



Technical Protocol for Evaluating the Natural Attenuation of MtBE

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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., 1999, (9) Schwarzenback et al., 2004, (10) Montgomery 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; DeVaul 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; DeVaul et al., 2003; Wilson, et al., 2005a, 2005b); and in methanogenic conditions (Yeh and Novak, 1994; Wilson et al., 2000; DeVaul 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; DeVaul 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 (DeVaul, 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 footprint² 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 $\delta^{13}\text{C}$ and $\delta^2\text{H}$ 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.

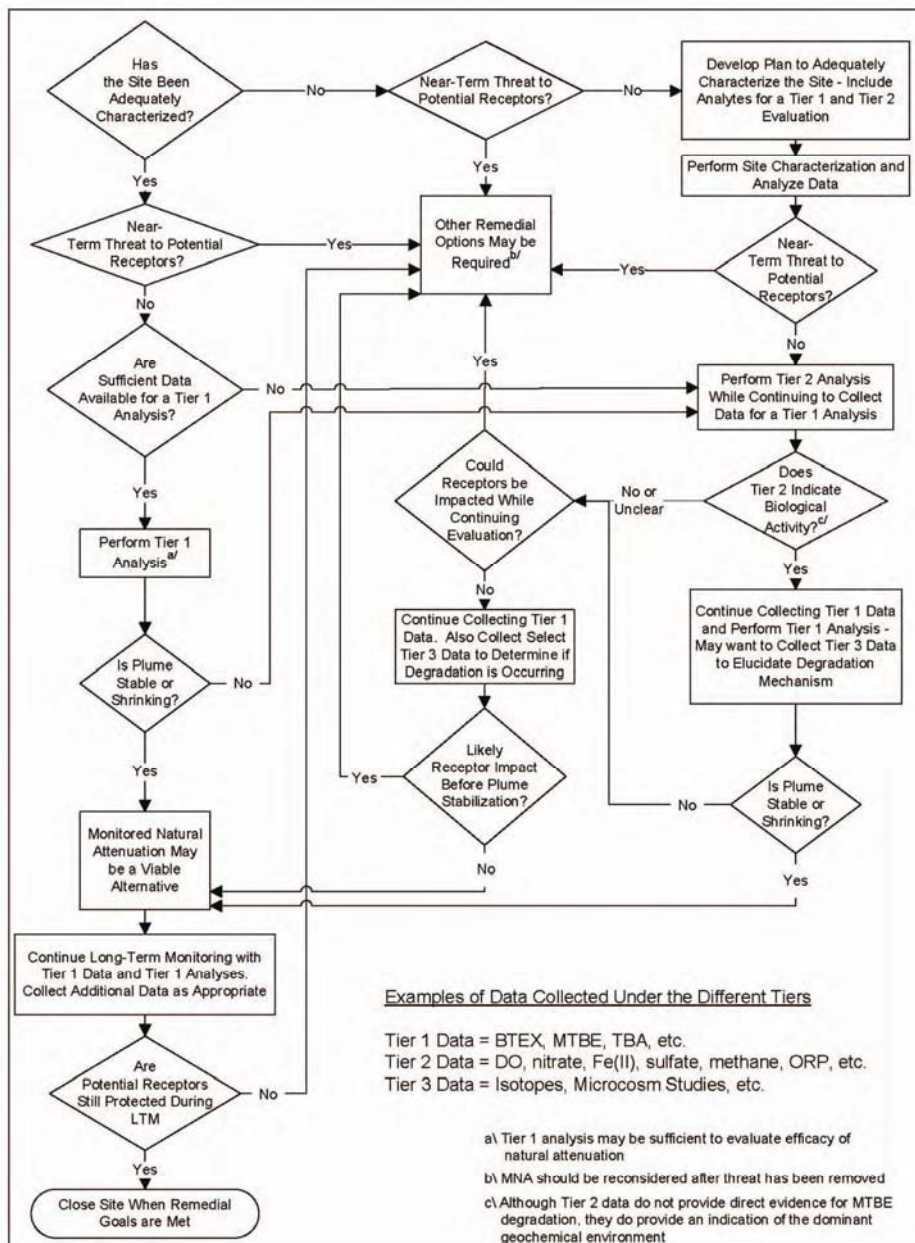


Figure 2-1 Flow Chart Showing Stepwise Approach for Using the Three Tiers of Data

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 $^{13}\text{C}/^{12}\text{C}$ or $^2\text{H}/^1\text{H}$ 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°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 MtBE³. 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 ₂ SO ₄ to pH<2, Cool 4°C	Plastic or Glass	100 ml	28 days
Nitrate	EPA 300.0 or equivalent	H ₂ SO 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 ₃ PO ₄ or H ₂ SO 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 - <i>Test Method for Evaluating Solid Waste, Physical and Chemical Methods</i>, SW-846, EPA, 3rd ed., 1986 and revisions. EPA/600/R-95-131 - <i>Methods for the Determination of Organic Compounds in Drinking Water-Supplement III</i> (EPA/600/R-95-131). c. Gas Chromatography Isotope Ratio Mass Spectrometry. d. Kampbell and Vandegrift, 1998. <i>J. Chromat. Sci.</i>, 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°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°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°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	Azeotropic 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 - <i>Test Method for Evaluating Solid Waste, Physical and Chemical Methods</i> , SW-846, EPA, 3 rd ed., 1986 and revisions EPA/600/R-95-131 - <i>Methods for the Determination of Organic Compounds in Drinking Water-Supplement III</i> (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 <i>Standard Method for the Examination of Water and Wastewater</i> , 18 th ed, 1992. “B” refers to <i>Method for Chemical Analysis of Water and Wastes</i> , EPA, 1983. “SW” refers to <i>Test Method for Evaluating Solid Waste, Physical and Chemical Methods</i> , SW-846, EPA, 3 rd 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 (¹³C to ¹²C) or hydrogen (²H to ¹H) 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 δ¹³C (“delta carbon 13”) or δD (“delta deuterium”) in units of per mil (‰). For example, ¹³C enrichment is calculated relative to internationally recognized standard materials⁴ 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).

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

The hydrogen isotope ratio δD is calculated in a similar manner using a water standard⁵ (Gray et al., 2002; Hunkeler et al., 2001).

Based on two surveys of commercial gasoline, the range of $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 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 tRFLP) 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.