Using Compound Specific Isotope Analysis (CSIA) in Groundwater Assessments

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A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)

Office of Research and Development
National Risk Management Research Laboratory, Ada, Oklahoma 74820

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Notice

The International Atomic Energy Agency, and the U.S. Environmental Protection Agency through its Office of Research and Development, funded and managed the development of this Guide. It has been subjected to U.S. Environmental Protection Agency peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

All research projects making conclusions or recommendations based on environmental data and funded by the U.S. Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project did not involve the collection and use of environmental data and, as such, did not require a Quality Assurance Plan.

Front Cover photos:

#1 an isotope ratio mass spectrometer

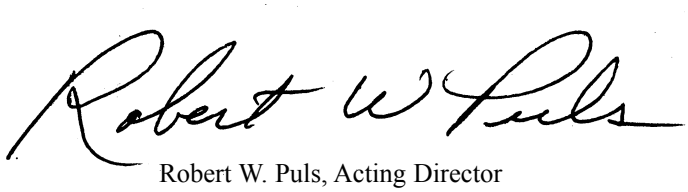
#2 purging a well to provide a sample of ground water for analysis

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.

As part of the U.S. EPA Quality System for Environmental Data and Technology, U.S. EPA requires that a Quality Assurance Project Plan (QAPP) be developed and approved before environmental samples are collected and analyzed. Compound Specific Isotope Analysis (CSIA) has only recently been applied to understand the degradation of organic compounds, or to identify the sources of ground water contamination at hazardous waste sites. As a result, there is little information available that can be used to develop, review, or approve a QAPP for the application of CSIA at a hazardous waste site. This Guide provides general recommendations on good practice for sampling ground water for CSIA, and quality assurance recommendations for measurement of isotope ratios. The Guide also provides recommendations for data evaluation and interpretation to use CSIA to document degradation of organic contaminants, or to associate plumes of contaminants in ground water with their sources.



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Acronyms

Bio-Sep – trade name for adsorptive particles used as substratum for microbial growth
BTEX – benzene, toluene, ethylbenzene, xylene
CSIA – compound specific isotope analysis
DCA – dichloroethylene
DCE – dichlorethene
$\delta^{13}C$ – delta ^{13}C [‰], carbon stable isotope ratio
DIC – dissolved inorganic carbon
DOC – dissolved organic carbon
DNAPL – dense non aqueous phase liquid
EA-IRMS – elemental analyser - isotope ratio mass spectrometer
ETBE – ethyl tertiary butyl ether
GC-IRMS – gas chromatograph - isotope ratio mass spectrometer
GC/MS – gas chromatography / mass spectrometry
H, C, O, N, Cl – hydrogen, carbon, oxygen, nitrogen, chlorine
IRMS – isotope ratio mass spectrometry
IUPAC – International Union of Pure and Applied Chemistry
LNAPL – light non aqueous phase liquid
MDL – method detection limits
MNA – monitored natural attenuation
MTBE – methyl tertiary butyl ether
NA – natural attenuation
NAPL – non aqueous phase liquid
PAH – polycyclic aromatic hydrocarbons
PCE – perchloroethylene
P&T – purge and trap
QA/QC – Quality Assurance/Quality Control
SIP - stable isotope probing
SPME – solid phase micro extraction
TAME – tertiary amyl methyl ether
TBA – tertiary butyl alcohol
TCE – trichloroethylene
TSP – trisodium phosphate dodecahydrate
U.S. EPA – United States Environmental Protection Agency
VC – vinyl chloride
VOA – volatile organic analyses
VOC – volatile organic compound
V-PDB – Vienna - PeeDee Belemnite reference standard
V-SMOW – Vienna - Standard Mean Ocean Water reference standard

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Executive Summary

Managing the risk associated with hazardous organic compounds in ground water at hazardous waste sites often requires detailed knowledge of the extent of degradation of the organic contaminants at the site. An evaluation of the contribution of natural biodegradation or abiotic transformation processes in ground water is usually crucial to the selection of Monitored Natural Attenuation (MNA) as a remedy for a site. Documentation that the organic contaminant is actually being degraded is important for performance monitoring of MNA, performance monitoring of active in situ bioremediation, and performance monitoring of many other active remedial technologies.

The traditional approach of monitoring a reduction in the concentrations of contaminants at sites often does not offer compelling documentation that the contaminants are actually being degraded. When data on concentrations are the only data available, it is difficult or impossible to exclude the possibility that the reduction in contaminant concentrations are caused by some other process such as dilution or dispersion, or that the monitoring wells failed to adequately sample the plume of contaminated ground water. Stable isotope analyses can provide unequivocal documentation that biodegradation or abiotic transformation processes actually destroyed the contaminant.

When organic contaminants are degraded in the environment, the ratio of stable isotopes will often change, and the extent of degradation can be recognized and predicted from the change in the ratio of stable isotopes. Recent advances in analytical chemistry make it possible to perform Compound Specific Isotope Analysis (CSIA) on dissolved organic contaminants such as chlorinated solvents, aromatic petroleum hydrocarbons, and fuel oxygenates, at concentrations in water that are near their regulatory standards.

At many hazardous waste sites, progress toward cleanup of contamination in ground water depends on successful identification of the true source of the contamination. Often, the ratio of stable isotopes in materials in commerce will vary, depending on the isotope ratio in the feed stock used for synthesis of the material, and on the particular chemical process used to manufacture the material. Different spills of the same material may have different isotopic "signatures" that can be used to associate a plume of contamination in ground water with a particular spill.

Because CSIA is a new approach, there are no widely accepted standards for accuracy, precision and sensitivity, and no established approaches to document accuracy, precision, sensitivity and representativeness. This Guide provides general recommendations on good practice for sampling ground water for CSIA, and quality assurance recommendations for measurement of isotope ratios. The Guide also provides recommendations for data evaluation and interpretation to use CSIA to document degradation of organic contaminants, or to associate plumes of contaminants in ground water with their sources.

This Guide is intended for managers of hazardous waste sites who must design sampling plans that will include CSIA and specify data quality objectives for CSIA analyses, for analytical chemists who must carry out the analyses, and for staff of regulatory agencies who must review and approve the sampling plans and data quality objectives, and who must review the data provided from the analyses.

1.0 Introduction

The atoms of a particular element must have the same number of protons and electrons, but they can have different numbers of neutrons. When atoms differ only in the number of neutrons, they are referred to as isotopes of each other. If a particular isotope is not radioactive, it is called a stable isotope. Because they differ in the number of neutrons, isotopes differ in mass, and they can be separated using a mass spectrometer. In recent years mass spectrometers have been joined to gas chromatographs to allow separation of individual organic compounds in a mixture, followed by combustion of each separate organic compound to carbon dioxide, and then determination of the ratio of isotopes in the carbon dioxide with a mass spectrometer. Even more recently, new techniques of sample preparation, such as purge and trap or solid phase micro-extraction, have made it possible to obtain adequate material for analyses from water with low concentrations of organic contaminants. For the first time, it is possible to perform Compound Specific Isotope Analysis (CSIA) on dissolved organic contaminants such as chlorinated solvents, aromatic petroleum hydrocarbons, and fuel oxygenates, at concentrations in water that are near their regulatory standards.

Biodegradation can come about through natural biological processes, or through active in situ bioremediation. When organic contaminants are degraded in the environment, the ratio of stable isotopes will often change, and the extent of degradation can be recognized and predicted from the change in the ratio of stable isotopes; CSIA has great promise to improve our understanding of the behavior of organic contaminants at hazardous waste sites. Better understanding can lead to better decisions on the remedies that are selected. CSIA can also be used to monitor the progress of natural attenuation or active biological remediation, and identify remedies that are not performing as expected.

The U.S. Environmental Protection Agency requires that data quality objectives be developed for the methods and procedures that are used to characterize hazardous waste sites. The U.S. EPA also requires that the data that are used to make decisions must meet predetermined goals for data quality, including the accuracy, precision, and sensitivity of the measurement, and the extent to which the sample submitted for analysis are representative of the environmental medium being sampled. Other regulatory agencies world-wide have similar expectations. Because CSIA is a new approach in environmental investigations, there are no widely accepted standards for accuracy, precision and sensitivity, and no established approaches to document accuracy, precision, sensitivity and representativeness.

This Guide is intended for managers of hazardous waste sites who must design sampling plans that will include CSIA and specify data quality objectives for CSIA analyses, for analytical chemists who must carry out the analyses, and for staff of regulatory agencies who must review and approve the sampling plans and data quality objectives, and who must review the data provided from the analyses. This Guide provides recommendations and suggestions to site managers, chemists and regulators. The recommendations and suggestions in this Guide are not legal guidance, and the site managers, chemists, and regulators may negotiate among themselves to develop objectives and approaches that are most appropriate for their site.

This Section describes the benefits and value of data provided by CSIA, and contrasts the information provided by CSIA to information provided by long-term monitoring of concentrations of contaminants, or information provided from techniques where specific stable isotopes are added to environmental samples.

Site investigations of soil and ground water contamination are carried out at industrial installations, at sites with leaking underground storage tanks, or at sites with accidental spills (Wiedemeier, et al., 1999). The goal of these investigations may include an evaluation of the *responsibility* for a release (environmental forensics) as well as an evaluation of the *necessity* for remedial actions.

Investigations to evaluate the responsibility for a release consider the timing of a release, the exact location of the source or sources, and the association of pollution in ground water with a particular source (Morrison, 2000). Although CSIA is an established approach in other areas of forensics such as the authenticity and purity of food stuffs and the control of doping in athletics (Aguilera et al., 2002; Asche, 2003; Rossmann, 2001) the application of CSIA in environmental forensics is

a recent development (Asche, 2003; Schmidt et al., 2004; Slater, 2003). CSIA has been used successfully at a variety of sites to distinguish between contaminant releases which occurred at different times and places at complex spill sites. This knowledge can be used to identify the parties that were responsible for the contamination (Hunkeler et al., 2004; Stark et al., 2003; Walker, et al., 2005) and CSIA has been accepted as one line of evidence in litigation.

To determine the need for active remediation, it is useful to have a good knowledge of the behavior of the contaminants in soil and ground water, including the extent of biodegradation and abiotic transformation. This is especially important for passive remedies such as Monitored Natural Attenuation that use naturally occurring processes to attenuate concentrations of contaminants (Wiedemeier et al., 1999).

Although natural attenuation has been the focus of many remediation investigations due to its expected economic benefits, it is often difficult to unequivocally prove that a contaminant is being transformed in ground water and that the extent of attenuation is sufficient to protect receptors that are down gradient of the source. The standard approach that is usually taken to characterize degradation in the field is to monitor the concentrations of the contaminant at selected wells and use mass balance calculations to estimate the extent of degradation. This approach has many shortcomings, and the shortcomings are particularly severe for common ground water pollutants that degrade slowly. The conventional approach requires a dense network of monitoring wells, monitoring that extends for long periods of time, and a rather homogeneous aquifer with well-understood hydrogeology. These requirements are rarely met at real sites, and even when they are, the evidence of degradation is only provided indirectly through a calculation of the missing mass of the contaminant after accounting for all the other processes that might reduce the concentration of the contaminant. These shortcomings have been nicely illustrated in a study of the natural biodegradation of methyl tertiary butyl ether in ground water at the Borden site in Canada (Schirmer and Barker, 1998).

New and different approaches will be required to gain wider acceptance of natural attenuation by regulatory authorities and by the public. If biodegradation or abiotic transformation produces a measurable change in the ratio of stable isotopes in the contaminant, CSIA may provide direct evidence of the degradation of the contaminant in ground water at the site (Hunkeler et al., 1999; Meckenstock et al., 1999; Sherwood Lollar et al., 1999). Over the past decade there have been numerous successful applications of CSIA that have demonstrated its potential to recognize and even quantify processes at field scale.

CSIA offers a new kind of information that has great economic value to site managers. The traditional approach for monitoring of concentrations of contaminants at sites often does not offer adequate information about the processes that are responsible for removal of the contaminants. Stable isotope analyses can provide an in-depth understanding of biodegradation or abiotic transformation processes in contaminated aquifers. This better understanding can improve the conceptual model of the site, which can lead to a more effective remedial strategy. The traditional approach of monitoring concentrations of contaminants can be very costly in the long run. The inclusion of CSIA in the monitoring plan can reduce overall costs by making it possible to reduce the amount of traditional monitoring.

Prior to the development of CSIA, isotope techniques relied on changes in the carbon isotope ratios of CO_2 or DIC (dissolved inorganic carbon) to evaluate the degradation of organic contaminants (Hunkeler et al., 1999). Although this earlier approach can be helpful, it was often difficult to resolve the signal of the carbon that was added to the pool of CO_2 or DIC by degradation of the contaminant from the influence of the many other carbon sources and sinks in the subsurface. Furthermore, the sensitivity of the comparison was dependent on the difference in the composition of carbon isotopes in the CO_2 produced by biodegradation of the contaminants compared to the isotope composition of the background CO_2. In addition, the sensitivity of the older technique was often limited by the slow rate of CO_2 production from degradation of the contaminant relative to the large pool of DIC in ground water (Dempster et al., 1997 and references therein). In contrast to the earlier techniques, CSIA provides a direct measurement of the isotope ratio in the individual organic contaminants. Interpretations of CSIA data are much less problematic.

There are several new techniques to study biodegradation in ground water that involve the addition of contaminants that are artificially labeled with a carbon isotope (usually ^{13}C-label). Examples include stable isotope probing (SIP) and Bio-Sep® beads amended with ^{13}C-labeled substrates. These techniques work in much the same way as radiocarbon labeling; the ^{13}C-label is used to track the transfer

of carbon from the substrate to its metabolites, or to the DIC pool, and its subsequent incorporation into the microbial biomass (Geyer et al., 2005; Stelzer et al., 2006). The disappearance of the label from the substrate pool is convincing evidence that the targeted compound is indeed degrading, and the identification of ^{13}C-label in microbial biomass is definitive proof that the compound was biologically degraded. There is an important caveat with these new techniques. Once a substrate with an isotope label has been added to a field site or to microcosms, the natural abundance of isotopes has been disturbed to an unpredictable extent, and a fundamental assumption in the CSIA approach is no longer valid. It is important to choose one approach or the other; they can not be used together.

To date, CSIA is most frequently applied to carbon isotopes, and CSIA for carbon isotopes can be considered to be a mature technique applicable on a routine basis for compounds containing less than ten carbon atoms. With current technology, the heaviest compounds that can be analysed for shifts in the ratio of stable carbon isotopes contain twelve to thirteen carbon atoms. In larger molecules, the isotope shifts are in the range of the experimental error of the isotope analysis (Morasch et al., 2004).

Although very promising, isotopic analysis of the other elements currently amenable to CSIA (hydrogen, oxygen, nitrogen and chlorine), has not been carried out to the same extent as CSIA for carbon isotopes; however, the other elements may become widely used (Berg et al., 2007; Hofstetter et al., 2008; Holmstrand et al., 2006; Sessions, 2006).

This guide is focused on biodegradation of organic contaminants in ground water because biodegradation represents the majority of applications to date. Nevertheless, the general principles of CSIA also apply to abiotic transformation reactions. They can be applied to natural materials or to engineered systems such as permeable reactive barriers. This guide can be used for a wide range of applications where reactive processes in ground water produce a change in the ratio of stable isotopes.

Currently, CSIA is in transition from a research tool to an applied method that is well integrated into comprehensive plans for management of contaminates sites. For this reason, the authors felt that it was timely to provide general guidance on good practice for sampling, for measurement, for data evaluation and for interpretation in CSIA based on our experience in research and consulting.

2.0
Data Quality Issues

Section 2 has primary application for analysts that will analyze samples for CSIA. It explains and defines the delta notation ($\delta^{13}C$ and $\delta^{2}H$) that is used to report the stable isotope ratio in a sample. This section explains the nature and source of the reference standards for stable isotope ratios of carbon and hydrogen in organic compounds, provides recommendations on the preparation of laboratory working standards, and the use of working standards to document accuracy, precision, and sensitivity of CSIA. It also explains the relationship between the linear range of the continuous flow isotope ratio mass spectrometer and the uncertainty of the determination of $\delta^{13}C$, identifies a threshold in signal strength below which the uncertainty of the determination of $\delta^{13}C$ is not stable, and recommends that the threshold be used as an operational method detection limit for determination of $\delta^{13}C$. Section 2 provides recommendations for the frequency of analysis of CO_2 working standards, compound specific working standards, and sample replicates. Finally this section reviews the sensitivity provided by various methods for preparation of the samples for analysis, and the effect of different methods that can be used to prepare the sample on the value of $\delta^{13}C$ that is determined by the isotope ratio mass spectrometer.

2.1. CSIA Principles

Compound specific isotope analysis (CSIA) involves a three step process, using a set of instrumentation typically referred to as GC-IRMS (gas chromatograph isotope ratio mass spectrometer): (1) separation of individual carbon-bearing compounds on a gas chromatograph, (2) quantitative conversion of each compound to CO_2 in a high temperature combustion oven, and (3) removal of H_2O produced in combustion and introduction of the CO_2 derived from each compound into the mass spectrometer for isotopic analysis (Figure 2.1). After ionization of CO_2, the mass spectrometer separates ions with different mass-to-charge ratios in space, allowing the simultaneous measurement of the ions with fixed Faraday cups. The high precision required in CSIA at the natural abundance level of stable isotopes can be achieved only with this simultaneous ion measurement.

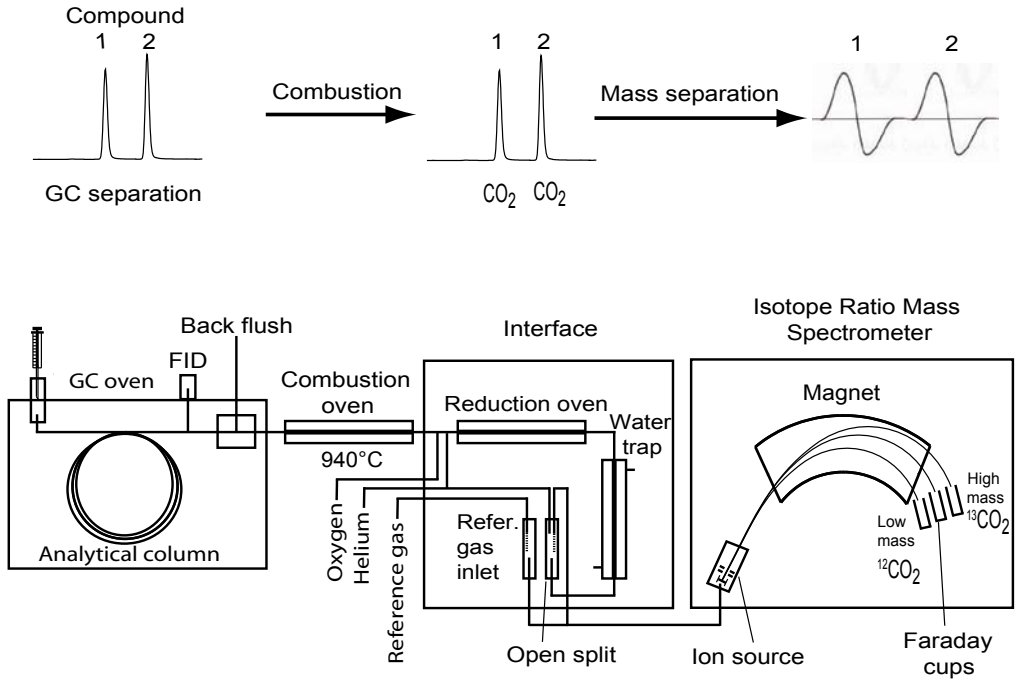

Figure 2.1. Schematic of the GC-IRMS and general procedure used in compound specific isotope analysis of carbon. The lower figure shows a schematic view of the instrumentation and the upper figure the respective output from the different steps.

2.2. Nomenclature and International Standards

Stable isotope analysis of carbon or hydrogen involves measurement of the relative abundance of the two stable isotopes of carbon (^{13}C and ^{12}C) or hydrogen (^{2}H and ^{1}H). In order to ensure inter-laboratory comparability and accuracy, these ratios are expressed relative to an international standard (typically V-PDB for carbon and V-SMOW for hydrogen). Measured values are reported as $\delta^{13}C$ and $\delta^{2}H$ respectively. These terms are defined in Equations 2.1 and 2.2 as follows:

$$\delta^{13}C\left(‰\right) = \left[\frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{standard}}{\left(^{13}C/^{12}C\right)_{standard}}\right] \times 1000 \quad \textit{2.1}$$

$$\delta^{2}H\left(‰\right) = \left[\frac{\left(^{2}H/^{1}H\right)_{sample} - \left(^{2}H/^{1}H\right)_{standard}}{\left(^{2}H/^{1}H\right)_{standard}}\right] \times 1000 \quad \textit{2.2}$$

Since the resulting δ values are very small (for $\delta^{13}C$ typically < 0.05), they are generally multiplied for convenience by 1000 and reported as parts per thousand or "per mill", indicated by the symbol ‰. Sometimes, the standard is explicitly indicated after the ‰ symbol, e.g. for carbon isotopes the values are reported as ‰ V-PDB. If no information is given, it can be assumed that the values are reported relative to the usual standard material.

For decades the International Atomic Energy Agency (IAEA) in Vienna, in conjunction with the National Bureau of Standards in the United States, has administered and overseen the storage and distribution of the key international stable isotope standards. Analysis and reporting of the other stable isotope systems (O, N, Cl, etc.) follow an analogous approach (Clark and Fritz, 1997).

In the common delta notion, the deviation of the stable isotope value of the sample from the standard will be either negative or positive. A negative value means that the sample is depleted in its ^{13}C-content relative to the $^{13}C/^{12}C$ content of the standard whereas a positive sign implies an enriched ^{13}C-content.

According to the IUPAC definition, compounds that only differ in their isotope composition (such as $^{12}CO_2$ and $^{13}CO_2$) are called isotopologues. The term "isotopomer" is used for isomers having the same number of each isotopic atom but differing in their positions. As an example, $Cl_2{}^{12}C$-$^{13}CHCl$ and $Cl_2{}^{13}C$-$^{12}CHCl$ are the two isotopomers of TCE with respect to carbon.

In the following sections we will focus on data quality issues of carbon isotope analysis since carbon is by far the most frequently measured element in CSIA to date.

2.3. Laboratory Working Standards

The following sections are intended primarily for laboratory staff that will actually analyze the samples, and for staff that will prepare or review Quality Assurance Project Plans.

2.3.1. CO_2 Reference Gas

Since the international standard materials are made available to each laboratory in limited amounts, they are not used for daily operations and measurements. For daily operations and standardization, each laboratory obtains pure CO_2 reference gas and cross-calibrates it against the international standard materials to develop in-house working standards. To obtain maximum accuracy, this cross-calibration should be done by the conventional dual inlet approach; alternatively, the isotope composition of working standards can be determined by an elemental analyser - isotope ratio mass spectrometer (EA-IRMS). Once the laboratory's working CO_2 standard is characterized (1) it should be used daily to calibrate the isotope ratio mass spectrometer, (2) the CO_2 working standard should be cross-checked against the international standard materials every few months to ensure continued accuracy, and (3) aliquots of the CO_2 working standard should be stored in glass ampoules so it can be available on a long term basis for calibration checks and quality control, and for inter-laboratory comparisons. See Coplen et al., (2006) and Qi et al., (2003).

2.3.2. Compound specific Working Standards

The CO_2 working standard is included in individual sample sets to act as an internal standard. Since organic compounds may behave differently in this analytical system than pure CO_2 (due to differences in chromatographic separation, combustion efficiency, peak shape, etc.), it is important for each laboratory to also characterize compound specific working standards for the target compounds that they typically analyze (Figure 2.2).

Isotopic characterization of the working standards should be done off-line using the sealed quartz tube combustion technique and conventional dual inlet mass spectrometry to ensure maximum precision and accuracy with respect to the international

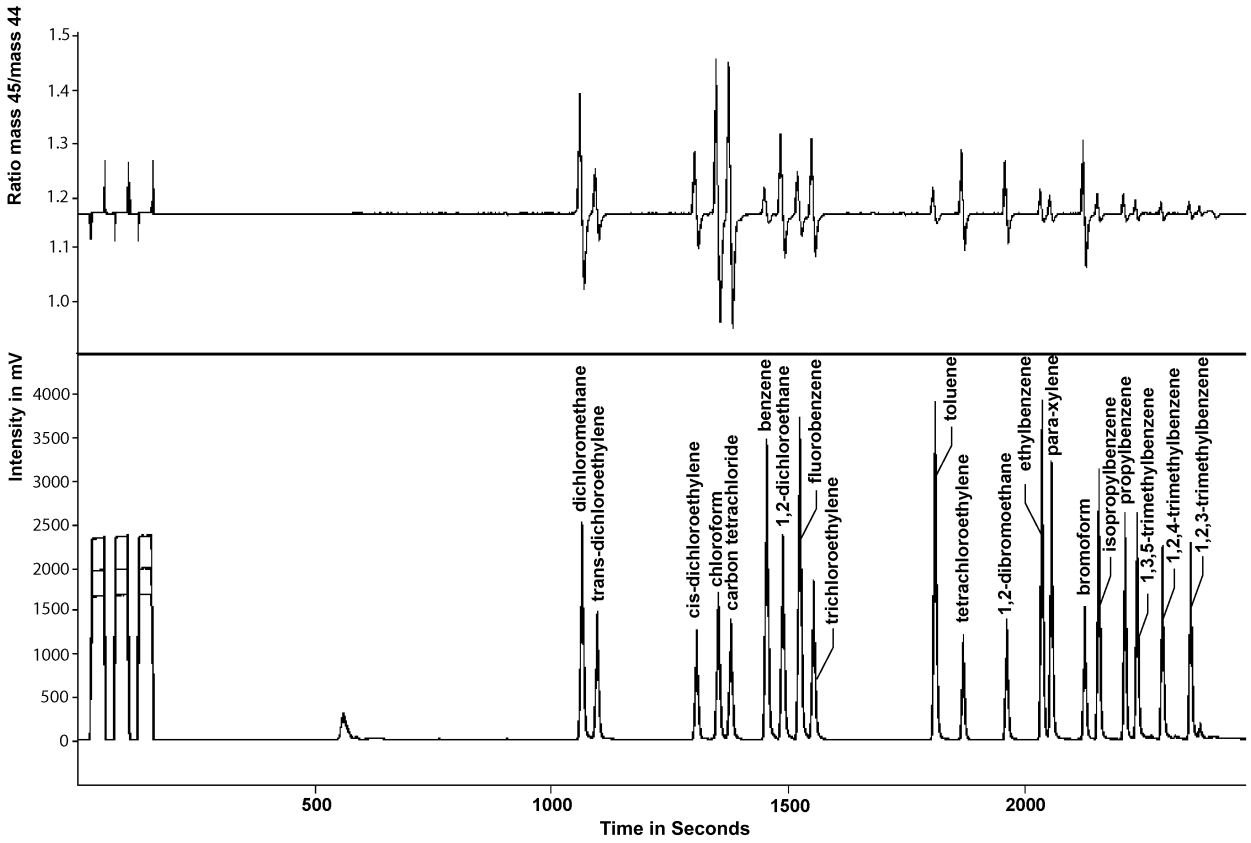

Figure 2.2. Example of a chromatogram obtained in GC-IRMS. Upper panel: Isotope ratio trace with the typical isotope swings due to the partial separation of isotopologues prior to on-line combustion caused by the inverse isotope effect in gas chromatography (modified after Jochmann et al. 2006). Lower panel: Gas chromatograph. Note that the CO_2 working standard produces the flat-top peaks at the start of the chromatogram.

standard (V-PDB) and the laboratory CO_2 working standard. Typical precision for this approach is ± 0.15‰ (Clark and Fritz, 1997). There is an increasing trend to measure the isotopic composition of organic working standards by an elemental analyser - isotope ratio mass spectrometer (EA-IRMS) which is appropriate as long as the careful procedures outlined in Qi et al. (2003) are followed.

