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Stable Isotope Forensics—When Isotopes Work

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Stable isotopic analysis—particularly compound specific stable carbon isotopic analysis—is being increasingly investigated and applied as a tool to investigate and monitor the sources and fates of contaminant compounds in the environment. Results of an increasing number of studies indicate that stable isotopic analysis is a promising tool in environmental chemistry. This paper discusses reported results and presents a case study of stable carbon isotopic analysis of volatile organic groundwater contaminants to illustrate the present abilities and limitations of the application of stable isotopic analysis to environmental contaminants. Though this paper focuses on stable carbon isotopic analysis of volatile organic compounds, the principles discussed herein are relevant to all applications of isotopic analysis as an environmental forensic tool.

Keywords: stable isotope, environment, compound specific, contaminant.

Introduction

Stable isotopic analysis is being increasingly applied to investigate and monitor the sources, transport, and fates of organic contaminants in environmental systems. This interest has been largely due to recent demonstrations of the ability of isotopic analysis to differentiate sources of, and to demonstrate the degradation of, contaminant organic compounds in the environment.

Isotopic analysis of a number of organic contaminants has shown that different sources of polycyclic aromatic hydrocarbons (PAHs) (O’Malley et al., 1996), polychlorinated biphenyls (PCBs) (Jarman et al., 1998; Reddy et al., 2000; Drenzek et al., 2002), methyl tert-butyl ether (MTBE) (Smallwood et al., 2001), chlorinated ethenes (van Warmerdam et al., 1995; Jendrzejewski et al., 2001), and BTEX (Benzene, Toluene, Ethyl-benzene, Xylenes) compounds (Dempster et al., 1997) can have different stable carbon and chlorine isotopic compositions or fingerprints. For semivolatile organic compounds (SVOCs) such as PAHs and PCBs, the stable carbon isotopic compositions are conserved during environmental processes such as degradation (O’Malley et al., 1994; Trust et al., 1995; Drenzek et al., 2001), leading to the suggestion that isotopic analysis can be used to differentiate sources of these compounds in the environment. In contrast, volatile organic compounds (VOCs) such as the chlorinated ethenes and BTEX compounds, which are some of the most commonly observed contaminants of groundwater systems, are strongly isotopically fractionated during degradation (Dayan et al., 1999; Heraty et al., 1999; Meckенstock et al., 1999; Sherwood Lollar et al., 1999; Bloom et al., 2000; Hunkeler, 2000; Ward, 2000; Bill et al., 2001; Hunkeler et al., 2001a; Morasch et al., 2001; Slater et al., 2001). Current evidence also shows that nondegradative subsurface processes such as volatilization (Harrington et al., 1999; Poulson and Drayer, 1999; Slater et al., 1999) and sorption (Slater et al., 2000) do not significantly fractionate these compounds relative to the fractionations induced by degradative processes. Thus isotopic analysis can offer definitive demonstration of the degradation of these compounds in the environment independent of mass loss due to nondegradative processes.

The ability of isotopic analysis to identify the occurrence of degradation makes it a potential tool for identifying and monitoring intrinsic bioremediation, or natural attenuation, of groundwater contaminants. This approach is being increasingly applied at field sites to identify the occurrence of degradation of groundwater contaminants (Kelley et al., 1997; Sturchio et al., 1998; Beneteau et al., 1999; Stehmeier et al., 1999; Sherwood Lollar et al., 2001; Mancini et al., 2002; Song et al., 2002). The results of these studies and of the expanding work with new compounds such as MTBE (Hunkeler et al., 2001b; Gray et al., 2002) and the n-alkanes (Pond et al., 2002) demonstrate that isotopic analysis has a great deal of potential as a tool in investigating and monitoring the sources and fates of organic contaminants in the environment.

With the increasing interest and application of isotopic analysis to environmental contamination, it is important also to consider the limits of this technology so that data from field applications is interpreted in a realistic and defensible way and

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to delineate areas where future research can further the understanding and application of isotopic analysis. With this in mind, this paper discusses the present abilities and limitations of stable carbon isotopic analysis to resolve sources and fates of contamination in the environment. The aim of this work is to illustrate key principles and issues involved in the application of stable isotopic analysis. Though what will be discussed is applicable to all isotopes and compounds of interest, this paper will focus primarily on stable carbon isotopic analysis of VOCs as groundwater contaminants, as this topic has been most thoroughly studied.

