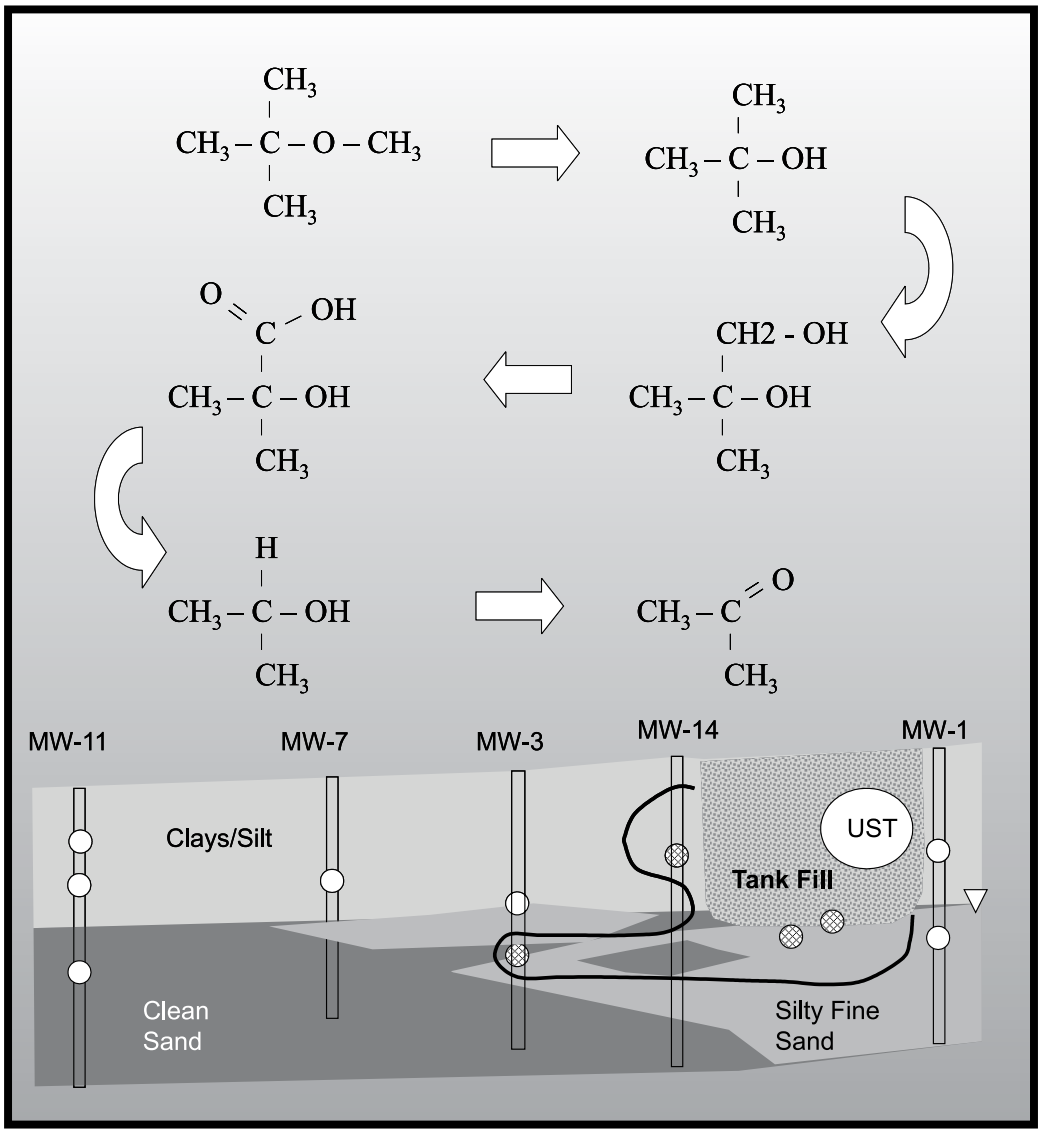
EPA United States Environmental Protection Agency

# Monitored Natural Attenuation of Tertiary Butyl Alcohol (TBA) in Ground Water at Gasoline Spill Sites



EPA/600/R-07/100
October 2007

# Monitored Natural Attenuation of Tertiary Butyl Alcohol (TBA) in Ground Water at Gasoline Spill Sites

John T. Wilson and Cherri Adair
U.S. Environmental Protection Agency
Office of Research and Development
National Risk Management Laboratory
Ada, Oklahoma 74820

Project Officer
John T. Wilson
Ground Water and Ecosystems Restoration Division
National Risk Management Research Laboratory
Ada, Oklahoma 74820

National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

# Notice

This document is intended for internal Agency use only. The U.S. Environmental Protection Agency through its Office of Research and Development funded portions of the research described here. Mention of trade names and commercial products does not constitute endorsement or recommendation for use. All research projects making conclusions and recommendations based on environmentally related measurements and funded by the Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project was conducted under a Quality Assurance Project Plan for Task 10013, Fate of Fuel Oxygenates in Aquifer Materials. Work performed by U.S. EPA employees or by the U.S. EPA on-site analytical contractor followed procedures specified in these plans without exception. Information on the plans and documentation of the quality assurance activities and results are available from Cherri Adair.

# Foreword

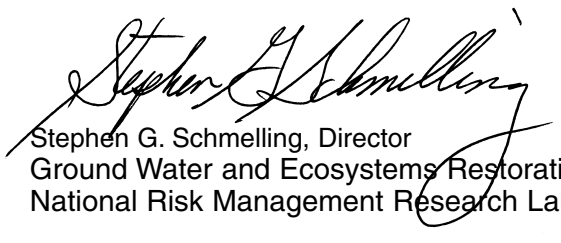
The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threatens human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Tertiary butyl alcohol (TBA) is one of the most widely distributed organic contaminants in ground water at gasoline spill sites. The U.S. EPA does not have a Maximum Contaminant Level (MCL) for TBA in drinking water. Nevertheless, many states have set standards for TBA in drinking water and clean up goals for TBA at gasoline spill sites. Because other contaminants, such as benzene and methyl tertiary butyl ether, are often biologically degraded in anaerobic ground water, the state agencies that implement the Under Ground Storage Tank program rely heavily on monitored natural attenuation to clean up these contaminants at gasoline spill sites. This report reviews the prospects for using monitored natural attenuation to manage the risk from TBA in ground water at gasoline spill sites.

The report reviews the distribution of TBA in ground water at gasoline spill sites, the process that produces TBA from anaerobic biodegradation of MTBE, and the prospects for natural biodegradation of TBA in ground water. The report presents data from a microcosm study conducted by U.S. EPA on TBA degradation in sediment from six gasoline spill sites distributed around the United States. Finally the report reviews the limited knowledge on use of stable carbon and stable hydrogen isotopes to evaluate natural biodegradation of TBA at field scale.



Stephen G. Schmelling, Director
Ground Water and Ecosystems Restoration Division
National Risk Management Research Laboratory

# Contents

# Figures

## Tables



## Appendix:

# Acknowledgments

This document was peer reviewed by Dr. Matthew Caldwell of the National Risk Management Laboratory, U.S. EPA, by Dr. Kevin T. Finneran of the University of Illinois, and by Dr. Yue Rong, Senior Environmental Scientist, California Environmental Protection Agency, California Regional Water Quality Control Board Los Angeles Region.

# Abstract

The state agencies that implement the Underground Storage Tank program rely heavily on Monitored Natural Attenuation (MNA) to clean up contaminants such as benzene and methyl tertiary butyl ether (MTBE) at gasoline spill sites. This is possible because the contaminants are biologically degraded in anaerobic ground water at the site. Tertiary Butyl Alcohol (TBA) is generally considered to be more readily degradable than MTBE, and there is a danger that the state agencies will consider contamination from TBA a good prospect for MNA. A close examination of the available information indicates that a default presumption that TBA is readily degraded in anaerobic ground water is not justified. Anaerobic biodegradation of TBA will require a supply of an electron acceptor such as sulfate or biologically available Iron(III) or Manganese(IV). The available survey data indicate that ground water in the source area of the majority of known plumes is devoid of sulfate. Although a procedure to estimate biologically available Iron(III) is commercially available, it is not routinely applied to gasoline spill sites. There is no established procedure to estimate biologically available Manganese(IV). To date, the performance of available approaches to document anaerobic biodegradation of TBA at specific field sites has been disappointing. These include field monitoring to show a statistically significant attenuation in concentration with distance along the flow path, microcosm studies conducted with sediment from the site, and analysis of stable isotope ratios in TBA in the plume.

# Introduction

Tertiary butyl alcohol (TBA) is one of the most widely distributed organic contaminants in ground water at gasoline spill sites. The U.S. EPA does not have a Maximum Contaminant Level (MCL) for TBA in drinking water. Nevertheless, many states have set standards for TBA in drinking water and clean up goals for TBA at gasoline spill sites.

The state agencies that implement the Underground Storage Tank program rely heavily on monitored natural attenuation to clean up organic contaminants in ground water at gasoline spill sites. There are a variety of processes that attenuate the concentrations of contaminants in ground water, including sorption, hydrodynamic dispersion, and biodegradation. At many gasoline spill sites, sorption and dispersion alone are not adequate to prevent the contaminants from reaching a receptor. To protect the receptor, the contaminant must be degraded in ground water. Monitored natural attenuation has been selected as a remedy, or part of the remedy, at certain fuel spill sites because it has shown that the contaminants of concern, such as benzene, are biologically degraded in anaerobic ground water (Parsons Engineering Science, 1999; Wiedemeier et al., 1999a; Wiedemeier et al., 1999b; Wiedemeier et al., 1999c).

Because TBA is very soluble in water, it dissolves readily out of spilled gasoline into ground water, and can reach high concentrations in ground water. Sorption of TBA to the solids in the aquifer matrix is negligible (Interstate Technology & Regulatory Council, 2005). To use monitored natural attenuation as a remedy for TBA contamination in ground water at many gasoline spill sites, it will be necessary to demonstrate that TBA is biologically degraded in the ground water at the site.

This report reviews the distribution of TBA in ground water at gasoline spill sites, the process that produces TBA from anaerobic biodegradation of MTBE, and the prospects for natural biodegradation of TBA in ground water. Additionally, the report evaluates available information on attenuation of TBA with distance along a flow path in ground water. This report reviews available information on the rates and extent of biodegradation of TBA in ground water. Data is presented from a microcosm study conducted by U.S. EPA on TBA degradation in sediment from six gasoline spill sites distributed around the United States. This report reviews the limited knowledge on the use of stable carbon and stable hydrogen isotopes to evaluate natural biodegradation of TBA at field scale. Finally, the report makes recommendations for using monitored natural attenuation to manage the risk posed by TBA in ground water at gasoline spill sites.

## Distribution of TBA, MTBE, and Benzene at Gasoline Spill Sites

Tertiary butyl alcohol (TBA) is widely distributed in ground water that is contaminated by spills of gasoline. Shih et al., (2004) compared the distribution of the maximum concentrations of TBA, methyl tertiary butyl ether (MTBE), di-isopropyl ether (DIPE), tertiary amyl methyl ether (TAME), ethyl tertiary butyl ether (ETBE) and benzene in ground water at 868 leaking fuel tank sites in Los Angeles, California. The geometric mean concentration of TBA was 1,730 µg/L. The geometric mean concentrations of MTBE, benzene, DIPE, TAME, and ETBE were 900, 700, 31, 24, and 7 µg/L. The concentration of TBA was slightly higher than the concentrations of MTBE and benzene, and substantially higher than the concentrations of DIPE, TAME, and ETBE. The frequency distribution of TBA, MTBE, and benzene as reported by Shih et al., (2004) is presented in Panel A of Figure 1.

The frequency distribution of concentrations of TBA and MTBE in Orange County, California are presented in Panel B of Figure 1 (Wilson et al., 2005a; personal communication Seth Daugherty, Supervising Hazardous Waste Specialist-Retired, Orange County Health Care Agency). Orange County is on the Pacific Coast in Southern California, just south of Los Angeles County. As might be expected, the distribution of concentrations of TBA and MTBE in Orange County are almost identical to the distribution in Los Angeles County.

Other regions of the United States have a similar distribution of concentrations of TBA. Panel C of Figure 1 presents data from a survey of 74 gasoline stations in the Eastern United States (Kolhatkar et al., 2000). A total of 41 sites from Pennsylvania, 8 sites from New Jersey, 5 sites from New York, 5 sites from Florida, 7 sites from Indiana, 3 sites from Maryland, 2 sites from Washington DC, and 3 sites from Ohio were included in the survey. At least six wells were sampled from each site. Panel C of Figure 1 presents the frequency distribution of the maximum concentration of TBA, MTBE, and benzene in any well at the 74 gasoline spill sites. There was no significant difference between the frequency distributions in the spill sites in the Eastern United States and the distribution reported by Shih et al., (2004) for Los Angeles, California. The concentrations of TBA were equivalent to the concentrations of MTBE. The geometric mean concentration of TBA was 1,512 µg/L while the geometric mean concentrations of MTBE and benzene were 1,724 µg/L and 447 µg/L respectively.

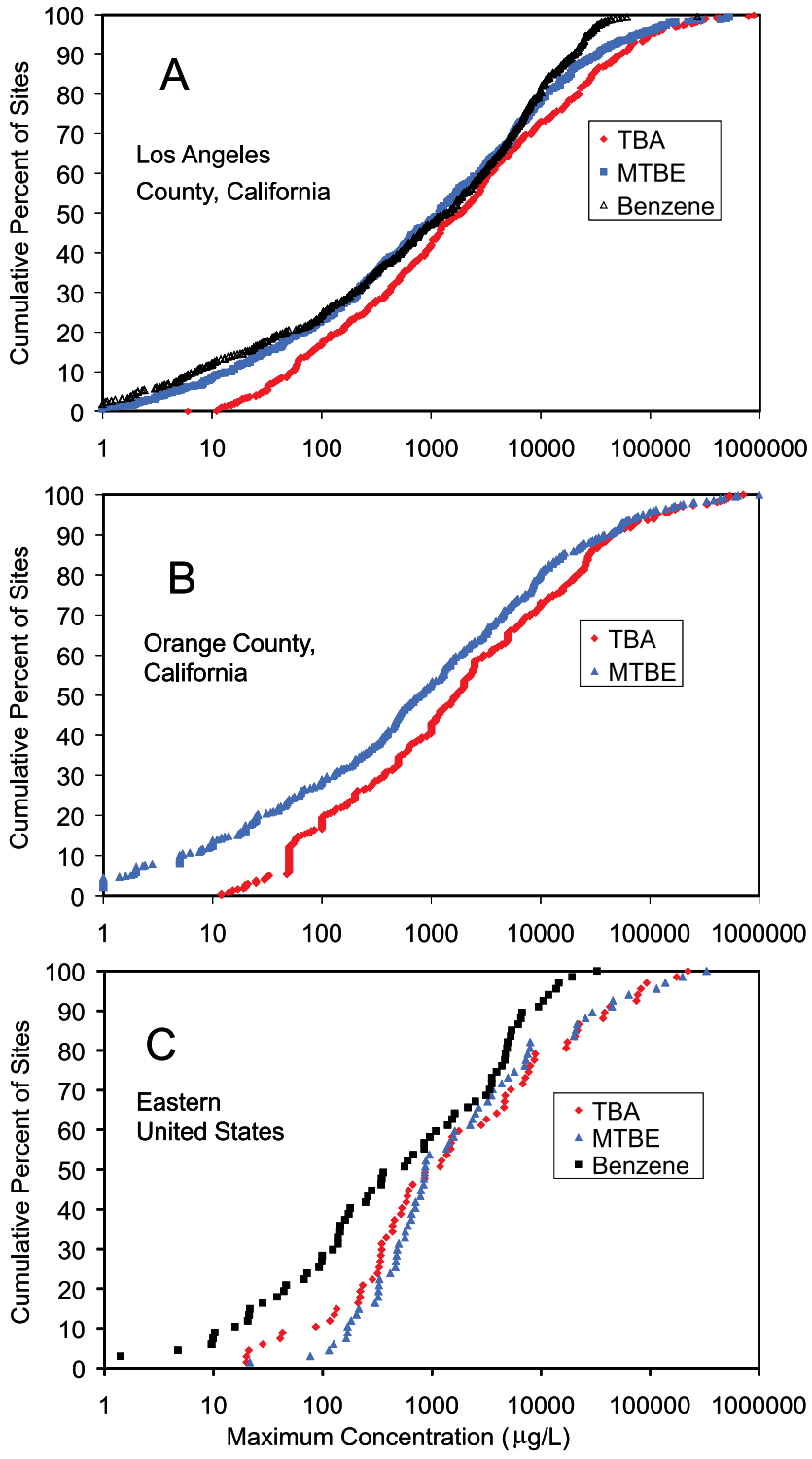



**Figure 1.** *Frequency distribution of the maximum concentrations of TBA, MTBE, and Benzene at gasoline spill sites. Panel A presents data from Los Angeles County, California. Panel B presents data from Orange County, California in 2002. Panel C presents data from the Eastern United States in 1999. Concentrations of TBA are represented by red diamonds, MTBE by blue triangles, and benzene by black squares.*

Although the frequency distribution of concentrations of TBA and MTBE are very similar in Southern California and the Eastern United States, the distributions of concentrations at individual gasoline spill sites are not similar. Panel A of Figure 2 compares the maximum concentration of TBA in any well at a site to the maximum concentration of MTBE in any well at a site in Orange County, California, in 2002 (Redrawn from Figure 1 in Wilson et al., 2005a). There was no correlation between concentrations of MTBE and TBA.

Wilson et al. (2005a) and Wilson et al. (2005b) used equilibrium partitioning theory to predict the concentrations of TBA and MTBE in ground water in contact with gasoline in an aquifer at reasonable values for residual gasoline. They assumed that gasoline spilled in Orange County was 11% MTBE by weight, and that the technical grade of MTBE used in the gasoline was 2% TBA by weight. The solid curved lines in Figure 2 are the range of concentrations of TBA and MTBE that would be expected if the residual concentration of gasoline in the spill varied from 1000 to 40,000 mg/kg total petroleum hydrocarbons. The dashed lines bound the range of concentrations that would be expected if concentrations of TBA or MTBE in contact with gasoline were attenuated by dilution and dispersion in ground water, and the TBA and MTBE did not sorb and were not biologically degraded in the ground water.

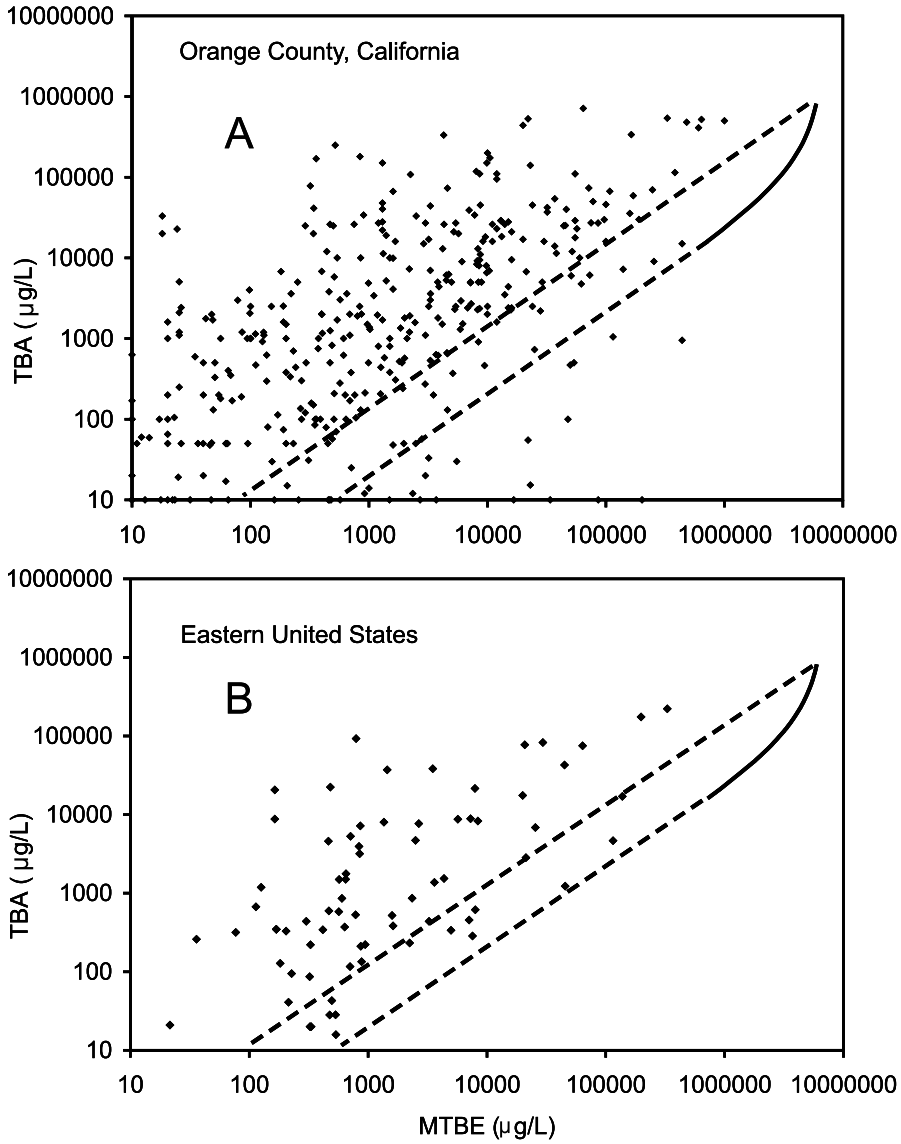



**Figure 2.** *Distribution of MTBE and TBA at gasoline spill sites. Panel A presents data from Orange County, California, in 2002. Panel B presents data from the Eastern United States in 1999. The figure compares the maximum concentration of TBA in any well at a particular gasoline spill site in any sampling period in the year against the maximum concentration of MTBE in any well at any sampling period in the year. The dashed straight lines and solid curved lines bound the range of concentrations of TBA and MTBE expected in water in contact with gasoline containing 11% MTBE and 0.22% TBA.*

Many of the data in Panel A of Figure 2 fall above the range of concentrations of TBA that would be expected for partitioning of TBA from gasoline. TBA is produced as the first biodegradation product during anaerobic biodegradation of MTBE (Kolhatkar et al., 2002); the most plausible source of the high concentrations of TBA is from natural anaerobic biodegradation of MTBE. Recent work makes it possible to recognize and track biodegradation of MTBE in ground water by a change in the ratio of the stable isotopes of carbon in MTBE (Hunkeler et al., 2001; Gray et al., 2002; Kolhatkar et al., 2002; Kuder et al., 2005; Wilson et al., 2005b; Zwank et al., 2005). Wilson et al., (2005a) used this approach to show that MTBE was being degraded at many of the sites in Orange County, California, and that the sites where MTBE was extensively degraded had high ratios of TBA to MTBE (Figure 3).