The following recommendations for archiving and storage of compound specific working standards are for compounds that are liquid at room temperature and pressure. Purchase pure product for each compound to be analyzed. Ensure highest purity as even small amounts of contaminants can affect the ability to accurately characterize the compound of interest. At least two dozen sealed glass ampoules should be set aside as ARCHIVED standards for use when needed to cross-check the isotopic value in the future, or for inter-laboratory comparisons. Set up a second set of thirty to forty sealed glass ampoules to be used as WORKING standards for daily standardization, controls on experiments, and for correcting problems with the performance of the instrument. It is advisable to test the procedure used to seal the ampoule to insure that the ampoules are flame-sealed quickly. If significant amounts of compound are lost through volatilization, this might change the isotopic ratio of the standard.

2.4. Method Testing, Quality Assurance and Quality Control

Conventional off-line preparation techniques and dual inlet isotope ratio mass spectrometry (IRMS) provide optimized analytical conditions to obtain maximum precision. In contrast, continuous flow IRMS provides for rapid analysis of complex mixtures of organic compounds, and for dissolved organic compounds in environmental samples. Continuous flow IRMS requires a sample size that is approximately four to five orders of magnitude smaller than the sample needed for off-line preparation techniques. Continuous flow IRMS however produces an inevitable loss of precision. The loss

of precision is related to a wide variety of factors which are beyond the scope of this Guide but which include a higher source pressure for the helium carrier gas, higher background concentrations of water, and the need to tune the source for optimum linearity rather than optimum sensitivity. In the following sections, we provide an approach to determine reasonable values for reproducibility, accuracy and the detection limit for compound specific working standards that are analyzed by continuous flow mass spectrometry, and provide attainable goals for reproducibility, accuracy and detection limits for CSIA data (for more detail see Sherwood Lollar et al., 2007; Jochmann et al., 2006).

2.4.1. Reproducibility

Reproducibility (or precision) refers to the ability to obtain the same value when the same sample or standard is analyzed repeatedly (Figure 2.3). In compound specific isotope analysis (CSIA), if a sample is run in duplicate or triplicate under constant operating conditions, the standard deviation of the mean of the replicate measurements is typically <0.1 to 0.3‰ for $\delta^{13}C$ values. While reproducibility is necessary to minimize uncertainty, it is not a sufficient expression of the total degree of uncertainty (error) in a measurement. As illustrated in Panel C of Figure 2.3, a measurement can be highly reproducible (precise) but nevertheless be inaccurate.

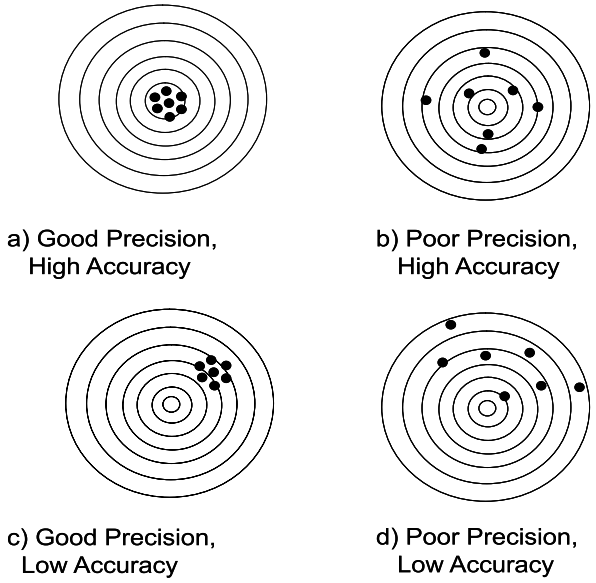

Figure 2.3. An illustration the difference between precision (reproducibility) and accuracy of several data points, using the bull's eye of a target as the goal for high accuracy and good precision.

2.4.2. Total Uncertainty and the Critical Role of Linearity

When a suite of real samples are analyzed by CSIA, all the operating conditions are not held constant from one run to the next as described above in the definition of analytical precision. In fact, one of the key advantages of continuous flow CSIA is that it permits, in the same analytical run, the measurement of the $\delta^{13}C$ values for several compounds that are present in the sample mixture at different concentrations. However, specific sample preparation parameters, such as split ratios, are often adjusted to bring the concentration of all of the analytes into the linear range of the instrument. In contrast to conventional dual inlet systems where the standard peak size must be carefully balanced to match the sample peak size, in continuous flow CSIA the standards can be input at one peak size (typically 1 to 2 Volts) and then used to characterize sample peaks that are either above or below that size. The linear range, the range over which accurate measurements are possible, varies from instrument to instrument. The linear range depends on the mass spectrometer itself as well as the chromatograph and combustion system.

As used in analytical chemistry, the term linearity usually refers to a linear increase of the signal with increasing amount of analyte. As applied to isotope analysis, linearity indicates that (within an acceptable range) the obtained isotope ratio is independent of the amount of compound injected. The following sections provide further details on how to establish the linearity of the CSIA analytical system and the implications that linearity has for documenting the uncertainty and detection limits associated with isotope ratios (see also Sherwood Lollar, et al., 2007).

Figure 2.4 shows the results of a typical linearity test. Multiple analyses of a laboratory working standard for TCE were run under identical operational parameters including constant concentration of TCE, and constant chromatographic conditions, combustion temperature, flow rate, and split setting. However, a wide range of different peak sizes (or signal sizes) were obtained by varying the amount of sample introduced. When this type of test is carried out, for any given measurement, the $\delta^{13}C$ value obtained is typically within ±0.5‰ of the value for the laboratory working standard obtained by off-line preparation techniques and dual inlet mass spectrometry. Based on these results a sample that is run under similar conditions should also have a total uncertainty of approximately ±0.5‰.

As illustrated in Figure 2.4, there is a threshold in the size of the signal below which the variation for

replicate values of the working standard increases significantly. The values of $\delta^{13}C$ that are measured below this threshold can be more enriched than the standard value, or less enriched than the standard value, and whether they are more or less than the standard value varies with time under constant operating conditions - hence no corrections should be attempted for values of $\delta^{13}C$ that are measured below the threshold. See also Jochmann, et al. (2006) and Sherwood Lollar, et al. (2007).

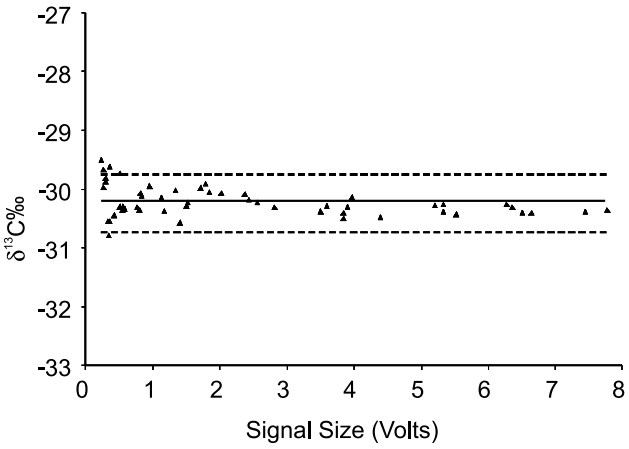

Figure 2.4. Typical linearity test of a laboratory working standard run by CSIA over a wide range of different peak sizes (or signal sizes) by varying the amount of analyte introduced. Modified after Sherwood Lollar et al. (2007). The solid line is the mean of all the replicate analyses of the working standard. The dotted lines are ± one standard deviation of the mean.

The largest effects on the value of $\delta^{13}C$ are typically attributable to the effects of sample size on linearity or to a change in a major parameter such as purposely changing the split setting. The effects of these changes vary somewhat from compound to compound. Therefore it is highly recommended to document the effect of changes in these parameters on the measured value of $\delta^{13}C$ for each compound specific working standard. The total uncertainty varies from compound to compound. Maintenance of a control chart to monitor this variation is an important part of good practice for Quality Assurance/Quality Control (QA/QC). In any laboratory inter-comparison, values measured from one laboratory to another should agree within ±0.5‰ (or other level of uncertainty specific to a particular compound) if each laboratory has properly calibrated their working standard to V-PDB. Sherwood Lollar et al. (2007) and references therein demonstrate that a total uncertainty of ±0.5‰ is typical for many hydrocarbon contaminants investigated to date, including alkanes, certain chlorinated ethenes, certain chlorinated ethanes and aromatic hydrocarbons. However, at the current state of practice for CSIA, an uncertainty of ±0.5‰ can not be routinely attained for analysis of carbon isotopes in every volatile organic compound that might be of regulatory interest. It is the responsibility of the analyst to provide documentation of the upper limit on uncertainty that is associated with a particular compound of interest when analyzed following a particular protocol for CSIA. It is the responsibility of the user of the data to determine whether the achieved upper limit on uncertainty is acceptable for their particular application.

2.4.3. Establishing Concentration Thresholds or "Detection limits"

Mass spectrometry can produce a $\delta^{13}C$ value for very small signals. However, as indicated above, at signal sizes below a certain threshold both the accuracy and reproducibility of $\delta^{13}C$ measurements deteriorate. We recommend that the operational detection limit be defined as that concentration of the compound in the water sample below which the accuracy and reproducibility of the value for $\delta^{13}C$ deteriorate beyond acceptable limits. The criterion for "acceptable limits" depends on the use of the data, and is dependent on the methods and the instruments used. As mentioned above, for many compounds of interest, most laboratories can attain a standard deviation of the mean of triplicate samples of ±0.5‰ for CSIA of carbon. Jochmann et al. (2006) compared the variation in $\delta^{13}C$ in triplicate analyses over a range of concentrations. They compared the data to identify the concentrations that met two criteria: the mean value of triplicate measurements at a particular concentration was within ±0.5‰ of the mean of all analyses over the range of concentrations, and the standard deviation of the triplicate analyses at a particular concentration was less than ±0.5‰, as is illustrated in Figure 2.5. They defined the method detection limit as the lowest concentration that satisfied both of the criteria.

The minimum quantity of sample necessary to keep the uncertainty in the determination of the isotopic ratio within acceptable limits will vary from compound to compound and may also depend on the technique used to prepare the samples for analysis. For any particular technique to prepare the sample, the minimum quantity will be associated with a minimum concentration necessary to keep uncertainty within acceptable limits. This minimum

concentration becomes the effective detection limit for determination of isotopic ratios. It therefore should be established separately for each compound and each injection/preconcentration technique using compound specific working standards. In the example shown in Figure 2.5, the MDL for benzene is 0.2 µg/L, which corresponds to a peak height of 250 mV.

As discussed above, it is not generally possible to "correct" for values run at smaller signal sizes. Ideally, improving detection limits for CSIA relies on increasing the efficiency of sample preparation and preconcentration steps to provide higher signal peaks (see below), and not by trying to "correct" values or simply to report values close to the threshold at which the accuracy of the determination will be compromised.

2.4.4. Application of These Principles to Other CSIA Measurements

For hydrogen isotopes, the principles for establishing reproducibility, total uncertainty and detection limit are the same as for carbon isotopes. However, there are few formal studies on the uncertainty associated with analysis of δ^2H in environmental investigations, and we will not make specific performance recommendations at this time. As a rule of thumb the total uncertainty for hydrogen is usually at least an order of magnitude greater than for carbon; total uncertainty for δ^2H is typically ±5‰ versus ±0.5‰ for $\delta^{13}C$ (Sherwood Lollar et al., 2007). For an example of this kind of method development for hydrogen isotope analysis see Gray et al. (2002). Similar principles will be applicable to H, N, Cl, S, and O using continuous-flow compound specific methods. See Sessions (2006) for an extensive review of analytical methods. As with any new method, there may be other important operational parameters in addition to those that affect carbon and hydrogen CSIA measurements and careful work is needed.

2.4.5. Extraction Methods for CSIA

Based on the requirements specified by the various manufacturers of isotope ratio mass spectrometers, it is necessary to inject approximately 1 nmol carbon or 8 nmol hydrogen on column to have adequate mass to provide an accurate and precise measurement of the isotope ratio. These criteria assume that the GC-IRMS instrument is tuned to maximum linearity, and that the chromatographic resolution (R_s) is greater than 1.5, which provides narrow peaks with good peak separation.

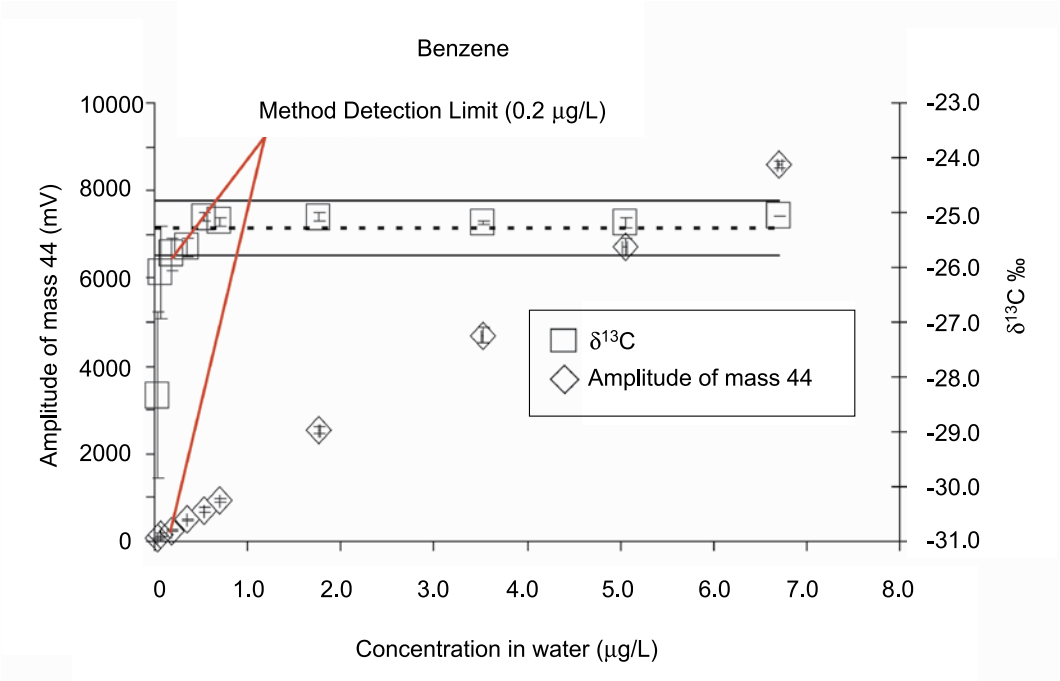

Figure 2.5. Example of the evaluation of method detection limits (MDLs) in CSIA. The squares represent the $\delta^{13}C$ values in ‰ and the diamonds show the amplitude of mass 44 in mV. Error bars indicate the standard deviation of triplicate measurements. The horizontal broken line represents the iteratively calculated mean value after the methods of Jochmann et al. (2006) and Sherwood Lollar et al. (2007). The solid lines around the mean value represent the standard deviation on the mean of triplicate measurements. Figure modified after Jochmann et al. (2006).

These criteria can be used to estimate the mass of individual compounds that must be delivered on column:

$$m_i = \frac{1\,nmol}{\mathrm{x}} M_i \quad \text{for carbon} \qquad \textbf{2.3}$$

and

$$m_i = \frac{8\,nmol}{\mathrm{x}} M_i \quad \text{for hydrogen} \qquad \textbf{2.4}$$

where m_i is the required mass in ng, x is the number of carbon or hydrogen atoms respectively in the compound, and M_i is the molecular weight of the compound in g/mole.

For methyl tertiary butyl ether, for example, this yields a minimum mass of 18 ng. Using a dimensionless air-water partition constant K_{aw} of 0.12 at 50 °C (Arp and Schmidt, 2004) for a typical headspace extraction (10 mL sample and 10 mL headspace, 1 mL gas injection, 50 °C) this is equivalent to a concentration in the water sample of 170 µg/L. For hydrogen, at least 8 nmol are required and the same calculation as above yields 59 ng or 550 µg/L.

These are calculated *minimum* numbers under optimum conditions and, as Table 2.1 shows, are often not achievable. Unfortunately, environmental concentrations of interest are frequently below these levels, especially at contaminated sites outside the plume core or if substantial degradation has occurred. Efficient extraction or preconcentration techniques must be integrated with GC-IRMS in order to fully exploit the potential of the method for a wide range of samples, in particular for elements other than carbon. To meet this need, over the past few years several studies have worked to lower the detection limits for CSIA by the use of sorptive extraction techniques such as solid-phase microextraction (SPME) or purge and trap (P&T). Note that almost all these studies have focused on compounds that are relatively water soluble and volatile, such as the BTEX compounds, MTBE, and chlorinated ethylenes. These compounds are among the most common industrial ground water pollutants.

The concentration thresholds or effective detection limits are constrained by the physical limits of the gas chromatograph isotope ratio mass spectrometer system, as well as by the technique used to prepare the sample. Ideally, the extraction or preconcentation technique will be free of isotope fractionation effects and will be adequate to concentrate enough material from each compound of interest to determine the isotopic ratio at concentrations that are relevant to plumes of contaminated ground water.

Table 2.1 summarizes the effective detection limit that has been reported for a variety of techniques that are used for sample preparation prior to CSIA of the common organic contaminants in ground water. As a first approximation, the sensitivity is correlated with the molar concentration of carbon (or any other element of interest) of the compound in the sample. Limits of detection should be determined based on the lower range of linearity of the instrument (see Section 2.4.3). However, Table 2.1 provides the concentration corresponding to the typical operational limits of detection, based on the criteria of 1 nmol carbon and 8 nmol hydrogen on column and our experience with the technique.

Detection limits for nitrogen or oxygen isotope analysis are provided by the manufacturer of the instrument. However, there are only a few methods available for extraction and preparation of samples, or protocols available for CSIA, that are applicable to isotopes of nitrogen, chlorine, and oxygen in ground water contaminants (Berg et al., 2007; Hartenbach et al., 2006; Holmstrand et al., 2006; Penning and Conrad, 2007). Studies for hydrogen isotope analysis of ground water contaminants are also still relatively limited (Kuder et al., 2005; Mancini et al., 2002; also Table 2.1).

A prerequisite for the selection of any extraction or preconcentration technique used to prepare samples for CSIA is adequate sensitivity. A further prerequisite is a negligible change in the value of $\delta^{13}C$ or $\delta^{2}H$ during the extraction or enrichment process, or at least a highly reproducible change. Before an extraction or preconcentration technique is implemented on a routine basis, it is mandatory to thoroughly evaluate the technique for changes in the value of $\delta^{13}C$ or $\delta^{2}H$ during sample preparation, rather than relying on data reported by others. The change in the value of $\delta^{13}C$ or $\delta^{2}H$ may vary depending on analytical conditions such as the split ratio and extraction time. Each compound that will be analyzed should be tested using working standards with a known isotopic composition (see Section 2.3). The evaluation should cover the typical range of operating conditions. The standard deviation of replicate analyses should typically be smaller than ±0.5‰ for carbon, otherwise the method is not suited for typical applications.

A number of extraction methods have been shown to provide accurate isotope ratios, while other methods change the value of $\delta^{13}C$ or $\delta^{2}H$ (Table 2.1). There are some general trends. Typically headspace and direct immersion SPME are not accompanied by a substantial changes in the value of $\delta^{13}C$ or $\delta^{2}H$ (Dayan et al., 1999; Slater et

al., 1999; Dias and Freeman, 1997; Hunkeler and Aravena, 2000b; Zwank et al., 2003). If changes in the value of $\delta^{13}C$ or $\delta^{2}H$ are observed with SPME, the analyte tends to be depleted in ^{13}C compared to pure phase standards, i.e., the lighter compound partitions more strongly into the fiber which is then subsequently analyzed. This resembles the same inverse isotope effect that is observed in gas chromatography. Although this effect is often quite small, for carbon tetrachloride, Zwank et al. (2003) found high deviation using direct immersion SPME, which could not be explained. Furthermore, Hunkeler et al. (2001a) found a significant ^{13}C-depletion of tertiary butyl alcohol extracted by SPME. Sometimes significant inverse isotope effects are seen during headspace equilibration with an aqueous sample. An enrichment of ^{13}C in the gas phase of up to 1.46‰, has been observed (Hunkeler and Aravena, 2000b), thus Hunkeler et al. (2005) applied corrections in subsequent work in order to allow for a comparison of isotope data generated with different methods.

There is no consistent pattern in the changes in the value of $\delta^{13}C$ or $\delta^{2}H$. Compounds other than tertiary butyl alcohol behave differently (Slater et al., 1999), which emphasizes the need for testing each individual compound during method validation. Dynamic extraction methods such as purge and trap and dynamic headspace extraction aim for a quantitative (100%) extraction of the sample with subsequent trapping and thermo-desorption of the analyte into the GC column. These dynamic extraction methods are more appropriate for isotope analysis at very low concentrations. In the various studies conducted to date that used an adequate purge time, no significant change in the value of $\delta^{13}C$ or $\delta^{2}H$ has been reported. Zwank et al. (2003) have shown for a number of volatile organic compounds that sample preparation does not compromise the analysis unless the extraction efficiencies drop below approximately 40%.

Table 2.1. Extraction or sample preparation techniques used in CSIA for volatile ground water pollutants. Adapted and updated from Schmidt et al. (2004).

Compound	**Injection/ preparation technique**	**Change in the value of $\delta^{13}C$ or $\delta^{2}H$ during analysis**	**Definition of the Detection Limit**	**Operational Detection limit [µg/L]**		**Reference**
				$\delta^{13}C$	$\delta^{2}H$	
Methyl Tertiary Butyl Ether	liquid injection[a]	OC[b] <0.3‰; SL[c] ~1‰	Amplitude > 0.5 V	24000	-	(Zwank et al., 2003)
	headspace injection	n.s.c.[e]	Amplitude > 0.5 V	5000	50000	(Gray et al., 2002)
			Amplitude > 0.5 V	4000 (TAME: 6000)	-	(Somsamak et al., 2005)
	headspace SPME	C: -0.9‰ H: -17‰ (both with resp. to HS injection)	Amplitude > 0.5 V	350	1000	(Gray et al., 2002)
	headspace SPME	Significant but small change (-0.67±0.21‰)	Amplitude > 0.75 V	11	-	(Hunkeler et al., 2001a)
	direct immersion SPME	Reproducible change (<0.5‰), but presence of BTEX concentrations >3 mg/L caused 2‰ deviation.	Amplitude > 0.5 V	16	-	(Zwank et al., 2003)

Compound	Injection/ preparation technique	Change in the value of $\delta^{13}C$ or $\delta^{2}H$ during analysis	Definition of the Detection Limit	Operational Detection limit [µg/L]		Reference
				$\delta^{13}C$	$\delta^{2}H$	
	P&T	Small shift of $\delta^{13}C$ values (+0.33‰)	n.r.[d]	15	-	(Smallwood et al., 2001)
	P&T	n.r.[d]	n.r.[d]	5	-	(Kolhatkar et al., 2002)
	P&T	n.s.c.[e]	Amplitude > 0.5 V	0.63	-	(Zwank et al., 2003)
	P&T	n.r.[d]	< 0.5‰ precision	2.5	20	(Kuder et al., 2005)
Benzene	liquid injection[a]	n.s.c.[e]	Amplitude > 0.5 V	19000	-	(Zwank et al., 2003)
	headspace injection	n.s.c.[e]	Amplitude > 0.5 V	500	-	(Mancini et al., 2003)
	direct immersion SPME	n.s.c.[e]	Amplitude > 0.5 V	22	-	(Zwank et al., 2003)
	P&T	n.s.c.[e]	Amplitude > 0.5 V	0.30	-	(Zwank et al., 2003)
	P&T	n.r.[d]	Moving mean within ± 0.5‰ interval and σ < 0.5‰	0.20	-	(Jochmann et al., 2006)
Toluene	liquid injection[a]	OC[b] n.s.c.[e] SL[c]~-1‰	Amplitude > 0.5 V	9500	-	(Zwank et al., 2003)
	headspace injection	n.s.c.[e]	Amplitude > 2 V	-	2000	(Ward et al., 2000)
	headspace injection	n.s.c.[e]	Amplitude > 0.2 V	100	-	(Slater et al., 1999)
	direct immersion SPME	n.s.c.[e]	Peak area equiv. to 50 pmol CO_2 at the ion source (ca. 0.7 Vs)	45	-	(Dias and Freeman, 1997)
	direct immersion SPME	n.s.c.[e]	Amplitude > 0.5 V	9	-	(Zwank et al., 2003)
	P&T	n.s.c.[e]	Amplitude > 0.5 V	0.25	-	(Zwank et al., 2003)
	P&T	n.s.c.[e]	Moving mean within ± 0.5‰ interval and σ < 0.5‰	0.07	-	(Jochmann et al., 2006)
Chlorinated Methanes	liquid injection[a]	$CHCl_3$, ~-1.5‰ CCl_4, OC[b] -3.31±0.34‰	Amplitude > 0.5 V	170000 to 220000	-	(Zwank et al., 2003)

Compound	Injection/ preparation technique	Change in the value of $\delta^{13}C$ or $\delta^{2}H$ during analysis	Definition of the Detection Limit	Operational Detection limit [µg/L]		Reference
				$\delta^{13}C$	$\delta^{2}H$	
	direct immersion SPME	$CHCl_3$, -1.8±0.28‰ CCl_4, -7.3±0.22‰	Amplitude > 0.5 V	170 to 280	-	(Zwank et al., 2003)
	direct immersion SPME	n.s.c.[e] -0.09 to 0.40 ‰	1.5 nmol C on column	360 to 2200	-	(Hunkeler and Aravena, 2000b)
	headspace injection	1.03 to 1.29 ‰	1.5 nmol C on column	800 to 3300	-	(Hunkeler and Aravena, 2000b)
	P&T	$CHCl_3$ and CCl_4, n.s.c.[e]	Amplitude > 0.5 V	≤5.0	-	(Zwank et al., 2003)
	P&T	$CHCl_3$, ~-1.5‰ CCl_4 and DCM, n.s.c.[e]	Moving mean within ± 0.5‰ interval and σ < 0.5‰	18 to 27	-	(Jochmann et al., 2006)
Chlorinated Ethenes	liquid injection[a]	Small but significant change observed for TCE and cis-DCE	Amplitude > 0.5 V	71000 to 84000	-	(Zwank et al., 2003)
	headspace injection	TCE, n.s.c.[e]	Amplitude > 0.2 V	400	-	(Slater et al., 1999)
	direct immersion SPME	n.s.c.[e] -0.37 to +0.06‰	1.5 nmol C on column	130 to 290	-	(Hunkeler and Aravena, 2000b)
	headspace injection	0.21 to 0.69 ‰	1.5 nmol C on column	170 to 1000	-	(Hunkeler and Aravena, 2000b)
	direct immersion SPME	Small (~1‰) but significant change observed for *cis*-DCE only	Amplitude > 0.5 V	66 to 130	-	(Zwank et al., 2003)
	P&T	n.s.c.[e]	Not given	5	-	(Song et al., 2002)
	P&T	Small (~0.7‰) but significant change observed for *cis*-DCE only	Amplitude > 0.5 V	1.1 to 3.6	-	(Zwank et al., 2003)

Compound	Injection/ preparation technique	Change in the value of $\delta^{13}C$ or $\delta^{2}H$ during analysis	Definition of the Detection Limit	Operational Detection limit [µg/L]		Reference
				$\delta^{13}C$	$\delta^{2}H$	
	P&T	n.s.c.[e]	Moving mean within ± 0.5‰ interval and σ < 0.5‰	0.8 to 5.1	-	(Jochmann et al., 2006)
	dynamic headspace extraction	n.s.c.[e]	Amplitude > 0.2 V	10 to 38	-	(Morrill et al., 2004)
Misc. Compounds						
Methyl-cyclohexane	direct immersion SPME	< 0.5 ‰	Peak area equiv. to 50 pmol CO_2 at the ion source (ca. 0.7 Vs)	24	-	(Dias and Freeman, 1997)
Alkylated Benzenes	P&T	n.s.c.e	Moving mean within ± 0.5‰ interval and s < 0.5‰	0.07 to 0.35	-	(Jochmann et al., 2006)
Hexanol	direct immersion SPME	< 0.5 ‰	Peak area equiv. to 50 pmol CO_2 at the ion source (ca. 0.7 Vs)	4200	-	(Dias and Freeman, 1997)
Tertiary Butyl Alcohol	direct immersion SPME	Significant change (-1.18±0.12‰)	Amplitude > 0.75 V	360	-	(Hunkeler et al., 2001a)
Tertiary Butyl Alcohol	P&T	n.r.d	< 0.5‰ precision	25	-	(Kuder et al., 2005)
Bromoform, Ethylene Dibromide	P&T	n.r.d	Moving mean within ± 0.5‰ interval and s < 0.5‰	14, 3.9	-	(Jochmann et al., 2006)
Nitro-aromatic compounds	direct immersion SPME	Significant change for some compounds (up to -1.3‰)	Amplitude > 0.5 V (equiv. to ca. 0.8 nmol C on column)	73 to 780	-	(Berg et al., 2007)
Anilines	direct immersion SPME	Significant change for some compounds (up to 1.1‰)	Amplitude > 0.5 V (equiv. to ca. 0.8 nmol C on column)	320 to 1600	-	(Berg et al., 2007)

[a] Analyte dissolved in solvent.
[b] On column injection.
[c] Splitless injection.
[d] Not reported in reference.
[e] No significant change (<0.5‰) observed.

