to reduce MtBE concentration in groundwater. Regardless of the efficacy of biodegradation, however, MNA can be protective of receptors.

1.4 MtBE Attenuation State-of-the-Science

Natural attenuation of MtBE results from several physical, chemical, and biological mechanisms. In general, the same physio-chemical mechanisms that affect other

chemicals dissolved in groundwater, including mechanical dispersion, diffusion, sorption, groundwater recharge, volatilization, and plant-mediated uptake, work to decrease the concentration of MtBE. Pathways for aerobic biodegradation of MtBE have been recently elucidated and MtBE biodegradation via this pathway has been documented at numerous field sites. Anaerobic biodegradation of MtBE has also been documented, although the specific pathways are less well understood.

The physio-chemical and biological processes that are especially dependent upon MtBE's unique properties are discussed briefly below. More detailed discussion of MtBE biodegradation and a summary of physio-chemical attenuation mechanisms are included in Appendices A and B, respectively.

1.4.1 Physio-Chemical Mechanisms of MtBE Attenuation

The physical and chemical properties of MtBE and common co-contaminants are listed in Table 1-1. A summary of physio-chemical attenuation mechanisms that act on solutes is provided in Appendix B. The attenuation mechanisms identified below include those that may act to a greater or lesser extent for MtBE than for other petroleum hydrocarbon compounds due to the unique properties of MtBE.

1.4.1.1 Sorption

The extent of attenuation of MtBE due to sorption is expected to be limited in most aquifers due to its high aqueous solubility and low organic carbon partitioning coefficient. Methyl tert-butyl ether may bind effectively to soil or sediments that have a high fraction of organic carbon such as some clays and wetland soils and sediments. However, MtBE sorption to organic matter is relatively weak and a less important attenuation mechanism for MtBE than for other fuel components such as BTEX compounds (Squillace et al., 1997).

Table 1-1 Physical Properties of MtBE and Other Constituents of Gasoline

		Compound							
		Methyl Tert-Butyl Ether (MTBE)	**Tert-Butyl Alcohol (TBA)**	**Ethyl Tert-Butyl Ether (ETBE)**	**Tert-Amyl Methyl Ether (TAME)**	**Tert-Amyl Alcohol (TAA)**	**Ethanol**	**Benzene**	**Di-isopropyl Ether (DIPE)**
		CAS Number							
		1634-04-4	75-65-0	637-92-3	994-05-8 (Methyl Tert-Pentyl Ether)	75-85-4 (Tert-Pentyl Alcohol)	64-17-5 (Ethyl Alcohol)	71-43-2	108-20-3 (Isopropyl Ether)
Property	**Unit**								
Molecular Mass	g/mol	88.15 (7)	74.12 (7)	102.17 (7)	102.17 (7)	88.15 (7)	46.07 (7)	78.11 (7)	102.17 (7)
Boiling Temperature	°C	55.2 (7)	82.41 (7)	69 to 73 (7)	86.3 (7)	102.5 (7)	78.5 (7)	80.1 (1, 7)	68.27 (7)
Melting Point	°C	-109 (1, 7)	25.6 (2, 7)	-94 (3, 7)	--	-11.9 (2)	-114.1 to -115.5 (2, 7)	5.53 (1, 7)	-85.5 to -85.89 (1, 7)
Specific Gravity	Dimensionless (@ 20°C)	0.74 (7)	0.79 (7)	0.74 (7)	0.77 (7)	0.808 (7)	0.789 (7)	0.879 (7)	0.728 (7)
Water Solubility	mg/l	43,000 to 54,300 (6, 7, 11)	Miscible[1] (6, 7, 11)	12,000 (7) 26,000 (6)	11,500 (7) 20,000 (6)	125,000 (7)	Miscible (6, 7)	1,650 to 1,780 (1, 6, 7, 9)	12,400 (7) 2,039 to 9,000 (6)
Vapor Pressure	mm Hg (@ 25°C unless noted)	245 to 256 (6, 7)	31 (12) 40 to 42 (6)	131 (7) 152 (6)	75.0 (7) 68.3 (6)	90.0	49 to 56.5 (6)	75 to 95.3 (1, 6, 9, 10)	149 to 151 (6) 158 (7)
Log K_{OC}	--	1.0 to 1.1 (6)	1.57 (6)	1.0 to 2.2 (6)	1.3 to 2.2 (6)	0.9	0.20 to 1.21 (6)	1.5 to 2.2 (6, 10)	1.46 to 1.82 (6)
Henry's Law Constant	Dimensionless (@ 20°C)	0.023 to 0.12 (6)	4.8×10^{-4} to 5.9×10^{-4} (6)	1.1×10^{-1} (6)	5.2×10^{-2} (6)	5.7×10^{-4}	2.1×10^{-4} to 5.9×10^{-4} (6)	2.2×10^{-1} (6)	0.195 to 0.41 (6)
Henry's Law Constant	atm· m^3/mol (@ 20°C)	5.5×10^{-4} to 2.9×10^{-3} (calculated)	1.2×10^{-5} to 1.4×10^{-5} (calculated)	2.6×10^{-3} (calculated)	1.3×10^{-3} (calculated)	1.4×10^{-5} (calculated)	5.0×10^{-6} to 1.4×10^{-5} (calculated)	5.4×10^{-3} (1)	4.7×10^{-3} to 9.9×10^{-3} (calculated)
Fuel-Water Partitioning Coefficient (12)	--	16 (5)	0.24 (5)	--	--	--	--	Log K_{ow} = 1.56 to 2.13 (8, 9, 10)	--

(1) Mackay et al., 1992, (2)Verschuren, 1996, (3) Chemsoft, 1999, (4) Squillace et al., 1997, (5) Schmidt et al., 2004, (6) Nichols et al., 2000, (7) Merck Index, 2001, (8) Schwarzenback et al., 1993, (9) Schwarzenback et al., 2004, (10) Mongomery and Welkom, 1990, (11) www.chemfinder.com, (12) Approximate, varies with fuel type.

[1] Some references report a solubility limit for TBA (MacKay et al., 1995). TBA solubility is highly non-ideal, especially near its melting point temperature.

1.4.1.2 Volatilization

Ethers such as MtBE generally have high vapor pressures but low Henry's law constants (Table 1-1) (Squillace et al., 1997; Nichols et al., 2000). These properties mean that MtBE will readily volatilize from non-aqueous phase liquid but will tend to remain in soil moisture and groundwater. The importance of volatilization as an attenuation mechanism for MtBE is therefore dependant primarily on the distribution of residual NAPL above and below the capillary zone. The rate of volatilization from the source depends further on soil permeability and the distribution of soil moisture within the unsaturated zone, which will retard vapor migration from the source. At sites where a substantial portion of the source remains above the capillary zone, surficial soils are permeable, and infiltration rates are low, source attenuation by volatilization may exceed the rate of mass loading of MtBE to groundwater from the source (Lahvis et al., 2004).

1.4.1.3 Plant Uptake

Because it is highly soluble in water, MtBE is readily taken up by several species of plants and trees within the active root zone (Zhang et al., 2001; Newman and Arnold, 2003). Although plants are biological systems, plant-mediated processes that attenuate MtBE are often primarily physical: the plants transport MtBE in water via the root system and then either sequester the MtBE in the plant material or transpire it to the atmosphere. It is also possible that MtBE is transformed within some plants (Newman and Arnold, 2003).

1.4.1.4 Abiotic Degradation

MtBE can undergo abiotic degradation through both oxidation and hydrolysis. In groundwater, these processes are expected to be of relatively minor importance because strong oxidants like peroxide are necessary, and hydrolysis is slow and acid-catalyzed. In the gaseous phase (Acero et al., 2001; O'Reilly et al., 2001), MtBE may be transformed by several abiotic chemical attenuation mechanisms. Gaseous-phase MtBE is reported to react with several atmospheric ions and is also subject to photolysis (Guillard et al., 2003; Japar et al., 1991; Wallington et al., 1988).

1.4.2 MtBE Biodegradation

MtBE and its intermediate degradation product, tert-butyl alcohol (TBA), have been reported to biodegrade in both *in situ* and ex-situ studies, under a wide range of aerobic and anaerobic geochemical conditions and examples of MtBE biodegradation are geographically widespread (Bradley et al., 2001). MtBE has been shown to biodegrade in natural soils and aquifer sediments under aerobic conditions (Bradley et al., 1999 and 2001; Salanitro et al., 2000; Hunkeler et al., 2001; Kane et al., 2001; Gray et al., 2002; Wilson et al., 2002; DeVaull et al., 2003; Schirmer et al., 2003); in anaerobic conditions, either with unspecified natural electron acceptors present or amendments, including

nitrate, iron III, or sulfate (Bradley et al., 2001; Finneran and Lovley, 2001; DeVaull et al., 2003; Wilson, et al., 2005a, 2005b); and in methanogenic conditions (Yeh and Novak, 1994; Wilson et al., 2000; DeVaull et al., 2003; Wilson et al., 2005b). TBA has been shown to degrade in natural soils and aquifer sediments under aerobic conditions (Novak, et al., 1985; Bradley et al., 1999; Hunkeler, et al., 2001; Kane, et al., 2001; Wilson, et al., 2002); or under anaerobic conditions with unspecified electron acceptors present (Novak, et al., 1985; Yeh and Novak, 1994; DeVaull et al., 2004; Wilson, et al., 2005b). Confirmed evidence for TBA biodegradation in highly reduced methanogenic conditions is lacking.

Biodegradation of MtBE may occur due to direct metabolism in which organisms directly derive energy from MtBE, or in co-metabolism with another chemical substrate. In direct aerobic metabolism, isolated microbial organisms have been observed to grow at a relatively slow rate (Deeb, et al., 2000). This slow rate of growth means that an initially low population of organisms capable of degrading MtBE may require a relatively long lag time (or acclimation time) before the exposed biomass has grown to a significant population for which a measurable rate of MtBE biodegradation can be observed.

We can infer that observed lag times in the environment may also be relatively long, depending on the initial *in situ* presence, population, and distribution of a biomass capable of degrading MtBE, as well as geochemical conditions and local MtBE concentrations. The time required for an adapted, natural, *in situ* MtBE-degrading biomass to adapt and grow to a population significant enough to show observed attenuation of MtBE can be up to many months in duration, following initial soil or groundwater exposure to MtBE (DeVaull, 2005; Wilson et al., 2005). In contrast, for BTEX chemicals, biomass adaptation times in soil can be on the order of hours to days. Once acclimation has occurred in the field, observed degradation rates for MtBE appear to be within the low end of the range of rates observed for BTEX degradation in groundwater plumes.

A detailed summary of biodegradation mechanisms for MtBE is provided in Appendix A.

2 DEVELOPING A NATURAL ATTENUATION EVALUATION STRATEGY

This section provides an overview of MNA as a remediation tool, including an overview of existing guidance, a quick review of the characteristics of sites where natural attenuation may or may not be appropriate, a tiered approach for evaluating natural attenuation, including a decision tree/stepwise approach for using the tiers, and a discussion of mass flux estimates.

2.1 Overview of MNA as a Remediation Tool

Natural attenuation of organic compounds occurs to some extent in all geologic and hydrogeologic environments, under both saturated and unsaturated conditions. Over the past decade, MNA has gained increasing regulatory acceptance as a remedial alternative and is now being applied to all or part of many sites with solute plumes.

2.1.1 Overview of Existing MNA Guidance

Over the past ten years, several protocols have been developed that guide the user through an evaluation of MNA. Table 2-1 presents a list of existing protocols developed to evaluate natural attenuation of various chemicals. With the exception of the Department of Energy document (Brady et al., 1998) which includes inorganics, these protocols only address petroleum hydrocarbons and chlorinated solvents. MtBE has been addressed in only one of these documents. All of the protocols are similar in that they rely on several types of data to evaluate the efficacy of natural attenuation as a remedial alternative. These data have typically been arranged into “lines” or “tiers” of evidence. Approaches for evaluating natural attenuation generally consist of using some combination of the following data:

- time series and spatial data to evaluate plume stability (often referred to as the “first” or “primary” line of evidence or Tier 1 analysis);
- evaluation of the geochemical environment and evidence of transformation products of degradation to help elucidate degradation mechanisms (often referred to as the “second” line of evidence or Tier 2 analysis); and
- microbiological or other laboratory data (often referred to as the “third” line of evidence, the Tier 3 analysis or as an “optional” line of evidence).

This document presents a tiered approach for evaluating the natural attenuation of MtBE. For many petroleum hydrocarbon compounds, the first and second tiers of data typically

are sufficient to evaluate natural attenuation. The geochemical footprint[2] that emerges from the analysis of Tier 2 data can result from hydrocarbon biodegradation and does not provide conclusive evidence that a particular terminal electron-accepting process (TEAP) is contributing to MtBE mass loss. Therefore, Tier 3 data may be needed to evaluate natural attenuation of MtBE at some sites. As the geochemical conditions for MtBE biodegradation become better understood over a range of site conditions, the analysis of Tier 3 data may become less important.

[2] The Geochemical Footprints of a plume is the area within which groundwater geochemistry is altered due to the release reflecting the activity of one or more physical, chemical, or biological process. Within a hydrocarbon plume the footprint is typically defined by a the sequential reduction of various electron acceptors due to the metabolism of added organic carbon. Section 4 discusses the nature of geochemical footprints in greater detail.

Table 2-1 Technical Protocols and Other Guidance Documents for Evaluating the Efficiency of Natural Attenuation

Organization	Chemicals	Title	Reference
AFCEE	Petroleum Hydrocarbons	Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Groundwater	Wiedemeier et al., 1995
AFCEE	Chlorinated Solvents	Technical Protocol for Evaluating the Natural Attenuation of Chlorinated Solvents in Groundwater	Wiedemeier et al., 1998
Amoco Oil Corporation	Petroleum Hydrocarbons	Natural Attenuation as a Remedial Alternative-- Technical Guidance	Amoco, 1995
ASTM	Petroleum Hydrocarbons	Guide for Remediation by Natural Attenuation at Petroleum Release Sites	ASTM, 1998
Department of Energy	Inorganic and organic contaminants	Draft Site Screening and Technical Guidance for Monitored Natural Attenuation at DOE Sites	Brady et al., 1998
Chevron Research and Technology Company	Petroleum Hydrocarbons	Protocol for Monitoring Intrinsic Bioremediation in Groundwater	Buscheck and O'Reilly, 1995
Chevron Research and Technology Company	Chlorinated Solvents	Protocol for Monitoring Natural Attenuation of Chlorinated Solvents in Groundwater	Buscheck and O'Reilly, 1997
Minnesota Pollution Control Agency	Chlorinated Solvents	Draft Guidelines-- Natural Attenuation of Chlorinated Solvents in Groundwater	MPCA, 1997
Mobile Oil Corporation	Petroleum Hydrocarbons	A Practical Approach To Evaluating Intrinsic Bioremediation Of Petroleum Hydrocarbons In Groundwater	Mobil, 1995
Netherlands Centre for Soil Quality Management and Knowledge Transfer	Chlorinated Solvents	SV-513 Protocol for the Determination of the Sustainability of the Natural Attenuation (S-NA) of Chlorinated Ethenes	Dijkhuis et al., 2003
New Jersey	Does Not Specify	Technical Requirements for Site Remediation and Classification Exception Areas: Final Guidance	NJDEP, 1995
RTDF	Chlorinated Solvents	Natural Attenuation of Chlorinated Solvents in Groundwater - Principles and Practices	RTDF, 1997
USEPA, Region 4	Chlorinated Solvents	Suggested Practices for Evaluation of a Site for Natural Attenuation of Chlorinated Solvents	USEPA, 1997
USEPA	Chlorinated Solvents	Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater	Wiedemeier et al., 1998
USEPA	Inorganic and organic contaminants	Use of Monitored Natural Attenuation at Superfund, RCRA Corrective Action, and Underground Storage Tank Sites	USEPA, 1999
US Navy	Petroleum Hydrocarbons and Chlorinated Solvents	Technical Guidelines for Evaluating Monitored Natural Attenuation of Petroleum Hydrocarbons and Chlorinated Solvents in Groundwater at Naval and Marine Corps Facilities	Wiedemeier and Chapelle, 2000
USEPA	MTBE	Monitored Natural Attenuation of MTBE as a Risk Management Option at Leaking Underground Storage Tank Sites	Wilson et al., 2005a.

Table 2-2 Summary of the Lines of Evidence Used to Evaluate Natural Attenuation and Enhanced Remediation

Tier	Parameters	Data Requirements	Applicability/Comments
Tier 1	Spatial and Temporal Trends in Contaminant Concentrations (and Mass-in-place if data are sufficient)	Hydrogeologic and historical contaminant data presented in Table 2-3.	Primary Line of Evidence. Older sites with good historical data; newer sites with limited historical data may require additional data collection.
Tier 2	Geochemical Data	Groundwater data evaluated using Table 2-4.	Secondary Line of Evidence. Use to evaluate geochemical environments within the geochemical footprint of the plume.
Tier 3	Various Supplemental Data	Compound-Specific Stable Isotope Analysis. Laboratory Microcosm Studies.	Supplemental Line(s) of Evidence. May be useful at sites where the predominant degradation mechanism(s) is (are) not readily apparent.

Table 2-3 Tier 1 Data

Analysis*	Method	Data Use	Comments
Groundwater Data			
MTBE	Environmental Sampling	Used to determine concentrations of MtBE and rates of attenuation.	Selection of analytical method requires consideration of DQOs, as discussed in Section 3.
TBA	Environmental Sampling	Depending on whether TBA has been released at the site, its presence or its changing ratio to MTBE can be used to assess presence of transformation compounds.	Selection of analytical method discussed in Section 3.
Soil Data			
MTBE	Environmental Sampling	Estimation of residual contaminant mass and source strength.	
Total Organic Carbon	Environmental Sampling	Sorption/solute retardation calculations.	Procedure must be accurate over the range of 0.1–5 percent TOC.
Bulk Density	Environmental Sampling	Sorption/solute retardation calculations.	In many cases, especially when dealing with unconsolidated sediments, bulk density can be adequately estimated using literature values.
Aquifer			
Hydraulic Gradient	Determine from site potentiometric surface maps.	Estimation of seepage velocity. Required for groundwater flow and solute transport models.	At least three measurement points required.
Hydraulic Conductivity	Slug tests or pumping tests.	Identification of primary flow pathways and seepage velocity. Required for groundwater flow and solute transport models.	Critical parameter with the potential for the most measurement error. Sensitivity analyses on this parameter may be useful when estimating seepage velocity.
Total and Effective Porosity	Tracer tests or estimates from literature values.	Estimation of seepage velocity. Required for groundwater flow and solute transport models.	Literature values typically are used.
* Where appropriate, samples of NAPL should also be analyzed**.**			

Table 2-4 Tier 3 Data

Analysis	Method	Data Use	Comments
Compound – Specific Isotope Analysis	Specialty analysis for determining $\delta^{13}C$ and $\delta^{2}H$ of MtBE.	Demonstrating MTBE biodegradation and estimating the extent of biodegradation.	Specialized laboratory analyses required.
Microcosm Studies	Specialized depending on the data needed.	Determine site-specific biodegradation processes under defined laboratory conditions.	May be anaerobic or aerobic; may utilize pure cultures or MTBE-contaminated material.

2.1.2 Applicability of Site Characteristics to MNA

The following subsections give a general overview of the characteristics of sites where natural attenuation is appropriate as a stand-alone remedy, where natural attenuation may be an appropriate secondary or follow-on remedial approach combined with some form of source reduction is, and those sites where natural attenuation is not appropriate.

Two definitions of source area are used in this document:

- Primary source: Nonaqueous-phase liquid that is the original cause of subsurface contamination. Examples include leaking underground storage tanks, leaking pipelines, etc.; and
- Secondary source: Soil impacted with residual NAPL left in the subsurface after the primary source has been removed and/or has become immobile. Secondary petroleum sources are often depleted in light-end hydrocarbons, volatiles, and soluble compounds. In the absence of NAPL in monitoring wells, concentration versus time plots of solute concentration may suggest the presence of residual NAPL.

2.1.2.1 Sites Where MNA is Appropriate as the Primary Remedial Strategy

Many sites where MNA is appropriate as the sole remedial approach will have the following characteristics:

- secondary source concentrations are stable and are expected to decrease over time or have been demonstrated to be decreasing;
- the solute plume is stable, shrinking, or expected to stabilize and shrink;
- no receptors are currently impacted, nor are any potential receptors likely to be impacted in the future;
- monitoring costs are not prohibitive; and
- stakeholders accept the approach.

2.1.2.2 Sites Where MNA is Appropriate as a Secondary or Follow-On Remedial Strategy

In some cases, MNA is appropriate for the solute plume, but remediation of NAPL present in the source area is required. This remedial strategy has spatial and temporal components and must be evaluated with this in mind (evaluation of LNAPL source removal as part of a remedy is the subject of a previous API publication [Huntley and Beckett, 2002]). Monitored Natural Attenuation may be applied to a portion of the plume during source reduction/remediation and then to the entire plume after source

remediation. Sites where MNA may be appropriate as a secondary or follow-on remedial strategy will likely have the following characteristics:

- the continuing source of contamination, either primary or secondary, has not been eliminated and/or solute concentrations in the source area are expected to remain stable or increase into the foreseeable future because of the residual NAPL;
- early in the plume lifecycle, the solute plume is expected to expand unless/until the source is reduced;
- no receptors are currently impacted and no potential receptors are likely to be impacted in the future by the distal portion of the solute plume if the source is reduced; and
- stakeholders accept the approach.

2.1.2.3 Sites Where MNA is Not an Appropriate Remedial Strategy

Sites where MNA for MtBE is not appropriate often have one or more of the following characteristics:

- a primary source of contamination persists;
- the solute plume is expected to expand and may impact potential receptors in the future;
- receptors have been impacted or are likely to be impacted; or
- stakeholders do not accept the approach.

After the continuing source has been depleted or remediated and receptors are no longer likely to be impacted, MNA may become a viable remedial alternative for the remaining MtBE.

2.2 Tiered Approach for Evaluating the Natural Attenuation of MtBE and Required Supporting Data

A tiered approach that relies on multiple, converging lines of evidence to evaluate MtBE natural attenuation is presented in this section. This approach is similar to that recommended in the protocols listed in Table 2-1, but perhaps most closely resembles the *"Standard Guide for Remediation of Groundwater by Natural Attenuation at Petroleum Release Sites"* (ASTM, 1998). Tier 1 involves the evaluation of contaminant trends and hydrogeologic data. Tier 2 involves the analysis of geochemical data to determine if biological activity is present. Tier 3 includes supplemental data to better define the contribution of biodegradation to natural attenuation. These detailed supplemental data may also be used to assist remedial investigations beyond MNA. It is anticipated that

Tier 3 data will be required for only a small percentage of sites where the natural attenuation of MtBE is being evaluated. Table 2-2 presents a summary of the different Tiers used to evaluate natural attenuation of MtBE. Figure 2-1 is a flow chart showing an integrated approach for using the three tiers of data. This flow chart is discussed further in Section 2.3.

2.2.1 Tier 1 – Evaluation of Plume Behavior

The data collected under Tier 1 is the primary line of evidence used to evaluate the natural attenuation of MtBE. The Tier 1 evaluation involves the analysis of hydrogeologic and historical concentration data to evaluate natural attenuation. In some cases, these data may be all that are needed to evaluate the efficacy of natural attenuation. Although the Tier 1 analysis does not differentiate between the various natural attenuation mechanisms (e.g., dispersion, sorption, biodegradation, etc.), it does provide a reliable and meaningful evaluation of plume behavior.

Data used for the Tier 1 analysis include both contaminant analytical data and hydrogeologic data. An historical database showing statistically significant plume stabilization and/or loss of contaminant mass over time can be used to make a very good case for natural attenuation, and is at the core of Tier 1 analysis. However, it is important to note that plume stabilization can occur with or without destructive attenuation mechanisms and even an expanding plume may stabilize and begin to shrink prior to impacting any receptors. Thus historical data are often coupled with an evaluation of groundwater seepage velocity and estimates of sorption and dispersion. Simple model simulations can then be used to infer attenuation rates and evaluate the potential for plume stabilization in the future. In some cases, nondestructive mechanisms of natural attenuation such as dispersion may be sufficient to cause the solute plume to reach steady-state equilibrium, or even recede if the strength of the NAPL source is decreasing due to natural weathering or engineered remediation.

Tier 1 data should be collected as part of any routine characterization and monitoring program. Data required for Tier 1 analysis are presented in Table 2-3. If geochemical data (Tier 2) are available at the onset of the monitored natural attenuation evaluation, they can be used at the same time to delineate basic biogeochemical processes within the plume. Protocols for collecting these data are described in Section 3.

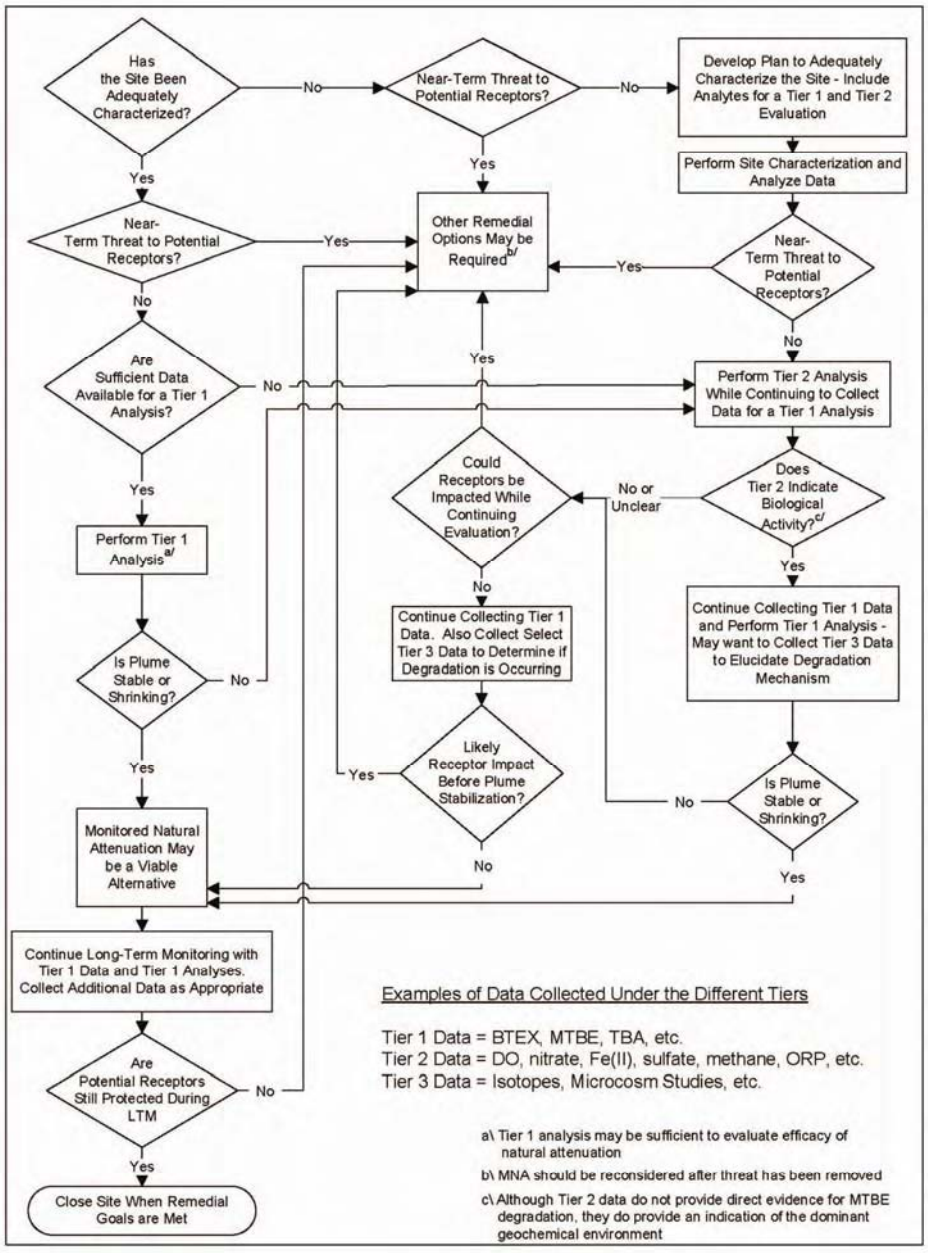


Figure 2-1 Flow Chart Showing Stepwise Approach for Using the Three Tiers of Data

Data interpretation to be completed under the Tier 1 analysis includes an evaluation of hydrogeologic conditions, preparing isopleth maps, preparing plots of concentration versus time and the associated statistical analysis, preparing plots of concentration versus distance, an evaluation of the ratio of TBA to MtBE (when possible) and an assessment of source attenuation over time. Tier 1 data analysis techniques are described in Section 4.

Buscheck and O'Reilly (2003) describe the use of a conceptual site model (CSM) for evaluating MNA. The remainder of this Subsection describes the use of the CSM consistent with this reference. A CSM provides the framework for site assessment and remediation decisions and can be developed based on Tier 1 data. The site assessment is intended to verify the CSM (i.e., sources, pathways, and receptors).

The contaminant source is critical in defining the CSM for an MtBE plume. If residual NAPL persists in the source area, dissolved MtBE concentrations in near-source monitoring wells may reflect the mass fraction of MtBE remaining in the NAPL, and can be estimated as described in Section 4.2.3. In heterogeneous stratigraphic settings or fine-grained soils, NAPL sources are subject to mass transfer limitations. Based on mass-transfer limitations, source zones may be long-lived, both for aromatics and oxygenates as the NAPL becomes depleted of MtBE, and if the primary source (ongoing release) has been eliminated, dissolved MtBE concentrations are expected to decline over time.

TBA is both an intermediate metabolite of MtBE and may also be present at low concentrations in MtBE-amended gasoline (Schmidt et al., 2004). Spatial and temporal concentration trends for TBA may be monitored similar to that of MtBE. Section 4.2.3 and 4.2.4 discuss evaluation of MtBE and TBA measurements in source zones and within the plume.

There are three CSMs for all solute plumes, including MtBE plumes:

1. Shrinking Plume – In a shrinking plume, MtBE concentrations decrease over time in source area and downgradient wells. A shrinking plume is evidence of natural attenuation and suggests a limited source. In a spatially shrinking plume, residual NAPL that may remain is likely depleted of MtBE. If MtBE remains in the NAPL, then the natural attenuation rate exceeds the MtBE dissolution rate from the NAPL.

2. Stable Plume – In a stable plume, MtBE concentrations remain relatively constant in source area and downgradient wells. A stable plume typically provides evidence of natural attenuation. However, because all groundwater systems are dynamic (i.e., groundwater elevations vary, etc.), few

contaminant plumes are truly "stable." In a stable plume, MtBE likely remains in residual NAPL, but the natural attenuation rate is approximately equal to the MtBE dissolution rate.

3. Expanding Plume – MtBE concentrations in the source area and most downgradient wells may increase in an expanding plume. The plume will continue to expand until the natural attenuation rate equals the MtBE dissolution rate. The NAPL source in an expanding plume may persist if not addressed. An ongoing primary source (NAPL or vapor) can contribute to an expanding plume.

A special case of an expanding plume is a detached plume in which the source is no longer contributing to the dissolved plume. The source may be depleted of NAPL or the NAPL may persist but is depleted of MtBE. Methyl tert-butyl ether concentrations in downgradient wells increase while concentrations decrease in source area wells. Detached plumes are rare and only occur in highly transmissive groundwater systems where residual NAPL sources are rapidly depleted (either naturally or through active remediation).

Demonstration of a stable or shrinking plume is the most important component (and typically a required component) of an MNA demonstration. Because Tier 1 data analysis allows determination of plume stability, it may be the only tier of analysis that is required. If the Tier 1 evaluation demonstrates an expanding plume, some of the Tier 2 and 3 methods may be useful for further evaluating natural attenuation or for selecting an alternative remedial alternative.

2.2.2 Tier 2 – Geochemical Data

Geochemical conditions influence, and are influenced by, microbial activity and are indicative of various microbiological processes. Differences in the geochemistry of unimpacted groundwater and that within the solute plume indicate that biodegradation processes are actively occurring in the aquifer. These data are used primarily to delineate the dominant microbial terminal electron-accepting process (TEAP). Definitive methods (Tier 3 data) are available to verify biodegradation processes and determine the related TEAP, but, in combination with Tier 1, geochemical data collected under the Tier 2 analysis are often sufficient for evaluating the natural attenuation of petroleum hydrocarbons. Data to be used for Tier 2 analysis include dissolved oxygen, nitrate, Fe (II), sulfate, methane, total alkalinity, oxidation-reduction potential (ORP), pH, temperature, and conductivity (ASTM, 1998). Protocols for collection of Tier 2 data are provided in Section 3, and data evaluation techniques are described in Section 4.

Because contaminants other than MtBE can exert a demand for electron acceptors, the Tier 2 analysis does not provide conclusive evidence to demonstrate that the particular TEAP is contributing to MtBE mass loss. Nevertheless, Tier 2 does provide an indication of the dominant geochemical environment, which may be helpful for identifying potential degradation mechanisms. Positive identification of active MtBE degradation cannot, however, be based simply on the determination of redox conditions from geochemical data.

2.2.3 Tier 3 – Supplemental Data

Tier 3 studies develop site specific data to evaluate whether the observed natural attenuation is caused by biodegradation. The Tier 3 analysis is intended to be used when the mechanisms responsible for natural attenuation have not been, or cannot, be determined through the Tier 2 data analysis and more definitive information regarding biodegradation of MtBE is necessary to develop a reasonably degree of certainty that MNA will be protective. Because they offer direct evidence of biodegradation, Tier 3 studies may be effective in convincing stakeholders that MNA is an appropriate remedy. As with other MNA data, Tier 3 data may also be useful for evaluating alternate remedial options including enhanced bioremediation. Tier 3 will likely be required for only a small percentage of sites. Examples of potential Tier 3 data are summarized in Table 2-4, and include:

- compound-specific stable isotope analysis (CSIA) to verify MtBE biodegradation at field sites. Changes in the $^{13}C/^{12}C$ or $^{2}H/^{1}H$ ratio of MtBE within a plume can be used to demonstrate biotransformation and also to (conservatively) estimate the extent of biodegradation; and
- laboratory microcosm studies, which can be conducted with sediment and/or groundwater samples to verify biodegradation under site-specific conditions. Although relative degradation rates can be determined in such studies, rates measured in the laboratory are often different from those measured in the field. While aerobic microcosm studies take only a few weeks, anaerobic studies can take many months. Very few laboratories are capable of conducting proper anaerobic microcosm studies.

2.3 An Integrated Approach for Utilizing the Three Tiers of Data

Figure 2-1 is a flow chart that illustrates the decision-making process to evaluate natural attenuation. The flow chart describes an integrated approach for using the three tiers of data. Adequate site characterization and the development of a robust CSM are critical to a natural attenuation evaluation. The following sections describe those conditions that

require each of the tiers of data and discuss the adequacy of the site characterization data and the CSM for evaluating MNA.

2.3.1 Tier 1 Data are Adequate to Evaluate Natural Attenuation

The left side of the flow chart illustrates the conditions where Tier 1 data are adequate to evaluate natural attenuation. For sites where there are no near-term threats to receptors and the Tier 1 data are sufficient to demonstrate the plume is either stable or shrinking, a long-term monitoring program may be adequate to demonstrate natural attenuation and to implement MNA. For such sites, Tier 2 data likely are not required.

2.3.2 Tier 2 Data are Collected

Those sites where Tier 1 data are insufficient and the plume can not yet be shown to be stable or shrinking will require the collection of additional Tier 1 data. Tier 2 data can also be collected to help evaluate natural attenuation, or to provide an additional line of evidence for evaluating natural attenuation. If Tier 2 data indicate biological activity, then the collection of Tier 1 data should be continued to evaluate plume stability. If the plume can be demonstrated to be stable or shrinking, MNA may be a viable alternative. If plume stability can not be demonstrated then the potential for receptor impacts should be evaluated.

2.3.3 Tier 3 Data are Collected

In some cases, Tier 3 data may be useful to further elucidate the degradation mechanism (the contribution of biodegradation to natural attenuation). If plume stability has not been demonstrated then collection of Tier 1 data should continue while Tier 3 data are collected. If, after integrating Tier 3 data, the CSM suggests the potential for completed exposure pathways and near-term threats to receptors exist, engineered remediation may be required.

2.3.4 Site Characterization and Conceptual Model Development

As shown in Figure 2-1, the first question to ask is if the site has been sufficiently characterized to allow reliable evaluation of natural attenuation. Consideration of this question involves review of *all* available site data and development of a preliminary conceptual model for the site. Development of a preliminary CSM will help identify data gaps and cost effective ways to fill them.

The degree of characterization required will be site specific and will depend upon, among other things, the velocity and direction of groundwater flow, the complexity of the hydrogeologic system, and the distance to potential receptors.

Development of the CSM should include review of:

- Nature, extent, and magnitude of contamination:
  - Nature and history of the contaminant release:

    --Catastrophic or gradual release of NAPL?
    --More than one source area possible or present?
    --Distinct or overlapping plumes?
  - Three-dimensional distribution of NAPL and dissolved contaminants. The distribution of NAPL is used to define the dissolved plume source area;
  - Groundwater and soil chemical data;
  - Historical water quality data showing variations in contaminant concentrations;
  - Chemical and physical characteristics of the contaminants; and
  - Potential for biodegradation of the contaminants.
- Geologic and hydrogeologic data (in three dimensions, if feasible):
  - Lithology and stratigraphic relationships (e.g., well boring logs, geologic cross-sections etc);
  - Grain-size distribution (sand *vs.* silt *vs.* clay);
  - Aquifer hydraulic conductivity;
  - Groundwater hydraulic gradients and potentiometric or water table surface maps (over several seasons, if possible);
  - Preferential flow paths (utility conduits, abandoned wells etc); and
  - Interactions between groundwater and surface water and rates of infiltration/ recharge.
- Locations of potential receptor exposure points:
  - Groundwater production wells;
  - Occupied buildings near sources or in areas where the water table is shallow; and
  - Downgradient and cross-gradient groundwater discharge points.

The CSM integrates these data to develop a representation of release mechanisms, the potential remaining sources and groundwater flow and solute transport, including transport pathways, exposure points, and receptors. After development, the CSM can be used to help determine optimal placement of additional monitoring points, as necessary,

to aid in the natural attenuation investigation and to develop a quantitative solute fate and transport model, if required. Contracting and management controls must be flexible enough to allow for the potential for revisions to the CSM and the associated data collection efforts. Successful CSM development involves:

- Definition of the problem to be solved (the existing and potential future nature, magnitude, and extent of contamination);
- Integration and presentation of available data, including:
  - local geologic and topographic maps;
  - geologic data;
  - hydraulic data;
  - geochemical data; and
  - contaminant concentration and distribution data.
- Determination of additional data requirements, including:
  - borehole locations and monitoring well spacing;
  - a sampling and analysis plan (SAP); and
  - any other remaining data requirements.

In some cases, available site-specific data are limited. As described in Section 2.3.6, initial characterization activities at such sites should include collection of both Tier 1 and Tier 2 data. Regardless of whether natural attenuation is selected as a sole remedial strategy or in conjunction with an engineered remediation system, the additional costs incurred by such data collection are likely to be outweighed by the cost savings that will be realized. Much of the data collected to evaluate natural attenuation through Tier 2 are useful to design and evaluate other remedial measures.

2.3.5 Sites That Have Adequate Site Characterization Data

If the site has been adequately characterized, then an assessment of the near-term threat to potential receptors should be made. If there is a near-term threat to potential receptors then other remedial alternatives may be required and should be evaluated and implemented if necessary. If there is not a near-term threat to potential receptors then available historical data should be used to evaluate solute plume behavior. If data are sufficient, a Tier 1 analysis should be performed and a determination of plume stability should be made. If the plume is shown to be stable or shrinking, natural attenuation may be a viable alternative. In many cases, however, a long-term monitoring program will still

be required. If, during long-term monitoring, it is determined that potential receptors are not being adequately protected then other remedial options may be necessary.

If there are not sufficient data to perform an adequate Tier 1 analysis and there is no near-term threat to receptors, then additional Tier 1 data should be collected and a Tier 2 analysis should be performed as discussed in Section 2.3.6.

2.3.6 Sites That Do Not Have Adequate Site Characterization Data

If the site has not been adequately characterized, a plan should be developed to perform further site characterization. If there is an obvious near-term threat to potential receptors, other remedial options may be required in the interim. The additional site characterization should include those analytes required to perform both Tier 1 and Tier 2 analyses. After site characterization is complete, the potential for near-term threat to potential receptors should be re-evaluated. If there is a near-term threat to potential receptors then other remedial options may be required.

If there is not a near-term threat to potential receptors, a Tier 2 analysis should be performed. If the Tier 2 data analysis indicates that there is biological activity, collection of Tier 1 data should continue until there are sufficient data to perform a Tier 1 analysis. In some cases it may make sense to collect some form of Tier 3 data to help elucidate degradation mechanisms while continuing to develop a Tier 1 evaluation.

If the Tier 2 data analysis shows that there is no biological activity, or is inconclusive, then a determination of potential long-term receptor impact should be made. If potential receptors could be impacted during the collection of additional Tier 1 data, other remedial options may be required. If no threat to potential receptors is expected during this period, additional Tier 1 data should be collected. In this case, some form of Tier 3 data may also be helpful to determine if degradation is occurring.

In cases where additional Tier 1 data are being collected, a Tier 1 analysis should be made once sufficient data are available. If the plume is expanding and is not likely to stabilize before a potential receptor is impacted, other remedial options may be required. If the plume is stable or shrinking or is likely to stabilize before receptor impact, natural attenuation may be a viable remedial alternative and a long-term monitoring program should be developed and implemented. If long-term monitoring data indicate that potential receptors are not being adequately protected, other remedial options may be necessary.

2.4 Mass Flux Estimates

In some cases, mass flux estimates can be a useful and practical way to evaluate the efficacy of natural attenuation. To estimate the change in contaminant mass flux across a plume and the potential for continued plume migration, mass flux calculations typically involve an estimate of the mass of contaminant flowing past two (or more) cross-sectional areas ("transects") oriented perpendicular to the direction of groundwater flow. Each transect must contain sufficient monitoring points to delineate both the horizontal and vertical extent of the solute plume at that location and to characterize preferential flow paths. The reliability of the mass flux calculation is directly dependent upon the amount and quality of hydrogeologic and geochemical data. The level of site characterization developed to support mass flux calculations should be commensurate with the level of certainty required for the analysis, which in turn is related to the magnitude of potential risk, remediation cost, and level of regulatory and stakeholder concern. Besides being used to evaluate natural attenuation, mass flux calculations can be useful for a variety of applications, including (Borden et al., 1997; Newell et al., 2003; Buscheck et al., 2003; Evans and Colsman, 2003; Nichols and Roth, 2004; Guilbeault et al., 2005):

- evaluation of potential impacts to receptors (i.e., surface water, pumping wells);
- estimation of remediation timeframes;
- evaluation of the design and performance of remediation systems; and
- site prioritization.