The distribution of TBA and MTBE in the survey of MTBE gasoline spill sites in the Eastern United States is presented in Panel B of Figure 2 (redrawn from Figure 3.10 in Wilson et al., 2003). The data are sparse, but the general distribution of concentrations is similar to the distribution in Southern California. As was the case in Southern California, the concentrations of TBA at many stations was from one hundred to one thousand-fold higher than would be expected from partitioning of TBA from gasoline. The high concentrations of TBA suggest that the TBA is largely produced by biodegradation of MTBE.

At this writing (June 2006), the standards set by individual states for TBA in ground water vary from 12 µg/L to 3,200 µg/L. Based on the frequency distribution of TBA in Orange County, California, approximately 5% of sites have at least one well with concentrations of TBA greater than 110,000 µg/L. In the data set reported by Shih et al. (2004) for Los Angeles, California, 5% of sites had TBA concentrations greater than 97,000 µg/L. A concentration of 110,000 µg/L would have to be diluted 10,000 fold to meet a standard of 12 µg/L, but only 34 fold to meet a standard of 3,200 µg/L. If natural attenuation is to be used as a remedy in those states that have clean up goals for TBA in the range of 12 to 140 µg/L, then natural biodegradation must make a significant contribution to natural attenuation. The remainder of this report evaluates the prospects for natural biodegradation of TBA in ground water at gasoline spill sites.

## Interpreting TBA Biodegradation at Field Scale

### *Attenuation with Distance from the Source*

The most direct approach to evaluate biodegradation of TBA at field scale is to compare the concentration of TBA in highly contaminated wells in the source area to concentrations in wells down gradient. Unfortunately, this approach rarely works well for TBA.

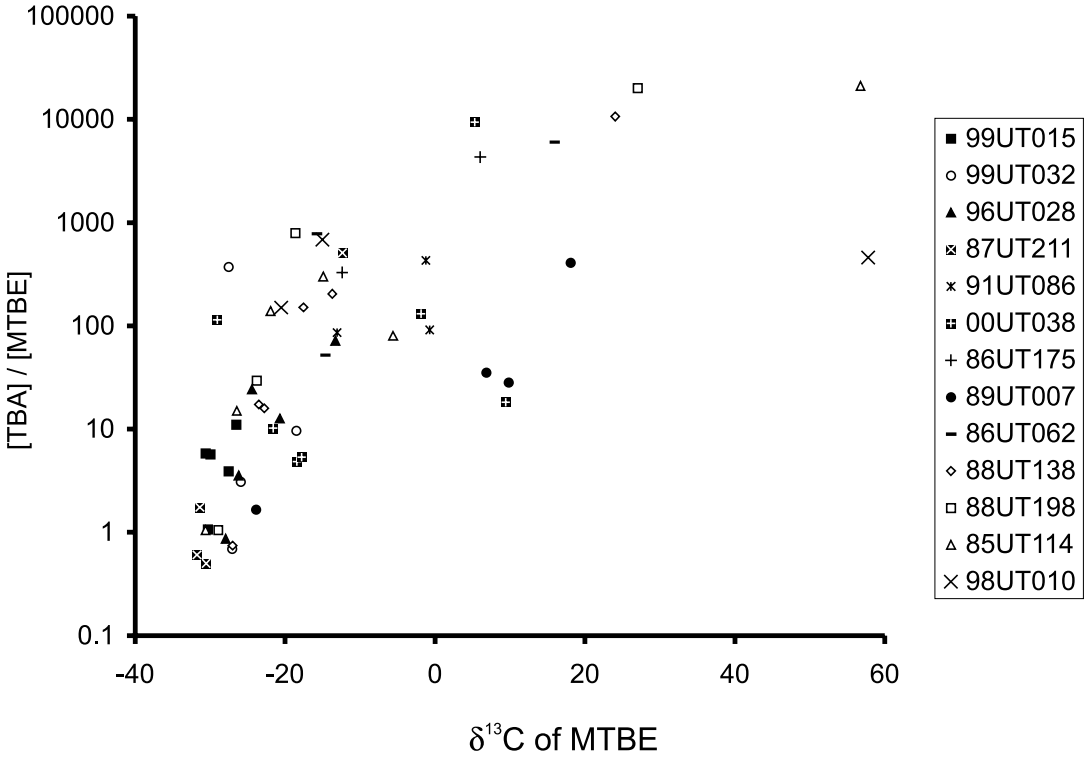



**Figure 3.** *Relationship between the fractionation of carbon isotopes in MTBE produced by biodegradation of MTBE to TBA and the ratio of TBA to MTBE in ground water in monitoring wells at 13 gasoline spill sites in Orange County, California. (Redrawn from Figure 3 in Wilson et al., 2005a)*

Buscheck and Alcantar (1995) developed an equation that could predict the first order rate of biodegradation of a compound along an inferred flow path in ground water from the changes in concentration in monitoring wells along the flow path and the seepage velocity of the contaminant along the flow path. The equation corrects for attenuation due to dispersion and sorption to aquifer solids. Kolhatkar et al., (2000) used the approach of Buscheck and Alcantar (1995) to evaluate TBA biodegradation in ground water at 74 gasoline spill sites. To correct for dilution effects in monitoring wells, Kolhatkar et al., (2000) divided the concentration of TBA by the concentration of methane in ground water.

In this approach the first-order biodegradation rate constant ($\lambda$) in a steady-state plume is estimated from (1) a linear regression of the natural logarithm of TBA concentration on distance along the flow path, (2) the coefficient of longitudinal dispersivity and (3) the flow velocity of the contaminant (equal to the seepage velocity of ground water divided by the retardation factor).

$$\lambda = \frac{v_c}{4\alpha_x}\left(\left[1+2\alpha_x\left(\frac{k}{v_x}\right)\right]^2 - 1\right)$$

Where: $\lambda$ = first-order biological degradation constant (per year),

$v_c$ = retarded contaminant velocity in the x-direction (meters per year),

$\alpha_x$ = longitudinal dispersivity (meters)

$k/v_x$ = rate of attenuation in contaminant concentration with distance along the flow path (per meter).

The rate of attenuation in contaminant concentration with distance along the flow path can be estimated as the negative of the slope of a regression of the natural logarithm of contaminant concentration on distance along the flow path (meters). Their criterion for biodegradation of TBA at a site was a rate of attenuation in contaminant concentration with distance that was statistically greater than zero. Specifically, the slope of the regression had to be greater than zero at 95% confidence.

Of 74 gasoline spill sites that they examined in the Eastern United States, they found only three sites where the regression for TBA was significant at 95% confidence. It is possible that TBA biodegradation was rare in this population of field sites. However, these sites were not research sites. The wells were installed at the direction of the regulatory authorities for monitoring to support a risk assessment and to select appropriate remedies. It is also possible that conventional practice for locating monitoring wells failed to recognize TBA biodegradation. In either case, monitoring using conventional practice provided little interpretable evidence that biodegradation of TBA was important at gasoline spill sites.

As a practical matter, it is difficult to distinguish changes in concentration due to biodegradation from changes due to purely physical processes. The concentration data may be affected by uncertainties associated with hydrological factors. If the ground water flow velocity is slow, the concentrations in down gradient wells may not have reached the maximum concentration. The plume may change flow direction, and carry the contaminated ground water away from the monitoring well. The down gradient well may be askew of the predominant flow path in the aquifer, and concentrations are lower because the well failed to sample the centerline of the plume. If there is variation in the lithology at the site, and the plume is confined to one flow zone and other zones produce clean ground water, concentrations in a down gradient well may be lower because the plume was diluted to a greater extent in the down gradient well. The concentrations of TBA can also be attenuated by hydrodynamic dispersion in the aquifer. Finally, the concentrations of TBA are related to the distribution of MTBE and BTEX contamination, particularly if the MTBE is being biodegraded to produce TBA.

### *Attenuation over Time in Individual Monitoring Wells*

Because it is often difficult to compare changes in concentration of TBA from one well to another, it is important to maintain a good continuous monitoring record of TBA concentrations over time in strategically located wells. If the general trend in concentrations over time is down, that is the best evidence available from conventional monitoring that natural attenuation processes are reducing concentrations. However, when the only data are TBA monitoring data, it is impossible to attribute natural attenuation to natural biodegradation. Very likely most of the reduction in concentration is due to physical weathering of TBA, or its parent compound MTBE from the source area of contamination at the spill site. If a transport and fate model is used as part of the risk evaluation, there is no justification to include biodegradation in the model. Including biodegradation in the model requires direct evidence that bacteria in the contaminated aquifer are capable of degrading TBA, or in fact, have degraded TBA. In the past, evidence that the aquifer harbored organisms that could degrade an organic contaminant was attained by conducting microcosm studies. In recent years, more direct evidence of biodegradation of benzene, other BTEX compounds, and MTBE has been attained from the analysis of stable isotope ratios (Schmidt et al., 2004b; Meckenstock et al., 2004).

The following sections review the literature on biodegradation of TBA, and then specifically consider anaerobic biodegradation of TBA in microcosms constructed from contaminated aquifer material. The final section evaluates the use of stable isotopes to recognize TBA biodegradation.

## Biodegradation of TBA

At many gasoline spill sites, TBA occurs at high concentrations in groundwater, and dilution and dispersion alone cannot be expected to bring concentrations of TBA to clean up goals before the ground water reaches a receptor. At these sites, Monitored Natural Attenuation will only be a

viable option if the TBA is biologically degraded to harmless materials.

Organic compounds in ground water can be biologically degraded through a variety of mechanisms. These mechanisms are biochemical reactions. In a chemical reaction, if an element or compound loses an electron it is said to be oxidized; if the element or compound receives an electron it is said to be reduced. This old nomenclature goes back to the chemistry of metal ores. Molecular oxygen tends to obtain additional electrons. The chemical that lost an electron to oxygen is said to be oxidized. When a metal ore is processed to recover the pure metal, the weight of the pure metal that is produced is less than the weight of the original ore. The ore was reduced to produce the pure metal. In the process the metal atoms in the ore received electrons.

Biological metabolism is a linked series of biochemical oxidation/reduction reactions where one compound loses electrons and is oxidized and the other compound receives electrons and is reduced. If the organic compound is entirely oxidized to carbon dioxide, the process is termed a respiration. If the organic compound is oxidized, something else must be reduced. That "something else" is described as the terminal electron acceptor. Oxygen, nitrate, sulfate, and Iron(III) minerals can serve as the terminal electron acceptors during respiration. When molecular oxygen is reduced to water, the process is termed aerobic respiration. When sulfate is reduced to sulfide, the process is termed sulfate reduction. When nitrate is reduced to ammonia or molecular nitrogen, the process is called nitrate reduction or denitrification. When insoluble Iron(III) minerals are reduced to form soluble Iron(II) salts, the process is termed iron reduction and when insoluble Manganese(IV) minerals are reduced to form soluble Manganese (II) salts, the process is termed manganese reduction.

In some cases, organic compounds can also serve as both an electron donor and an electron acceptor. These reactions are called fermentations. In a fermentation reaction, an organic compound is the terminal electron acceptor. Sometimes it is the same compound that acts as the electron donor. The most straightforward example of fermentation is the anaerobic biodegradation of acetate to produce methane and carbon dioxide. One of the carbon atoms in acetate is oxidized to carbon dioxide; the other is reduced to form methane.

Bacteria that carry out aerobic respiration are widely distributed in soil and sediment and have a great metabolic diversity. However, oxygen has limited solubility in water and is usually unavailable in contaminated aquifer sediments. Ambient concentrations of nitrate are usually low. As a result, the most important electron acceptors in contaminated ground water are sulfate and Iron(III) minerals in the aquifer sediment (Wiedemeier et al., 1999a).

Biodegradation of TBA has been reviewed by Schmidt et al., (2004a). Degradation of TBA has been reported with a variety of terminal electron acceptors including oxygen, nitrate, sulfate, Iron(III) and Manganese(IV). Biodegradation of TBA is also theoretically possible through fermentation where part of the TBA molecule is reduced to methane or hydrogen and part is oxidized to carbon dioxide. Schmidt et al. (2004a) calculated the free energy yield for biodegradation of TBA with oxygen, nitrate, sulfate, or Iron(III) as an electron acceptor and for the fermentation of TBA to methane. The free energy yield under environmental conditions, $\Delta rG^0(w)$, was -2659 kilojoules per mole for oxygen as electron acceptor, -2360 kilojoules per mole for nitrate as electron acceptor, -814 kilojoules per mole for Iron(III) as electron acceptor, -171 kilojoules per mole for sulfate as electron acceptor, and -72.06 kilojoules per mole during methanogenesis.

If the value for $\Delta rG^0(w)$ is negative, then the reaction is theoretically possible. Based on the thermodynamics of the reactions, biodegradation of TBA should be theoretically possible under aerobic, nitrate-reducing, sulfate-reducing, Iron(III)-reducing, and methanogenic conditions. The more negative the value for $\Delta rG^0(w)$, the greater the amount of energy available from biodegradation. Much more energy is available from aerobic biodegradation and nitrate reduction compared to iron reduction, sulfate reduction, and methanogenesis.

When oxygen or nitrate is available, biodegradation of TBA in sediment is rapid and extensive. Bradley et al., (2002) compared biodegradation of radio-labeled TBA in surface water sediments. When sediment from Charleston, South Carolina, was amended with nitrate and incubated at 23°C for 198 days, 28 ± 5% of the label was recovered as carbon dioxide. When the sediment was amended with oxygen, 99 ± 3% of the label was recovered as carbon dioxide. When sediment from Laurens, South Carolina, was amended with nitrate or oxygen, the recovery was 70 ± 10% and 99 ± 2% respectively.

Schirmer et al., (2003) constructed laboratory microcosms using sediment from the Borden field site in Ontario, Canada. The sediment was collected from a region in the aquifer where there was evidence of field scale biodegradation of MTBE. After 22 days of incubation at 10°C, the sediment degraded 650 µg/L TBA to a concentration of less than 5 µg/L. Hunkeler et al., (2001) showed transitory accumulation of TBA in laboratory cultures derived from sediment from the Borden field site. During aerobic biodegradation of 10 mg/L MTBE, up to 4 mg/L of TBA accumulated. After the MTBE was completely degraded, the TBA began to degrade. The TBA was completely degraded within 15 days after the MTBE was no longer available.

Kane et al., (2001) evaluated MTBE biodegradation under aerobic conditions in sediment from fuel spill sites in California. They added 4.5 mg/L of MTBE to sediment from a spill site in Palo Alto, California. When the MTBE was degraded, they respiked the sediment with 4.5 mg/L MTBE. As the MTBE was degraded, TBA accumulated to a maximum concentration near 2.0 mg/L. Within 25 days, the TBA was degraded.

Aerobic conditions may exist at the down gradient margin of a plume, or in sediment where a plume discharges to aero-

bic surface water; however, ground water is often devoid of oxygen in the source area of plumes where concentrations of TBA are high. If Monitored Natural Attenuation is to be a viable remedy for TBA, then TBA must be biologically degraded in the absence of oxygen.

White et al., (1986) evaluated biodegradation of TBA under anaerobic conditions in sediment from a refinery near the Schuykill River in Philadelphia, Pennsylvania. They incubated 12 grams of sediment and 4 ml of sterile ground water without headspace in test tube microcosms at 12°C. After a period of acclimation, the TBA was completely removed.

Zenker et al., (1999) constructed microcosms using sediment from a site in North Carolina that had been exposed to MTBE. The microcosms were constructed under anaerobic conditions. After 200 days of incubation, TBA was completely consumed in each of triplicate microcosms.

Bradley et al., (2001) added radio-labeled [$^{14}$C] TBA to microcosms prepared using stream and lake-bed sediments from sites in Charleston, South Carolina; Jacksonville, Florida; and Picatinny Arsenal, New Jersey. When the microcosms were prepared to simulate anaerobic conditions, 8%± 1%, 11% ± 1%, and 8% ± 1% of the label was recovered as carbon dioxide after 166 days of incubation.

Finneran and Lovley (2001) reported rapid and extensive degradation of TBA under mixed iron(III)-reducing, sulfate-reducing, and methanogenic conditions in sediment from the Potomac River. They added radio-labeled [$^{14}$C] TBA to the sediment. After 62 days of incubation, approximately 7% of the label was recovered as methane and 25% of the label was recovered as carbon dioxide. If the methane had been produced by fermentation of TBA, the molar ratio of methane produced to carbon dioxide produced should have been three to one. If TBA is degraded by sulfate-reducing bacteria or Iron(III)-reducing bacteria, all the label should go to carbon dioxide. The actual ratio was near 0.3 to one. Finneran and Lovley (2003) concluded that TBA in the sediment from the Potomac River was being degraded by a variety of terminal electron accepting processes, which included sulfate reduction and Iron(III) reduction.

In a subsequent experiment, Finneran and Lovly (2003) added additional sulfate to the sediment from the Potomac River. If the addition of sulfate allowed the sulfate-reducing bacteria to compete with methanogenic bacteria for the TBA, the fraction of radio-label going to methane should be smaller. When sulfate was not added, approximately 24% of the label was recovered as carbon dioxide and 10% of the label was recovered as methane. The ratio of label in methane to label in carbon dioxide was near 0.4 to one. When 960 mg/L of sulfate was added to sediment from the Potomac River, approximately 26% of the label was recovered as carbon dioxide and approximately 3% of the label was recovered as methane, and the ratio of label in methane to label in carbon dioxide was near 0.1 to one. The addition of sulfate inhibited the production of methane. Molybdate is a specific inhibitor of sulfate reduction. When Finneran and Lovley (2003) added sulfate and molybdate to sediment from the Potomac River, approximately 23% of the label was recovered as carbon dioxide and approximately 3% of the label was recovered as methane. Because the molybdate did not produce a substantial reduction in the amount of label in carbon dioxide, Finneran and Lovley (2003) concluded These results indicate that two processes are probably responsible for the [$^{14}$C]-TBA mineralization – sulfate reduction and Fe(III) reduction (because nitrate was not detected in these sediments).

Bradley et al., (2002) compared anaerobic biodegradation of radio-labeled [$^{14}$C] TBA in surface water sediments under various electron accepting conditions. In sediment from Charleston, South Carolina, the sediment as collected was methanogenic. After 200 days of incubation, none of the $^{14}$C from TBA was recovered as carbon dioxide. They should have been able to detect 2% of the original label as carbon dioxide. When the sediment was amended with Iron(III) or Manganese(IV), no $^{14}$C from TBA was detected as carbon dioxide. However, when the sediment was amended with sulfate, 4 ± 1% of the $^{14}$C from TBA was recovered as carbon dioxide.

In sediment from a site in Laurens, South Carolina, the unamended sediment was also methanogenic. After 200 days of incubation, none of the labeled TBA was recovered as carbon dioxide. When the sediment was amended with Iron(III), none of the labeled TBA was recovered as carbon dioxide. When the sediment was amended with Manganese(IV), 75 ± 20% of the label was recovered as carbon dioxide. When the sediment was amended with sulfate, 5 ± 1% of the label was recovered as carbon dioxide.

Finneran and Lovley (2003) added radio-labeled [$^{14}$C] acetate to material from a contaminated aquifer near Bemidji, Minnesota. Based on the distribution of label between methane and carbon dioxide, they concluded that methanogenesis was the only important electron accepting process in the sediment. They added radio-label [$^{14}$C] TBA to this sediment. After 23 days of incubation, approximately 5% of the $^{14}$C from TBA was recovered as methane and approximately 9% was recovered as carbon dioxide.

The interpretation of their experiments on TBA biodegradation under sulfate reducing and methanogenic conditions is not straightforward. Some portion of the $^{14}$C from TBA is recovered as methane, indicating that at a minimum some transformation product of TBA can be degraded under methanogenic conditions. However, the yield of methane is much lower than would be expected from the complete fermentation of TBA under methanogenic conditions.

$$C_4H_{10}O + H_2O \rightarrow 3CH_4 + CO_2$$

If TBA is fermented to methane according to the stoichiometry above, 75% of label in [$^{14}$C] TBA should be recovered as methane. In sediment where TBA is degraded in the absence of oxygen or nitrate, approximately 36% to 10% of the label is recovered as methane. This would suggest that some portion of the TBA is being oxidized by sulfate, Iron(III), or Manganese(IV).

There is not unequivocal evidence that TBA can be metabolized under strictly fermentative conditions. In their review, Schmidt et al., (2004a) concluded *Thus, in contrast to MTBE degradation, there is general consensus that TBA is recalcitrant under methanogenic conditions.* If this is the case, either sulfate or biologically available Iron(III) or Manganese(IV) is required for biodegradation of TBA in the absence of oxygen or nitrate.

As part of a study to explain the distribution and behavior of TBA in ground water at gasoline spill sites in Orange County, California, DeVaull et al., (2003) collected sediment from three locations at each of three gasoline spill sites. The sediment was used to construct microcosms. One experimental treatment in their report simulated the natural anaerobic conditions in the aquifer at their study sites. A 160 ml glass vial was filled with 75 ml of water and 50 g of wet sediment. The headspace was purged with nitrogen for 30 minutes and then the vial was sealed with a septum. The microcosms were spiked with MTBE and TBA to bring the concentrations in the pore water to approximately 10 mg/L. The ratio of water to solids in the experimental system was approximately 2.1 to 1 (gm/gm). The ratio of water to solids in a sandy aquifer is near 0.25 to 1 (gm/gm). Because the experimental system had a high water to solids ratio, and because both the TBA and soluble electron acceptors such as sulfate were supplied in water, the microcosm system was more sensitive than a natural aquifer to the contribution of soluble electron acceptors and less sensitive to the contribution of Iron(III).