2.5. Avoiding Some Pitfalls in CSIA Measurements

Sessions (2006) gives an excellent overview of requirements for successful isotope analysis. Blessing et al. (2008) recently discussed potential pitfalls in CSIA of environmental samples. Some of their recommendations are summarized here.

Many analysts nowadays are accustomed to the selectivity provided by mass spectrometric detectors in quantitative analysis. However, the continuous flow GC-IRMS is non-selective. In the case of carbon, all of the compounds are converted to CO_2 before analysis of the isotopic ratio. The system "sees" all the carbon (or other element) eluted from the column. Therefore, it is of the utmost importance to remove coeluting non-target compounds as completely as possible or to modify separation methods to allow a baseline separation.

Samples should be screened by GC/MS or GC/FID prior to CSIA measurements to avoid overloading of the GC-IRMS system with non-target analytes. If the interfering compounds are sufficiently separated from the target analytes, they can be eliminated by switching a valve installed between the GC column and the combustion oven. The valve diverts the flow of carrier gas with the interfering compounds away from the combustion oven.

Peak integration should be closely monitored and adjusted manually if necessary. This is a much more delicate task than in quantitative analysis because shifting the peak delimiters can significantly change the calculated isotope values due to the partial chromatographic separation of isotopologues. Isotope swings can serve as good indicators of peak quality.

A correction can be applied with care to account for material that bleeds from the GC column. Use of CO_2 standards within the sample run is helpful to provide the "ground-truth" for such corrections. Data can be automatically corrected using various algorithms that are available for this purpose in commercial instruments. However, at the time of this writing (Spring 2008) a thorough comparison of the various methods has not been conducted (Sessions, 2006).

2.6. Recommended Routine for Daily Laboratory Quality Assurance/ Quality Control (QA/QC) for Carbon Isotope Analysis

Test the linearity and sensitivity of the instrument with the CO_2 working standard. Then test the linearity of the instrument with the compound specific working standards over a typical range of operating conditions that will be used for the day's samples. Operating conditions include the range of concentrations, split or flow settings, and the technique used to prepare the samples, such as a headspace sampler, SPME or purge and trap. Values of $\delta^{13}C$ for each standard typically should remain within $\pm 0.5‰$ ($1\ \sigma$) of the previously determined isotopic working standard value to ensure both accuracy and reproducibility. Plotting these values on a control chart will allow for continuous monitoring of QA/QC over the long term.

Analyze samples under the same conditions as above, ensuring baseline separation for the target compounds. Requirements for excellent chromatography are even more stringent than for concentration analysis.

At a minimum, the CO_2 working standard should be analyzed at the beginning of each sample run. At least every fifth sample should be a replicate. At least every tenth sample should be the compound specific working standard.

All samples should stay within the previously established range of acceptable linearity and above the established threshold limit. If a sample falls outside the acceptable range, the concentrations of the analytes should be adjusted, if possible, to bring the sample within the established range, and the sample analyzed a second time. Follow the specifications provided by the manufacturer of the instrument for the upper limit of the range of linearity.

3.0
Collection, Preservation and Storage of Samples

This section provides specific recommendations for collection, preservation and storage of ground water samples that are intended for CSIA analysis. The section has application to the development of Quality Assurance Project Plans for site characterization, and is intended for contractors that sample ground water, and for site managers and regulators that develop and approve sampling plans.

3.1. Collection of Ground Water from Monitoring Wells

Procedures and conditions that would compromise a sample intended for analysis of concentrations of a particular organic contaminant will also compromise the sample for CSIA analysis. Procedures and conditions that sustain and maintain the suitability of a sample for analysis of concentrations of a particular organic contaminant will also sustain and maintain the suitability of the sample for CSIA analysis. As a general rule, established good practice for acquisition of samples for analysis of concentrations of organic contaminants can be accepted as good practice for samples intended for CSIA analysis. In particular, criteria for methods of preservation, for methods for shipping samples from the field site to the laboratory, and criteria for holding times and holding temperatures to store samples that have been established for analysis of concentrations of organic contaminants are also applicable to CSIA analyses.

U.S. EPA has provided recommendations and guidance on collecting ground water samples (Barcelona et al., 1985; Yeskis and Zavala, 2002) and a comprehensive description of good practice for ground water sampling is available in Nielsen (2006).

Ground water can be collected from monitoring wells by a variety of devices including bailers, above ground peristaltic pumps with plastic sampling tubes inserted into the well, and down-hole pumps (Nielsen, 2006). Any of the devices can produce an adequate sample if they are used appropriately. To minimize the loss of volatile analytes, the device should be used in a manner that minimizes the exposure of the ground water sample to the atmosphere during sampling.

If a well is not purged before sampling, the water pumped from the well may or may not be representative of the ground water in the aquifer being sampled (Nielsen, 2006). Volatile compounds can be lost to the headspace above the water column in the well. Oxygen from the air above the water in the well can diffuse into the water and support aerobic biodegradation of organic compounds in the water in the well that might not occur in the ground water in anoxic aquifers.

There are two general approaches to purge the water from the well before it is sampled for chemical analysis. In the first approach, the volume of water contained in the casing of the well is calculated from the depth of the water column in the well and the diameter of the well, then two or three casing volumes of water are purged from the well before the well water is sampled. In the second approach, field instruments are used to continuously monitor sensitive parameters such as temperature, redox potential, and concentrations of dissolved oxygen in the water purged from the well. The samples are taken after the sensitive parameters become stable (Yeskis and Zavala, 2002).

Depending on the vertical interval screened by a monitoring well, on the vertical distribution of hydraulic conductivity, and on the vertical extent of concentrations of contaminants in ground water, the concentrations of contaminants in well water may change over time as a well is purged. Figure 3.1 presents a common scenario where this situation might be expected. Ground water contamination is present in an aquifer in the flood plain of a major river. The land surface is comprised of silts and clays of the flood plain, while sands and gravels from old meanders of the river occur at depth. The water table occurs in the silts and clays, and the monitoring well is screened in the silts and clays. Contaminated ground water moves through the layers of sand and gravel because they have higher hydraulic conductivity.

When the monitoring well is pumped to a modest extent, it will produce water from the silts and clays. This water often is recent recharge water from precipitation, and is free of contamination. When the well is purged more extensively, contaminated water is drawn in from the deeper, more

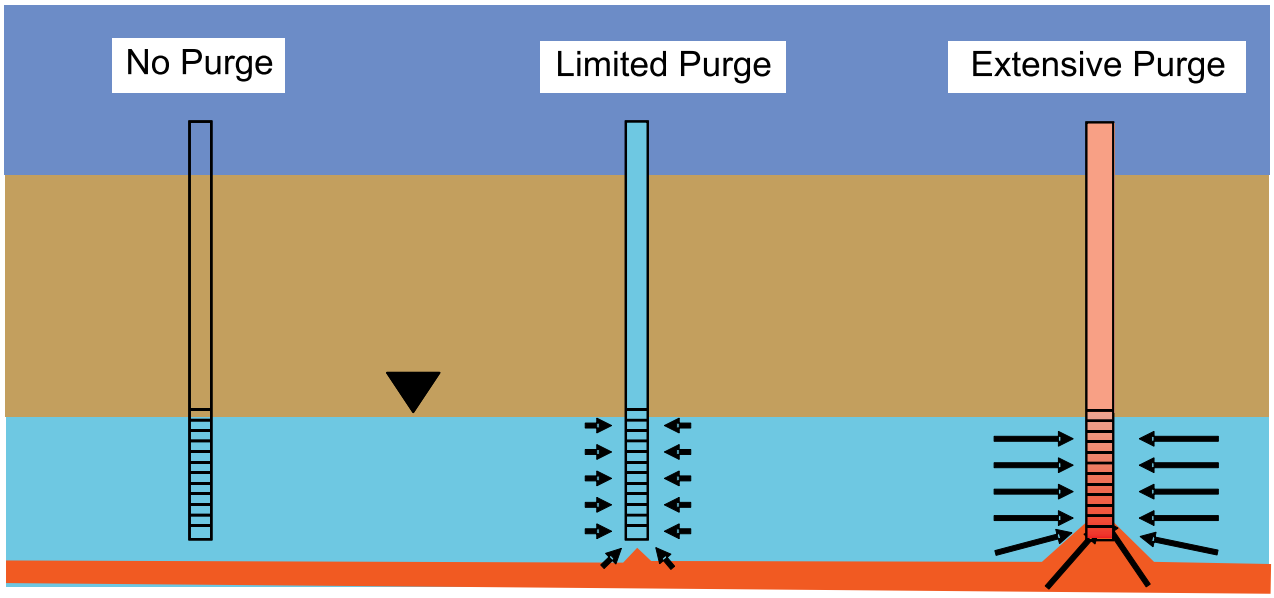

Figure 3.1 Effect of the extent of purging and vertical heterogeneity on concentrations of contaminants sampled by a monitoring well.

hydraulically conductive intervals. The actual physical relationship between water bearing units that are sampled after modest purging and the units sampled after extensive purging will vary from one site to another. However, all aquifers are to some extent heterogeneous, and similar effects can be expected at most sites. The concentrations of contaminants can go up or down as a well is purged more extensively. Aquifer heterogeneity is discussed in more detail in Section 4.5.

To select appropriate wells for concentration and isotope analysis, it is necessary to know the relationship between the screen of the monitoring well and the various units in the aquifer that can yield ground water to the monitoring well. If a well has a short screen that is installed near the center of a transmissive sand and gravel unit, the geochemical parameters will likely stabilize when the well is purged, and the concentrations of contaminants in the well water will be representative of the aquifer unit that is being sampled. If a well has a long screen that extends between layers of sand or gravel and layers of silt or clay, the well will sample different aquifer units that may have different concentrations of contaminants and different values for the ratio of stable isotopes in the contaminants. The water from the well is a composite of the water from the different units, and the relative contribution of the different aquifer units may vary over time as the well is purged. When this is the case, there may be a problem in interpretation of both concentrations and stable isotope ratios in organic contaminants and one should consider the heterogeneities.

When water from a monitoring well comes from several different aquifer units, the ground water often is not in geochemical equilibrium. The water may have measurable concentrations of iron (II) or sulfide, or a low redox potential, but also have oxygen or nitrate concentrations greater than 1 mg/L. Oxygen or nitrate should not occur along with reduced species of iron or sulphur in the same ground water. If they occur together, this is strong evidence that ground waters from aerobic and anaerobic geochemical environments have been mixed together in the monitoring well.

It is difficult to fully avoid contamination of a ground water sample with oxygen from the atmosphere. The simultaneous presence of oxygen and reduced species of iron and sulphur may be an artifact of sampling. However, this is not the case with nitrate. The simultaneous presence of nitrate and reduced species of iron or sulphur is an unequivocal indication that the water produced from the well came from different geochemical environments in the aquifer.

3.2. Need to Replicate Samples

Once the monitoring well has been adequately purged, the sample can be taken into appropriate containers such as small glass vials marketed for Volatile Organic Analyses. These VOA vials have a volume of 40 ml, and are sealed with Teflon®-faced silicone rubber septa secured in place with

screw caps. They are appropriate for water samples that will be prepared for analysis by purge and trap or by headspace extraction.

It is good practice to collect several replicate VOA vials from each monitoring well. This is necessary for the regular measurement of sample replicates as discussed in Section 2.6. The dynamic range of an isotope ratio mass spectrometer is relatively narrow. In order to determine the appropriate concentration for determination of the stable isotope ratio, as recommended earlier, many laboratories will first determine the concentrations of analytes using conventional analytical techniques. If an adequate number of replicate samples are collected, a replicate VOA vial can be opened as needed for each separate analysis, for laboratory duplicates, and to provide a spare sample in case there is an instrument failure and an analysis must be repeated. Any replicates that are not needed for an analysis can be discarded once the necessary data have been collected. In the authors' experience, a minimum of four replicate VOA samples should be acquired from each well sampled. The replicates should be packaged separately. If the samples are shipped to the laboratory for analysis, and the samples are particularly critical, half the replicates should be shipped in one container and half in another.

If the samples are prepared by liquid extraction with pentane or cyclohexane (e.g. samples for analysis of BTEX or PAH), the water can be collected in a larger container to avoid the need to handle small volumes of volatile solvents. A 1-liter glass bottle is convenient. Again samples should be taken in replicate. At least two replicate samples should be acquired from each well.

3.3. Requirement for Sample Preservation

It is the practice in some laboratories to forgo the use of chemical preservatives, and rely on cooling of the sample at 4°C or 10°C to prevent biodegradation of analytes. This practice is not recommended. The ambient temperature of ground water at many sites in the temperate parts of the Earth is only a few degrees warmer than that in refrigerators. The micro-organisms in these aquifers are already acclimated to the lower temperatures. As an example, Bradley and Chapelle (1995) reported that micro-organisms in sediment from a contaminated aquifer at Adak, Alaska metabolized toluene under aerobic conditions with a first order degradation rate near 11% per day at 5°C. Bradley et al. (2005) documented anaerobic reductive dechlorination of trichloroethylene, *cis*-dichloroethylene, and vinyl chloride at 4°C in aquifer sediments and river sediments from Alaska. It is prudent to chemically preserve the samples.

The most widely used preservative is the addition of a solution of 36% hydrochloric acid diluted 1:1 in water to produce a pH < 2 in the sample. For most ground water samples, only three to five drops of the 1:1 dilution are necessary to preserve a 40 ml sample. Purge and trap methods that are approved by U.S. EPA specify that samples be preserved by addition of hydrochloric acid to obtain a pH < 2 (U.S. EPA, 1984; U.S. EPA, 1990; U.S. EPA, 1996).

Hydrochloric acid was generally considered a safe "Universal Preservative" until O'Reilly et al. (2001) reported that methyl tertiary butyl ether (MTBE) was hydrolyzed at pH near 2. Following the recommendation of Kovacs and Kampbell (1999), McLoughlin, et al. (2004) proposed using trisodium phosphate dodecahydrate (TSP) at a concentration of 1% as a preservative for ethers such as MTBE. This concentration of TSP will buffer the water sample to a pH near 10.5. Other alternatives for preservatives include sodium hydroxide at a concentration of 0.1%, sodium azide, and mercury salts. Sodium hydroxide is a useful preservative; however, it may hydrolyze chloroethanes (Jeffers, et al., 1989; Pagan, et al., 1998).

If sodium azide or mercury salts are used as preservative, the preserved water sample becomes a hazardous waste when the analysis is completed. These compounds are not recommended. Hydrochloric acid, trisodium phosphate, and sodium hydroxide act by maintaining the pH in a range that is not tolerated by most micro-organisms. When the analysis is completed, it is a simple matter to neutralize the preservative before the samples are disposed.

Kovacs and Kampbell (1999) demonstrated that several volatile organic compounds sorbed to the Teflon-faced septum used to seal a conventional VOA vial. The sorption of longer chain aliphatic compounds such as 2,3-dimethylpentane, 2,4-dimethylhexane, and 2,2,5-trimethylhexane was substantial. From 20% to 30% of the material originally present would sorb within 21 days storage at 4°C. To prevent sorption to the Teflon-faced septum, they covered the septum with lead foil (3M® Company, Lead Foil Tape 420). The foil was cut into circles with a device used to bore holes in rubber stoppers, and then the circles were attached to the Teflon-face of the septum. To prevent dissolution of the lead foil by hydrochloric acid used as preservative, they preserved the samples with 1% trisodium phosphate dodecahydrate (TSP).

3.4. Performance of HCl and TSP as Preservatives During Storage of Samples

Preservatives prevent biodegradation or abiotic transformation of analytes. If a preservative functions as intended, the concentrations of the analyte of concern will not change during storage. Science can not be used to prove a negative. Experimental trials with preservatives can not be used to prove that a preservative is universally effective. One sample of ground water may have active micro-organisms, or have reactive chemical species such as Fe^{+2} or HS^{-1}, while another does not. There is no way to know whether concentrations of the analytes of concern were stable in a trial because the preservative was effective, or because the samples of water submitted to the evaluation of the preservative did not contain active micro-organisms or reactive chemicals.

Most evaluations of preservatives have been conducted with drinking water. Tap water would not be expected to contain organisms that degrade organic contaminants, or contain reactive chemicals other than chlorine and dissolved oxygen. In particular, tap water would not be expected to have organisms that are capable of anaerobic biodegradation of organic contaminants. To evaluate the performance of hydrochloric acid to pH <2 or 1% trisodium phosphate as preservatives for contaminated ground water, common ground water contaminants were spiked into ground water acquired from monitoring wells at hazardous waste sites. The ground water used in the trial was collected from monitoring wells at a gasoline spill site, an industrial landfill, and a municipal solid waste landfill. Three replicate water samples were analyzed immediately, and three replicate water samples were stored at 4°C for 28 days, and then analyzed. The difference in the average concentrations of the contaminants was evaluated statistically.

For most of the contaminants, the variance in the analytical data made it possible to detect any removal of the contaminant that was greater than 20% of the initial concentration at 95% confidence. If the removal was less than 20% of the initial concentration this was considered as evidence of adequate preservation. It has to be noted that the study may not be representative for all hydrochemical conditions. Depending on specific conditions (e.g. presence of reactive species Fe^{2+}, HS^{1-}), sample alterations may occur even if preservatives are added. Table 3.1 and 3.2 provides a summary of the performance of hydrochloric acid and trisodium phosphate in conserving organic contaminant samples.

If biodegradation in the samples is stopped by the addition of preservatives and appropriate measures are taken to prevent analyte losses by evaporation or decay, then isotope values can be stable over time periods of 1 to 3 months and even longer in some cases. This was shown for BTEX compounds after a holding time of 4 weeks (Hammer et al., 1998) and PCE after a holding time of 4 months (Blessing et al., 2008). However, systematic studies of holding times in CSIA under varying storage conditions have not been published to date. The stability of the compounds in samples collected for CSIA should be carefully evaluated. In the absence of other information, adopt containers, methods of shipping, conditions for storage, and holding times for samples that are intended for CSIA of contaminants in ground water that are the same as the containers, methods of shipping, conditions for storage, and holding times that are acceptable to the regulatory authority for the analysis of concentrations of the contaminants.

Table 3.1. Compounds that are adequately preserved in ground water with Hydrochloric Acid to pH <2 or with 1% Trisodium Phosphate. Less than 20% of the initial concentration should be lost from ground water stored in at 4°C for 28 days in 40-ml VOA vials with a Teflon-faced silicone rubber septum.

Hydrocarbons, alcohols, aldehydes and ethers	**Halogenated compounds**
benzene	methylene chloride
toluene	chloroform
ethyl benzene	carbon tetrachloride
m+*p*-xylene	bromoform
o-xylene	dibromochloromethane
1,2,3-trimethylbenzene	1,1-dichloroethane
1,2,4-trimethylbenzene	1,2-dichloroethane
1,3,5-trimethylbenzene	tetrachloroethene
nitrobenzene	trichloroethene
naphthalene	*trans*-1,2-dichloroethene
methyl tertiary butyl ether	*cis*-1,2-dichloroethene
ethyl tertiary butyl ether	1,1-dichloroethene
tertiary amyl methyl ether	vinyl chloride
di-isopropyl ether	chlorobenzene
tertiary butyl alcohol	1,2-dichlorobenzene
tertiary amyl alcohol	1,3-dichlorobenzene
acetone	1,4-dichlorobenzene
isopropyl alcohol	
2-butanone	
4-methyl-2-pentanone	
1,4-dioxane	

Table 3.2. Compounds that are adequately preserved in ground water with Hydrochloric Acid to pH <2, but are not adequately preserved with 1% Trisodium Phosphate. More than 20% of the initial concentration might be lost from ground water stored in at 4°C for 28 days in 40-ml VOA vials with a Teflon-faced silicone rubber septum.

Hydrocarbons, alcohols, aldehydes and ethers	**Halogenated compounds**
Adequately preserved with Hydrochloric Acid, not adequately preserved with Trisodium Phosphate	
	dibromofluoromethane
	1,2-dibromo-3-chloropropane
	1,2-dibromoethane
	1,1,1-trichloroethane
	1,1,2-trichloroethane
	1,1,1,2-tetrachloroethane
	1,1,2,2-tetrachloroethane
Not adequately preserved with either Hydrochloric Acid or Trisodium Phosphate	
styrene	chloromethane
	bromomethane

3.5. Avoid Isotopically Labelled Surrogate Compounds and Internal Standards

In the analysis of contaminant concentrations, surrogate compounds and or internal standards are typically introduced to the samples during the sample preparation process to allow corrections for losses of analytes and for variability in the response of the instrument which might be caused by factors such as slight differences in the injection volume or in the flow rate of the carrier gas. It is essential that there is base line separation of the peaks of the added surrogate or internal standard and the peaks of any target analyte. This in particular precludes the use of isotopically labelled surrogate compounds and or internal standards, which are common in GC/MS analysis. If such isotopically labelled surrogate compounds are used for analysis of contaminant concentrations, additional samples must be provided for CSIA that did not receive the isotopically labelled surrogates or internal standards.

4.0
Interpretation of Stable Isotope Data from Field Sites

This section is intended for contractors and consultants that will evaluate data on stable isotope ratios, and produce a report for the site manager and the regulatory staff. It is also intended for regulators who will review the report. This section presents a simple equation (the Rayleigh equation) that may be used to predict the extent of biodegradation of an organic compound from changes in the value of the stable isotopic ratio ($\delta^{13}C$ or $\delta^{2}H$). This section discusses conditions that are necessary to apply the Rayleigh equation to predict biodegradation of an organic contaminant in ground water samples from field sites. It discusses the different assumptions that are necessary to calculate the extent of biodegradation, and evaluates situations where the various assumptions are most appropriate. It compares rates of biodegradation extracted from concentration data from monitoring wells to rates of biodegradation extracted from CSIA analyses. The section illustrates the use of CSIA analyses to estimate field-scale rates of biodegradation when it is impossible or misleading to extract the rates from data on attenuation of concentrations. It discusses the effect of heterogeneity of flow and of the rate of biodegradation on stable isotopic ratios, and it provides recommendations to minimize the confounding effect of heterogeneity on the estimate of biodegradation.

4.1. Prerequisites for Application of Isotope Data to Demonstrate and Quantify Biodegradation

Sherwood Lollar et al. (1999) suggested four criteria that must be met to apply CSIA to provide evidence for biodegradation in the field. These original criteria, with two additional criteria, form the basis for the recommendations below and hence will be discussed in some detail.

In the course of many biochemical and abiotic reactions, molecules containing the lighter isotopes exclusively (i.e. ^{12}C) tend to react more rapidly compared to molecules containing the heavy stable isotope (i.e. ^{13}C). As the reaction proceeds, the ratio of stable isotopes in the material that remains behind, in the material that has not gone through the reaction, will therefore change. The more the reaction proceeds the more pronounced the isotope shift in the ratio of ^{13}C to ^{12}C will be. This change in the ratio of stable isotopes is called stable isotope fractionation and can be expressed as the stable isotope fractionation factor alpha (α) as described in Equation 4.1:

$$\alpha = R_a / R_b = \left(1000 + \delta^{13}C_a\right) \Big/ \left(1000 + \delta^{13}C_b\right) \qquad \textbf{4.1}$$

where R is the stable isotope ratio ($^{13}C/^{12}C$) of the compound, and the subscripts *a* and *b* may represent a compound at time zero (t_0) and at a later point (t) in a reaction; or a compound in a source zone, versus a down gradient well. For many organic contaminants, stable isotope fractionation during biotic and abiotic degradation can also often be quantitatively described by the Rayleigh equation (Equation 4.2)

$$R = R_0 \, f^{(\alpha - 1)} \qquad \textbf{4.2}$$

where R is the stable isotope ratio ($^{13}C/^{12}C$) of the compound at time t, R_0 is the initial isotope value of the compound and *f* is the ratio (C/C_0) of the concentrations of the compound at time t and zero.

As discussed in Section 2, the stable isotope ratio is reported in the delta notation, where the ratio is normalized to the ratio in a standard.