Section 4 presents the techniques used to complete a mass flux analysis. Appendix C includes an example mass-flux calculation.

3 SAMPLE COLLECTION AND ANALYSIS

This section provides procedures and analytical methods for collecting the data required to support an evaluation of MtBE natural attenuation. Additional detailed information on data collection techniques is provided in Appendix D. A brief summary of data quality issues is included as Appendix E.

3.1 Sampling Location and Frequency

The adequacy of a monitoring well network should be assessed with respect to the network's ability to support the data quality objectives (DQOs) associated with the current phase of natural attenuation monitoring. The monitoring well network should evolve from its initial use for characterization and validation of natural attenuation processes to long-term monitoring (Wiedemeier and Haas, 2002; Wilson and Kolhatkar, 2002). Due to the site-specificity of this process, no single guideline specifying the number and location of wells can be applied to all sites. Generally, wells are added to a network through the validation stage, and removed during the long-term monitoring stage.

The field tools and approach to developing information during the validation stage has been described in detail in *Strategies for Characterizing Subsurface Releases of Gasoline Containing MTBE* (Nichols et al., 2000). The DQOs associated with long-term groundwater monitoring can generally be satisfied with wells located along the center line of the oxygenate plume, with wells located in three areas: immediately downgradient of the source (or former source); the plume interior; and the plume edges. Additionally, monitoring upgradient of the source is required to characterize background groundwater geochemistry and to identify any potential contribution from off-site sources. These well locations provide the data necessary to support evaluation of plume behavior (i.e., expanding, stable, or contracting) and to provide corroborating lines of evidence for the Tier 2 and 3 analyses (Section 2.2). The number of wells required for long-term monitoring will be site-specific. Ideally, wells would be located along the axis of the plume in the direction of groundwater flow. This well distribution facilitates graphical analysis of Tier 1 data. Monitoring should also be adequate to define the vertical extent of contamination while minimizing the potential for cross-contamination.

The frequency of groundwater sampling to support MNA is an important consideration. Monitored Natural Attenuation programs can be divided into three phases: site-characterization monitoring; validation monitoring; and long-term monitoring (Wiedemeier and Haas, 2002). The recommended frequency of sampling is dependent upon the objectives of each phase. During site characterization monitoring, quarterly sampling is recommended to assess spatial distribution of MtBE and characterize

seasonal fluctuations in groundwater levels and flow regime. Seasonal variations in recharge can cause significant changes in flow direction, contaminant concentrations and groundwater geochemistry that must be taken into account when evaluating plume attenuation. Once the spatial distribution of MtBE and seasonal variability in flow direction is understood, validation monitoring should be undertaken. Validation monitoring should consist of semi-annual sampling of wells along the plume centerline to characterize longer term temporal trends in the dissolved plume as well as the groundwater flow regime. During validation monitoring, annual sampling of wells that are not used for plume center-line monitoring should be considered where regional groundwater characterization is of continued interest (if an identified DQO). Once the dynamics of the MtBE plume have been established, a long-term monitoring program should be implemented. Depending on the groundwater seepage velocity, annual or semi-annual sampling of selected monitoring wells generally suffices for long-term monitoring. Wiedemeier and Haas (2002) discuss methods to determine sampling frequency.

3.2 Sample Preservation

Table 3-1 summarizes preservation and holding times for MtBE, TBA and other chemicals of interest to be analyzed in the laboratory. The analytical methods that are listed in this table are further discussed in Section 3.3 (laboratory analytical methods). Samples to be analyzed in the field using test kits generally do not require preservation if analyzed immediately; however, if these samples must be stored prior to analysis for any reason, method-appropriate preservation should be used.

Soil samples to be analyzed for MtBE and other volatile organic compounds can be preserved in several ways, including the addition of methanol or sodium bisulfate in the field and the collection and storage of the sample in a closed-system container such as an En Core® sampler (or equivalent). EPA Method 5035A (July 2002, Draft Revision 1) presents a discussion of sample preservation methods for soil samples.

Groundwater samples to be analyzed for volatile organic compounds such as BTEX are typically preserved by acidification with hydrochloric acid (HCl) to pH less than 2 standard units and refrigerated at a temperature of 4°C. However, at low pH (<2) and elevated temperatures, MtBE can undergo acid-catalyzed hydrolysis to form tert-butyl alcohol (TBA) and methanol (O'Reilly et al., 2001). These results have raised concerns about the quality of MtBE and TBA data obtained from acid-preserved groundwater samples.

The potential for MtBE hydrolysis appears to be limited to samples that are analyzed by a heated-headspace method (e.g., EPA Method 5021A) instead of the more common

ambient-temperature purge-and-trap (EPA Method 5030C). White and others (USEPA, 1999) saw significant hydrolysis in acid-preserved samples when they used heated headspace analysis. Lin et al., (2003) observed losses of MtBE and formation of TBA from acid-preserved samples undergoing heated headspace analysis at 80^{o}C, but saw no losses in alkaline-preserved samples and recommended alkaline preservation to avoid hydrolysis in heated headspace analyses. Rong and Kerfoot (2003) presented data that showed no significant difference between MtBE results for acid-preserved samples and alkaline-preserved samples prepared using ambient-temperature purge and trap (EPA Method 5030C). A recent EPA publication reports that the rate of hydrolysis is slow in refrigerated samples, leading to less than 5% MtBE hydrolysis over 30 days at 10 degrees C. (Wilson et al., 2005a). The most recent revisions of EPA Methods 5021A and 5035A now recommend that samples containing MtBE or other ether oxygenates be prepared by heated methods and must not be preserved with acid. EPA Method 5021A recommends pH adjustment with sodium trisodium phosphate to pH 10 or higher, or acid preservation in the field but then adjustment to pH 10 or higher prior to initiation of the headspace analysis. Acid preservation is acceptable for samples to be prepared using standard ambient-temperature purge and trap methods, which is the default method employed by commercial laboratories when water analysis is requested for “8021” or “8260” without specifying a preparation method.

3.3 Laboratory Analytical Methods

3.3.1 MtBE

Methyl tert-butyl ether and other oxygenates can be quantified in water or solid media by standard laboratory analytical methods for volatile organic compounds using EPA Methods 8015, 8020, 8021, and 8260B with appropriate sample preparation procedures. EPA Methods 8015 and 8260B are the currently recommended methods for MtBE analysis. EPA Method 8020 is an older method that is generally no longer used, but may have been used for historic data. EPA Method 8021 is commonly used for BTEX and MtBE; however, this method is more appropriate for aromatic compounds such as BTEX. Other methods such as ASTM Method D4815 have been developed for MtBE but may not be widely available commercially or accepted by regulators. Drinking water methods (EPA 502.2 and 524.2) are similar to Methods 8021 and 8260B and are used for potable water supplies. Table 3-2 summarizes these methods, as well as sample preparation methods commonly used with these methods.

EPA Methods 8015, 8020, 8021, and 8260B are standard gas chromatography (GC) methods that differ in their detection methods. Method 8260B uses a mass spectrometry (MS) detector that is capable of positive identification of MtBE and is suitable for all data uses. EPA Method 8015, uses a flame ionization detector (FID) that can quantify MtBE

and other organics. EPA Methods 8020 and 8021 use photoionization detectors (PID) that are most sensitive to compounds such as BTEX that contain double bonds, and can also quantify MtBE[3]. ASTM Method D4815 also uses a flame ionization detector (FID), which is sensitive to organic compounds including ethers. However, compound identification for these non-MS methods is primarily determined by retention time and can result in overestimation of MtBE concentrations or false-positive detection of MtBE, due to interference by other gasoline constituents. Comparisons of these methods of detection for MtBE have been conducted by various groups (Happel et al., 1998). EPA Method 8015 is generally preferred over EPA Method 8021, but both of these lower-cost methods are generally acceptable for routine monitoring. In areas of the site with elevated gasoline concentrations (e.g., greater than 5,000 ug/L total petroleum hydrocarbons) and for data usages with stringent DQOs, confirmation of MtBE detection and concentration by EPA Method 8260B is recommended (Nichols et al., 2000).

[3] EPA Methods 8020 and 8021 also use an electrolytic conductivity detector (HECD) that is used primarily for the detection of halogenated analytes.

Table 3-1 Sample Preservation and Hold Times

Analytes	Method [b]	Preservative	Container Type	Volume	Hold Time
Water Samples					
MtBE, TBA, and BTEX	EPA 8260B, 8021, 8015, 502, or 524.2 with ambient temperature preparation	HCl to pH<2, cool to 4°C[a]	Glass vials	3 X 40 ml	14 days
MtBE and other Ethers	EPA 8260B, 8021, 8015 with 5021A or other heated preparation method	Trisodium phosphate (TSP) to pH>10, cool to 4°C	Glass vials	3 X 40 ml	14 days
pH	EPA 150.1 or equivalent	Cool to 4°C	Plastic or glass	50 ml	Immediately
Conductivity	EPA 120.1 or equivalent	Cool to 4°C	Plastic or glass	250 ml	Immediately
Dissolved oxygen	EPA 360.1 or equivalent	Cool to 4°C	Glass	1000 ml	Immediately
Ammonia nitrogen	EPA 350.3 or equivalent	H_2SO_4 to pH<2, Cool 4°C	Plastic or Glass	100 ml	28 days
Nitrate	EPA 300.0 or equivalent	H_2SO_4 to pH<2, Cool 4°C	Plastic or Glass	100 ml	28 days
Sulfate	EPA 300.0 or equivalent	Cool to 4°C	Plastic or Glass	2 X 500 ml	28 days
Alkalinity	EPA 310.1 or equivalent	Cool to 4°C	Plastic	500 ml	14 days
Total Organic Carbon	EPA 415.1/SW9060	H_3PO_4 or H_2SO_4 to pH<2, cool to 4°C	Glass	100 ml	28 days
Total Inorganic Carbon	EPA 310.1 or equivalent	Cool to 4°C	Plastic	500 ml	14 days
Dissolved Gases including Methane	RSK175[d] EPA 8015B Mod.	HCl to pH<2, cool to 4°C[a]	Glass vial with Teflon® caps	2 X 40 ml	14 days
Aqueous Fe(II)	3500 Fe D Mod.	HCl to pH<2, cool to 4°C[a]	Glass	500 ml	Immediately
Iron, manganese	EPA 200.7, 6010B	$HNO_3$to pH<2, cool to 4°C	Plastic	2 X 500 ml	180 days
Compound-specific Stable Isotope Analysis (CSIA)	GCIRMS[c]	Trisodium phosphate (TSP) to pH>10, cool to 4°C	Glass vials	lab-specific	14 days
Microcosm studies	Various	Cool to 4°C	Glass	lab-specific	na
Soil or Sediment Samples					
Total Organic Carbon	SW9060A	Cool to 4°C	Glass w/Teflon®	100 g	28 days
Total Bioavailable Iron	Ferrozine Assay	HCl to pH<2 or maintain anaerobic conditions, cool to 4°C	G preferred; P acceptable	at least 10 g	Immediately
Microcosm studies	Various	Cool to 4°C	Glass	lab-specific	na

a. Typically, 4 drops HCL for a 40 mL vial.

b. EPA SW-846 - *Test Method for Evaluating Solid Waste, Physical and Chemical Methods*, SW-846, EPA, 3rd ed., 1986 and revisions. EPA/600/R-95-131 - Methods for the Determination of Organic Compounds in Drinking Water-Supplement III (EPA/600/R-95-131).

c. Gas Chromatography Isotope Ratio Mass Spectrometry.

d. Kampbell and Vandegrift, 1998. J. Chromat. Sci., 36:253-256. Developed by EPA, but not an EPA Method. Sold as “modified RSK-175” or “modified 8015”, but should a headspace extraction consistent with RSK-175 and an FID.

Water samples submitted to commercial laboratories for analysis by EPA Methods 8015, 8020, 8021, or 8260 without specifying the preparation method are almost universally prepared using ambient-temperature purge and trap by EPA Method 5030C. The purge and trap method strips the volatiles from the water sample and then traps (i.e., concentrates) them on a sorbent material prior to analysis. Other methods of preparation can be used with these GC methods, but must be specifically requested and may not be widely available at commercial laboratories.

Due to the high solubility of MtBE, other sample preparation methods, such as heated (80^{o}C) purge and trap (a recommended method modification in EPA Method 5030C for improving recoveries of MtBE at low concentrations), headed headspace analysis (EPA Method 5021A), or direct aqueous injection (DAI) may provide additional sensitivity. As discussed in Section 3.2, methods involving heat should not be used unless the sample has been preserved with an alkali solution (pH>10) or, if acid-preserved in the field, adjusted to pH >10 prior to the heating step. Other preparation methods such as solid phase microextraction (SPME), in which volatile compounds are concentrated onto a sorbent fiber can provide lower detection limits but have limited commercial availability.

Purge and trap using EPA Method 5035A is also applied to solid samples, such as soil and sediment. Samples can be prepared by methanol extraction of the solid sample and then a purge of the extract using Method 5030A, or the sample can be placed in the purging apparatus with deionized water, heated to 40^{o}C and purged to disperse and strip the volatiles.

NAPL samples can also be analysed by direct injection after dilution with a solvent (e.g., EPA Method 3585).

3.3.2 Breakdown Products of MtBE and Other Associated Chemicals

Other oxygenates and degradation products derived from MTBE can generally be analyzed by the same methods as MtBE, as indicated in Table 3-2. Since the degradation products are generally highly soluble or miscible in water, they are typically associated with aqueous samples, rather than soil samples.

Environmental Protection Agency Methods 8015 and 8260B can also be used for TBA; however, only EPA Method 8015 has been validated for analysis of TBA. Method 8015 also recommends that sample preparation for highly water soluble compounds such as TBA be performed by direct aqueous injection or azeotropic distillation (EPA Method 5031) and not by the standard ambient-temperature purge and trap method. Tert-butyl alcohol, ethanol, and other soluble compounds have poor purging efficiencies that can result in both elevated detection limits and poor reproducibility. The standard purge-and-trap method (EPA Method 5030C) also now recommends heated purging at 80^{o}C for

TBA. The headed headspace method (EPA Method 5021A) has also been validated for TBA.

Tert-butyl formate (TBF), which is also highly soluble, can be analyzed using direction aqueous injection (Einarson and Mackay, 2001). Since TBF is not a standard analyte of any of the methodologies discussed above, analysis by Method 8260B with MS detection is recommended. EPA Method 8015 may be acceptable for some data uses if TBF standards are included.

The other known metabolites of MtBE discussed in Appendix A include hydroxy isobutyric acid (HIBA) and 2-methyl-2-hydroxy 1-propanol (MHP), for which analytical methods have not yet been published.

Other oxygenates such as tert-amyl methyl ether (TAME), ethyl tert-butyl ether (ETBE), and tert-amyl alcohol (TAA) have properties similar to MtBE and can generally be analyzed using the same methods discussed for MtBE in Section 3.2.1. Ethanol, which like TBA, is miscible in water, is a validated analyte under EPA Method 8015 and sample preparation by direct injection or azeotropic distillation (Method 5031) is recommended.

3.3.3 Geochemical Data

Methods of analysis for geochemical parameters are listed in Table 3-3. The standard geochemical analytes include nitrate, ammonium, total and dissolved iron, total and dissolved manganese and sulfate. As discussed in Section 3.2.3, some of these parameters such as nitrate and sulfate may be collected either as field or laboratory measurements. The majority of these parameters are only measured on aqueous samples. Total iron and total manganese can be analyzed in both soils and water. The geochemical analytes generally fall under the Tier 2 analysis.

Methods are also available for dissolved oxygen, and methane, as listed in Table 3-2. Analysis of methane concentrations in groundwater should be conducted by a qualified laboratory which can obtain the appropriate detection limit. To achieve the required low detection limit of 1 μg/L or less, methane is generally analyzed by GC with an FID.

Other analytes that may be collected include dissolved organic carbon (DOC), biochemical oxygen demand (BOD), and total inorganic carbon (TIC). Humic substances, which are a component of TOC and DOC, can also be analyzed using either microbial cell suspension and Fe(III) or a spectrophotometer assay (Nevin and Lovley, 2002). However, these assays are not commercially available and an analysis for TOC will be sufficient for most sites.

Table 3-2 Methods for Analysis of MtBE TBA and Other Volatile Organics of Interest

Methods for Sample Preparation			
Method	**Description**	**Comment**	**Reference**
EPA Method 5021A	Heated headspace	Samples must be adjusted to pH >10 in the field or prior to analysis; recommended for TBA, MtBE, and acetone	EPA SW-846
EPA Method 5030C	Purge and trap	Common method for MtBE and acetone, but may have poor purging efficiency	EPA SW-846
EPA Method 5031	Azeotrophic distillation	Recommended for TBA and ethanol; may be appropriate for TBF	
EPA Method 5035A	Purge and trap	Common method for MtBE and acetone, but may have poor purging efficiency	EPA SW-846
EPA Method 3585	Direct injection with dilution with hexadecane	Can be used for NAPL samples	EPA SW-846
Direct injection	Direct injection	Recommended for TBA, ethanol and TBF	EPA SW-846
Solid phase microextraction (SPME)	Sorbent fiber in contact with sample	Limited commercial availability; lower detection limits	

Analytical Methods for MTBE				
Method	**Detector**	**Sensitivity**	**Comment**	**References**
EPA Method 8015	FID		Validated for acetone, TBA and ethanol; may be appropriate for TBF	EPA SW-846
EPA Method 8021B	PID/ELCD	0.1 μg/l MDL	Overestimation or false-positive detection of MtBE and other oxygenates may occur	EPA SW-846
EPA Method 8260B	MS	0.5 μg/l MDL	Recommended method for all MtBE, TBA and acetone data uses	EPA SW-846
EPA Method 502.2 Rev 2.1	PID/ELCD		This method is similar to Method 8021; includes purge and trap	EPA/600/R-95-131
EPA Method 524.2	MS		This method is similar to Method 8260B; includes purge and trap	EPA/600/R-95-131
ASTM D 4815	FID			ASTM

References:
EPA SW-846 - *Test Method for Evaluating Solid Waste, Physical and Chemical Methods*, SW-846, EPA, 3rd ed., 1986 and revisions
EPA/600/R-95-131 - Methods for the Determination of Organic Compounds in Drinking Water-Supplement III (EPA/600/R-95-131)
Abbreviations:
PID – photoionization detector ELCD – electrolytic conductivity detector FID – flame ionization detector
MS – mass spectrometry detector GC – gas chromatography RL – reporting limit; lower limit of accurate quantitation
MDL – method detection limit; lower limit of accurate identification
"A" refers to *Standard Method for the Examination of Water and Wastewater*, 18th ed, 1992.
"B" refers to *Method for Chemical Analysis of Water and Wastes*, EPA, 1983.
"SW" refers to *Test Method for Evaluating Solid Waste, Physical and Chemical Methods*, SW-846, EPA, 3rd ed., 1986.

Table 3-3 Laboratory Analyses for Tier 2 Geochemical Parameters

Target Chemical	Method/Reference	Minimum Limit of Quantification	Potential Issues/Comments
pH	EPA 150.1		
Conductivity	EPA 120.1/SW9050		
Dissolved oxygen	EPA 360.1 Dissolved oxygen electrode	1 mg/L	Avoid exposure of sample to atmospheric oxygen
Dissolved iron (Fe II)		0.5 mg/L	Interference from Turbidity Sensitive to sunlight Requires immediate analysis
Major Cations	SW6010 for some cations	1 mg/L	Interference from colloids
Nitrate	EPA 300 (IC)	10 µg/L	
Sulfate (SO_4^{-2})	EPA 300	1 mg/L	Maximum concentration of 80 mg/L Temperature sensitive; keep cool Filter if necessary to mitigate turbidity interferences
Dissolved methane	RSK-175 GC/FID	1 µg/L	Use tubing such as LDPE or C-Flex®, which have low permeable to light gasses Avoid exposure of sample to atmospheric oxygen
Alkalinity	EPA 310.1	1.0 mg/l	Use plastic bottles and do not exceed hold time of 14 days
Bulk density	ASTM D 5057-90B		Common geotechnical analysis, requires 100 – 200 g of soil

3.3.4 Compound Specific Stable Isotope Analyses

Compound specific isotope analysis (CSIA) determines the ratio of heavy to light stable carbon (^{13}C to ^{12}C) or hydrogen (^{2}H to ^{1}H) isotopes in MtBE or other compounds (e.g., TBA) present in a soil or water sample (Gray et al., 2002; Hunkeler et al., 2001). The compounds of interest in the sample are separated on a gas chromatographic column, followed by high-temperature conversion to carbon dioxide and water. The stable carbon and hydrogen isotopes in carbon dioxide and water are determined using isotope ratio mass spectrometry. The analytical results of CSIA analysis are calculated as enrichment of the heavy isotope with respect to the lighter isotope and are expressed as $\delta^{13}C$ ("delta carbon 13") or δD ("delta deuterium") in units of per mil (‰). For example, ^{13}C enrichment is calculated relative to internationally recognized standard materials[4] for carbon isotopic ratios and is, calculated as follows:

[4] The international standard material for carbon stable isotope ratio analysis is Cretaceous belemnite from the PeeDee formation (PDB) or Vienna Pee Dee Belemnite (VPDB).

$$\delta^{13}C = \left(\frac{{}^{13}C/{}^{12}C_{\,sample}}{{}^{13}C/{}^{12}C_{\,standard}} - 1 \right) \times 1000$$

The hydrogen isotope ratio δD is calculated in a similar manner using a water standard[5] (Gray et al., 2002; Hunkeler et al., 2001).

Based on two surveys of commercial gasoline, the range of $\delta^{13}C$ for MtBE in gasoline measured to date is between -27.5‰ and -33‰ (Smallwood et al., 2001; O'Sullivan et al., 2003). To date δD values of MtBE in gasoline and some source area water samples range from -80‰ to -125‰ (Kuder et al., 2005). Quantitation limits as low as 2.5 ppb for $\delta^{13}C$ and 25 ppb for δD have been reported for MtBE in groundwater samples.

CSIA is currently available in the US from a number of research laboratories including the University of Oklahoma, University of Waterloo and University of Toronto; however, this capability is being further developed and is becoming available commercially. (Kuder et al., 2004, 2005; Kolhatkar et al., 2002; Gray et al., 2002, Hunkeler et al., 2001.)

3.3.5 Laboratory Microcosms

Laboratory incubations can support the development of an MNA strategy by providing site-specific data under controlled conditions that are not readily available in the field as part of a Tier 3 evaluation. These incubations can be designed to provide simple "proof-of-concept" data that MtBE degrading microorganisms are present in site materials, or to simulate field conditions and provide kinetic data that approximate field biodegradation rates. Detailed guidance on conducting microcosms is beyond the scope of this document and requires the services of a qualified laboratory. Variations on techniques are many; a recent summary of background information on petroleum degradation is provided in Magot and Olliver (2005).

In microcosms, the general intent is to culture the microorganisms present in site media (soil, sediment, groundwater), with the chemical under study as the sole carbon source and in the presence of a dominant electron acceptor. Chemical-specific analysis aids the data interpretation. Depending on objectives, isotope-labeled chemicals and monitoring may be used. Successful execution of anaerobic microcosms, in particular, requires specialized techniques and experience, especially with respect to exclusion of oxygen. Some discussion on anaerobic microcosms is provided in the USEPA Technical Protocol for evaluating chlorinated solvent MNA (Wiedemeier et al., 1998). The value and

[5] The international standard material for stable hydrogen isotope ratio analysis is the Vienna Standard Mean Ocean Water (VSMOW).

limitations of microcosm studies for MtBE are discussed briefly below. Guidance on evaluating and presenting microcosm data is provided in Section 4.4.

Laboratory batch incubations (also called bottle incubations or microcosm studies) are useful to determine MtBE biodegradation parameters under controlled conditions. Field conditions vary across a site as a result of the variation in many different hydrogeologic parameters that together influence observed chemical distributions in time and space. Laboratory incubations can be designed to test the effects of one parameter or a few parameters at a time, regardless of the prevailing site conditions. If constructed appropriately, the laboratory incubations can be constructed to test the effects of: electron acceptor concentration, MtBE concentration, pH, temperature, or the presence/absence of specific microbial populations. These data can be used to estimate biodegradation rates under the test conditions, verify that certain biodegradation reactions are occurring in the field, and infer the extent and rate of these reactions *in situ*.

Laboratory studies have limited direct value for MNA studies because laboratory conditions only approximate *in situ* processes. Variability among replicate microcosms is often observed, providing evidence that even in controlled laboratory conditions, the influence of specific parameters on the degradation of chemicals often cannot be isolated. Further, because *in situ* conditions cannot be precisely duplicated in the lab, laboratory degradation rates may be biased either high or low relative to *in situ* conditions. Bias may arise from avoidable errors such as sample disturbance or introduction of oxygen to an anaerobic microcosm or unavoidable causes such as the inevitable discrepancy between lab and field scale which limits the overall biogeochemical diversity of microcosms. Rate constants derived from laboratory studies, therefore, must be verified before application to field scale predictions. In addition, effective laboratory studies may require multiple batch experiments representative of a variety of conditions encountered across a site.

Because of the possibility of long acclimation times for MtBE and TBA biodegradation, microcosm study design and planning should account for potential delays and results should always report acclimation time and degradation rate, as well as supporting information including concentration levels and total incubation period.

3.3.6 Molecular Microbial Community Analysis

Although not included by this protocol as a Tier 3 method, analysis of MtBE-degrading microbial communities using molecular tools is becoming more accessible and may eventually provide a common line of evidence for MtBE MNA studies. The state of the science of such tools is summarized in this section.

Aerobic microorganisms have been identified in pure culture and environmental samples that degrade MtBE efficiently. Molecular biology tools are available to determine

whether specific organisms involved in this reaction are present in environmental media. For the most part, these tools are based on bacterial identification using the 16S rRNA gene a which, while not directly involved in MtBE degradation, is widely used in microbial identification as a sort of "molecular fingerprint" for grouping and differentiating types of bacteria. 16S rRNA based tools are used to identify MtBE degrading microorganisms in samples by genetically relating them to known MtBE-degrading microorganisms. Furthermore, the number of these microorganisms present in a sample can be estimated by using quantitative (real-time) PCR. Hristova et al. (2001) developed a quantitative PCR test for a MtBE degrader *Rubrivivax gelatinosus* strain PM1 which is commercially available. Note however, that this test is targeted at one particular strain of bacteria, when in fact other strains or genera of bacteria may also contribute to MtBE degradation in the field.

In addition to 16S rRNA gene tests other molecular tests use so-called "functional genes" these genes produce the enzymes that are directly involved in the reaction in question. When compared to 16S rRNA based assays, functional gene tests provide increased confidence that the desired metabolic activity is present in the sample, regardless of the specific type of bacteria present. For certain compounds, for example the chlorinated ethenes, functional genes have been determined and specific PCR based tests developed (e.g., Muller et al., 2004). A number of genes directly involved in MtBE degradation have been identified (Fayolle et al., 2001; Johnson et al., 2004). Functional gene tests in relation to evaluating MtBE degradation in soil and groundwater, however, have not yet been routinely applied.

The general methods utilized in molecular microbiology are widely described in the literature (e.g., Maniatis et al., 1989, Ausubel et al., 1992) and are briefly summarized below.

16S rRNA based molecular tests on environmental samples typically involve:

- DNA extraction from environmental samples
- amplification (copying) of specific DNA fragments using the polymerase chain reaction (PCR)
- separating unique DNA fragments within the amplified total DNA
- sequencing the DNA to determine the nearest phylogenetic relative

Total microbial DNA is extracted directly from environmental water or solid samples, typically using commercially available extraction kits. The DNA can be immediately analyzed or may be archived (frozen) for subsequent manipulations. Extracted DNA is

amplified using the polymerase chain reaction (PCR) a DNA copying technology widely described in the literature (Ausubel et al., 1992).

PCR assays often make use of or specific primers (short synthetic pieces of DNA that initiate the point of DNA replication) that only amplify 16S genes of particular organisms (Löffler et al., 2000, Hendrickson et al., 2002; Hristova et al., 2001). In the case of specific primers a successful PCR reaction indicates the presence of the organism, whereas an unsuccessful PCR indicates its absence. Specific primers can also be used to quantify specific organisms of interest.

Alternatively, PCR testing with universal primers amplifies the 16S rRNA genes from all bacteria present (Marchesi et al., 1998, Muyzer et al., 1993). Universal primer methods are useful for determining the overall microbial composition of samples. In this case each individual type of PCR product (each produced by a specific bacterial species) must be separated from the pool of PCR amplification products (amplicons) in order to obtain interpretable data. The most commonly employed DNA separation methods are denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993), amplified ribosomal DNA restriction analyses (ARDRA), and terminal restriction fragment length polymorphism analysis (tRFLP). These techniques produce a pattern of gel bands (or fluorescence peaks in the case of tRLFP) each corresponding to a unique PCR amplicon. In some cases these fragments can be purified and the DNA sequenced at commercial facilities. Molecular cloning (Maniatis et al., 1989) is ideal for generating high quality DNA sequences, however, it is also the most expensive and time consuming to perform. The sequences generated by the above methods may be used to screen gene databases (e.g., GenBank at National Institutes of Health) to compare them to tens of thousands of published microbial 16S rRNA gene sequences which then allows the identification of the organism in question by relating it to the known organisms in the database.

4 MTBE MNA DATA EVALUATION AND PRESENTATION

This section discusses procedures to evaluate the predominant physical, chemical, and biological processes that cause the natural attenuation of MtBE and other fuel oxygenates. In addition, presentation techniques commonly used for evaluating the natural attenuation of MtBE are discussed. Examples of data evaluation and presentation tools and techniques are included as appendices to this document, and are referenced below.

4.1 Tiered Approach for Evaluating the Natural Attenuation of MtBE

This section generally follows the flow chart provided in Figure 2-1. As discussed in Section 2, the tiered approach described in this protocol relies on one or more of three converging lines of evidence (Tiers) to evaluate the natural attenuation of MtBE. Tier 1 involves the evaluation of hydrologic and contaminant data trends. Tier 2 involves the collection and analysis of geochemical data to determine if biological activity is occurring. Tier 3 includes collecting and analyzing supplemental data to help define specific microbiological natural attenuation mechanisms to understand plume behavior. The following sections describe data evaluation procedures and presentation techniques for each of the three tiers of data.

In addition to guiding the evaluation of MNA, the tiered approach described in this protocol provides for development of the CSM, which is the fundamental building block upon which any remedial strategy is developed. Accordingly, the CSM is critical in assessing the viability of monitored natural attenuation for the remediation of MtBE.

A CSM may be effectively represented by a schematic or diagram (Figure 4-1). Although the number of schematics used must be limited, at times they are indispensable to describe new concepts. Schematic usage is a function of the audience as well as the processes depicted. Community groups or other lay people may appreciate a basic hydrogeologic cycle schematic including MtBE as it moves through the various parts of the cycle. An aquifer sketch may be used to depict MtBE migration and fate, as well as potential receptors.

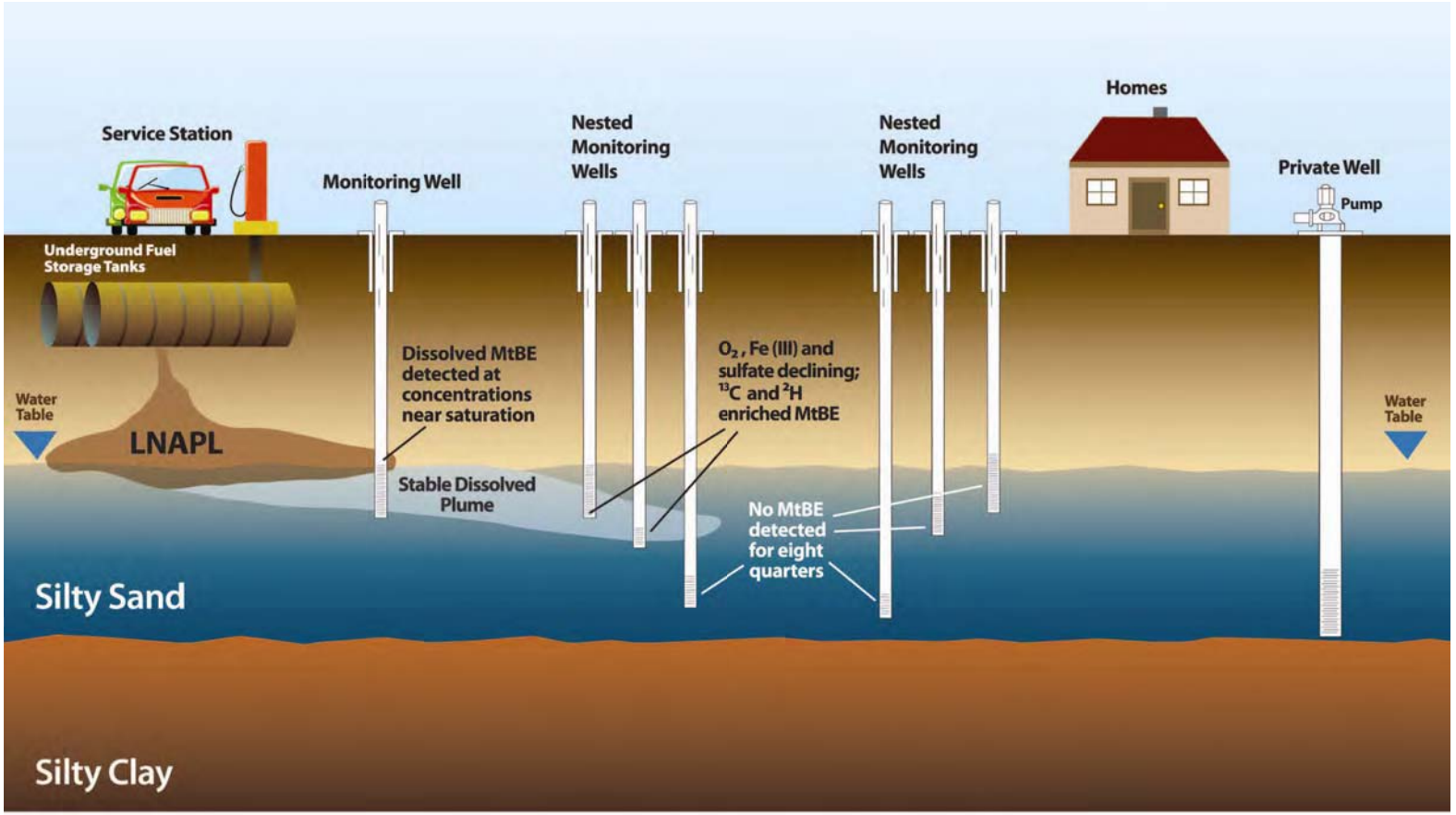


Figure 4-1 Example Conceptual Site Model

4.2 Tier 1 Data Analysis

The Tier 1 data analysis is one of the initial steps in creating the CSM and is focused on describing and evaluating plume behavior. The foundation for Tier 1 analysis is a strong understanding of the groundwater flow characteristics of the site. This understanding is particularly important because variation in the direction and velocity of groundwater flow is an important control on the distribution of contaminants.

After the hydrogeology of the site is understood, both qualitative and quantitative techniques can be used to evaluate source strength and plume behavior. In addition, the ratio of TBA to MtBE can be used to support an initial assessment of whether degradation of MtBE is evident. Similarly, mass flux analyses may be used in Tier 1 analysis to evaluate the natural attenuation of MtBE. Calculation of bulk attenuation rates may also be completed during Tier 1 analysis to help quantify the rate at which MtBE is attenuating. Ultimately, some mix of qualitative and quantitative techniques likely will be used to characterize plume behavior and complete a Tier 1 analysis.

4.2.1 Hydrogeologic Evaluation

The evaluation of site-specific hydrogeologic conditions is described in many prior documents (Nichols et al., 2000; Wilson et al., 2005a; Wiedemeier et al., 1995). The following tasks should be included in the hydrogeologic evaluation:

- assessment of groundwater elevations, flow directions and gradients over time;
- preparation of groundwater elevation contour maps;
- preparation of hydrogeologic cross-sections;
- identification of the location of potential receptors relative to the plume; and
- evaluation of the source, nature and extent of contamination including:
    - nature and history of the contaminant release;
    - evaluation of historic contaminant analytical data; and
    - three-dimensional distribution of NAPL and dissolved contamination.

To assist with hydrogeologic data evaluation, the EPA has published a software program called the Optimal Well Locator (OWL) to help evaluate groundwater flow directions from existing monitoring well networks and to assist in the selection of new monitoring well locations. This program can be found at http://www.epa.gov/ada/csmos/models/owl.html. The OWL program uses groundwater elevation measurements to evaluate variations in ground-water flow magnitude and direction over time and to calculate corresponding plume migration paths.

4.2.2 Hydrogeologic Data Presentation

Data presentation techniques that are useful for illustrating the flow characteristics of the site include traditional groundwater contour plans, rose diagram plots and cross sections. While groundwater contour plans allow rapid visualization of potential flow pathways, each plan represents only one set of measurements, and does not provide information on the variability in gradient, which can result in flow pathways that are substantially different than those interpreted from any one contour plan. Until historical data are available that demonstrate otherwise, the potential for variations in hydraulic gradient over time should be considered when evaluating contaminant migration pathways.

A rose diagram plot illustrates the variability of horizontal hydraulic gradients and direction over time and may be overlain on a location map of monitoring wells. Rose diagram plots are constructed by measuring the direction and magnitude of the hydraulic gradient at a particular location at a number equally spaced time intervals, and representing these measurements graphically. Typical types of rose diagram plots are shown in Figures 4-2a and 4-2b.

Figure 4-2a illustrates a groundwater contour map generated based on current quarterly sampling results and a rose diagram plot of historical groundwater flow direction and gradients from 34 different sampling events.

Figure 4-2b illustrates the use of vectors to show changes in groundwater flow direction over eight quarters of monitoring in a rose diagram superimposed directly on the Site map. Each vector represents one quarterly monitoring event. The lengths of the vectors are proportional to the gradient (and thus groundwater seepage velocity) and equal to the distance groundwater would travel in one year.

Rose diagram plots allow an assessment of the adequacy of the monitoring network by identifying important migration pathways where monitoring points may be sparse or absent. They also provide a practical and concise summary of historical groundwater flow patterns that may be used in discussing the efficacy of MNA with regulators or multiple stakeholders, and eliminate the need for using numerous groundwater elevation contour maps or tables of data to illustrate variations in groundwater flow characteristics.

Cross-sections are useful for displaying hydrogeologic data and evaluating vertical hydraulic gradients. A cross section should illustrate the lithology of the various formations present, monitoring well/boring positions and total depths, depths of monitoring well screen intervals, current and historical high and low groundwater table elevations, potentiometric contours, analytical results of selected contaminants of concern, and all relevant site-related infrastructure such as drain lines and other utilities. Superimposing these various data is necessary to develop an adequate understanding of the effects of hydrogeology on contaminant transport, and the importance of vertical flow

and transport. Cross sections are also useful for identifying the proper well screen intervals for additional well installations.