The concentrations of TBA, MTBE, methane, and sulfate in monitoring wells at the sites that provided sediment for the microcosm study of DeVaull et al., (2003) are presented in Table 1. The water samples were collected in 2002 and 2003. All the wells at all three sites had much higher concentrations of TBA than MTBE. There were high concentrations of methane in at least one well from each site. As will be discussed later, the oxidation of TBA by sulfate reduction requires 3.9 mg/L sulfate for each 1.0 mg/L of TBA. All the wells at the site at Fountain Valley, California, and Laguna Niguel, California, had adequate concentrations of sulfate to support complete metabolism of TBA by sulfate reduction. The site at San Juan Capistrano, California, did not have adequate sulfate to meet the demand associated with the TBA.

The terminal electron accepting processes (TEAP) were not defined in the treatment that simulated natural anaerobic conditions in the aquifer. Sulfate and nitrate were available at the beginning of the experiment, but DeVaull et al., (2003) did not present data on depletion of nitrate or sulfate, or accumulation of Iron(II).

In microcosms constructed with three different sediment samples from a site in Fountain Valley, California, MTBE was degraded in all three microcosms after 91 days of incubation. In two of the three microcosms, TBA was either completely degraded or substantially degraded within 161 days of incubation (Panel A of Figure 4 presents data from one of the microcosms).

In microcosms constructed with three different sediment samples from a site in San Juan Capistrano, California, TBA was completely degraded in two of the three microcosms within 114 days, but MTBE did not degrade within 154 days. In the third microcosm, degradation followed the opposite pattern; MTBE degraded within 70 days, but TBA did not degrade within 154 days.

In microcosms constructed with three different sediment samples from a site in Laguna Niguel, California, TBA degraded within 90 days in one microcosm and 145 days

**Table 1.** Distribution of TBA, MTBE, Methane, and Sulfate in Monitoring Wells at the three Gasoline Service Stations in Orange County, California that Provided Sediment for the Microcosm Study of DeVaull et al. (2003).

| Station | Well | TBA | MTBE | Methane | Sulfate |
|---|---|---|---|---|---|
| | | ml/L | ml/L | ml/L | ml/L |
| | | | | | |
| Fountain Valley | 16969 mw-6 | 28.0 | 0.079 | 0.0982 | 183 |
| Fountain Valley | 16969 mw-15 | 2.0 | 0.015 | 0.0293 | 128 |
| Fountain Valley | 16969 mw-13 | 14.8 | 0.012 | 0.535 | 79 |
| | | | | | |
| Laguna Niguel | 30011 mw-7 | 45.9 | 0.042 | 1.02 | 1290 |
| Laguna Niguel | 30011 mw-10 | 33.2 | 0.028 | 0.953 | 2640 |
| Laguna Niguel | 30011 mw-11 | 40.3 | 0.229 | 0.0176 | 2680 |
| | | | | | |
| San Juan Capistrano | 27101 w-2 | 10.8 | 0.230 | 4 | 11 |
| San Juan Capistrano | 27101 mw-8 | 12.0 | 0.002 | 1.56 | 17 |
| San Juan Capistrano | 27101 mw-11 | 69.9 | 0.084 | 0.835 | 6 |

in a second microcosm, but MTBE did not degrade within 196 days in either of the two microcosms. In the third microcosm, degradation followed the opposite pattern; TBA did not degrade within 196 days, but MTBE completely degraded within 90 days (Panel B of Figure 4).

The results of the microcosm studies reported by DeVaull et al., (2003) suggest that conditions that are favorable for anaerobic biodegradation of TBA are widespread in Orange County, California. At least one microcosm from each of the three sites degraded TBA under anaerobic conditions. However, the microcosm studies also suggest that the capacity to degrade TBA may be heterogeneously distributed at particular sites; TBA failed to degrade in at least one of the microcosms constructed with one of the three different sediment samples from each of the three sites.

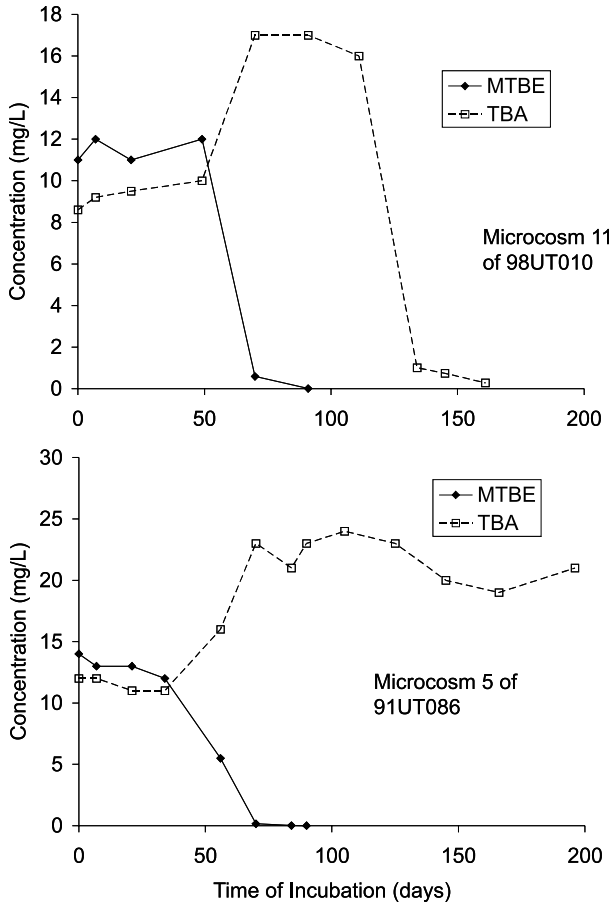



**Figure 4.** *Removal of MTBE and production or removal of TBA in anaerobic sediment from a gasoline spill site in Fountain Valley, California (98UT010) and a gasoline spill site in Laguna Niguel, California (91UT086). Plotted from data in DeVaull et al. (2003). Data are plotted from one of three microcosm studies constructed with sediment from each of the sites.*

## Rates of Biodegradation of TBA in Ground Water

The rates of attenuation of TBA in sediment under aerobic conditions are rapid. Schirmer et al., (2003) reported a first order rate of removal of TBA in sediment from the Borden field site of 0.12 per day. The initial concentration of TBA was 0.7 mg/L. Wilson et al., (2002) conducted a microcosm study with sediment from a gasoline spill site at Vandenberg AFB, California. The sediment was spiked with MTBE, and then spiked again six more times after the MTBE was degraded. The spiked concentrations of MTBE were as high as 16 mg/L. The first order rates of removal of MTBE were 0.05, 0.15, 0.03, 0.07, and 0.08 per day. TBA was produced by metabolism of MTBE. In each cycle of spike and removal of MTBE, the TBA never accumulated to any significant extent. The rate of removal of MTBE was the rate of production of TBA. TBA would be expected to accumulate unless the rate of removal of TBA was faster than the rate of removal of MTBE.

Kane et al., (2001) spiked and respiked MTBE into sediment from a gasoline spill site in Palo Alto, California. Each spike of MTBE was near 5 mg/L. As the MTBE degraded, TBA accumulated to concentrations near 2 mg/L. Once MTBE was entirely degraded, the concentrations of TBA started to decline. The rate of removal of TBA corresponded to first order rates of degradation of 0.10 and 0.13 per day.

The rates of aerobic biodegradation are so fast that they are effectively instantaneous in the context of ground water movement at field scale. Shih et al., (2004) reported the distribution of the maximum concentration of TBA in monitoring wells at gasoline spill sites in Los Angeles, California. The median of the TBA concentrations was 1,880 µg/L. A first order rate of aerobic biodegradation of 0.1 per day would bring the median concentration of TBA to the California Action Limit of 12 µg/L in fifty days.

The reported first order rates of biodegradation of TBA under anaerobic conditions (as reported in Table 2) are slower than under aerobic conditions. In their study of TBA degradation in sediment from a refinery near Philadelphia, Pennsylvania, White et al., (1986) reported their data as a zero order rate of utilization. At initial concentrations of 1.7, 13.5, and 150 mg/L, the utilization rates were 0.057, 0.52 and 2.00 mg/L/day respectively. A first order rate constant can be thought of as a zero order rate of change in concentration ($\Delta C/\Delta t$) normalized to the concentration at the particular instance in time over which the rate is operating. By definition of a first order rate constant (k), first order rate = $\Delta C/\Delta t = k*C$; therefore $k = (\Delta C/\Delta t)/C$. We estimated the first order rate constants for attenuation of TBA at the beginning of the experiment by dividing the reported zero order rates of utilization by the initial concentration of TBA in the pore space of the microcosms. At an initial concentration of 1.7 mg/L and a zero order rate of utilization of 0.057 mg/L/day, the first order rate constant for degradation was 0.057 mg/L/day divided by 1.7 mg/L or 0.0335 per day, equivalent to a rate of 12 per year. At an initial concentration of 13.5 mg/L, the first order rate constant was 14 per year.

**Table 2.** Rates of Anaerobic Biodegradation of TBA in Aquifer Sediments or Field Scale Plumes at Gasoline Spill Sites. If More than one Rate is Reported from Microcosm Experiments Constructed from Material from One Location at a Site, all the Rates are Reported in the Table.

| Source of Material | Field Rate | Lab Rate | First Order Rate of Biodegradation (per year) | Reference |
|---|---|---|---|---|
| Fountain Valley, California | | X | 26, 17 | DeVaull et al. (2003) |
| Laguna Niguel, California | | X | 18, 5.1 | DeVaull et al. (2003) |
| Potomac River | | X | 15 | Finneran and Lovley (2001) |
| Philadelphia, Pennsylvania | | X | 14, 12, 4.9 | White et al. (1986) |
| New York | X | | 8.8 | Kolhatkar et al. (2000) |
| Florida | X | | 7.3 | Kolhatkar et al. (2000) |
| Pennsylvania | X | | 7.2 | Kolhatkar et al. (2000) |
| San Juan Capistrano, California | | X | 5.1 3.5 | DeVaull et al. (2003) |
| Pasadena, Texas flow path from location 150 | X | | 1.1 | Day and Gulliver (2003) |
| Pasadena, Texas flow path from location 57 to | X | | 0.97 | Day and Gulliver (2003) |
| Pasadena, Texas flow path from location 165 | X | | 0.26 | Day and Gulliver (2003) |

At an initial concentration of 150 mg/L, the first order rate constant was 4.9 per year. As would be expected for a first order process, the estimated first order rates of attenuation were essentially independent of concentration.

Finneran and Lovley (2001) conducted spike and respike experiments with TBA in sediment from the Potomac River. The spiked concentrations were approximately 50 mg/L. The time course of TBA degradation reported in Figure 3 of Finneran and Lovley (2001) corresponds to a first order rate constant of 15 per year.

In the microcosm experiment conducted by DeVaull et al., (2003) the rates of TBA biodegradation in the anaerobic microcosms where TBA degraded were in the range of 26 to 3.5 per year (Table 2). The rates in sediment samples from Fountain Valley, California, were 26 and 17 per year. The rates in sediment samples from San Juan Capistrano, California, were 3.5 and 5.1 per year. The rates in sediments from Laguna Niguel, California were 18 and 5.1 per year.

There are also a few reports on the rate of TBA biodegradation under anaerobic conditions at field scale (Table 2). Kolhatkar et al., (2000) evaluated the distribution of TBA at 74 gasoline spill sites in the Eastern United States. They used the approach of Buscheck and Alcantar (1995) to extract first order rate constants for biodegradation of TBA along the flow path in the plume. They were able to extract rate constants that were statistically significant at 95% confidence from three of the 74 plumes. The rate of TBA biodegradation in a plume in New York was 8.8 per year, the rate in a plume in Pennsylvania was 7.26 per year, and the rate in a plume in Florida was 7.3 per year.

Day and Gulliver (2003) evaluated the natural attenuation of a large plume of TBA at a chemical manufacturing plant at Pasadena, Texas. They compared the reduction in concentration of TBA with distance along the flow path to the reduction in concentration of the co-occurring contaminants 1,1-dichloroethene and 1,1-dichloroethane. The attenuation of TBA was significantly faster than that

of 1,1-dichloroethene and 1,1-dichloroethane. They used the approach of Buscheck and Alcantar (1995) to extract first order rate constants for natural biodegradation of TBA along three flow paths in the aquifer. The rate constants were 0.97, 0.26, and 1.1 per year.

The rates of TBA biodegradation under anaerobic conditions (when biodegradation occurs) vary over two orders of magnitude (Table 2). The rates are faster than the rates reported for anaerobic biodegradation of MTBE or for benzene, which is the usual "risk-driver" at gasoline spill sites (Table 3). If the microbial community at a particular spill site acclimates to anaerobic biodegradation of TBA, and if there is an adequate supply of the metabolic requirements for anaerobic degradation, then natural anaerobic biodegradation of TBA can provide a substantial contribution to natural attenuation of TBA.

## Extent of Biodegradation of TBA

The extent of TBA biodegradation by aerobic respiration, nitrate reduction, Iron(III) reduction or sulfate reduction will depend on the supply of these electron acceptors in the ground water or aquifer sediment. Wiedemeier et al., (1995) compared the concentrations of oxygen, nitrate, Iron(II) and sulfate in ground water in the interior of 25 fuel spills to the concentrations in the surrounding ground water that had not been affected by the fuel spill. They calculated the consumption of each of the soluble electron acceptors and the production of soluble Iron(II) in the ground water from insoluble Iron(III) compounds in the aquifer solids. Then they compared the concentrations of electrons that were transferred by each of the electron accepting processes on a milliequivalent per liter basis. Their results are presented in Figure 5. Sulfate-reduction was the dominant electron accepting process.

Figure 6 presents data on the concentration of sulfate in monitoring wells at 77 gasoline spill sites in the Eastern United States (unpublished data from study published in Kolhatkar et al., 2000). The figure compares the maximum sulfate concentration in any well at each site and the minimum sulfate concentration in any well at each site. The maximum concentration is an estimate of the ambient concentration in ground water in the aquifer. The minimum concentration is an estimate of the sulfate concentration in the LNAPL source area of the gasoline spill. At 75% of sites, the ambient concentration of sulfate was at least 56 mg/L, at 50% of sites the concentration was at least 107 mg/L, and at 25% of sites the concentration was at least 304 mg/L.

Oxidation of TBA by sulfate reduction requires 3.9 mg/L sulfate for each 1.0 mg/L of TBA:

$$C_4H_{10}O + 3SO_4^{-2} \rightarrow 3S^{-2} + 4CO_2 + 5H_2O$$

Ambient concentrations of 56, 107 and 304 mg/L sulfate can support degradation of 14, 27, and 78 mg/L of TBA.

In the most typical scenario at a gasoline spill site, a plume of MTBE in ground water is produced and sustained by continual dissolution of MTBE from the residual gasoline to ground water. The major portion of the TBA is produced by biodegradation of the MTBE once it is dissolved in ground water. Other soluble components of gasoline, such as the BTEX compounds also dissolve in ground water. The demand for oxygen, nitrate, and sulfate for biodegradation of the other components of the gasoline depletes sulfate and other soluble electron acceptors from the ground water that are in contact with the residual gasoline.

Based on the supply of electron acceptors, and the free energy available from the reaction, anaerobic regions in aquifers tend to develop distinct areas dominated by different electron acceptors (Finneran and Lovley, 2003; Wiedemeier et al., 1999a). When gasoline is spilled into an aquifer, oxygen is depleted first, then nitrate is depleted, then sulfate is depleted, and finally the terminal electron accepting process transitions to methanogenesis. As a consequence, the ground water immediately adjacent to a gasoline spill is often methanogenic. This region is surrounded by ground water that is sulfate reducing, which in turn is surrounded by ground water that is Iron(III)-reducing, which in turn is surrounded by ground water that is nitrate reducing. Although one electron accepting process tends to dominate, they often proceed concomitantly. If the supply of biologically available Iron(III) is adequate, Iron(III) reduction

**Table 3.** Comparison of First Order Rates of Anaerobic Biodegradation of TBA, MTBE, and Benzene.

| | TBA | MTBE | Benzene |
|---|---|---|---|
| number of rates | 14 | 10 | 20 |
| mean (per year) | 9.2 | 1.0 | 3.7 |
| median (per year) | 7.3 | 0.41 | Not Provided |
| Reference | this report | Wilson (2003) | Suarez and Rifai (1999) |

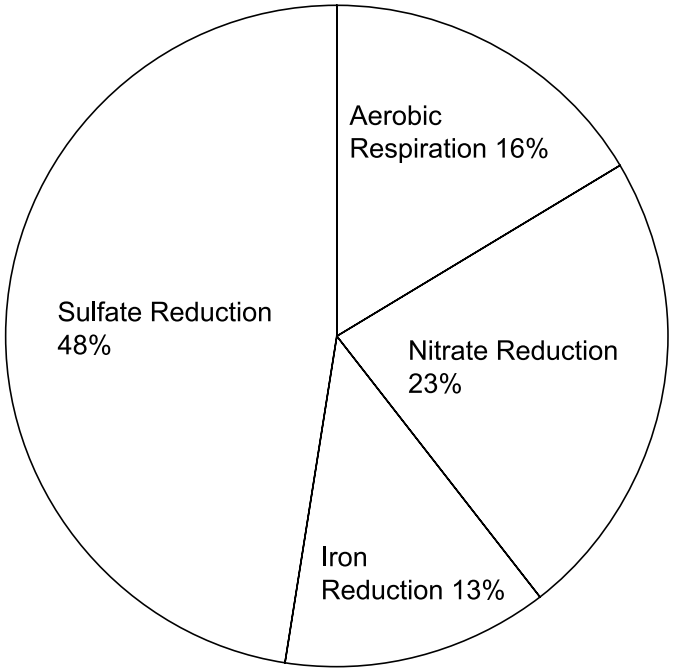



**Figure 5.** *Relative importance of electron acceptors at 25 fuel spill sites in North America. Adapted from Wiedemeier et al., 1995.*

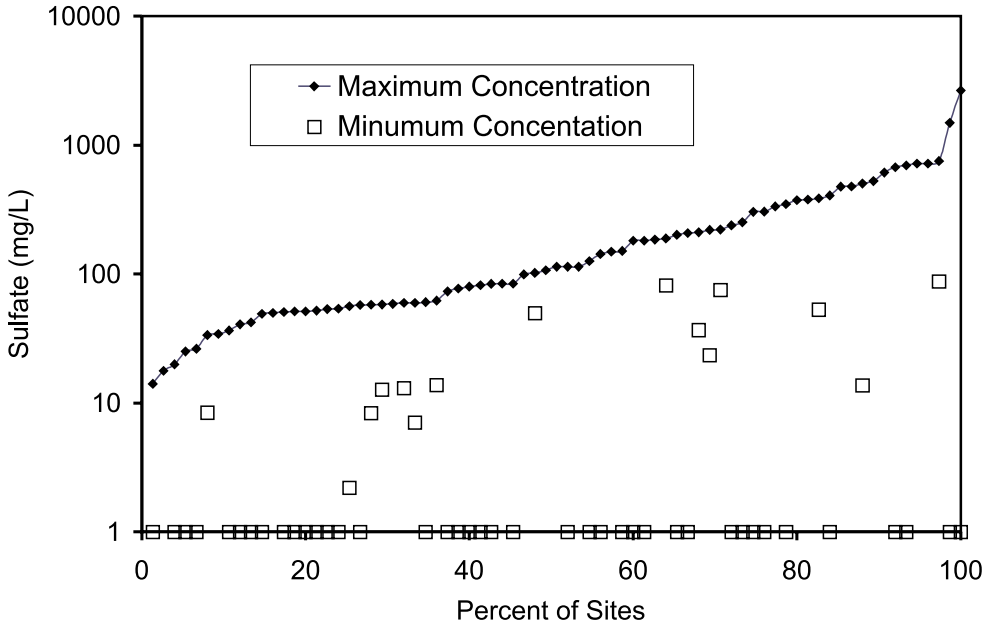



**Figure 6.** *Distribution of sulfate at 77 gasoline spill sites in the Eastern United States. The maximum concentration represents the likely concentration in ambient ground water that was not impacted by the spill. The minimum concentration represents the concentration in the LNAPL source area of ground water contamination. Unpublished data from Kolhatkar et al. (2000).*

can occur in ground water that is also sulfate reducing or is methanogenic. Sulfate reduction and methanogenesis can occur together, particularly at lower concentrations of sulfate. These relationships are depicted diagrammatically in Figure 7.

The concentration of sulfate at which sulfate becomes limiting for sulfate reduction varies widely from one organism to the next and with different environmental conditions. Ingvorsen and Jørgensen (1984) found that the half saturation constant for sulfate reduction in four strains of bacteria varied from 0.5 mg/L to 7 mg/L. Fukui and Takii (1994) showed that the half saturation constant for sulfate reduction of a Desulfovibrio desulfuricans was 0.8 mg/L when the cells were associated with particles of FeS, and 24 mg/L when the cells were free living. Concentrations of sulfate less than 5 mg/L are considered sulfate poor (Fukui and Takii, 1994), and flow of electrons can be expected to transition to methanogenesis at sulfate concentrations less than 40 mg/L (Personal Communication Kevin T. Finneran, University of Illinois, August, 2006).

If sulfate has been depleted in contaminated ground water, it is reasonable to presume that nitrate and oxygen are also depleted (Finneran and Lovley, 2003; Lovley, et al., 1994; Wiedemeier et al., 1999a). In 62 of the 77 sites in the survey of gasoline spills in the Eastern United States, the concentration of sulfate was less than 1.0 mg/L in the most contaminated wells in the LNAPL source area (Figure 6). Based on the available knowledge of anaerobic biodegradation of TBA, biodegradation of TBA supported by aerobic respiration, nitrate reduction, or sulfate reduction would not be expected in the source area of these plumes.