Equation 4.2 can be rearranged to produce Equation 4.3 (Section 7 for details)

$$f = e^{(\delta^{13}C_{groundwater} - \delta^{13}C_{source})/\varepsilon)} \qquad \textbf{4.3}$$

where $\delta^{13}C_{groundwater}$ is the measure of the isotope ratio in the organic contaminant in the sample of ground water, $\delta^{13}C_{source}$ is the isotopic ratio in the un-fractionated organic contaminant before biodegradation has occurred, and epsilon (ε) is the stable isotope enrichment factor as defined in Equation 4.4.

$$\varepsilon = (\alpha - 1) * 1000 \qquad \textbf{4.4}$$

The larger the fractionation during the reaction, the more negative is the corresponding value of epsilon. Throughout this Guide we will use the

stable isotope enrichment factor (ε) to make all the data easily comparable.

The next few sections discuss in detail the criteria that must be met to apply CSIA to provide evidence for biodegradation in the field.

4.1.1. Does Biodegradation of the Compound Produce Isotope Fractionation?

For CSIA to be useful, laboratory studies must have demonstrated that significant fractionation does occur during biodegradation (see Table 8.1 for a compendium of information on enrichment factors during biodegradation). While this basic principle has been established for a wide range of organic contaminants (including chlorinated ethylenes and ethanes, aromatic hydrocarbons such as the BTEX compounds, lower molecular weight alkanes, MTBE, TBA, and some PAHs), it is not true for all compounds under all circumstances. For example, high molecular weight petroleum hydrocarbons tend to be isotopically conservative because any fractionation due to biodegradation is generally "diluted" by the large number of non-reactive carbon atoms. Similarly, for some compounds under specific conditions (i.e. aerobic toluene biodegradation) significant carbon isotope fractionation is observed only if the degradation pathway proceeds by reactions that attack the methyl group rather than reactions that attack the benzene ring (Morasch et al., 2002).

4.1.2. Is the Observed Extent of Fractionation Significant?

To be significant, the extent of fractionation must be greater than the total analytical uncertainty. In addition, the observed difference in the values of $\delta^{13}C$ must exceed the spatial and temporal variability introduced by different sources of contamination at the site, by the mixing of ground water flow lines, and by what are typically the minor effects of processes such as sorption or volatilization. As demonstrated in Section 2.4, the total analytical uncertainty for $\delta^{13}C$ analyses is typically ± 0.5‰. As a result, the observed fractionation must be at a minimum > 1‰. To ensure reliable interpretation, we recommend that fractionation on the order of 2‰ be used as a criterion for positive identification of degradation in order to minimize the possibility of an erroneous interpretation. Provided that other causes for the differences in the stable isotope values can be excluded, there is a qualitative indication of biodegradation or transformation along a flow path in ground water when the values of $\delta^{13}C$ in the compounds of interest in the down gradient wells are enriched (less negative) by 2‰ compared to values of $\delta^{13}C$ in the up gradient well.

It is important to appreciate that this criterion of 2‰ will be met at very different levels of biodegradation, depending on the extent of fractionation during degradation of a given compound. For example, due to the large enrichment factors (ε) associated with reductive dechlorination of TCE, observed fractionation exceeds 2‰ at a very early stage of biodegradation, when < 20% is degraded or > 80% is still remaining (Panel A of Figure 4.1). In contrast, for petroleum hydrocarbons such as benzene and toluene, the important but more subtle carbon isotope effects observed during degradation are such that significant fractionation > 2‰ is only discernable when biodegradation has proceeded more extensively and almost 60% of the original contaminant mass has been degraded, as illustrated in Panel B of Figure 4.1 (Ahad et al., 2000; Mancini et al., 2003; Meckenstock et al., 1999; Morasch et al., 2004). Several studies suggest that for compounds with small enrichment factors for carbon, such as the aromatic hydrocarbons, the larger enrichment factors (ε) associated with hydrogen isotope fractionation may make coupling of CSIA for carbon and hydrogen the best approach to identify biodegradation (Fischer et al., 2008; Gray et al., 2002; Mancini et al., 2003; Mancini et al., 2008a).

Once biodegradation is documented in a qualitative fashion, the next step is an evaluation of whether isotopic variation can be used to quantitatively calculate the extent of biodegradation and to derive biodegradation rates based on the CSIA data.

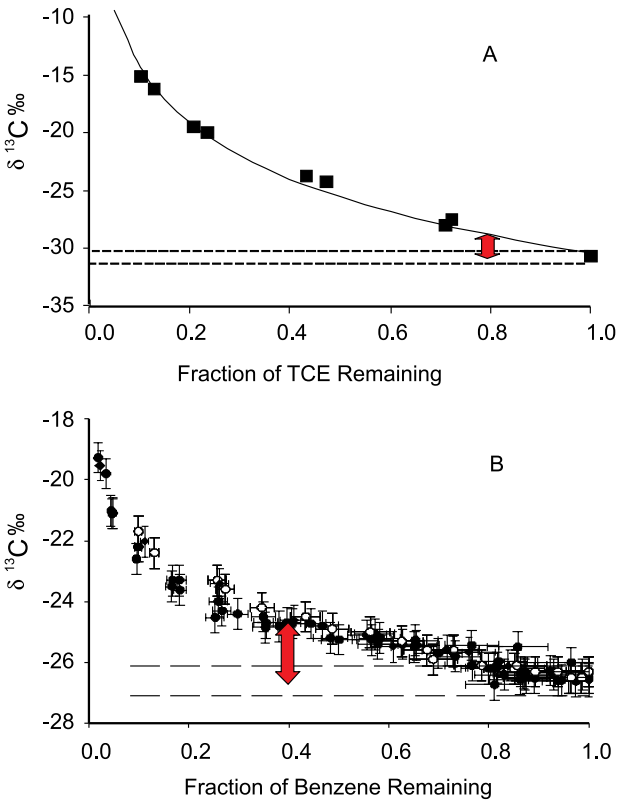

Figure 4.1. Degradation of (A) TCE and (B) benzene by enrichment cultures. The stable carbon isotope ratios in the substrate that remains after biodegradation are plotted against the fraction of the original concentration remaining. Data for TCE degradation are after Sherwood Lollar et al. (1999) and for benzene after Mancini et al. (2003). Dotted lines represent ± 0.5 ‰ around the $\delta^{13}C_0$ value of TCE and of benzene, respectively. The vertical solid red arrow represents the extent of fractionation necessary to recognize biodegradation in field data (2‰).

4.1.3. Is the Observed Fractionation Reproducibly and Accurately Correlated to a Distinct Process?

If fractionation is to be used to predict degradation, the isotopic enrichment factor for a particular contaminant that is degraded by a particular process or pathway must be reproducible from one study to the next. The results of extensive research have shown this criterion to be necessary for valid interpretation of data on $\delta^{13}C$.

Published information on laboratory-derived, compound specific enrichment factors that were determined for biodegradation processes under various redox conditions is available from recent review articles (Elsner et al., 2005; Mancini et al., 2003; Meckenstock et al., 2004; Morrill et al., 2006; Schmidt et al., 2004). Many values are summarized in Table 8.1. Enrichment factors are also available on the internet at www.isodetect.de, and this website will provide updated information over time. The web page is available in either German or English. For the English Language website; select the link for the English Website from the menu, follow the link to *isotope enrichment*, and follow the link to *table Isofrac*. In the experiments listed in Table 8.1, either single strains or mixed bacterial cultures degraded the compounds as the sole carbon source using a single electron acceptor (e.g. oxygen, nitrogen, sulfate). For the same compound and the same biochemical pathway of degradation, the agreement among the enrichment factors determined by the different studies is quite good, reflecting the fact that, to first approximation, the main controlling influence on fractionation is the reaction mechanism (e.g. bond breakage).

For many of the compounds in Table 8.1, different laboratories and different studies report a range of enrichment factors for the same biodegradation process. Table 4.1 below summarizes data from Table 8.1 to compare the total range of values published to date for reductive dechlorination of chlorinated ethenes. Carbon isotope fractionation during reductive dechlorination of chlorinated ethenes is perhaps the most extensively studied system to date, with the values in Table 4.1 reflecting experiments done by a large number of different groups worldwide with a wide range of different microbial consortia and microcosm conditions.

Table 4.1. Ranges of carbon isotope enrichment factors for microbial reductive dechlorination of chlorinated ethenes published in the literature to date (Bloom et al., 2000; Cichocka et al., 2007; Hunkeler et al., 2002; Lee et al., 2007; Slater et al., 2001). See also Table 8.1.

Compound	Range of α values	Range of ε (‰)
TCE	0.9975 to 0.9771	-2.5 to –22.9
cis-DCE	0.9859 to 0.9789	-14.1 to –21.1
VC	0.9785 to 0.9689	-21.5 to –31.1

While variation in the range of published enrichment factors for a given degradation reaction are very important from the point of view of

understanding the details of the reaction mechanism, the variation in published values does not necessarily introduce a large uncertainty into the estimate of the fraction remaining after degradation (*f*) as calculated using Equation 4.2 or 4.3.

For instance, the total analytical uncertainty in measured $\delta^{13}C$ values is typically ± 0.5‰ for carbon CSIA for many of the hydrocarbon contaminants investigated to date. Total uncertainty in (*f*), the fraction of contaminant remaining, is at a minimum the analytical uncertainty associated with typical VOC concentration analyses. While under optimized performance, VOC concentrations can be determined to a precision of ± 5%; typically, commercial VOC analyses are ± 20 to 30%. In the estimate of (*f*) using Equation 4.2, uncertainty in the second or third decimal place in the exponent (α-1) does not contribute as much uncertainty as does the uncertainty in the direct calculation of (*f*) caused by uncertainty in the analysis of VOC concentrations. This can be shown by calculating the propagation of errors for the individual parameters in the Rayleigh equation (Griebler et al., 2004b). More examples and discussion are provided in Section 4.2.3.4a.

Data published to date suggests that the rate of biodegradation does not seem to significantly impact the observed enrichment factor ε (Mancini et al., 2006; Morasch et al., 2001). The dominant controlling parameter on fractionation is the reaction mechanism. As is predicted from theoretical principles of isotope fractionation, degradation pathways or reaction mechanisms can have characteristic stable isotope enrichment factors based on the bonds that are broken. Variations between the stable isotope enrichment factors for one pathway compared to another are one of the most important factors influencing stable isotope fractionation during biodegradation. This principle is a well-established foundation of stable isotope geochemistry, having been demonstrated for microbial methanogenesis via different pathways in a landmark paper in 1985 (Whiticar and Faber, 1985) and elucidated for photosynthesis by C3 versus C4 metabolic pathways more than twenty years ago (O'Leary, 1981). It follows that conditions that control the dominant degradation pathway can control the characteristic fractionation pattern, and the value of the isotopic enrichment factor. For compounds that degrade under different reaction mechanisms under aerobic versus anaerobic conditions, the characteristic isotopic fractionation observed varies with redox conditions. This has been quite extensively studied for MTBE (Hunkeler et al., 2001a; Kolhatkar et al., 2002; Kuder et al., 2002; Kuder et al., 2005; Rosell et al., 2007; Zwank et al., 2005), benzene and toluene (Ahad et al., 2000; Fischer et al., 2007; Fischer et al., 2008; Hunkeler et al., 2001b; Mancini et al., 2003; Mancini et al., 2008a; Meckenstock et al., 2004; Morasch et al., 2001; Morasch et al., 2002; Morasch et al., 2004) and is recently being elucidated for the chlorinated ethenes (Chartrand et al., 2005; Chu et al., 2004). Even under similar redox conditions, if different microbial populations use different degradation pathways, each can result in a reproducible and distinct value for the isotopic enrichment factor, as has been shown for aerobic biodegradation of 1,2-dichlorethane (Hirschorn et al., 2004), aerobic biodegradation of toluene (Morasch et al., 2002), and aerobic biodegradation of MTBE (Rosell et al., 2007).

In most aerobic degradation pathways, the first step is usually an activation of the molecules by an oxygenase reaction to introduce hydroxyl, epoxide or other reactive oxygen-containing groups. For some compounds, there are several types of oxygenase reactions, and the extent of isotope fractionation can depend on the particular oxygenase reaction that is responsible for biodegradation. In the case of aromatic hydrocarbons this may range from undetectable fractionation of stable isotopes of carbon for reactions that are carried out by dioxygenase enzymes that attack the π-electron system of the aromatic ring to strong fractionation caused by reactions carried out by monooxygenase enzymes that attack the ring or methyl groups. Practical recommendations for assessing the uncertainty introduced by the range of available fractionation factors are discussed in detail in Section 4.4.

4.1.4. Do Non-Degradative Processes Influence the Observed Isotope Fractionation?

In order to use CSIA to understand the degradation of contaminants, the isotope fractionation during degradation must be readily discernable from isotope effects associated with other subsurface processes that do not destroy the contaminant, such as volatilization, dissolution and sorption. Isotope fractionation during volatilization (Harrington et al., 1999; Ward et al., 2000); dissolution (Dempster et al., 1997; Hunkeler et al., 2004; Slater et al., 1999; Ward et al., 2000); diffusion (Hunkeler et al., 2004; Bouchard et al., 2008) and sorption (Harrington et al., 1999; Kopinke et al., 2005; Meckenstock et al., 1999; Schuth et al., 2003; Slater et al., 2000) is typically small or is indiscernible outside of the analytical uncertainty typical for CSIA (± 0.5‰ for carbon isotopes; ± 5‰ for hydrogen). During sorption of contaminants to carbonaceous material,

a hydrogen isotope shift of only 8‰ was observed after 95% of the contaminant was sorbed (Schuth et al., 2003). Significant hydrogen isotopic effects were only observed in laboratory experiments where aromatic hydrocarbons underwent near complete vaporization or sorption, in excess of 95% removal (Schuth et al., 2003; Wang and Huang, 2003). Hence, Wang and Huang (2003) noted that large isotopic shifts might be relevant to processes such as air sparging and to studies in the unsaturated zone, but large isotopic shifts are not likely to be significant in most natural systems where extensive mass loss due to volatilization or sorption is unusual. In a recent study documenting carbon isotope fractionation due to diffusion, Bouchard et al. (2008) demonstrated that even in the unsaturated zone where diffusive effects on isotope composition might be expected to be most pronounced compared to the saturated zone, diffusive effects were only observable if measured within a few days of the spill, and where measurements could be done at a very fine spatial scale.

4.1.5. Do Abiotic Degradation Processes Occur and Produce Isotope Effects for the Compound of Interest?

The relative importance of biodegradation versus processes of abiotic degradation at the site must be considered. In the past few years, the principles of Rayleigh controlled isotope fractionation of organic contaminants in ground water have been shown to apply to abiotic degradation as well as biodegradation (Bill et al., 2001; Elsner et al., 2007a; Elsner et al., 2008; Hofstetter et al., 2008; Slater et al., 2002; VanStone et al., 2004; VanStone et al., 2008; Zwank, et al., 2005). Zero valent iron is widely used in active remediation of ground water contamination. While much research is still underway to understand the precise reaction mechanisms associated with degradation of chlorinated ethenes on zero valent iron, CSIA indicates that the mechanisms are similar to the familiar mechanisms associated with biodegradation, and that different abiotic degradation mechanisms are associated with different characteristic patterns of fractionation.

Traditionally, rates of natural abiotic degradation in ground water were thought to be insignificant unless they were enhanced through abiotic remediation schemes such as the addition of zero valent iron. This view is changing. There have been several recent studies of the role of abiotic reactions with minerals and the role of microbially-mediated abiotic reactions at field sites (Bradley and Chapelle, 1997; Butler and Hayes, 1999; Cervini-Silva et al., 2001; Ferrey et al., 2004; Lee and Batchelor, 2002; McCormick and Adrians, 2004).

The possibility of abiotic degradation introduces the challenge of distinguishing between the effects of abiotic and biotic isotopic fractionation in any system where both types of degradation may be significant. Liang et al. (2007) noted that the isotope fractionation during abiotic degradation of PCE and TCE by FeS was much greater than the fractionation during anaerobic biodegradation of PCE and TCE. Reduced iron sulfides such as FeS can be an important component of aquifer sediments at hazardous waste sites. Liang et al. (2007) warn that the use of an enrichment factor appropriate for biodegradation instead of the factor appropriate for the abiotic mechanism may overestimate the true extent of degradation at field scale. A similar pattern of smaller biological enrichment factors compared to abiotically-mediated degradation has been identified for MTBE and 1,1,1-TCA (Elsner et al., 2007a; 2007b) and PCE (Lee et al., 2007; Nijenhuis et al., 2005; Slater et al., 2001; Slater et al., 2003), suggesting that additional rate-limiting factors in biochemical reactions require more in depth research. VanStone et al., (2008) and Elsner et al., (2008) discuss the potential of using CSIA to distinguish between abiotic and biodegradation processes where both types of processes may be important.

4.1.6. Is the Rayleigh Equation an Appropriate Model to Describe the Data Set?

For compounds that are intermediates in degradation pathways, such as the products of reductive dechlorination of chlorinated ethylenes, a straightforward application of the Rayleigh equation (Equation 4.2) is not strictly possible. The isotope ratio in the intermediate compound will change due to the combined effects of isotopic fractionation during its production from the parent compound and isotopic fractionation due to its own continuing degradation. There is one important exception. The Rayleigh equation can be used when complete transformation of the parent compound occurs prior to further degradation of the intermediate compound (Morrill et al., 2005).

When production and degradation of the intermediate compound occurs simultaneously, a more complex isotope evolution occurs that can be evaluated using multistep reactive transport models (van Breukelen et al., 2005; Morrill et al., 2006). Quantitative information on biodegradation can be obtained by fitting an analytical model (Beranger et al., 2005) or numerical model (van Breukelen,

et al., 2005) that describes the isotope evolution during sequential processes to the measured isotope data. Van Breukelen et al. (2005) used a simple one dimensional model to provide insight in the rates of transformation of parent and intermediate compounds. The simulation of different degradation scenarios such as various degrees of degradation or different relative rates of biodegradation for different steps in a multi-step process can also be very useful as a benchmark for a semi-quantitative interpretation of isotope data.

For certain chlorinated solvents the situation is even more complex because the degradation pathways of different compounds can converge and produce the same daughter products (Kirtland et al., 2003). For example, trichloroethylene (TCE) can be produced from biological reductive dechlorination of tetrachlorethylene (PCE), or through an abiotic reaction from 1,1,2,2-tetrachlorethane. If several potential parent compounds are present in ground water at the same time, it is difficult to interpret the behaviour of the compound from CSIA. Due to these complexities, the conceptual model for biodegradation at a site should distinguish those compounds that are only present as parent compounds from those compounds which might be present both as parent and daughter compounds. See for example Hirschorn et al., (2007). Section 4.2 covers the appropriateness of a Rayleigh model in more detail with respect to field data.

4.2. Recommended Steps for the Quantification of Biodegradation Based on CSIA

4.2.1. Site Characterization

Use of CSIA is no silver bullet and will be most useful and cost effective when applied within the context of the hydrological, geological, geochemical and microbiological parameters at the site. The factors that affect contaminant transport and degradation over time as well as space must be identified and evaluated. This includes the important geohydrological parameters (ground water flow direction, hydraulic conductivity, hydraulic gradient) and geochemical conditions (concentrations of oxygen, nitrate or sulfate within the plume). Ultimately, a conceptual site model can be developed that will reveal practicable remediation goals that are capable of protecting existing or potential receptors from contamination. Iterative generation and interpretation of field data from a general survey is necessary to identify the major compartments of the plume (the source, the fringe, the center line, and the mixing zones) as well as the most relevant processes that contribute to natural attenuation.

4.2.2. Evaluate Field Data for the Fit to the Rayleigh Model

The Rayleigh model (Equation 4.2 and 4.2) predicts that a plot of $\delta^{13}C$ or $\delta^{2}H$ on the logarithm of the concentration remaining should be a straight line. If field data are plotted as described above, and the data follow a straight line, then a single process for biodegradation or abiotic transformation likely controls the concentrations at field scale, as illustrated in Figure 4.2. This is called a Rayleigh correlation. Dilution, dispersion, sorption, volatilization, and mixing between contaminant sources with different values of $\delta^{13}C$ or $\delta^{2}H$ will cause the data to fall off of the straight line.

Given the importance of dilution and dispersion at field scale, it might intuitively seem likely that no set of realistic field data would show a Rayleigh correlation. However, case studies and evolving field experience have in fact shown that a significant number of sites do have field data that fit the Rayleigh model (Abe and Hunkeler, 2006; Griebler et al., 2004b; Kolhatkar et al., 2002; Morrill et al., 2005). The existence of such a correlation indicates that biodegradation or abiotic transformation is the significant process that controls changes in concentrations of contaminants.

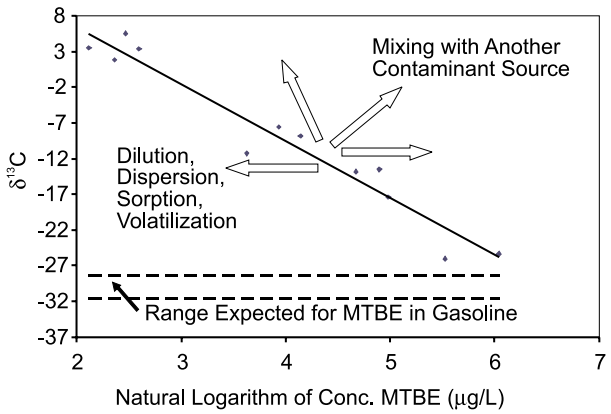

Figure 4.2. Testing field data on CSIA and concentrations of contaminants for fit to the Rayleigh equation. Deviations from a straight regression line in the plot of $\delta^{13}C$ on the natural logarithm of concentration can indicate that processes other than degradation control the concentrations of contaminants. Example data plotted for Table 1 of Kolhatkar et al. (2002). The dotted lines bound the values of $\delta^{13}C$ that are expected for MTBE that was blended into gasoline.

The first recommendation for using CSIA to quantify biodegradation is to plot the $\delta^{13}C$ of the compound against the natural logarithm of the concentration of the compound to determine if these parameters show a Rayleigh correlation as illustrated in Figure 4.2. This "test" is simply the first step in determining if the Rayleigh controlled fractionation inherent in Equation 4.2 is an appropriate model for the site. There is no need to take the location of the respective wells into account on this level when performing the Rayleigh analysis because the location does not influence the calculation. However, data points that drastically fall of the straight regression line can be identified and might be evaluated further for other processes that influence the compound apart from biodegradation, such as dilution in the monitoring well, dispersion along the flow path, or volatilization. A strong correlation to the Rayleigh model adds considerable confidence to the application of CSIA data to understand the behaviour of a contaminant at a site.

Variations in the length and elevation of the screened interval of monitoring wells can cause a well to produce ground water that is either dominated by the plume of contamination, or cause the well to produce water that has a small contribution from the plume and a major contribution from clean ground water above or below the plume. Details of well construction can have a strong effect on the concentration of the organic compound in water produced by the well. A poor correlation to the Rayleigh model may be due to these incidental perturbations in the concentration that are created by the monitoring wells. As a result, a poor correlation does not automatically disqualify a site for the application of CSIA to understand the transformation processes.

4.2.3. Determination of the Primary Isotope Signature ($\delta^{13}C_{source}$ or $\delta^{2}H_{source}$)

The primary isotopic signature is the isotopic ratio of the organic contaminant of concern before it is fractionated by biodegradation processes or abiotic transformations. The ideal approach would be to measure the isotopic signature of the primary contaminant that was spilled at the site. However, this is rarely feasible. Nor is measurement of $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$ for the most recent spill necessarily relevant at many sites where there has been a history of multiple spills or leakage.

There are three basic approaches to determination of $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$. One approach compares values of $\delta^{13}C$ or $\delta^{2}H$ for contaminants in ground water to values of $\delta^{13}C$ or $\delta^{2}H$ reported in the literature. The second and third approaches are entirely site specific. They compare $\delta^{13}C$ or $\delta^{2}H$ for contaminants in different samples of ground water to determine the extent of degradation between points in space (between different wells) or points in time (temporal variation within a single well) at a specific site.

4.2.3.1. Value of $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$ Based on Literature.

In the routine case where samples of the actual spilled material are neither available nor relevant, the approach is to make an assumption for $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$ based on published values in the literature for undegraded pure product. This is not unreasonable for petroleum hydrocarbons, or for anthropogenic compounds such as chlorinated ethenes produced from petroleum hydrocarbon feedstocks, because the range of $\delta^{13}C$ for petroleum hydrocarbons is well characterized and relatively well constrained. As degradation proceeds, a point is reached where the value of $\delta^{13}C$ or $\delta^{2}H$ may be more positive (more enriched in ^{13}C or ^{2}H) than any reported value from commercially available products. When the value of $\delta^{13}C$ or $\delta^{2}H$ in the field is more positive than the range in the pure substance, degradation at the site is evident (compare Figure 4.2).

4.2.3.2. Values of $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$ Based on Most Negative Value at the Site

Because biodegradation induces a shift of the residual compound to less negative values of $\delta^{13}C$ or $\delta^{2}H$, the most negative values measured for the organic contaminant in ground water at the site can be the best estimate of the original values of $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$. While this approach can work well for compounds for which the fractionation due to biodegradation is large (tens of ‰) relative to the variation in assumed $\delta^{13}C_{source}$, the approach is not recommended for compounds such as benzene and toluene for which the error in the assumption of $\delta^{13}C_{source}$ will be large with respect to a relatively small changes in $\delta^{13}C$ caused by biodegradation.

4.2.3.3. Values of $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$ Based on Point to Point or Time to Time Comparisons

Quantifying the relative amount of biodegradation between wells, or in a given well over time, is compelling since it involves fewer assumptions than the literature-based approach. It does, however, require a good hydrogeological and geological understanding of the site. In this approach, one can select wells for $\delta^{13}C_{source}$ or $\delta^{2}H_{source}$ that sample the known

source zone. As an example, the wells might be screened across an interval with non aqueous phase liquids (NAPL) that act as the source of ground water contaminant. Wells in the source area would be expected to produce water with the highest concentrations of contaminants. Since biodegradation produces more enriched (less negative) $\delta^{13}C$ values, such wells may be assumed to represent the least degraded material at the site.

It is important to note that this approach will provide a conservative estimate of the extent of biodegradation. If undegraded compound is in fact being added to the plume through mixing, desorption, or continued dissolution of NAPL, the addition of this more isotopically negative $\delta^{13}C$ material will minimize the observed fractionation effects produced by biodegradation (Abe and Hunkeler, 2006, Morrill et al., 2005). While continued dissolution of NAPL close to the source zone may result in a complete suppression of the fractionation signal of biodegradation, the calculation can at least provide a conservative upper boundary on C/Co. The true fraction remaining may be less than the estimate.

The most thorough approach would be to calculate the extent of biodegradation using all three approaches for determining $\delta^{13}C_{source}$. If the three estimates agree, the extent of biodegradation is well constrained. In several case studies this was indeed the situation because the source well $\delta^{13}C$ values were not only the most negative $\delta^{13}C$ values at the site, but they were within the published range for undegraded pure product (Sherwood Lollar et al., 2001).