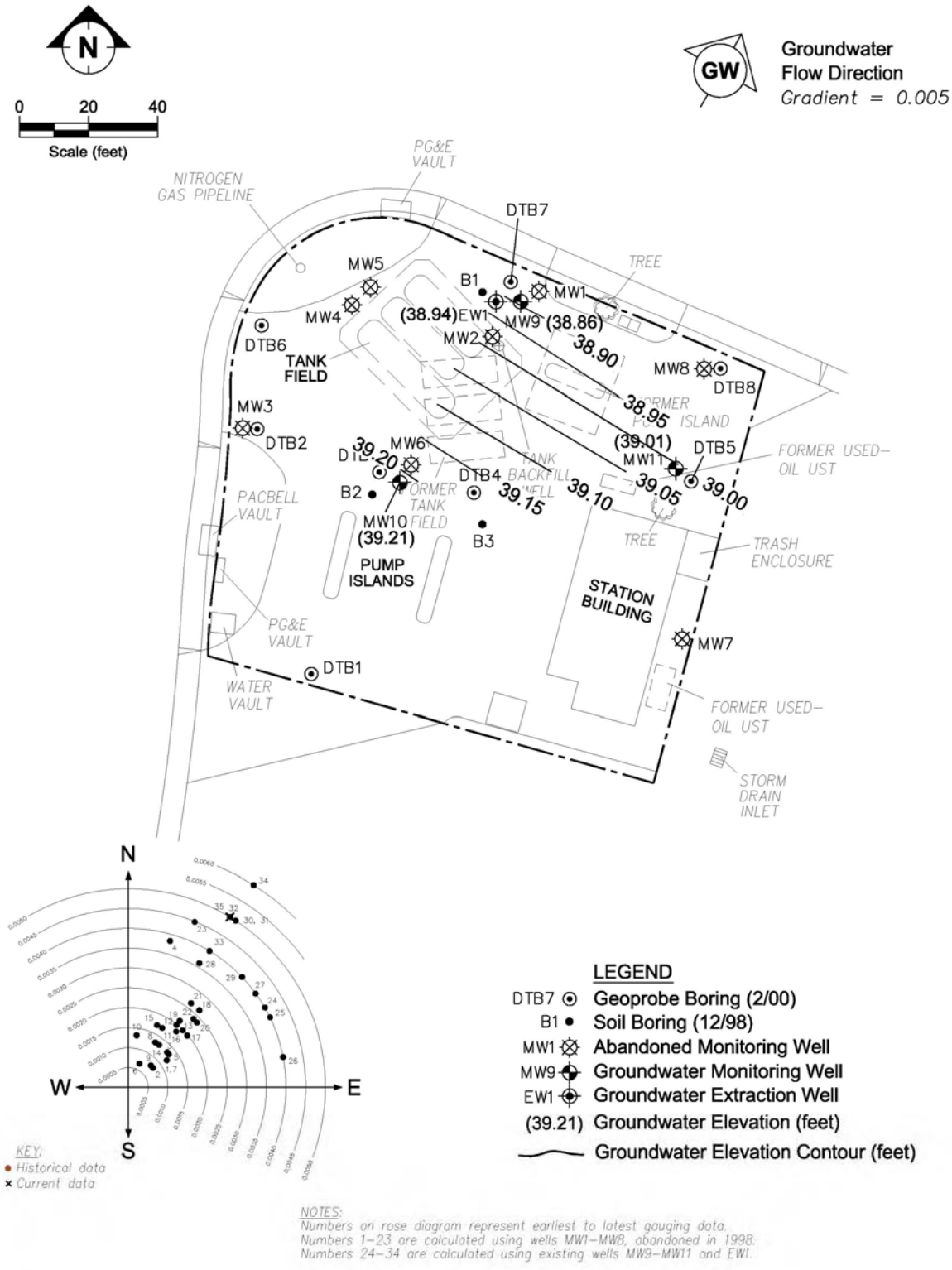


Figure 4-2a Example Rose Plot

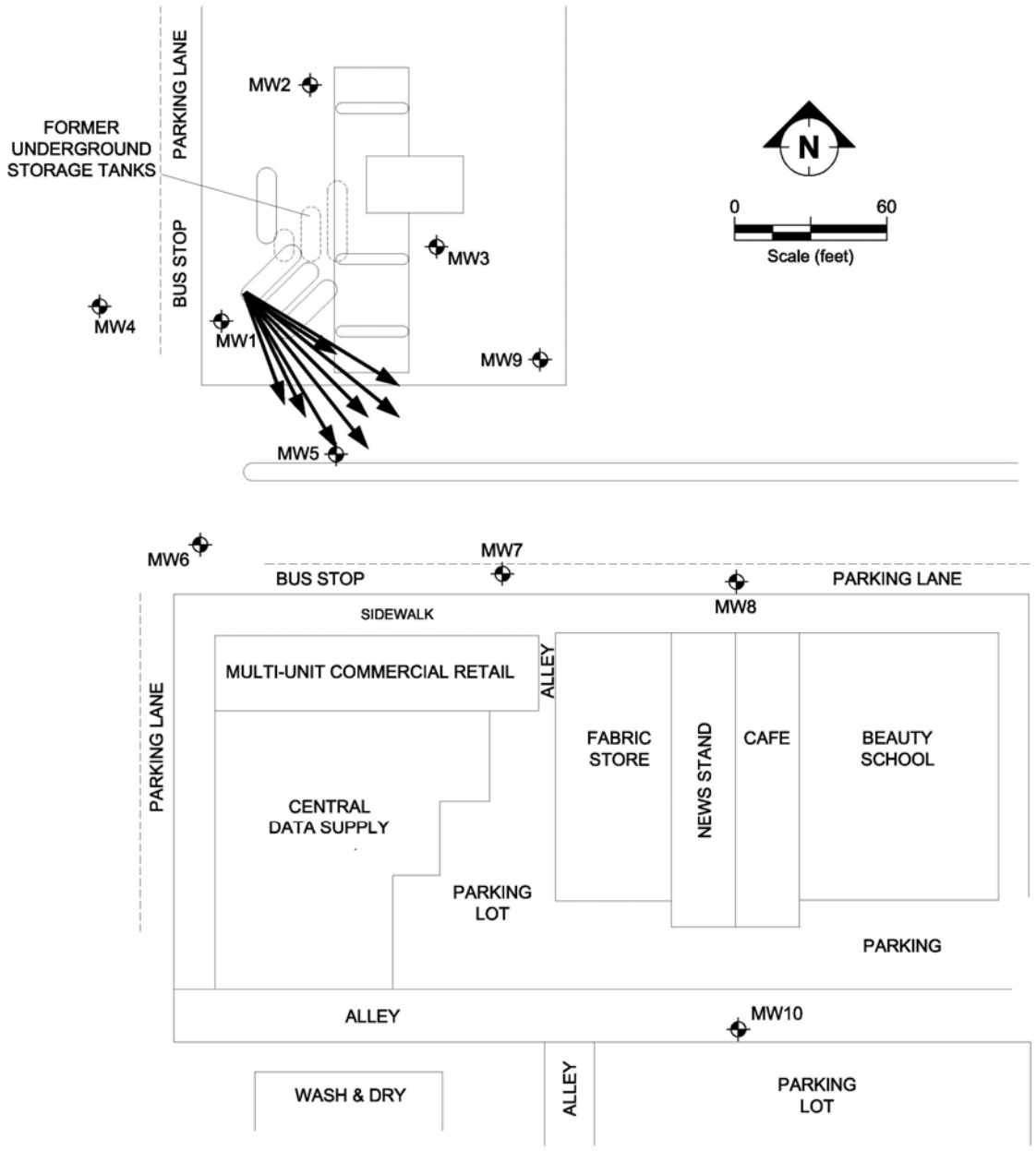


Figure 4-2b Example Rose Plot

4.2.3 Evaluation of Source Strength and Composition

A NAPL source may be evaluated to estimate plume stability and remediation timeframes. Chemical analyses of the NAPL can also be helpful in establishing the composition of the oxygenated fuel released at a site. A prior API publication has provides additional useful guidance on MtBE source zone evaluation (Nichols et al., 2000).

4.2.3.1 Source Strength

At most petroleum release sites, in the absence of source removal actions, the rate of contaminant attenuation in the source area is slower than the rate of attenuation in locations down gradient from the source area. In these cases the longevity of the plume is controlled by the attenuation rate in the source area. The concentration of MtBE in groundwater in contact with gasoline in the source area can be estimated using Raoult's Law as follows:

$$C_{MtBE-w} = C_{MtBE-f} \cdot \left[\frac{MW_f}{MW_{MtBE}} S \right]$$

where:

$C_{MtBE\text{-}W}$ = concentration of MtBE in groundwater;

$C_{MtBE\text{-}F}$ = concentration of MtBE in the fuel (g/g);

MW_f = average molecular weight of the fuel (~90.1 g/mol);

MW_{MtBE} = molecular weight of MtBE (88.15 g/mol); and

S = pure chemical aqueous solubility of MtBE (50,000 mg/L)

For example, for gasoline containing 11% MtBE the estimated MtBE concentration in groundwater in equilibrium with this gasoline is 5,620 mg/L

A more relevant estimate of equilibrium MtBE concentration in groundwater near a NAPL source accounts for the limited volume of NAPL relative to groundwater:

$$C_{MtBE-w} = \frac{C_{o,f}}{K_{fw} + \frac{\theta_w}{\theta_f}}$$

where:

$C_{MtBE\text{-}W}$ = concentration of MtBE in groundwater in equilibrium with the NAPL phase;

$C_{o,f}$ = initial concentration of MtBE in the fuel (85,800 g/L for an MtBE content of 11%);

K_{fw} = nominal partitioning coefficient for MtBE between fuel and water (16, see Table 1-1);

Θ_w = water saturation; and

Θ_f = fuel (NAPL) saturation.

For example, soil with a porosity of 0.3 and a residual NAPL saturation of 10% or 0.03 would result in a lower equilibrium MtBE concentration of 1,560 mg/L.

Concentrations of MtBE in groundwater as high as either of these predictions are rarely observed in the subsurface, and are typically 10 to 100 times lower (Wilson et al., 2005a). The reasons for this discrepancy include:

- the prevalence of lower (<10%) residual NAPL saturations in source areas;
- preferential dissolution or volatilization of MtBE from the NAPL over time which acts to decrease the *in situ* MtBE content;
- mass transfer limitations that prevent equilibrium conditions from developing in the monitoring zone; and
- biological activity occurring in the source zone that acts to reduce the concentration of MtBE in groundwater.

Therefore, one should not assume that a residual NAPL results an MtBE source to groundwater that is similar to its effective solubility limit. When source zone monitoring data are available, source strength may be evaluated by plotting concentration profiles of source zone wells over time as explained in Section 4.2.4.

Although TBA may be released as a trace constituent in fuel-grade MtBE, TBA is not known to be currently used as a significant gasoline additive in the USA.[6]At sites where high concentrations of TBA relative to MtBE are detected in groundwater in the source area TBA may in fact be a transformation product of MtBE (see Section 4.2.4.2).

4.2.3.2 NAPL vs. Vapor Sources

It is important to distinguish the type of source (NAPL versus vapor) in developing the CSM and making natural attenuation decisions. If NAPL is not in contact with groundwater, it is more slowly depleted of soluble compounds such as MtBE. Figure 4-3 illustrates monitoring well data for dissolved constituents emanating from a NAPL source. In this case, BTEX and MtBE concentrations trend together. This “railroad tracks” pattern suggests dissolution from NAPL that retains each of the soluble constituents. In contrast, the MtBE concentrations in Figure 4-4 are significantly greater

[6] In the evaluation of source area or NAPL chemistry, careful attention should be paid to typically elevated detection limits, which can mask the presence of minor or low solubility components of the release.

than those of BTEX; MtBE concentrations also vary dramatically over time. The concentration trends shown in Figure 4-4 suggest that the dissolved constituents in the vicinity of the monitoring well resulted from a vapor and/or leachate, or a small volume release. MtBE's partitioning characteristics (i.e., vapor pressure and Henry's Law constant) can generate solute concentrations exceeding 1 ppm, while the BTEX compounds (lower vapor pressures and higher Henry's Law constants), if detected at all, are found at concentrations below 100 ppb (Lahvis and Rehmann, 2000; Dakhel et al., 2003).

4.2.4 Techniques for Evaluating Plume Stability

Tier 1 data can be evaluated both qualitatively and quantitatively. Several methods for evaluating plume stability are discussed in the following subsections.

4.2.4.1 Temporal and Spatial Trends in Contaminant Concentrations

Contaminant concentration data should be evaluated with regard to temporal and spatial trends (plume stability). The use of plots of concentration versus time and concentration versus distance and isopleth maps can facilitate evaluation of plume behavior and the potential for MtBE attenuation. Evaluation of TBA/MtBE ratios can also provide insight into potential degradation processes.

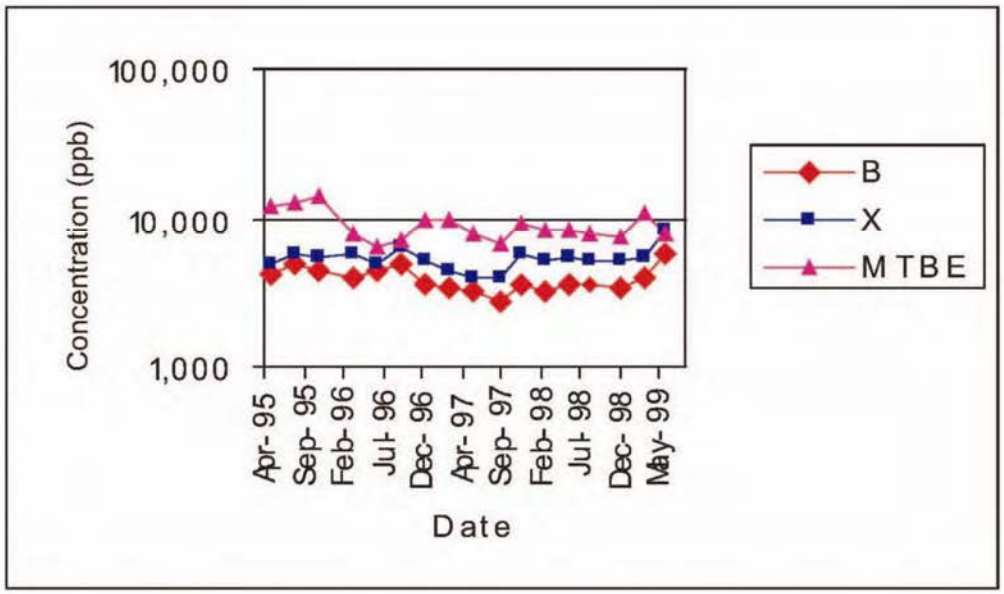


Figure 4-3 Concentrations of MtBE, Benzene, and Xylene in Groundwater in a Monitoring Well with a NAPL Source

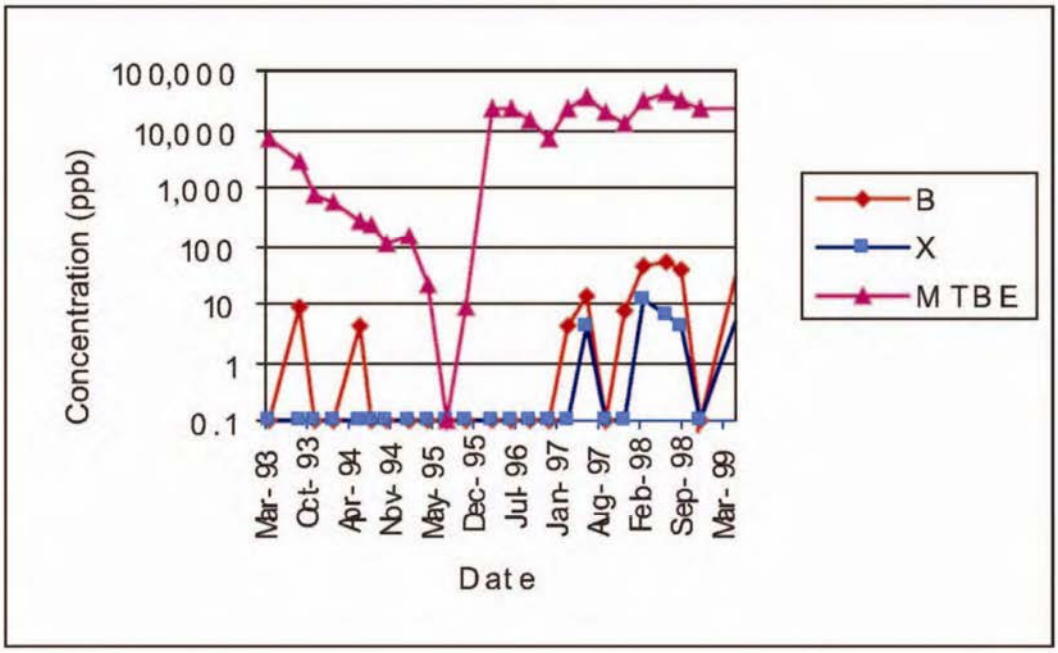


Figure 4-4 Concentrations of MtBE, Benzene, and Xylene in Groundwater in a Monitoring Well with a Vaport Source

4.2.4.1.1 Plots of Concentration versus Time or Distance

Plots of MtBE concentration versus time and distance may be particularly useful for pattern identification, and are typically the basis for the evaluation of plume stability. Figure 4-5 illustrates a typical plot of concentration vs. time. The following should be considered when plotting concentration versus time and distance data:

- trend plots must contain at least three data points; however, five or more points are preferable to evaluate temporal data trends;
- when only several data points are available, bar charts or box plots are best suited to describe temporal relationships. An example of appropriate time trend bar chart is a plot with two time points: time equals zero (t_0) and time equals final (t_f);
- multiple X-axis scales (i.e., distance and travel time equivalent) can help illustrate the evolution of groundwater quality along the flow path. Important features/events should be indicated on these scales (i.e., locations of barriers, sources, pumping wells, timing of initiation of remedial measures, etc.);
- plotting groundwater elevations along with concentration data on multiple Y-axis scales facilitates assessment of the impact of fluctuating water levels on concentrations of COCs;
- a vertical line plot illustrating well screen and filter pack elevations is useful to ensure that monitoring well(s) are properly constructed;
- plotting the concentration data on a logarithmic scale against time or distance on an arithmetic (linear) scale may be necessary to capture both large changes (i.e., thousand-fold) changes in concentration while still capturing smaller variations that may be important in distal portions of the plume or at later times;
- if plotting concentration over distance from multiple well locations it may be useful to plot mean concentrations for sub-groups of wells at similar travel times from the source to depict general trends while averaging out natural variability;
- plot data only from those wells within the geochemical footprint of the plume;
- plot MtBE and its intermediate transformation products, such as TBA;
- it is important that the degradation of other chemicals that may indicate the occurrence of natural attenuation and/or biodegradation is not overlooked. For example, if benzene is present in the system, then its behavior should be evaluated;
- when plotting concentration data from individual monitoring wells it is important to ensure that the data are from monitoring wells located along similar flow paths (e.g., centerline of the plume);

- bar charts are useful to depict multiple chemical concentrations for different sampling locations in concentration versus distance plots. When plotting concentrations, a different bar should be used for each individual compound (e.g., MtBE and TBA). However, when plotting proportions on a percent scale (0 to 100%), then a single bar may be used for multiple compounds, with each bar segmented to represent the proportion of each compound present; and
- plotting MtBE and BTEX concentrations from source zone wells versus time will help assess nature of source, determine "source strength" and possibly the estimated source duration.

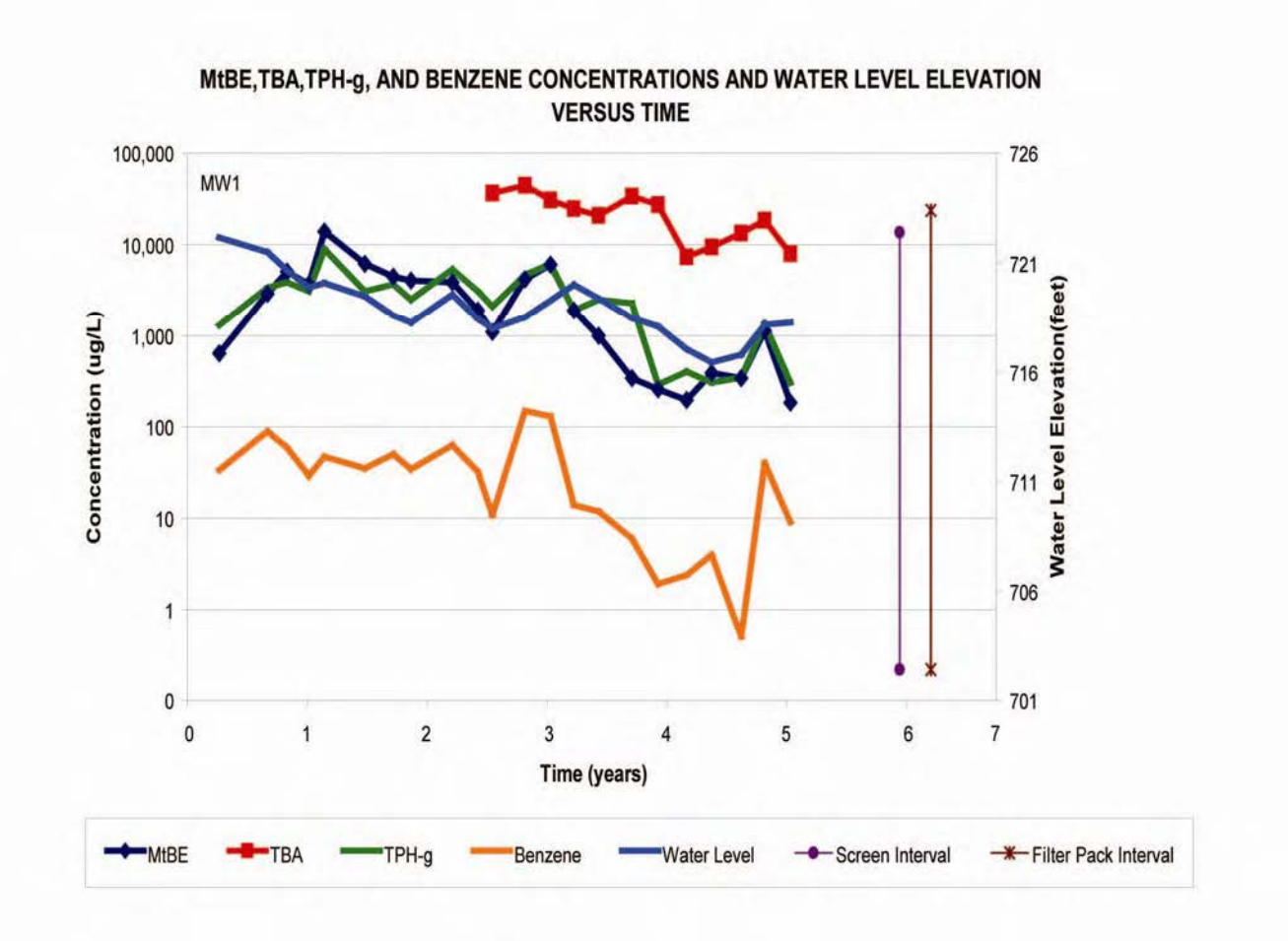


Figure 4-5 Typical Plot of Concentration vs. Time

4.2.4.1.2 Isopleth Maps

Isopleth maps showing the spatial distribution of MtBE and its transformation products can be prepared. Where historic data are available, a series of maps can be prepared to show the change in distribution of dissolved chemicals over time. Mass-in-place estimates can also be derived from isopleth maps (i.e., based on the average chemical concentration over an interpolated area) and used to calculate attenuation rates as described in Section 4.2.4.5. Maps using samples collected at specific depths can be used

to show the variation in spatial distribution with depth and the depth of maximum contaminant (or solute) concentration.

Seasonal variations in concentrations should be taken into account and, when appropriate, data used for isopleth map preparation should be collected during the same season of the year, usually when solute concentrations are at their maximum. Example isopleth maps based on multiple monitoring events are shown in Figure 4-6.

The following should be considered when developing isopleth maps:

- isopleth maps should be generated for each COC and time interval of interest. They are not the venue for presenting all available site data. Rather, isopleth maps are used for comparing plume morphology over time, and are typically focused on specific compounds within specific time frames;
- color may be useful in differentiating between contours;
- dashed lines should be used to illustrate uncertainty in the contours;
- contouring is an interpretive procedure (mathematical or subjective) where point measurements are converted to two-dimensional (or three-dimensional) representations of subsurface features. Contouring software has made mathematical contouring easier and more common. These mathematical algorithms can help the investigator use data to the fullest extent possible, and provide the "best" interpretation of the data from an objective mathematical or statistical perspective. The disadvantage of these techniques is that they typically do not consider groundwater flow characteristics or other site features, and can result in interpretations that defy hydrogeologic common sense. Users must carefully scrutinize the input data and understand the assumptions or mathematical algorithms inherent to the contouring software. The output of contouring routines must often be altered using engineering judgment to obtain a useful interpretation of the data;
- if flow paths are highly three-dimensional (i.e., migration occurs vertically between layers or across a thick stratigraphic unit), care should be used when representing data in plan view. In this case, cross section isopleth maps may be useful to infer migration pathways and evaluate plume dynamics; and
- in addition to isopleth maps, pie charts representing the relative proportion of several chemicals in a groundwater sample can be overlain on plan view maps for specific time intervals. Such maps may be particularly useful to visualize the evolution of groundwater quality along migrations paths from the source area. The overall size of each pie chart can also be scaled to indicate the total concentration range. Although such scaling is useful for illustrative purposes, scaled pie charts should not be used for quantitative interpretation of concentration data.

Visual inspection of plotted data for concentration versus time and/or distance or isopleth maps can yield qualitative information on the presence of trends that are indicative of solute plume behavior. While such plots and maps are recommended for most plume stability analyses, discerning trends in the plotted data through visual means is a subjective process, particularly if the data are variable. Quantitative trend analyses may also be warranted, as discussed below.

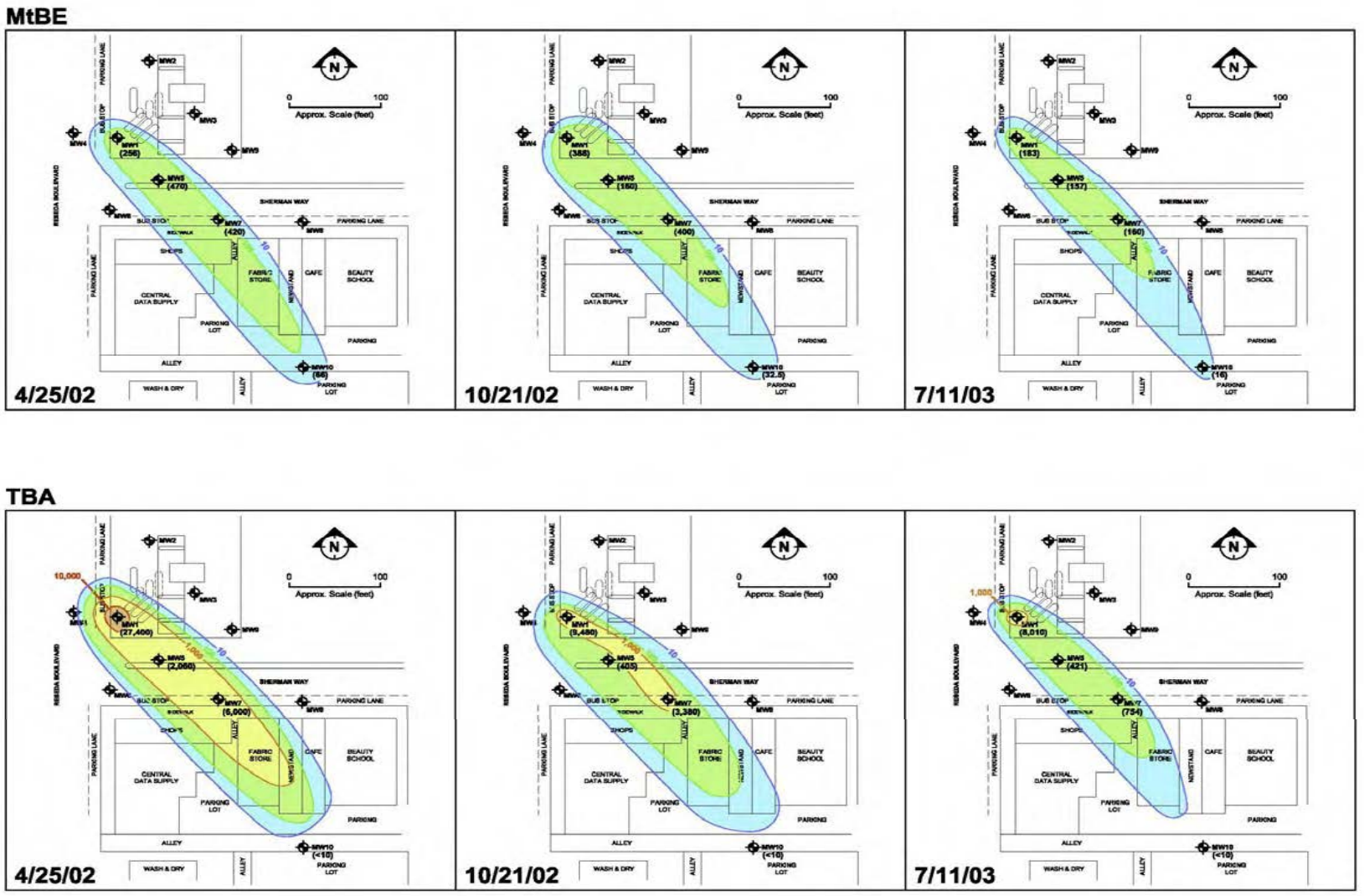


Figure 4-6 Typical Isopleth Maps

4.2.4.2 TBA/MtBE Ratios

Because MtBE may be metabolized to TBA under both aerobic and anaerobic conditions, increases in the TBA/MtBE ratio can be used in some cases to infer transformation of MtBE to TBA. If TBA is not present in the oxygenated fuel that was released, then any detection of TBA provides direct evidence of MtBE biodegradation.[7] As explained in Section 4.2.3, TBA, although present as a trace impurity in MtBE, is not currently used in the United States as a gasoline additive (DeVaull et al., 2003). Based on measured fuel-water partitioning coefficients for MtBE and TBA and the typical range of TBA concentration in fuel, the possible range of TBA concentrations can be predicted similar to the method used in Section 4.2.3. Figure 4-7 shows hypothetical source concentrations of MtBE and TBA as a function of NAPL saturation for various TBA concentrations in fuel resulting from its presence in MtBE. As apparent in this figure, expected source TBA concentrations are low, and substantially lower than corresponding MTBE concentrations for reasonable values of residual NAPL saturation (1 – 10%, or 0.003 to 0.03). Note that these hypothetical predictions are subject to the same caveats cited in Section 4.2.3-actual source area concentrations are typically lower than predicted.

Increasing ratios of TBA to MtBE (especially TBA/MtBE greater than 1), either over time at a single location or between two hydraulically connected locations, also provides evidence for biodegradation, even if TBA was present in the released fuel (Zwank et al., 2002; Wilson et al., 2005a). Plots of TBA/MtBE concentration ratios over distance from the source or over time in individual locations may indicate a time interval or location where a significant shift in ratio is observed and conversion to TBA is therefore evidenced. However, lack of increasing TBA to MtBE ratios or even lack of detection of TBA does not mean that MtBE biodegradation is absent. At some sites, TBA may degrade more quickly than MtBE. Where this is the case, TBA may not accumulate to any significant degree; even though the parent MtBE is continuously degrading.

[7] As noted earlier, elevated detection limits, especially near NAPL source areas, can mask the presence of minor source components such as TBA.

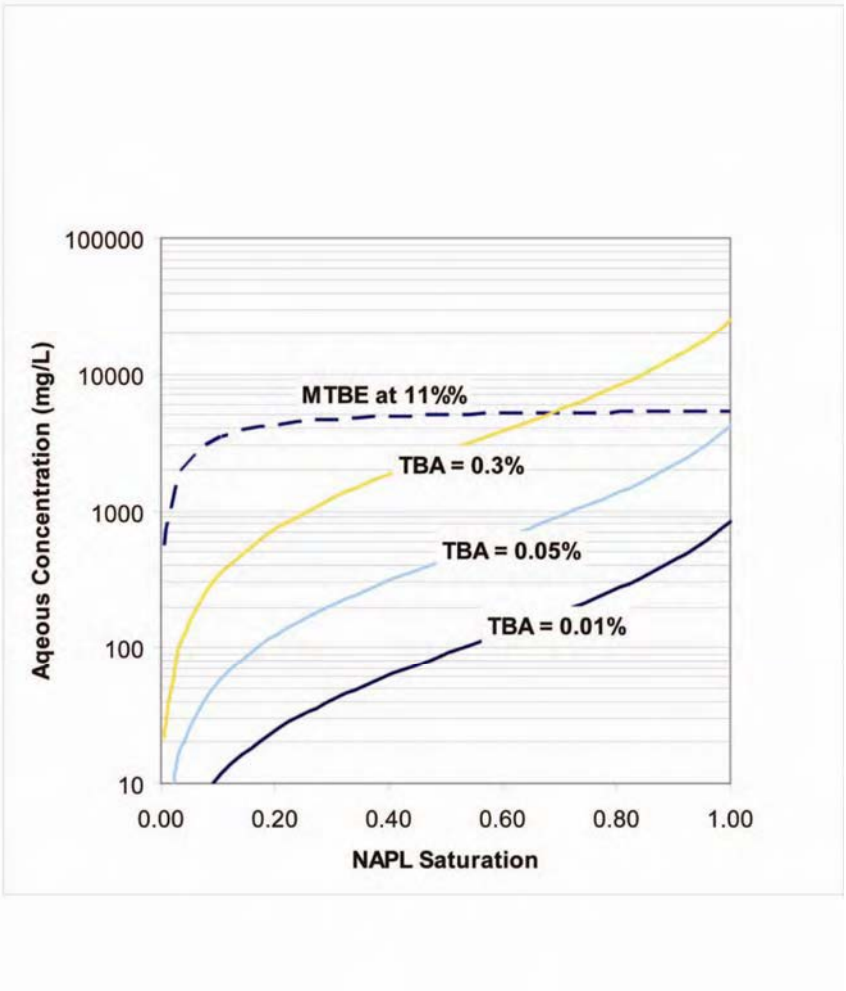


Figure 4-7 Hypothetical MtBE and TBA Source Area Concentration as a Function of NAPL Saturation and TBA Content of Fuel (MtBE at 11% in Fuel, porosity = 0.3, KMTBE = 16, KTBA = 0.24).
Adapted from Rixey et al., 2004 (Appendix A).

4.2.4.3 Statistical Methods

Statistical methods for identifying trends in concentration data are powerful tools that provide an unbiased evaluation of time-series or distance-series data. The use of non-parametric techniques is generally preferred for environmental concentration data and some commonly used methods are described briefly below. Non-parametric statistical methods do not depend on assumptions regarding the underlying data distribution and can accommodate missing data points, outliers, and non-detect values that are common in groundwater concentration data sets. These methods rely on the relative magnitudes of the data rather than the actual values and are fairly straight-forward to use. A general consideration for the use of statistical methods in identifying trends and evaluating solute

plume behavior is that statistical significance does not necessarily imply real-world significance and statistical test results can provide a false sense of assurance regarding conclusions (Barden, 2003). It is important to always relate statistical results and evaluation back to the physical problem in the field to ensure that the results are meaningful.

The Mann-Whitney U test, also called the Wilcoxon rank sum test, is a non-parametric analog of the Student's *t*-test that is based on the relative ranks of the data points rather than the actual concentration values. This method tests whether measurements from one population are consistently larger or smaller than those from another population. The typical application of the Mann-Whitney test is to compare concentrations from individual monitoring points for one time period to those for another time period (e.g., quarterly monitoring results for one year to those for another year). Such a comparison can identify whether a group of variable concentration data demonstrate a decrease or increase relative to another group.

The Mann-Whitney test is based on two independent populations. However, in some cases, the data for the two populations can be considered paired by "seasons" and are not really independent. In such situations, a paired-sample test, such as the "Sign test" or the "Wilcoxon signed rank test" (not to be confused with the Wilcoxon rank sum test), might be more appropriate (Gilbert, 1987).

The Mann-Kendall test for trend is used to determine the presence or absence of a trend in concentration over time or distance for individual monitoring points. It is a test for zero slope of time- or distance- ordered data that is based on a non-parametric analog of linear regression. The basic methodology and its variants (such as the Seasonal Mann-Kendall test) are described in Gilbert (1987). Four or more independent sampling events (or locations) are required to implement the Mann-Kendall test. The results of the Mann-Kendall test indicate the presence or absence of a statistically significant increasing or decreasing trend in concentrations over time at a monitoring point (or over distance at multiple points). These results can be used to help evaluate whether the solute plume is shrinking, expanding, or stable. A general consideration for using the Mann-Kendall test is that the absence of a significant increasing or decreasing trend can not be interpreted to mean that the plume is stable (Barden, 2003).

A useful variation on the Mann-Kendall test is a test for Homogeneity of Stations. This test pools the results for tests at individual monitoring points and allows conclusions to be made about consistency of trends throughout the plume or portions of the plume (e.g., whether the trends at all monitoring points are in the same direction; i.e., all increasing or all decreasing). Such a general statement about the presence or absence of monotonic trends is useful for making interpretations of the overall behavior of the entire plume or

specific portions of the plume. Appendix F provides a step by step approach for evaluating concentration trends using the Mann-Kendall approach.

Sen's nonparametric estimator for trend (Sen, 1968; Gibbons and Coleman, 2001; Gilbert, 1987) is closely related to the Mann-Kendall test, and is applied on similar time- or distance- ordered data sets. It may be applied for linear [y = c] or log-transformed [y = ln(c)] data. In addition to a test for zero slope, it also yields an estimate of slope. Sen's test is not greatly affected by outliers or gross data errors. In application, for an ordered series of values [x, y], a set of slope estimates $(y_j - y_i)/(x_j - x_i)$ is calculated for all data pairs with $x_j > x_i$. The median value of the distribution of slopes is taken as the best estimate of slope. A two-sided confidence interval on the slope is estimated from a calculated variance. A one-sided confidence interval is used in testing for zero or decreasing slope.

4.2.4.4 Mass Flux Evaluation

As discussed in Section 2.4, an evaluation of mass flux can be very useful for evaluating natural attenuation. This section describes the mathematics of mass flux calculations and the data required to complete them. An example mass flux calculation is provided in Appendix C. In addition, Buscheck (2002), Nichols and Roth (2004), and Buscheck et al., (2003) present examples of mass flux calculations, and API has provided detailed guidance in prior publications (Newell et al., 2003).

The flux of water across a given cross-sectional area of an aquifer is given by Darcy's Law which states:

$$Q = K \cdot i \cdot A$$

where:

Q = Hydraulic Flux [L^3/T]

K = Hydraulic Conductivity [L/T]

i = Hydraulic Gradient [L/L]

i = Cross-Sectional Area of Flow [L^2]

The cross-sectional area of flow, *A* is given by:

$$A = w \cdot b$$

where:

A = Cross-Sectional Area of Flow [L^2]

w = Width of Flow [L]

b = Depth of Flow, or Saturated Thickness [L]

Given the above, the mass flux across a specified cross-sectional area is calculated as:

$$MF = Q \cdot C$$

where:

MF = Mass Flux [M/T]

Q = Hydraulic Flux [L^3/T]

C = Average Total VOC Concentration Across Cross-Sectional Area of Flow [M/L^3]

Multiplying the flux of water, Q, by the average concentration of a given contaminant across the cross-sectional area of flow gives the mass flux in units of mass per unit time. An estimate of the amount of mass lost (or gained) between two cross-sectional areas of flow (transects) along the same flow path is given by subtracting the mass flux across the downgradient transect from the mass flux across the upgradient transect. The rate of change in plume mass flux over time may be estimated from sequential estimates of flux at the same transect. In order to more accurately estimate the mass flux, the plume should be discretized along transects oriented perpendicular to the direction of groundwater flow. When this is done the relationship expressing the total mass flux becomes:

$$MF_{total} = \sum_{i}^{n} Q_i \overline{C_i}$$

where:

MF_{total} = Total Mass Flux Along Transect [M/T]

Q_i = Hydraulic Flux Across Area i [L^3/T]

$\overline{C_i}$ = Average Solute Concentration Across Cross-Sectional Area of Flow i [M/L^3]

The transect approach for estimating mass flux is typically used for natural attenuation evaluations. This approach requires at least two (2) transects oriented perpendicular to the direction of solute transport, which is typically the same as the direction of groundwater flow. Each transect should delineate the entire width and depth of the solute plume and as much as practical should enable horizontal and vertical discretization of the plume. Figure 4-8 is a simplified example of how to discretize a plume transect. Because of the nature of environmental characterization, the horizontal distribution of contaminants is typically better known than the vertical distribution. If the well screen intercepts the entire vertical depth of the plume at a point, then the horizontal distribution at a point will

be an average of the vertical distribution, thus making the mass flux calculation a fair approximation.

Because it is directly proportional to the amount of groundwater flux and contaminant concentrations, an accurate estimate of mass flux is dependent on the quality of site characterization data, including both hydrogeologic and contaminant data. Aquifer heterogeneity will complicate the calculation and should be taken into account. Also, if the plume has a three-dimensional monitoring network with wells screened at different depths in the aquifer, then a more detailed discretization can be considered. In general, the more data available to discretize the site, the more accurate the mass flux calculation.

4.2.4.5 Presentation of Mass Flux Data

Estimating mass flux estimates over time and space is a useful method of assessing plume stability and potential impacts to receptors. The following should be considered when presenting such data:

- to illustrate the “transect methodology” for estimating mass flux it is useful to use isopleth maps with the various transects overlain as shown in Figure 4-9;
- differences in mass flux across various transects over time may be illustrated as shown is Figure 4-9; and
- predominate flow direction as determined by potentiometric contours and rose diagram plots described in Section 4.2.2 should also be illustrated on these figures, as mass flux estimates are based on transects orthogonal to groundwater flow directions.

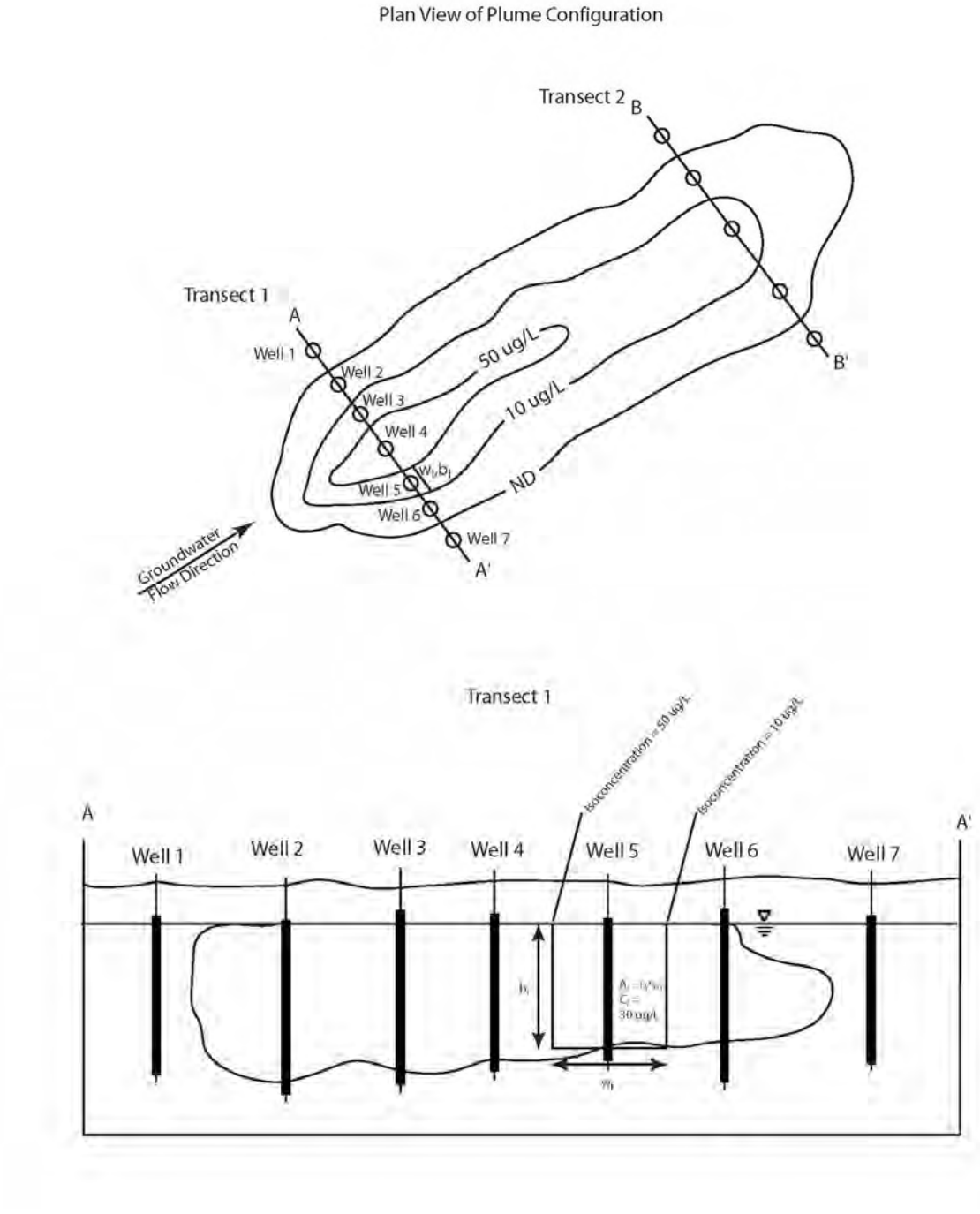
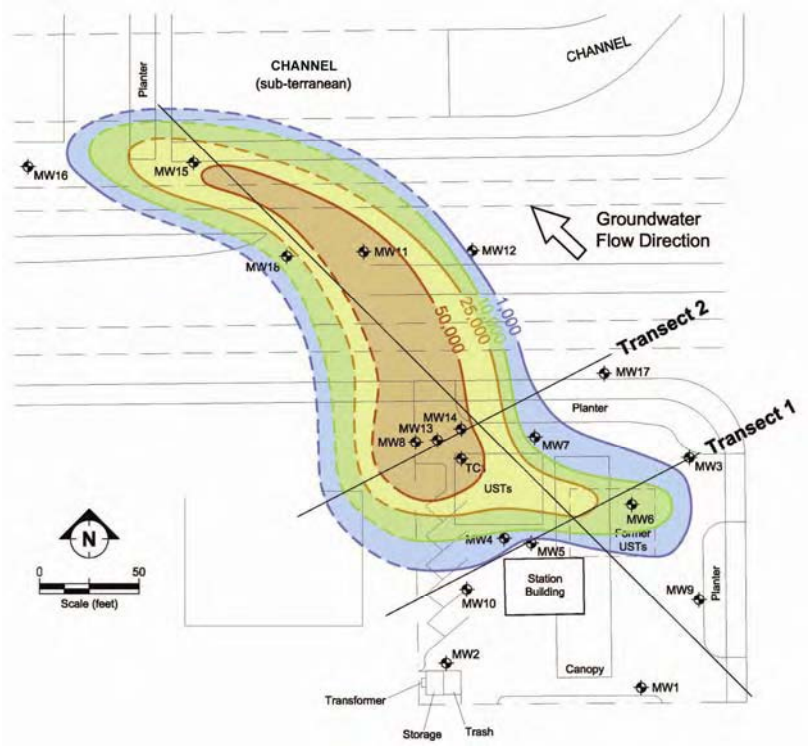


Figure 4-8 Example of How to Discretize a Plume Transect



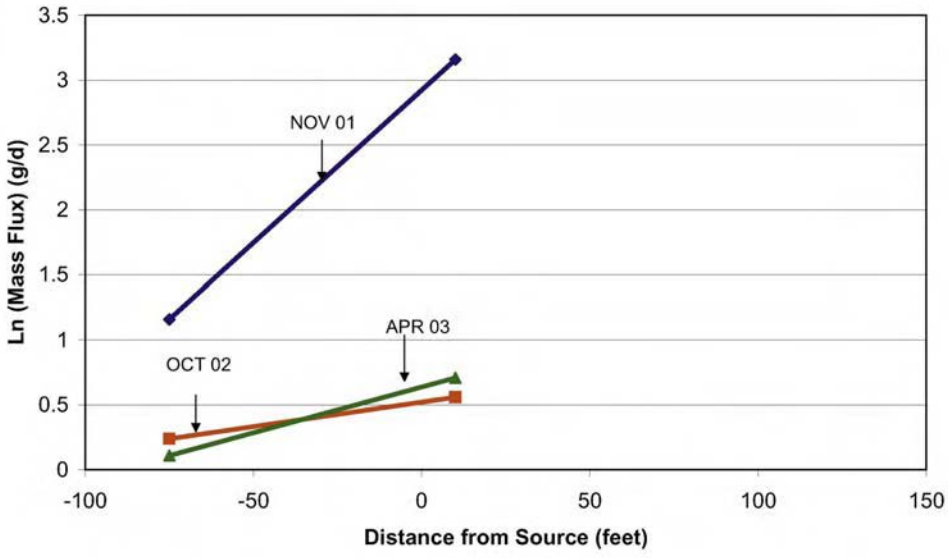


Figure 4-9 Presentation of Mass Flux Data

4.2.4.6 Estimating Natural Attenuation Rate Constants

A Tier 1 evaluation is typically focused on evaluating plume stability based on plume size, concentration, and/or mass through empirical means, without regard to the specific mechanisms responsible for attenuation. The data available for Tier 1 analysis typically are not sufficient to identify or quantify biological attenuation mechanisms. However, the action of such mechanisms may in some cases be inferred from Tier 1 data. If Tier 1 data are sufficient to demonstrate decreasing mass-in-place, a logical inference is that degradation is occurring. Decreasing mass may be apparent from a detailed monitoring network that allows calculation of mass-in-place estimates for several different times, or detailed hydrogeologic knowledge that allows quantitative evaluation of transport along flow paths and comparison to transport predictions derived from models. If the observed MtBE distribution is less extensive than predicted by a validated model, degradation may be inferred and its rate estimated.