If the biodegradation of TBA in ground water is limited by the supply of sulfate, there should be an inverse relationship between the concentration of TBA in ground water and the concentration of sulfate. Figure 8 compares the concentration of sulfate to the concentration of TBA in 58 wells at 13 gasoline spill sites in Orange County, California. These are the same wells sampled by Wilson et al., (2005c) to determine whether stable isotope ratios in MTBE could be used to predict MTBE biodegradation in ground water. In general, an inverse correlation did apply to the data set from these wells (Figure 8), but there is more scatter than would be expected.

Part of the scatter of the data may be an artifact caused by mixing of ground water from different plumes in monitoring wells. Most conventional monitoring wells are screened over ten or fifteen vertical feet (3.05 to 4.57 meters) in the aquifer. Many plumes at gasoline spill sites are vertically heterogeneous over ten to fifteen feet. This relationship is illustrated in Figure 9 using data from a gasoline spill site at Port Hueneme, California. The plume was in a layer of sands and gravels. Water samples were taken with temporary push wells with a vertical screened interval of 1.5 feet (0.46 meters). Samples started just below the water table and extended to a clay confining layer. The ground water in the shallow intervals had low concentrations of sulfate and high concentrations of TBA while water in the deeper interval had high concentrations of sulfate and low concentrations of TBA. However, water from a well screened across this aquifer would have intermediate concentrations of both TBA and sulfate. The concentrations of TBA and sulfate in the water produced by the monitoring well would

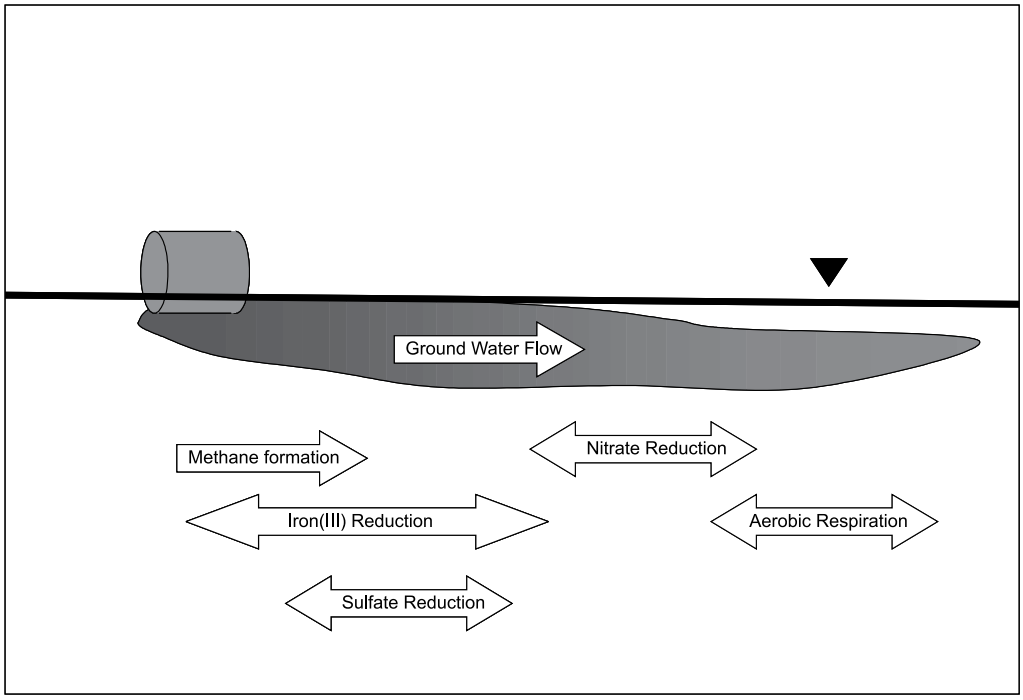



**Figure 7.** *General distribution of terminal electron accepting processes (TEAPs) in ground water down gradient from a spill of gasoline.*

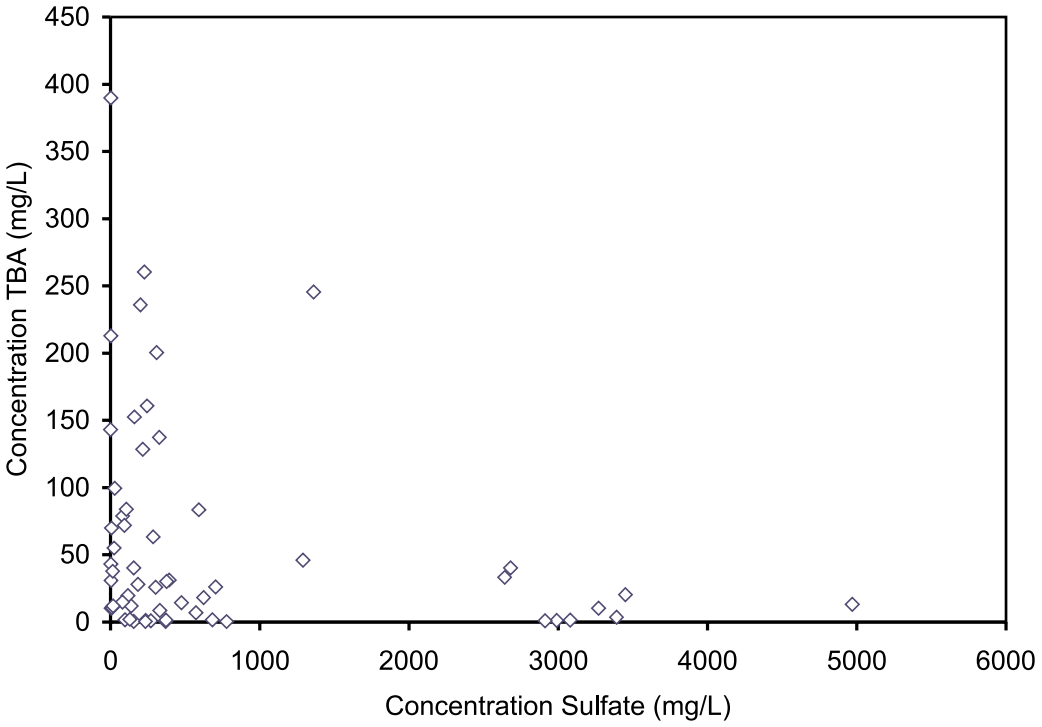



**Figure 8.** *Distribution of the concentrations of TBA and sulfate in selected monitoring wells at gasoline spill sites in Orange County, California.*

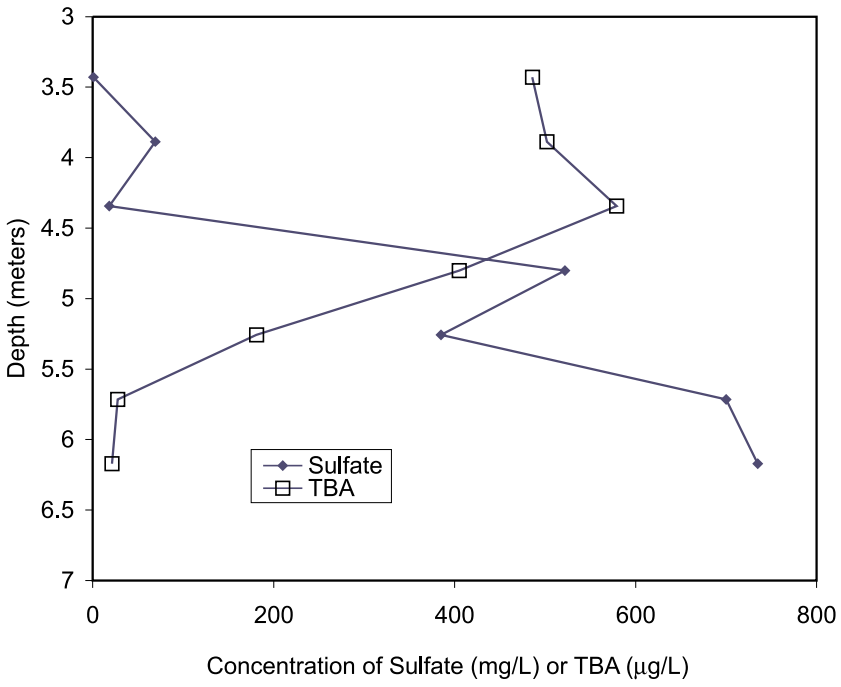



**Figure 9.** *Relationship between depth below the water table and the concentrations of TBA and sulfate in ground water at a gasoline spill site in Port Hueneme, California.*

suggest that sulfate was available for anaerobic biodegradation of TBA.

The TBA at this site was produced through biodegradation of MTBE that dissolved out of residual gasoline near the water table (data not shown). Biodegradation of alkylbenzenes (the BTEX compounds) associated with the residual gasoline is probably responsible for the lower concentrations of sulfate near the water table. The mixed water sample produced by a conventional monitoring well in this aquifer would not have produced a water sample that was representative of the water surrounding a particular sulfate reducing bacterium.

In general, if water produced from a monitoring well is devoid of sulfate, then sulfate would not be available to bacteria in the ground water sampled by the well. The inverse is not necessarily true. The presence of both sulfate and TBA in water produced by a monitoring well does not mean that sulfate is available for organisms to degrade the TBA. The sulfate and TBA may have come from different depth intervals.

If sulfate is depleted in the source area of a gasoline spill, anaerobic biodegradation of TBA will require admixture of TBA in the contaminated ground water in the plume with sulfate in the receiving ground water in the aquifer down gradient. Mixing by dispersion is controlled by flow of ground water and the geometry of the plume. The mixing occurs across the interface between the plume and the ambient ground water. If a plume has a large volume relative to the surface area presented to the ambient ground water, then mixing by dispersion will be a slow process, and adequate admixture of sulfate to meet the stoichiometric demand for biodegradation of TBA will likely occur at some distance away from the source area.

At most gasoline spill sites, the monitoring wells are on the property of the gasoline station or on the property of the immediate neighbors. The existing monitoring wells may not be located in the portion of the plume where sulfate is available to support biological degradation of TBA. At many gasoline spill sites, it may be impossible to use monitoring wells that are in or near the source area to document natural biodegradation of TBA carried out by sulfate reducing bacteria.

If TBA is not biologically degraded under methanogenic conditions, the only plausible agents for biodegradation of TBA in ground water that has been depleted of sulfate and the other soluble electron acceptors are Iron(III) reducing bacteria or Manganese(IV) reducing bacteria. If sulfate and the other soluble electron acceptors are depleted, the extent of biodegradation of TBA will be limited by the supply of biologically available Iron(III) or Manganese(IV) in the aquifer sediments. At present there is a commercially available assay for biologically available Iron(III) in sediment, (Bioavailable Ferric Iron (BAFe III) Assay, New Horizons Diagnostics Corp., 9110 Red Branch Road, Columbia, Maryland USA 21045, 1-800-888-5015 ext. 0 or 235, fax: 410-992-0328, NHDiag@aol.com ). The performance of the assay to predict biologically available Iron(III) has been evaluated by the Environmental Security and Technology Certification Program (ESTCP, 2005). However, to the authors' knowledge, the assay has never been used to evaluate natural attenuation of TBA in ground water. There is no commercially available assay for biologically available Manganese(IV). Until techniques to directly evaluate the supply of biologically available Iron(III) and Manganese(IV) in aquifer sediment become standard practice, the potential contribution of Iron(III)-reducing and Manganese(IV)-reducing bacteria to the natural attenuation of TBA cannot be characterized. It is inappropriate to attribute apparent disappearance of TBA from ground water to Iron(III)-reducing or Manganese(IV)-reducing bacteria simply because Iron(II) or Manganese(II) accumulates in ground water.

## EPA microcosm study of anaerobic TBA biodegradation

To provide an independent evaluation of the natural anaerobic biodegradation of TBA in ground water at gasoline spill sites, EPA/ORD conducted microcosm studies using material from several gasoline spill sites around the United States. Sediment was collected from BP gasoline stations at Petaluma, California; Deer Park, New York; Parsippany, New Jersey; and Boca Raton, Florida. The microcosms were part of a larger study that examined the anaerobic biodegradation of MTBE and ethanol. Sediment was also collected from a motor gasoline spill site at a U.S. Navy Base at Port Hueneme, California, and a motor gasoline spill site at Vandenberg Air Force Base, California. All the samples were from shallow aquifers in sandy unconsolidated sediments. Sediment from Deer Park, Parsippany, and Boca Raton were selected for the microcosm study because field data collected in 1999 indicated that the rates of degradation of MTBE and TBA in ground water, using the method of Buscheck and Alcantar (1995), were statistically significant at 80% confidence. Sediment from Parsippany was selected because the field data indicated that MTBE was being degraded, and TBA was not accumulating. The sediments from Port Hueneme and Vandenberg AFB were selected as negative control sites. At the time the microcosms were constructed, there was no evidence of biodegradation of MTBE or TBA in the plumes at Port Hueneme and Vandenberg AFB.

### *Construction of Microcosms*

The sediment was collected and stored in 1-L glass jars. To protect the anaerobic microorganisms that might be present in the samples from oxygen in the atmosphere, the head space above the sediment was replaced with ground water. The sediment samples were shipped by air freight and were stored at 4 °C until they were used to construct microcosms.

All manipulations to prepare the microcosms were carried out aseptically in an anaerobic glove box. An oxygen meter indicated that the concentration of oxygen in the atmosphere of the glove box was less than 1 ppm by volume. Microcosms were prepared in sterile glass serum bottles with a volume of 25 mL. When available, ground water from monitoring wells at the sites was added to the sediment to

make a slurry, and the sediment samples were stirred to blend well. If ground water was not available, the slurry was made with autoclaved reverse osmosis water. The added water was 5% or less of the final volume of the slurry. The slurry was transferred to the serum bottles with a sterile scoop. Each microcosm received approximately 45 gm of slurry and 1.0 mL of an aqueous dosing solution containing a sterile aqueous solution of TBA. The concentration of TBA in the dose solution for microcosms constructed with sediment from Deer Park, New York; Petaluma, California; Vandenberg AFB, California; Parsippany, New Jersey; and Boca Raton, Florida, varied from 13 to 15 mg/L. The microcosms constructed with sediment from Port Hueneme, California, had 80 mg/L TBA. The microcosms were sealed with a sterile Teflon-faced gray butyl rubber septum and a crimp cap. The microcosms were stored at room temperature (20 to 22 °C) in the same glove box, under an atmosphere containing 2% to 5% v/v hydrogen.

They were incubated from eighteen months to two years. Every two to three months, triplicate microcosms were selected for analysis. Prior to sampling, the contents of each microcosm were mixed with a vortex mixer while the microcosms were still sealed, and then the sediment was allowed to settle. The septum was removed and approximately 1 mL of the standing water was taken and diluted in 14 ml of distilled water containing 1% trisodium phosphate as a preservative. The diluted samples contained approximately 15 ml of diluted water and 6.0 ml of head space. The diluted samples were sealed with a septum and crimp cap, and then shaken to bring the water and head space to equilibrium.

## *Laboratory Analytical Procedures*

The concentrations of TBA were determined by head space gas chromatography/mass spectrometry (GC/MS) using a modification of EPA Method 5021A, “Volatile Organic Compounds in Various Sample Matrices using Equilibrium Headspace Analysis,” June 2003. Samples were collected for analysis with an automated static headspace sampler. Analytes were determined by gas chromatography/mass spectrometry using an Ion Trap Detector. The lowest calibration standard was 10 µg/L; the method detection limit was 2.4 µg/L. Concentrations of sulfate were determined with a Waters Quanta 4000 Capillary Ion Analyzer, using a modification of EPA Method 6500, “Dissolved Inorganic Anions in Aqueous Matrices by Capillary Ion Electrophoresis,” January 1998. The method detection limit for sulfate was 0.172 mg/L.

Depending on the amount of standing water that was sampled from each microcosm, the pore water in the microcosms was diluted in a range between 15:1 and 30:1 before analysis. In the samples with concentrations of TBA below the lower calibration limit, the pore water in the microcosms was diluted 15:1 before analysis. As a result, the effective lower limit for calibration of TBA in the original pore water of the microcosms was 150 µg/L, and the effective detection limit was 36 µg/L. The effective method detection limits for sulfate in diluted samples were 1.2 mg/L.

## *Data Quality*

Laboratory analyses for data presented in Panel C of Figure 1, in Panel B of Figure 2, Figure 6, Figures 8 through 13, and Table 1 were conducted at the R.S. Kerr Environmental Research Center in accordance with a Quality Assurance Project Plan prepared for in-house task 10013 (Fate of Fuel Oxygenates in Aquifer Material).

Major quality assurance and quality control (QA/QC) evaluations for the analyses included method blank (MB), continuing calibration check (CCC), second source check (QC) using a sample obtained from the second source as identified by their designated names, laboratory duplicates (LD), and matrix spike (MS). A method blank was analyzed in the beginning and end of a sample set. Continuing calibration check standards (CCC) were analyzed every ten samples as well as in the beginning and end of a sample set. QC checks were analyzed every ten samples. Lab duplicates were analyzed every ten samples. Matrix spikes were analyzed every twenty samples.

The data quality objectives for TBA were as follows: The target analyte in the method blank would be below method detection limit. The reported concentration of the continuing calibration check standard (CCC) would agree with the expected concentration plus or minus 20% of the known concentration: the matrix spike would agree with the expected concentration plus or minus 30% of the known concentration (i.e., Recovery of the expected value would be in the range of 70-130%). Laboratory duplicates would agree with each other with a relative percent difference of ± 25%.

The data quality objectives for sulfate were as follows: The target analyte in the method blank would be below method detection limit. The reported concentration of the continuing calibration check standard (CCC) and the QC check would agree with the expected concentration plus or minus 10% of the known concentration, the matrix spike would agree with the expected concentration plus or minus 20% of the known concentration (i.e., Recovery of the expected value would be in the range of 80-120%). Laboratory duplicates would agree with each other with a relative percent difference of ± 10%.

Performance of the Quality Checks is presented in Tables 4 and 5 in the Appendix. For analysis of TBA, 80 of the 81 continuing calibration check standards met the goal. One of the standards was 126% of the nominal concentration. For analysis of TBA, 16 of 16 matrix spikes met the goal. For the analysis of TBA, 16 of 18 laboratory duplicates met the goal; the relative percent difference of one duplicate was ± 25.2% and the relative percent difference of another duplicate was ± 29.5%. In the 38 blanks for analysis of TBA, reported concentrations were less than the lower calibration limit, or less than the method detection limit, depending on which concentration was reported by the analyst.

For analysis of sulfate, 92 of 93 continuing calibration check standards met the goal. For the analysis of sulfate, 19 of 19 matrix spikes met the goal. For the analysis of sulfate

19 of 19 laboratory duplicates met the goal. The reported concentration of all 19 blanks was less than the method detection limit.

There were 19 sample sets for analysis of sulfate; 10 of the sets were analyzed within 30 days, and the maximum holding time for any set was 165 days. There were 20 sample sets for analysis of TBA; 12 of the sets were analyzed within 30 days, and the maximum holding time for any set was 145 days. Based on the reproducibility of data in the container controls (as presented in Figures 10, 11, 12 and 13) the excess holding times do not appear to have compromised the data quality for TBA. All the data were determined to be of acceptable quality, and the data were used in the report.

## *Biodegradation of TBA in Microcosms*

Of the six sites in the survey, TBA only degraded in microcosms constructed from sediment from Petaluma, California (Figure 10). There were three experimental treatments in the study: microcosms constructed with sediment as collected, microcosms constructed with sediment that had been autoclaved to kill organisms that could biodegrade TBA, and container controls that did not contain sediment. Live microcosms were spiked to initial concentrations of approximately 1,400 µg/L. Reductions in concentrations were apparent after 91 days of incubation. After 182 days of incubation, the concentration was down to the effective detection limit in two of the three replicate microcosms sampled. After 273, 314 and 456 days of incubation, the concentration of TBA was below the detection limit in all the microcosms sampled. Disregarding the lag, the rate of removal of TBA was 6.5 ± 4.1 per year at 90% confidence. This rate is in good agreement with the rates presented in Table 2.

In one of the treatments, the sediment was autoclaved at 121 °C overnight in an attempt to sterilize the sediment and then dosed. There was not significant removal of TBA over 734 days of incubation in the autoclaved control microcosms. One of the experimental treatments was a control for loss from the microcosm container. This control consisted of the sterile glass serum bottle filled with sterile water and dose solution, and sealed with a sterile septum and crimp cap. There was no sediment in the container control. As expected, there was no loss of TBA from the container control.

The pore water of the live microcosms contained 11.7 ± 0.2 mg/L sulfate at the start of the experiment. At the time when the TBA was entirely consumed, the sulfate concentration was 15.9 ± 0.1 mg/L. As was discussed earlier, oxidation of TBA by sulfate reduction requires 3.9 mg/L sulfate for each 1.0 mg/L of TBA. The sulfate demand to oxidize 1.4 mg/L TBA in the live microcosms would be 5.5 mg/L. It is not entirely clear why sulfate concentrations appeared to increase over time. The method for analysis of sulfate is recalibrated when calibration check standards are off by 10%. It is possible that sulfate desorbed from the anion exchange complex in the sediment. In any case, there were adequate concentrations of sulfate to meet the theoretical demand for sulfate reduction of the TBA. However, the sulfate concentrations were low. They were near the half saturation constant for sulfate reduction. It is equally plausible that TBA in the sediment from Petaluma was degraded by Iron(III) or Manganese(IV) reducing bacteria.

Panel A of Figure 11 presents data for the sediment from Deer Park, New York. In contrast to the behavior of TBA in sediment from Petaluma, California, there was no sustained or significant removal of TBA in the live microcosms, in the control microcosms, or in the container controls. The estimated rate of MTBE biodegradation in the plume at Deer Park was 5.3 ± 3 per year at 95% confidence (Kolhatkar et al., 2001). However, the companion microcosm study on anaerobic biodegradation of MTBE also failed to show any degradation of MTBE (Wilson et al., 2005b).