4.2.3.4. Selection of an Appropriate Enrichment Factor

This Guide assumes that the isotope enrichment factors derived from laboratory microcosm studies are applicable to the field. In contaminant hydrology, the removal of organic contaminants in traditional laboratory microcosm studies is commonly used to predict the removal in field scale plumes. The assumptions made in extrapolating isotope enrichment factors to the field are equivalent to the assumptions made in extrapolating data on contaminant degradation from laboratory microcosm studies to predict the behaviour of a plume at field scale.

As discussed in Section 4.1.3, there are two important sources of uncertainty in extrapolation of enrichment factors. The value of the enrichment factor is sensitive to the biodegradation pathway (and hence to parameters such as redox conditions and microbial populations) and to the reproducibility of fractionation factors under any given set of conditions for any given biodegradation reaction pathway. The selection and evaluation of enrichment factors from the literature is a two step process. First, use site specific data on geochemical parameters to determine the most probable pathway for metabolism (or abiotic transformation) of the contaminant at field scale. Then search the literature (Table 8.1) for published enrichment factors for the compound of interest under the relevant redox conditions.

The variation in published enrichment factors for a given set of conditions is a measure of the reproducibility of the enrichment factor. One option to deal with the variation in published enrichment factors is to select the largest enrichment factor in the literature to estimate the extent of biodegradation at field scale. In this case, the "largest" enrichment factor is the most negative factor, the factor with the largest absolute value. For a given change in the value of $\delta^{13}C$ or $\delta^{2}H$, the largest enrichment factor will predict the largest value for the fraction remaining after biodegradation and will predict the smallest extent of biodegradation. As a result, the largest value for the enrichment factors will provide the most conservative estimate of the extent of biodegradation. When the difference in values of $\delta^{13}C$ between the source and the down gradient monitoring wells is small (2‰ to 5‰), the value selected for the enrichment factor can have a stronger influence on the extent of biodegradation predicted from Equation 4.3.

A second option to deal with variation in the published values of the enrichment factors is to calculate a lower boundary on the extent of biodegradation using the highest published enrichment factor, an upper boundary using the lowest published enrichment factor, and a best estimate of bioremediation using the mean of all the enrichment factors, then compare the predictions of the extent of bioremediation. When this approach was applied to data from studies of bioremediation of TCE and *cis*-DCE at spill sites at Dover Air Force Base in Dover, Delaware, USA and at Kelly Air Force Base in San Antonio, Texas, USA, the difference between the upper and lower boundaries on the extent of biodegradation was small (Morrill et al., 2005, Sherwood Lollar et al., 2001).

A third option is to calculate the mean and the standard deviation of the enrichment factors, and then use statistical techniques to estimate propagation of error to determine the effect of the variation in published values for the enrichment factor on the estimate of the extent of biodegradation.

Reactions with large fractionation factors (ε more than an absolute value of 3‰) allow a more sensitive quantification of biodegradation, while reactions with small fractionation factors (ε smaller than an absolute value of 1‰) require a large degree of biodegradation (>90%) before a significant isotopic difference between source and monitoring wells can be resolved (Ahad et al., 2000). As a general principle, as the difference between $\delta^{13}C$ and $\delta^{13}C_{source}$ becomes larger, the uncertainty in the calculation of the extent of biodegradation becomes smaller.

Figure 4.3 compares the relative effect of the value of the isotopic enrichment factor, and the value of $\delta^{13}C_{source}$, on the predicted extent of biodegradation. When the value of $\delta^{13}C$ is close to the value of $\delta^{13}C_{source}$, the estimate of the extent of biodegradation is more sensitive to the value of $\delta^{13}C$. When the value of $\delta^{13}C$ is further away from the value of $\delta^{13}C_{source}$, the estimate of the extent of biodegradation is more sensitive to the value of the enrichment factor ε.

One may be tempted to use fractionation data from a contaminated field site to determine implicit isotope enrichment factors. Although some scientific studies have practiced this approach (Steinbach et al., 2004), it cannot be recommended as a general procedure. The complexity of hydrogeological and microbial processes in the field will give only a crude estimate of the enrichment factor compared to well-controlled laboratory experiments and will certainly introduce additional uncertainty. Therefore, it is advisable to take appropriate laboratory-derived enrichment factors from the literature.

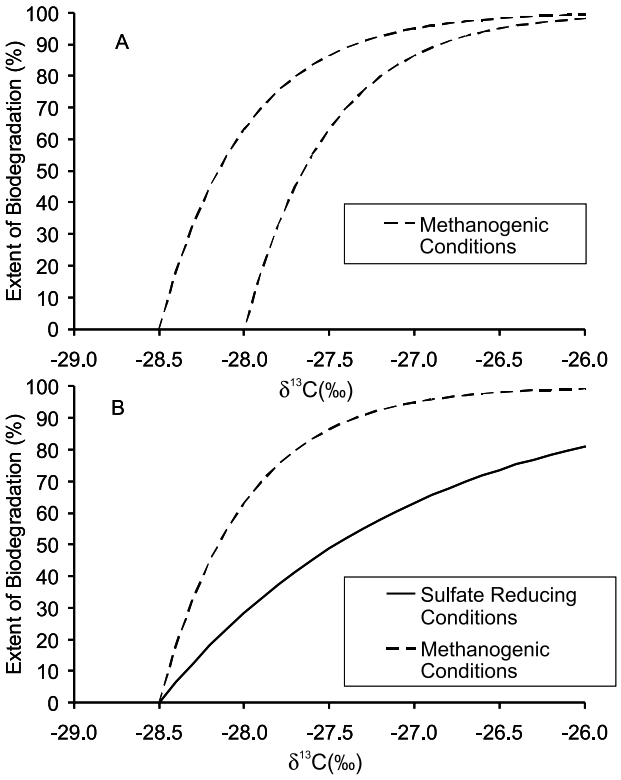

Figure 4.3. Relative influence of different values for $\delta^{13}C_{source}$ (Panel A) and different values for the isotopic enrichment factor ε (Panel B) on the calculated extent of toluene biodegradation. The extent of biodegradation is expressed in percent of the material originally present, calculated as B = (1-*f*), where *f* is the fraction remaining as calculated from Equation 4.3. The dashed lines are estimates of the extent of biodegradation from $\delta^{13}C$ for biodegradation of toluene under methanogenic conditions where ε = -0.5‰ with two different values for $\delta^{13}C_{source}$. The solid line is an estimate of the extent of biodegradation under sulfate-reducing conditions where ε = -1.5‰.

4.2.3.5. Estimating an Enrichment Factor when none is Available.

Although the literature on isotope enrichment factors is expanding rapidly, there may be occasions when an isotopic enrichment factor for a particular compound is not available in the literature. The following material describes an approach that may be used to estimate an isotopic enrichment factor from the data available for similar compounds.

Stable isotopic fractionation occurs at a distinct chemical bond within a molecule, where the enzymatic reaction takes place. A heavy isotope at an adjacent position might still affect the reaction but to a much lower extent (referred to as a secondary isotope effect) and can usually be neglected. Heavy atoms further distant from the reactive position have no influence on isotope fractionation. As a first approximation, only the atom in the reactive position of the molecule undergoes isotope fractionation. However, in CSIA the isotopic composition of all of the atoms of a respective element in the molecule is measured (e.g. all carbon atoms).

The stable isotope effect is therefore "diluted" by the number of atoms at non-reactive positions of a compound. One can distinguish between the intrinsic isotope enrichment factor (ε_i) which considers only the isotope shifts at the reactive position and the overall isotope enrichment factor (ε) which determines the isotope fractionation of the entire molecule. Details of this approach can be found in Elsner et al. (2005) and Morasch et al. (2004). The relation of (ε_i) and (ε) follows Equation 4.5, where (n) is the total number of atoms of a particular element in the molecule.

$$\varepsilon = \varepsilon_i / n \qquad \textit{4.5}$$

From the stable isotope enrichment factors and the intrinsic factors published for anaerobic or aerobic degradation of mineral oil constituents and chlorinated solvents it is apparent that CSIA can be successfully applied to recognize isotope fractionation in compounds with no more than twelve to thirteen carbon atoms. For larger molecules, the expected isotope shifts will be so strongly diluted that they fall into the range of the experimental error of the isotope analysis (Morasch et al., 2004).

Expressing fractionation as the intrinsic enrichment factor (ε_i) reveals that the same biochemical reactions produce similar intrinsic enrichment factors for different compounds. Anaerobic degradation of BTEX compounds and methylnaphthalene provide a good example. The primary enzyme reaction in the anaerobic degradation pathways of methylated aromatic hydrocarbons (toluene, xylene, methylnaphthalene) is always a fumarate addition to the methyl group by glycyl radical enzymes. The intrinsic carbon isotope enrichment factors have been shown to be similar (Morasch et al., 2004).

If there is no published value for the isotope enrichment factor (ε) for a compound, but the biochemical reaction of the primary degradation step is known, it should be possible to use literature values for the intrinsic enrichment factors (ε_i) of similar compounds to estimate an isotope enrichment factor (ε) for the compound. Such estimates have been shown to be in the same range of accuracy as those obtained from laboratory experiments with the respective compounds (Meckenstock et al., 2004; Morasch et al., 2004; Zwank et al., 2005).

As an example, a representative carbon isotope enrichment factor for toluene which can be taken from the literature is -1.7‰. As toluene contains 7 carbon atoms the intrinsic enrichment factor ε_i for the reactive carbon position is -1.7‰ * 7 = -11.9‰ (Table 8.1). Imagine that we require an enrichment factor for xylene. Because the initial reaction of the degradation pathway of xylene is similar to toluene degradation we will make an assumption that the intrinsic enrichment factor ε_i for xylene is the same as for toluene (ε_i = -11.9‰). For the overall enrichment factor ε we divide the estimate of ε_i by 8 (xylene contains 8 carbon atoms) to produce an estimate for the enrichment factor of -11.9‰ / 8 = - 1.5‰. This estimate is exactly equivalent to the only value that is available in the literature for a pure culture study of the anaerobic biodegradation of xylene (Morasch et al., 2004; Table 8.1). However, Table 8.1 also reveals that the variation of fractionation factors determined for anaerobic xylene degradation is quite large.

4.2.3.6. Concurrent Application of CSIA Analysis for Different Elements (Two-Dimensional Analysis).

For some contaminants, such as MTBE and benzene, there is a fundamental difference in the enzymatic mechanism for biodegradation under aerobic and anaerobic conditions, and the difference in enzymatic mechanism is reflected in a large difference in the values of the enrichment factors (ε) under aerobic or anaerobic conditions. As discussed in Section 4.1.5 and Section 4.2.3.4, depending on the compound, different values of ε may have an effect on the predicted extent of biodegradation, and the implications should be investigated as in the example in Figure 4.3.

If field measurements of $\delta^{13}C$ are to be used to estimate the extent of degradation, it is necessary to know the mechanism of degradation to be able to select the correct value of ε. Frequently it is not possible from conventional site characterization data to unequivocally associate biodegradation with either the aerobic mechanism or the anaerobic mechanism. However, it may be possible to identify the mechanism of degradation from the concurrent enrichment of both carbon and hydrogen isotopes. Kuder et al. (2005) compared the enrichment of carbon and hydrogen during biodegradation of MTBE in anaerobic microcosms and in field samples from gasoline spill sites in the USA. In a plot of δ^2H for MTBE against $\delta^{13}C$ for MTBE, the data from the field sites had the same distribution as the distribution of the data from the anaerobic microcosm study (Figure 4.4).

Zwank et al. (2005) made the same comparisons of δ^2H against $\delta^{13}C$ for MTBE contamination in ground water at a former industrial landfill in South America, and established that MTBE degraded under anaerobic conditions at that site as well. Zwank et al. (2005) applied the term "two-dimensional analysis" to describe the concurrent CSIA

for both carbon and hydrogen, and offered the approach as a useful tool to distinguish the pathway of biodegradation of MTBE in ground water at field scale.

The dotted line in Figure 4.4 projects the values of δ^2H and $\delta^{13}C$ for MTBE that would be expected from values of ε_C of -2.4‰ and ε_H of -30‰. These values are the extremes in the range reported in Gray et al. (2002) for aerobic biodegradation of MTBE by strain PM1 or mixed cultures that resembled PM1 in their behavior. These organisms degrade MTBE by oxidation of the methyl group with an oxygenase enzyme. Because oxygenase enzymes act by extracting a proton from the methyl group, there is a very strong enrichment of deuterium in the residual MTBE. To provide the most conservative estimate in Figure 4.4, the projections of δ^2H and $\delta^{13}C$ expected in MTBE in ground water start from the most positive values of δ^2H and $\delta^{13}C$ determined in MTBE in gasoline as reported in Kuder et al. (2005).

The actual distribution of δ^2H against $\delta^{13}C$ for MTBE at field scale was very different than the distribution that would be expected from aerobic biodegradation of MTBE. The actual distribution of δ^2H corresponds to ε_H of -11.5‰. Zwank et al. (2005) reported an estimate of ε_H of -15.6‰ at the site in South America. The actual distribution of $\delta^{13}C$ corresponds to a value of ε_C in the range -8.9‰ to -10.2‰.

The first step in anaerobic biodegradation of MTBE is hydrolysis of the ether bond (Kuder et al., 2005; Zwank et al., 2005). In the hydrolysis reaction, there is strong enrichment of ^{13}C in the carbon atoms involved in the ether bond. Because the hydrogen atoms are not directly involved, there is much less fractionation of hydrogen.

Rosell et al. (2007) compared the distribution of δ^2H against $\delta^{13}C$ for MTBE during aerobic degradation by two cultures that metabolized MTBE through a different pathway that involves attack on the ether bond. The value of ε_H was -0.2‰ for strain L108 and +5‰ for strain IFP2001. In these organisms, the values of ε_H are much lower than is the case for organisms like PM1. Enrichment of δ^2H and $\delta^{13}C$ during aerobic biodegradation by these organisms is projected as the solid line in Figure 4.4. The values used in the projection were ε_C of -1.48‰ and ε_H of -0.2‰. There was considerable overlap of the field data of Kuder et al. (2005) and plausible values of δ^2H and $\delta^{13}C$ that would be expected from aerobic biodegradation of MTBE by organisms similar to strains L108 and IFP2001. As a consequence, Rosell et al. (2007) warn against uncritical comparison of δ^2H and $\delta^{13}C$

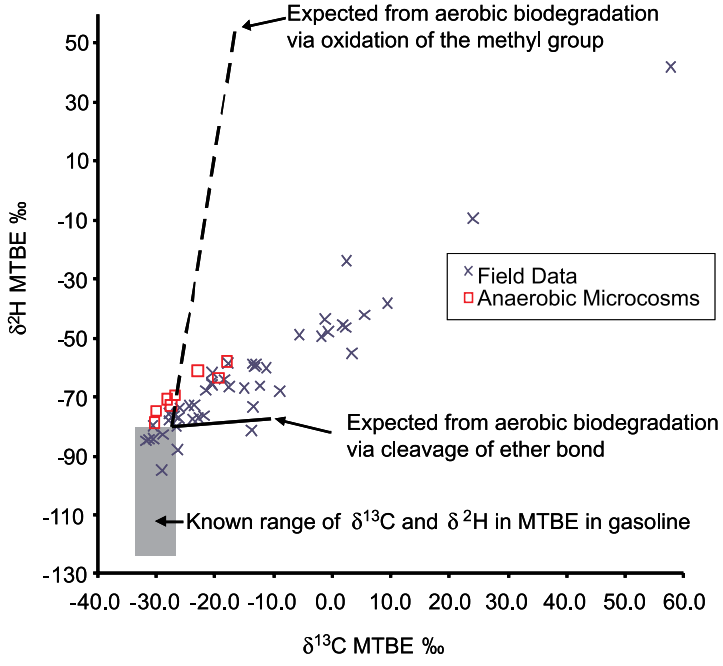

Figure 4.4. Concurrent analysis of $\delta^{13}C$ in MTBE and δ^2H in MTBE in ground water to associate natural biodegradation of MTBE in ground water with an anaerobic process, which allows the selection of an appropriate value for the enrichment factor (ε) to be used to estimate the extent of biodegradation of MTBE.

in MTBE in the field to infer the primary pathway for biodegradation.

The length of the solid line in Figure 2.4 is the range of values of δ^2H and $\delta^{13}C$ that would be expected in MTBE when MTBE is degraded from an initial high concentration of 100,000 µg/L to 1 µg/L. Only 5% of gasoline spill sites in the USA have initial concentrations of MTBE above 100,000 µg/L, and 1 µg/L is the lower limit for determination of δ^2H and $\delta^{13}C$ in MTBE in water samples. The solid line in Figure 4.4 represents the plausible range of δ^2H and $\delta^{13}C$ that would be expected during aerobic biodegradation of MTBE by organisms similar to strains L108 and IFP2001. By examination of Figure 4.4, and allowing for uncertainty in the estimation of δ^2H of 10‰ and $\delta^{13}C$ of 0.5‰, the two dimensional approach proposed by Zwank et al. (2005) can be used to distinguish anaerobic biodegradation of MTBE whenever the value of δ^2H in MTBE in the field sample is more positive than -67‰ and $\delta^{13}C$ is more positive than -9‰.

Similar success has been reported recently for determining benzene biodegradation pathways (Fischer et al., 2007; Fischer et al., 2008; Mancini et al., 2008a). The approach will find wider use, and have more validity, as more data are available on the concurrent enrichment of δ^2H and $\delta^{13}C$ in organic contaminants by different microorganisms under different geochemical conditions.

4.3. Conversion of Calculated Extent of Biodegradation (1-f) to Biodegradation Rates

At many hazardous waste sites, mathematical models are used to predict the transport of contaminants in ground water from source areas to potential receptors such as drinking water wells. These models are calibrated using estimates of the rate of biodegradation of the contaminant in ground water. Most commonly the rates of biodegradation are extracted from field monitoring data. These conventional approaches compare changes in concentration of the contaminant with travel time along a flow path in an aquifer.

One valuable application of CSIA is an independent evaluation of the rates of biodegradation of contaminants. Section 7.3 derives equations that can be used to calculate the rates of biodegradation or abiotic transformation at field scale from an estimate of the fraction remaining after biodegradation (C/Co) and from some assumptions about flow paths and ground water flow rates for the site. This approach combines the uncertainty in the estimates of the hydrogeological parameters with any uncertainties in the estimate of the extent of biodegradation based on CSIA and Equation 4.3. Nonetheless, several recent case studies have shown good agreement between biodegradation rates extracted from isotope studies and rates derived by conventional approaches that are based on changes in concentrations in monitoring wells along a flow path in the aquifer (van Breukelen et al., 2005; Fischer et al., 2006; Hirschorn et al., 2007; Morrill et al., 2005). A key point to emphasize is that CSIA typically provides a more conservative estimate of the degradation rate compared to the conventional approach (Abe and Hunkeler, 2006; Chartrand et al., 2005; Morrill et al., 2005).

4.4. Using Estimates of Rates of Biodegradation to Predict Plume Behaviour

In the conventional approach, the extent of removal along a flow path is estimated by dividing the concentration of contaminant in a down gradient well (C_t) by the concentration in an up gradient well (C_0). Often at field scale, monitoring wells are screened vertically across plumes, and produce samples of the contaminated plume that are diluted with clean water from above or below the plume. Occasionally a well will only sample the top or bottom of a plume. In this case the apparent attenuation of concentrations of contaminants has a strong component of dilution, and data on concentrations cannot be used in the conventional approaches to estimate the extent of removal.

Fischer et al. (2006) provided an approach for solving this problem by taking the concentration that is actually measured in the down gradient well C_t and the measured values of $\delta^{13}C$ in the two wells, to calculate a theoretical value for C_0 using the Rayleigh equation. The difference between the calculated theoretical value of C_0 and the measured value of C_t provides an estimate of the amount of compound that was degraded that is independent of dilution or other non destructive processes that can lead to a reduction of the contaminant concentration (Fischer et al., 2006). Because the estimate of the extent of biodegradation provided by CSIA is independent of the concentration of the contaminant in the ground water sample, the extent of biodegradation from the CSIA analyses and the estimated travel time from the source of contaminant to a well can be used to estimate the rate of biodegradation along the flow path.

The behaviour of contaminants in most plumes is heterogeneous, with extensive biodegradation

in some regions and little or no biodegradation in others. When a plume is heterogeneous, it is best to consider the behaviour of the contaminant in each flow path, instead of trying to predict the average behaviour of the entire plume. The approach will be illustrated with data from a plume of MTBE from a gasoline spill at a site in Dana Point, California, USA (Figure 4.5). Additional details of this case study are described in section 6 of an EPA report (Wilson et al., 2005a).

The direction of ground water flow for separate rounds of sampling is presented as flow arrows in Figure 4.5. The length of each arrow is proportional to the distance ground water would move in one year under the hydraulic gradient during that particular round of sampling. The length was calculated by multiplying the hydraulic gradient by the average hydraulic conductivity (11 meters per day), then dividing by an estimate of porosity (0.25).

After the spill of gasoline was discovered, the leaking underground storage tanks and most of the surrounding fill material were excavated. However, residual gasoline in the aquifer acts as a continuing source of MTBE in ground water. The highest concentrations of MTBE are immediately down gradient of the underground storage tanks (Figure 4.5). A second source is associated with the distribution lines to the south-eastern dispenser island.

Table 4.2 compares the concentrations of MTBE in selected monitoring wells to the fraction of MTBE remaining as predicted from Equation 4.3 using the $\delta^{13}C$ of MTBE in the ground water in each well and a value of -27.4‰ for the $\delta^{13}C$ that would be expected for MTBE in gasoline. This value is the most positive $\delta^{13}C$ value that has been published for MTBE in gasoline (O'Sullivan et al., 2003). To be conservative, the most negative enrichment factor available in the literature was used in the calculations (ε = -14.6‰; Somsamak et al., 2006). This approach provided the most conservative estimate

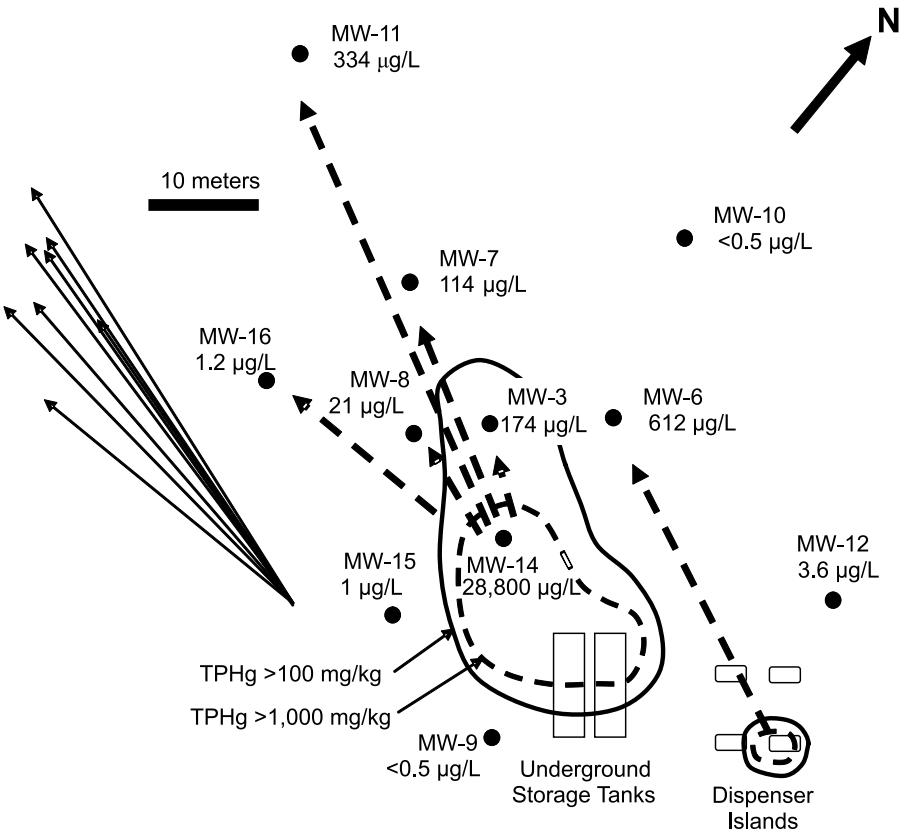

Figure 4.5. Concentration of MTBE in selected monitoring wells at a gasoline spill site in Dana Point, California, USA in 2004. The cluster of arrows is a flow rose indicating the direction and distance ground water would move in one year based on the elevation of the water table in monitoring wells on particular sampling dates. The dashed arrows indicate possible flow paths between wells. Concentrations are MTBE in ground water. TPHg is the Total Petroleum Hydrocarbons in the range of constituents of gasoline.

of the fraction of MTBE remaining compared to the MTBE that was originally present in the gasoline spilled to the aquifer.

Table 4.2 reveals that the most conservative approach to calculate C/Co must have underestimated the true extent of biodegradation at this site. Well MW-11 had a value of $\delta^{13}C$ for MTBE that was even more negative than the value assumed for MTBE in the gasoline that was spilled. The true value of $\delta^{13}C$ for MTBE in the gasoline that was spilled may have been even more negative than -28.9‰. This most conservative approach was taken because this study was conducted as part of a risk evaluation, and the rates extracted from the CSIA analyses were the only rates available. If the purpose of the study were to validate other rates of biodegradation that were extracted from conventional approaches, it would have been appropriate to use estimates of $\delta^{13}C_{source}$ that were more likely to be representative of the true $\delta^{13}C_{source}$.

The most contaminated well at the site (MW-14 in Figure 4.5) is located in an area that had 9,000 mg/kg of Total Petroleum Hydrocarbons in the range of constituents of gasoline (TPHg). Wells MW-3 and MW-8 are further down gradient of the source of MTBE associated with the underground storage tanks (Figure 4.5). The $\delta^{13}C$ of MTBE in wells MW-3 and MW-8 is much more enriched in ^{13}C than MTBE in gasoline with values of +8.5‰ and +38.0‰ respectively. The fraction remaining corresponds to 91% and 99% biodegradation of MTBE. The attenuation in concentration of MTBE in wells MW-3 and MW-8 compared to well MW-14 can be attributed to biodegradation.

Well MW-6 appears to be cross gradient to the source of MTBE associated with the underground storage tanks (compare the flow arrows in Figure 4.5). However, well MW-6 is directly down gradient of the secondary source associated with the dispenser islands. The behaviour of MTBE in well MW-6 is very similar to wells MW-3 and MW-8. The $\delta^{13}C$ of MTBE (-1.6‰) is highly enriched relative to MTBE in gasoline, and the predicted fraction remaining corresponds to 83% biodegradation of MTBE.

Wells MW-7 and MW-11 are even further down gradient of the source of MTBE. The concentrations of MTBE are low, and it would be tempting to attribute the low concentrations to biodegradation. However, the $\delta^{13}C$ of MTBE in these wells is even more depleted in ^{13}C (-27.3‰, -28.9‰) than the $\delta^{13}C$ in MW-14, the most contaminated well. The $\delta^{13}C$ of MTBE in these wells falls near or within the range of $\delta^{13}C$ expected for MTBE in gasoline. Hence, there is no evidence from the $\delta^{13}C$ of MTBE that biodegradation contributed to attenuation of MTBE in these two down gradient wells.