Calculating MtBE attenuation rates is a site-specific endeavor. Typically, Tier 1 data are used to estimate a bulk attenuation rate for the compound of interest. Observed rates of attenuation reflect the combined influence of physical, chemical, and biological processes as summarized in Appendices A and B. For MtBE, observed bulk attenuation rates can yield over-estimates of the degradation rate because the contributions of other physical processes can be significant relative to biological mechanisms.

Isolating the effect of biodegradation on overall attenuation and quantifying its contribution requires measurement or estimation of other non-biological processes. Several methods have been described that allow the effects of multiple processes to be incorporated into rate estimation, and the effects of biodegradation distinguished. Simple transport models such as the method of Buscheck and Alcantar (1995) allow dispersion, retardation, and degradation to be simulated explicitly. Other methods simply apply a tracer correction to observed contaminant concentrations to distinguish physical processes of dilution and dispersion from degradation. These methods can be useful if an appropriate conservative (non-degradable) chemical has been co-released with MtBE. Briefly, the tracer correction to field measurements is implemented as follows:

$$C_{i,corr} = C_i \left[T_i / T_o \right]$$

where:

$C_{i,cor}$ = corrected contaminant concentration
C_i = measured contaminant concentration
T_i = measured tracer concentration at the same point along the flow path
T_o = the tracer concentration in the source area.

Use of models and tracer correction methods to estimate degradation rates is explained elsewhere and not described further here. The reader is referred to Newell et al., 2002 (http://www.epa.gov/ada/download/issue/ 540S02500.pdf), Wiedemeier, et al., 1996, Wilson and Kolhatkar, 2002 and USEPA, 1998 (http://www.epa.gov/superfund/ resources/ gwdocs/ protocol.htm) for a more detailed description of these approaches.

In many cases, it may not be important to discern the contributions of various different processes. Estimation of bulk attenuation rates can be accomplished using simple regression analysis of readily available data. Data required for such an analysis include concentration measurements located along a known flow path and an estimate of the travel times along that flow path, or time series concentration data for a source area or plume. It is important to only include data from monitoring wells that lie within the geochemical footprint of the plume and to ensure that the plume lies within the monitoring network for all time points used in the analysis.

For flow path analysis, point measurements or average concentrations must be calculated for points at known or estimated travel times along the flow path. Travel times can be estimated based on one of the methods identified in Appendix B. If data permit, use of average concentrations developed for monitoring fences (i.e., as used for mass flux monitoring) or groups of wells located at similar travel times along a flow path help mitigate the errors associated with uncertainty relative to flow path definition. However, this approach can also be applied to point measurements provided the hydrogeology is well described and sufficient data are available.

For time series analysis, a group of monitoring wells can be used to develop mass-in-place estimates for several points in time. An example of this approach can be found in a case study for the Eielson Air Force Base in Alaska (Dupont et al., 1997). Total plume mass can be estimated from contouring, kriging, or other method. This approach is relatively simple and is easily applied, and errors due to dispersion, dilution or poor flow path definition are minimized. Alternatively, data from individual wells can be used if located in the source area.

Buscheck and Alcantar (1995), Wiedemeier et al., (1996), USEPA (1998) Suarez and Rifai (1999), Wiedemeier et al., (1999), and USEPA (2002) contain an examples which illustrate calculation of first order attenuation rate constants from concentration data along a flow path or from mass-in-place estimates made for several times.

A basic understanding of regression analysis is required to ensure proper use of this technique. Attenuation processes are typically first order, resulting in an exponential decay curve. To help satisfy the assumption inherent in regression analysis that data are normally distributed, concentration data should be log-transformed prior to performing a

linear regression to estimate the first order rate constant. The commonly-cited "goodness-of-fit" or "R-squared" statistic is less important than the confidence intervals on the rate constant estimate itself. If the range of estimates for the chosen confidence interval includes zero, then a conclusion that attenuation is occurring cannot be made to that level of confidence. Wilson et al., (2005a) provide useful detailed guidance on this topic.

If Tier 1 data indicates attenuation may be occurring, Tier 2 analyses may be used to determine if subsurface conditions are representative of environments known to be conducive to MtBE biodegradation.

4.3 Tier 2 Data Analysis

Tier 2 data analysis consists of measuring the geochemical parameters listed in Table 3-3 and evaluating the changes in groundwater geochemistry in the vicinity of the solute plume. Tier 2 evaluation is intended to delineate the geochemical footprint of the solute plume, identify the range of biological processes occurring within the footprint. If spatial or temporal relationships between geochemical conditions and the distribution and migration of MtBE and its metabolites exist, these may form another line of evidence for MtBE and/or TBA atteunuation.

Table 4-1 presents the basis for defining plume geochemistry for a variety of anaerobic conditions (Kolhatkar et al., 2000; McLoughlin et al., 2001). The groundwater analytical results for methane, sulfate, and nitrate are used for the column 3 designation in Table 2-4. Sites with dissolved methane concentrations greater than 0.5 ppm are defined as methanogenic (M). Based on the presence or absence of sulfate in the plume as compared to background (i.e., upgradient), sites are classified as sulfate depleted (SD). Sites that do not contain detectable methane but have depleted nitrate in the plume compared to background are defined as nitrate depleted (ND).

Techniques used to evaluate changes in groundwater geochemistry include: 1) inclusion of key geochemical parameters on the plots of concentration versus distance prepared during Tier 1 analysis and, 2) comparison of the isopleth maps prepared during Tier 1 for correlation of geochemical trends with contaminant distribution. The Tier 2 analysis can also include an evaluation of the dominant terminal electron-accepting process (TEAP) in an aquifer as described in Wiedemeier et al. (1999).

Table 4-1 Definition of Plume Geochemistry for Anaerobic Conditions*

Analyte	Concentration in Impacted Wells	Designation
Minimum Nitrate	Non-detect or << maximum nitrate	Nitrate depleted (ND)
Maximum Iron (II)	Elevated or >>minimum iron (II)	Iron-reducing
Minimum Sulfate	Non-detect or << maximum sulfate (upgradient)	Sulfate depleted (SD)
Maximum Methane	> 0.5 ppm	Methanogenic (M)
*Kolhatkar et al., 2000; McLoughlin et al., 2001		

4.3.1 Biogeochemistry Evaluation

The geochemistry of groundwater typically changes quickly after a release of oxidizable organic carbon such as the compounds found in gasoline. Easily monitored parameters such as ORP can change as the microbial community shifts from aerobic to anaerobic.

More detailed evaluation of geochemical trends indicative of microbial metabolism that may oxidize MTBE involves field or laboratory-based analysis of other redox-sensitive parameters, as described below. Microbial oxidation of MtBE consumes electron acceptors (oxidants). Thus the concentration of electron acceptors such as dissolved oxygen, nitrate, Fe(III) and sulfate will decrease over time and along flow paths as oxidation of MtBE or other substrates proceeds. In some cases, the reduced counterpart of the electron acceptor (such as Fe[II] and methane) may also accumulate as evidence of the reaction. The primary redox reactions (in order of decreasing ORP) that indicate microbial activity are:

- aerobic oxidation: reduction of oxygen to water;
- nitrate reduction: reduction of nitrate to ammonium, N_2 and/or nitrite (because of its extremely transitory nature, N_2 cannot be accurately quantified in environmental media);
- manganese reduction: reduction of Mn(IV) to Mn(II) (Mn[IV] is present on aquifer solids);
- iron reduction: reduction of Fe(III) to Fe(II) (Fe[III] is present on aquifer solids);
- sulfate reduction: reduction of sulfate to sulfide (sulfide rarely accumulates in groundwater, as it is scavenged effectively by Fe[II] to form such minerals are FeS); and
- methanogenesis: native and anthropogenic organic carbon reduction to methane.

The reduction of these electron acceptors is evidence of microbial activity and can often be correlated to decreases in MtBE concentration; however, they do not positively identify MtBE biodegradation coupled to a specific process. Numerous other substrates (e.g., petroleum hydrocarbons) can promote reducing conditions that may or may not reflect, or cause, MtBE degradation. Further, some aquifers are naturally or artificially in a reduced redox state due to high organic loading (e.g., marshes, bogs, swamps, filled areas with high organic matter, leach fields). Observation of trends in ORP can, however, directly demonstrate microbial activity and can often be correlated to decreases in MtBE concentration and increases in metabolite concentrations, thus generating a weight of evidence that MtBE is biodegrading. In some cases, redox state may help in assessing the fate of MtBE intermediate degradation products such as TBA, which is apparently recalcitrant under methanogenic conditions but degradable in less reducing environments[8].

Because the redox state and geochemical characteristics of groundwater vary based on natural factors (e.g., availability of oxygen, presence of natural organic matter, presence of sulfate, iron, or other mineralogical constituents), a meaningful geochemical evaluation must also include groundwater that is unimpacted by the contaminant release. As with the characterization of background conditions for any contaminant, this effort requires the collection of representative groundwater samples that are upgradient or cross-gradient from the plume, but within similar hydrogeologic units. The geochemical footprint of the plume can then be defined relative to site background conditions to infer the presence of biological activity related to the release.

Geochemical data may also be used to estimate the dominant terminal electron-accepting process (TEAP). Because the energy gained from the oxidation of MtBE or other hydrocarbons using the various electron acceptors is so different, the more favorable electron acceptors are typically depleted before the less favorable acceptors are utilized; thus, distinct zones often arise within an aquifer in which a specific type of microbial metabolism (e.g., nitrate-reducing) dominates. However, using typical groundwater sampling techniques, it is often difficult to discern small-scale variations in TEAPs. Once biodegradation activity has been identified, determining the dominant TEAP is useful to help identify the major degradation mechanism. If the dominant TEAP is an anaerobic process at some locations and aerobic at others, biodegradation rates may vary at different locations throughout the contaminated environment. TEAP zone determination

[8] Although degradation of TBA has been observed under methanogenic conditions in microcosms (Finneran and Lovely, 2001) is has not been observed to degrade readily in situ in methanogenic conditions.

is described further in Wiedemeier et al., 1999. Table 4-1 presents a set of criteria for determining basic plume biogeochemistry.

4.3.2 Presentation of Spatial/Temporal Changes in Geochemical Parameters

Spatial and temporal changes in geochemical parameters can be evaluated by several methods including plotting geochemical parameters versus time and/or distance and preparing isopleth maps.

4.3.2.1 Plots of Geochemical Parameters Concentrations versus Distance

A plot of geochemical parameters versus distance is typically used to evaluate the microbial processes occurring at different zones within the plume. The same recommendations discussed in Section 4.2.4 for concentration trend plots apply to geochemical plots. Figure 4-10 illustrates a typical plot of geochemical data versus distance. Additionally, the following should be considered when presenting such data:

- geochemical parameters to be plotted in this manner include dissolved oxygen, nitrate, Fe(II), sulfate, methane, and ORP;
- data from a background well should be included to facilitate comparisons to source, mid-plume and distal portions of the plume;
- concentrations of significant COCs (e.g., BTEX, MtBE, and TBA) may be included on these plots to help assess the availability or depletion of COCs and their correlation with specific TEAPS;
- one data point located a short distance downgradient of the solute plume may be plotted to ensure that groundwater geochemistry along the entire flow path is included; and
- geochemical data can be used to confirm that downgradient wells are sampling groundwater that was once contaminated with organic compounds and has since been remediated. In such wells, geochemical evidence of the release (i.e., oxygen or nitrate depletion, presence of excess alkalinity) will remain, while regulated COCs will be absent above local background concentrations. This approach is further discussed in Wiedemeier et al. (2002).

4.3.2.2 Isopleth Maps

Isopleth maps showing the spatial distribution of geochemical parameters in relation to the BTEX plume and MtBE and its transformation products can be useful for evaluating natural attenuation. If historic data are available, a series of maps can be prepared showing the distribution of these parameters over time. As with MtBE isopleth maps,

seasonal variations in solute concentrations should be taken into account and, when appropriate, data used for isopleth map preparation should be collected during the same season, usually when solute concentrations are at their maximum.

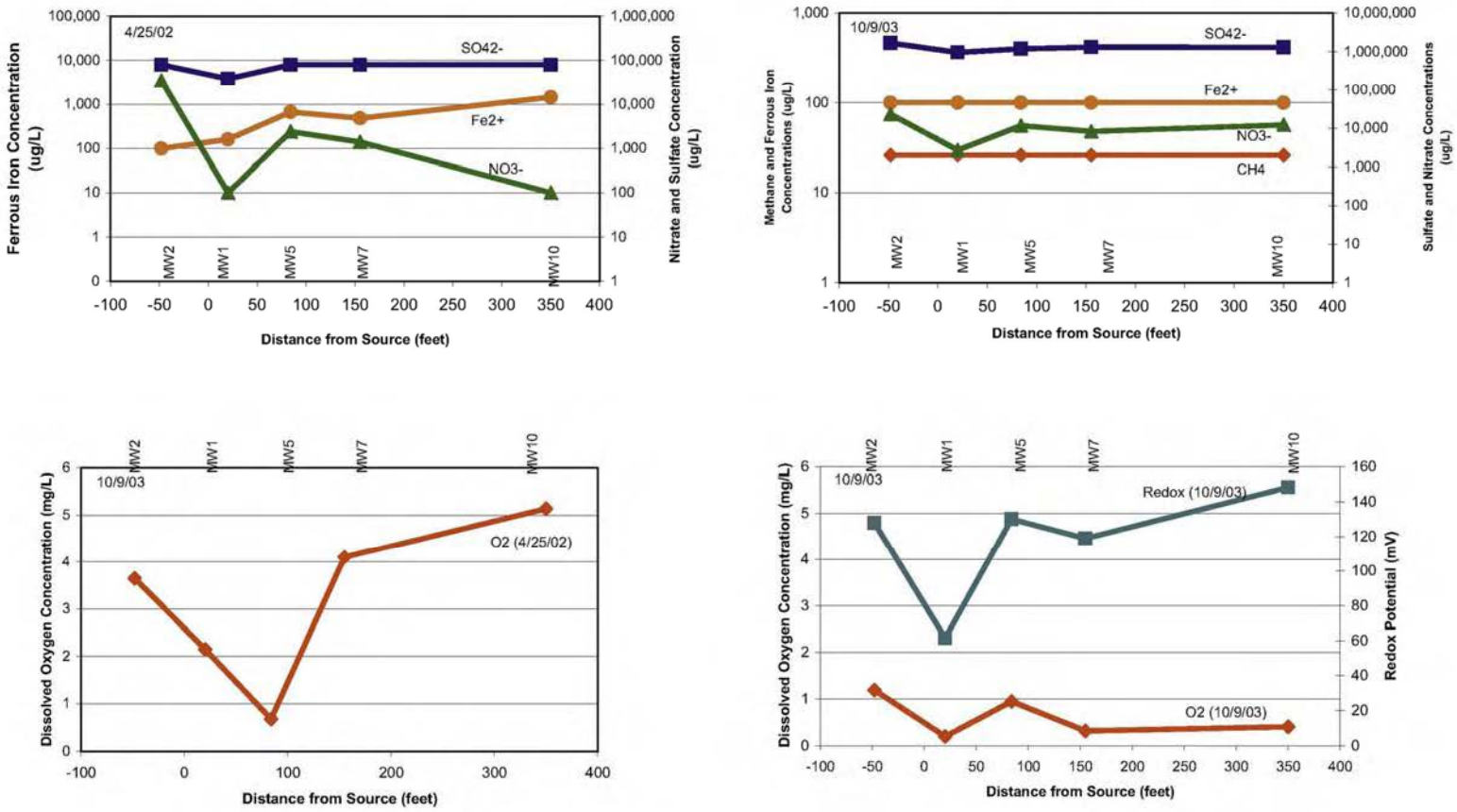


Figure 4-10 Typical Plots of Geochemical Data

4.4 Tier 3 Data Analysis

The Tier 3 analysis is intended to be used at sites where the predominant attenuation mechanism(s) have not been, or cannot be determined through the Tier 2 analysis. This Tier of data can also be used when additional measures are required to convince stakeholders of the efficacy of biodegradation in MNA. As with other data collected to evaluate natural attenuation, Tier 3 data may be useful for evaluating alternate remedial options including enhanced bioremediation. This level of effort will likely be required for only a small percentage of sites. Examples of potential Tier 3 data include compound specific isotope analysis (CSIA) and laboratory microcosm studies. Evaluation of these data is described in the following sections.

4.4.1 Compound Specific Isotope Analysis

CSIA data can sometimes be used to provide definitive evidence that MtBE biodegradation is occurring. The analytical protocol for CSIA is discussed in Section 3.3.4 (Kolhatkar et al., 2002).

Microorganisms preferentially degrade MtBE and TBA molecules that contain the lighter isotopes of carbon and hydrogen (i.e., ^{12}C and ^{1}H, respectively), while the isotopically heavier molecules [containing ^{13}C and ^{2}H (deuterium)] become enriched in the residual (undegraded) contaminant pool. This fractionation increases the isotopic ratios of ^{13}C to ^{12}C and ^{2}H (deuterium) to ^{1}H in the residual MtBE. These ratios are expressed as $\delta^{13}C$ ("delta carbon 13") and δD ("delta deuterium"), and increase (i.e., become less negative) as the heavier isotopes are enriched during biodegradation.

CSIA data can be used both quantitatively and qualitatively to infer MtBE biodegradation, with several caveats:

- the sensitivity of the method is typically substantially less in aerobic applications than anaerobic ones, due to the lower enrichment factor observed for the aerobic degradation pathways; and
- observed isotopic ratios will be affected if multiple releases occurred over time that continue to introduce undergraded MtBE and/or TBA to groundwater. When using CSIA, knowledge of the release history must be considered in this regard.

Quantitative Trend Analysis and Data Presentation

The relationship between the decrease in MtBE concentration and the associated change in $\delta^{13}C$ of MtBE is described by the Rayleigh distillation equation shown below.

$$1000 * \ln\left(\frac{10^{-3} * \delta^{13}C + 1}{10^{-3} * \delta^{13}C_0 + 1}\right) = \varepsilon * \ln\left(\frac{[MtBE]}{[MtBE]_0}\right)$$

where:

$\delta^{13}C$ and $\delta^{13}C_0$ represent the carbon isotopic composition of MtBE at time t and t=0, respectively; ε is the isotopic enrichment factor for carbon in MtBE; and, MtBE and $MtBE_0$ represent MtBE concentrations at times t and t=0, respectively.

A similar equation is used for analyzing δD data.

For enrichment factors $|\varepsilon| < 20$ ‰ (typical for $\delta^{13}C$ data), the following simplified equation can be used to estimate isotopic enrichment factor in laboratory microcosms (ε_{lab}) (Mariotti et al., 1981).

$$\delta^{13}C = \delta^{13}C_0 + \varepsilon * \ln\left(\frac{[MtBE]}{[MtBE]_0}\right)$$

Tracking temporal changes in stable isotope ratio of MtBE using the Rayleigh equation is more relevant for analyzing laboratory microcosm data than for field studies evaluating relatively stable plumes. The above equation can also be used to estimate ε_{field} using groundwater samples collected from various regions of a stable or a shrinking plume in the general direction of solute transport. If the simplified Rayleigh equation describes the data, then a linear regression of $\delta^{13}C$ of MtBE versus natural log of MtBE concentration will yield a straight line with slope ε and a Y-intercept of $[\delta^{13}C_0 - \ln (MtBE)_0]$. Such a graph demonstrating natural biodegradation of MtBE at a retail site is shown in Figure 4-11 (Kolhatkar et al., 2002).

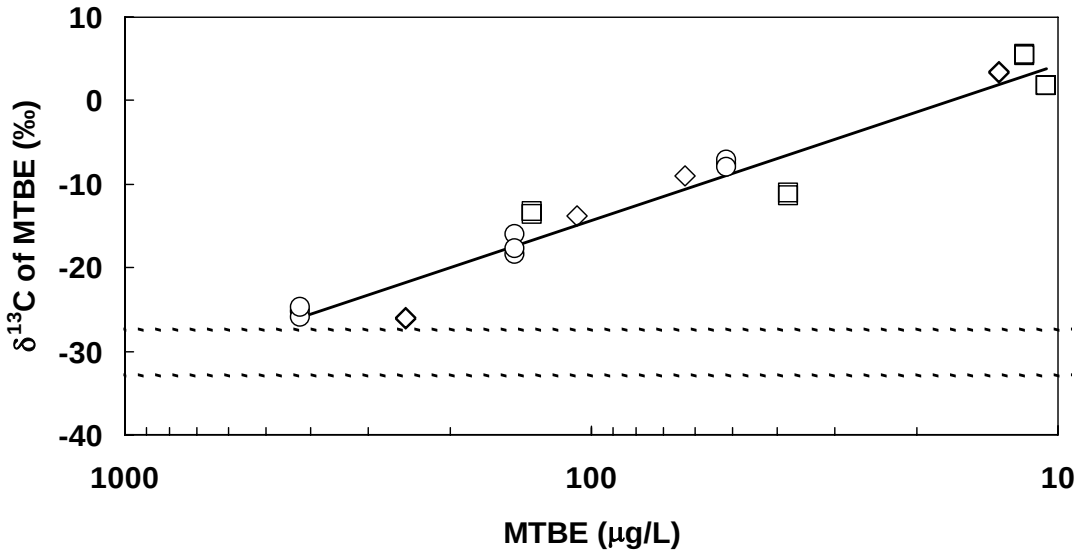


Figure 4-11 Plot of Isotopic Data Demonstrating Natural Biodegradation of MtBE

$\delta^{13}C$ (‰) values for MTBE in groundwater samples collected in 2000 (○), 2001 (□) and, 2002 (◇) as a function of MTBE concentrations (Rayleigh type plot). Methyl tert-butyl ether concentrations decrease by a factor of 40 along the flow path and concurrently $\delta^{13}C$ values for MTBE increase by 30 parts per thousand (‰). The dotted lines represent the range of typical $\delta^{13}C$ values for MTBE in gasoline (-27.5 ‰ to –33.0 ‰). The slope of the Rayleigh plot is the isotopic enrichment factor observed in the field (ε_{field} = -8.10 ± 0.85).

Quantitative analysis at this level of detail is possible only if samples are collected from portions of the plume covering two to three orders of magnitude difference in the MtBE concentrations. However, at many sites this may not be feasible or even necessary and the following qualitative approach is sufficient.

Qualitative Analysis

The qualitative approach involves comparing stable isotopic values of MtBE within the source area samples (LNAPL or groundwater) to the stable isotopic values of MtBE in the downgradient portions of the plume. Heavier (less negative) values of MtBE $\delta^{13}C$ (or δD) in samples with lower MtBE concentration than MtBE $\delta^{13}C$ (or δD) in the source area samples (higher MtBE concentration) indicate biodegradation of MtBE. This approach is likely to be more robust for $\delta^{13}C$ MtBE data than δD MtBE data, because the range of $\delta^{13}C$ MtBE in source gasoline (-27.5‰ and -33‰) (Smallwood et al., 2001, and O'Sullivan et al., 2003) is much narrower than the corresponding δD range (-80‰ to -

125‰) (Kuder, 2005). In the absence of a "true" source area sample, it is proposed that $\delta^{13}C$ MtBE value greater than -26.5‰ or δD MtBE value greater than -60‰ be considered as conservative indicators of MtBE biodegradation. These thresholds account for instrumental error and site-specific variability in CSIA.

Detecting Aerobic Degradation Using δD

Recent data (Kuder, 2004) suggest that aerobic MtBE biodegradation fractionates the hydrogen isotopes more readily than the carbon isotopes due to the initial aerobic enzymatic mechanism of cleaving the molecule. These data also suggest that carbon isotopes may be fractionated more readily under anaerobic conditions. If analysis of *δ*D is judged to have potential value, an adequate number (six) of replicate groundwater samples should be collected. One set of samples can be analyzed only for $\delta^{13}C$ MtBE initially, and if a isotopic shift is measured, further analyses of δD MtBE may not be necessary. However, if a shift in $\delta^{13}C$ MtBE is not observed, analysis of δD MtBE may be warranted to evaluate if aerobic degradation is favoring fractionation of hydrogen isotopes. This strategy may eventually be used with geochemical data to identify not only biodegradation, but also the biodegradation mechanism.

4.4.2 Microcosm Study Data

Microcosm studies can support the evaluation of MNA by providing definitive site-specific data that biodegradation of MtBE is occurring in site soil and groundwater. These studies can be designed at varying levels of complexity ranging from simple "proof-of-concept" for MtBE biodegradation to detailed simulation of field conditions to provide kinetic data that approximate field biodegradation rates. Microcosms can be constructed under both aerobic and anaerobic conditions. Methyl tert-butyl ether depletion and appearance of transformation products can be monitored to show degradation. Studies can also be conducted using radiolabeled [^{14}C] MtBE and monitored for production of ^{14}C-carbon dioxide to confirm complete oxidation of MtBE.

Laboratory experiments allow stakeholders to assess site-specific biodegradation issues within a controlled laboratory setting. However, they generally are not conducted within the scope of a typical site assessment or most natural attenuation evaluations. Laboratory experiments are only recommended when one of the following two conditions are met:

1. The monitoring network is too small to yield meaningful data about plume dynamics and historical data are limited. In this case laboratory experiments are useful to fill in gaps regarding biodegradation mechanisms.

2. Site data are not sufficient to provide an adequate evaluation of attenuation mechanisms because data are highly variable in time and/or space. In this case

laboratory experiments may provide an approach to isolate potential mechanisms that may explain the field data.

Laboratory experiments can also be useful to estimate biodegradation rates. Note, however, that laboratory derived rates represent only an estimate of the potential rate *in situ* which can be higher or lower.

As explained in Section 1.4.2 and Appendix A, the potential for long biomass acclimation times and low growth rates means that initial incubation periods may last several months to a year. Actual acclimation times may vary significantly for a specific site; therefore, multiple samples and microcosms are recommended to increase the chance that samples of acclimated zone are obtained. If site biomass levels capable of degrading MtBE or TBA are already significant, positive results may be achieved within a time period of several weeks to several months.

4.4.3 Presentation of Microcosm Study Data

Depending on the sophistication of the study, diagrams or sketches may be used to depict metabolic pathways. The level of detail used in such a presentation should be specific to the audience; understanding of many attenuation processes requires basic scientific knowledge. Flow charts may also be used to represent multi-component processes associated with a natural attenuation strategy. Metabolic pathways, such as the putative MtBE biodegradation pathway, can also be described using flow charts.

Presentation of microcosm study results may need to communicate both the conclusion that biodegradation is occurring, and also the rate at which it occurs. Demonstration of the biodegradation process is typically accomplished using time versus concentration plots depicting MtBE concentration in active vs. “killed” control microcosms. In addition, the concentration of transformation products such as TBA can be depicted to further demonstrate the degradation pathway. If degradation rates can be derived from these data, they are typically presented in tabular format as first order rate constants or half-lives. Observed lag times should be reported separately. Degradation rate is proportional to the time rate of decrease (slope) once the initial concentration starts to decrease, not measured from time zero. Tables should also indicate the MtBE (or TBA) concentration ranges, the fraction degraded, the total incubation period, the observed lag time, and geochemical characteristics of each microcosm for which a rate is reported. Where appropriate, average rates for replicate microcosms should also be reported in the tables. An example tabular presentation of microcosm data is provided as Table 4-2.

Table 4-2 Example of Microcosm Data Table

description	vial	Date: / Day:	18-Dec 0	22-Dec 4	29-Dec 11	8-Jan 21	15-Jan 28	22-Jan 35	29-Jan 42	5-Feb 49	13-Feb 58	26-Feb 71	10-Mar 84	24-Mar 98
		chemical	concentration (mg/L) - water sample analysis result											
sterile control: (BMW-04 soil sample with added sodium azide)	1A	MTBE	8.9	8.9	9.3	9.5	11	9.7	10	9.8	11	10	11	10
	1B	MTBE	9.3	8.9	9.7	9.6	11	9.7	10	9.6	11	11	11	10
	1A	TBA	10	10	10	9.9	10	10	10	10	11	10	11	10
	1B	TBA	9.9	9.9	10	9.8	10	9.9	10	9.6	11	11	10	10
active control: BMW-04 soil sample plus added MC culture	2A	MTBE	9.2	2.3	<0.005									
	2B	MTBE	9.5	3.5	<0.005									
	2A	TBA	10	2.1	<0.02									
	2B	TBA	10	3	<0.02									
soil sample: BMW-04	3A	MTBE	9.4	NS	9.6	9	8.5	9.6	10	10				
	3B	MTBE	9.6	NS	9.8	9.7	9.7	9.7	10	10				
	3A	TBA	10	NS	10	10	10	10	10	10				
	3B	TBA	10	NS	10	10	10	9.8	10	10				
soil sample: BMW-05	4A	MTBE	9.2	NS	9.5	9.8	9.4	9.4	10	9.8				
	4B	MTBE	9.1	NS	9.3	9.6	10	9	9.5	8.7				
	4A	TBA	10	NS	10	10	9.8	9.6	10	9.7				
	4B	TBA	10	NS	9.9	10	9.6	9.4	10	10				
soil sample: BMW-08	5A	MTBE	9	NS	9.6	9.9	10	8.7	6.6	4.2	2.1	<0.02	0.008	<0.005
	5B	MTBE	9	NS	9.3	10	10	6.6	2.9	1	0.23	0.039	0.026	<0.005
	5A	TBA	9.7	NS	10	10	9.5	9.8	11	11	11	3.7	<0.02	<0.02
	5B	TBA	9.8	NS	9.6	10	9.7	11	13	13	12	4.4	0.1	<0.02
soil sample: BMW-11	6A	MTBE	8.8	NS	9.5	9.6	10	8.3	8.2	6.6	4.3	2.2	1.3	0.012
	6B	MTBE	8.7	NS	9.2	9.4	8.3	8.7	8.1	7	6.1	3.5	2.8	2.2
	6A	TBA	9.8	NS	10	10	9.7	8.8	7.9	6.1	4	1	0.063	<.02
	6B	TBA	9.5	NS	9.7	9.5	9.6	9.4	9.9	9.9	11	5.8	4	2.4

Notes: NS – not sampled.

Table 4-2 Example of Microcosm Results Table (continued)

description	incubation	vial	chemical	degradation evident?	incubation duration (days)	observed lag time range (days)	overall change in concentration (%)	transient increase in TBA observed?	estimated first-order degradation rate (1/day)	estimated half-life (days)	observed concentration range (mg/L)
sterile control: (BMW-04 soil sample with added sodium azide)	aerobic	1A	MtBE	no		--	4%				8.9 to 11
		1B		no		--	4%				
		1A	TBA	no		--	2%				9.6 to 11
		1B		no	98	--	2%				
active control: BMW-04 soil sample plus added MC culture	aerobic	2A	MtBE	yes		0 to 4	– 100%		0.61	1.1	<0.005 to 9.5
		2B		yes		0 to 4	– 100%		0.59	1.2	
		2A	TBA	yes		0 to 4	– 100%	no	0.53	1.3	<0.02 to 10
		2B		yes	11	0 to 4	– 100%	no	0.51	1.4	
soil sample: BMW-04	aerobic	3A	MtBE	no		>49	4%				8.5 to 10
		3B		no		>49	1%				
		3A	TBA	no		>49	0%	--			9.8 to 10
		3B		no	49	>49	0%	--			
soil sample: BMW-05	aerobic	4A	MtBE	no		>49	2%				8.7 to 10
		4B		no		>49	3%				
		4A	TBA	no		>49	1%	--			9.4 to 10
		4B		no	49	>49	2%	--			
soil sample: BMW-08	aerobic	5A	MtBE	yes		28 to 35	– 100%		0.095	7.3	<0.005 to 10
		5B		yes		28 to 35	– 100%		0.11	6.2	
		5A	TBA	yes		58 to 71	– 100%	yes	0.24	2.9	<0.02 to 13
		5B		yes	98	58 to 71	– 100%	yes	0.16	4.3	
soil sample: BMW-11	aerobic	6A	MtBE	yes		28 to 35	– 100%		0.076	9.1	
		6B		yes		35 to 42	– 77%		0.023	31	0.012 to 10
		6A	TBA	yes		28 to 35	– 100%	no	0.073	9.4	
		6B		yes	98	58 to 71	~ 78%	yes	0.038	18	<0.02 to 11

Notes: First order degradation rate is estimated as average of –ln[c(n+1) – c(n)] / [t(n+1) – t(n)] during period of decreasing concentration, n = 1, 2, 3… .
Half-life = ln [2] / (first-order rate)

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APPENDIX A
BIODEGRADATION MECHANISMS

A-1 BIODEGRADATION OF MtBE AND TBA

MtBE and its intermediate degradation product, TBA, have been reported to biodegrade in both *in situ* and ex-situ studies, under a wide range of aerobic and anaerobic geochemical conditions. The following subsections summarize aerobic and anaerobic degradation mechanisms, MtBE metabolites, and related chemicals.

A-1.1 Aerobic Biodegradation

Aerobic respiration is the process of biologically mediated substrate (electron donor) oxidation with molecular oxygen (O_2) serving as the sole terminal electron acceptor. MtBE, petroleum hydrocarbons, and other sources of oxidizable carbon serve as substrates in aerobic respiration. Aerobic processes will dominate observed biodegradation when sufficient oxygen is locally present (greater than approximately 2.0 milligrams per liter [mg/l]) (Finneran and Lovley, 2003).

MtBE has been shown to biodegrade in natural soils and aquifer sediments under aerobic conditions (Bradley et al., 1999; Salanitro et al., 2000; Bradley et al, 2001; Wilson et al., 2002; Gray et al., 2002; Hunkeler et al., 2001; Kane et al., 2001; Schirmer et al, 2003; DeVaull et al., 2004). TBA, which can be an intermediate metabolite of MtBE, has also been shown to degrade in natural soils and aquifer sediments under aerobic conditions (Novak, et al., 1985; Bradley et al., 1999; Hunkeler, et al, 2001; Kane, et al, 2001; Wilson, et al., 2002).

Pure microbial cultures and enrichment cultures have been isolated in laboratory tests and shown to metabolize MtBE, either directly or as a co-metabolite. (Finneran and Lovely, 2003; Wilson, 2005; Hanson et al., 1999; Hardison et al., 1997; Salanitro et al., 1994; Steffan et al., 1997). The availability of pure cultures has also allowed elucidation of metabolic pathways, enzyme mechanisms, and intermediate degradation products in aerobic MtBE biodegradation. Note that observed rates of biodegradation in laboratory microcosms using pure cultures or enrichments are not directly applicable in estimating unqualified in situ biodegradation rates.

In aerobic direct metabolism organisms derive energy directly from use of MtBE as an electron donor. In co-metabolism, enzymatic degradation of MtBE occurs in conjunction with another chemical substrate. Co-substrates reported to stimulate MtBE oxidation include propane, butane, and methane (CH_4) (Wilson, 2003; Steffan et al., 1997). These aerobic processes are summarized in Table A-1.

Intermediate compounds that have been observed in laboratory study of direct metabolism or co-metabolism of MtBE include Tert-Butyl alcohol (TBA), hydroxyl

isobutyric acid (HIBA), tert-butyl formate (TBF), 2-methyl-2-hydroxy-1-propanol (MHP), and acetone. These intermediate degradation products may show transient increases (and later decreases) in some aerobic laboratory incubations. Martienssen, et al. (2006) reports production, accumulation and degradation of several of these MtBE metabolites and by-products measured in a groundwater plume down gradient of a large MtBE release. Persistence of these intermediate degradation products will be variable, depending on the rate-limiting step in their production and degradation, geochemical conditions and the microbial community composition *in situ*. The presence of any of these compounds may indicate MtBE biodegradation, with TBA being the most significant and easily-detected indicator.

In direct aerobic metabolism of MtBE, isolated microbial organisms have been observed to grow at a relatively slow rate (Deeb, et al., 2000). This slow rate of growth means that an initially low population of organisms capable of degrading MtBE may require a relatively long lag time (or acclimation time) before the exposed biomass has grown to a population sufficient to affect a measurable rate of MtBE biodegradation.

Observed lag times in the environment may also be relatively long, depending on the initial in situ presence, population, and distribution of a biomass capable of degrading MtBE, as well as geochemical conditions and local MtBE concentrations. The time required for an adapted, natural, in situ MtBE-degrading biomass to adapt and grow to a population capable of readily detectable degradation of MtBE can be up to many months from initial soil or groundwater exposure to MtBE. Acclimation times for organisms capable of degrading MtBE co-metabolically times can be shorter, but have not been widely quantified. In comparison, the acclimation times for BTEX degrading organisms can be on the order of hours to days. Once acclimation has occurred in the field, observed degradation rates for MtBE appear to be within the low end of the range of rates observed for BTEX degradation in soluble groundwater plumes.

In groundwater plumes of soluble hydrocarbons, high concentrations of the mixture of biodegrading petroleum chemicals will deplete the plume core of oxygen and other electron acceptors. Ongoing significant biodegradation of hydrocarbons will then occur at the plume margins, where both soluble hydrocarbons and oxygen (and other electron acceptors) are present. In such cases, the observed rate of aerobic biodegradationin the plume core may be quite low. In *in situ* tests of oxygen addition within a soluble MtBE plume, the rate of aerobic MtBE degradation has been reported to decrease at low oxygen levels, while the observed biodegradation rate increases if more oxygen is added (Wilson and Mackay, 2002). The availability of nutrients such as nitrogen, phosphorus, and potassium may limit degradation in laboratory cultures at high substrate concentrations, but has not been found to be a limiting factor at most field sites with relatively low substrate concentrations.

Several aerobic microorganisms that degrade MtBE have been isolated and characterized in pure cultures as listed in Table A-2. The majority of these cultures degrade MtBE co-metabolically, and not as a primary substrate. Although most cultures have been isolated through laboratory enrichment techniques and many have been only used in bioaugmentation approaches, at least one laboratory isolate has been identified in MtBE-contaminated aquifer material using molecular techniques (Burns et al., 2001; Hristova et al., 2001). This pure culture is a good model system for studying aerobic MtBE degradation as it occurs naturally and likely is involved in aerobic biodegradation of MtBE in the environment. Bacteria and fungi that co-metabolize MtBE with substrates such as propane and butane have also been isolated (Hardison et al., 1997; Smith et al., 2003; Steffan et al., 1997). Finally, molecular probes have been developed to identify aerobic MTBE degrading populations *in situ*. A number of identified MtBE degrading organisms are listed in Table A-2.

A-1.2 Anaerobic Biodegradation

Anaerobic biodegradation may occur either with electron acceptors other than oxygen, or in highly-reduced methanogenic conditions in which electron acceptors have been depleted.

Anaerobic conditions with alternate electron acceptors present

Alternate electron acceptors may include nitrate, manganese (Mn[IV]), ferric iron (Fe[III]), , and sulfate. These electron acceptors are reduced to various end products, for example, Fe(II) and sulfide, during biodegradation. MtBE and petroleum hydrocarbons can serve as electron donors for these reactions. Anaerobic processes are summarized in Table A-3.