The maximum concentration of TBA in the live microcosms was 350 µg/L. If 10% of the TBA is converted to microbial biomass, and if the average dry weight of a microbial cell is $10^{-12}$ g, then complete consumption of 350 µg/L would produce 3.8 x $10^{7}$ cells per liter. The microcosms were incubated for 740 days. If initially, there was only a single TBA degrading bacterium in each microcosm, and the growth rate of the organism was at least 3% per day, the organisms would have grown to consume the TBA in the incubation period.

The concentration of sulfate in the pore water of the Deer Park microcosms was less than 1.0 mg/L at the beginning of the incubation period and at the end of the incubation period. However, the theoretical demand for sulfate for TBA biodegradation would have only been 1.4 mg/L. It is highly likely, but not definitely established, that concentrations of sulfate limited TBA biodegradation in the microcosms constructed with sediment from the Deer Park site.

In microcosms constructed from sediment from Parsippany, New Jersey, the concentration of TBA in the live microcosms increased approximately tenfold during 744 days of incubation (Panel B of Figure 11). This was probably due to TBA formed by anaerobic biodegradation of MTBE in residual gasoline in the sediment used to construct the microcosms (Wilson et al., 2005c). If TBA was degraded in the sediment, the rate did not exceed the rate of TBA production from biodegradation of MTBE. As expected, there was no loss of TBA from the killed control microcosms or the container controls.

Similarly, there was no evidence of removal of TBA over 730 days of incubation in microcosms constructed with sediment from the Boca Raton site (Figure 12). The initial concentration of TBA was 1.6 mg/L. The theoretical demand for sulfate was 6.2 mg/L. The initial concentration of sulfate was 4.8 ± 1.9 mg/L. The final concentration of sulfate was 3.4, 1.3, and <0.3 mg/L respectively in the triplicate microcosms. It is likely that sulfate was limiting for TBA biodegradation in the microcosms constructed with sediment from the Boca Raton site.

Finally, there was no evidence of TBA biodegradation in microcosms constructed with sediment from the Port Hueneme site (Panel A of Figure 13) or the Vandenberg AFB site

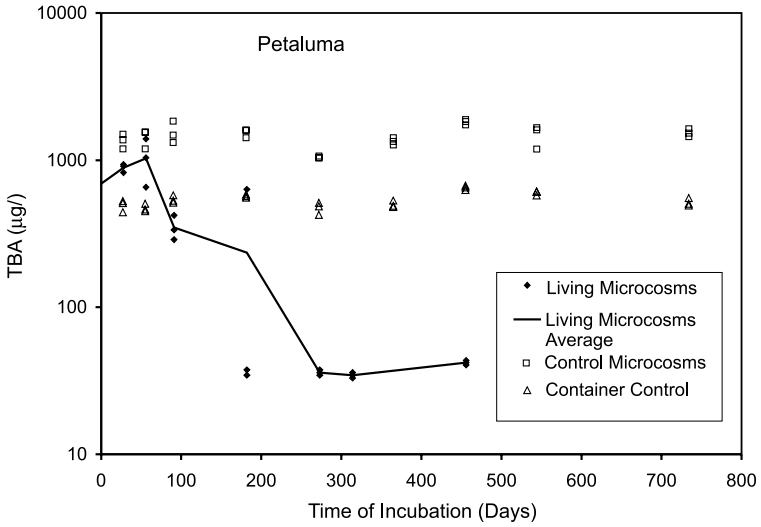



**Figure 10.** *Removal of TBA in microcosms constructed with material from a gasoline spill site in Petaluma, California.*

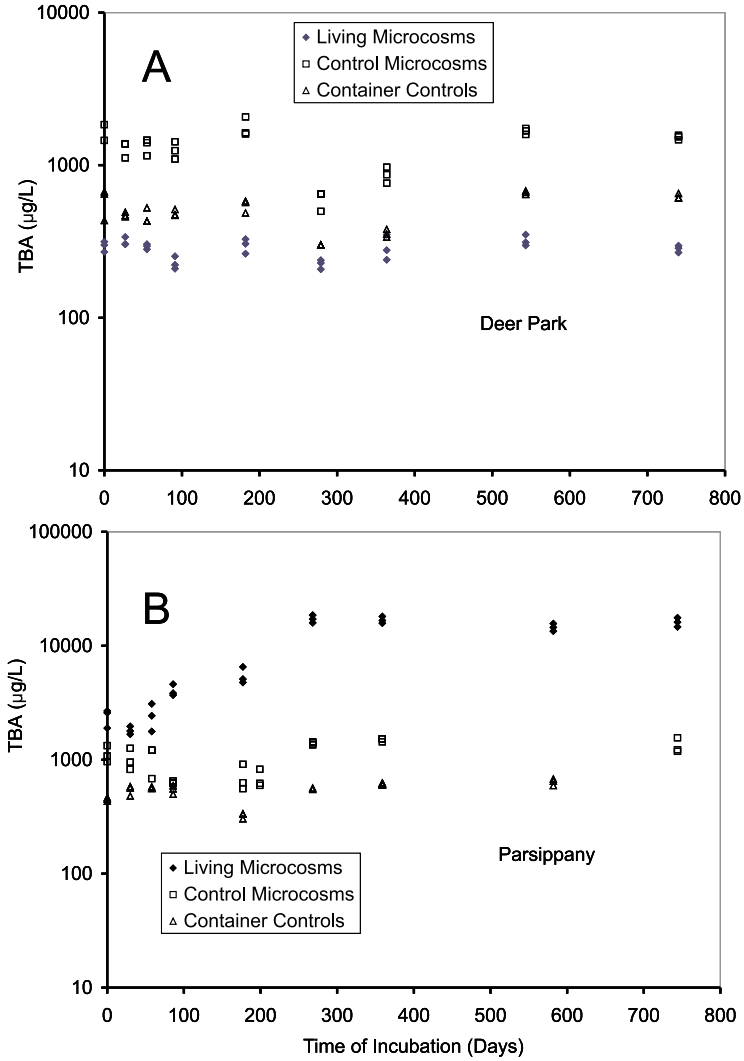



**Figure 11.** *Behavior of TBA in microcosms constructed with material from gasoline spill sites in Deer Park, New York and Parsippany, New Jersey.*

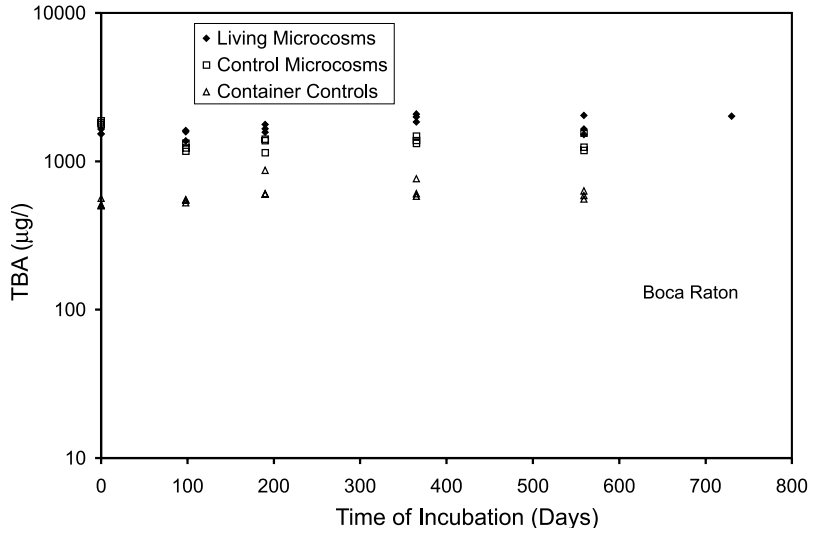



**Figure 12.** *Behavior of TBA in microcosms constructed with material from a gasoline spill site in Boca Raton, Florida.*

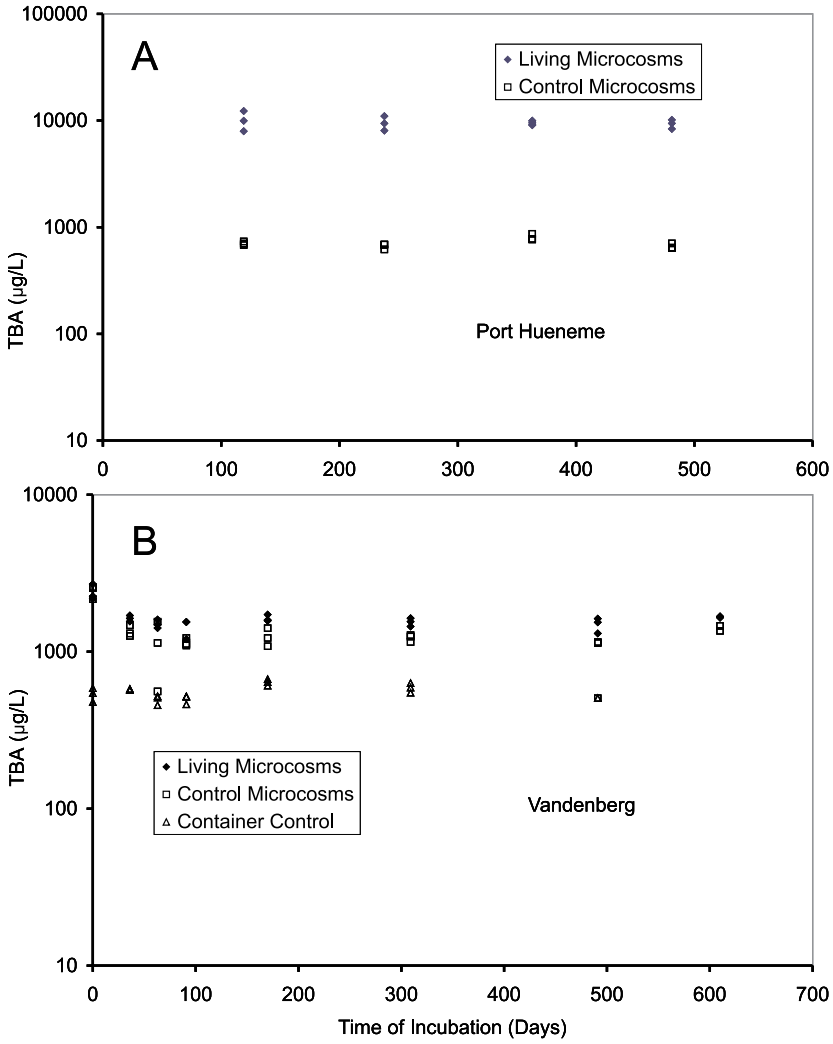



**Figure 13.** *Behavior of TBA in microcosms constructed with material from a gasoline spill site at Port Hueneme, California and a site at Vandenberg AFB, California. These are sites where natural anaerobic biodegradation of TBA was not expected.*

(Panel B of Figure 13). Both of these aquifers are naturally anaerobic and sulfate reduction is the dominant electron accepting process. Unfortunately, data on concentrations of sulfate were not collected in the microcosm experiments.

In summary, the microcosm experiments did not present compelling evidence that anaerobic TBA biodegradation was widespread at gasoline spill sites. At three of the sites, the microcosm studies failed to confirm initial expectations that TBA was being degraded at the site based on field monitoring data.

## *Use of Stable Isotope Ratios*

In recent years an alternate approach has been developed to recognize natural biodegradation of MTBE in ground water (Hunkeler et al., 2001; Gray et al., 2002; Kolhatkar et al., 2002; Kuder et al., 2005; Wilson et al., 2005b; Zwank et al., 2005). Organic compounds contain two stable isotopes of carbon. Carbon with a weight of twelve daltons ($^{12}C$) is approximately one hundred times more abundant than carbon with a weight of thirteen daltons ($^{13}C$). During biodegradation, MTBE molecules with $^{12}C$ in the methyl group are degraded more rapidly than MTBE molecules with $^{13}C$ in the methyl group. Over time $^{13}C$ accumulates in the residual MTBE molecules that have not been degraded, and the extent of biodegradation of MTBE can be inferred by an increase in the ratio of $^{13}C$ to $^{12}C$ in the residual MTBE.

The use of stable isotopes has two important advantages. It uses the compound of interest as its own tracer. Molecules composed with $^{13}C$ and molecules with $^{12}C$ share a common source and should have the same behavior with respect to sorption, hydrodynamic dispersion, or dilution in a monitoring well. A second and more important advantage is that the stable isotope ratios recognize and validate biodegradation that has actually occurred in the aquifer. Microcosm studies done in the laboratory with sediment samples only establish a potential for biodegradation in the aquifer.

The behavior of stable isotopes is described by two parameters: $\delta^{13}C$ and $\varepsilon$. The $\delta^{13}C$ of a compound is a measure of the ratio of $^{13}C$ atoms to $^{12}C$ atoms in the molecule. It will be defined formally later in the text. The usual pronunciation of $\delta^{13}C$ is “delta thirteen sea.” During the course of biodegradation, the compound that is still remaining will have more of the heavy isotope $^{13}C$, and the value of $\delta^{13}C$ will become more positive. The value of $\varepsilon$ (epsilon) relates the change in $\delta^{13}C$ to the fraction of the original contaminant that is still remaining. As a consequence, $\varepsilon$ is often called the isotopic enrichment factor.

The ratio of isotopes is determined with an isotope ratio mass spectrometer. The mass spectrometer does not measure the ratio of the stable carbon isotopes to each other. Rather, it measures the deviation of the ratio in the sample from the ratio of a standard used to calibrate the instrument. The substance used as the international standard for stable carbon isotopes has a ratio of $^{13}C$ to $^{12}C$ of 0.0112372.

The conventional notation for the ratio of $^{13}C$ to $^{12}C$ in a sample ($\delta^{13}C$) reports the ratio in terms of its deviation from the ratio in the standard.

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

The units for $\delta^{13}C$ are parts per thousand, often represented as ‰, or per mil, or per mill.

The extent of isotopic fractionation is typically determined by a linear regression of the $\delta^{13}C$ in MTBE on the natural logarithm of the fraction of MTBE remaining after biodegradation. The slope of the regression line is termed the isotopic enrichment factor ($\varepsilon$). Fractionation of carbon in MTBE is much greater during anaerobic biodegradation compared to biodegradation during aerobic respiration. Somsamak et al., (2006) reported values for $\varepsilon$ during biodegradation of MTBE under methanogenic conditions of -13.3‰ to -14.6‰, and values under sulfate reducing conditions of -13.4‰ to -14.6‰. In contrast, Hunkeler et al., (2001) reported enrichment factors for aerobic biodegradation that varied from –1.52‰ ± 0.06‰ to –1.97‰ ± 0.05‰, and Gray et al. (2002) determined the enrichment factors that varied from –1.4‰ ± 0.1‰ to –2.4‰ ± 0.3‰.

Figure 14 compares the extent of fractionation of MTBE that would be expected during biodegradation under aerobic and anaerobic conditions. The most negative value for $\varepsilon$ was plotted because this value will predict the least biodegradation of MTBE with an increase in the value of $\delta^{13}C$ in MTBE. The most negative value for $\varepsilon$ is the most conservative value.

Any change in the value of $\delta^{13}C$ in MTBE caused by biodegradation must be compared to the normal variation of $\delta^{13}C$ in MTBE used to make gasoline. Smallwood et al., (2001) reported that the normal range of $\delta^{13}C$ for MTBE in gasoline is from –28.3‰ to –31.6‰; more recent surveys indicate that the normal range extends between –27.5‰ and –33‰ (O’Sullivan et al., 2003). This natural variation is represented as a filled arrow in Figure 14. The natural variation in $\delta^{13}C$ in MTBE is of the same order as the fractionation that would be expected after 99% of the original amount of MTBE had been degraded under aerobic conditions. As a consequence of this weak fractionation, it has been difficult to document natural aerobic biodegradation of MTBE at field scale. However, the variation in $\delta^{13}C$ during anaerobic biodegradation is much larger than the variation in $\delta^{13}C$ in MTBE in gasoline, and determinations of $\delta^{13}C$ in MTBE have been used to document natural anaerobic biodegradation of MTBE at field scale (Kolhatkar et al., 2002; Kuder et al., 2005; Wilson et al., 2005a; Wilson et al., 2005b; Zwank et al., 2005).

Wilson et al., (2005a) used stable isotope ratios to evaluate production of TBA from natural biodegradation of MTBE at thirteen gasoline spill sites in Orange County, California. At

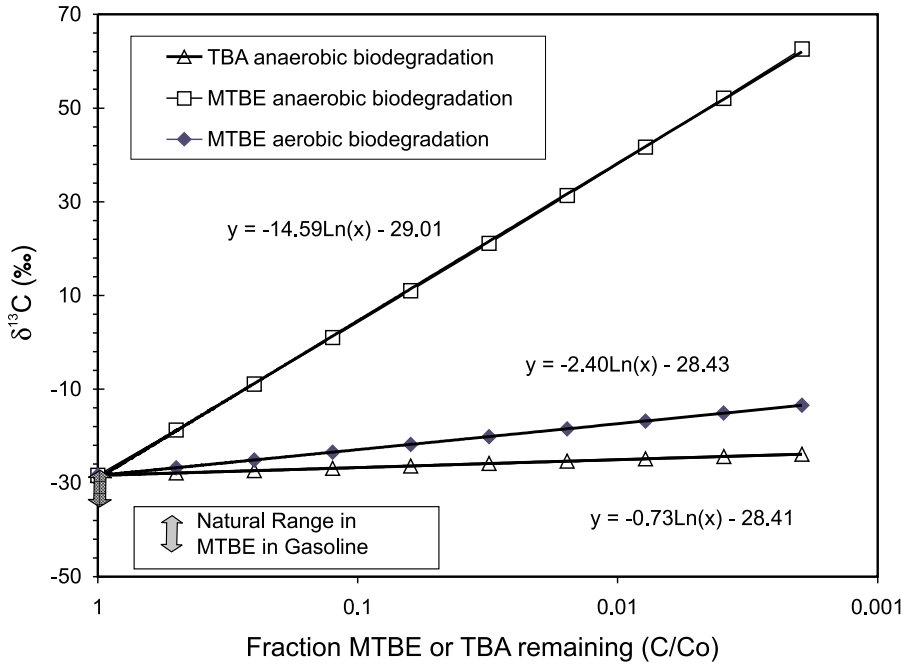



**Figure 14.** *Expected relationship between the ratio of stable isotopes of carbon in MTBE or TBA and the extent of biodegradation of MTBE or TBA.*

these thirteen sites in Orange County, the concentrations of MTBE and TBA are not constant over time. Figures 15 and 16 present data from wells at three separate sites that illustrate common patterns. At some wells, at some sites, concentrations of MTBE are greater than concentrations of TBA at the beginning of the monitoring record. Over time, the concentrations of MTBE go down by orders of magnitude and the concentrations of TBA increase by orders of magnitude (Figure 15). Occasionally there was an almost stoichiometric replacement of MTBE with TBA (Figure 16 and 17). An evaluation of the ratio of stable isotopes of carbon in the residual MTBE demonstrated that biodegradation of MTBE to TBA could explain the increase in concentrations of TBA (Wilson et al., 2005a).

In some wells, at four of the thirteen sites, the high concentrations of TBA persisted for a period and then the concentrations of TBA also declined over time (illustrated by Figure 17). The pattern was very similar to the pattern seen in the microcosm studies conducted with sediment from sites in Orange County, California (Figure 4, presenting data from DeVaull et al., 2003).

Studies with enrichment cultures typically follow the same pattern (Bradley et al., 1997; Suflita et al., 1997; Walter, 1997). The initial density of active organisms in the inoculum is low and the activity of the organisms has no perceptible effect on the concentration of their substrate. As the organisms grow and increase in number, they eventually reach a density where their activity has a perceptible effect on the concentration of substrate. As they continue to grow and increase in numbers they eventually exhaust the substrate.

The pattern shown in Figure 4 for a laboratory microcosm and in Figure 17 for a monitoring well at field scale can be explained as a lag period of MTBE biodegradation during microbial acclimation to MTBE, followed by biodegradation of MTBE to TBA, then a lag during microbial acclimation to TBA, followed by biodegradation of TBA. However, there are other explanations for the pattern at field scale. The supply of MTBE may have been exhausted in the source area and as a result there was no continuing supply of MTBE to produce TBA. As a result, the concentrations of TBA decreased because the TBA that was previously produced was swept away by advective flow of ground water in the aquifer. It is also possible that the direction of ground water flow in the aquifer changed and the new flow path to monitoring well MW-4S at site 88UT138 did not contain MTBE or TBA. Because there are alternative explanations for the pattern illustrated in Figure 17, field monitoring data cannot provide unequivocal evidence that TBA is being biologically degraded in ground water.