Methods

Stable isotopic analysis involves determining the ratio of two stable isotopes in a sample. For carbon this is the ratio \( R = ^{13}\text{C} / ^{12}\text{C} \). This ratio is expressed in delta notation, which relates the isotopic ratio of a sample to an internationally accepted standard by the formula

\[
\delta^{13}\text{C} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

(1)

in units of permil. In the case of carbon, the internationally accepted standard is Vienna Pee Dee Belemnite (VPDB). Similar ratios for other common isotopes in environmental applications are \(^2\text{H} / ^1\text{H}, ^{18}\text{O} / ^{16}\text{O}, \) and \(^{37}\text{Cl} / ^{35}\text{Cl} \), which are reported relative to the international standards SMOW (Standard Mean Ocean Water) and SMOC (Standard Mean Ocean Chloride). The application of isotopic analysis to investigate sources and fates of contaminants in the environment depends on the ability of this technique to reliably resolve differences in these isotopic ratios between samples. One source of such a difference is isotopic fractionation of a sample during an environmental process. During an isotopically fractionating process molecules containing the lighter isotope react at a slightly faster rate than those containing the heavier isotope. This difference in reaction rate is caused by the greater energy required to break bonds containing the heavier isotope (Galimov, 1985). The result of this difference in reaction rate is that the residual reactant pool becomes relatively enriched in the heavier isotope, while the product pool is relatively depleted in the heavier isotope. That is, the \( \delta \) value of the reactant pool becomes more positive than its initial \( \delta \) value, and the \( \delta \) value of the product pool is correspondingly more negative than the initial \( \delta \) of the reactant pool. This type of isotopic fractionation is known as a kinetic isotope effect (Galimov, 1985). Kinetic isotope effects can often be described by the Rayleigh model (Equation (2)), where the change in the isotopic ratio of the reactant pool, \( R \), from its initial ratio, \( R_0 \), is related to the fraction of reactant remaining, \( f \), by a fractionation factor, \( \alpha \).

\[
R / R_0 = \left( \frac{\alpha}{1} \right)
\]

(2)

A detailed development of the applicability of the Rayleigh model to a one-step irreversible reaction, such as a biological enzymatic reaction, was carried out by Mariotti et al. (1981), who demonstrated that the fractionation factor for such a process could be determined from the equation

\[
(\alpha - 1) \ln f = \ln \left( \frac{(\delta^{13}\text{C}_r/1000) + 1}{((\delta^{13}\text{C}_r/1000) + 1)} \right)
\]

(3)

where \( \delta^{13}\text{C}_r \) represents the initial isotopic composition of the substrate and \( \delta^{13}\text{C}_r \) represents the isotopic composition of the substrate at a fraction remaining \( f \). A least-squares regression of a plot of lnf versus ln((\delta^{13}\text{C}_r/1000) + 1)/(((\delta^{13}\text{C}_r/1000) + 1)) yields a slope of \( \alpha - 1 \). This fractionation factor can then be converted to a permil enrichment factor, \( \varepsilon = 1000 (\alpha - 1) \), for easier comparison.

The effect of isotopic fractionation on the \( \delta^{13}\text{C} \) of a closed reaction system is illustrated in Figure 1. The upper line on this figure shows the changes in \( \delta^{13}\text{C} \) of the residual reactant, \( \delta^{13}\text{C}_r \), relative to the initial \( \delta^{13}\text{C}_r \), for a reaction with an enrichment factor of \( \varepsilon = -7.1\% \) (\( \alpha = 0.9929 \)). The \( \delta^{13}\text{C}_r \) is isotopically enriched (becomes more positive) relative to the \( \delta^{13}\text{C}_r \) as the reaction proceeds from a fraction remaining of 1 to 0. The instantaneous product, \( \delta^{13}\text{C}_{ip} \), refers to the \( \delta^{13}\text{C} \) of the product of any instantaneous step of the reaction. This value is isotopically depleted relative to (more negative than) the \( \delta^{13}\text{C}_r \) by a constant amount which is represented by the enrichment factor \( \varepsilon \). However, this value can rarely be measured; in general what is measured is the

\[
\delta^{13}\text{C}_{ap} = \delta^{13}\text{C}_r - \varepsilon (\ln f / (1 - f))
\]

(4)