Anaerobic MtBE biodegradation has been reported under conditions with each of these alternate electron acceptors are present (Finneran and Lovley, 2001; Finneran and Lovley, 2003; Finneran et al., 2001). Nitrate-dependent MtBE oxidation has been reported for stream-bed sediment and aquifer material, in which MtBE was completely mineralized to CO_2 (Bradley et al., 2001). Mn(IV)-dependent MtBE degradation has been reported for surface water sediments. The extent of degradation was lower under these conditions with approximately 11% of the MtBE mineralized to CO_2 (Bradley et al., 2001). Fe(III) and humic substance reduction are tightly coupled to anaerobic MtBE oxidation due to their role as electron shuttles and both processes have been reported to promote MtBE degradation in freshwater river sediment and aquifer material, in which approximately 30% of the MtBE was mineralized to CO_2 (Finneran and Lovley, 2001). Sulfate-dependent MtBE degradation has been reported in freshwater and marine sediment, as well as aquifer material (Finneran and Lovley, 2001; Somsamak et al., 2001). Sulfate reduction is reported to be a significant process for attenuating MtBE and

petroleum hydrocarbons within petroleum-impacted source areas (Finneran and Lovley, 2003).

TBA has been reported to degrade in anaerobic conditions, either with unspecified natural electron acceptors present or amendments, including nitrate, Fe(III), or sulfate (Novak, et al., 1985; Yeh and Novak, 1994; Wilson, et al., 2005; DeVaull et al., 2003).

No specific pure cultures have yet been isolated for MtBE biodegradation in anaerobic conditions. Metabolic processes and intermediate degradation products in anaerobic conditions are not completely identified.

Methanogenic conditions

Methanogenesis is the production of methane and carbon dioxide by methanogens from simple organic compounds. Methanogenesis predominates in environments where electron acceptors have been depleted. Fermentation is the energy-yielding anaerobic metabolic breakdown of a nutrient, without net oxidation, by anaerobic microorganisms. Fermentive organisms facilitate breakdown of larger organic molecules to smaller molecules; some of these smaller molecules may be utilized by methanogens in production of methane. Fermentation may occur in anaerobic conditions (particularly sulfate-reducing) as well as methanogenic conditions. Biodegradation of MtBE under methanogenic conditions has been reported in both the laboratory and the field. (Wilson et al., 2000; Wilson and Kolhatkar, 2002, Yeh and Novak, 1994; Wilson et al., 2005; DeVaull et al., 2003). Confirmed evidence for further biodegradation of TBA produced in methanogenic conditions is lacking.

Recent data indicate that TBA may accumulate at some sites, especially methanogenic sites, but will further biodegrade to CO_2 (Bradley et al., 2002; Finneran and Lovley, 2001) if electron acceptors are present.

Specific mechanisms for MtBE biodegradation in anaerobic conditions (including methanogenic conditions) have not been identified. Homoacetogenic bacteria capable of anaerobic growth on methyl-aromatic ethers have been isolated from a variety of anoxic environments, including sewage sludge, sediments, and soil (White et al., 1996). Methyl-aromatic ethers occur as terminal molecular groups in a number of natural polymeric molecules, including peat lignin, and cellulose, as well as in paper mill effluents. A first step in metabolism of the methyl-aromatic ethers is scission of the methyl-O bond. These acetogenic bacteria may also be responsible for a similar demethylation of MtBE to produce TBA. Other ethers with terminal methyl ethers groups may show similar degradation (TAME to TAA, for example).

Laboratory-derived rates for anaerobic degradation must be used cautiously for MNA applications since they may over- or under-estimate the degradation for a particular site. No pure cultures of anaerobic MtBE-degrading microorganisms have been isolated.

A-1.3 MtBE-Metabolites and Co-contaminants

Tert-butyl alcohol (TBA) has been identified as the primary metabolite of MtBE biodegradation and can also be present in reformulated gasoline as a co-contaminant (Devaull et al., 2003). In general, alcohols are miscible in water and partition less strongly from water than ether compounds (Moyer, 2003). Like MtBE, TBA adsorbs poorly to aquifer solids and tends to remain in the aqueous phase. Depending on geochemical conditions and the local microbial community composition, TBA may biodegrade locally either faster or slower than MtBE. Overall, however, it does biodegrade in subsurface environments (Wilson, 2003; Finneran and Lovley, 2003; Wilson, 2005; Bradley et al., 2002; Finneran and Lovley 2001). The physical and chemical properties of TBA are summarized in Table 1-1 of the main body of this document. Biodegradation of TBA is summarized in Table A-4.

TBA biodegradation is site-specific, and reports indicate that TBA degrades rapidly in some subsurface environments while it accumulates in other environments (depending on geochemical conditions and the local microbial community composition). Laboratory data demonstrate that TBA will degrade aerobically and anaerobically with all relevant subsurface electron acceptors (Finneran and Lovley, 2003). These laboratory studies suggest that anaerobic processes can degrade TBA at the same rate and to the same extent as aerobic processes. However, field data indicate that TBA accumulates in some anaerobic environments. Rapid *in situ* TBA biodegradation has only been reported for aerobic portions of oxygenate-contaminated groundwater plumes. These data are limited, and it is likely that TBA will biodegrade *in situ* under aerobic and anaerobic conditions.

Few data have been reported regarding TBA biodegradation mechanisms; putative biodegradation pathways under aerobic or anaerobic conditions have been suggested but data supporting these pathways have not been reported to date. The aerobic microorganisms that have been isolated which degrade MtBE also degrade TBA under identical conditions (Hanson et al., 1999). Anaerobic studies indicate that Fe(III) reduction or sulfate reduction are the most favorable processes for anaerobic TBA biodegradation (Bradley et al., 2002; Finneran and Lovley, 2001). Confirmed TBA biodegradation under solely methanogenic conditions has not been reported.

Other metabolites of MtBE include tert-butyl formate (TBF), 2-methyl-2-hydroxy-1-propanol (MHP), 2-hydroxyisobutyric acid (HIBA), and acetone (Wilson, 2003). Of these metabolites, HIBA is the only compound that may accumulate at a significant concentration; although transient acetone accumulation has been reported *in situ* (Wilson, 2003). Information on the biodegradation of these intermediates is reported as the following:

- Tert-butyl formate (TBF): degraded by an pathway via TBA as a potential product; may be directly mineralized to CO_2

- 2-Methyl-2-Hydroxy-1-Propanol (MHP): degraded by a pathway via HIBA and acetone; may be directly mineralized to CO_2
- 2-Hydroxyisobutyric Acid (HIBA), degraded by an pathway via 2-propanol and acetone; may be directly mineralized to CO_2
- Acetone: oxidized directly to CO_2 under both aerobic and anaerobic conditions

BTEX is usually a co-contaminant at MtBE sites. Several laboratory and field studies indicate that BTEX compounds, ethanol, TBA, and endogenous organic matter may inhibit MtBE biodegradation. These processes are summarized in Table A-5. BTEX, ethanol, and natural organic compounds degrade quickly under both aerobic and anaerobic conditions (Finneran and Lovley, 2001). Microorganisms preferentially utilize these relatively labile compounds for energy and carbon at the expense of MtBE. These compounds may prevent the appropriate MtBE-degrading microbial community from developing, or the MtBE-degrading microorganisms may not oxidize MtBE until the alternate substrates are depleted. Electron acceptor concentration influences these processes; several substrates may be degraded concurrently when electron acceptor concentration is high. However, rapid BTEX biodegradation consumes the available electron acceptors and, despite the potential presence of MtBE-degrading microorganisms, the electron acceptor concentration may be too low to promote MtBE biodegradation. This is true whether the electron acceptor is oxygen or one of the aerobic electron acceptors. One report indicates that BTEX or other substrates may facilitate MtBE biodegradation by increasing the total biomass, which fortuitously increases MtBE-degrader biomass (Deeb et al., 2001). Although this enhancement is possible, most data indicate that co-contaminants inhibit MtBE biodegradation.

Other co-contaminants and related chemicals include tert-amyl methyl ether (TAME), ethyl tert-butyl ether (ETBE), tert-amyl alcohol (TAA), and ethanol, which has replaced MtBE as a gasoline oxygenate in certain geographic areas. The properties of these chemicals are provided in Table 1-1 of the body of this document. A discussion of sampling and analytical issues for these chemicals is provided in Section 3 of the main document.

Table A-1 Aerobic Respiration Processes for MtBE

Process	Description	Reported Metabolites and Degradation Products	Reference
Direct Aerobic Oxidation	MtBE is utilized as the sole source of energy and is likely used as the primary carbon source, although supplemental carbon may be required for microbial biomass. Few microorganisms catalyze this reaction. Metabolites may accumulate at a lower concentration as intermediate carbon compounds are oxidized to form cellular components. Limited by oxygen concentration *in situ*.	TBA, HIBA, MHP, Acetone	(Wilson, 2003)
Aerobic Co-Metabolism (Bacterial)	MtBE is degraded as a secondary substrate by an oxygenase enzyme in a fortuitous side reaction, while a primary substrate is oxidized for carbon and/or energy by the same oxygenase. Primary substrates can include butane, propane, iso-butane, pentane, and cyclohexane. Limited by oxygen concentration and primary substrate concentration *in situ*; primary substrates may also act as competitive inhibitors of MtBE degradation.	TBA, TBF	
Aerobic Co-Metabolism (Fungal)	MtBE is degraded as a secondary substrate by oxygenase enzymes in a fortuitous side reaction, while the primary substrate (reportedly butane) is oxidized for carbon and/or energy. Limited by oxygen concentration and butane concentration *in situ*; butane may also act as competitive inhibitors of MtBE degradation.	TBA, TBF	

Table A-2 MtBE Degrading Microorganisms

Strain or Scientific Name	Pure or Enrichment Culture	Description	Reported Degradation Rates in Laboratory Incubations	Reference(s)
MC1, MC2, MC3	Enrichment	Contains *Bacillus* spp., *Acinetobacter* spp., and undefined gram-positive cells	$k = 1.66 \times 10^{-2}$ to 1.79×10^{-1} day^{-1} mg cell mass^{-1}	(Acuna-Askar et al., 2000)
PM1	Pure	Closely related to the *Leptothrix* subgroup of the Proteobacteria; identified using molecular techniques in sediment that actively degraded MtBE, which indicates this organism is environmentally relevant	0.07 to 3.56 g ml^{-1} hr^{-1}	(Hanson et al., 1999)
Biotrickling Filter Consortium	Enrichment	Enrichment culture continuously mineralized approximately 97% of added MtBE to CO_2; microbial phylogeny was not determined	5 to 10 mg hr^{-1} g cell mass^{-1}	(Fortin et al., 2001)
IFP 2012	Pure	*Mycobacterium austroafricanum*	0.6 mmol hr^{-1} g cell mass^{-1}	(Francois et al., 2002)
Pseudomonas aeruginosa	Pure	Co-metabolic biodegradation of MtBE with pentane as primary substrate	$k = 0.19$ hr^{-1}	(Garnier et al., 1999)
ENV 375	Pure	*Hydrogenophaga flava*	86 nmol min^{-1} mg cell protein^{-1}	(Steffan et al., 1997)
Gasoline-Contaminated Soil Consortium	Enrichment	Enrichment culture that degraded MtBE, TBA, ETBE, and TAME; microbial phylogeny was not determined	Rates reported for ETBE	(Kharoune et al., 2002)
ATCC 27778	Pure	*Arthrobacter* sp. which co-metabolizes MtBE when grown on butane as the primary carbon and energy source	$k = 0.43$ day^{-1} mg cell mass^{-1}	(Liu et al., 2001)
Ghinko Tree Isolates	Pure	*Arthrobacter* sp., *Methylobacterium* sp., and *Rhodococcus* sp.; MtBE was degraded as the primary substrate	Not Calculated	(Mo et al., 1997)
Membrane Bioreactor Consortium	Enrichment	An enrichment culture developed on a membrane reactor continually fed 5mg/l MtBE degraded 99.9% MtBE; microbial phylogeny was not determined	Not Calculated	(Morrison et al., 2002)
BC-1	Enrichment	An enrichment culture developed from bioreactor sludge which directly oxidized MtBE with TBA accumulation; individual species have not been isolated; microbial phylogeny has not been determined; utilized in engineered biobarriers	$k = 0.8$ day^{-1} g cell mass^{-1}	(Salanitro et al., 1994)
JOB 5	Pure	*Mycobacterium vaccae*; co-metabolic bacterium that oxidizes MtBE with propane; the oxygenase mediated catalysis is well characterized; Utilized in ex situ bioreactors; environmental relevance has not been established	10.4 to 24.0 nmol min^{-1} mg cell protein^{-1}	(Smith et al., 2003)
ENV 452	Pure	*Rhodococcus ruber*; co-metabolic bacterium that oxidizes MtBE with propane	$k = 0.25$ day^{-1} g cell mass^{-1}	(Steffan et al., 2000)

Table A-3 Anaerobic Processes for MtBE

Process	Description	Putative Metabolites and Degradation Products	References
Nitrate Reduction and/or Denitrification	MtBE is completely mineralized to CO_2. Nitrate respiration is prevalent in stream-bed sediments and aquifer material near the aerobic fringe.	TBA, N_2, NH_4^+	(Bradley et al., 2001)
Mn(IV) Reduction	MtBE is utilized as the energy source (electron donor). Mn(IV) reduction has been reported for stream-bed sediment and shallow aquifer material.	TBA, Mn(II)	(Bradley et al., 2001)
Fe(III) and Humic Substance Reduction	MtBE is utilized as the energy source (electron donor); Fe(III) is the terminal electron acceptor. Humic substances are electron shuttling compounds that accelerate the rate and extent of Fe(III) reduction, thereby increasing MtBE oxidation. This process has been reported in freshwater sediment and shallow aquifer material.	TBA, Fe(II)	(Finneran and Lovley, 2001)
Sulfate Reduction	MtBE is utilized as the energy source (electron donor); sulfate is the sole terminal electron acceptor. This process is often coupled with MtBE degradation via methanogenesis. Laboratory studies indicate that molybdate (a sulfate-reduction inhibitor) limits MtBE degradation with sulfate-reducing sediment. Intermediate accumulation is site-specific. Sulfate-dependent MtBE biodegradation has been reported in aquifer material, freshwater sediment, and marine sediment.	TBA, S_2^-, FeS	(Finneran and Lovley, 2001, Somsamak et al., 2001)
Methanogenesis	MtBE is utilized as the energy source (electron donor). Although intermediates in the catabolic pathway may be reduced (e.g. methanol); MtBE itself does not likely accept electrons as the initial step. This process has been reported in aquifer material and freshwater sediment.	CH_4	(Finneran and Lovley, 2001, Wilson et al., 2000, Wilson and Kolhatkar, 2002)

Table A-4 Biodegradation of TBA

Process	Description	Reported Metabolites	References
Aerobic Biodegradation	TBA degraded by a microbial consortium enriched from gasoline-contaminated soil	N/A	(Kharoune et al., 2001)
	TBA mineralized in surface water sediments with oxygen as the terminal electron acceptor	CO_2	(Bradley et al., 2002)
	Microbial consortium degraded TBA in a continuously stirred tank reactor	N/A	(Wilson, 2003)
	Pure culture that utilized TBA as the sole carbon and energy source	N/A	(Piveteau et al., 2001)
	TBA degradation was identified in gasoline-contaminated aquifer material using compound specific stable isotope analyses	CO_2	(Hunkeler et al., 2001)
	Pure culture of propane-oxidizing microorganisms that degraded TBA co-metabolically	CO_2	(Hatzinger et al., 2001)
	TBA mineralized by a microbial consortium that utilized TBA as the sole source of energy	CO_2	(Fortin et al., 2001)
	Pure culture of *Mycobacterium vaccae* that degraded TBA co-metabolically with propane as the primary substrate	N/A	N/A
Anaerobic Biodegradation	TBA mineralized in aquifer sediments with Fe(III) or sulfate as terminal electron acceptors, and under methanogenic conditions	CO_2, CH_4	(Finneran and Lovley, 2003)
	TBA mineralized in surface water sediments with nitrate, Mn(IV), or sulfate as terminal electron acceptors	CO_2	(Bradley et al., 2002)
	TBA mineralized in freshwater sediments with Fe(III) or sulfate as terminal electron acceptors, and under methanogenic conditions	CO_2, CH_4	(Finneran and Lovley, 2001)
	TBA degraded in soil incubations with nitrate as the likely electron acceptor	Not Reported	(Yeh and Novak, 1994)

Table A-5 MtBE-Specific Attenuation Issues

Interaction Process	Description	References
BTEX Interaction	Reports indicate that BTEX compounds may inhibit MtBE biodegradation; particularly benzene and toluene. BTEX compounds are preferential substrates for aerobic and anaerobic metabolism. BTEX degraders also deplete available electron acceptors. Previous reports also indicate that geochemical changes attributed to MtBE degradation may have been facilitated by BTEX biodegradation. However, one report indicated that the aerobic microbial community that actively degraded BTEX also degraded MtBE, and that BTEX degradation increased the cell mass for MtBE metabolism.	(Deeb et al., 2001)
TBA Interaction	Reports indicate that TBA degradation is site-specific, and that it will accumulate in some environments and will degrade quickly in other environments. When present within the initial spill, TBA is likely a preferred substrate relative to MtBE. However, when it is produced as MtBE degrades it will not likely inhibit MtBE degradation.	(Bradley et al., 1999,Deeb et al., 2001, Finneran and Lovley, 2001)
Ethanol Interaction	Reports indicate that ethanol inhibits MtBE and BTEX biodegradation in aerobic and anaerobic environments. Ethanol is relatively labile compared to all fuel hydrocarbons. However, ethanol may enrich microbial cell mass that can eventually degrade MtBE or BTEX.	(Da Silva and Alvarez, 2002)
Labile Organic Carbon Interaction	Data indicate that all labile organic carbon (sugars, alcohols, and low molecular weight organic acids) can inhibit MtBE and BTEX biodegradation in aerobic and anaerobic environments or pure culture.	(Salanitro, 1995)

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APPENDIX B
PHYSIOCHEMICAL ATTENUATION MECHANISMS

B.1 PHYSIOCHEMICAL ATTENUATION MECHANISMS

Physio-chemical processes such as advection, dispersion, groundwater recharge (dilution), volatilization, plant-mediated uptake, sorption, and abiotic degradation affect the fate and transport of organic chemicals dissolved in groundwater. This section discusses ways to identify and evaluate the impact of physical attenuation processes on the attenuation of MtBE. Because of its high aqueous solubility and its low soil sorption coefficient, advection and dispersion typically are the most important physical processes affecting MtBE dissolved in groundwater. Table B-1 summarizes physical and chemical attenuation mechanisms.

B.1.1 Advective Transport of Solutes

The transport of solutes by the bulk movement of groundwater is termed advection. In most subsurface environments, advection is the most important process causing the downgradient migration of dissolved contaminants. Advective transport is quantified using the groundwater seepage velocity. This section describes the calculations used to estimate the seepage velocity of groundwater. Seepage velocity is a key parameter in natural attenuation studies because it can be used to estimate the time of travel of a contaminant front and is required for degradation rate constant and mass flux calculations.

The seepage velocity of groundwater and dissolved chemicals influenced by advective transport only is given by:

$$v_x = -\frac{K}{n_e}\frac{dH}{dL}$$

where:

v_x	=	seepage velocity [L/T]
K	=	hydraulic conductivity [L/T]
n_e	=	effective porosity [L^3/L^3]
dH/dL	=	hydraulic gradient [L/L]

Table B-2 summarizes this and various other methods to estimate seepage velocity.

B.1.2 Hydrodynamic Dispersion

Hydrodynamic dispersion, which includes both molecular diffusion and mechanical dispersion, is the process whereby a contaminant plume migrates from areas of higher concentration to lower concentration, spreading out in directions that are longitudinal

(parallel) and transverse (perpendicular, both horizontal and vertical) to the direction of groundwater flow. Dispersion results in reduced contaminant concentrations, and introduces contaminants into relatively pristine portions of the aquifer where they may admix with more electron acceptors crossgradient and downgradient from the direction of groundwater flow. Mechanical dispersion is the dominant mechanism causing hydrodynamic dispersion at typical groundwater velocities. At extremely low groundwater seepage velocities (typically less than about 5 feet per year), molecular diffusion (discussed below) can become the dominant mechanism of hydrodynamic dispersion.

Molecular Diffusion

Molecular diffusion is described by Fick's laws, which described the migration of chemicals due to molecular scale mixing along concentration gradients from zones of higher concentration to zones of lower concentration. Molecular diffusion is slow in groundwater and is overwhelmed by mechanical dispersion except at extremely low groundwater seepage velocities (See Table B-1). Molecular diffusion can generally be ignored for most groundwater studies.

Mechanical Dispersion

Mechanical dispersion involves the spreading of solutes caused by velocity variations in the porous media. This three-dimensional process is described by the same mathematical relationship as Fick's law (See Table B-1) that results in the spreading of the solute in directions that are both longitudinal (parallel) and transverse to the direction of groundwater flow. With time, a given volume of solute gradually will become more dispersed as different portions of the mass are transported at differing velocities. The primary cause of variations in both the rate and direction of transport velocities is the heterogeneity of the porous aquifer medium. These heterogeneities are present at scales from the micro-scale (i.e., pore scale [mm]) to the macro-scale (i.e., formation scale [m to km]).

The overall result of dispersion is spreading and mixing of the contaminant plume with uncontaminated groundwater. The component of hydrodynamic dispersion contributed by mechanical dispersion is given by the relationship:

$$\text{Mechanical Dispersion} = \alpha_x v_x$$

where:

v_x = average seepage velocity of groundwater [L/T]

α_x = dispersivity [L]

Dispersivity is a parameter that is characteristic of the porous medium through which the solute migrates and can vary over three orders of magnitude depending on the variability

of the subsurface. It is also now commonly accepted (on the basis of empirical evidence) that, as the scale of the plume or the system being studied increases, the value of dispersivity increases. Therefore, not only does dispersivity vary based on geology, but dispersivity is also scale-dependent.

Several approaches can be used to estimate longitudinal dispersivity, α_x, at the field scale. One technique involves conducting a tracer test. Although a tracer test is potentially the most reliable method, it is time consuming and costly. Methods for estimating dispersivity from tracer tests are reported elsewhere (Zou and Parr, 1994; Molz et al., 1986; Davis et al., 1985).

Dispersivity values found in the literature are reported and evaluated by several authors and in many texts (Newell et al., 1996; Gelhar et al., 1992, Fetter, 1993). Pickins and Grisak (1981) report a simplified relationship between dispersivity and transport scale whereby dispersivity is estimated as 0.1 times the plume length. Xu and Eckstein (1995) evaluated the same data presented by Gelhar et al., (1992) and, by using a weighted least-squares method, developed the following relationship for estimating dispersivity:

$$\alpha_x = 0.83(Log_{10} L_P)^{2.414}$$

where:

α_x = longitudinal dispersivity [L]

L_p = plume length [L]

Another recent and very comprehensive review of the literature on this topic described empirical power laws for estimating longitudinal dispersivity for a unconsolidated materials and a variety of bedrock types (Schulze-Makuch, 2005). This reference provides the individual study results of 109 authors and a description of each.

In any case, the value derived for longitudinal dispersivity will be an estimate at best, given the great variability in dispersivity for a given plume length. In addition to estimating longitudinal dispersivity, it may be necessary to estimate the transverse and vertical dispersivities (α_Y and α_Z, respectively) for a given site. Several empirical relationships between longitudinal dispersivity and transverse and vertical dispersivities have been described. Commonly, α_Y is estimated as $0.1\alpha_x$, or as $0.33\alpha_x$. Vertical dispersivity, α_Z, may be estimated as $0.05\alpha_x$, or as $0.025\alpha_x$ to $0.1\alpha_x$ (Gelhar et al., 1992).

B.1.3 Groundwater Recharge (Dilution)

Groundwater recharge can be defined as the entry into the saturated zone of water made available at the water-table surface. In recharge areas, flow near the water table is generally downward. Recharge defined in this manner may therefore include not only precipitation that infiltrates through the vadose zone, but water entering the groundwater

system via discharge from surface water bodies (e.g., rivers, streams, and lakes). Where a surface water body is in contact with, or is part of, the groundwater system, the definition of recharge above is stretched slightly. However, such bodies often are referred to as recharging lakes or rivers/streams. Recharge of a water table aquifer has two effects on the natural attenuation of a dissolved contaminant plume. Additional water entering the system due to infiltration of precipitation or from surface water will contribute to dilution of the plume, and the influx of relatively fresh, electron-acceptor-charged water will alter geochemical processes, and in some cases facilitate additional biodegradation.

Recharge from infiltrating precipitation is the result of a complex series of processes in the unsaturated zone. Description of these processes is beyond the scope of this discussion; however, it is worth noting that the infiltration of precipitation through the vadose zone brings the water into contact with the soil, and thus may allow dissolution of additional electron acceptors and possibly organic soil matter (a potential source of electron donors). Infiltration, therefore, provides fluxes of water, inorganic species, and possibly organic species into the groundwater. Recharge from surface water bodies occurs when the hydraulic head of the water body is greater than that of the adjacent groundwater. The surface water may be a connected part of the groundwater system, or may be perched above the water table. In either case, the water entering the groundwater system will not only aid in dilution of a contaminant plume, but it may also add electron acceptors and possibly electron donors to the groundwater.

The relationship for estimating the amount of dilution caused by recharge is:

$$C_L = C_o \exp - \left(\frac{I \times W \times \left(\frac{L}{V_D} \right)}{W \times T_h \times V_D} \right)$$

Eliminating the width and rearranging, gives:

$$C_L = C_o \exp - \left(\frac{I \times L}{T_h \times \left(V_D \right)^2} \right)$$

where:

C_L	=	Concentration at distance L from origin assuming complete mixing of recharge with groundwater (mg/L)
C_0	=	Concentration at origin or at distance L = 0 (mg/L)
I	=	Recharge mixing with groundwater (ft/yr)
W	=	Width of area where recharge is mixing with groundwater (ft)
L	=	Length of area where recharge is mixing with groundwater (ft)

T_h	=	Thickness of aquifer where groundwater flow is assumed to completely mix with recharge (ft)
V_D	=	Darcy velocity of groundwater (ft/yr)

Note that this relationship is an approximation and valid only if recharge is significantly less than ambient groundwater flow and recharge water is completely mixed with groundwater. Mixing typically happens only over large scales because vertical dispersion in aquifers is usually weak. More typically a "blanket" of recharge forms overlying a plume as it migrates beneath areas receiving recharge. Provided that infiltrating water does not leach contaminants from the vadose zone, shallow groundwater may remain relatively un-impacted downgradient of sources, which has important implications for the vapor intrusion pathway and, in some cases, the viability of MNA.

B.1.4 Volatilization

Volatilization removes volatile chemicals from groundwater and surface water, although it is not a destructive attenuation mechanism. In general, factors affecting the volatilization of chemicals from groundwater into soil gas include the concentration, the change in concentration with depth, the Henry's Law constant and diffusion coefficient of the compound, mass transport coefficients for the chemical in water and soil gas, sorption, and the temperature of the water (Larson and Weber, 1994).

Partitioning of a chemical between the liquid phase and the gaseous phase is governed by Henry's Law. Thus, the Henry's Law constant of a chemical determines its tendency to volatilize from groundwater into the soil gas, and surface water to the atmosphere. Henry's Law states that the concentration of a contaminant in the gaseous phase is directly proportional to the compound's concentration in the liquid phase and is a constant characteristic of the compound. Stated mathematically, Henry's Law is given by (Lyman et al., 1992):

$$C_a = HC_w$$

where:

H	=	Henry's Law Constant (atm m^3/mol)
C_a	=	concentration in air (atm)
C_w	=	concentration in water (mol/m^3)

Henry's Law constants for MtBE, BTEX, and other fuel oxygenates are given in Table 1-1 of the main document. Because of its low Henry's law constant (1.5×10^{-3} atm m^3/g mol or 0.023 to 0.12 dimensionless), volatilization from groundwater typically is not important for MtBE, although volatilization may be a significant mechanism for MtBE in surface water.

Because of its high volatility, however, MtBE has little chance to enter the hydrologic cycle from a surface spill unless it is immediately entrained in surface runoff or infiltrating rainfall, or the depth to groundwater is small and the spill is large (Moyer, 2003). Vapor-phase MtBE in the atmosphere can subsequently be degraded by several chemical mechanisms. Gasoline containing MtBE will also volatilize quickly from dry soil (Lahvis et al., 2004). A recent study based on transport modeling at a site in North Carolina concluded that while volatilization of MtBE from a 230 m long plume is likely insignificant, volatilization from the source zone was potentially the most important natural attenuation pathway (Lahvis et al., 2004).

B.1.5 Plant-Mediated Uptake

Several reports indicate that some plants including pine trees can remove MtBE from groundwater through root uptake (Borden et al., 1997). MtBE is then either accumulated and/or transpired depending on the species of plant. Plant-mediated attenuation can be directly or indirectly quantified.

Pine trees have little transpiration surface area (needles rather than leaves) and, therefore, will tend to sequester MtBE in the plant material. For plants such as pine trees that accumulate MtBE, plant tissue can be analyzed directly and the data can be correlated between mass loss and plant uptake. These data provide direct evidence for plant root uptake.

Rooted, vascular plants such as hybrid poplar trees are reported to transpire MtBE after uptake from the root zone. Data on poplar MtBE uptake have been reported during engineered phytoremediation applications (Moyer, 2003; Zhang et al., 2001). If MtBE is trapped via hydrogen bonding in transpiration water, then the plant will not accumulate MtBE in its tissue. Although the plant is actively removing MtBE from the subsurface, tissue analyses of such plants will not detect MtBE. If MtBE mass becomes depleted within a root zone and cannot be explained by physio-chemical mechanisms then plant uptake is a potential mechanism.

Limited data suggest that some plants can transform MtBE once taken into the roots or within the vascular plant tissue (Moyer, 2003). Plants have cytochrome P-450 enzyme systems, which can oxidize organic compounds for use in plant metabolism. These enzymes are similar to microbial oxygenase enzymes in that they are non-specific and may interact co-metabolically with MtBE (despite the fact that oxidase and oxygenase enzymes are mechanistically different.) Detailed guidance on quantifying plant-mediated attenuation mechanisms is beyond the scope of this document. Further information can be found at http://www.rtdf.org/public/phyto/.

B.1.6 Sorption

As stated in Section 1.4.1.1 of the main document, sorption is typically less important for MtBE than for other chemicals. The organic carbon partitioning coefficient, Log K_{OC}, is a measure of the tendency of a compound to bind to organic carbon such as the organic fraction of aquifer solids or sediments. As listed in Table 1-1, MtBE has a log K_{OC} of approximately 1.1 (which is low compared to other constituents of gasoline (Table 1-1) (1). K_{OC} is often used to calculate a linear sorption coefficient, or solid-water distribution coefficient, (K_D) for organic chemicals, which is a measure of the tendency of a chemical to bind to aquifer solids (see Table B-1):

$$K_D = K_{OC} f_{OC} = \frac{C_S}{C_W}$$

where:

f_{oc}	=	the fraction of organic carbon of the solids;
C_s	=	the sorbed concentration of the chemical; and
C_w	=	the dissolved concentration of the chemical.

Sorption has the effect of retarding the migration of a chemical in groundwater relative to advection alone. The retardation coefficient (R) can be calculated as:

$$R = 1 + \frac{\rho_b K_D}{n_e}$$

where:

ρ_b	=	the bulk density of the aquifer solids; and
n_e	=	the effective porosity.

The chemical transport velocity, v_t, is then calculated as:

$$v_t = \frac{v_x}{R}$$

where:

v_x	=	the seepage velocity;

Due to its low soil sorption coefficient (K_{OC}) and typically low distribution coefficient (K_D), the migration of MtBE in aquifers is only slightly retarded as compared to groundwater flow. For instance, in a sandy aquifer with a moderate organic carbon content of 0.1%, most MtBE will remain in the aqueous phase, and the retardation coefficient would be 1.09 (based on bulk density of 1.75 kg/L and effective porosity of 0.25) (Nichols, et al., 2000).

Because MtBE and other fuel oxygenate are highly water-soluble and have low organic carbon partitioning coefficients, sorption typically is not an important process. In some organic rich environments such as sediments and wetland soils, sorption of MtBE may be important. Other chemicals that may be co-released with MtBE (i.e., BTEX) typically sorb more strongly to the aquifer matrix and can be significantly retarded.

B.1.7 Abiotic Degradation

As explained in Section 1.4.2 of the main document, ethers such as MtBE can undergo both oxidation and hydrolysis. In groundwater, chemical degradation mechanisms are typically less important than other attenuation mechanisms. In the vapor-phase, MtBE can be oxidized by several atmospheric gases and also be photolytically degraded. Identifying the potential role of these reactions in MtBE attenuation is discussed below.

MtBE can be degraded by acid hydrolysis under certain conditions. Most groundwaters are unlikely to be at sufficiently low pH for the process to occur to an appreciable degree. If the MtBE plume enters an acidic environment (i.e., pH below approximately 3.0 standard units) then acid hydrolysis of MtBE could occur.

Under engineered conditions MtBE can be oxidized by hydrogen peroxide (H_2O_2) and by ozone (API, 2000; Karpel et al., 1994). For MNA, this transformation is limited by the concentration of naturally occurring ozone or hydrogen peroxide. Ozone is not present in groundwater, but hydrogen peroxide may form naturally at very low concentrations in subsurface environments due to indigenous microbial activity (primarily fungal) (Tonon and Odier, 1998). It is unknown whether this low concentration of natural hydrogen peroxide reacts effectively with dissolved MtBE *in situ* but it may present an additional chemical attenuation mechanism in aquifer material or surface soils.

Atmospheric hydroxyl radicals (free OH•) will react quickly with atmospheric MtBE. The half-life of MtBE in the presence of OH• may be as short as three days (Humberto et al., 1991). The primary transformation products of this reaction include tert-butyl formate, formaldehyde, methyl acetate, and acetone (Humberto et al., 1991). Formaldehyde, which has been reported to constitute approximately 37% of the transformation products and can be a health concern, undergoes subsequent rapid photolysis and does not persist (Humberto et al., 1991). The other intermediates may persist in the gaseous phase, although at lower concentrations than MtBE. Formaldehyde is a health concern at high concentration. Hydroxyl radicals can form when methyl nitrite is oxidized by UV light in the presence of nitrogen oxides. Free hydroxyl radicals are abundant in the upper atmosphere due to reactivity among UV light, nitrogen and sulfur oxides, and ozone. OH-mediated MtBE transformation is a significant attenuation mechanism for gas-phase MtBE.

Partitioning of vapor-phase MtBE into rain droplets can limit the extent of its transformation in the atmosphere, and allow MtBE to re-enter the hydrologic cycle (Moyer, 2003). MtBE has been reported in atmospheric washout at 3 micrograms per liter (μg/l) with the concentration conserved as the washout re-entered surface water and groundwater. Atmospheric washout may be more rapid than OH-mediated transformation in high rainfall, low sunlight systems (Moyer, 2003).

The evaluation of chemical degradation mechanisms for MtBE should be pursued only when the attenuation of MtBE cannot be adequately explained by other mechanisms, when site conditions suggest it could be an important mechanism, or as part of laboratory microcosm studies, where abiotic degradation would be quantified as part of a controlled experiment (see Section 3.4 of the main document).

Site conditions that suggest MtBE may be undergoing significant abiotic degradation include highly acidic environments, environments that have a high concentration of hydrogen peroxide, or environments in which oxygen free radicals are high. These conditions are not common, but they may exist in subsurface environments depending on the prevailing microbial community (i.e., fungi produce peroxide and oxygen free radicals). Atmospheric chemical attenuation is likely given the high concentration of atmospheric ozone and oxygen free radicals resulting from breakdown of nitrogen and sulfur oxides.

If these conditions exist, the rate of transformation may be estimated as follows:

- Fitting site data to a simple transport model with a degradation term. Linear regression techniques and rate estimation are detailed in Appendix G;
- Rates can be extrapolated from literature values if the environment is similar to the site in question; however, rates obtained in this manner are merely estimates and may not indicate site-specific values;
- Rates may also be estimated based on accumulation of MtBE-specific metabolites resulting from abiotic degradation. Studies of atmospheric MtBE and engineered chemical oxidation indicate that TBF and TBA are the most common breakdown products of MtBE with oxygen free radicals as the oxidant. Rates can be derived from TBF and TBA accumulation provided that a concomitant loss of MtBE can also be quantified.

Table B-1 Physiochemical MtBE Attenuation Mechanisms

Process	Description	Dependencies	Effect on Transport	Parameter Estimation
Advection	Movement of solute by bulk ground-water movement. Parameter: seepage velocity	Dependent on aquifer properties, such as hydraulic conductivity, effective porosity, and hydraulic gradient; independent of contaminant properties.	Primary mechanism driving contaminant movement in groundwater.	$v_x = -\frac{K}{n_e}\frac{dH}{dL}$
Dispersion	Fluid mixing due to ground-water movement and aquifer heterogeneities. Parameter: dispersivity	Dependent on aquifer properties and scale of observation; independent of contaminant properties. The dispersion coefficient is the product of dispersivity (α) and groundwater velocity (υ_x)	Causes longitudinal, transverse, and vertical spreading of the plume. Reduces solute concentration. Dispersive flux (F) is dependant on concentration gradient $F = -\alpha v_x \frac{dC}{dx}$	Estimated by: $\alpha_x = 0.83 * (Log_{10} L_P)^{2.414}$ or $\alpha_x = 0.1\ L_p$ where L_p is plume length in meters
Diffusion	Spreading and dilution of contaminant due to molecular diffusion. Parameter: diffusivity	Diffusivity in water is a chemical property. Effective diffusivity is the product of Diffusivity and tortuosity (τ)	Diffusion of contaminant from areas of relatively high concentration to areas of relatively low concentration according to Fick's Law. $F = -D\frac{dC}{dx}$	Diffusive flux is generally unimportant unless groundwater velocity is very low
Sorption	Reaction between aquifer matrix and solute whereby relatively hydrophobic organic compounds become sorbed to organic carbon or clay minerals. Parameter: solid-water distribution coefficient	Dependent on aquifer matrix properties organic carbon content (f_{oc}) and contaminant properties (organic carbon partitioning coefficient).	Reduces apparent solute transport velocity and removes solutes from the groundwater via sorption to the aquifer matrix. A retardation coefficient can be calculated from the distribution coefficient, aquifer bulk density, and effective porosity: $R = 1 + \frac{\rho_b K_d}{n_e}$	The solid water distribution coefficient (K_d) can be calculated from the organic carbon distribution coefficient for the chemical (k_{oc}) and the fraction organic carbon in the aquifer (f_{oc}). $K_d = K_{oc} f_{oc}$
Recharge	Movement of water across the water table into the saturated zone. Parameter: recharge	Dependent on topography, vegetation, vadose zone soil properties, depth to groundwater, and climate.	Causes dilution of the contaminant plume and may replenish electron acceptor concentrations, especially dissolved oxygen.	1. Measurement of rainfall and estimates of interception, runoff, and evapotranspiration. 2. Water balance.