It would greatly simplify the evaluation of natural biodegradation of TBA at field scale if the ratios of stable isotopes could be used to recognize and quantify TBA biodegradation in ground water. However, the authors are not aware of any report comparing fractionation of TBA under anaerobic conditions in either microcosm studies or enrichment cultures. The only data available on the fractionation of carbon isotopes during biodegradation of TBA are from a study of a field-scale plume and the data suggest that the fractionation coefficient is small. Day et al., (2002) and Day and Gulliver (2003) describe a detailed evaluation of natural TBA biodegradation in ground water at a chemical

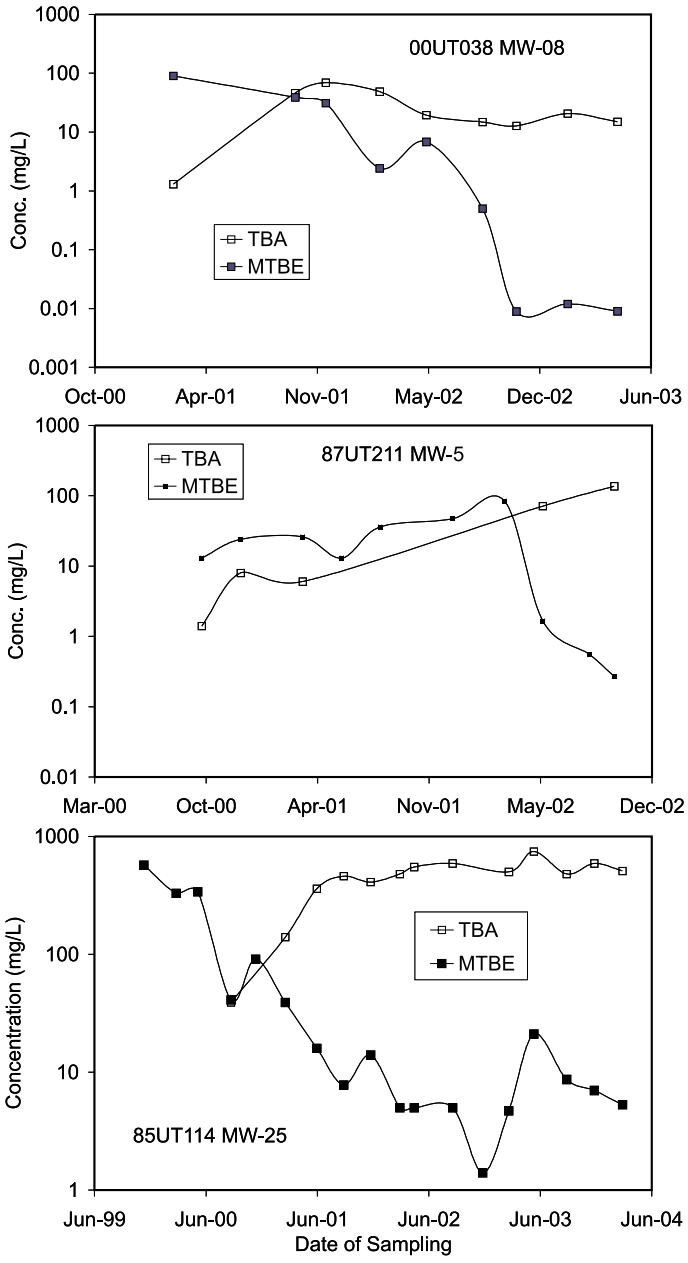



**Figure 15.** *Changes in concentrations of MTBE and TBA in monitoring wells over time at three sites in Orange County, California, data plotted on logarithmic scale.*

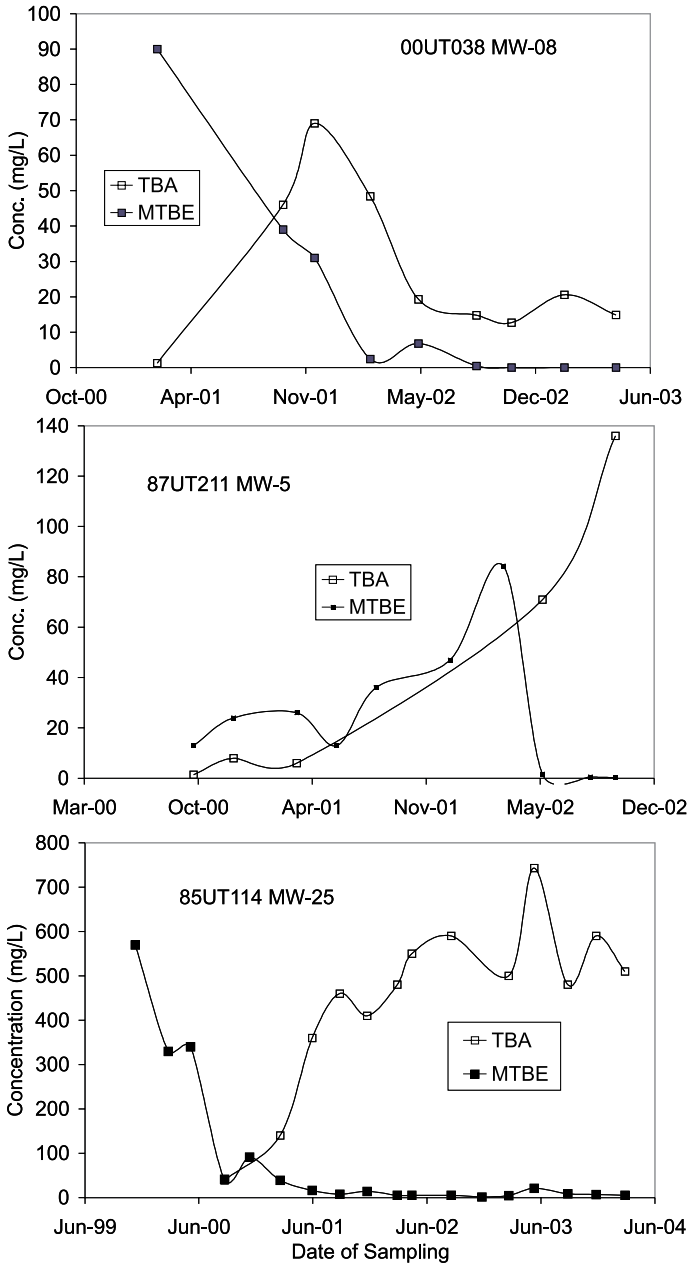



**Figure 16.** *Changes in concentrations of MTBE and TBA in monitoring wells over time at three sites in Orange County, California, data plotted on arithmetic scale.*

manufacturing plant at Pasadena, Texas. They compared the concentrations of TBA in the plume to the $\delta^{13}C$ of TBA. Figure 18 in this report plots the data provided in Figure 30-7 of Day and Gulliver (2003). Following the approach of Kolhatkar et al., (2002), a regression of $\delta^{13}C$ of TBA on the natural logarithm of the concentrations of TBA in the plume was used to estimate the fractionation coefficient ($\varepsilon$) for biodegradation of TBA at field scale. The value of $\varepsilon$ was -0.73‰.

Figure 14 compares the extent of fractionation of TBA that would be expected during biodegradation under anaerobic conditions to the extent of fractionation of MTBE that would be expected under aerobic and anaerobic conditions. If the value of the enrichment coefficient during anaerobic biodegradation of TBA is -0.73‰, then minimal change can be expected in the value of $\delta^{13}C$ for TBA, even when the mass of TBA is reduced by two orders of magnitude through biodegradation of TBA.

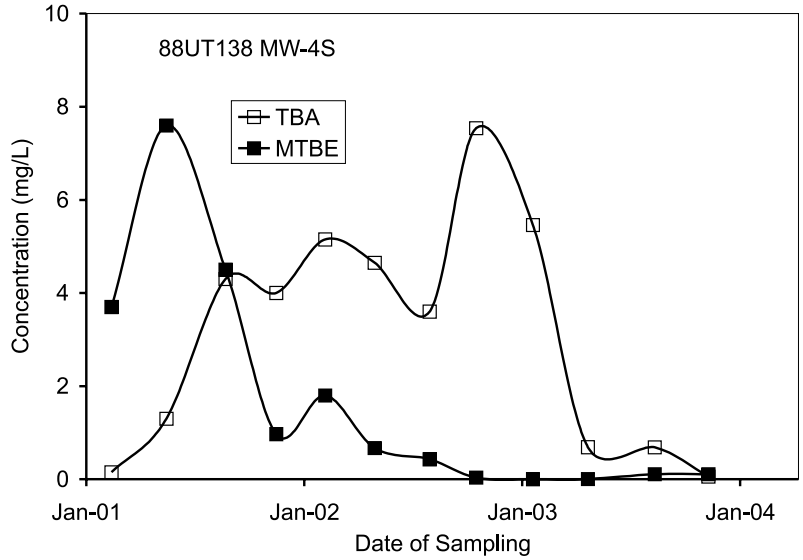



**Figure 17.** *Changes in concentrations of MTBE and TBA in monitoring wells over time at a site in Orange County, California, where a decline in concentration of MTBE was followed at a later time by a decline in concentration of TBA.*

The prediction of the enrichment factor $\varepsilon$ for anaerobic biodegradation of TBA in Figure 18, as projected in Figure 14, is consistent with field observations of $\delta^{13}C$ for MTBE and $\delta^{13}C$ for TBA at field sites in Orange County, California, (Kuder et al., 2005) and a large field site in South America (Zwank et al., 2005). Figure 19 compares the total variation in $\delta^{13}C$ in TBA in ground water samples in monitoring wells to the variation in $\delta^{13}C$ in MTBE in the same well. In the data reported by Kuder et al., (2005) for Orange County, the total variation of $\delta^{13}C$ for TBA was 6.76‰ while the total variation of $\delta^{13}C$ for MTBE was 89.5‰ (Panel A of Figure 19). In the data reported by Zwank et al., (2005) for South America, the total variation of $\delta^{13}C$ for TBA was 5.6‰ while the total variation of $\delta^{13}C$ for MTBE was 66.7‰ (Panel B of Figure 19). There was substantial anaerobic biodegradation of MTBE in these studies, as documented by extensive fractionation of carbon isotopes in MTBE. There was little fractionation of TBA. This may have been a result of little biodegradation of TBA. It may also have been the result of the much weaker fractionation of TBA during anaerobic biodegradation.

To properly interpret the variation of $\delta^{13}C$ for TBA in ground water, it will be necessary to compare the measured value of $\delta^{13}C$ for TBA to the natural variation of $\delta^{13}C$ for TBA. To the authors' knowledge, data on the natural variation in $\delta^{13}C$ for TBA has not been published. In fact, two distributions on natural variation would be necessary to interpret $\delta^{13}C$ for TBA in ground water, (1) the natural variation of $\delta^{13}C$ in TBA in gasoline, and (2) the natural variation of $\delta^{13}C$ in the tertiary butyl functional group of MTBE in gasoline. Zwank et al., (2005) determined the $\delta^{13}C$ of a sample of MTBE, then hydrolyzed the MTBE to TBA and determined the $\delta^{13}C$ of the TBA produced. The $\delta^{13}C$ of the MTBE was -28.13 ± 0.15‰, while the $\delta^{13}C$ of the TBA was -25.49 ± 0.10‰. The difference between the $\delta^{13}C$ of MTBE and the $\delta^{13}C$ of the tertiary butyl functional group in MTBE is one half as wide as the entire reported range of variation of $\delta^{13}C$ of MTBE in gasoline (–27.5‰ to –33‰; reported in O'Sullivan et al., 2003). It will not be appropriate to use the variation of $\delta^{13}C$ of MTBE in gasoline as a surrogate for the variation of $\delta^{13}C$ of TBA.

It is possible that future research will show that the true coefficient of fractionation of TBA during anaerobic biodegradation is considerably more negative than -0.73‰. Until that work is published, and until the distribution of the natural variation of $\delta^{13}C$ of TBA is published, it will be difficult or impossible to use measurements of $\delta^{13}C$ of TBA in ground water to estimate the extent of biodegradation of TBA at field sites.

In contrast to the limited variation in $\delta^{13}C$ of the TBA, there was much wider variation of isotope ratios of hydrogen in TBA (expressed as $\delta$ D, the ratio of deuterium to hydrogen one as measured against a standard reference material for hydrogen isotopes). Data reported by Zwank et al., (2005) are presented in Figure 20. Although values of $\delta^{13}C$ in TBA varied by 3.9‰ in the population of wells, values of $\delta$ D in TBA varied by 67.9‰. Future research may establish a relationship between $\delta$ D and the extent of biodegradation of TBA in ground water; however, there are concerns that isotopic exchange of the hydrogen in the alcohol functional group with hydrogen in water may alter $\delta$ D in TBA, making a straightforward interpretation of $\delta$ D in TBA difficult (Zwank et al., 2005).

In summary, there is not a good technique to determine biodegradation of TBA at field scale. At most field sites there will not be enough monitoring wells, or the wells will not be in the right place to document anaerobic biodegradation along a flow path in the aquifer. The application of stable isotope ratios to TBA in ground water (at the present level of development) cannot resolve TBA biodegradation at field scale.

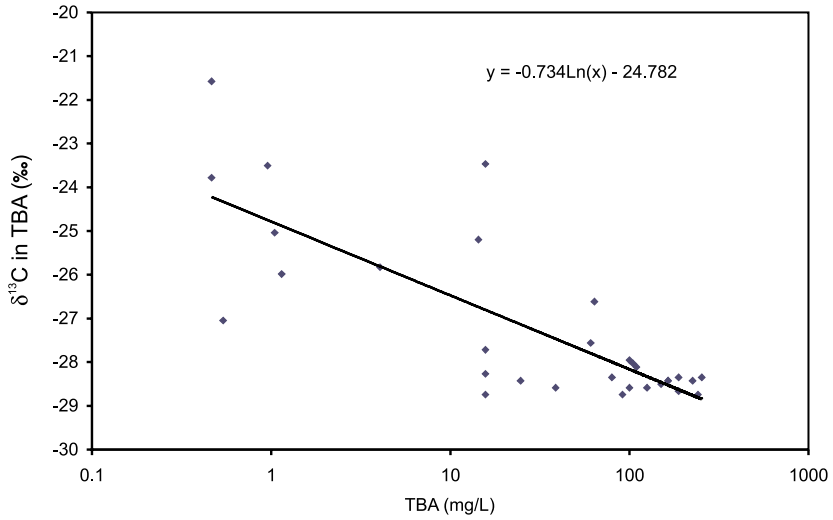



**Figure 18.** *Relationship between the isotopic ratio of carbon in TBA in ground water at a manufacturing facility in Pasadena, Texas, and the concentration of TBA. The slope of the line is an indirect estimate of the isotopic fractionation factor (ε) for anaerobic biodegradation of TBA at the site. Adapted from Day and Gulliver (2003).*

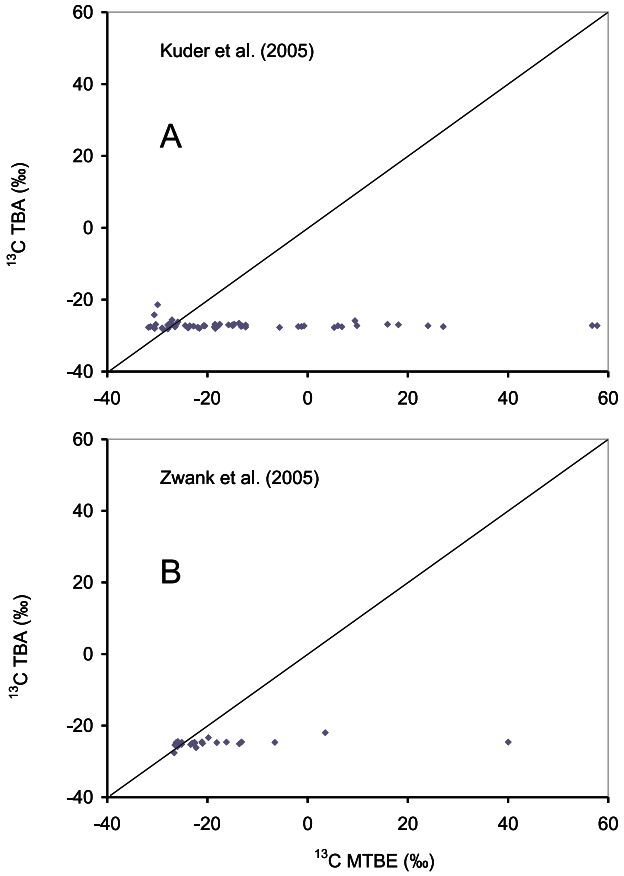



**Figure 19.** *Comparison of the range of variation in the isotope ratio of carbon ($\delta^{13}C$) in TBA at monitoring wells at sites in Orange County, California, (Kuder et al., 2005) and at a site in South America (Zwank et al., 2005) to the range of variation in the isotope ratio of carbon ($\delta^{13}C$) in MTBE in the same monitoring wells.*

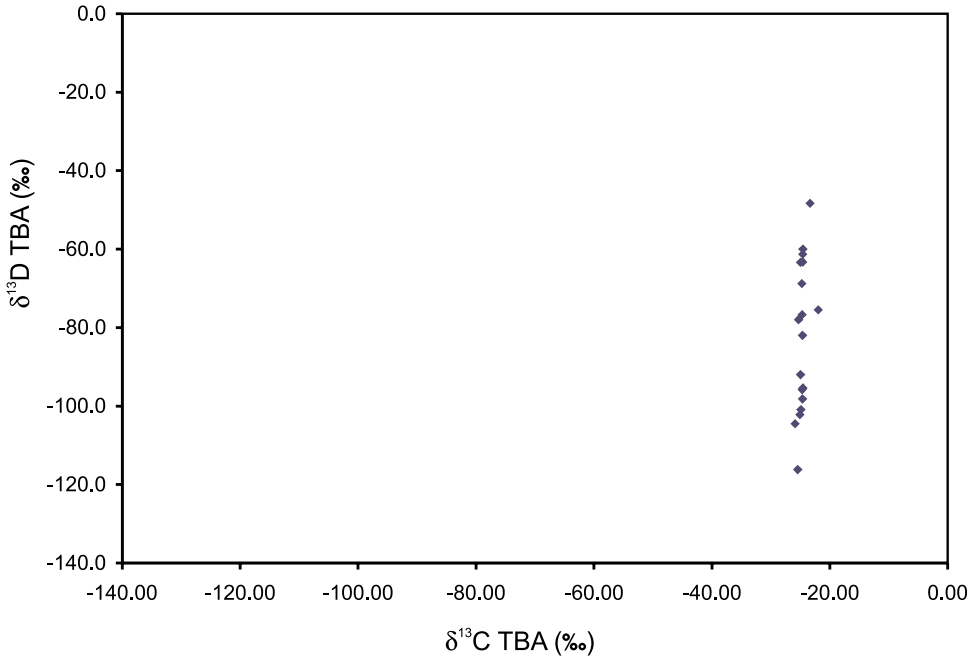



**Figure 20.** *Comparison of the range of variation in the isotope ratio of hydrogen (δD) in TBA at monitoring wells at a site in South America (Zwank et al., 2005) to the range of variation in the isotope ratio of carbon ($\delta^{13}C$) in MTBE in the same monitoring wells.*

# *Summary*

Tertiary butyl alcohol (TBA) is widely distributed at gasoline spill sites, and is present at high concentrations. As an example, Shih et al., (2004) reported that TBA was detected in ground water at 61.1% of sites in Los Angeles County, California. The concentration of TBA was equivalent to the concentrations of methyl tertiary butyl ether (MTBE) and benzene. The mean and median concentration of TBA was 30,100 and 1,880 µg/L, compared to 44,800 and 1,200 µg/L for MTBE and 83,800 and 1,370 µg/L for benzene.

At a major fraction of sites, the concentration of TBA in ground water is greater than can plausibly be expected from the TBA that was a constituent in the gasoline that was originally spilled (Wilson, 2003; Wilson et al., 2005a; this report Figure 2). Based on the ratio of TBA to MTBE at gasoline spill sites in Orange County, California, Wilson et al., (2005a) determined that TBA resulting from the biodegradation of MTBE could explain the concentrations of TBA at 85% of the sites.

A review of available literature indicated that microorganisms can degrade TBA using oxygen, nitrate, Iron(III), Manganese(IV), or sulfate as a terminal electron acceptor (Bradley et al., 2002). The current consensus opinion is that an electron acceptor is necessary, and that TBA cannot be directly fermented to produce methane (Schmidt et al., 2004a). Although TBA does not degrade under anaerobic conditions in many laboratory experimental systems, the average rate of anaerobic biodegradation of TBA (when it does occur) is faster than the average rate of anaerobic biodegradation of MTBE. The average first order rate of anaerobic biodegradation of TBA in the studies reported in Table 2 was 9.2 per year compared to an average rate of anaerobic biodegradation of MTBE of 1.0 per year (Wilson, 2003; Table 3).

Data on the availability of electron acceptors in ambient ground water and the concentrations of electron acceptors in the source area of plumes indicates that sulfate is the most important electron acceptor at fuel spill sites (Wiedemeier et al., 1995). Data collected for a study published by Kolhatkar et al., (2000), but presented for the first time in this report, indicates that sulfate, and presumably oxygen and nitrate, are entirely depleted in the source area of approximately 80% of gasoline spill sites.

As a consequence, anaerobic biodegradation of TBA at many particular field sites will be limited by the availability of sulfate and the rate of TBA biodegradation will be limited by the rate that sulfate is supplied to the plume by diffusion and dispersion. This rate of supply can be orders of magnitude slower than the rate of TBA biodegradation in laboratory microcosm studies where sulfate is not limiting.

There is a strong possibility that Iron(III) reducing and Manganese(IV) reducing bacteria degrade TBA in ground water that is depleted of soluble electron acceptors. However, at the current state of practice, it is impossible to evaluate the contribution of Iron(III) reducing and Manganese(IV) reducing bacteria to the natural biodegradation of TBA in ground water at gasoline spill sites.

The OSWER Directive on MNA (U.S. Environmental Protection Agency, 1999) identifies three lines of evidence that can be used to support the selection of MNA as a remedy. The first line of evidence is *Historical groundwater and/or soil chemistry data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points.* The second line of evidence is *Hydrogeologic and geochemical data that can be used to demonstrate indirectly the type(s) of natural attenuation processes active at the site, and the rate at which such processes will reduce contaminant concentrations to required levels.* Until techniques are applied that can estimate the supply of biologically available Iron(III) or Manganese(IV) in aquifer sediment, it is not possible to compare the supply of these insoluble electron acceptors to the demand for electron acceptors provided by TBA or other organic materials in contaminated ground water. It is not possible to provide the second line of evidence for natural biodegradation of TBA by iron-reducing or manganese-reducing bacteria.