Because the isotope fractionation provides a direct estimate of the fraction of contaminant remaining after biodegradation, the rate constant for biodegradation can be calculated from the removal

Table 4.2. Rates of natural biodegradation of MTBE in ground water moving along a flow path to monitoring wells. The rates were calculated from the estimated seepage velocity of ground water and the fraction of MTBE remaining after biodegradation.

Well	MTBE (µg/L)	$\delta^{13}C$ MTBE (‰)	Fraction MTBE Remaining (C/C_o)	Distance from MW-14 (meters)	Rate of Degradation with Distance (per meter)	Rate of Degradation with Time (per year)
MW-14	28,800	-21.6	0.67	0		
MW-3	174	8.5	0.085	9.6	0.26	9.4
MW-8	21	38.0	0.0113	11.7	0.38	14.1
MW-7	114	-27.3	0.995	23.0	0.00021	0.0077
MW11	334	-28.9	1.11	44.1	0	0
				Distance from Dispenser Island (meters)		
MW-6	612	-1.6	0.171	31.1	0.057	2.1

of contaminant along the flow path in the aquifer, the distance between wells, and an estimate of the interstitial seepage velocity. If biodegradation follows a pseudo first order rate law, the rate of attenuation can be expressed directly as a first order rate of attenuation with distance, or the rate of attenuation with distance can be multiplied by an estimate of the seepage velocity of ground water to calculate a rate of attenuation with time of travel. The rate of attenuation with distance is calculated following Equation 4.6. Attenuation with time follows Equation 4.7.

$$\lambda_{\text{with distance}} = -\ln(f)/d \qquad \textbf{4.6}$$

$$\lambda_{\text{with time}} = -\ln(f) * v/d \qquad \textbf{4.7}$$

In Equation 4.6 and 4.5, λ is the rate of natural biodegradation, f is the fraction of contaminant remaining predicted from Equation 4.3, d is the distance along the flow path between the up gradient well and the down gradient well, and v is the ground water seepage velocity.

The average hydraulic conductivity at the site in Dana Point, California is 11 meters per day. The average hydraulic gradient over eight rounds of sampling was 0.0023 meter per meter. Assuming the effective porosity is 0.25, the average ground water seepage velocity should be near 37 meters per year. Table 4.2 presents the rates of biodegradation of MTBE along flow paths between the most contaminated well (MW-14), and down gradient wells MW-3, MW-7, MW-8, and MW-11, and between the secondary source at the pump island and down gradient well MW-6. In wells MW-3 and MW-8, the first order rate of degradation is rapid, on the order of 0.3 per meter of travel, or 10 per year of residence time. In well MW-6, the rate of biodegradation is about ten fold slower. In well MW-7, the rate of biodegradation was one thousand fold slower, and in well MW-11 biodegradation was not detected at all.

The field rates estimated for wells MW-3, MW-6 and MW-8 are in good agreement with laboratory rates reported in the literature. The rate of anaerobic biodegradation of MTBE in a microcosm study constructed with material from a gasoline spill in Parsippany, New Jersey, varied from 11 ± 2.3 per year to 12 ± 2.9 per year (Wilson et al., 2005b). The rate of anaerobic MTBE biodegradation in a microcosms study constructed with core material from a JP-4 jet fuel spill in Elizabeth City, North Carolina, was 3.02 ± 0.52 per year and 3.5 ± 0.65 per year (Wilson et al., 2000).

The distance travelled before the concentration of contaminant reaches a particular goal (d_{further}) can be calculated by rearranging Equation 4.7 to produce Equation 4.8. In Equation 4.8, F is the ratio of the goal to the existing concentration in the monitoring well.

$$d_{\text{further}} = -\ln(F)/\lambda_{\text{with distance}} \qquad \textbf{4.8}$$

If F is calculated by dividing the U.S. EPA advisory limit of 20 µg/L by the concentration of MTBE remaining in monitoring wells MW-3, MW-6, or MW-8 (Table 4.2), and if the first order rates of biodegradation also apply to the flow path that is down gradient of the monitoring wells, then the plume would move 8.4 meters past MW-3, and 60 meters past MW-6. Biodegradation had essentially already brought the concentration of MTBE to the limit in well MW-8.

In contrast, the first order rate of biodegradation in well MW-7 (Table 4.2) is much slower. At a rate of 0.00021 per meter, starting at a concentration of 114 µg/L, the MTBE plume would be expected to move 8,300 meters further down gradient before it reached the advisory limit of 20 µg/L.

In well MW-11, biodegradation of MTBE could not be established based on the $\delta^{13}C$ for MTBE in the ground water. The concentration of dissolved oxygen in water from well MW-1 was 0.65 mg/L, the concentration of Iron(II) was 0.2 mg/L, and the concentration of methane was 0.018 mg/L. Conditions were not favourable for aerobic biodegradation. The only processes that can be reasonably expected to attenuate MTBE further down gradient of MW-11 are dilution and dispersion. It would appear that while the biodegradation of MTBE in the core of the plume was rapid and extensive, MTBE in the periphery of the plume was not degraded.

As a consequence of the spatial heterogeneity in the rate of biodegradation, the extent of the plume would be seriously underestimated if a single rate constant for biodegradation was applied to the maximum concentration of MTBE in the source area. On the other hand, the maximum extent of the plume was seriously overestimated if biodegradation was ignored. At this point in the evolution of risk evaluation, a conservative course of action is to recognize that plumes are heterogeneous. An independent estimate of the extent of MTBE contamination further down gradient should be made for each well used in the risk evaluation, based on the concentration of MTBE in each well, and the rate of biodegradation in the flow path leading to each well.

4.5. Effect of Heterogeneity in Biodegradation in the Aquifer on Stable Isotope Ratios

The rate and extent of biodegradation may be heterogeneously distributed in an aquifer. As ground water moves away from a source of contamination, the organic contaminants are removed in flow paths where biodegradation is rapid and extensive, and persist in flow paths where biodegradation is weak or absent. This effect can confuse the interpretation of a shift in the isotopic ratio in the residual organic contaminant. As the contaminant is degraded in the flow paths where biodegradation is rapid and extensive, the residual contaminant is fractionated. However, the concentration of the contaminant that is fractionated is reduced much faster than the concentration of contaminant that is not fractionated. With time and distance away from the source area, the total mass of contaminant that is contributed by the flow paths that degrade the contaminant will decline compared to the flow paths that do not degrade the contaminant. Eventually, the contribution of the fractionated contaminant to the total concentration of contaminant is negligible. Even though a large proportion of the total mass of contaminant has been removed in the aquifer, the stable isotope ratio of the residual material closely resembles the ratio in the material that was released from the source. An analysis of stable isotope ratios in contaminants in water from a monitoring well that blended the flow paths would suggest that the contaminant had not fractionated, and had not been biologically degraded. This situation is illustrated in Figure 4.6.

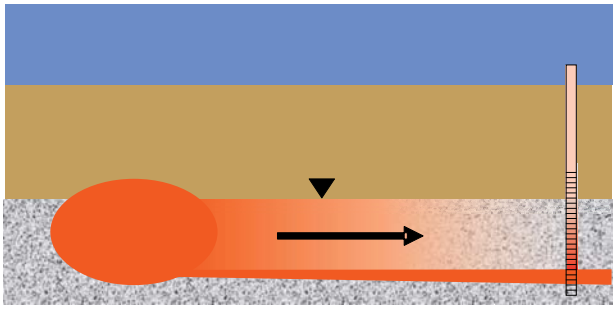

Figure 4.6. Hypothetical illustration of a heterogeneous plume, where a monitoring well that produces ground water from some flow paths where biodegradation of an organic contaminant is rapid and extensive (upper part of the saturated zone), and other flow paths where biodegradation of the organic contaminant is absent.

Figure 4.7 presents a thought experiment that illustrates the effect. In the thought experiment, the isotope enrichment factor for anaerobic biodegradation of MTBE in an aquifer is -12‰, and MTBE in various proportions of the ground water is not degraded. Initially, the $\delta^{13}C$ in the total mass of MTBE increases as biodegradation progresses in the aquifer. Eventually, the total mass of ^{13}C in MTBE in the regions where MTBE is degrading becomes less than the total mass of ^{13}C in MTBE in the regions where MTBE is not degrading. From that point forward, the $\delta^{13}C$ in the total mass of MTBE decreases as biodegradation proceeds in the aquifer. Eventually the $\delta^{13}C$ in residual MTBE returns to the initial $\delta^{13}C$, even though a small fraction of the original mass of MTBE remains. If a shift in the stable isotope ratio was the only criterion to estimate biodegradation, the contribution of biodegradation could be seriously underestimated or missed altogether.

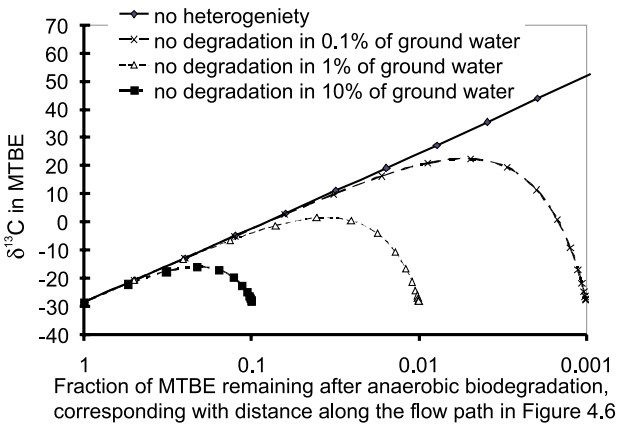

Figure 4.7. Theoretical experiment of the effect of heterogeneity in biodegradation on the stable isotope ratio for carbon in residual MTBE in water produced from a monitoring well, when MTBE does not degrade in certain portions of the aquifer as depicted in Figure 4.6. The Y-axis shows the calculated values for $\delta^{13}C$ of MTBE that is a mixture of MTBE from a flow path with biodegradation and MTBE from a flow path with no biodegradation.

The scenario described above is an extreme case where we have either 100% biodegradation or 0% in different sections of the aquifer. However, there have been recent publications that tried to assess the problem of heterogeneity in a mathematical model, and an *in situ* tracer test where biodegradation was monitored by several methods and the estimate based on stable isotope fractionation was verified in the field (Abe and Hunkeler, 2006; Fischer et al., 2006). Both studies concluded that the influence of spatial heterogeneities in a gravel sediment aquifer was not significant for the calculation of biodegradation. For other sites with more complex heterogeneity, these potential effects should be considered; biodegradation might be significantly underestimated. In any case, situations that protect a portion of the contaminant from fractionation, such as unreactive flow paths or sorption to organic matter, will cause an underestimate of the extent of biodegradation (Kopinke et al., 2005).

4.6. Recommended Practices to Minimize the Confounding Effects of Heterogeneity

Water samples for determination of stable isotope ratios should be acquired from wells with short screen intervals, or from temporary push wells or from cluster wells with small screens. Whenever possible, the depth interval of the well screen should be compared to the lithology of the aquifer, and only wells that are screened across a single unit in the aquifer should be sampled. Often, the very top layer of an anoxic contaminated aquifer will be oxic. This can result from diffusion of oxygen into the ground water from the capillary fringe, or from recharge of aerobic uncontaminated ground water from surface precipitation. Avoid sampling wells that are screened across the water table.

Wells should be purged to the minimum extent necessary to bring geochemical parameters to stability. If the geochemical parameters do not stabilize after three casing volumes have been purged, purging should stop at that point and the ground water should be sampled. If the well water is not in geochemical equilibrium, there is reasonable chance that the well will blend organic contaminants that have been fractionated to different extents.

Use geochemical parameters to recognize the "footprint" of a contaminant plume when the contaminant of interest has been extensively degraded and may not be present at high concentrations in the ground water. As an example, the "footprint" of a plume from a fuel spill often has high concentrations of methane, alkalinity and iron(II), and low concentrations of soluble electron acceptors such as sulfate, nitrate, or oxygen. The "footprints" are expressed in aquifers in both horizontal view (two dimensional space) and with depth. Select locations and depth intervals for CSIA where the geochemical parameters indicate that they are in the "footprint" of the plume, even though they may have lower concentrations of the contaminant of concern.

5.0
Strategies for Field Investigations

Sampling and analysis by CSIA can be expensive, which produces a financial incentive to minimize the number of samples analyzed by CSIA. As a result, there is risk that too few samples will be acquired and analyzed to adequately describe the behaviour of the contaminants at the site. This section discusses important considerations in the design of a sampling strategy that will allow an adequate characterization of the degradation of organic contaminants in ground water at a particular site. This section is primarily intended for consultants who will devise sampling strategies and consultants and regulatory staff who will review reports provided by others on the degradation of contaminants in ground water.

5.1. Design of Stable Isotope Fractionation Studies

According to the standard of the U.S. EPA (1999) at least one of three lines of evidence should be provided to demonstrate natural attenuation in contaminated aquifers: first, field data should show a reasonable decrease of contaminant concentration or mass; second, hydrogeological and geochemical data should indirectly reveal the type and the rate of attenuation; and third, microcosm studies in field or laboratory should demonstrate the occurrence of substantial attenuation processes at the site. Isotope fractionation is a tool that can contribute to all three of the lines of evidence for monitored natural attenuation. It can demonstrate contaminant mass loss due to biodegradation as part of the first line of evidence. It can provide information for direct calculation of biodegradation rates for use in the second line of evidence. Finally, isotope fractionation can provide direct unequivocal evidence of biodegradation in the aquifer.

5.2. Temporal Design

The design of a stable isotope study always depends on a site conceptual model that is unique to every site. This section can only depict the general frame work for the design of CSIA studies.

Before a major investment is made in CSIA, it is prudent to get an indication of the utility of CSIA to understand the behavior of contaminants at the site. First, a snapshot of stable isotope fractionation should be made during a sampling event that is routinely performed for measurement of contaminant concentrations. Such a preliminary study would concentrate on the monitoring of four to six wells with CSIA. Of course, this would provide a limited data set which is not adequate to interpret the biodegradation potential on the site in a serious way. Nevertheless, a preinvestigation might provide sufficient evidence to justify a detailed and extensive stable isotope survey.

In order to provide reliable data for interpretation of biodegradation on the site, a comprehensive survey of CSIA across the entire plume is recommended, which usually requires monitoring of twelve to twenty wells depending on the size and complexity of the site. A second sampling event after two to three months is particularly necessary in highly variable plumes to insure reproducibility of the data from the CSIA. At least the important wells that have been identified in the first sampling event should be sampled a second time after two to three months. A complete investigation will require approximately four to seven months. Once the contribution of biodegradation has been established using CSIA, the long-term behaviour and stability of fractionation within the plume should be evaluated in a final isotope survey conducted one to three years after the first survey (Figure 5.1).

Before CSIA of the samples, the concentrations of the contaminants should be determined by conventional methods such as GC/MS. This information is necessary to select the appropriate concentrations of the samples that will bring the analytes within the linear range of the isotope ratio mass spectrometer, and to ensure that the intended method of sample preparation (such as purge and trap, or SPME) provides adequate sensitivity.

5.3. Spatial Sampling Design

The information needed for the design of a sampling strategy includes the location and extent of the source, the direction of the ground water flow, and the extent of the plume. The sampling pattern should cover each of the compartments of the plume (the source, the plume center line, and the fringes) with an adequate number of monitoring wells. As a general rule, isotope data from twelve to twenty wells would be appropriate for a reasonable and detailed evaluation of biodegradation at a typical site. These numbers depend on the extent

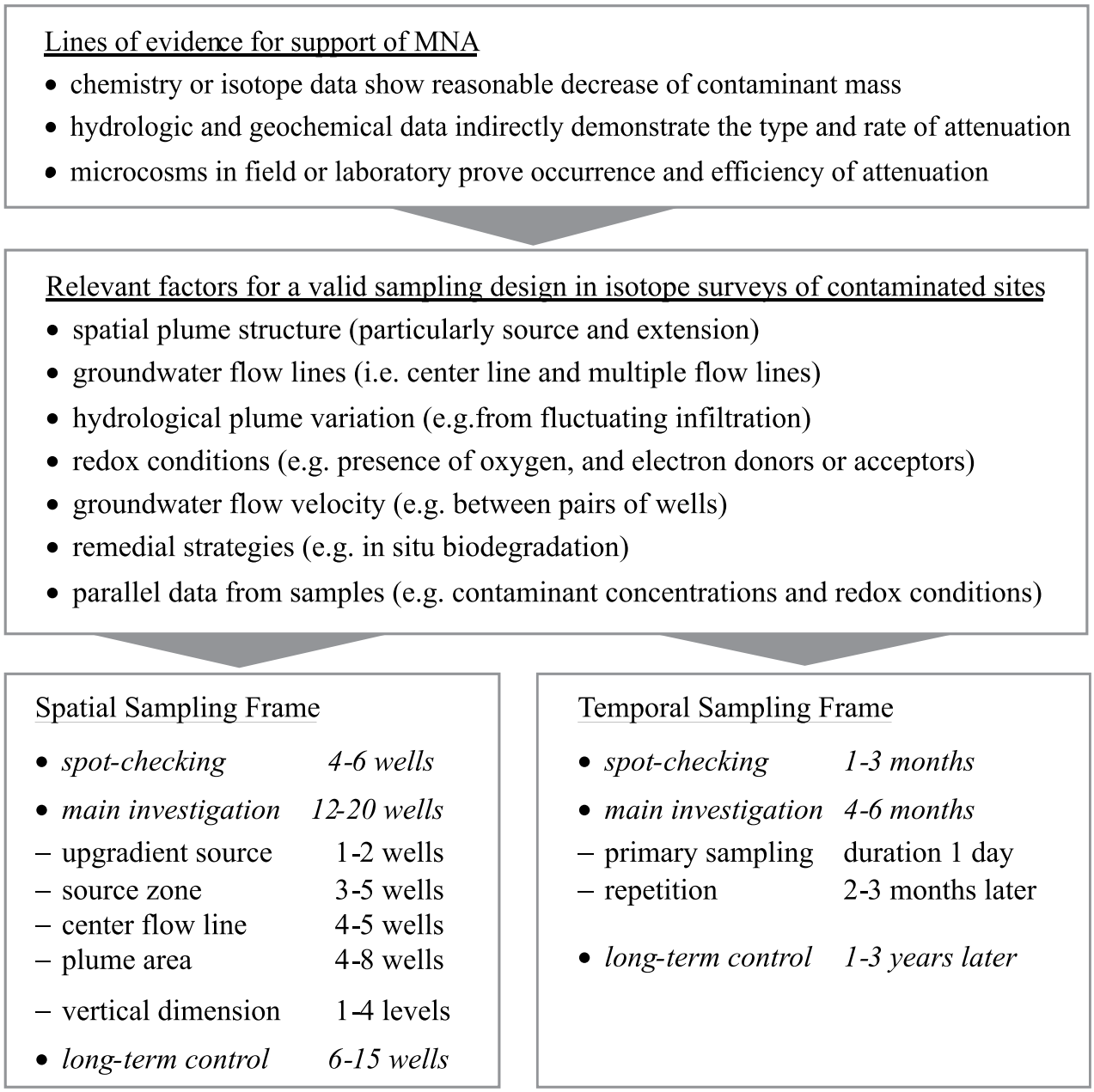

Figure 5.1. Development of a spatial and temporal sampling design for CSIA surveys to evaluate MNA. The number of wells are offered as an example for an optimal study of contamination in a single aquifer. The design of a real survey should be adapted to the specific conditions at the site.

and the complexity of the plume; multiple sources require more samples. The authors recommend taking samples for chemical and isotope analysis from every well and storing aliquots for isotope analysis as recommended in Section 3.3 above. Analyze a water sample from each of the wells for the concentration of the contaminants, and then use the information on concentrations to select the subset of wells that will be subjected to CSIA.

As discussed in Section 6.1, the value of $\delta^{13}C$ or $\delta^{2}H$ in the feed stock used to manufacture an industrial chemical may vary depending on the source of the feed stock, and as a consequence, the value of $\delta^{13}C$ or $\delta^{2}H$ in the industrial chemical can vary from one batch to another. The carbon and hydrogen in the feed stock may be fractionated during the manufacturing processes, and the values of $\delta^{13}C$ or $\delta^{2}H$ in the industrial chemical can vary if different

processes were used to manufacture the chemical. The value of $\delta^{13}C$ or $\delta^{2}H$ for an industrial chemical may vary from one batch to another, or from one manufacturer to another, and different releases of a an industrial chemical may have different values of $\delta^{13}C$ or $\delta^{2}H$.

If there is more than one source of contamination at a site, each of the sources must be identified and delineated, because the contaminants in each of the different releases may have started with different values of $\delta^{13}C$ or $\delta^{2}H$. If there are multiple sources, it is possible that $\delta^{13}C$ or $\delta^{2}H$ from a well in one source will be compared to the $\delta^{13}C$ or $\delta^{2}H$ plume produced by a second source, and the difference in $\delta^{13}C$ or $\delta^{2}H$ might give the false impression of biodegradation and lead to misinterpretations. In order to be able to make a reliable quantification of biodegradation processes, it is necessary to determine the values of $\delta^{13}C$ or $\delta^{2}H$ of the contaminants in three to five wells in each of the the source zones.

Often, it will not be possible to assess the source zone directly because it might be buried below buildings, roads, or other infrastructure. In this case, it will be sufficient to take the first monitoring well that is accessible down gradient of the source area and use the concentrations and values of $\delta^{13}C$ or $\delta^{2}H$ as the initial values (primary signature) for the interpretation of data from wells located further down gradient. If an analysis of the data on concentrations indicates that the ground water upgradient of the source is already slightly contaminated, isotope measurements from an upgradient well should be performed as a control for the interpretation of isotope values in fringe areas of the main plume (one to two wells). The clearest picture for isotope fractionation analysis can generally be derived from isotope data at the center flow line of plumes where there is a better understanding of the geohydrology of the plume. The center flow line should be sampled in at least four to five wells and more if possible.

A comprehensive analysis should consider the entire extent of the plume, realizing that the distribution of contamination at the plume fringe is often insufficiently defined. It should be taken into account that some of the monitoring wells may not be hydrologically connected. At least four to eight wells should be sampled in the central parts of the plume in addition to the four to eight center line wells. Several wells should also be sampled in the fringes of the plume. It is important to sample the downgradient margin of the plume because this portion of the plume is most important for the prediction of future migration of the contaminant.

If multi-level wells, or multi-level well clusters are available to provide vertical resolution in the distribution of contaminants and electron acceptors, the wells can be sampled to evaluate any vertical differences in the extent of biodegradation in the plume.

In common practice, the number of monitoring wells that are available for a CSIA survey of a site will be often fewer than the twelve to twenty wells that we recommend. It may be necessary to acquire water samples from temporary push wells to adequately delineate and characterize the plume. The fewer the number of wells that are analysed in the study, the higher the risk of misinterpreations. In such cases, the comprehensive interpretation of many different lines of evidence becomes more important. As a result, we can not offer a general design for CSIA studies which is applicable to sites with only a few monitoring wells.

6.0 Use of Stable Isotopes for Source Differentiation

In industrial and urban areas, multiple sources of the same contaminant frequently occur. This is particularly true for releases of chlorinated solvents and petroleum hydrocarbons. This section discusses the application of isotope data to identify different sources of the same contaminant and to link sources to contaminated ground water down gradient of the source. This section is primarily intended for consultants who will devise sampling strategies to associate particular contaminants in ground water plumes with particular sources, and consultants and regulatory staff who will review reports provided by others on the source of contaminants in ground water.

6.1. Variability of Isotope Ratios of Different Sources

The isotopic composition of synthetic organic compounds depends on the isotope ratio of the source materials and on isotope fractionation during production of the compounds. For example, chlorinated methanes sold in commerce generally have more negative values of $\delta^{13}C$ compared to chlorinated ethanes and ethylenes because they are produced from methane in natural gas. The methane in natural gas is formed when heat and pressure deep in the earth pyrolyze native organic matter in sediments. Because of the strong fractionation during pyrolysis, the methane is depleted in ^{13}C (Whiticar, 1999; Whiticar and Faber, 1985).

The values of $\delta^{13}C$ and $\delta^{37}Cl$ for a particular chlorinated compound in commerce can vary from one manufacturer to another and also between different production batches produced by the same manufacturer (Beneteau et al., 1999; Jendrzejewski et al., 2001; Shouakar-Stash et al., 2003; van Warmerdam et al., 1995). Data on the variation in the isotopes of carbon and chlorine in chlorinated solvents and chlorinated production chemicals are summarized in Figure 6.1. Even larger variations have been reported for isotopes of hydrogen in trichloroethylene and 1,1,1-trichloroethane (Shouakar-Stash et al., 2003). Similarly, Smallwood et al. (2001) observed differences in carbon and hydrogen isotope ratios for a range of different natural hydrocarbons in gasoline as well as methyl tertiary butyl ether (MTBE). The variation in isotope ratios of compounds in gasoline reflects variations in the origin of the crude oil.

All the studies discussed above were concerned with variations between commercial products. Any variation in the source and isotopic composition of the material that is spilled adds additional complexity at field scale. A contiguous source of ground water contamination can be heterogeneous with respect to its isotopic composition if it is the result of several different spill events over time, and if the compound that was spilled had different sources with different isotopic compositions. It is also possible that different spill events in different locations can have the same isotopic composition. To distinguish these possibilities, it is helpful to perform CSIA for several elements at the same time, such as carbon, hydrogen, and chlorine, and in cases of multi-component mixtures, several compounds. This is especially true for compounds of larger molecular mass as they undergo smaller shifts in stable isotope ratios during biodegradation because the reactive atom is "diluted" with greater numbers of other atoms (see section 4.2.3.5). Larger molecules of multi-component spills such as mineral oil products can therefore be used as conservative tracers when isotope fingerprinting is applied.

6.2. Contaminated Sites Scenarios

Isotope analysis is especially useful when there are multiple sources of the same ground water contaminants. Table 6.1 summarizes several common scenarios that may be encountered and outlines questions to be addressed, and potential sampling strategies. The actual strategy can vary depending on the complexity of the site and the available information about source location and transport mechanisms. While it may frequently be possible to clearly locate and sample source zones for LNAPLs (Light Non-Aqueous Phase Liquids), this is often not the case for DNAPLs (Dense Non-Aqueous Phase Liquids) where source zones are often inferred from high concentrations of the contaminant in ground water.