Table B-2 Methods for Inferring Groundwater Flow and Chemical Transport

	Estimate	Definition	Calculation	Units
From Theory	Darcy velocity (Darcy's Law)	Groundwater flow rate per unit width and thickness of aquifer is equal to the product of the hydraulic conductivity and the hydraulic gradient ($\partial h / \partial \ell$).	$q = K \frac{\partial h}{\partial l}$	L/T (i.e., ft/day, ft/year). Volumetric flux, Q, is the product of q and aquifer thickness and width (L^3/T)
	Seepage velocity	The velocity at which groundwater and conservative tracers move through an aquifer and depends on effective porosity (n_e).	$v_s = \frac{K}{n_e} \frac{\partial h}{\partial l}$	L/T (i.e., ft/day, ft/year)
	Chemical transport velocity by advection	The velocity of chemical transport through an aquifer is the seepage velocity divided by the retardation coefficient (*R*).	$v_a = \frac{v_s}{R} = \frac{K}{n_e R} \frac{\partial h}{\partial l}$	L/T (i.e., ft/day, ft/year).
	Chemical mass discharge by advection	Mass of chemical transported per time within a defined flow zone of width *w* and thickness *B*, based on existing chemical concentration *C* and expected retardation factor *R*.	$\dot{M}_a = \frac{q}{R} \cdot w \cdot B \cdot C$ $\dot{M}_a = \frac{Q}{R} \cdot C$	M/T (i.e., g/day, Kg/yr)
	Chemical mass discharge by diffusion	Mass of chemical transported per time through a zone of width *w* and thickness *B* solely by a difference in concentration and independent of groundwater flow direction.	$\dot{M}_D = D^* \frac{\partial C}{\partial l} \cdot w \cdot B$	M/T (i.e., g/day, Kg/yr), $\dot{M}_D$ is usually less than $\dot{M}_a$ except when v_a is small.
From Mass Balance	Groundwater flux from aerial recharge	If watershed area (*A*) above zone of interest can be defined and recharge rate (*I*) estimated, the volumetric rate of water recharging groundwater (Q) can be estimated.	$Q = I \cdot A$	L^3/T (i.e., ft^3/day, gal/day)
	Groundwater flux from baseflow increase	If flow in a fully-penetrating stream can be estimated at two locations (Q_{S1} and Q_{S2}), groundwater contributing to streamflow (Q) can be estimated.	$Q = Q_{S2} - Q_{S1}$	L^3/T (i.e., ft^3/day, gal/day)
From Observation	Chemical transport velocity from historical data	If a release date (t_r) is known, and historical monitoring data can be used to define an arrival time (t_1) at some distance (*l*) down-gradient, transport velocity for chemical 1 can be estimated.	$v_1 = \frac{l}{(t_1 - t_r)}$	L/T (i.e., ft/day, ft/yr) Multiply by aquifer thickness, width of flow path, and concentration along the flow path to calculate mass rate of transport for the flowpath.
	Transport velocity of dissimilar chemicals	The velocity of chemicals (v_1, v_2, and v_3) with different retardation factors (R_1, R_2, and R_3) can be predicted if retardation factors are known and the velocity of any is known.	$V_1 R_1 = V_2 R_2 = V_3 R_3$	L/T (i.e., ft/day, ft/yr)

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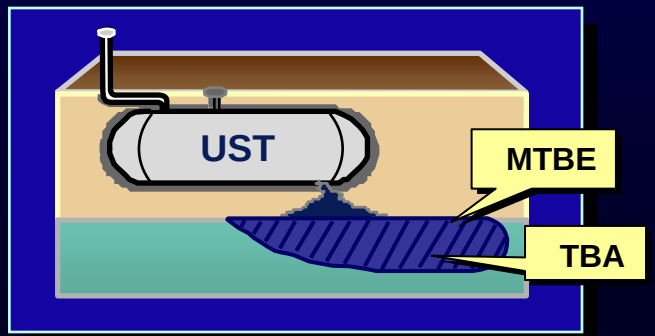
APPENDIX C
ESTIMATING MASS FLUX

ESTIMATING MASS FLUX

A Tool to Assess Natural Attenuation, Remediation Alternatives, and Potential Threat to Water-Supply Wells



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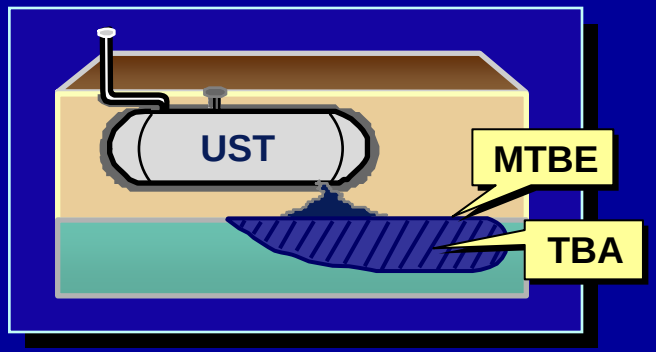
Mass Flux Approach to Site Evaluation

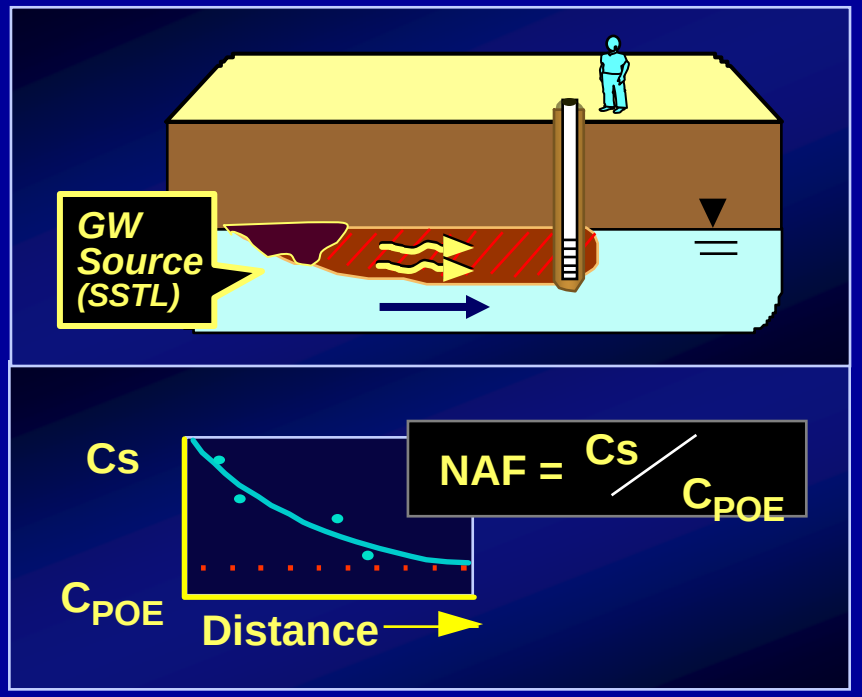
- **Assessing Potential Receptor Impacts**
- **Assessing Applicability of MNA**
- **Selection of Remedial Options**



Traditional RBCA Evaluation

Traditional risk-based decisions at corrective action sites are based on concentration, including:

- **Potential for Impact to Receptor and Need for Remedy**
- **Remedy Effectiveness**
- **Evaluation of Natural Attenuation**



KEY POINT: **Highest measured concentration often used to assess risk to down-gradient receptor.**

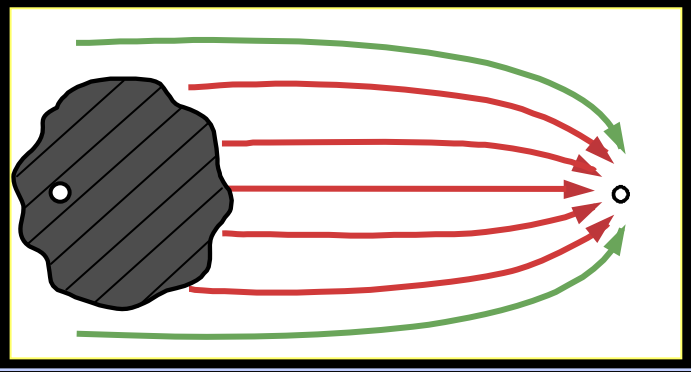
C_S = COC conc. at source; C_{POE} = COC conc. at Point of Exposure; GW = Groundwater
RBCA = Risk-Based Corrective Action; SSTL = Site-Specific Target Levels

Limitations of Traditional Approach

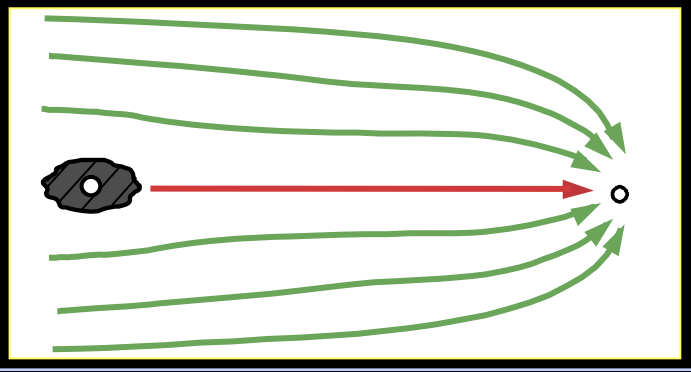
Concentration-based approach may not account for important site characteristics:

- Large vs. small release (Especially important for MTBE/TBA)
- Pumping rate at receptor well

Case A: Large Release
High Max. Conc. and High Mass Flux



Case B: Small Release
High Max. Conc. and Low Mass Flux

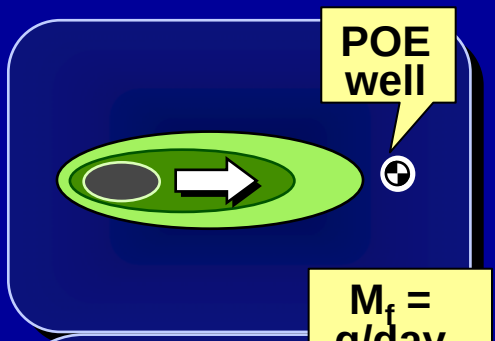


KEY POINT: **Evaluation of mass flux can increase understanding of site and be an important component of the site conceptual model.**

Mass Flux vs. Traditional Approach

Traditional Approach

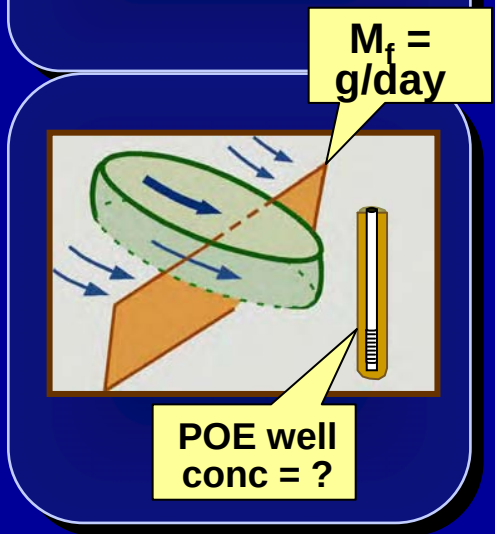
- Measure existing plume ***concentrations*** to assess:
  - Impact on receptor wells
  - Natural attenuation rates
  - Remedial options



Mass Flux Approach

- Define rate of ***mass*** flux across specified cross-sectional areas of plume to assess:
  - Impact on receptor wells
  - Natural attenuation rates
  - Remedial options

Mass flux approach based on
Einarson and Mackay (2001) ES&T, 35(3): 67A-73A



KEY BENEFITS:

Mass flux approach <u>sometimes</u> offers a better understanding of potential impacts on receptors, natural attenuation rates, and remedial options.

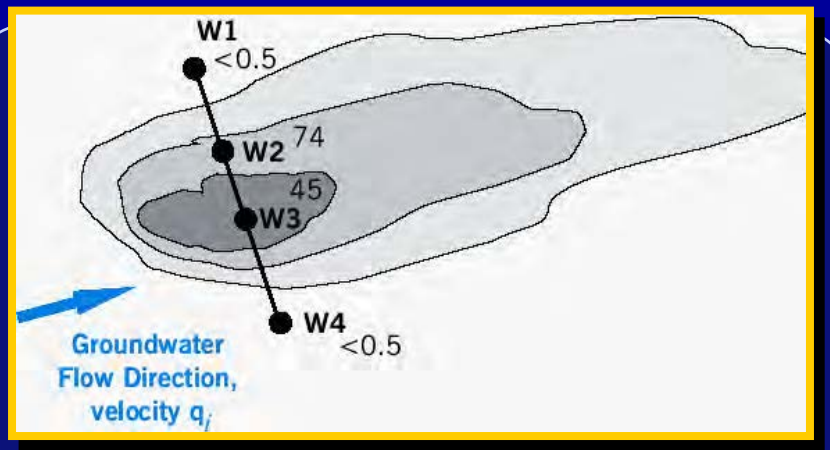
Mass Flux Calculation: *Transect Method*

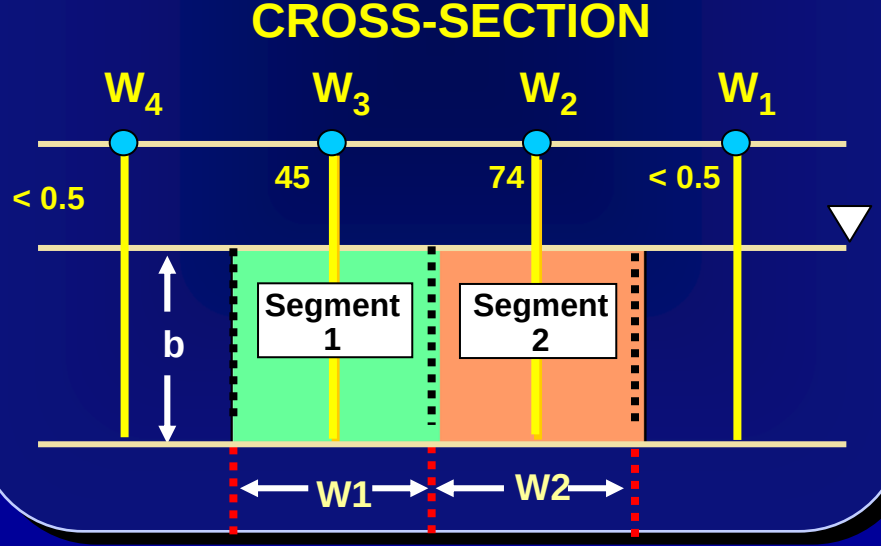
Step-By-Step Approach

1. Measure groundwater concentrations in transect across plume
2. Calculated average plume concentrations in cross-sectional areas between each well
3. Sum Total Mass Flux as:

$$M_f = \sum C_i \times A_i \times q$$

$$q = K \times I$$





M_f = Mass flux; C_i = concentration in segment i ; A_i = Area of segment i ;
I = Hydraulic gradient ; k = Hydraulic conductivity; q = Groundwater Darcy velocity (k x I)

Nichols and Roth, 2004

Concentration Term: *Option 1*

Well Data

For each transect of wells, concentration in each segment is equal to measured well concentration:

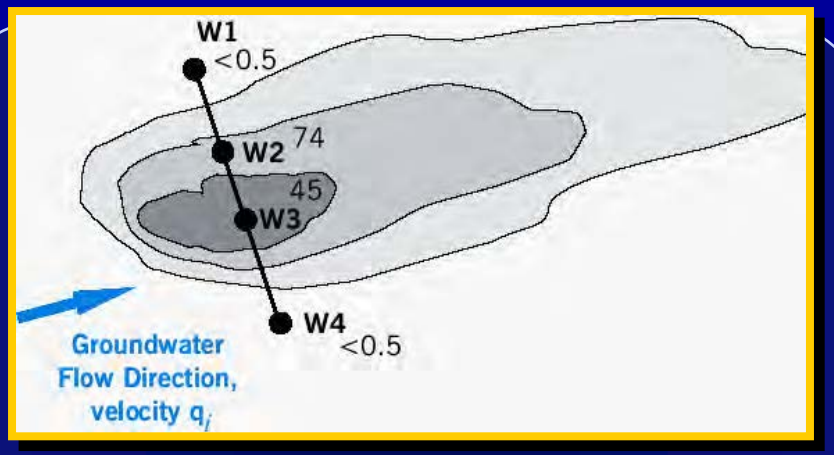
C_1 = 45 ug/L (based on well W_3)

C_2 = 74 ug/L (based on well W_2)

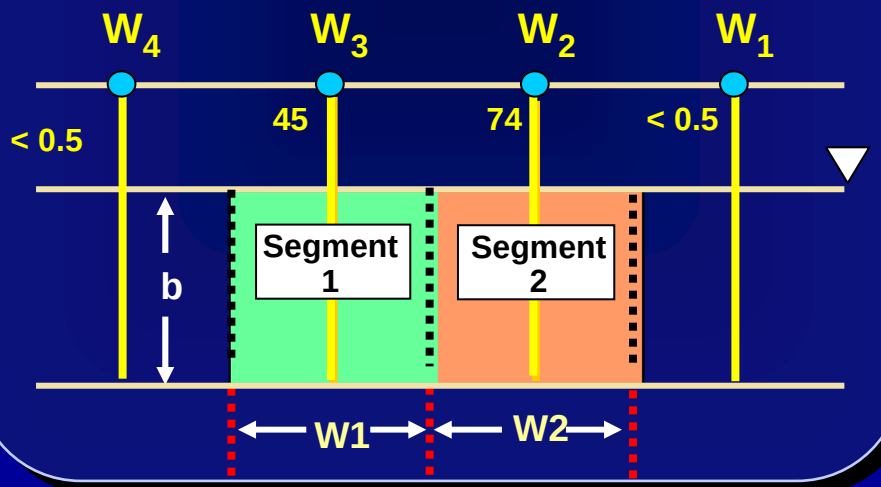
Segment Area:

$$A_i = w_i \times b$$

A_i = Area of segment i ; W_i = Width of segment i ;
b = plume thickness



CROSS-SECTION



Nichols and Roth, 2004

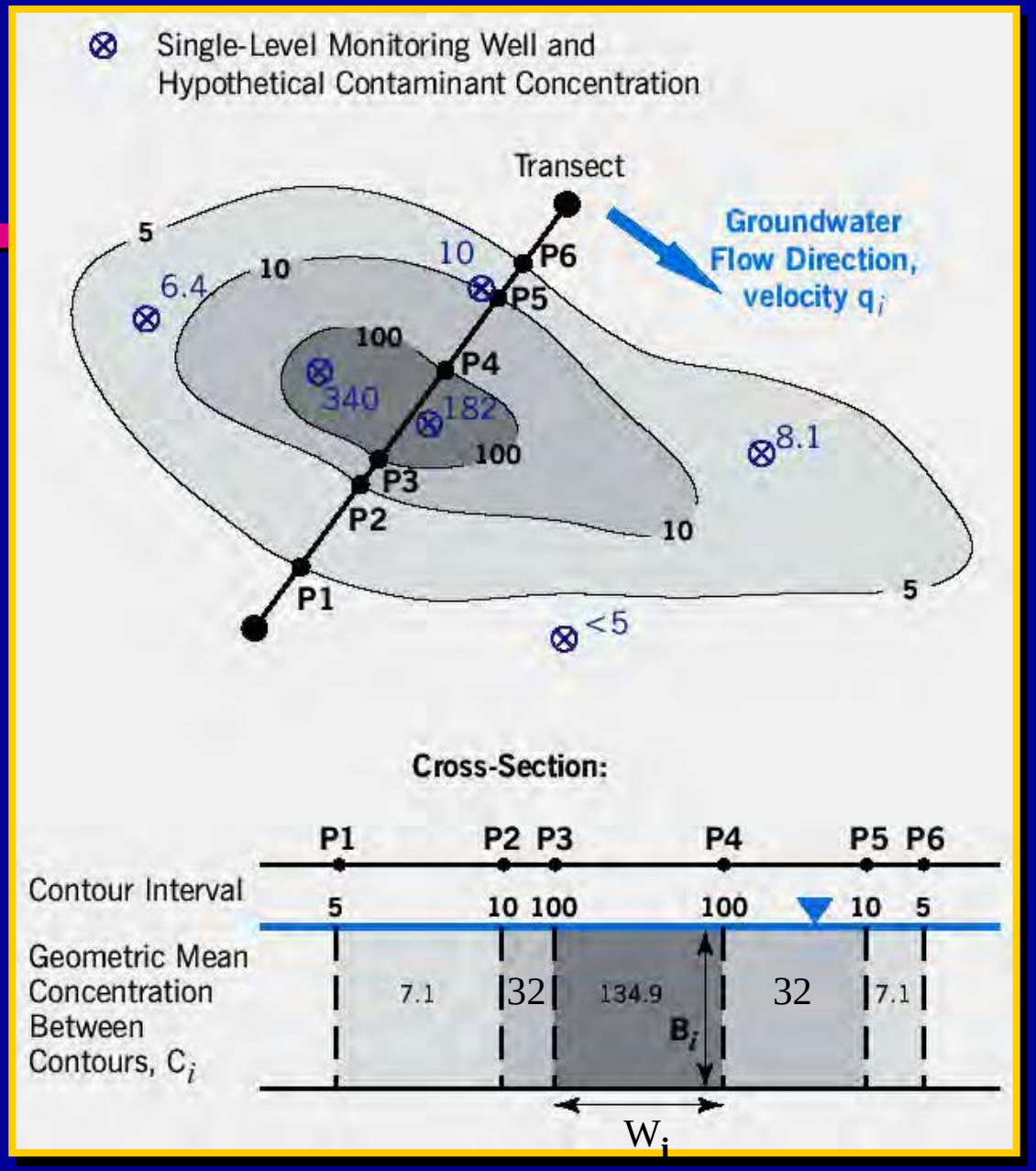
Calculating Mass Flux Concentration Term: *Option 2*

Plume Contour Data

1. Draw contours from existing well data.
2. For each transect drawn, concentration in each segment is equal to geometric mean concentration between contours.

Segment Area:

$$A_i = w_i \times b$$



Nichols and Roth, 2004

Calculating Mass Flux Concentration Term: *Option 3*

Multi-Level Sampling

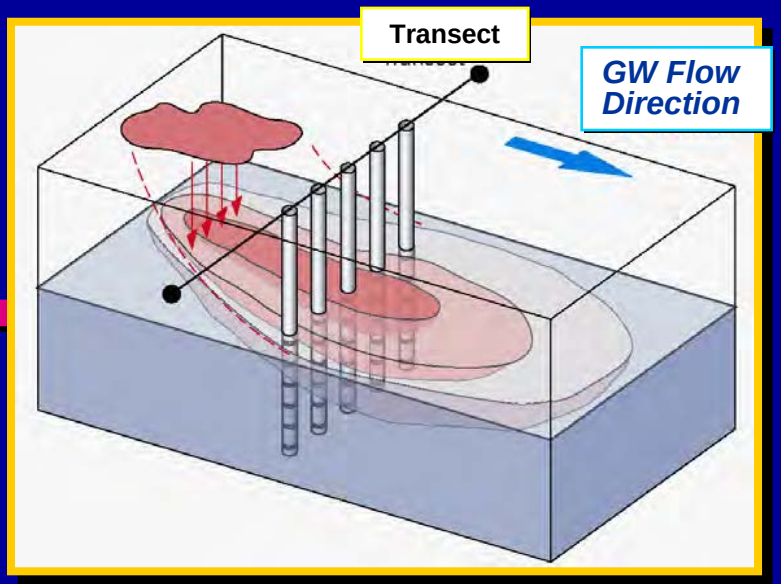
Can be used to increase accuracy of mass-flux calculation.

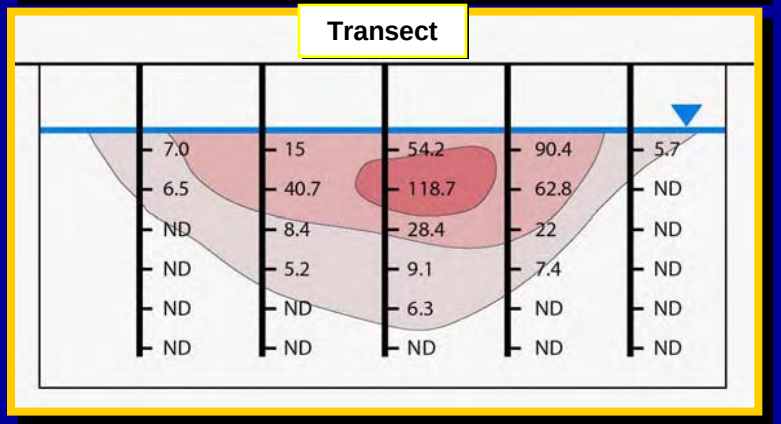
Segment Area:

$$A_{ij} = w_i \times b_j$$

EXAMPLE:

Well data approach applied to results of multilevel analysis





	w_1	w_2	w_3	w_4	w_5
b_1	7.0	15	54.2	90.4	5.7
b_2	6.5	40.7	118.7	62.8	0
b_3	0	8.4	28.4	22	0
b_4	0	5.2	9.1	7.4	0
b_5	0	0	6.3	0	0
b_6	0	0	0	0	0

Nichols and Roth, 2004

Calculating Mass Flux: *Groundwater Darcy Velocity Term (q)*

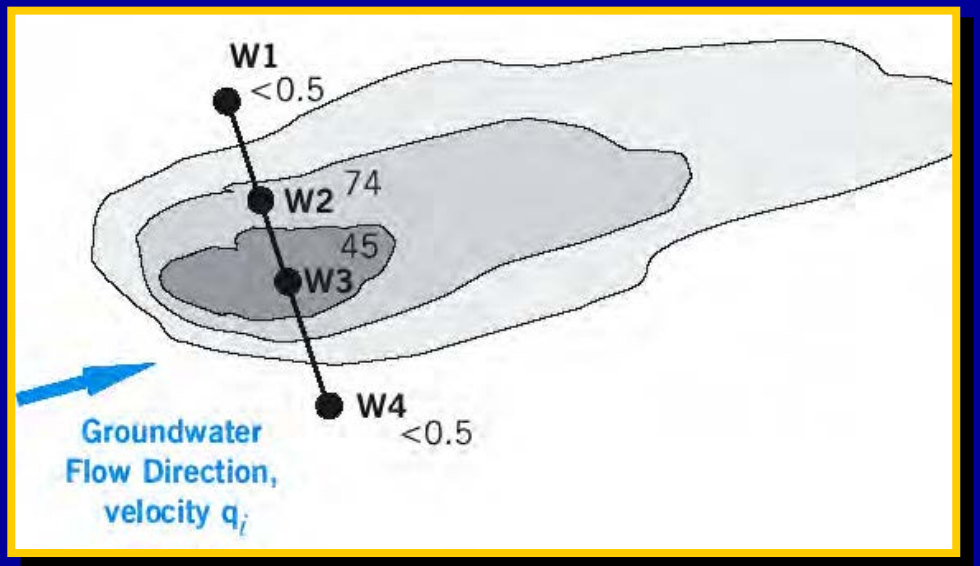
$$M_f = \sum C_i \times A_i \times q$$

Calculation of Darcy Velocity

$$q = K \times I$$

q = Groundwater Darcy velocity;
I = Hydraulic gradient;
K = Hydraulic conductivity*

* Hydraulic conductivity can be determined by pumping test, slug test, or estimated based on soil type



Variability in GW Velocity

Typically, a single groundwater Darcy velocity will be used for a site. However, different values may be used for different cross-section segments if sufficient data are available.

Mass Flux Calculation:
Option 4 - Pumping Well Method

Pumping Well Data

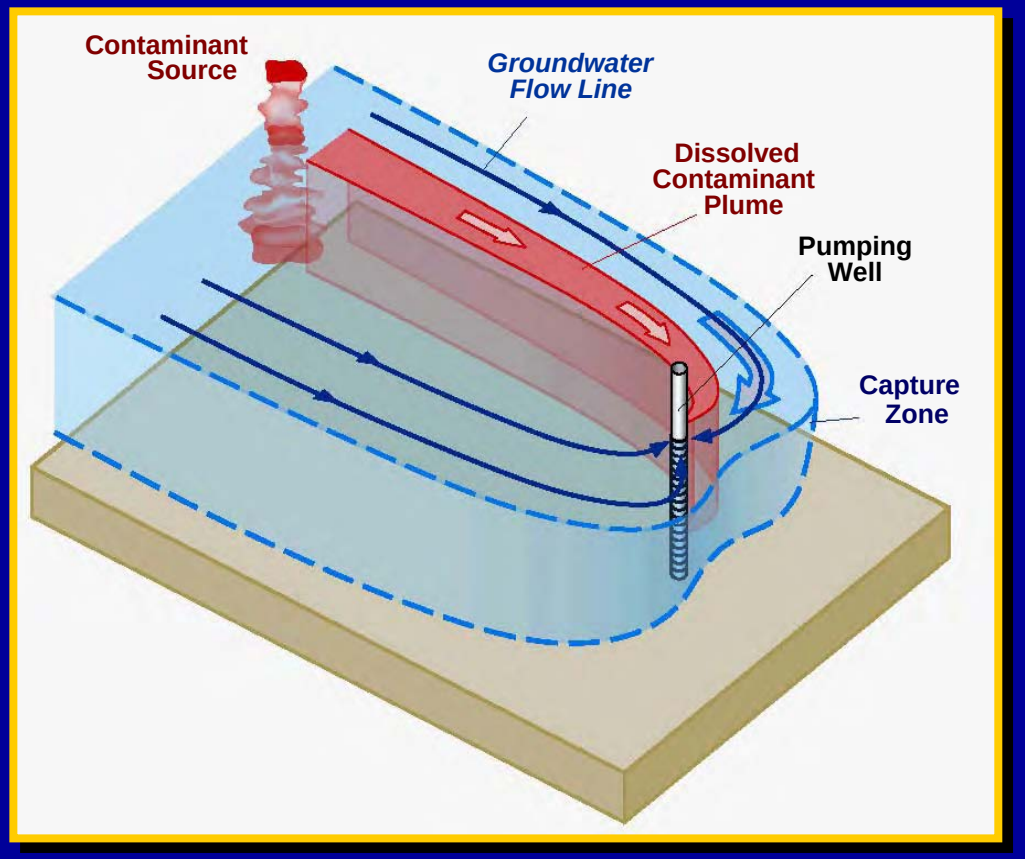
Calculate mass flux based on capture of plume by pumping system.

$$M_f = Q \times C_{well}$$

M_f = Mass flux;

C_{well} = concentration in recovery well effluent;

Q = Recovery well pumping rate



NOTE: Analysis assumes plume is completely captured by pumping well(s)

Nichols and Roth, 2004

Mass Flux Calculations for Various Sites

Site	Contaminant	Mass Flux (g/d)	Reference
Sampson County, North Carolina	MTBE	0.6 - 2	(Borden et al, 1997)
Vandenberg AFB, California	MTBE	4 to 7	Unpublished
Unnamed Site	MTBE	4	Unpublished
Elizabeth City, NC	MTBE	7.6	Wilson, 2000
St. Joseph, Michigan	TCE	167	(Semprini et al, 1995)
Dover AFB, Delaware	CVOCs	630	(RTDF 1998)

Table adapted from Einarson and Mackay (2001) ES&T, 35(3): 67A-73A

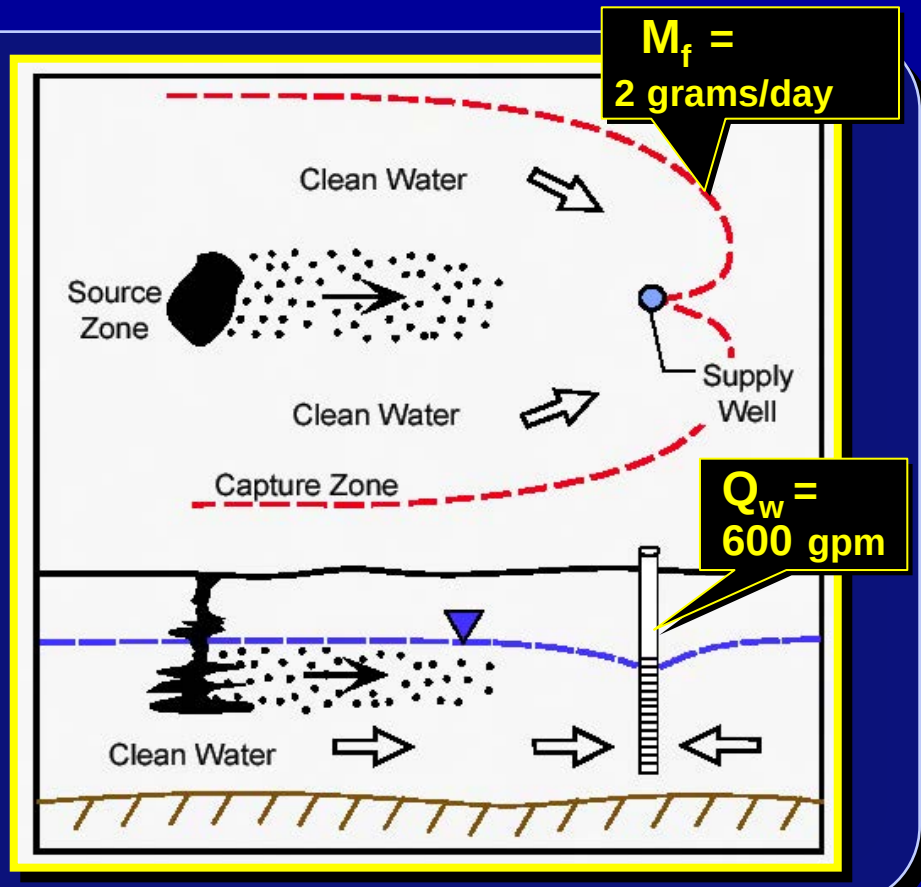
Using Mass Flux: *Estimating Well Impacts*

What

Use Mass Flux for plume to predict COC concentration in downgradient water supply well.

How

$$C_{well} = M_f \times \frac{1}{Q_{Well}}$$

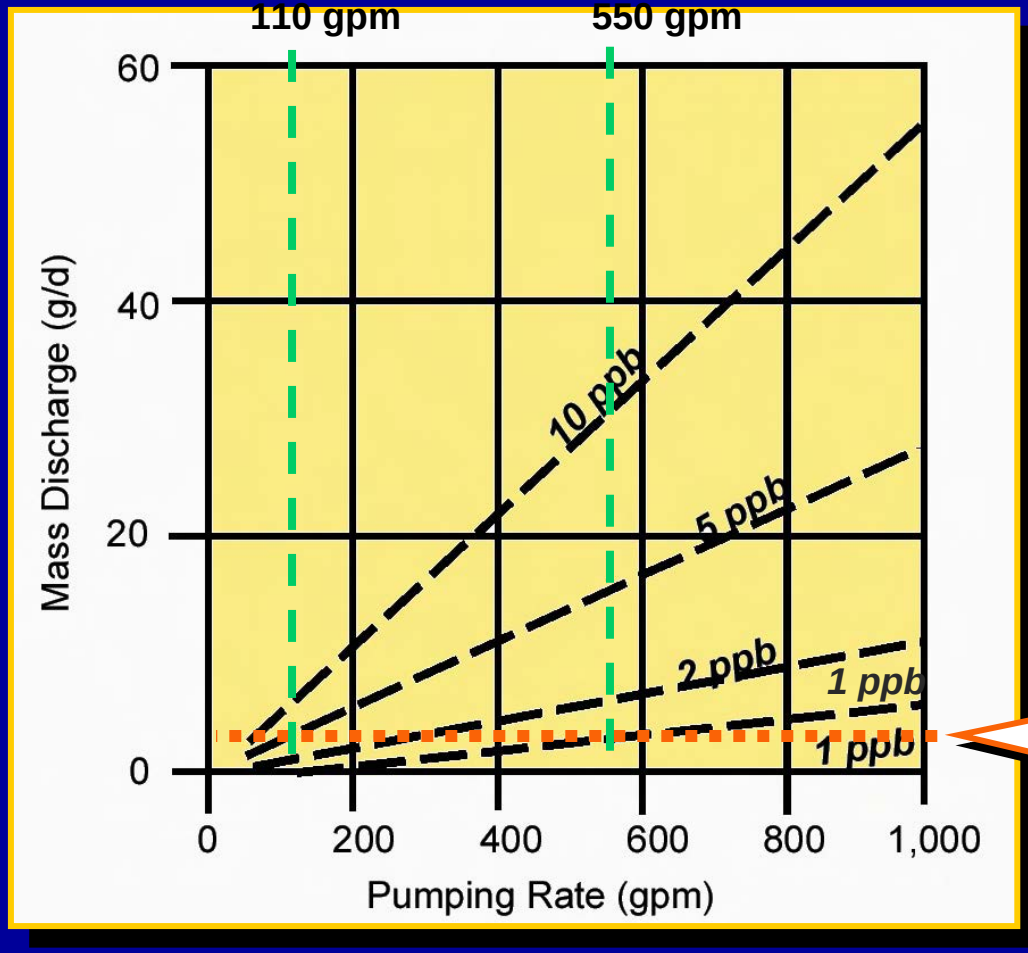


$$2\ g/day \times \frac{1}{600\ gpm} \times \frac{1day}{1440\ min} \times \frac{1\ gal}{3.79\ L} \times \frac{10^6 ug}{g} = \text{< 1 ug /L}$$

COC = Constituent of Concern; M_f = Plume mass flux; C_{well} = Concentration in supply well; Q_{well} = Pumping rate for supply well

Einarson and Mackay, 2001

Graphical Evaluation of Well Impact



Einarson and Mackay, 2001

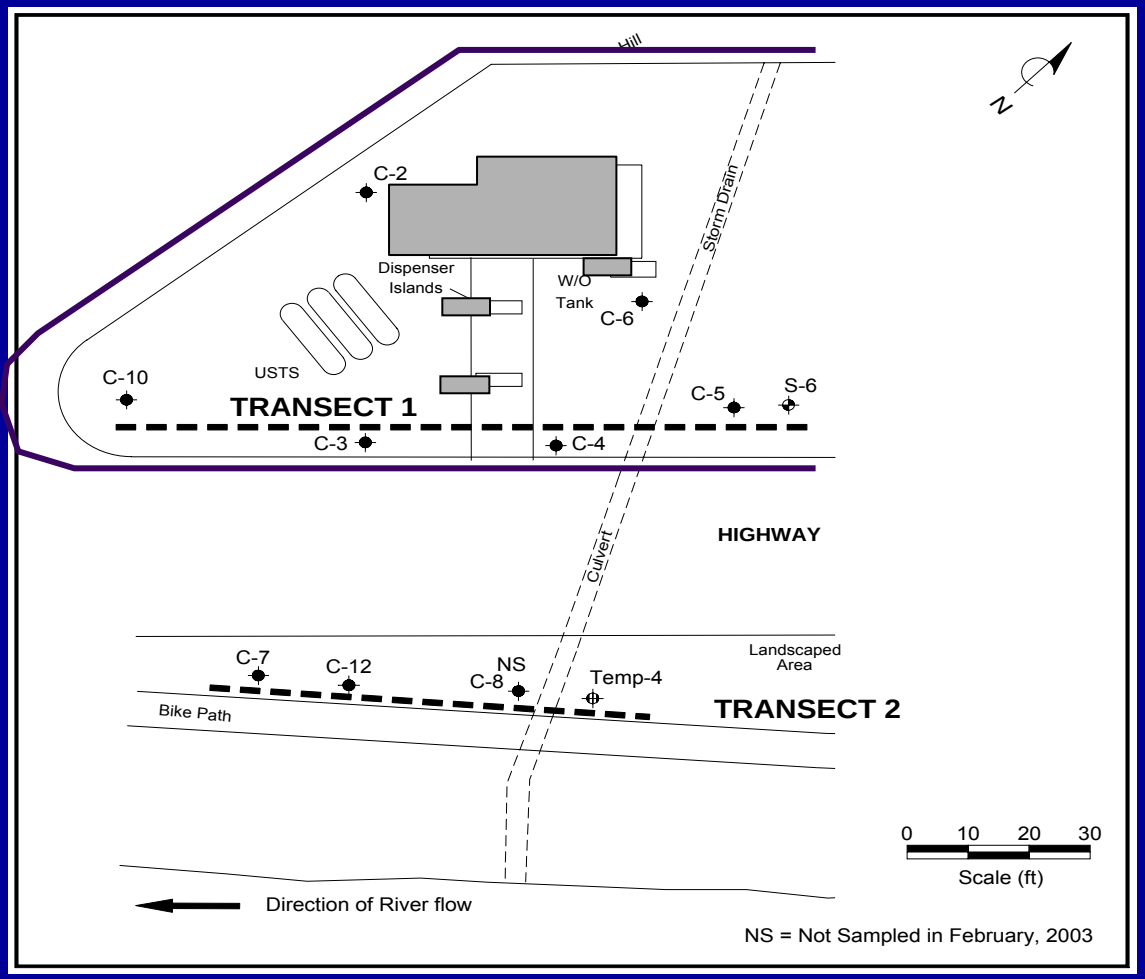
Target Concentration Limit in Well	Required Pumping Rate
5 ppb	110 gpm
<1 ppb	550 gpm

NOTE: Analysis assumes no Natural Attenuation (i.e., worst case scenario)

Sampson County Site: Mass Flux = 2 g/day

Borden et al., 1997

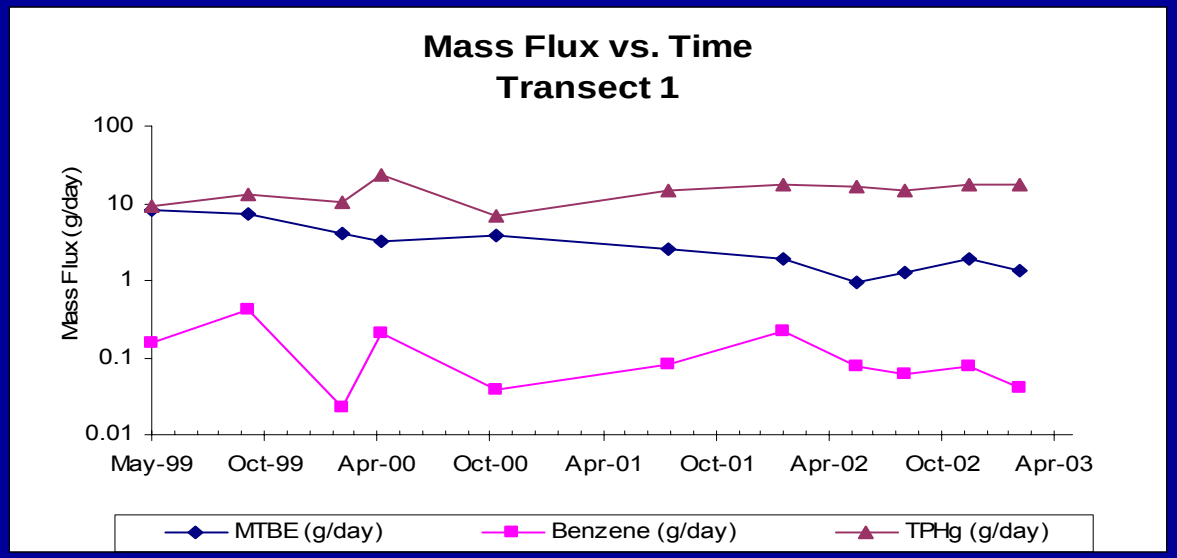
Evaluation of Potential Receptors - Tahoe City

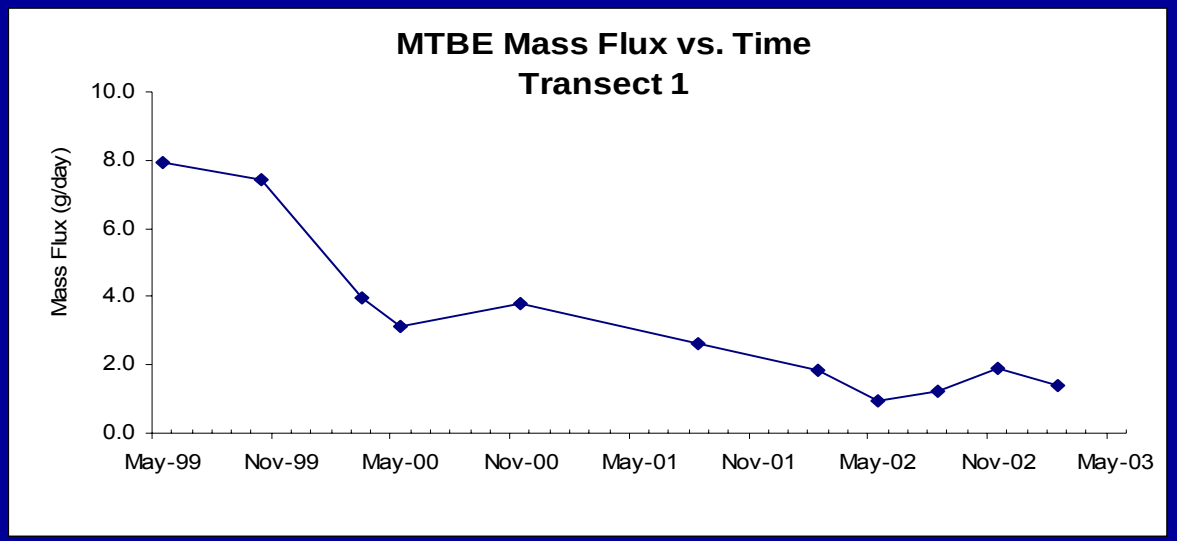


- Service station 200 feet upgradient of river
- Silt and clay sediments, interfingered with sand units
- Groundwater velocity - 0.2 ft/day (pump tests), depth to groundwater ~ 4 ft
- Air sparging system began operation in December 2000
- Transect 1 includes five monitoring wells
- Transect 2 includes three off-site monitoring wells (first sampled February 2003)

Buscheck et al., 2003

Tahoe City Mass Flux Versus Time





Assumptions:

- River is a "horizontal well" pumping at its min. flow rate: 3.5 ft^3/s = 1600 gpm
- Between the transect and the river there is no mass loss and no additional contaminant sources

Estimate potential MTBE concentrations in river:

May 99: M_D = 8 g/day, C_r = 0.9 ppb

Feb 02: M_D = 2 g/day, C_r = 0.2 ppb

Aug 02: M_D= 1.3 g/day, C_r = 0.1 ppb

Buscheck et al., 2003

Tahoe City
Summary of Mass Flux Estimates

- **Transect 1:**
  MTBE mass flux varies between 1 and 2 g/day between February 2002 and February 2003.
- **Transect 2:**
  MTBE mass flux is 0.1 g/day (based on February 2003 event), at least 10 times smaller than the Transect 1 estimates, suggesting the role of natural attenuation.
- **Mass flux estimates suggest MTBE from this site will not impact the river.**

Buscheck et al., 2003

Using Mass Flux: *Evaluation of Evaluation of Remedial Systems*

Example Site: No receptors within 0.5 miles of site, but regulatory project manager requires treatment of source area.