Data on the fractionation of carbon isotopes in TBA during anaerobic biodegradation are indirect data from a field study instead of direct measurements from controlled laboratory studies and the data are available from only one site (Day et al., 2002; Day and Gulliver, 2003). The fractionation reported at the one site available in the literature is weak; suggesting that fractionation of carbon isotopes will not be generally useful to recognize anaerobic biodegradation of TBA at field sites. Fractionation of hydrogen isotopes in TBA is much stronger (Zwank et al., 2005) and may be useful in the future. However, there is a concern about isotopic exchange of the hydrogen in the –OH function of TBA with hydrogen in water. At this writing, there is not a consensus on the appropriate interpretation of shifts in the ratio of hydrogen isotopes of TBA. However, isotopic fractionation during biodegradation is an active area of research and advances in the state of knowledge can be expected in the future.

# Recommendations

If it is necessary to evaluate natural biodegradation of TBA at a gasoline spill site, do not rely on the data from conventional monitoring wells. It is necessary to obtain data on the vertical distribution of the concentrations of TBA, sulfate, benzene, and methane in ground water. This can be accomplished using the following steps. Collect ground water with push tools that sample a narrow vertical interval (six inches to two feet; 0.15 m to 0.61 m). Extend the vertical profile from the water table into clean water below the plume. Use the concentrations of benzene as a tracer for the contaminated ground water that might contain TBA. If the benzene has been biologically degraded and is not present in the ground water, use concentrations of methane as a tracer for the plume. The strongest and most direct evidence for natural biodegradation of TBA is a series of sampling locations or monitoring wells down gradient of the source area that have high concentrations of tracer compounds but are devoid of TBA.

If unacceptable concentrations of TBA are still present in ground water from the down gradient wells, compare the concentrations of TBA to the supply of sulfate as an electron acceptor for biodegradation of TBA. As a loose rule of thumb, the stoichiometric demand for sulfate as an electron acceptor for complete oxidation of a fuel component is four times the concentration of the fuel component. There is a reasonable prospect for natural attenuation of TBA through natural anaerobic biodegradation if the concentration of sulfate exceeds four multiplied by the sum of the concentrations of TBA, MTBE, benzene, toluene, ethylbenzene, xylenes, and trimethylbenzenes.

Many risk evaluations at gasoline spill sites use a simple transport and fate model such as BIOSCREEN (Newell et al., 1996) or the calculations in ASTM E-1739, Risk-Based Corrective Action (RBCA) at Petroleum Release Sites, issued by the American Society for Testing and Materials Standards (2002). Unless or until it has been shown that a sufficient supply of electron acceptor is available to meet the stoichiometric demand for TBA and the other organic compounds in the plume, assume that TBA will not be biologically degraded in the ground water plume and that natural attenuation of TBA will be due exclusively to hydrodynamic dispersion.

Do not assume that TBA is being biologically degraded under anaerobic conditions in ground water because there is evidence that natural anaerobic biodegradation of MTBE is occurring. The same organisms are not degrading MTBE and TBA. The initial step in anaerobic MTBE biodegradation is carried out by acetogenic bacteria that use MTBE to consume molecular hydrogen as part of their energy metabolism. The anaerobic biodegradation of TBA is most commonly carried out by sulfate reducing bacteria.

A risk evaluation for TBA may indicate that it is necessary to remediate TBA in ground water. Long term monitoring data may indicate that the plume of TBA is continuing to expand down gradient. Farther expansion of the TBA plume may put a down gradient receptor at risk, or exceed some concentration-based goal at a down gradient point of compliance. Depending on the remedial goals, on the nature of the source area for TBA in ground water, and on the geochemistry of the ground water, Monitored Natural Attenuation may have a role in the overall strategy for risk management. Small and Weaver (1999) reasoned that plumes of MTBE and benzene expand because the MTBE or benzene is transferred from the source area to flowing ground water faster than natural biodegradation can remove MTBE or benzene from the flowing ground water. Plumes of TBA should follow the same pattern.

The capacity for biodegradation of TBA is limited by the concentration of sulfate in ground water entering the source area and the amount of biologically available Iron(III) or Manganese(IV) associated with the aquifer sediments. The only remedial approach that can tip balance between the release of TBA to ground water and the degradation of TBA in ground water is to reduce the transfer of TBA from the source to flowing ground water in the aquifer.

There are two common situations that lead to a continuing source of TBA contamination. In the first situation, MTBE in residual gasoline is slowly released to flowing ground water over time, and the MTBE is biologically degraded to TBA once it comes into solution in ground water. In the second situation, TBA that is present in residual gasoline, as an oxygenate, is slowly released to flowing ground water over time because the residual gasoline is held in clay or silt by capillary attraction. As a result the TBA must escape the clay or silt by diffusion through pore water before it can enter the major channel of ground water flow in the aquifer. In either situation, the first remedial response should be removal of the residual gasoline by excavation, or some effective technique for in situ remediation. At some sites, air sparging has proved effective to remove sources of MTBE in residual gasoline (Hattan et al., 2003). Because air sparging can effectively supply oxygen to ground water, air sparging should effectively remove TBA as well.

After the source has been remediated, it may be necessary to pump and treat the ground water that still contains high concentrations of TBA. It is not reasonable to expect natural biodegradation of TBA to remediate TBA in a plume unless the concentration of sulfate in the plume can meet the stoichiometric demand for complete metabolism of TBA and other organic compounds in the plume. If a pump and treat remedy is put in place, it should continue until the concentrations of sulfate in the plume are adequate to degrade TBA and all the other organic compounds in the ground water.

## *Research needs*

More research and field studies are needed on the contribution of Iron(III) reducing and Manganese(IV) reducing bacteria to the natural anaerobic biodegradation of TBA at gasoline spill sites. In particular, techniques are needed to evaluate the supply of biologically available Iron(III) and Manganese(IV).

More research is also needed on techniques to determine the presence and activity of naturally occurring microbes in ground water that can degrade TBA. Recently, new techniques have been developed to sample and evaluate the microorganisms in water in monitoring wells (Biggerstaff, 2007; Geyer et al., 2005; Sublette et al., 2006). These techniques are built around the use of Bio-Sep® beads (Microbial Insights, Rockford, TN). These beads are constructed from a composite of 25% aramid polymer (Nomex®, DuPont, Wilmington, DE) and 75% powdered activated carbon. The beads are from 2 to 4 mm in diameter. They have a high porosity (74%) and high specific surface area (600 $m^2$ /g). The Bio-Sep® beads provide a surface for the microorganisms to colonize and grow. After a period of incubation, in ground water in a monitoring well, the beads are retrieved and the microorganisms that grew in the beads are extracted and analyzed.

One particularly compelling approach is to amend Bio-Sep® beads with an organic compound that is mass labeled with the stable carbon isotope $^{13}C$. If the compound is biologically degraded, some portion of the mass label should find its way into the biomass that develops in the bead. Geyer et al., (2005) amended the beads with $^{13}C$ labeled benzene or toluene by sorbing vapors of benzene or toluene to the powdered activated carbon. The beads were installed in a monitoring well at a contaminated site for 32 days and then recovered. The phospholipid fatty acids in the biomass were extracted and the concentration of $^{13}C$ in the fatty acids was determined using compound specific isotope ratio mass spectrometry. Selected fatty acids were highly enriched in $^{13}C$ and the mass label could only have come from metabolism of the mass labeled benzene or toluene incorporated into the Bio-Sep® beads before they were deployed to the well. As of this writing, EPA funded research is applying the same approach to evaluate the biodegradation of TBA in contaminated ground water.

Finally, more effort is needed to monitor the lifecycle of TBA plumes in ground water at gasoline spill sites and to document the contribution of natural attenuation processes, including natural biodegradation and dilution and dispersion.

# References


American Society for Testing and Materials Standards. ASTM E-1739-95; Standard Guide for Risk-Based Corrective Action (RBCA) Applied at Petroleum Release Sites (2002). Available at http://webstore.ansi.org/ansidocstore/default.asp.

Biggerstaff, J.P., M. Le Puil, B.L. Weidow, J. Leblanc-Gridley, E. Jennings, J. Busch-Harris, K.L. Sublette, D.C. White and R.S. Alberte. A novel and in situ technique for the quantitative detection of MTBE and benzene degrading bacteria in contaminated matrices. Journal of Microbiological Methods 68(2): 437-441 (2007).

Bradley, S.N., T.B. Hammill, and R.L. Crawford. "Biodegradation of agricultural chemicals." In Manual of Environmental Microbiology, by C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter: American Society for Microbiology (ASM) Press, Washington, DC, 815-821, (1997).

Bradley, P.M., F.H. Chapelle, and J.E. Landmeyer. Effect of redox conditions on MTBE biodegradation in surface water sediments. Environmental Science & Technology 35 (23): 4643-4647 (2001).

Bradley, P.M., J.E. Landmeyer, and F.H. Chapelle. TBA biodegradation in surface-water sediments under aerobic and anaerobic conditions. Environmental Science & Technology, 36 (19): 4087-4090 (2002).

Buscheck, T.E., and C.M. Alcantar. "Regression techniques and analytical solutions to demonstrate intrinsic bioremediation." In Intrinsic Bioremediation, Proceedings of the Third International In Situ and On-Site Bioremediation Symposium, San Diego, CA, 109-116, (1995).

Day, M., R. Aravena, D. Hunkeler, and T. Gulliver. Application of carbon isotopes to document biodegradation of tert-butyl alcohol under field conditions. Contaminated Soil Sediment and Water. Oxygenated Fuels Issue, July/August 2002, 88-92.

Day, M.J. and T. Gulliver. "Natural attenuation of tert butyl alcohol at a Texas chemical plant." In MTBE Remediation Handbook, by E.E. Moyer and P.T. Kostecki, Amherst, Massachusetts: Amherst Scientific Publishers, 2003, 541-560.

DeVaull, G.E., P.T. Sun, I.A.L. Rhodes, and D.F. Walsh. Study of tert-butyl alcohol (TBA) at selected underground storage tank remediation project sites in Orange County, California, Final Report, prepared by Shell Global Solutions US, Inc., for Seth Daugherty, Orange County Health Care Agency, November 13, 2003, 25 pages. Available by sending a request to George DeVaull, Shell Global Solutions US, Inc., HSC Consultancy, 3333 Highway 6 South, Houston, Texas 77082 or by sending e-mail to Wilson.johnt@epa.gov.

Environmental Security Technology Certification Program (ESTCP). Cost and Performance Report Bioavailable Ferric Iron (BAFeIII) Assay, ESTCP Project Number CU-0009 (June 2005). http://www.estcp.org/viewfile.cfm?Doc=ER%2D0009%2DC%26P%2DFerricIron%2Epdf. Accessed July 16, 2007.

Finneran, K.T. and D.R. Lovley. Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA). Environmental Science & Technology, 35 (9): 1785-1790 (2001).

Finneran, K.T. and D.R. Lovley. "Anaerobic In Situ Bioremediation." In MTBE Remediation Handbook, by E.E. Moyer and P.T. Kostecki, Amherst, Massachusetts: Amherst Scientific Publishers, 2003, 265-277.

Fukui, M., and T.S. Takii. Kinetics of sulfate respiration by free-living and particle-associated sulfate-reducing bacteria. FEMS Microbiology Ecology 13 (4): 241-247 (1994).

Geyer, R., A.D. Peacock, A. Miltner, H.H. Richnow, D.C. White, K.L. Sublette, and M. Kästner. In Situ Assessment of Biodegradation Potential Using Biotraps Amended with 13C-labeled Benzene or Toluene. Environmental Science and Technology 39(13):4983-4989 (2005).

Gray, J.R., G. Lacrampe-Couloume, D. Gandhi, K.M. Scow, R.D. Wilson, D.M. Mackay, and B.S. Lollar. Carbon and hydrogen isotopic fractionation during biodegradation of methyl tert-butyl ether. Environmental Science & Technology 36 (9): 1931-1938 (2002).

Hattan, G., B. Wilson, and J.T. Wilson. Performance of Conventional Remedial Technology for Treatment of MTBE and Benzene at UST Sites in Kansas. Remediation: The Journal of Environmental Cleanup Costs, Technologies & Techniques 14(1):85-94. (2003).

Hunkeler, D., B.J. Butler, R. Aravena, and J.F. Barker. Monitoring biodegradation of methyl tert-butyl ether (MTBE) using compound-specific carbon isotope analysis. Environmental Science & Technology 35 (4): 676-681 (2001).

Ingvorsen, K. and B.B. Jørgensen. Kinetics of sulfate uptake by freshwater and marine species of Desulfovibrio. Archives of Microbiology 139 (1): 61-66 (1984).

Interstate Technology & Regulatory Council. Overview of Groundwater Remediation Technologies for MTBE and TBA, by The Interstate Technology & Regulatory Council, MTBE and Other Fuel Oxygenates Team, 2005. available http://www.itrcweb.org/Documents/MTBE-1.pdf

Kane, S.R., H.R. Beller, T.C. Legler, C.J. Koester, H.C. Pinkart, R.U. Halden, and A.M. Happel. Aerobic biodegradation of methyl tert-butyl ether by aquifer bacteria from leaking underground storage tank sites. Applied and Environmental Microbiology. 67 (12): 5824-5829 (2001).

Kolhatkar, R., J.T. Wilson, and L.E. Dunlap. "Evaluating Natural Biodegradation of MTBE at multiple UST sites." In Petroleum and Organic Chemicals in Ground Water: Prevention, Detection and Remediation Conference & Exposition Anaheim, California November 15-17, 2000, 32-49.

Kolhatkar, R., J.T. Wilson, and G. Hinshalwood. "Natural biodegradation of MTBE at a site on Long Island, NY." In Proceedings of the Sixth International In Situ and On-Site Bioremediation Symposium, San Diego, California, June 4-7, 2001, Bioremediation of MTBE, Alcohols, and Ethers 6(1):43-50. Battelle Press, Columbus Ohio (2001).

Kolhatkar, R., T. Kuder, P. Philp, J. Allen, and J.T. Wilson. Use of compound-specific stable carbon isotope analyses to demonstrate anaerobic biodegradation of MTBE in groundwater at a gasoline release site. Environmental Science & Technology 36 (23): 5139-5146 (2002).

Kuder, T., J.T. Wilson, P. Kaiser, R. Kolhatkar, P. Philp, and J. Allen. Enrichment of stable carbon and hydrogen isotopes during anaerobic biodegradation of MTBE: microcosm and field evidence. Environmental Science & Technology 39 (1): 213-220 (2005).

Lovley, D.R., F.H. Chapelle and J.C. Woodward. Use of dissolved H2 concentrations to determine distribution of microbially catalyzed redox reactions in anoxic groundwater. Environmental Science & Technology 28 (7): 1205-1210 (1994).

Meckenstock, R., B. Morasch, C. Griebler, and H.H. Richnow. Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated aquifers. Journal of Contaminant Hydrology 75: 215-255 (2004).

Newell, C.J., R.K. McLeod, and J.R. Gonzales. BIOSCREEN natural attenuation decision support system, EPA/600/R-96/087, Cincinnati, OH: U.S. Environmental Protection Agency, 1996.

O'Sullivan, G., G. Boshoff, A. Downey, and R.M. Kalin. Carbon isotope effect during the abiotic oxidation of methyl-tert-butyl ether (MTBE). In Proceedings of the Seventh International In Situ and On-Site Bioremediation Symposium, Orlando, FL: Battelle Press, Columbus, OH, 2003, I-10.

Parsons Engineering Science. Streamlined Risk-Based Closure of Petroleum Contaminated Sites, Performance and Cost Results from Multiple Air Force Demonstration Sites, Technology Demonstration Summary Report, Air Force Center for Environmental Excellence, Technology Transfer Division, Brooks AFB, San Antonio, Texas. 1999. available http://www.afcee.brooks.af.mil/products/techtrans/riskbased.asp.

Schirmer, M., B.J. Butler, C.D. Church, J.F. Barker, and N. Nadarajah. Laboratory evidence of MTBE biodegradation in Borden aquifer material. Journal of Contaminant Hydrology 60 (2003): 229-249 (2003).

Schmidt, T., M. Schirmer, H. Weiss, and S.B. Haderlein. Microbial degradation of methyl tert-butyl ether and tert-butyl alcohol in the subsurface. Journal of Contaminant Hydrology 70 (2004): 173-203 (2004a).

Schmidt, T.C., L. Zwank, M. Elsner, M. Berg, R.U. Meckenstock, and S.B. Haderlein. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. Analytical and Bioanalytical Chemistry 378:283-300 (2004b).

Shih, T., Y. Rong, T. Harmon, and M. Suffet. Evaluation of the impact of fuel hydrocarbons and oxygenates on groundwater resources. Environmental Science & Technology 38 (1): 42-48 (2004).

Small, M.C. and J. Weaver. An updated conceptual model for subsurface fate and transport of MTBE and benzene. In: Proceedings of the Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection, and Remediation Conference. API, NGWA, STEP Conference and Exposition. November 17-19, Houston, Texas, pages 209-220 (1999).

Smallwood, B.J., R.P. Philp, T.W. Burgoyne, and J.D. Allen. The use of stable isotopes to differentiate specific source markers for MTBE. Environmental Forensics 2 (3): 215-221 (2001).

Somsamak, P., H.H. Richnow and M.H. Häggblom. Carbon isotope fractionation during anaerobic degradation of methyl tert-butyl ether under sulfate-reducing and methanogenic conditions. Applied and Environmental Microbiology 72 (2): 1157-1163 (2006).

Suarez, M.P. and H.S. Rifai. Biodegradation rates for fuel hydrocarbons and chlorinated solvents in groundwater. Bioremediation Journal 3 (4): 337-362 (1999).

Sublette, K., A. Peacock, D. White, G. Davis, D. Ogles, D. Cook, R. Kolhatkar, D. Beckmann, and X. Yang. Monitoring subsurface microbial ecology in a sulfate-amended, gasoline-contaminated aquifer. Ground Water Monitoring & Remediation 26(2):70-78 (2006).

Suflita, J.M., K.L. Londry, and G.A. Ulrich. "Determination of anaerobic biodegradation activity." In Manual of Environmental Microbiology, by C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter, Washington, DC: American Society for Microbiology (ASM) Press, 1997, 790-801.

U. S. Environmental Protection Agency. Use of monitored natural attenuation at superfund, RCRA corrective action, and underground storage tank sites, EPA 540/ R-99/009. EPA Office of Solid Waste and Emergency Response Directive, 9200.4-17P, 1999.

Walter, M.V. "Bioaugmentation." In Manual of Environmental Microbiology, by C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach, and M.V. Walter, Washington, DC: American Society for Microbiology (ASM) Press, 1997, 753-757.

White, K.D., J.T. Novak, C.D. Goldsmith, and S. Bevan. "Microbial degradation kinetics of alcohols in subsurface systems." In: NWWA/API Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water-- Prevention, Detection, and Restoration, November 13-15, 1985, The Westin Galleria, Houston, TX,140-159, National Water Well Association, Dublin, Ohio, 1986.

Wiedemeier, T.H., R.N. Miller, J.T. Wilson, and D.H. Kampbell. "Significance of anaerobic processes for the intrinsic bioremediation of fuel hydrocarbons." In Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection, and Remediation Conference, November 29-December 1, 1995, Houston, TX, 49-61, National Ground Water Association, Dublin, Ohio, 1995.

Wiedemeier, T.H., H.S. Rifai, C.J. Newell, and J.T. Wilson. "Intrinsic Bioremediation of Petroleum Hydrocarbons." In Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface, New York: John Wiley & Sons, 1999a., 189-240.

Wiedemeier, T., J.T. Wilson, D.H. Kampbell, R.N. Miller, and J.E. Hansen. Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Ground Water Volume I, Air Force Center for Environmental Excellence, Technology Transfer Division, Brooks AFB, San Antonio, Texas. (1999b) available http://www.afcee.brooks.af.mil/products/ techtrans/download/A324248.pdf.

Wiedemeier, T., J.T. Wilson, D.H. Kampbell, R.N. Miller, and J.E. Hansen. Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Ground Water Volume II, Air Force Center for Environmental Excellence, Technology Transfer Division, Brooks AFB, San Antonio, Texas. 1999c. available http://www.afcee.brooks.af.mil/products/ techtrans/download/A324247a.pdf. and http://www. afcee.brooks.af.mil/products/techtrans/download/ A324247b.pdf.

Wilson, J.T. "Fate and transport of MTBE and other gasoline components." In MTBE Remediation Handbook, by E.E. Moyer and P.T. Kostecki, Amherst, Massachusetts: Amherst Scientific Publishers, 2003, 19-61.

Wilson, J.T., R. Kolhatkar, T. Kuder, P. Philp, and S.J. Daugherty. Stable isotope analysis of MTBE to evaluate the source of TBA in ground water. Ground Water Monitoring and Remediation 25 (4): 108-116 (2005a).

Wilson, J.T., P.M. Kaiser, and C. Adair. Monitored Natural Attenuation of MTBE as a Risk Management Option at Leaking Underground Storage Tank Sites, EPA-600/ R-04/179. Cincinnati, OH: U.S. Environmental Protection Agency, 2005b

Wilson, J.T., C. Adair, P.M. Kaiser, and R. Kolhatkar. Anaerobic biodegradation of MTBE at a gasoline spill site. Ground Water Monitoring and Remediation 25 (3): 103-115 (2005c).

Wilson, R.D., D.M. Mackay, and K.M. Scow. In situ MTBE degradation supported by diffusive oxygen release. Environmental Science & Technology 36 (2): 190-199 (2002).

Zenker, M.J., R.C. Borden, and M.A. Barlaz. "Investigation of the intrinsic biodegradation of alkyl and cyclic ethers." In In Situ Bioremediation of Petroleum Hydrocarbon and Other Organic Compounds. The Fifth International In Situ and On-Site Bioremediation Symposium, San Diego, CA, 1999: Battelle Press, Columbus, OH, 1999, Volume (1): 165-170.

Zwank, L., M. Berg, M. Elsner, T.C. Schmidt, R.P. Schwarzenbach, and S.B. Haderlein. New evaluation scheme for two-dimensional isotope analysis to decipher biodegradation processes: application to groundwater contamination by MTBE. Environmental Science & Technology 39 (4): 1018-1029 (2005).