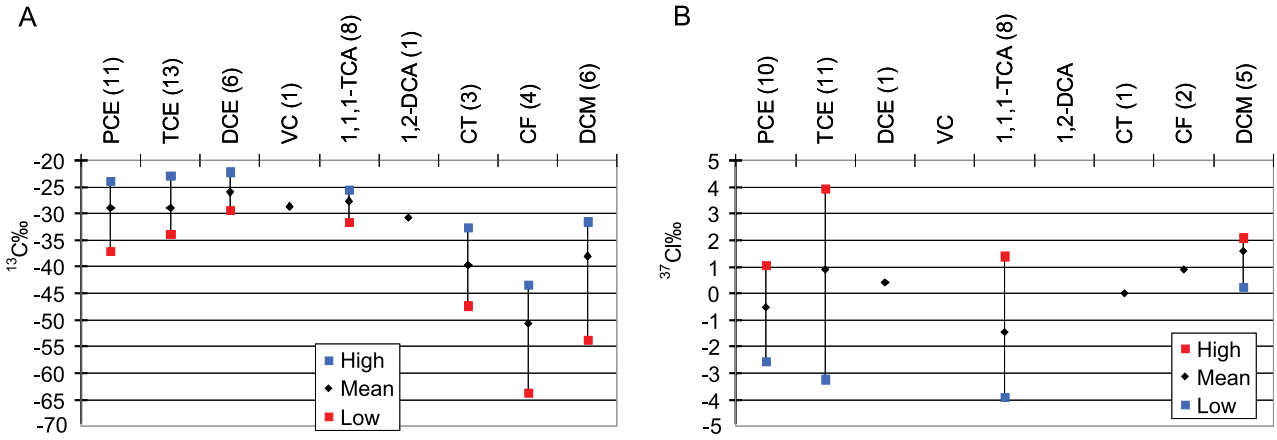

Figure 6.1. Minimum, maximum and mean carbon (A) and chlorine (B) isotope ratio of chlorinated hydrocarbons from different manufacturers and production batches measured to date. The number in parentheses following each compound name indicates the number of samples analyzed for that compound. PCE is tetrachloroethylene, TCE is trichloroethylene, DCE is dichloroethylene, 1,1,1-TCA is 1,1,1-trichloroethane, 1,2-DCA is 1,2-dichloroethane, CT is carbon tetrachloride (tetrachloromethane), CF is chloroform (trichloromethane) and DCM is dichloromethane. Data compiled from (Beneteau et al., 1999; Holt et al., 1997; Hunkeler and Aravena, 2000c; Jendrzejewski et al., 1997; Jendrzejewski et al., 2001; Shouakar-Stash et al., 2003; van Warmerdam et al., 1995; Zwank et al., 2003).

6.3. Evaluating the Relevance of Biodegradation

Generally, there is little significant isotopic fractionation caused by transport and partitioning processes (See Section 4 for a discussion of the exceptions). As a consequence, transport and partitioning processes will not mask the variation in stable isotope ratios that are associated with different sources. Because the differences in isotope ratios of different sources are commonly on the order of several ‰, the change in the isotope ratio due to biotic or abiotic degradation can rapidly become more important than variations due to different sources. Before carrying out a source differentiation study using CSIA, complementary data such as the concentrations of daughter products and the redox conditions should be evaluated to determine whether degradation processes can be expected to cause changes in the isotope ratios, or whether the ratios of the compounds of interest are conservative.

For highly chlorinated hydrocarbons such as PCE and TCE, little biodegradation occurs as long as oxygen is present and the redox potential is elevated. The relevance of biodegradation can be evaluated by characterizing redox conditions. The absence of degradation products such as *cis*-DCE and VC can serve as an additional indicator for conservative behaviour. For less chlorinated hydrocarbons and for petroleum hydrocarbons, biodegradation should be expected under a range of redox conditions.

If the isotope fractionation factor and the degree of biodegradation are known, it should theoretically be possible to make a correction for isotope fractionation due to biodegradation. However, in most cases the extent of biodegradation cannot be estimated independently (which is the reason why isotopes are used to assess biodegradation) and any such correction becomes very uncertain. In the case of reductive dechlorination of chlorinated ethenes, the original isotope ratio of carbon in the parent compound can be estimated based on a mass balance of carbon in parent and daughter compounds assuming that carbon is conserved during degradation. However, the uncertainty of the calculated value is larger than is the case when only the parent compound is present. In the future, with increased possibilities for dual isotope measurement, it may be possible to distinguish shifts due to different sources from shifts due to biodegradation because shifts due to biodegradation follow a systematic trend. Such an approach has been used to track the sources of nitrate in ground water (Widory et al., 2005), and of benzene at a contaminated site with multiple source zones (Mancini et al., 2008b).

6.4. Designing a Sampling Strategy to Distinguish Sources

Recommended sampling strategies for different scenarios are summarized in Table 6.1 and Figure 6.2. The design of the sampling strategy should be based on a conceptual model of the site that summarizes the actual or potential location of the sources and the pathways of contaminant migration. If possible, for each presumed source and associated plume segment, at least three samples should be taken to evaluate within-source variability and to facilitate data evaluation. Take into account that different sources may coincidentally have the same isotopic composition. Therefore, it is advisable to carry out preliminary sampling to evaluate differences in isotope ratios between different sources or plume segments before carrying out more extensive sampling to delineate different plumes.

Table 6.1. Recommended sampling strategies for the use of CSIA to evaluate the origin of ground water contamination

Scenario	Plume and several known source zones	Plume and one known source zone	Up gradient and down gradient pollution	Extended plume but no identified source zone
Question	What is contribution of the different sources to the plume(s)?	Are there additional sources contributing to the plume?	Does the site contribute to down gradient contamination?	Is plume linked to one or several sources?
Sampling	**Source characterization** Take NAPL samples of different sources; if not available, take three ground water samples in high concentration zone close to each source. **Plume characterization** (only if sources have different isotopic composition) Take at least three samples in each of the plume segments presumably linked to the each of the sources.	**Source characterization** Take NAPL sample of source; if not available take ground water samples in high concentration zone close to the source. **Plume characterization** Take at least three samples in each plume or plume segment.	Take at least three ground water samples up gradient and down gradient of the location of the potential source.	Take at least three ground water samples in each plume or plume segment.

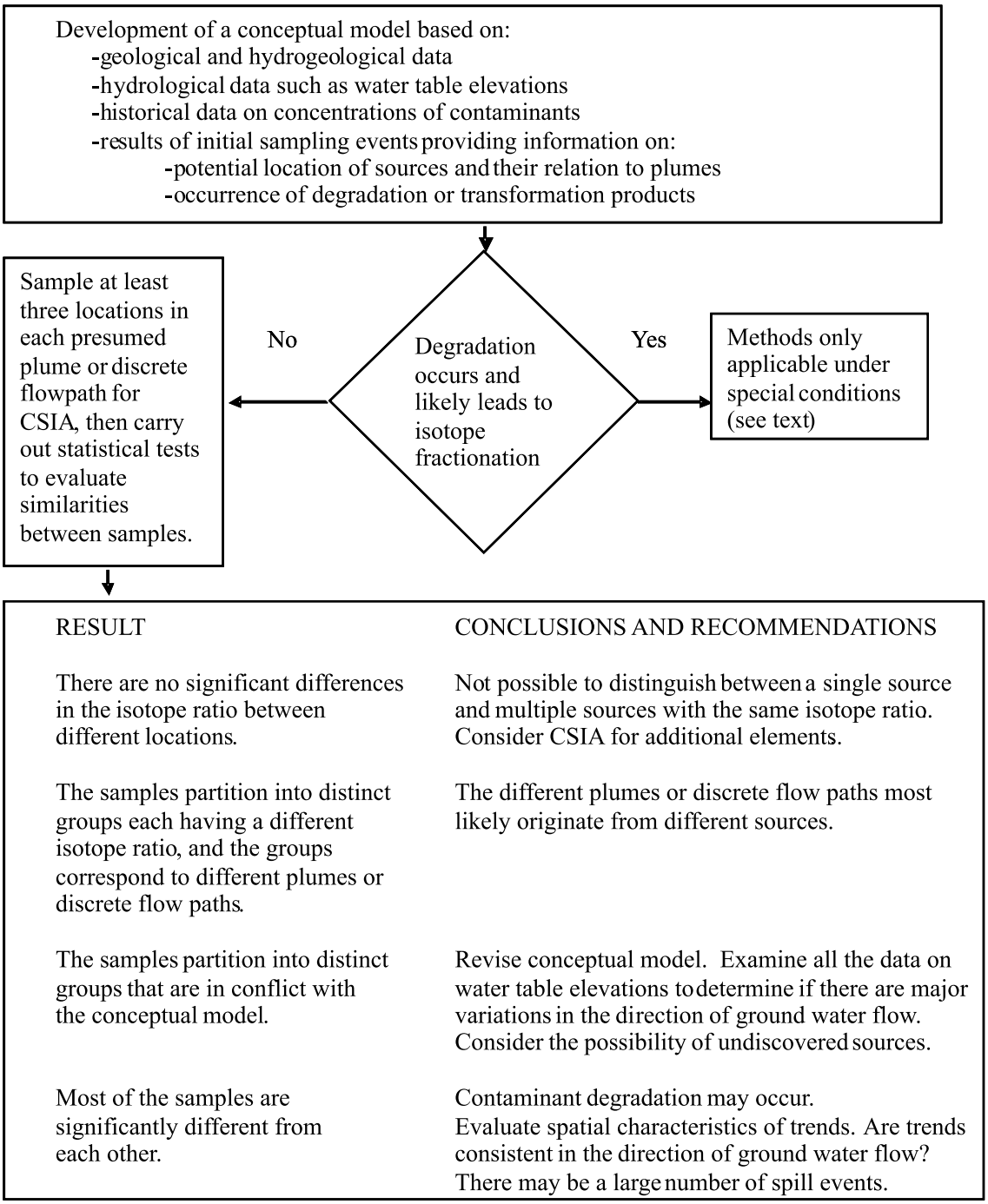

Figure 6.2. Flow chart for the design and evaluation of a source identification strategy based on stable isotope analysis.

6.5. Data Evaluation to Distinguish Sources

The stable isotope data evaluation can involve different levels of complexity:

If only one or a few compounds were analyzed, as is typically the case for chlorinated hydrocarbons, and if the different sources or plume segments can be clearly distinguished, calculate the mean and standard deviation for each source and/or plume segment. Compare the means using a Student's t-test based on the calculated standard deviations.

If the site conceptual model is not sufficiently detailed to group the samples into different sources or plumes, an analysis of variance (ANOVA) can be carried out to determine whether there are significant differences between any of the samples. If the ANOVA indicates that there are significant differences between samples, then the Newman-Keuls test or other equivalent test that makes pairwise comparisons can be used to identify whether any particular sample is different from any other particular sample. Details on the calculations are available in many introductory statistics textbooks.

In the case of petroleum hydrocarbons, isotope data are often available for a number of compounds in the same water sample. One possibility for comparing these data for pairs of samples is to carry out a student *t*-test for each of the compounds, as was done by Smallwood et al. (2002). However, for a series of *t*-tests the probability of a type I error increases (falsely rejecting the hypothesis that isotope values are the same), and samples may be determined to be different by the test when in reality there is no difference. To circumvent this problem, reduce the number of variables using Principle Component Analysis and then test the transformed variables for similarity (Boyd et al., 2006).

Once the similarities and differences are evaluated, the data should be compared with the conceptual model of the site. Different situations can be envisaged. There may be no significant differences in the isotope ratios for different sources and plume segments. In this case it is not possible to distinguish between a single source and multiple sources. The isotope data are consistent with the hypothesis that there is only one source but do not demonstrate it. Consider using CSIA for other elements such as hydrogen or chlorine.

The samples may partition into different groups, with each group having different isotope ratios which correspond to different sources and plume segments. In this case, the isotope data provide strong evidence that the different plume segments originate from different sources.

The samples may partition into different groups that are in conflict with the conceptual model. Reconsider the conceptual model with respect to the potential for additional sources or spill events, additional contaminant migration pathways, or the possibility of reactive processes that change isotope ratios in some zones. Revisit the assumptions behind the interpretation of the isotopic data, such as conservative behaviour and lack of fractionation due to biodegradation.

Most of the samples may be significantly different from each other, making it difficult to exclude a wide variety of possible interpretations. There may be a substantial number of different spill events or the compounds may be affected by reactive processes. If reactive processes occur, the isotope ratios may show a trend with distance from the source.

6.6. A Case Study of Source Differentiation

A PCE plume was characterized in a sandy aquifer of a small town (Angus) in Ontario, Canada (Hunkeler et al., 2004). The plume was very wide (60 m) close to the presumed source area, and there were several discrete zones with high concentrations of PCE, which raised the question of whether there were one or several sources for the plume (Figure 6.3). The carbon isotope ratio of PCE was determined for a large number of samples from multilevel samplers in two transects at two different distances down gradient from the presumed source area. In the up gradient transect, three different plume cores with significantly different isotope ratios can be distinguished (contained within intervals A, B, and C in Transect 1 as presented in Figure 6.3). This observation together with the considerable width of the plume indicates that the plume likely originates from several spatially separated sources. The relatively large width of interval C may be due to lateral migration of DNAPL in the source zone. Two of the three plume cores can still be identified at the down gradient transect (Transect 2 in Figure 6.3), while on either side of the plume core, there are zones with more enriched values for ^{13}C compared to corresponding locations in the up gradient transect, the enriched values indicate biodegradation of PCE in the flow path between the two transects. The trend towards more negative values in the low concentration fringes observed in some multilevel samples may be due to a small (1-2‰) diffusive effect (Hunkeler et al., 2004).

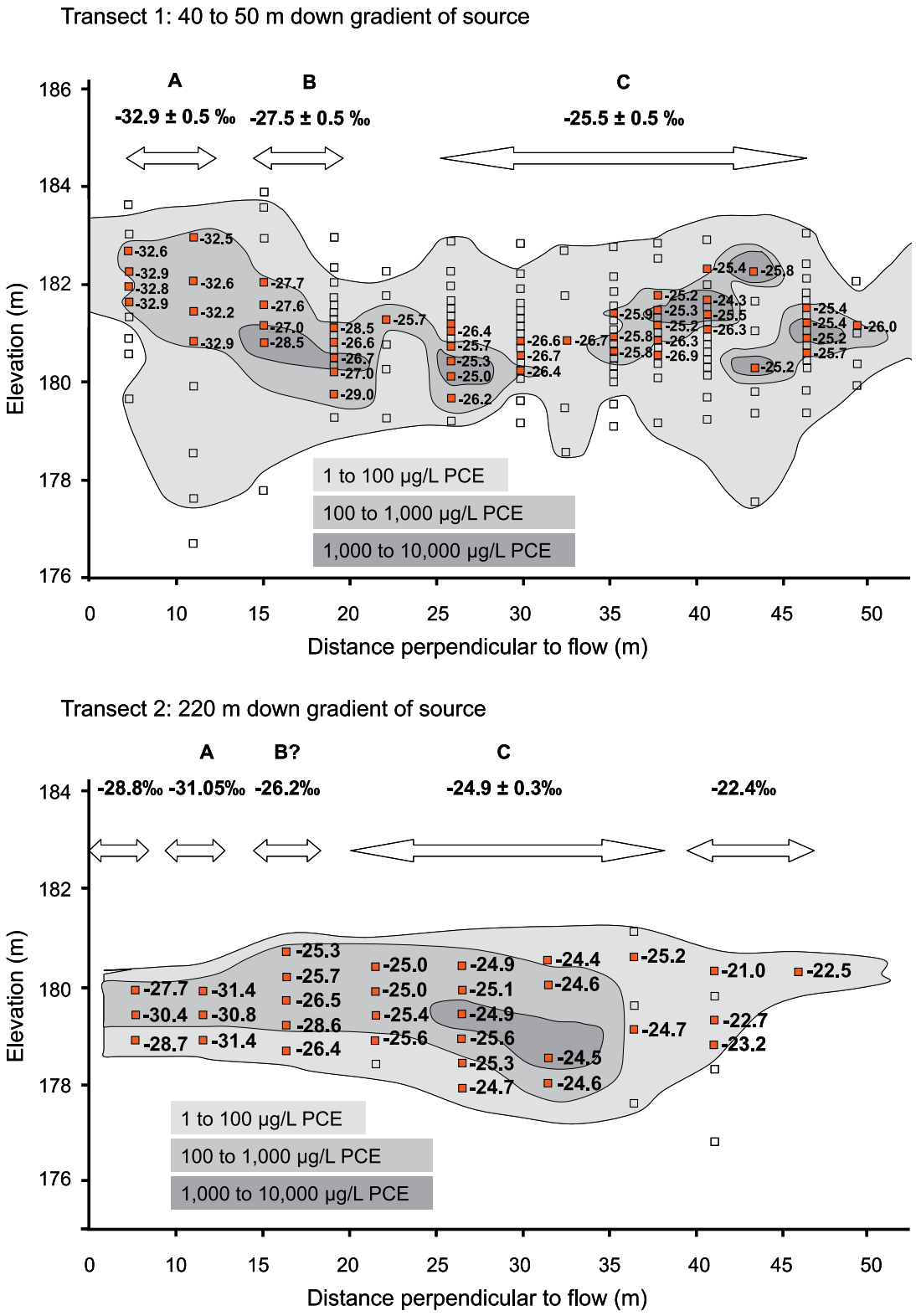

Figure 6.3. Concentrations and carbon isotope ratios of PCE in two transects downgradient of unidentified PCE sources. All values are given in ‰ relative to the V-PDB standard. Filled squares are depths sampled for determination of both concentration and $\delta^{13}C$. Open squares are depths sampled for concentration only. The figure is modified after Hunkeler et al. (2004).

7.0 Derivation of Equations to Describe Isotope Fractionation

This section is intended for contractors and consultants that will work up data on stable isotope ratios, and produce a report for the site manager and the regulatory staff. It is also intended for regulators who will review the report. This section derives the equations that are used to calculate the extent of biodegradation from the change in the stable isotope ratio, and the rate of biodegradation from the extent of biodegradation.

7.1. Expressing and Quantifying Isotope Fractionation

In a kinetic reaction, isotope fractionation occurs due to slight differences in the reaction rates of molecules with a heavy and light isotope, respectively, at the reactive site of the molecule. The magnitude of isotope fractionation is usually expressed by the isotope fractionation factor (α) that quantifies the difference in isotope ratio between the product that is formed at a given time (IP - instantaneous product), and the reactant (R).

For a general reaction: R → P

The isotope fractionation factor is given by:

$$\alpha_{PR} = \frac{R_{IP}}{R_R} \qquad 7.1$$

with

$$R_{IP} = \frac{dH_P}{dL_P} \text{ and } R_R = \frac{H_R}{L_R}$$

where

α_{PR} = the isotope fractionation factor,

R_R =the isotope ratio of the reactant

R_{IP} = the isotope ratio of the product (instantaneous basis)

$\delta^i E_R$ = the isotope ratio in ‰ of reactant

$\delta^i E_{IP}$ = the isotope ratio in ‰ of product (instantaneous basis)

H_R = the amount of the heavy isotope in reactant

dH_P = the instantaneous rate of production of the heavy isotope in the product

L_R =the amount of the light isotope in reactant

dL_P = the instantaneous rate of production of the light isotope in the product.

Rearranging Equation 2.1, where R_S is the isotope ratio of the standard:

$$\delta^i E_R * 1000 = R_R / R_S$$

and

$$\delta^i E_{IP} * 1000 = R_{IP} / R_S$$

then

$$\alpha_{PR} = \frac{R_{IP}}{R_R} = \frac{\delta^i E_{IP} + 1000}{\delta^i E_R + 1000} \qquad 7.2$$

Often isotope fractionation is expressed on a ‰ scale, using the isotope enrichment factor (ε) which is defined as:

$$\varepsilon_{PR} = (\alpha_{PR} - 1) \cdot 1000 \qquad 7.3$$

For reactions of any order, the isotope fractionation factor corresponds to the ratio of the rate constant for reaction of molecules with a heavy isotope (K_H) anywhere in the molecule compared to the rate constant for reaction of molecules with light isotopes only (K_L) (Bigeleisen and Wolfsberg, 1959).

$$\alpha_{PR} = \frac{k_H}{k_L} \qquad 7.4$$

A constant fractionation factor throughout the degradation process is also expected for Monod kinetics (Simon and Palm, 1966).

7.2. The Rayleigh Equation

For evaluating laboratory and field data, an equation is required that describes the changes in the isotope ratio as the reaction progresses. Such an equation can be derived starting from Equation 7.1, the definition of the fractionation factor:

$$\alpha_{PR} = \frac{dH_P / dL_P}{H_R / L_R} \qquad 7.5$$

For mass balance reasons

$$dH_P = -dH_R \qquad \textbf{7.6}$$
$$dL_P = -dL_R$$

Combining equation 7.5 and equation 7.6 and rearranging leads to

$$\frac{dH_R}{H_R} = \alpha_{PR} \cdot \frac{dL_R}{L_R} \qquad \textbf{7.7}$$

Integration of equation 7.7 from $H_{R,0}$ to H_R and $L_{R,0}$ to L_R, where $H_{R,0}$ = the initial amount of heavy isotope and $L_{R,0}$= the initial amount of light isotope yields:

$$\ln \frac{H_R}{H_{R,0}} = \alpha_{PR} \cdot \ln \frac{L_R}{L_{R,0}}$$

or

$$\frac{H_R}{H_{R,0}} = \left(\frac{L_R}{L_{R,0}} \right)^{\alpha_{PR}}$$

Dividing both sides by $L_R/L_{R,0}$ yields

$$\frac{R_R}{R_0} = \left(\frac{L_R}{L_{R,0}} \right)^{(\alpha_{PR}-1)} \qquad \textbf{7.8}$$

where R_R and R_0 are the isotope ratios at a given time t and at time zero, respectively.

The fraction of substrate that has not reacted (*f*) at time t is given by:

$$f = \frac{C_t}{C_0} = \frac{H_R + L_R}{H_{R,0} + L_{R,0}} \qquad \textbf{7.9}$$

From equation 7.9, if the amount of heavy isotope (H_R and $H_{R,0}$) is small compared to the amount of the light isotope (L_R and $L_{R,0}$), as is typical for studies at the natural abundance of isotopes, (L_R/ $L_{R,0}$) can be approximated by (*f*), and Equation 7.8 transforms to

$$f = \frac{C_t}{C_0} = \frac{H_R + L_R}{H_{R,0} + L_{R,0}} = \frac{L_R \cdot (1+R)}{L_{R,0} \cdot (1+R_0)} \qquad \textbf{7.10}$$

Equation 7.10 is usually denoted as the Rayleigh equation and describes the evolution of the isotope ratio of the reactant as a function of the progress of the reaction (Clark and Fritz, 1997; Mariotti et al., 1981).

Sometimes, the isotope fractionation factor is expressed as the inverse of the ratio given in Equation 7.1. If this is the case, the value of α will be larger than 1.0, and accordingly the exponent in Equation 7.10 corresponds to (1/α)-1.

Equation 7.10 is often expressed using the delta notation for isotope ratios and the isotope enrichment factor instead of the isotope fractionation factor:

$$1000 \cdot \ln \frac{\delta^i E / 1000 + 1}{\delta^i E_0 / 1000 + 1} = \varepsilon_{PR} \cdot \ln f \qquad \textbf{7.11}$$

where $\delta^i E_0$ and $\delta^i E$ are the initial isotope ratio of the compound and the isotope ratio at a moment in time, respectively.

Because ln(1+u) corresponds approximately to u when u is small compared to 1, as can be shown using a Taylor series expansion, Equation 7.11 can be further simplified to:

$$\delta^i E = \delta^i E_0 + \varepsilon_{PR} \cdot \ln f \qquad \textbf{7.12}$$

An equation for the accumulated product can be derived from equation 7.12 using an isotope mass balance equation that links the isotope ratio of reactant and accumulated product:

$$R_o = R_R \cdot f + (1-f) \cdot \overline{R}_B \qquad \textbf{7.13}$$

where

$\overline{R}_B$ = the isotope ratio of the accumulated product

R_R = the isotope ratio of the reactant at the time of measurement

R_0 = the initial isotope ratio of the reactant

Inserting Equation 7.10 into Equation 7.13 followed by rearrangement leads to:

$$\overline{R}_B = R_0 \cdot \frac{1 - f^{\alpha_{PR}}}{1-f} \qquad \textbf{7.14}$$

The corresponding approximate equation for the accumulated product can be derived by combined an isotope mass balance equation in δ-notation analogous to Equation 7.13 with Equation 7.12:

$$\delta^i E_P = \delta^i E_0 + \varepsilon_{PR} \cdot \frac{f \cdot \ln f}{1-f} \qquad \textbf{7.15}$$

Following Equation 7.15, the value of $\delta^i E_{IP}$ for the instantaneous product is always offset by ε_{PR} compared to the $\delta^i E_R$ of the reactant in a Rayleigh controlled system, while the $\delta^i E_P$ of the accumulated product approaches the initial isotope ratio of the parent compound ($\delta^i E_0$) as the reaction proceeds (Figure 7.1).

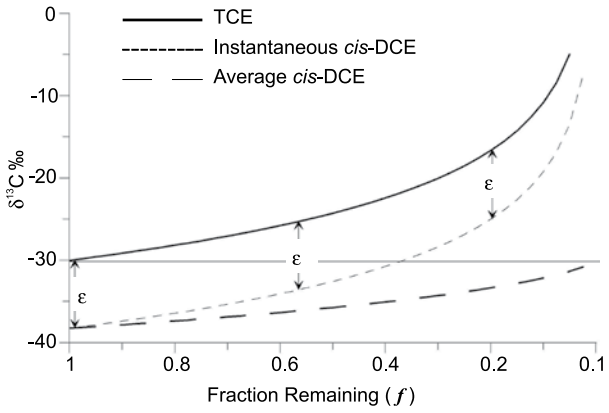

Figure 7.1. Simulated evolution of carbon isotope ratios of reactant (TCE) and degradation product (*cis*-DCE) according to the Rayleigh equation. The isotope evolution of the product that is formed at a certain moment in time (instantaneous product) as well as the average isotope ratio of the accumulation product is shown. The average *cis*-DCE deviates from the instantaneous product to more depleted values as it is a mixture of product that accumulates from the start of the reaction. As the instantaneous product becomes heavier, the accumulated product becomes heavier. The enrichment factor ε stays constant over time. An isotope enrichment factor of -8.5‰ was assumed in the simulation.

7.3. Quantification of Isotope Fractionation in Laboratory Studies

Laboratory data should be evaluated using the full Rayleigh equation (Equation 7.10) unless the experiments were carried out with labelled compounds. In this case, it is necessary to use an equation derived from Equation 7.8 without simplifications. When the uncertainty of the measurement is in the same range as the uncertainty of the isotope ratio of the reference gas, the following linearized form of Equation 7.10 is recommended to quantify α_{PR}:

$$\ln\frac{R}{R_0} = \ln\frac{\delta^i E/1000+1}{\delta^i E_0/1000+1} = (\alpha_{PR}-1)\cdot\ln f \qquad \textbf{7.16}$$

The linear regression should be carried out without forcing the regression line through the origin. Laboratory experiments are often carried out in replicates, which raises the question of how the data should be combined to obtain a representative fractionation factor. The non-weighted average fractionation factor should only be used if the replicates consist of a similar number of observations spread over a similar range in f. Otherwise, Scott et al. (2004) propose a method based on linear regression with dummy variable and a method based on a Pitman estimator.