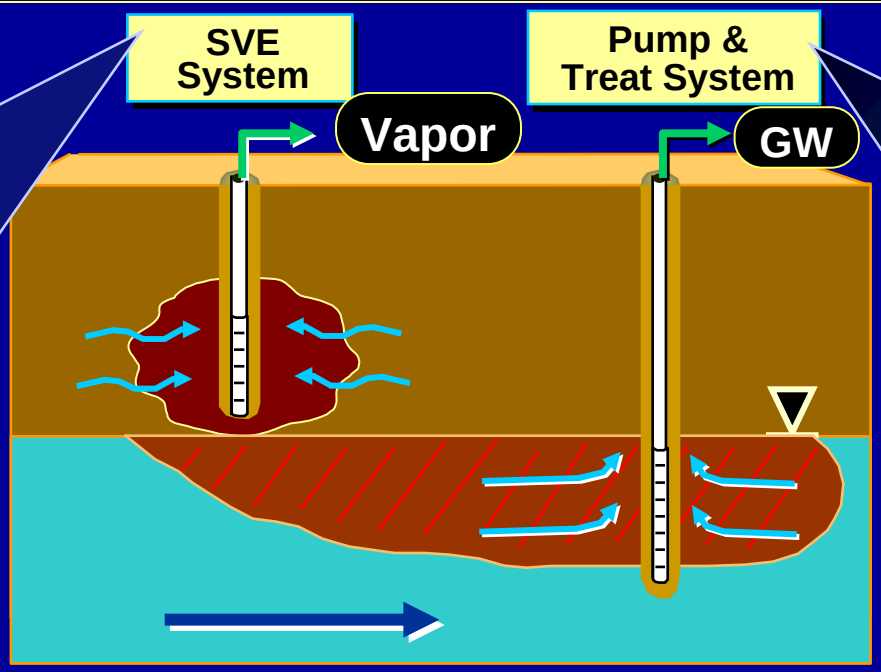
SVE System:

Current Mass Removal:

620 g/day

Cumulative Mass Removal:

1400 kg



Pump & Treat System

Current Mass Removal:

3 g/day

Cumulative Mass Removal:

40 kg

CONCLUSION: Terminate operation of P&T System due to limited mass removal. Continued operation of SVE system may be appropriate. Assess plot of SVE mass removal over time.

Benefits of Mass Flux Calculations

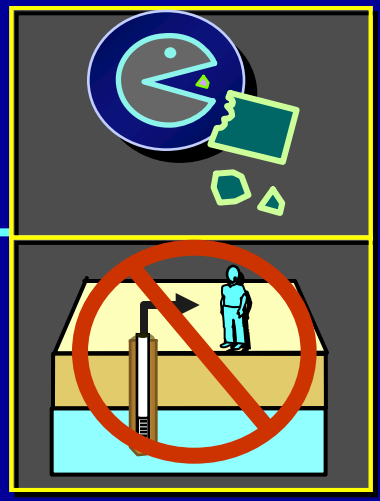
Impact to Receptor	***"Flux estimate across the boundary to a receptor is the best estimate of loading to a receptor."***
Natural Attenuation	***"The reduction in the flux along the flowpath is the best estimate of natural attenuation of the plume as a whole."***
Remedy Evaluation	***"The flux is the best estimate of the amount of contaminant leaving the source area. This information would be needed to scale an active remedy if necessary."***

Source: USEPA MNA Seminar Notes, 1998

Mass Flux: *Applications*

MNA

- ***Shows*** effect of natural attenuation

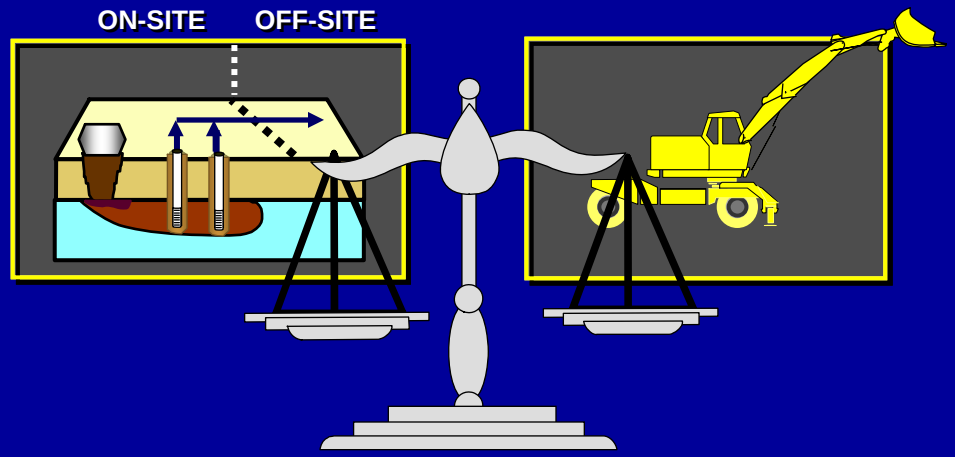


Well Impacts

- ***Quantifies*** potential impacts to wells, streams

Remedy Selection

- ***Guides*** where remediation is needed



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APPENDIX D
FIELD DATA COLLECTION PROTOCOLS

D-1 FIELD DATA COLLECTION PROTOCOLS

This appendix summarizes selected procedures and test methods for collecting the field data required to support an evaluation of MtBE natural attenuation. The data collection procedures discussed in this appendix span the three Tiers of Evaluation discussed in Section 2. Laboratory analytical methods applied to field samples are not discussed here, but are found in Section 3 of the main document.

D-1.1 Hydraulic Data

In most cases, the viability of MNA depends on the ability of naturally-occurring attenuation processes (including physical processes) to result in adequate mass or concentration reduction before chemicals migrate to receptors. Therefore, hydraulic data describing the migration of chemicals, as well as data that quantify physical attenuation processes, are paramount to any MNA investigation. MNA evaluations often require more detailed characterization of site hydraulics than required in support of other remedies because of the dependence of MNA on passive *in situ* processes to attenuate chemicals before they migrate to receptors. An understanding of site hydraulics is also required to quantify migration and fate processes along preferred flow paths.

The hydraulic data required to evaluate the physical attenuation mechanisms described in Section 4.2 of the main document include water levels (and resulting hydraulic gradients), hydraulic conductivity, and effective porosity. In order to estimate the hydraulic gradient at a given site, at least three measurement points are necessary. However, additional measurements are generally necessary to make an accurate assessment of the hydraulic gradient and groundwater flow and solute migration pathways at a site.

Hydraulic data generally are collected early in an MNA investigation. Table D-1 lists accepted methods for measuring or estimating these data, key method references, and special considerations for MNA evaluations.

Estimation of the groundwater flow rate (seepage velocity) and direction is the primary goal of collecting hydraulic data such as water levels, hydraulic conductivity, and effective porosity. Groundwater flow rate and direction can sometimes be directly inferred from site observations such as solute plume configuration as well as calculated from the parameters of gradient, hydraulic conductivity, and effective porosity, as described in Section 4.2 of the main document and in Appendix B.

D-1.2 Sampling Techniques and Equipment

The objective of sampling is to obtain a soil or water sample that is representative of the localized surface water, groundwater, or soil matrix quality under ambient conditions. After an adequate initial stratigraphic and hydraulic evaluation, water level gauging, sampling and chemical analyses typically become the most important types of data for the ongoing evaluation of MNA. Time-series plots of these groundwater quality data showing plume behavior (Tier 1) typically provide the foundation of any MNA evaluation. Preservation methods are important for MtBE analysis of water samples and are discussed in Section 3.3. Soil samples are collected less often than water samples, but they provide critical data for hydrogeology, including preferential pathways, sorption, or geochemistry.

Representative groundwater samples should contain the average concentration of all chemical constituents present in the target aquifer volume; constituents in the same phase and chemical speciation as present *in situ*; and only the chemical constituents that are mobile under ambient groundwater flow conditions. Sampling technique affects the quality of data collected. Groundwater must be collected while minimizing artifacts that can be introduced by the collection method.

The choice of groundwater sampling technique is site specific and may be dictated by regulatory requirements and guidance. Acceptable and appropriate sampling techniques may include conventional purge-and-sample, low flow sampling, micropurge sampling, and no purge (grab) sampling. Passive diffusion bags are not effective for MtBE and related chemicals because of their slow rate of diffusion through polyethylene diffusion bags. New diffusion bag materials, if developed, may make this method viable for these chemicals.

Conventional purge and sample techniques are appropriate for medium to high yield wells. Protocols for this type of sample collection specify that drawdown during purging be limited to 10% of the saturated screen thickness (Wiedemeier et al., 1998). At higher flow rates, it may not be possible to use commercially available flow-through cells for field monitoring during high flow purging, as they are designed for low (0.1 – 1 l/min) flow rates. In these cases a larger capacity flow-through cell can be made from readily available materials.

The low-flow sampling technique (Puls and Barcelona, 1996) is appropriate and widely accepted for collecting samples for VOCs, metals, and geochemical parameters, and for making field measurements of parameters such as ORP and dissolved oxygen (see Table D-3). The low flow protocol limits drawdown to 0.1 m in ideal circumstances, typically resulting in flow rates of 0.l to 0.5 l/min[1]. In low-yield formations, it may not be possible to limit drawdown during sampling. Samples collected for stable isotope analyses (CSIA) and dissolved methane can also collected using standard low-flow techniques.

Micropurge or no-purge (grab) (Parker and Clark, 2004) sampling can also be employed if water in the well casing is representative of formation water (this condition requires a reasonable ambient flow of water across or through the well screen). Although accurate measurement of geochemical parameters, including field measurements such as ORP, dissolved oxygen, and pH may not be possible with this method, it can be a cost effective way of obtaining monitoring data for other constituents.

Sampling equipment may be simple or specialized depending on hydrogeology, well construction, data quality objectives, the number/volume of samples, and the timeframe and cost of the sampling event. Various sampling devices are described in Table D-2 below. The choice of groundwater sampling device for low flow sampling should consider the ability to pump at a regulated low flow rate, ability to retrieve samples from deep screens and below the suction limit, repeatability, and cost.

D-1.3 Field Analyses and Sampling Requirements

Field measurements that support MNA evaluations typically include pH, temperature, specific conductance, dissolved oxygen, and oxidation/reduction potential (Wiedemeier et al., 1998). Multi-parameter test probes and flow-through cells are readily available for making these measurements accurately and reliably in the field. Field instruments must be properly maintained and regularly calibrated per the manufacturer's recommendations to provide reliable data.

Other Tier 2 geochemical data that can analyzed in the field include major anions (nitrate, and sulfate), ammonium, ferrous iron (Fe[II]), alkalinity and others. Field test kits are available for these analytes and can generate data that meet DQOs for most uses of geochemical data. Field test methods are summarized in Table D-3. Laboratory methods for these parameters are discussed in Section 3.3 of the main document.

Analyses for MtBE, TBA, and other volatile organics can also be conducted in the field; however, these analyses generally require a field laboratory and are outside the scope of this document.

[1] The low flow sampling protocol was designed, in part, to limit hydraulic disturbance in the area of the well screen that could entrain aquifer solids and lead to sample bias for otherwise immobile low solubility constituents. This consideration is less important for highly soluble analytes such as MtBE, and more important for low solubility analytes such as iron, manganese, and other metals.

Table D-1 Measurement Methods for Hydraulic Data

Hydraulic Data	Methods	Method References
Hydraulic Head	Water level meter or product/water interface meter Staff Gauge	Sanders (1998) http://www.ert.org/media_resrcs/media_resrcs.asp
Horizontal Hydraulic Gradient	Determine head difference between monitoring points in same geologic strata; divide by distance between points parallel to groundwater flow direction. Often inferred from potentiometric surface maps.	Freeze and Cherry, 1979. Fetter, 1993.
Vertical Hydraulic Gradient	Determine head difference between co-located monitoring points at different depths; divide by vertical distance between the mid-point of each screen interval	Freeze and Cherry, 1979. Fetter, 1993.
Hydraulic Conductivity	Slug Tests	Butler, 1998. Kruseman and de Ridder, 1991.
	Step Tests	Driscoll, 1986.
	Constant rate of discharge tests	http://www.epa.gov/ada/download/issue/issue15a.pdf Kruseman and de Ridder, 1991.
	Literature estimation	Freeze and Cherry, 1979, Table 2.3.
Porosity	Literature estimation	NAVFAC, 1986. Freeze and Cherry, 1979, Table 2.4
	Laboratory measurement	ASTM, 2004.
	Tracer tests	Fetter, 1993. Molz et al., 1986. Davis et al., 1985. Hall et al., 1991.

Table D-2 Groundwater Sampling Equipment

Item	Est. Cost (2005 USD)	Notes
Peristaltic pump	$25/day (rental) $500-1500 (purchase)	Only effective when depth to water < 25 ft Simple to operate, easy to decontaminate Requires power source
Bladder pump	$50/day (rental) $2000-3000 (purchase)	Effective for all depths Requires an air compressor
Inertial pump (Waterra™ pump)	< $50 (purchase)	Simple and inexpensive, can be dedicated to the well Available down to < 0.5 in. diameter Does not produce steady flow, and can increase turbidity in the well casing
Positive displacement pumps	$50-$150/day (rental) $500 – $5,000 (purchase)	Effective for all depths, requires power source Pump size must be matched to desired flow rate to avoid overheating
Flow through cell and field parameter meters	$150/day (rental) $5,000 - $10,000 (purchase)	Includes a multiparameter meter for dissolved oxygen, oxidation-reduction potential, temperature, pH, and conductivity
Other Meters		
Water level probe	$400 – 600 (purchase)	
Turbidity	$25/day (rental)	
Oil/water interface probe	$1150 (purchase)	
Tubing	$110/25ft	For use in the pump head of peristaltic pumps
Polyethylene tubing	$25/100 ft	For use inside a well casing
Generator	$45/day (rental)	For electric pumps
Sample vials	$125/100	Includes Teflon® septum screw caps, amber or clear

Table D-3 Field Test Methods for Groundwater Analysis

Target Chemical	Method/Reference	Resolution	Precision	Potential Issues/Comments
Dissolved Oxygen (DO)	direct reading field probe or CHEMetrics® kit	0.01 mg/L	0.2 mg/L	• Requires proper calibration to oxygen-saturated conditions and zero DO, and periodic re-calibration • Electrode is error prone if not maintained properly • Must ensure that bubbles are eliminated from behind membrane
Temperature	Field probe with direct reading meter; EPA 170.1	0.01^{o}C	15^{o}C	• Time sensitive
pH	Field probe with a direct reading meter or CHEMetrics® kit; EPA 150.1	0.01 standard units	0.2 standard units	• Requires proper calibration and re-calibration • Sensitive to de-gassing—measurement must be made promptly
Oxidation/ Reduction Potential (ORP)	Field probe with direct reading field probe	0.1 mV	20 mV	• Requires proper calibration and periodic re-calibration • Correction must be applied that is specific to electrode used • Electrode is error prone if not maintained properly • Sensitive to atmospheric oxygen, bubbles, or fouling, flow rate of water across electrode
Conductivity	Direct reading meter	1 µS/cm^{2}	1 to 100 µS/cm^{2}	• Requires proper calibration and periodic re-calibration
Alkalinity	Hach® alkalinity test kit, model AL-AP or CHEMetrics kit®	5 to 20 mg/L	5 to 20 mg/L	• Volatilization during sampling and biodegradation during transport • Note 1
Iron II	Colorimetric Hach® Method #8146 or CHEMetrics® kit	0.01 to 0.2 mg/L	0.012 to 0.04 mg/L	• Interference from turbidity • Sensitive to sunlight • Requires filtration with 0.45 micron filter and immediate analysis • Note 1
Ammonia	Hach® Mid-Range or Low-Range test kit or CHEMetrics kit®			• Note 1
Nitrate/Nitrite	Hach® Nitrate/Nitrite test kit (color disk method) or CHEMetrics kit®	0.05 mg/L (low range) 2.5 - 5 mg/L (medium range)	0.05 mg/L (low range) 2.5 - 5 mg/L (medium range)	• Note 1
Sulfate	Hach® Sulfate test kit #8051 (Colorimetric method)	1 mg/L	2 mg/L	• Note 1 • Maximum concentration of 70 mg/L**** • Temperature sensitive; keep cool • Filter if necessary to mitigate turbidity interferences

* Hach® model number from http://www.hach.com

** CHEMetrics® kits are prepared ampules of an indicator solution added to a sample. Concentration is inferred from a hand-held meter or color change. See: http://www.chemetrics.com

*** Kampbell, D., Wilson, J., and McInnes, D. M.. (1998) Determining Dissolved Hydrogen, Methane, and Vinyl Chloride Concentrations in Aqueous Solution on a Nanomolar Scale with the Bubble Strip Method. Proceedings of the 1998 Conference on Hazardous Waste Research. Snowbird, Utah, May 18-21.

**** Other kits are available for higher concentration ranges.

Note 1: Test kits are economical and user friendly. However, lab analytical data may be required to quantify test results. Sources of error are attributable to improper QA/QC on the operation of the test kits. Other sources of error are from inconsistent visual quantification of sample results.

Note 2: The resolution and precision for dissolved oxygen, temperature, pH, ORP, and conductivity are typical for multi-parameter electronic field probes. The resolution and accuracy for alkalinity, iron II, ammonia, nitrate/nitrite and sulfate are typical for field tests kits. The resolution and precision of field test kits may vary depending on the manufacturer of the kit and range of analysis required (i.e., some tests kits can be used for high, medium or low ranges of concentration by diluting the sample).

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APPENDIX F
EXAMPLE MANN-KENDALL ANALYSIS

This section presents a step-by-step approach for evaluating concentration trends using the Mann-Kendall approach. The approach shown in this example requires between 4 and 40 independent sampling events. Figure F-1 is a worksheet that can be used to facilitate the Mann-Kendall analysis. This worksheet is used by completing the following 4 steps:

Step 1: Well Data: Enter contaminant concentrations for each sampling event. Include only events for which numeric or non detect (ND) values are available.

Step 2 Data Comparisons: Complete Row 1, comparing the results of Events 2, 3, etc. to Event 1, as follows:

- Concentration of Event x > Event 1: Enter 1
- Concentration of Event x = Event 1: Enter 0
- Concentration of Event x < Event 1: Enter -1

Complete all rows in same manner until all sampling events are complete.

Step 3: Mann-Kendall Statistic: Sum across each row (e.g., 0 + 0 + -1 + -1 + 0 = -2) and record in far right hand column. Sum the right hand column down to get the TOTAL sum. This TOTAL value represents Mann-Kendall Statistic "S" for the data from this well.

Step 4: Results: Use the Confidence Level Chart in Figure F-1 to determine percent confidence in plume trend based on the S value and the number of sampling events.

Step 5: Analysis: Compare results from monitoring wells and evaluate overall plume stability.

Tables F-1a through F-1c show the results of an example Mann-Kendall statistical analysis. Figures F-2a through F-2c show concentration versus time plots for these same wells. The first example (Table F-1a, Figure F-2a) shows a well with MtBE concentrations that are obviously decreasing over time. In this case a Mann-Kendall Statistical analysis may not be necessary. Example 2 shows (Table F-1b, Figure F-2b) a well where MtBE concentrations are decreasing but the trend is not obvious. This is an example where the Mann-Kendall analysis is helpful. The Mann-Kendall analysis also is helpful for the data presented in Example 3 (Table F-1c, Figure F-2c) where there is no statistically-significant trend although visually it may appear that concentrations are increasing.

MANN-KENDALL ANALYSIS OF PLUME: **MONITORING WELL NO. _____** **CONTAMINANT_____**

	Event 1	Event 2	Event 3	Event 4	Event 5	Event 6	Event 7	Event 8	Event 9	Event 10	Sum Rows
Total BTEX (mg/L)											
Row 1: *Compare to Event 1:* →											
Row 2: *Compare to Event 2:* →											
Row 3: *Compare to Event 3:* →											
Row 4: *Compare to Event 4:* →											
Row 5: *Compare to Event 5:* →											
Row 6: *Compare to Event 6:* →											
Row 7: *Compare to Event 7:* →											
Row 8: *Compare to Event 8:* →											
Row 9: *Compare to Event 9:* →											
Mann-Kendall Statistic (S) = TOTAL											

CHARACTERIZATION OF GROUNDWATER PLUME BASED ON DATA FROM THIS WELL

Use the Confidence Level Chart with the Mann-Kendall Statistic computed above (S) and the number of sampling events to estimate confidence level in the presence of a plume trend (i.e., expanding plume or diminishing plume):

Confidence Level Chart

S Value	Total No. of Sampling Events						
	4	5	6	7	8	9	10
0							
± 1			No Trend Indicated				
± 2							
± 3							
± 4							
± 5							
± 6							
± 7							
± 8							
± 9							
± 10							
± 11							
± 12							
± 13							
± 14			Trend Probablv Present (≥90% Confidence)				
± 15							
± 16							
± 17							
± 18							
± 19							
≥ 20							

Stability Evaluation Results

- o **No Trend Indicated** · Plume Not Dimishing or Expanding (Plume is Stable)
- o **Trend Is Present** (≥90% Confidence
  - o S < 0 · Diminishing Plume
  - o S > 0 · Expanding Plume

Figure F-1 Worksheet for Concentration Trend Analysis using the Mann-Kendall Test (From Wiedemeier et al., 1999)

Table F-1a Mann-Kendall Analysis for MtBE at Well with Obviously Decreasing Trend

Date	Event 1 1/22/1987	Event 2 10/11/1988	Event 3 9/21/1989	Event 4 4/14/1990	Event 5 9/7/1990	Event 6 4/4/1991	Event 7 11/8/1991	Event 8 4/9/1992	Event 9 11/5/1992	Event 10 4/8/1993	Event 11 10/25/1993	SUM OF ROWS
Concentration	126	68.3	18.4	16.6	17.9	8.16	8.07	8	8.2	3.5	6.1	
Compare to Event 1		-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-10
Compare to Event 2			-1	-1	-1	-1	-1	-1	-1	-1	-1	-9
Compare to Event 3				-1	-1	-1	-1	-1	-1	-1	-1	-8
Compare to Event 4					1	-1	-1	-1	-1	-1	-1	-5
Compare to Event 5						-1	-1	-1	-1	-1	-1	-6
Compare to Event 6							-1	-1	1	-1	-1	-3
Compare to Event 7								-1	1	-1	-1	-2
Compare to Event 8									1	-1	-1	-1
Compare to Event 9										-1	-1	-2
Compare to Event 10											1	1
									Mann-Kendal Statistic (S)			-45

Table F-1b Mann-Kendall Analysis for MtBE at Well with Slightly Decreasing Trend

	Event 1	Event 2	Event 3	Event 4	Event 5	Event 6	Event 7	Event 8	Event 9	Event 10	Event 11	Event 12	Event 13	Event 14	Event 15	SUM OF
Date	**10/28/1988**	**9/19/1989**	**4/12/1990**	**6/6/1990**	**9/7/1990**	**1/8/1991**	**4/3/1991**	**11/5/1991**	**12/17/1991**	**4/8/1992**	**11/5/1992**	**4/5/1993**	**10/26/1993**	**8/18/1994**	**11/17/1995**	**ROWS**
Concentration	111	144	105	118	123	88.9	92.9	110	95.6	87.6	66	130	94	92	87	
Compare to Event 1		1	-1	1	1	-1	-1	-1	-1	-1	-1	1	-1	-1	-1	-6
Compare to Event 2			-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-13
Compare to Event 3				1	1	-1	-1	1	-1	-1	-1	1	-1	-1	-1	-4
Compare to Event 4					1	-1	-1	-1	-1	-1	-1	1	-1	-1	-1	-7
Compare to Event 5						-1	-1	-1	-1	-1	-1	1	-1	-1	-1	-8
Compare to Event 6							1	1	1	-1	-1	1	1	1	-1	3
Compare to Event 7								1	1	-1	-1	1	1	-1	-1	0
Compare to Event 8									-1	-1	-1	1	-1	-1	-1	-5
Compare to Event 9										-1	-1	1	-1	-1	-1	-4
Compare to Event 10											-1	1	1	1	-1	1
Compare to Event 11												1	1	1	1	4
Compare to Event 12													-1	-1	-1	-3
Compare to Event 13														-1	-1	-2
Compare to Event 14															-1	-1
														Mann-Kendal Statistic (S)		-45

Table F-1c Mann-Kendall Analysis for MtBE at Well with No Trend

	Event 1*	Event 2	Event 3	Event 4	Event 5	Event 6	Event 7	Event 8	Event 9	Event 10	SUM OF
Date	**12/7/1989**	**4/17/1990**	**9/11/1990**	**1/22/1991**	**5/2/1991**	**6/21/1991**	**9/17/1991**	**11/6/1991**	**4/8/1992**	**4/7/1993**	**ROWS**
Concentration	3.3	4.18	1.52	3.63	6.75	10.8	1.81	2.49	6.6	7.2	
Compare to Event 1		1	-1	1	1	1	-1	-1	1	1	3
Compare to Event 2			-1	-1	1	1	-1	-1	1	1	0
Compare to Event 3				1	1	1	1	1	1	1	7
Compare to Event 4					1	1	-1	-1	1	1	2
Compare to Event 5						1	-1	-1	-1	1	-1
Compare to Event 6							-1	-1	-1	-1	-4
Compare to Event 7								1	1	1	3
Compare to Event 8									1	1	2
Compare to Event 9										1	1
											13

* Average of 2 samples collected on the same day.

Figure F-2a MtBE Concentration Versus Time for Well with Obviously Decreasing Trend

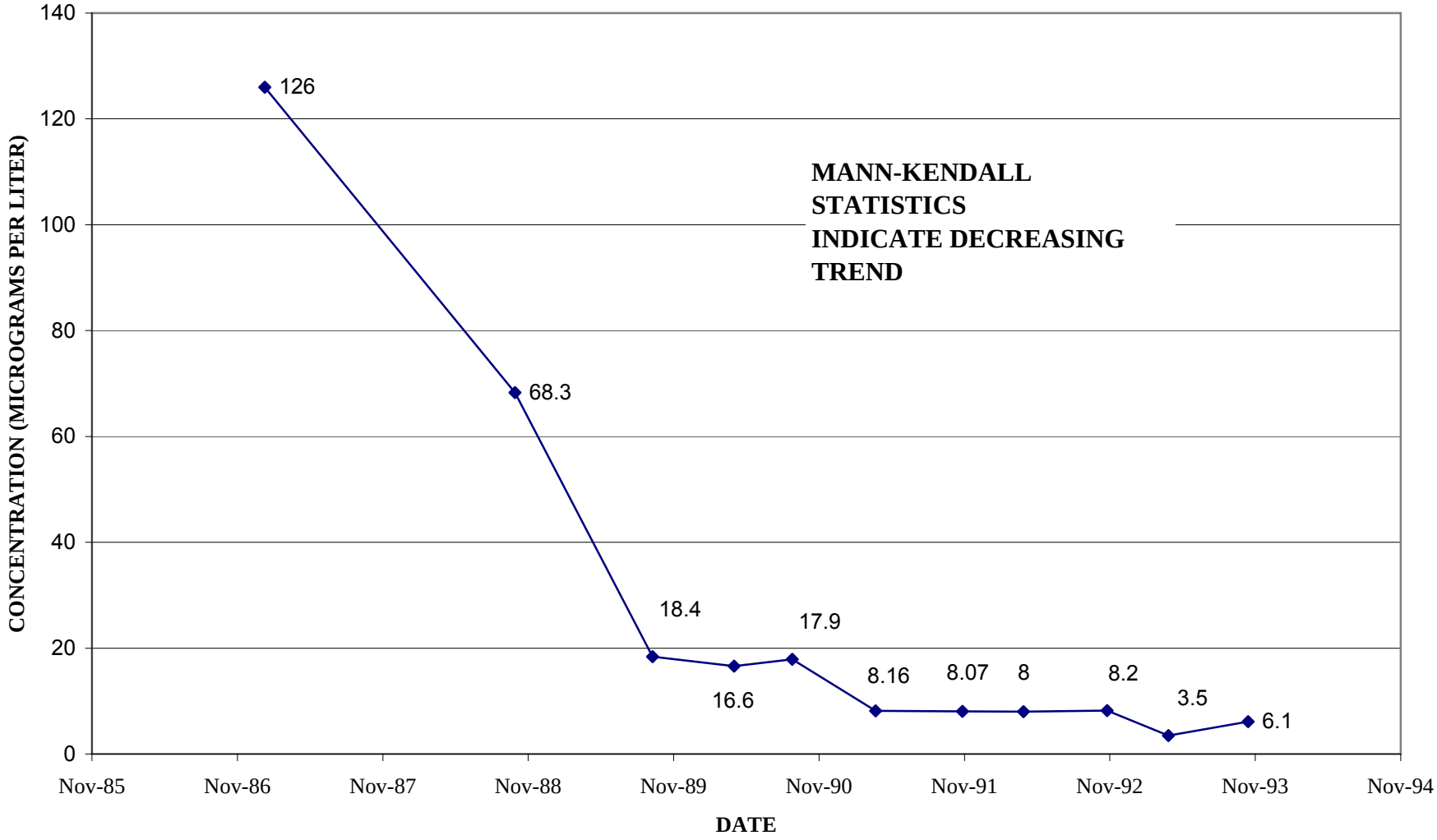


Figure F-2b MtBE Concentration Versus Time for Well with Slightly Decreasing Trend

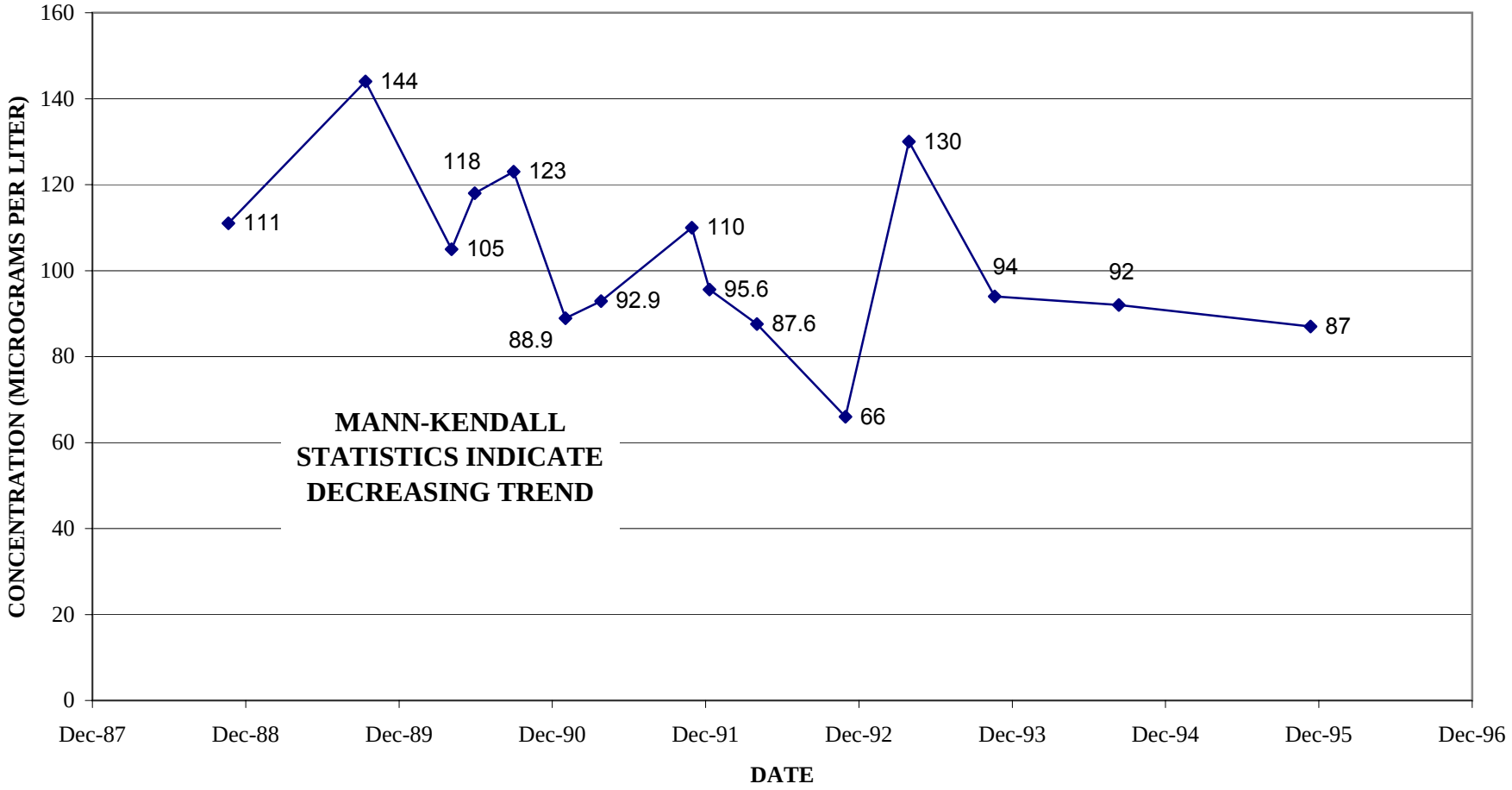
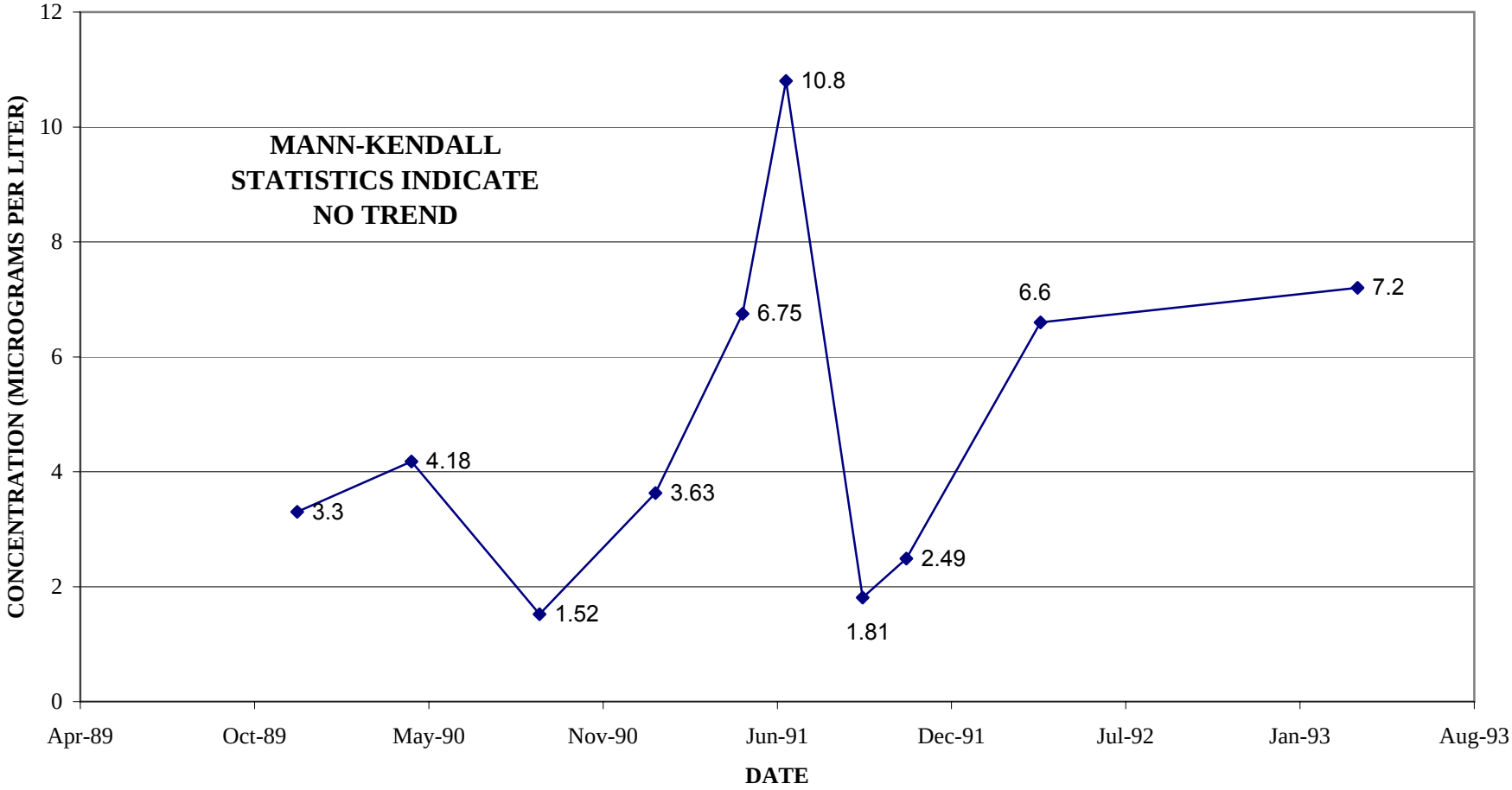


Figure F-2c MtBE Concentration Versus Time for Well with No Trend



APPENDIX G
EXAMPLE FIRST ORDER RATE CALCULATION

A first-order model is typically used to fit flow path or time series data, as it is often the most appropriate for describing biodegradation and other chemical transformation reactions that tend to dominate the loss of chemical mass at most sites. The basis of the first order model is the differential equation:

$$\frac{dC}{dt} = -kC$$

where:

C	=	contaminant concentration
t	=	time
k	=	the attenuation rate constant [time^{-1}].

This relationship states simply that the rate of concentration change (by attenuation) is directly proportional to the value of concentration. An attenuation rate coefficient (k) describes the magnitude of the rate relative to the concentration. This equation can be solved and rearranged to show that the ratio of any two observed concentrations is exponentially related (by the reaction rate constant) to the elapsed time between the two observations (t).

$$\frac{C_B}{C_A} = \exp(-kt)$$

This solution can also be written as:

$$\log\big(C(t)\big) = \log\big(C_o\big) - kt\,,$$

which can also be written as:

$$C = C_o e^{-kt}$$

which is a log-linear equation that relates the concentration at any time t (i.e., $C(t)$) to a previous or initial concentration (C_o): the logarithms of concentration will fall on a straight line of slope k when plotted against elapsed time. Using multiple observations allows the impacts of field and measurement variability to be mitigated by fitting the data to this function (i.e., regression), and deriving an average value for k.

The example provided in Figure G-1 depicts how a first-order model can be fit to field data. Concentration at points A and three points (B, C, and D) downgradient of A are plotted on the y-axis as a log-scale and contaminant travel time from A to all other

points (transport distance divided by the transport velocity) is plotted on the x-axis as a linear-scale. A straight line is regressed through the data and the slope of this line is k. Many software programs will perform the regression automatically. These programs will also report the equation for this line (i.e., *k*) and the regression coefficient. To fit these data using a linear concentration axis (y-axis), the data must first be converted to natural log (ln) of the actual remaining concentration (C/C_0) at point B, C, and D, and fitted to the straight line equation y = mx + b (where the rate constant is equal to the slope (m). Figure G-1 shows linear regression results for a hypothetical data set located along a flow path.

Sample	Distance (feet)	Travel Time (days)[1]	Concentration (mg/l)	Natural Log C/C_0	k (attenuation rate)
A	0	0	97	0	0.0054 $days^{-1}$
B	50	100	91	-0.063851472	
C	145	290	64	-0.415827895	
D	205	410	8	-2.495269437	
[1] Calculated based on an average groundwater velocity of 0.5 feet/day					

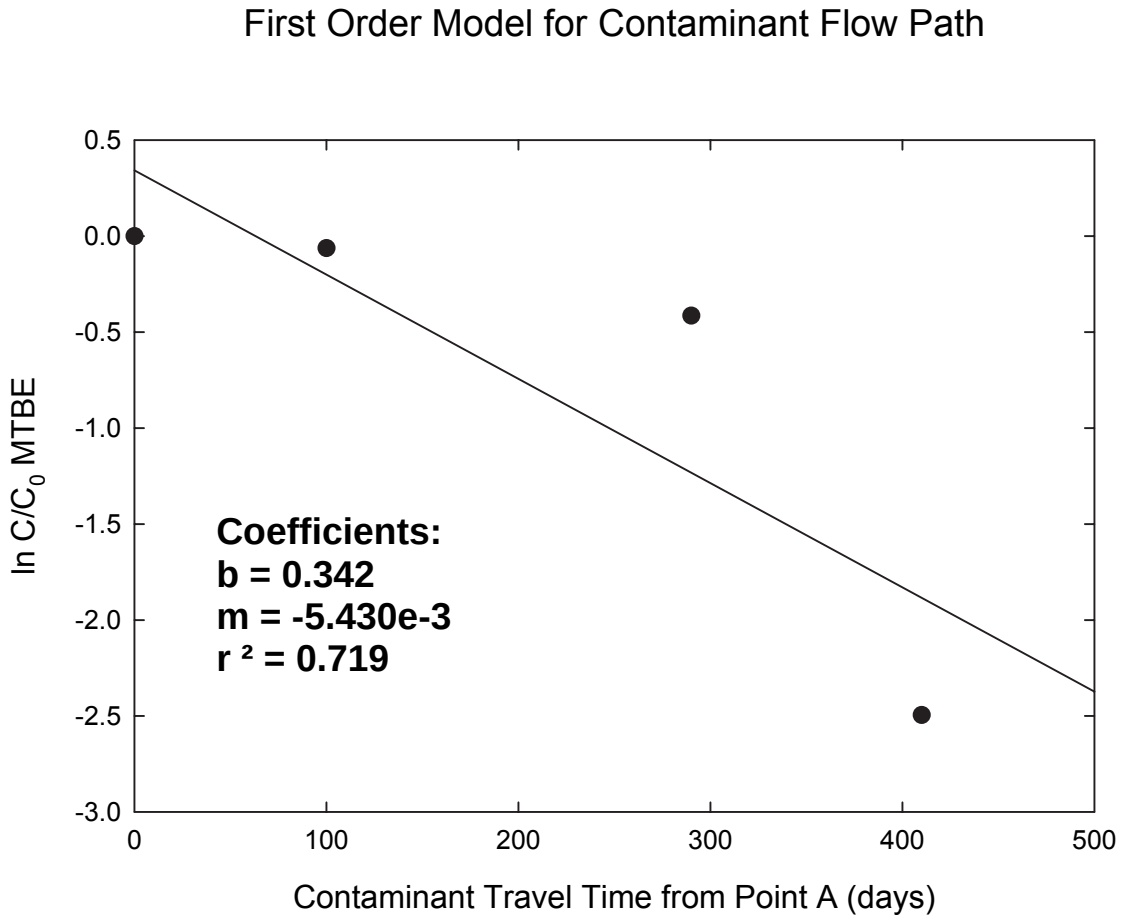


Figure G-1 Attenuation Rate Sample Calculation

APPENDIX H
Summary of the Results of TBA NAPL/Aqueous Partitioning Experiments (Period 9/16/03 – 3/15/04)

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In our last summary (9/15/03), experiments were reported for the partitioning of TBA between water and three different NAPLs: n-octane, 1,2,4-trimethylbenzene (TMB), and a six-component NAPL mixture. These experiments were for an initial concentration of TBA in NAPL of 0.5 wt.%, and were conducted to obtain equilibrium partitioning data in the lower TBA concentration range. The n-octane and TMB experiments were reported for 24 ^{o}C, and the NAPL mixture experiments were reported for 24 ^{o}C and preliminary experiments were reported for 10 ^{o}C. Experiments with an initial TBA concentration in NAPL of 5.0 wt. % were reported in our first summary (5/02/03). These experiments were conducted to obtain equilibrium partitioning data in a higher TBA concentration range.