# Appendix

**Table 4** part 1. Typical Quality Performance Data for Analysis of TBA in Water. All Values are µg/L Unless Otherwise Indicated.

| Date Collected | 02/01/01 | 04/06/01 | 05/31/01 | 07/06/01 | 09/05/01 |
|---|---|---|---|---|---|
| Date Analyzed | 03/22/01 & 03/26/01 | 06/07-14/01 | 09/27/01, 10/18-23/01 | 08/28-31/01 & 09/19/01 | 11/02-06/01 |
| | | | | | |
| CCC Standard Nominal | 200 | 200 | 200 | 200 | 20.0 |
| CCC Standard Measured | 194 | 188 | 217 | 198 | 19.8 |
| Percent of Check Standard | 97.0% | 94.0% | 109% | 99.0% | 99.0% |
| | | | | | |
| CCC Standard Nominal | 200 | 20.0 | 20.0 | 20.0 | 200 |
| CCC Standard Measured | 177 | 18.0 | 22.4 | 20.5 | 171 |
| Percent of Check Standard | 89.0% | 90.0% | 112% | 103% | 85.5% |
| | | | | | |
| CCC Standard Nominal | 20.0 | 200 | | 200 | 200 |
| CCC Standard Measured | 19.0 | 177 | | 204 | 182 |
| Percent of Check Standard | 95.0% | 88.5% | | 102% | 91.0% |
| | | | | | |
| QC Standard Nominal | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| QC Standard Measured | 17.4 | 16.6 | 18.9 | 21.1 | 19.0 |
| Percent of Check Standard | 87.0% | 83.0% | 94.5% | 106% | 95.0% |
| | | | | | |
| QC Standard Nominal | 200 | 200 | 200 | 200 | 200 |
| QC Standard Measured | 194 | 190 | 189 | 209 | 210 |
| Percent of Check Standard | 97.0% | 95.0% | 94.5% | 105% | 105% |
| | | | | | |
| Blank 1 | <10* | <10* | <10* | <10* | <10* |
| Blank 2 | | <10* | <10* | <10* | <10* |
| | | | | | |
| Sample Analysis | 31.2 | 34.2 | | 20.8 | 91.6 |
| Laboratory Duplicate | 33.8 | 33.4 | | 19.8 | 118 |
| Relative Percent Difference | 8.00% | 2.37% | | 4.93% | 25.2% |
| | | | | | |
| Spike Concentration | NP | NP | | NP | 200 |
| Sample Concentration | 112 | <1.00 | | 17.1 | 31.4 |
| Spike Recovery (Percent) | 83.0% | 89.0% | | 100% | 99.0% |

CCC: Continuing Calibration Check; QC: Second Source Check; NP: Not Provided
* Lower Calibration Limit

**Table 4** part 2. Typical Quality Performance Data for Analysis of TBA in Water. All Values are µg /L Unless Otherwise Indicated.

| **Date Collected** | **10/11/01** | **10/25/01** | **11/16/01** | **12/05/01** | **01/04/02** |
|---|---|---|---|---|---|
| Date Analyzed | 11/28-30/01 | 11/30/01-12/04/01 | 11/26 - 27/01 | 01/04-09/02 | 01/18/02 |
| | | | | | |
| CCC Standard Nozminal | 20.0 | 200 | 200 | 200 | 200 |
| CCC Standard Measured | 19.7 | 226 | 241 | 219 | 213 |
| Percent of Check Standard | 98.5% | 113% | 121% | 110% | 107% |
| | | | | | |
| CCC Standard Nominal | 20.0 | 20.0 | | 20.0 | 20.0 |
| CCC Standard Measured | 19.5 | 24.3 | | 21.5 | 24.0 |
| Percent of Check Standard | 97.5% | 122% | | 108% | 120% |
| | | | | | |
| CCC Standard Nominal | 200 | | | 20.0 | 200 |
| CCC Standard Measured | 194 | | | 20.9 | 201 |
| Percent of Check Standard | 97.0% | | | 105% | 101% |
| | | | | | |
| QC Standard Nominal | 200 | 200 | 200 | 20.0 | 20.0 |
| QC Standard Measured | 219 | 237 | 252 | 20.3 | 22.2 |
| Percent of Check Standard | 110% | 119% | 126% | 102% | 111% |
| | | | | | |
| QC Standard Nominal | | | | 200 | |
| QC Standard Measured | | | | 198 | |
| Percent of Check Standard | | | | 99.0% | |
| | | | | | |
| Blank 1 | <10* | <10* | <20* | <10* | <10* |
| Blank 2 | <10* | <10* | | <10* | <10* |
| | | | | | |
| Sample Analysis | <20.0 | 55.2 | 113 | | 57.9 |
| Laboratory Duplicate | <20.0 | 53.6 | 110 | | 67.3 |
| Relative Percent Difference | 0.00% | 2.94% | 3.05% | | 15.0% |
| | | | | | |
| Spike Concentration | NP | NP | NP | | NP |
| Sample Concentration | <20.0 | 42.4 | 71.8 | | 53.9 |
| Spike Recovery (Percent) | 97.0% | 126% | 121% | | 104% |

CCC: Continuing Calibration Check; QC: Second Source Check; NP: Not Provided

**Table 4** part 3. Typical Quality Performance Data for Analysis of TBA in Water. All Values are µg /L Unless Otherwise Indicated.

| **Date Collected** | **1/15/02** | **2/12/02** | **3/12/02** | **4/16/02** | **4/25/02** |
|---|---|---|---|---|---|
| Date Analyzed | 1/26/02 | 3/6/02 | 4/8/02 | 5/7/02 | 4/25-26/02 |
| | | | | | |
| CCC Standard Nominal | 100 | 20.0 | 200 | 200 | 200 |
| CCC Standard Measured | 88.7 | 19.3 | 189 | 213 | 223 |
| Percent of Check Standard | 88.7% | 96.5% | 94.5% | 107% | 112% |
| | | | | | |
| CCC Standard Nominal | 200 | 100 | 200 | 20.0 | 20.0 |

| Date Collected | 1/15/02 | 2/12/02 | 3/12/02 | 4/16/02 | 4/25/02 |
|---|---|---|---|---|---|
| CCC Standard Measured | 160 | 93.7 | 200 | 21.9 | 22.5 |
| Percent of Check Standard | 80.0% | 93.7% | 100% | 110% | 113% |
| | | | | | |
| CCC Standard Nominal | 20.0 | 100 | 100 | 200 | |
| CCC Standard Measured | 17.9 | 93.3 | 116 | 205 | |
| Percent of Check Standard | 89.5% | 93.3% | 116% | 103% | |
| | | | | | |
| QC Standard Nominal | 200 | 200 | 20.0 | 100 | 200 |
| QC Standard Measured | 162 | 208 | 17.5 | 117 | 239 |
| Percent of Check Standard | 81.0% | 104% | 87.5% | 117% | 120% |
| | | | | | |
| QC Standard Nominal | 100 | 20.0 | | 1000 | |
| QC Standard Measured | 84.9 | 19.1 | | 962 | |
| Percent of Check Standard | 84.9% | 95.5% | | 96.2% | |
| | | | | | |
| Blank 1 | <10* | <2.4** | <2.4** | <2.4** | <2.4** |
| Blank 2 | <10* | <2.4** | <2.4** | <2.4** | <2.4** |
| | | | | | |
| Sample Analysis | 19.8 | <2.40 | <2.40 | 88.1 | 118 |
| Laboratory Duplicate | 20.3 | <2.40 | <2.40 | 90.0 | 105 |
| Relative Percent Difference | 2.49% | 0.00% | 0.00% | 2.13% | 11.7% |
| | | | | | |
| Spike Concentration | 92.6 | 98.8 | 94.9 | 114 | 290 |
| Sample Concentration | 4.20 | <2.40 | <2.40 | <2.40 | 276 |
| Spike Recovery (Percent) | 88.0% | 98.8% | 94.9% | 114% | 109% |

CCC: Continuing Calibration Check; QC: Second Source Check

**Table 4** part 4. Typical Quality Performance Data for Analysis of TBA in Water. All Values are µg /L Unless Otherwise Indicated.

| Date Collected | 5/29/02 | 7/16/02 | 12/04/02 | 12/11/02 | 5/15/03 |
|---|---|---|---|---|---|
| Date Analyzed | 6/6/02 to | 8/7/02 | 12/12/02 | 12/18/02 | 5/16/03 |
| | | | | | |
| CCC Standard Nominal | 200 | 200 | | 100 | 200 |
| CCC Standard Measured | 225 | 240 | | 112 | 200 |
| Percent of Check Standard | 113% | 120% | | 112% | 100% |
| | | | | | |
| CCC Standard Nominal | | 100 | | 100 | 20.0 |
| CCC Standard Measured | | 116 | | 101 | 22.5 |
| Percent of Check Standard | | 116% | | 101% | 113% |
| | | | | | |
| CCC Standard Nominal | | 100 | | 50.0 | |
| CCC Standard Measured | | 106 | | 54.5 | |
| Percent of Check Standard | | 106% | | 109% | |
| | | | | | |
| QC Standard Nominal | 20.0 | 200 | 20.0 | 100 | 100 |
| QC Standard Measured | 20.8 | 224 | 20.9 | 92.5 | 98.6 |
| Percent of Check Standard | 104% | 112% | 105% | 93.0% | 98.6% |

| Date Collected | 5/29/02 | 7/16/02 | 12/04/02 | 12/11/02 | 5/15/03 |
|---|---|---|---|---|---|
| QC Standard Nominal | 200 | 100 | 200 | 50.0 | 200 |
| QC Standard Measured | 220 | 102 | 238 | 49.4 | 200 |
| Percent of Check Standard | 110% | 102% | 119% | 99.0% | 100% |
| | | | | | |
| Blank 1 | <2.4** | <2.4** | <2.4** | <2.4** | <2.4** |
| Blank 2 | <2.4** | <2.4** | <2.4** | <2.4** | <2.4** |
| | | | | | |
| Sample Analysis | 38.8 | <2.40 | 259 | 39.5 | 270 |
| Laboratory Duplicate | 52.2 | <2.40 | 283 | 43.5 | 270 |
| Relative Percent Difference | 29.5% | 0.00% | 8.90% | 9.64% | 0.00% |
| | | | | | |
| Spike Concentration | 200 | 109 | 256 | | |
| Sample Concentration | 40.4 | <2.40 | 251 | | |
| Spike Recovery (Percent) | 94.0% | 109% | 103% | | |

CCC: Continuing Calibration Check; QC: Second Source Check

**Table 5** part 1. Typical Quality Performance Data for Analysis of Sulfate in Water. All Values are mg/L Unless Otherwise Indicated.

| Date Collected | 01/05/01 | 5/31/01 | 5/31/01 | 6/28/01 | 07/06/01 |
|---|---|---|---|---|---|
| Date Analyzed | 4/15-26/01 | 11/8-12/01 | 10/3-4/01 | 10/4-5/01 | 9/26-27/01 |
| | | | | | |
| CCC Standard Nominal | 29.8 | 25.0 | 28.3 | 28.3 | 28.3 |
| CCC Standard Measured | 30.8 | 25.5 | 28.1 | 27.1 | 28.2 |
| Percent of Check Standard | 103% | 102% | 99.3% | 95.8% | 99.6% |
| | | | | | |
| CCC Standard Nominal | 1.00 | 5.00 | 5.00 | 5.00 | 1.00 |
| CCC Standard Measured | 1.08 | 4.87 | 4.93 | 4.67 | 0.95 |
| Percent of Check Standard | 108% | 97.4% | 98.6% | 93.4% | 95.0% |
| | | | | | |
| CCC Standard Nominal | 25.0 | 25.0 | 5.00 | 5.00 | 50.0 |
| CCC Standard Measured | 25.2 | 26.4 | 4.93 | 4.78 | 47.9 |
| Percent of Check Standard | 101% | 106% | 98.6% | 95.6% | 95.8% |
| | | | | | |
| QC Standard Nominal | 29.8 | 5.00 | 5.00 | 5.00 | 28.3 |
| QC Standard Measured | 29.5 | 5.24 | 4.95 | 4.92 | 29.0 |
| Percent of Check Standard | 99.0% | 105% | 99.0% | 98.4% | 103% |
| | | | | | |
| QC Standard Nominal | 5.00 | 25.0 | 28.3 | 5.00 | 50.0 |
| QC Standard Measured | 5.25 | 27.4 | 28.1 | 5.45 | 48.3 |
| Percent of Check Standard | 105% | 110% | 99.3% | 109% | 96.6% |
| | | | | | |
| Blank 1 | <0.50 | <1.00 | <1.00 | <1.00 | <1.00 |
| Blank 2 | <0.50 | | | | <1.00 |
| | | | | | |
| Sample Analysis | 2.85 | 16.0 | 2.82 | 2.50 | 2.04 |
| Laboratory Duplicate | 2.82 | 15.9 | 2.84 | 2.53 | 2.01 |
| Relative Percent Difference | 1.06% | 0.63% | 0.71% | 1.19% | 1.48% |
| | | | | | |
| Spike Concentration | NP | NP | NP | NP | 50.0 |

| Date Collected | 01/05/01 | 5/31/01 | 5/31/01 | 6/28/01 | 07/06/01 |
|---|---|---|---|---|---|
| Sample Concentration | 3.49 | 12.8 | 2.77 | 2.39 | 2.28 |
| Spike Recovery (Percent) | 100% | 101% | 95.0% | 96.0% | 98.6% |

CCC: Continuing Calibration Check; QC: Second Source Check; NP: Not Provided

**Table 5** part 2. Typical Quality Performance Data for Analysis of Sulfate in Water. All Values are mg/L Unless Otherwise Indicated.

| Date Collected | 7/26/01 | 9/05/01 | 10/11/01 | 10/25/01 | 12/05/01 |
|---|---|---|---|---|---|
| Date Analyzed | 10/10/01 | 12/12-14/01 | 10/25/01 | 11/9-12/01 | 1/7/02 |
| | | | | | |
| CCC Standard Nominal | 28.3 | 9.87 | 28.3 | 28.3 | 9.87 |
| CCC Standard Measured | 28.2 | 9.09 | 27.6 | 28.6 | 9.32 |
| Percent of Check Standard | 99.6% | 92.1% | 97.5% | 101% | 94.4% |
| | | | | | |
| CCC Standard Nominal | 5.00 | 25.0 | 25.0 | 5.00 | 25.0 |
| CCC Standard Measured | 4.83 | 24.9 | 24.8 | 5.18 | 24.4 |
| Percent of Check Standard | 96.6% | 99.6% | 99.2% | 104% | 97.6% |
| | | | | | |
| CCC Standard Nominal | 5.00 | 5.00 | 25.0 | 25.0 | 5.00 |
| CCC Standard Measured | 5.00 | 4.97 | 24.9 | 25.8 | 4.93 |
| Percent of Check Standard | 100% | 99.4% | 99.6% | 103% | 98.6% |
| | | | | | |
| QC Standard Nominal | 5.00 | 25.0 | 5.00 | 5.00 | 25.0 |
| QC Standard Measured | 5.31 | 24.8 | 4.85 | 5.24 | 25.1 |
| Percent of Check Standard | 106% | 99.2% | 97.0% | 105% | 100% |
| | | | | | |
| QC Standard Nominal | 5.00 | 5.00 | 25.0 | 25.0 | 5.00 |
| QC Standard Measured | 5.27 | 5.11 | 25.9 | 26.1 | 5.01 |
| Percent of Check Standard | 105% | 102% | 104% | 104% | 100% |
| | | | | | |
| Blank 1 | <1.00 | <1.00 | <1.00 | <1.00 | <1.00 |
| Blank 2 | | | | | |
| | | | | | |
| Sample Analysis | 2.70 | 3.25 | 2.00 | 2.69 | 4.49 |
| Laboratory Duplicate | 2.75 | 3.34 | 1.99 | 2.61 | 4.49 |
| Relative Percent Difference | 1.83% | 2.73% | 0.50% | 3.02% | 0.00% |
| | | | | | |
| Spike Concentration | NP | NP | NP | NP | NP |
| Sample Concentration | 2.81 | 4.97 | 1.99 | 2.78 | 2.27 |
| Spike Recovery (Percent) | 98.0% | 102% | 95.0% | 97.0% | 94.0% |

CCC: Continuing Calibration Check; QC: Second Source Check; NP: Not Provided

**Table 5** part 3. Typical Quality Performance Data for Analysis of Sulfate in Water. All Values are mg/L Unless Otherwise Indicated.

| Date Collected | 01/04/02 | 1/15/02 | 02/07/02 | 2/12/02 | 3/12/02 |
|---|---|---|---|---|---|
| Date Analyzed | 02/06/02 | 2/4-5/02 | 03/01/02 | 3/2/02 | 4/11/02 |
| | | | | | |
| CCC Standard Nominal | 9.87 | 9.87 | 9.87 | 9.87 | 9.87 |
| CCC Standard Measured | 9.18 | 8.91 | 8.95 | 8.32 | 9.24 |

| Date Collected | 01/04/02 | 1/15/02 | 02/07/02 | 2/12/02 | 3/12/02 |
|---|---|---|---|---|---|
| Percent of Check Standard | 93.0% | 90.3% | 90.7% | 84.3% | 93.6% |
| | | | | | |
| CCC Standard Nominal | 25.0 | 25.0 | | 25.0 | 25.0 |
| CCC Standard Measured | 24.5 | 25.1 | | 25.1 | 25.3 |
| Percent of Check Standard | 98.0% | 100% | | 100% | 101% |
| | | | | | |
| CCC Standard Nominal | 5.00 | 5.00 | | 5.00 | 5.00 |
| CCC Standard Measured | 4.84 | 4.83 | | 5.00 | 5.04 |
| Percent of Check Standard | 96.8% | 96.6% | | 100% | 101% |
| | | | | | |
| QC Standard Nominal | 25.0 | 25.0 | 5.00 | 25.0 | 25.0 |
| QC Standard Measured | 25.2 | 25.4 | 5.14 | 25.4 | 26.7 |
| Percent of Check Standard | 101% | 102% | 103% | 102% | 107% |
| | | | | | |
| QC Standard Nominal | 5.00 | 5.00 | 25.0 | 5.00 | 5.00 |
| QC Standard Measured | 5.05 | 5.16 | 25.8 | 5.25 | 5.20 |
| Percent of Check Standard | 101% | 103% | 103% | 105% | 104% |
| | | | | | |
| Blank 1 | <1.00 | <1.00 | <1.00 | <1.00 | <1.00 |
| Blank 2 | | | | | |
| | | | | | |
| Sample Analysis | 2.65 | 12.4 | 6.60 | 10.6 | 8.16 |
| Laboratory Duplicate | 2.59 | 12.3 | 6.64 | 10.7 | 8.15 |
| Relative Percent Difference | 2.29% | 0.81% | 0.60% | 0.94% | 1.23% |
| | | | | | |
| Spike Concentration | 3.58 | 3.67 | 13.4 | 16.8 | 8.49 |
| Sample Concentration | 2.54 | 2.74 | 1.16 | 9.16 | 12.1 |
| Spike Recovery (Percent) | 92.0% | 92.0% | 103% | 98.0% | 98.0% |

CCC: Continuing Calibration Check; QC: Second Source Check

**Table 5** part 4. Typical Quality Performance Data for Analysis of Sulfate in Water. All Values are mg/L Unless Otherwise Indicated.

| Date Collected | 4/16/02 | 4/25/02 | 5/29/02 | 7/16/02 |
|---|---|---|---|---|
| Date Analyzed | 5/14-15/02 | 5/23/02 | 6/25/02 | 8/15/02 |
| | | | | |
| CCC Standard Nominal | 9.87 | 18.6 | 18.6 | 18.6 |
| CCC Standard Measured | 9.04 | 17.0 | 17.6 | 17.9 |
| Percent of Check Standard | 91.6% | 91.4% | 94.6% | 96.2% |
| | | | | |
| CCC Standard Nominal | 25.0 | 25.0 | 25.0 | 5.00 |
| CCC Standard Measured | 25.0 | 24.6 | 24.7 | 5.09 |
| Percent of Check Standard | 100% | 98.4% | 98.8% | 102% |
| | | | | |
| CCC Standard Nominal | 5.00 | 5.00 | 5.00 | 25.0 |
| CCC Standard Measured | 4.97 | 4.91 | 4.85 | 25.7 |
| Percent of Check Standard | 99.4% | 98.2% | 97.0% | 103% |
| | | | | |
| QC Standard Nominal | 25.0 | 25.0 | 25.0 | 5.00 |
| QC Standard Measured | 25.7 | 25.8 | 24.5 | 5.04 |

| Date Collected | 4/16/02 | 4/25/02 | 5/29/02 | 7/16/02 |
|---|---|---|---|---|
| Percent of Check Standard | 103% | 103% | 98.0% | 101% |
| | | | | |
| QC Standard Nominal | 5.00 | 5.00 | 5.00 | 25.0 |
| QC Standard Measured | 5.05 | 4.91 | 4.91 | 25.5 |
| Percent of Check Standard | 101% | 98.2% | 98.2% | 102% |
| | | | | |
| Blank 1 | <1.00 | <1.00 | <0.33 | <1.00 |
| Blank 2 | | | | |
| | | | | |
| Sample Analysis | 10.7 | 0.53 | 9.78 | 13.4 |
| Laboratory Duplicate | 10.7 | 0.51 | 9.78 | 13.5 |
| Relative Percent Difference | 0.00% | 3.85% | 0.00% | 0.74% |
| | | | | |
| Spike Concentration | 18.1 | 11.5 | 11.8 | 18.7 |
| Sample Concentration | 10.5 | 0.53 | <0.331 | 12.1 |
| Spike Recovery (Percent) | 103% | 90.0% | 94.0% | 101% |

United States
Environmental Protection
Agency

Please make all necessary changes on the below label, detach or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE ☐; detach, or copy this cover, and return to the address in the upper left-hand corner.

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

National Risk Management
Research Laboratory
Cincinnati, OH 45268

Official Business
Penalty for Private Use
$300

EPA/600/R-07/100
October 2007

Recycled/Recyclable
Printed with vegetable-based ink on paper that contains a minimum of 50% post-consumer fiber content processed chlorine free