7.4. Equations to Evaluate Field Isotope Data

Under certain conditions (see Section 3), the degree of biodegradation or the first order rate constant for biodegradation can be quantified for the zone between the source and a monitoring point, or between two monitoring points along a flow path. By rearrangement of equation 7.16, the following equation is obtained to quantify the fraction remaining (f):

$$f = \left(\frac{R}{R_0}\right)^{\frac{1}{\alpha_{PR}-1}} = \left(\frac{\delta^i E+1000}{\delta^i E_0+1000}\right)^{\frac{1000}{\varepsilon_{PR}}} \qquad \textbf{7.17}$$

where

$\delta^i E$ = the isotope ratio at the downgradient monitoring point, and

$\delta^i E_0$ = the isotope ratio at the source or upgradient monitoring point.

The amount of biodegradation or abiotic transformation (in percent of the material originally present) is given by:

$$B = (1-f)\cdot 100 \qquad \textbf{7.18}$$

The change in the isotope ratio from the source area to a monitoring well or from well to well can be used for two purposes. The CSIA data can be used to test the hypothesis that the concentration decrease is predominantly due to biodegradation or abiotic transformation, and the data can be used to extrapolate the removal that would be expected further along the flow path. The expected concentration at a down gradient monitoring point can be calculated using:

$$C_{\exp} = C_o \cdot f \qquad \textbf{7.19}$$

where

C_{exp} = the expected concentration at the down gradient monitoring point

C_0 = the concentration at the source or the up gradient monitoring point along a flow path.

If the reductions in concentrations are due to a particular process that has a characteristic value for ε_{PR} , then the value of (*f*) as obtained from Equation 7.17, should substitute into Equation 7.19 to predict a value for C_{exp} that is in good agreement with the measured concentration in the down gradient monitoring well. If the values are not in good agreement, then it is possible that other processes such as dilution in the well or dispersion along the flow path has a stronger influence on the measured concentration. If is also possible that there is an error in the conceptual model, that some other process is responsible for destruction of the compound, and the assumed value of ε_{PR} is in error.

The CSIA data can also be used to extrapolate contaminant degradation further down the flow path. The first order rate constant for contaminant removal can be estimated by combining Equation 7.10 with the equation describing first-order degradation of a substance:

$$f = \frac{C}{C_0} = \exp(-\lambda_t \cdot T) \qquad \textbf{7.20}$$

where:

T = the average travel time of the compounds of interest between source and monitoring point or between two monitoring points along a flow line. For retarded compounds, the travel time is given by $T=R_T \cdot T_w$ where R_T is the retardation factor and T_w is the average travel time of water,

λ_t = the first order rate constant for reduction in concentration due to biodegradation or abiotic transformation.

Solving Equation 7.20 for λ_t, and then substituting Equation 7.17 for *f*, produces Equation 7.21.

$$\lambda_t = -\frac{\frac{1}{\alpha_{PR}-1}\cdot \ln\frac{R}{R_0}}{T} = -\frac{\frac{1000}{\varepsilon_{PR}}\cdot \ln\frac{\delta^i E + 1000}{\delta^i E_0 + 1000}}{T} \qquad \textbf{7.21}$$

The calculated rate constant represents the rate of removal from biodegradation or abiotic transformation. The rate of removal is distinct from the bulk attenuation rate k that is calculated by plotting the natural logarithm of the concentrations against the time of travel along the flow path (Newell et al., 2002). The bulk attenuation rate also includes the effect of dilution through dispersion on the concentration in addition to the effect of removal.

Equation 7.20 can be solved for the travel time required along the flow path ($T_{required}$) to attain any desired concentration ($C_{required}$) at the field-scale rate of removal λ_t.

$$T_{required} = -\ln\left(\frac{C_{required}}{C_0}\right) / \lambda_t \qquad \textbf{7.22}$$

Multiplication of ($T_{required}$) by the contaminant velocity ($V_{seepage}$) yields an equation for the distance along the flow path from the source or the up gradient monitoring well that is required to reduce the contaminant concentration to the desired concentration ($L_{required}$).

$$L_{required} = -\ln\left(\frac{C_{required}}{C_0}\right) * (V_{seepage}) / \lambda_t \qquad \textbf{7.23}$$

where

$V_{seepage}$ = the actual seepage velocity of the contaminant in ground water along the flow path.

The seepage velocity is usually estimated by dividing the Darcy velocity by the effective porosity, and then dividing by the retardation factor for the contaminant.

If the value of $C_{required}$ is a Maximum Contaminant Level (MCL), a clean up goal, or other regulatory standard, a value for $L_{required}$ can be used to estimate a perimeter beyond which the concentrations of a contaminant should no longer be of regulatory concern. The value of $L_{required}$ can be calculated without knowledge of the ground water flow velocity. The calculation of $L_{required}$ is conservative because it does not include reductions in concentrations caused by dilution or dispersion.

8.0
Stable Isotope Enrichment Factors

This section summarizes the isotope enrichment factors that are available in the literature at the time this section was written. However, the literature on isotope fractionation is growing rapidly, and this section is only offered as a point of departure. The reader should perform a literature search to update the information needed for a particular application.

Table 8.1. Isotope enrichment factors (ε) for aerobic and anaerobic biodegradation of selected ground water pollutants. Intrinsic enrichment factors (ε_i) for carbon isotope fractionation have been calculated following Morasch et al. (2004), where $\varepsilon_i = \varepsilon * n$. Values of ε_i are provided in the third column to illustrate the isotope effect at the atom where the reaction takes place.

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fractionation of other elements	Conditions	Bacteria	Reference
BTEX Compounds						
Benzene	ε = -1.46	ε_i = -8.8	$^{2}H/^{1}H$: ε = -12	Oxic	*Acinetobacter sp*.	(Hunkeler et al., 2001b)
Benzene	ε = -3.53	ε_i = -21.2	$^{2}H/^{1}H$: ε = -11.	Oxic	*Burkholderia sp*.	(Hunkeler et al., 2001b)
Benzene	ε = -2.4 ε = -2.0	ε_i = -14.4 ε_i = -12	$^{2}H/^{1}H$: ε = -29 ε = -35	Nitrate-reducing	Enrichment culture	(Mancini et al., 2003)
Benzene	ε = -3.6	ε_i = -21.6	$^{2}H/^{1}H$: ε = -79	Sulfate-reducing	Enrichment culture	(Mancini et al., 2003)
Benzene	ε = -1.9 ε = -2.1	ε_i = -11.4 ε_i = -12.6	$^{2}H/^{1}H$: ε = -60	Methanogenic	Enrichment culture	(Mancini et al., 2002) (Mancini et al., 2003)
Ethylbenzene	ε = -2.2	ε_i = -17.6		Nitrate-reducing	Strain EBN1	(Meckenstock et al., 2004)
Ethylbenzene	ε = -3.7	ε_i = -30		Sulfate-reducing	Enrichment culture	(Wilkes et al., 2000)
Toluene	Not significant			Oxic	Microcosms	(Sherwood Lollar et al., 1999)
Toluene	ε = -3.3	ε_i = -23.1		Oxic	*Pseudomonas putida* strain mt-2	(Morasch et al., 2002)

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fractionation of other elements	Conditions	Bacteria	Reference
Toluene	ε = -1.7 (high iron) ε = -2.5 (low iron)	ε_i = -11.9 ε_i = -17.5	$^{2}H/^{1}H$: ε = -77 (high iron) ε = -159 (low iron)	Oxic	*Pseudomonas putida* strain mt-2	(Mancini et al., 2006)
Toluene	ε = -1.1	ε_i = -7.7		Oxic	*Ralstonia pickettii* strain PKO1	(Morasch et al., 2002)
Toluene	ε = -0.4	ε_i = -2.8		Oxic	*Pseudomonas putida* strain F1	(Morasch et al., 2002)
Toluene	ε = -1.7	ε_i = -11.9		Nitrate-reducing	*Thauera aromatica*	(Meckenstock et al., 1999)
Toluene	ε = -1.8	ε_i = -12.6		Fe(III)-reducing	*Geobacter metallireducens*	(Meckenstock et al., 1999)
Toluene	ε = -0.8	ε_i = -5.6		Sulfate-reducing	Enrichment culture	(Ahad et al., 2000)
Toluene	ε = -1.5	ε_i = -10.5		Sulfate-reducing	Column experiment	(Meckenstock et al., 1999)
Toluene	ε = -2.2	ε_i = -15.4		Sulfate-reducing	*Desulfobacterium cetonicum*	(Morasch et al., 2001)
Toluene	ε = -1.7	ε_i = -11.9		Sulfate-reducing	Strain TRM1	(Meckenstock et al., 1999)
Toluene			$^{2}H/^{1}H$: ε = -728	Sulfate-reducing	strain TRM1	(Morasch et al., 2001)
Toluene			$^{2}H/^{1}H$: ε = –198	Sulfate-reducing	*Desulfobacterium cetonicum*	(Morasch,et al., 2001)
Toluene	ε = -0.5	ε_i = -3.5		Methanogenic	Enrichment culture	(Ahad et al., 2000)
Toluene			$^{2}H/^{1}H$: ε = -12 ε = -65	Methanogenic	Consortium	(Ward et al., 2000)
m-Xylene	ε = -1.7	ε_i = -13.6		Oxic	*Pseudomonas putida* strain mt-2	(Morasch et al., 2002)
m-Xylene	ε = -1.8	ε_i = -14.4		Sulfate-reducing	Strain OX39	(Morasch et al., 2004)
p-Xylene	ε = -2.3	ε_i = -18.4		Oxic	*Pseudomonas putida* strain mt-2	(Morasch et al., 2002)
o-Xylene	ε = -1.5	ε_i = -12		Sulfate-reducing	Strain OX39	(Morasch et al., 2004)

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fraction-ation of other elements	Conditions	Bacteria	Reference
o-Xylene	ε = -1.1	ε_i = -8.8		Sulfate-reducing	Column experiment	(Richnow et al., 2003)
o-Xylene	ε = -3.2	ε_i = -25.6		Sulfate-reducing	Enrichment culture	(Wilkes et al., 2000)
m-Cresol	ε = -3.9	ε_i = -27.3		Sulfate-reducing	*Desulfobacterium cetonicum*	(Morasch,et al., 2004)
p-Cresol	ε = -1.6	ε_i = -11.2		Sulfate-reducing	*Desulfobacterium cetonicum*	(Morasch et al., 2004)
Polyaromatic Hydrocarbons						
Naphthalene	ε = -0.1	ε_i = -1.1		Oxic	*Pseudomonas putida* strain NCIB9816	(Morasch et al., 2002)
Naphthalene	ε = -1.1	ε_i = -11		Sulfate-reducing	Enrichment culture N47	(Griebler et al., 2004b)
2-Methyl-naphthalene	ε = -0.9	ε_i = -9.9		Sulfate-reducing	Enrichment culture N47	(Griebler et al., 2004b)
2-Methyl-phenanthrene	No enrichment			Oxic	*Sphingomonas sp*. strain 2MPII	(Mazeas and Budzinski, 2002)
Fluoranthene	No enrichment			Oxic	*Sphingomonas paucimobilis*	(Hammer et al., 1998)
Chlorinated Hydrocarbons						
PCE			$^{37}Cl/^{35}Cl$ ε = -10	Anoxic, dehalogenating	Strain T, consortium N, consortium F	(Numata et al., 2002)
PCE	ε = -5.2	ε_i = -10.4		Anoxic, dehalogenating	Consortium (butyric acid)	(Slater et al., 2001)
PCE	Enrichment estimated 2‰			Anoxic, dehalogenating	Microcosm experiment	(Hunkeler et al., 1999)
PCE	ε = -5.2 to -8.8	ε_i = -10.4 to -17.6		Anoxic, dehalogenating	*Desulfitobacterium* sp. PCE-S	(Nijenhuis et al., 2005)
PCE	ε = -0.42 to -1.7	ε_i = -0.84 to -3.4		Anoxic, dehalogenating	*Sulfurospirillum multivorans*	(Nijenhuis et al., 2005)
PCE	ε = -0.46 to -3.2	ε_i = -0.92 to -6.4		Anoxic, dehalogenating	*Sulfurospirillum halorespirans*	(Cichocka et al., 2007)
TCE	ε = -18.2	ε_i = -36.4		Oxic	*Burkholderia cepacia* strain G4	(Bill et al., 2001)

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fraction-ation of other elements	Conditions	Bacteria	Reference
TCE	ε = -1.1	ε_i = -2.2		Oxic, cometabolic	*Methylosinus trichosporium* OB3b	(Chu et al., 2004)
TCE	ε = -10.9 to -12.2	ε_i = -21.8 to -24.4		Anoxic, dehalogenating	*Desulfitobacterium* sp. PCE-S	(Cichocka et al., 2007)
TCE	ε = -13.2 to -18.7	ε_i = -26.4 to -37.4		Anoxic, dehalogenating	*Sulfurospirillum multivorans*	(Cichocka et al., 2007)
TCE	ε = -18.7 to -22.9	ε_i = -37.4 to -45.8		Anoxic, deha-logenating	*Sulfurospirillum halorespirans*	(Cichocka et al., 2007)
TCE	ε = -16.4	ε_i = -32.8		Anoxic, dehalogenating	*Sulfurospirillum multivorans*	(Lee et al., 2007)
TCE	ε = -3.3	ε_i = -6.6		Anoxic, dehalogenating	*Dehalobacter restrictus* strain PER-K23	(Lee et al., 2007)
TCE	ε = -9.6	ε_i = -19.2		Anoxic, dehalogenating	*Dehalococcoides ethenogenes* 195	(Lee et al., 2007)
TCE	ε = -6.6; ε = -2.5	ε_i = -13.2 ε_i = -5		Methanogenic, dehalogenating	Enrichment culture	(Bloom et al., 2000)
TCE	ε = -7.1	ε_i = -14.2		Anoxic, dehalogenating	Mixed facultative anaerobic culture	(Sherwood Lollar et al., 1999)
TCE	ε = -13.8	ε_i = -27.6		Anoxic, dehalogenating	Consortium (MeOH)	(Slater et al., 2001)
TCE	Enrichment estimated 4‰			Anoxic, dehalogenating	Microcosm experiment	(Hunkeler et al., 1999)
TCE			$^{37}Cl/^{35}Cl$ ε = -5.5	Sulfate-reducing, dehalogenating	Strain T	(Numata et al., 2002)
TCE			$^{37}Cl/^{35}Cl$ ε = -5.6	Anoxic, dehalogenating	Consortium N	(Numata et al., 2002)
TCE			$^{37}Cl/^{35}Cl$ ε = -5.7	Anoxic, dehalogenating	Consortium F, nitrate reducing	(Numata et al., 2002)
TCE			$^{37}Cl/^{35}Cl$ ε = -30	Anoxic, dehalogenating	Strain T, consortium N, consortium F	(Numata et al., 2002)
cis-DCE	No enrichment			Oxic, cometabolic	*Methylosinus trichosporium* OB3b	(Chu et al., 2004)
cis-DCE	ε = -21.1	ε_i = -42.2		Anoxic, dehalogenating	*Dehalococcoides ethenogenes* 195	(Lee et al., 2007)

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fraction-ation of other elements	Conditions	Bacteria	Reference
cis-DCE	ε = -16.9	ε_i = -33.8		Anoxic, dehalogenating	*Dehalococcoides* sp. Strain BAV1	(Lee et al., 2007)
cis-DCE	ε = -14.1 ε = -16.1	ε_i = -28.2 ε_i = -32.2		Methanogenic, dehalogenating	Enrichment culture	(Bloom et al., 2000)
cis-DCE	ε = -19.9	ε_i = -39.8		Anoxic, dehalogenating	Microcosms	(Hunkeler et al., 2002)
cis-DCE	ε = -20.4	ε_i = -40.8		Anoxic, dehalogenating	Consortium (MeOH)	(Slater et al., 2001)
cis-DCE	Enrichment estimated 12‰			Anoxic, dehalogenating	Microcosm experiment	(Hunkeler et al., 1999)
trans-DCE	ε = -3.5	ε_i = -7		Oxic, cometabolic	*Methylomonas methanica*	(Brungard et al., 2003)
trans-DCE	ε = -6.7	ε_i = -13.4		Oxic, cometabolic	*Methylosinus trichosporium* OB3b	(Brungard et al., 2003)
trans-DCE	ε = -21.4	ε_i = -42.8		Anoxic, dehalogenating	*Dehalococcoides* sp. Strain BAV1	(Lee et al., 2007)
trans-DCE	ε = -30.3	ε_i = -60.6		Anoxic, dehalogenating	Microcosms	(Hunkeler et al., 2002)
1,1-DCE	ε = -7.3	ε_i = -14.6		Anoxic, dehalogenating	Microcosms	(Hunkeler et al., 2002)
1,1-DCE	ε = -5.8	ε_i = -11.2		Anoxic, dehalogenating	*Dehalococcoides ethenogenes* 195	(Lee et al., 2007)
1,1-DCE	ε = -8.4	ε_i = -16.8		Anoxic, dehalogenating	*Dehalococcoides* sp. Strain BAV1	(Lee et al., 2007)
VC	ε = -5.7	ε_i = -11.4		Oxic, metabolic	*Mycobacterium aurum* L1	(Chu et al., 2004)
VC	ε = -3.2	ε_i = -6.4		Oxic, cometabolic	*Methylosinus trichosporium* OB3b	(Chu et al., 2004)
VC	ε = -4.8	ε_i = -9.6		Oxic, cometabolic	*Mycobacterium vaccae* JOB5	(Chu et al., 2004)
VC	ε = -4.5	ε_i = -9.0		Oxic, cometabolic	Enrichment culture Travis	(Chu et al., 2004)
VC	ε = -5.5	ε_i = -11		Oxic, cometabolic	Enrichment culture Alameda	(Chu et al., 2004)
VC	ε = -8.2	ε_i = -16.4		Oxic	*Mycobacterium* sp. JS60	(Chartrand et al., 2005)
VC	ε = -7.1	ε_i = -14.2		Oxic	*Mycobacterium* sp. JS61	(Chartrand et al., 2005)
VC	ε = -7.1	ε_i = -14.2		Oxic	*Mycobacterium* sp. JS617	(Chartrand et al., 2005)

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fractionation of other elements	Conditions	Bacteria	Reference
VC	ε = -7.6	ε_i = -15.2		Oxic	*Nocardioides* sp. JS614	(Chartrand et al., 2005)
VC	ε = -24.0	ε_i = -48		Anoxic, dehalogenating	*Dehalococcoides* sp. Strain BAV1	(Lee et al., 2007)
VC	ε = -21.5	ε_i = -43		Methanogenic, dehalogenating	Enrichment culture	(Bloom et al., 2000)
VC	ε = -22.4	ε_i = -44.8		Anoxic, dehalogenating	Consortium (MeOH)	(Slater et al., 2001)
VC	ε = -31.1	ε_i = -62.2		Anoxic, dehalogenating	Microcosms	(Hunkeler et al., 2002)
VC	ε estimated -26			Anoxic, dehalogenating	Microcosm experiment	(Hunkeler et al., 1999)
Dichloromethane			$^{37}Cl/^{35}Cl$ ε = -3.8	Oxic	Biodegradation	(Holt et al., 1997)
Dichloromethane	ε = –41 to –66	ε_i = –41 to –66		Oxic	Various methylotrophic bacteria	(Nikolausz et al., 2006)
Dichloromethane	ε = –46 to –61	ε_i = –45 to –61		Denitrifying	Various methylotrophic bacteria	(Nikolausz et al., 2006)
1,2-Dichloroethane	ε = -32 ε = -27	ε_i = -64 ε_i = -54		Oxic	*Xanthobacter autotrophicus*	(Hunkeler and Aravena, 2000a)
1,2-Dichloroethane	ε = -32.1	ε_i = -64.2		Anoxic, dehalogenating	Microcosms	(Hunkeler et al., 2002)
1,2-Dichloroethane	ε = -32.3	ε_i = -64.6		Oxic	*Xanthobacter autotrophicus* GJ10	(Hirschorn et al., 2004)
1,2-Dichloroethane	ε = -32.1	ε_i = -64.2		Oxic	*Ancylobacter aquaticus* AD20	(Hirschorn et al., 2004)
1,2-Dichloroethane	ε = -3.0	ε_i = -6.0		Oxic	*Pseudomonas* sp. StrainDCA1	(Hirschorn et al., 2004)
1,1,2-Trichloroethane	ε = -2.0	ε_i = -4		Anoxic, dehalogenating	Microcosms	(Hunkeler et al., 2002)
Chlorobenzene	ε = -0.4	ε = -2.4		Oxic	*Ralstonia sp.*	(Kaschl et al., 2005)
Chlorobenzene	ε = -0.3	ε = -1.8		Oxic	*R. erythropolis*	(Kaschl et al., 2005)
Chlorobenzene	ε = -0.2	ε = -1.2		Oxic	*P. veronii*	(Kaschl et al., 2005)
Chlorobenzene	ε = -0.1	ε = -0.6		Oxic	*A. facilis*	(Kaschl et al., 2005)
1,2,4-Trichlorobenzene	Not significant			Oxic	*Pseudomonas sp.* strain P51	(Griebler et al., 2004a)

Compound	$^{13}C/^{12}C$ fractionation	Intrinsic $^{13}C/^{12}C$ fractionation	Fraction-ation of other elements	Conditions	Bacteria	Reference
1,2,4-Trichloro-benzene	ε = -3.2	ε_i = -19.2		Anoxic, dehalogenating	*Dehalococcoides sp.* strain CBDB1	(Griebler et al., 2004a)
1,2,3-Trichloro-benzene	ε = -3.5	ε_i = -21		Anoxic, dehalogenating	*Dehalococcoides sp.* strain CBDB1	(Griebler et al., 2004a)
Fuel Oxygenates						
MTBE	ε = -2 ε = -2.4	ε_i = -10 ε_i = -12	$^{2}H/^{1}H$ ε = -36	Oxic	Strain PM1	(Gray et al., 2002)
MTBE	ε = -1.5 ε = -1.8	ε_i = -7.5 ε_i = -9	$^{2}H/^{1}H$ ε =-66 ε =-29	Oxic	Enrichment culture	(Gray et al., 2002)
MTBE	ε = -1.52 ε = -1.	ε_i = -7.6 ε_i = -5		Oxic	Microcosm experiments	(Hunkeler et al., 2001a)
MTBE	ε = -0.48	ε_i = -2.4	$^{2}H/^{1}H$ no enrich-ment	Oxic	Strain L108	(Rosell et al., 2007)
MTBE	ε = -0.28	ε_i = -1.4	$^{2}H/^{1}H$ no enrich-ment	Oxic	Strain IFP2001 (resting cells)	(Rosell et al., 2007)
MTBE	ε = -2.4	ε_i = -11.8	$^{2}H/^{1}H$ ε = -42	Oxic	Strain R8	(Rosell et al., 2007)
MTBE	Estimated ε = -9.2 ε = -14.2 ε = -4.2	ε_i = -45.8 ε_i = -70.8 ε_i = -20.8		Anoxic	Microcosms	(Kolhatkar et al., 2002)
MTBE	ε = -13	ε_i = -65	$^{2}H/^{1}H$ ε = -16	Anoxic	Enrichment culture	(Kuder et al., 2005)
MTBE	ε = -15.6	ε_i = -78		Methanogenic	Microcosm experiments	(Somsamak et al., 2005)
MTBE	ε = -14.4	ε_i = -78		Methanogenic and sulfate-reducing	Enrichment cultures	(Somsamak et al., 2006)
ETBE	ε = -0.68	ε_i = -4.1	$^{2}H/^{1}H$ ε = -14	Oxic	Strain L108	(Rosell et al., 2007)
ETBE	ε = -0.8	ε_i = -4.6	$^{2}H/^{1}H$ ε = -11	Oxic	Strain L108 (resting cells)	(Rosell et al., 2007)
ETBE	ε = -0.8	ε_i = -4.4	$^{2}H/^{1}H$ ε = -11	Oxic	Strain IFP2001 (resting cells)	(Rosell et al., 2007)
TAME	ε = -13.7	ε_i = -68.5		Methanogenic	Microcosm experiments	(Somsamak et al., 2005)
TBA	ε = -4.2	ε_i = -16.8		Oxic	Microcosm experiments	(Hunkeler et al., 2001a)

9.0
Recommendations for the Application of CSIA

Compound Specific Isotope Analysis (CSIA) provides another dimension of information on pollutants in the environment to supplement knowledge of their chemical identity and their concentration. CSIA has matured into a technique that can be used on a routine basis, in particular for carbon isotope analysis, for a wide range of organic contaminants relevant to hydrogeology and environmental geochemistry. Modern instrumentation can provide valid determinations of isotope ratios at low concentrations of contaminants that are near their regulatory standards or clean-up goals.

Application of CSIA at a contaminated site should start with a clear idea of the information that is sought from stable isotope analysis. Basically, there are three distinct goals: (i) source characterization or differentiation; (ii) qualitative proof of biodegradation or abiotic transformation; or, (iii) quantification of biodegradation or abiotic transformation processes. Advantages and limitations of the use of CSIA for these purposes have been discussed in detail throughout this guideline. If the specific interest is *quantification* of degradation, the first step is to consult the literature (summarized in Table 8.1) to determine whether an appropriate isotopic enrichment factor is available. Using the enrichment factor, the second step is to estimate whether the observed changes in concentration of the contaminant at the field site are sufficient to produce a measurable change in the isotope ratio. If these two prerequisites are met there is a good chance that it will be possible to put a conservative boundary on the extent of biodegradation or abiotic transformation at the field site.

On a per sample basis, the cost of an individual isotope analysis is substantially higher than the cost of a VOC analysis to identify the chemical, and determine its concentration. This is due to the price of the equipment required to perform CSIA, the costs of consumables, the level of training and experience required for the analytical chemist, and the number of standards and sample duplicates that are needed to ensure reliable data as discussed in section 2.4. However, a simple comparison of direct costs for analyses is not very meaningful. Instead, consider the total cost of a site investigation. If the additional information from CSIA leads to a robust conceptual model for a site, it can lead to savings in conventional monitoring. If there is greater faith in the site conceptual model, adequate monitoring can be attained with fewer rounds of sampling and analysis, and fewer monitoring wells.

Furthermore, CSIA can guide decisions on selection and implementation of remediation strategies, and can be used to monitor the performance of remedial technology in an early stage of implementation. Thus, the huge waste in resources that are associated with the selection of an unsuitable remediation strategy might be avoided.

Compound specific isotope analysis can be applied on a routine basis when precautions are taken to ensure high quality data and the appropriate interpretation of the data. However, CSIA cannot replace a proper hydrological and geochemical characterization or measurements of contaminant concentrations. Multiple lines of evidence will continue to be necessary to come to a meaningful assessment of the risks associate with the contaminants and the selection of an appropriate remedy.

It is our hope that this Guide will be a useful introduction for beginners in environmental isotope analysis. We expect that CSIA will have a growing role in investigations at hazardous waste sites. The growth in the application of CSIA is driven by continued improvements in analytical methods, by more widespread availability of the instruments used in CSIA, by an increasing number of publications showing the broad applicability of CSIA to a variety of contaminants, and by an increasing appreciation for the unique information provided by CSIA. We have only "scratched the surface" of the potential of CSIA to provide a better understanding of the source, distribution, and behavior of organic compounds at contaminated field sites.

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