In this current summary results for the following additional experiments are reported: n-octane and TMB at 10 ^{o}C, and n-octanol at 23 ^{o}C. The initial TBA concentration in NAPL was 0.5 wt.%. Also some of the experiments reported in the previous summaries were repeated (at 23 ^{o}C and 10 ^{o}C) in this latest round of experiments in order to obtain better mass balances for the experimental data. The TBA mass balances, i.e., initial TBA mass in NAPL vs. TBA mass in both phases at equilibrium, for all data are now within ±10%.

Again all experiments were conducted in triplicate. Also included in this new summary are comparisons of measured vs. predicted partition coefficients using UNIFAC (Fredenslund et al., 1975; Prausnitz et al., 1999).

All of the results for this project are included in this summary for completeness.

Materials and Methods

A series of aqueous-NAPL batch partitioning experiments was conducted using a six-component NAPL mixture to quantify the equilibrium partitioning of TBA between the organic phase and aqueous phase as a function of varying TBA concentrations in the aqueous and NAPL phases. These experiments were conducted with an initial TBA concentration of 0.5 wt. % at temperatures of 23 ^{o}C and 10 ^{o}C.

Additional experiments were also conducted with n-octane and 1,2,4 trimethylbenzene as the NAPL phase. These experiments were also conducted with an initial TBA concentration of 0.5 wt. % at 23 ^{o}C and 10 ^{o}C.

NAPL mixture experiments:

The initial composition (in weight fraction) of the NAPL mixture for these experiments was as follows: TBA, 0.005 benzene, 0.005; toluene, 0.05; m-xylene, 0.12; 1,2,4-trimethylbenzene

(TMB), 0.30; n-octane, 0.52. The NAPL mixture was equilibrated with varying amounts of 0.005 M $CaCl_2$ solution in 50 ml VOA glass vials. The volume ratios of NAPL/water in glass vials were from 0.1 to 4. The glass vials were kept at either 10 deg C±0.5 ^oC or 23±1 ^oC for one week before sampling. The concentrations of TBA, benzene, toluene, m-xylene, TMB and n-octane in both the organic phase and the aqueous phase were analyzed by a HP 6890 GC system equipped with a OI FID detector. Each set of the partitioning experiments at a given volume ratio was conducted in triplicate. The procedure for these experiments is similar to that for the batch experiments described previously (Rixey and He, 2001; He, 2002).

n-octane, 1,2,4-trimethylbenzene, and n-octanol NAPL experiments:

The initial compositions (in weight fraction) of these experiments were as follows: TBA, 0.005 and n-octane, 0.995; TBA, 0.005 and 1,2,4-trimethylbenzene, 0.995; TBA, 0.005 and n-octanol, 0.995. Similar to the NAPL mixture experiments, the NAPLs were equilibrated with varying amounts of 0.005 M $CaCl_2$ solution in 50 ml VOA glass vials. The volume ratios of NAPL/water in glass vials were from 0.1 to 4. The glass vials were kept at kept at either 10 ^oC±0.5 ^oC or 23±1 ^oC for one week before sampling. (Experiments for n-octanol were conducted at 23 ^oC only.) The concentrations of TBA in both the NAPL and the aqueous phase were analyzed by a HP 6890 GC system equipped with a OI FID detector. Each set of the partitioning experiments at a given volume ratio was conducted in triplicate.

Results and Discussion

Measured NAPL/water Partition Coefficients at 23 ^oC :

The partition coefficients, K [(g_i/cm^3-o)/(g_i/cm^3-w)] where 'o' denotes oil phase or NAPL, for TBA between the NAPLs and the aqueous phase vs. TBA equilibrium aqueous concentrations are shown in Figures 1 and 2. The partition coefficient for TBA was determined from the measured TBA concentration in the NAPL phase (g/cm^3-o) at equilibrium divided by its measured aqueous concentration (g/cm^3-w) at equilibrium. TBA mass balances, i.e., initial TBA mass in NAPL vs. TBA mass in both phases at equilibrium, for these data were all within 10%.

The partition coefficients for TBA were relatively constant vs. TBA equilibrium concentration as shown in Figures 1 and 2. Theoretical calculations using UNIFAC (discussed below) predict a slightly decreasing partition coefficient with increasing concentration over this same concentration range.

The partition coefficient, K [(g_i/cm^3-o)/(g_i/cm^3-w)], was highest for 1,2,4-trimethylbenzene (0.145), lowest for n-octane (0.065) with an intermediate value of 0.12 for the NAPL mixture. Calculations using UNIFAC yield similar differences in partition coefficients among these three NAPLs. The differences in partition coefficients for the three NAPLs are largely due to differences in NAPL phase activity coefficients. Results of these calculations are discussed below.

Effect of Temperature on TBA Partition Coefficients:

Partition coefficients were also measured at 10 ^{o}C and are shown in Figures 3a-c. Partition coefficients at 10^{o}C were approximately a factor of two lower than values at 23 ^{o}C.

Comparison of Measured and Predicted (UNIFAC) Partition Coefficients:

Estimated partition coefficients, $K_o=C_o/C_w$, were calculated using the following equation (Garg and Rixey, 1999):

$$K_o = \frac{\gamma_w \rho_o MW_w}{\gamma_o \rho_w MW_o}$$

where:

K_o = NAPL/water partition coefficient (L-w/L-o)
ρ_o = density of NAPL (kg/L-o)
ρ_w = density of aqueous phase (kg/L-w)
MW_o = molecular weight of NAPL
MW_w = molecular weight of aqueous phase
γ_o = activity coefficient for TBA in NAPL (unitless)
γ_w = activity coefficient for TBA in aqueous phase (unitless)

Activity coefficients for TBA in the NAPL phase were estimated using UNIFAC (Fredenslund et al., 1975; Prausnitz et al., 1999). UNIFAC (*uni*versal *f*unctional *a*ctivity *c*oefficient) is based on the UNIQUAC (*uni*versal *qua*si-*c*hemical theory) equation (Abrams, 1975). It uses a group contribution method approach for estimating the molecule-molecule interaction parameters for UNIQUAC. In group contribution-based methods, molecules are divided into functional groups, and a given functional group is assumed to behave in a manner independent of the molecule in which it appears. For our calculations we used the software (UNIFAC Activity Coefficient Calculator) developed by Choy and Reible, 1996. This calculator is available at http://www.hsrc-ssw.org/ssw-downloads.html

Activity coefficients for water obtained from the literature (Whitehead and Sandler, 1999) were used in our calculations of K_o, since UNIFAC significantly over predicts the activity coefficient for TBA in water as shown in Figure 6.

As shown in Figure 4, UNIFAC provided reasonable predictions of partition coefficients for TBA for the three NAPLs when measured (literature) values for activity coefficients in water were used.[1] UNIFAC predictions yielded values approximately equal to measured values of K_o for n-octane. Predicted values for the NAPL mixture were 10 to 20% greater than measured values, and predicted values for TMB were 30 to 45% greater than measured values.

[1] Partition coefficients based on UNIFAC calculations for activity coefficients in both the NAPL and aqueous phases were up to 7 times greater than the measured values.

The differences in partition coefficients for the three NAPLs are largely due to differences in NAPL phase activity coefficients. The differences in NAPL molecular weights and densities are a smaller contribution to the observed change in partition coefficients. The calculated and measured activity coefficients for TBA in the three NAPLs are shown in Figure 5. The relative differences between predicted and measured activity coefficients are the same as that reported above for the predicted vs. measured activity coefficients. Note that the NAPL phase activity coefficients for TBA range from 8 to 22 for the various NAPLs, thus significant non-ideality (relative to Raoult's Law) for TBA in the NAPL phase is observed.

These calculations indicate that UNIFAC reasonably estimates (within 50%) the partitioning of TBA in different NAPL mixtures, and provides an understanding of the magnitude of the observed partition coefficients and of the differences in the values observed for the various NAPLs.

Implications for Relative Concentrations of TBA/MTBE in Ground Water near a NAPL Source:

In Figure 7 TBA concentrations in water are compared with MTBE concentrations for various NAPL/water ratios. Our experimental data for the 6-component NAPL mixture are compared with calculations assuming constant NAPL/water partition coefficients of 0.12 and 0.06 L-w/L-NAPL (from Figure 3b) at 23 ^{o}C and 10 ^{o}C, respectively. The curve for MTBE is based on K_{NAPL} = 16 L-w/L-NAPL for a similar NAPL (Rixey and Joshi, 2000). The initial concentration in the NAPL mixture, $C_{o,NAPL}$, was 4,000 mg/L-NAPL for the TBA experiments. The curve for MTBE is based on assuming an initial concentration of 100,000 mg/L-NAPL. (The initial ratio of TBA/MTBE in the NAPL = 0.04 for this figure).

Figure 7 illustrates the potential relative concentrations of TBA and MTBE that could be observed near a NAPL source for different NAPL to water ratios. When free-product NAPL is present as a source of contamination, NAPL saturations are high (corresponds to high V_{NAPL}/V_w). For NAPL saturations, $S_{NAPL} > 0.80$ ($V_{NAPL}/V_w > 4$; assumes saturated zone, i.e., $S_{NAPL}+S_W=1$), TBA concentrations in water in equilibrium with the NAPL are significantly greater than MTBE concentrations in water.

Figures 8 a & b also illustrate the relative concentrations of TBA and MTBE in water. Curves in Figure 8a are shown for two values of the initial ratio of TBA/MTBE in NAPL of 0.02 and 0.2, assuming V_{NAPL}/V_w =1 (corresponds to S_{NAPL}=0.5) and a partition coefficient, K_{NAPL}=0.24 L-w/L-NAPL. These curves are reproduced from Kolhatkar, 2003. In Figure 8b, the curves of Figure 8a are shown along with two other curves assuming 100% NAPL saturation (S_{NAPL}=1) and partition coefficients measured in this study (K_{NAPL}=0.12 at 23 ^{o}C and 0.06 at 10 ^{o}C). The additional curves in Figure 8b increase the previous predictions of TBA concentrations relative to MTBE by up to a factor of 10.

The curves of Figure 8 represent the relationship between concentrations of TBA and MTBE in water at various points downstream of a highly concentrated NAPL source where both MTBE and TBA attenuate only by dilution due to dispersion in ground water. Observed values of TBA above these lines therefore could represent the possible degradation of MTBE to TBA as

indicated in Figure 8a. Observed values of TBA near these lines could represent that TBA is coming from the NAPL source, particularly for the high concentration region.

TBA Partition Coefficients at Higher Aqueous Concentrations:

Experiments were also conducted for initial TBA concentrations of 5 wt.% in the NAPL mixture in order to obtain partition coefficients at higher TBA concentrations. These experimental results were reported previously (5/2/03) and are reproduced in Figures 9a & b. (Lower concentration data reported in Figure 3b are not included, but the values do converge to the same value of K at the lower concentrations). Partition coefficients are relatively constant at low TBA concentrations in NAPL (< 0.02 g/cm^3 or 0.25 wt. %), then increase significantly at higher concentrations. This needs to be considered when predicting groundwater impacts for NAPLs containing higher levels of TBA.

Conclusions

1) Measured partition coefficients for TBA varied from 0.065 to 0.145 L-w/L-o at 23 ^oC for three NAPLs (K_{NAPL}=0.12 for a 6-component model gasoline). These values are of the same order of magnitude as previously reported values (Kolhatkar, 2003) for similar NAPLs. For comparison these values are significantly lower than the measured value for n-octanol/water of 1.8 L-w/L-octanol.

2) Temperature had a significant effect on measured partition coefficients for TBA. Partition coefficients at 10 ^oC were two times lower than values at 23 ^oC.

3) UNIFAC provided reasonable predictions of partition coefficients for TBA for the three NAPLs when measured (literature) values for activity coefficients in water were used. Partition coefficients based on UNIFAC calculations for both the NAPL and aqueous phases were up to 5 times greater than the measured values. These calculations indicate that UNIFAC reasonably estimates (within 50%) the partitioning of TBA in different NAPL mixtures, and provides an understanding of the magnitude of the observed partition coefficients and of the differences in the values observed for the various NAPLs.

4) Partition coefficients are relatively constant at low TBA concentrations in NAPL (< 0.5 wt. %), but increase significantly at higher concentrations. This needs to be considered when predicting groundwater impacts for NAPLs containing higher levels of TBA.

5) The use of lower partition coefficients measured in this study (K_{NAPL}=0.12 at 23 ^oC and 0.06 at 10 ^oC) and higher NAPL saturations (higher NAPL/water volume ratios) resulted in an increase of TBA concentrations relative to MTBE by up to a factor of 10 over previous predictions. This is significant when assessing the contribution of biodegradation to TBA concentrations in ground water.

Future Work

These results complete the current proposed scope of work for this project. The following are suggestions for additional work:

a) additional NAPL/water partition coefficient measurements for TBA for the higher aqueous concentration range at 10 ^{o}C,
b) direct measurement of vapor phase concentrations in equilibrium with NAPLs containing TBA and MTBE (our measured NAPL activity coefficients coupled with readily available vapor pressure data can be used to calculate vapor concentrations for TBA),
c) measurement of MTBE partition coefficients at 10 ^{o}C,
d) partition coefficient measurements for other oxygenates of interest, and/or
e) UNIFAC calculations for other oxygenates of interest.

In addition an API Technical Bulletin on NAPL/water partitioning for TBA will be prepared. The possibility of co-writing this technical bulletin with members of the soil/groundwater task force (e.g., particularly Dr. Ravi Kolhatkar of BP) will be pursued.

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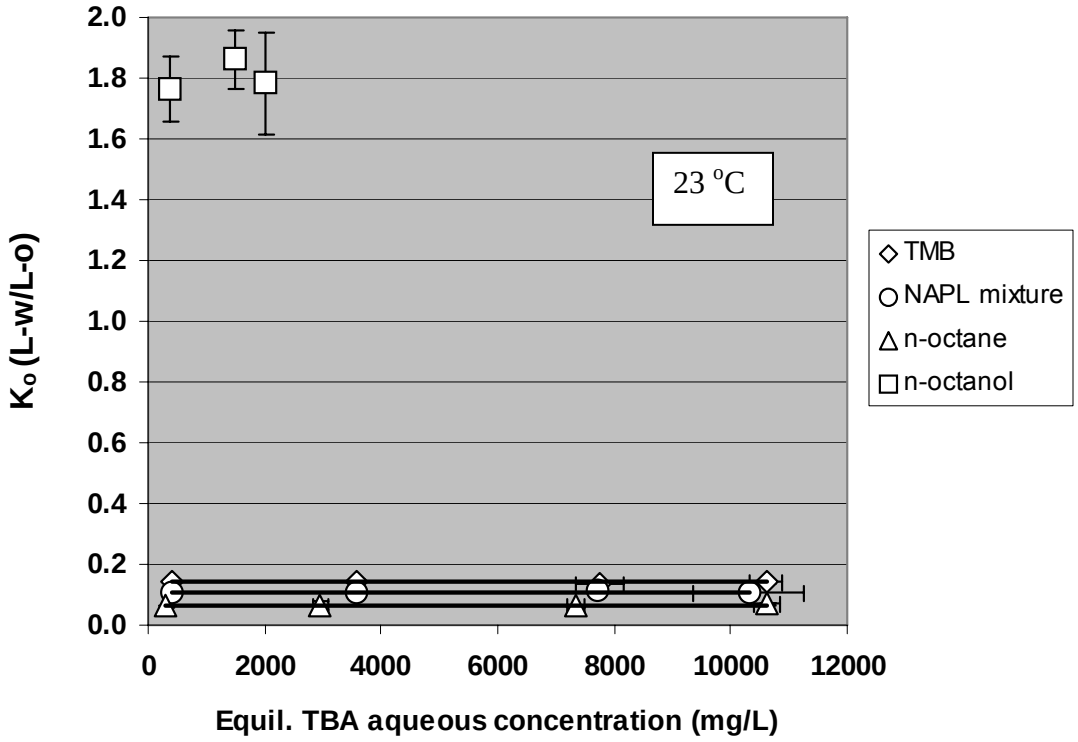


Figure 1. Partition coefficients for TBA partitioning between various NAPLs and an aqueous phase. T=23 °C. Partition coefficients are plotted vs. TBA equilibrium aqueous phase concentrations.

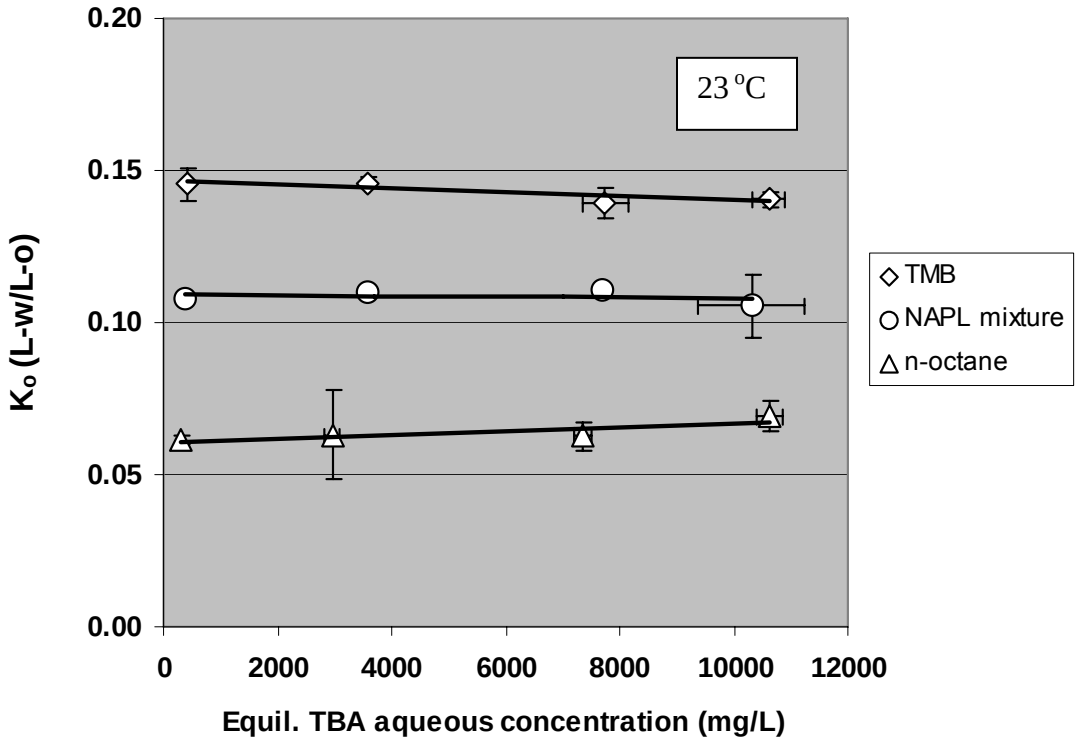
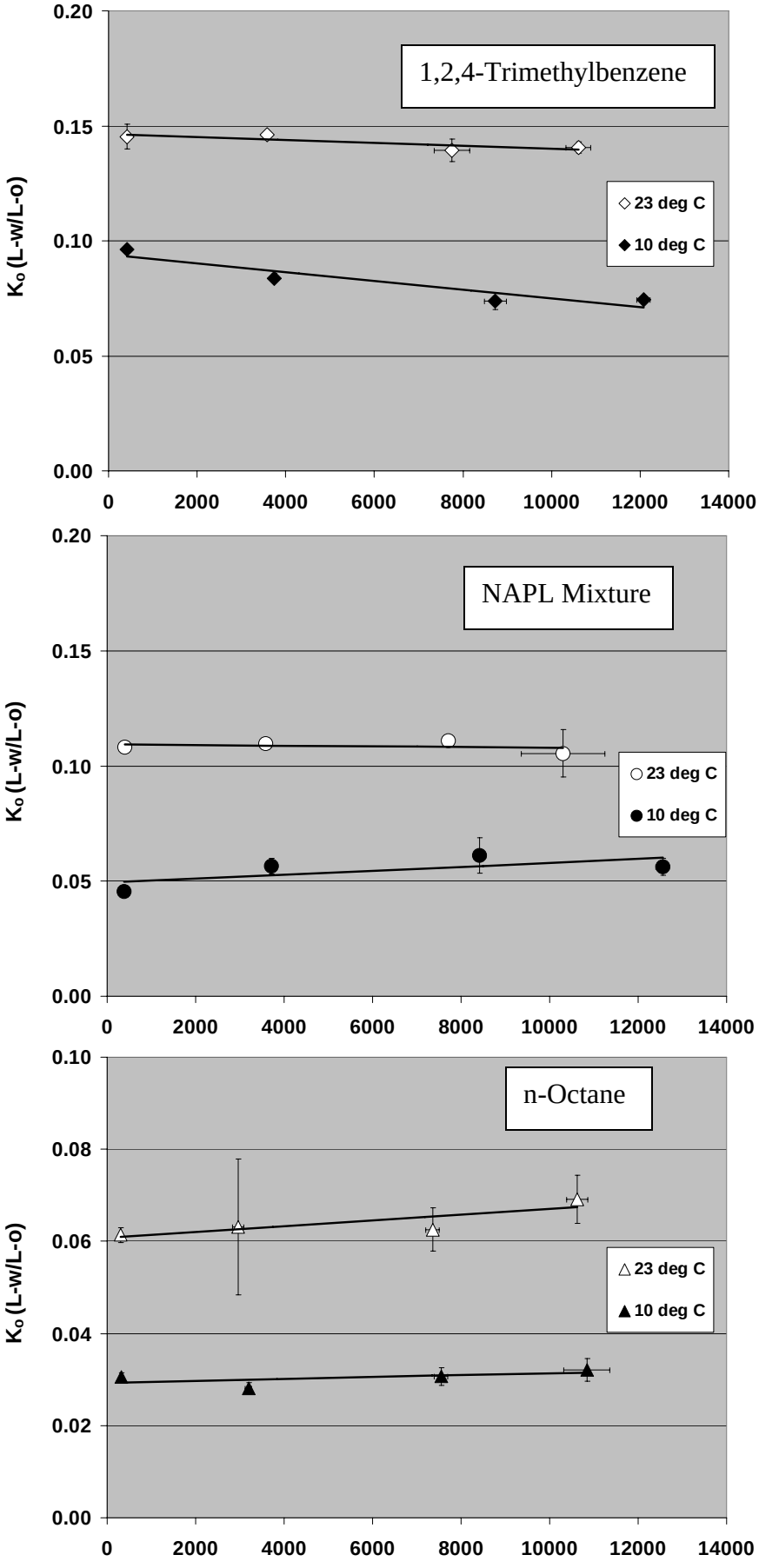


Figure 2. Same as Figure 1 except for expanded scale for TMB, NAPL mixture, and n-Octane.



(a)

(b)

(c)

Figures 3a-c. Partition coefficients for TBA at 23 ^{o}C and 10 ^{o}C.

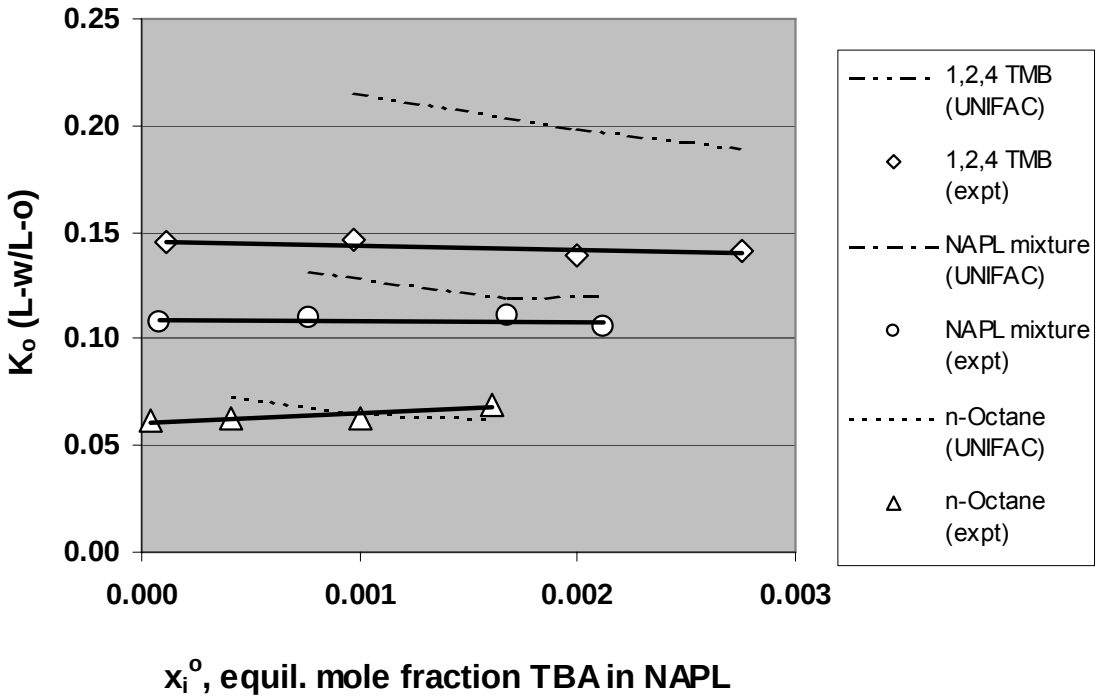


Figure 4. Comparison of UNIFAC calculated and measured partition coefficients for TBA partitioning between various NAPLs and an aqueous phase at 23 ^{o}C. Aqueous phase activity coefficients based on literature values (Whitehead and Sandler, 1999); NAPL activity coefficients based on UNIFAC. Aqueous concentration range for UNIFAC calculated curves is limited to the range of literature values for aqueous phase activity coefficients.

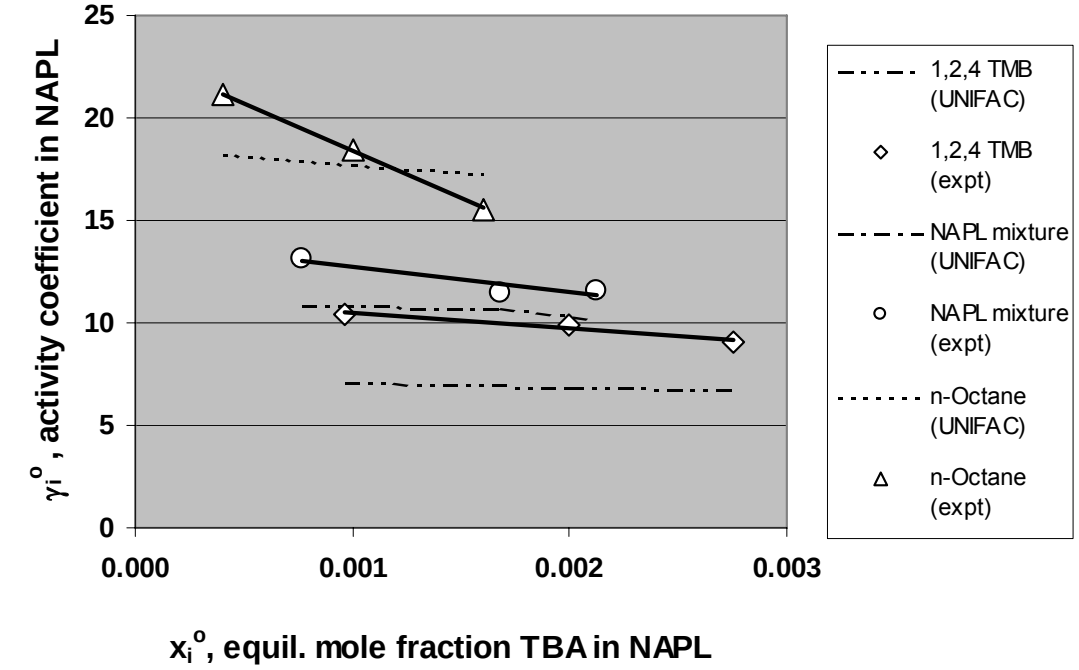


Figure 5. Comparison of UNIFAC calculated and measured activity coefficients for TBA in various NAPLs at 23 ^{o}C.

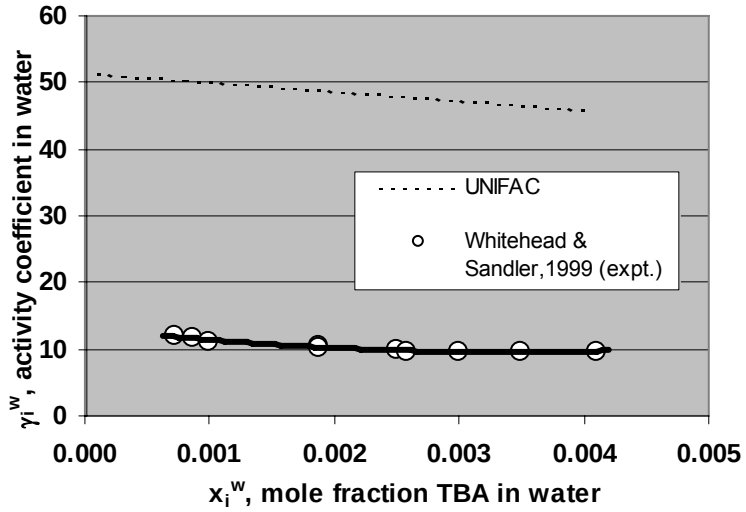


Figure 6. Comparison of UNIFAC calculated and measured activity coefficients for TBA in water at 23 ^{o}C.

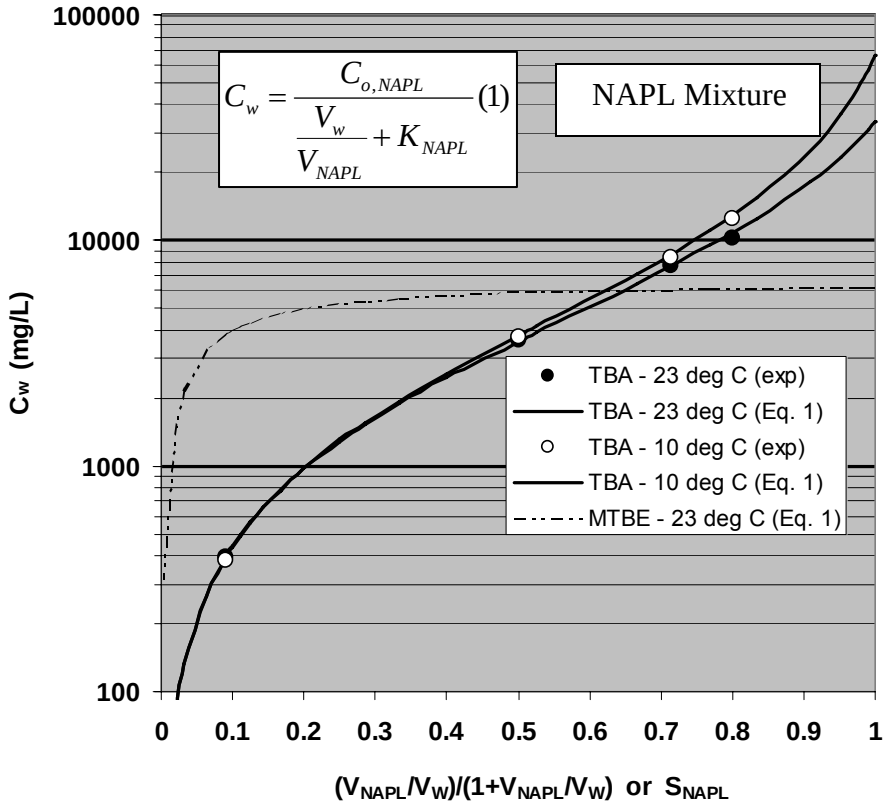
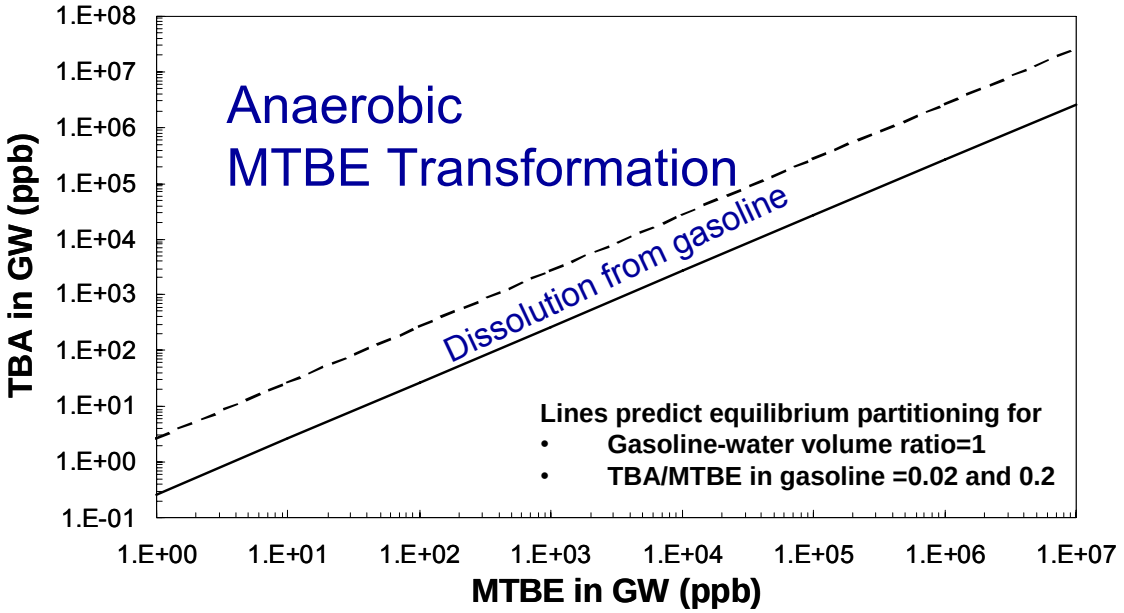
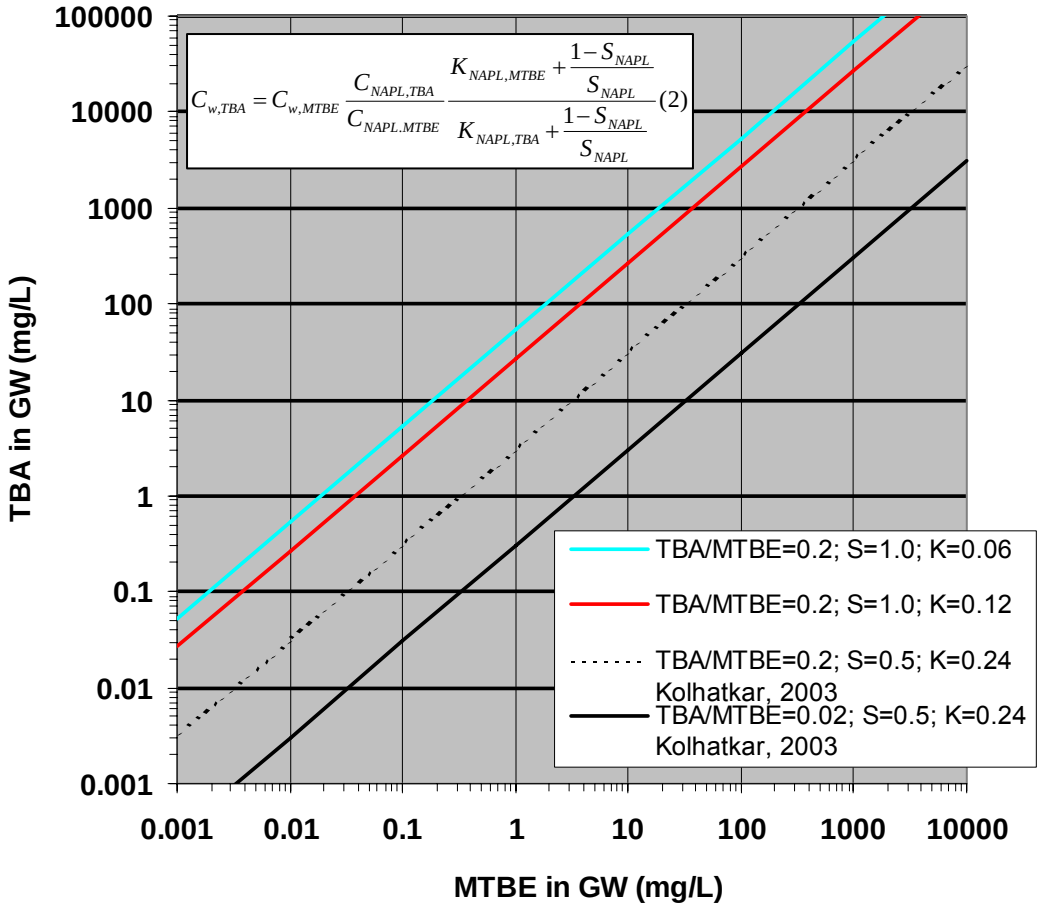


Figure 7. Concentrations of TBA in water for various NAPL/water volume ratios. The TBA experimental values for the 6-component NAPL mixture are compared with calculated values using Equation 1 with constant K_{NAPL} values of 0.12 (23 deg C) and 0.06 (10 deg C) L-w/L-o. The curve for MTBE is based on K_{NAPL} = 16 L-w/L-o for a similar NAPL (Rixey and Joshi, 2000). The initial concentration in the NAPL mixture, $C_{o,NAPL}$, was 4,000 mg/L-o for the TBA experiments. The curve for MTBE is based on assuming an initial concentration of 100,000 mg/L-o in the NAPL. Note: K_{NAPL} is the same as K_o in previous figures.

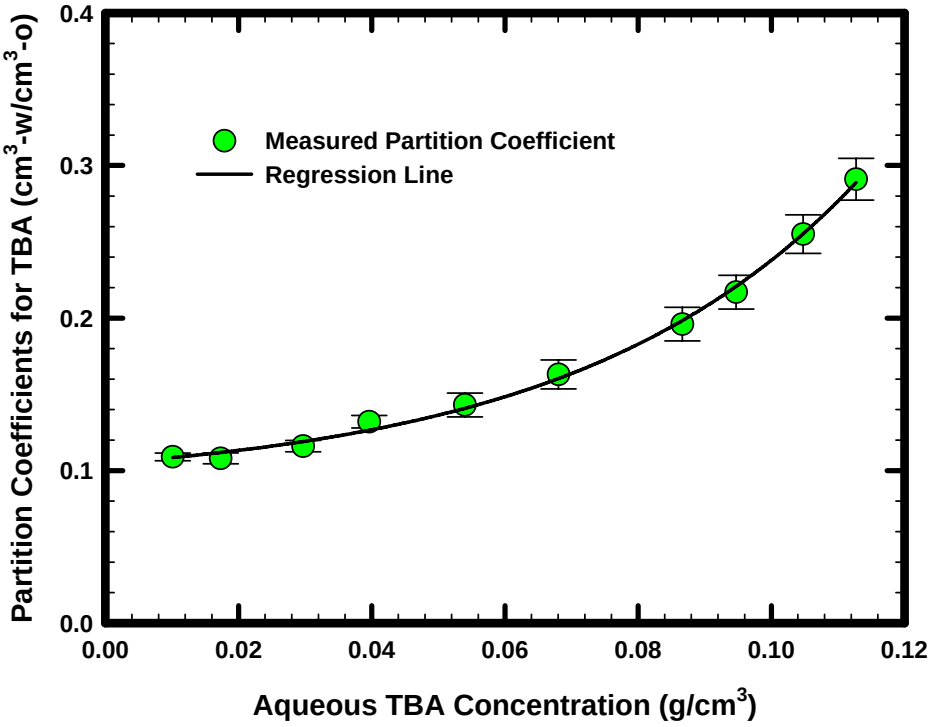


(a)

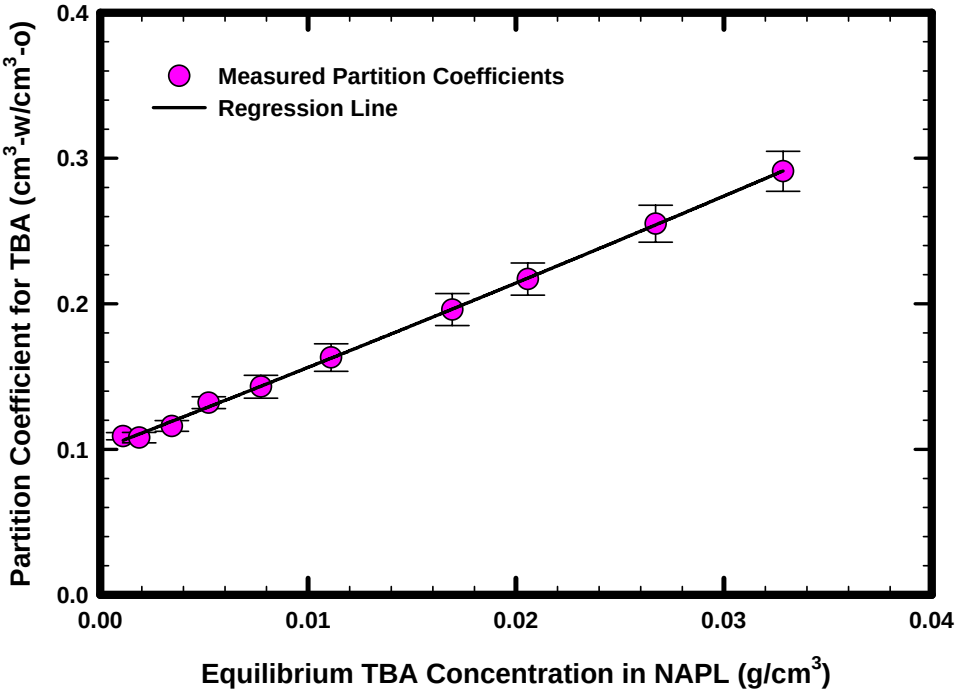


(b)

Figures 8a-b. Calculated concentrations of TBA in water vs. MTBE concentrations in ground water near a NAPL source. Calculations are based on Equation 2 for various values of K_{NAPL} for TBA and ratios of concentrations of TBA to MTBE in the NAPL. Figure 8a is reproduced from Kolhatkar, 2003. Curves from 8a are also shown in 8b. Note S_{NAPL}=0.5 corresponds to V_{NAPL}/V_w=1; S_{NAPL}=1 corresponds to V_{NAPL}/V_w=∞.



(a)



(b)

Figure 9a-b. Effect of higher concentrations on partition coefficients for TBA partitioning between a NAPL mixture and an aqueous phase at 24 ^{o}C. Partition coefficients are plotted vs. equilibrium aqueous concentration (a) and NAPL concentration (b).

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