Figure 1. Theoretical change in reactant and product isotopic compositions in a single-step irreversible reaction with an enrichment factor, \( \varepsilon = -7.1\% \). \( \delta^{13}\text{C}_r \) refers to initial isotopic composition; \( \delta^{13}\text{C}_r \) refers to residual reactant isotopic composition as reaction goes from a fraction remaining, \( f \), of 1 to 0. \( \delta^{13}\text{C}_{ip} \) refers to the isotopic composition of the instantaneous product of any reaction step. \( \delta^{13}\text{C}_{ap} \) refers to the isotopic composition of the accumulated product (after Mariotti et al., 1981).
accumulated product pool, $\delta^{13}C_{ap}$, which has an isotopic composition which is a result of an isotopic mass balance of the entire instantaneous product that has been produced up to that point in the reaction. In a closed system, once quantitative conversion of the reactant to product has occurred the $\delta^{13}C_{ap}$ will equal the $\delta^{13}C_{ro}$. The equations that describe the isotopic compositions of these pools are shown on Figure 1 and correspond to those developed in detail by Mariotti et al. (1981). Present applications of isotopic analysis to field situations primarily focus on looking for the enrichment trends in the isotopic composition of the residual reactant as degradation occurs.

Application of isotopic analysis to environmental problems such as groundwater contamination has in some cases been criticized for being too costly, requiring greater interpretation, and being too variable from site to site (Brady et al., 1998). However, this criticism is more applicable to the initial applications of isotopic analysis to the $\delta^{13}C$ of soil gas CO$_2$ as a tool to monitor the degradation of petroleum hydrocarbons (Suchomel et al., 1990; Aggarwal and Hinchee, 1991). Indeed, this approach does suffer from some of these factors. In particular, this approach has difficulty resolving CO$_2$ produced during degradation of petroleum hydrocarbon from that produced during the respiration of natural organic matter, since both have $\delta^{13}C$ signatures in the range of $-25$ to $-35\%$ as a result of primarily being produced via the C$_3$ photosynthetic pathway. This illustrates an important point in the application of isotopic analysis to environmental applications. In order to resolve an isotopic difference between two pools of a compound, the difference between the pools must be greater than the variability of either of the end-members. In the case of CO$_2$, this lack of resolution can be overcome by applying this approach in situations where a resolvable difference exists between natural organic matter and contaminant compounds. Natural organic matter in ecosystems dominated by plants using the C$_4$ photosynthetic pathway, such as salt marsh systems, have $\delta^{13}C$ signatures for organic matter in the range of $-12$ to $-19\%$, thus CO$_2$ produced during degradation of petroleum contamination ($-25$ to $-35\%$) can be resolved from that produced during degradation of the natural organic matter in the system (Jackson et al., 1996).

The development of online gas chromatography–isotope ratio mass spectrometry (GC–IRMS) and current research into its application as a tool in contaminant hydrogeology has overcome many of the criticisms of isotopic analysis. GC–IRMS enables compound specific isotope analysis (CSIA) of individual organic compounds through the use of the GC interface directly coupled to an isotope ratio mass spectrometer (IRMS) (Matthews and Hayes, 1978). Previous to the development of GC–IRMS technology, stable carbon isotopic analysis of organic materials was carried out by combusting bulk samples, or individual compounds purified in extensive preparation procedures, in quartz breakseals with copper oxide. The CO$_2$ generated was then transferred to the dual inlet system of an IRMS. The online nature of GC–IRMS increased the sensitivity of isotopic analysis by 4 to 5 orders of magnitude relative to the traditional sealed tube combustion method of isotopic analysis of organic compounds. This greater sensitivity, added to the compound-specific nature of this technique, greatly outweighs the slight loss in precision due to GC-IRMS (from standard deviations generally better than 0.1‰ for off-line techniques to a range of 0.1 to 0.3‰ for on-line methods) and has made application of isotopic analysis to organic environmental contaminants possible. Additionally, GC-IRMS provides determination of the $\delta^{13}C$ of individual compounds in a complex mixture and thus minimizes interferences by other organic matter in the system, which can be a problem in the bulk analysis or soil gas CO$_2$ approach. The development of preparation techniques which allow isotopic analysis of organic compounds at concentrations relevant to environmental contamination levels such as pentane extraction (Dempster et al., 1997), headspace analysis (Slater et al., 1999), Solid Phase MicroExtraction (SPME) (Dias and Freeman, 1997; Hunkeler and Aravena, 2000), and purge and trap techniques (Song et al., 2002) have made sample preparation requirements and timeframes for CSIA analogous to those for concentration analysis. And while the cost of isotopic analysis can still be higher than concentration analysis due to the use of specialized instrumentation and expertise, these costs are coming down. Further, the information which can be gained from a suite of isotopic analyses can far outweigh the slight increase in costs, which is generally less than the cost to install one additional monitoring well at a site.

Though isotopic analysis is still in its early development and there are no established protocols for its application at field sites, nevertheless reputable academic laboratories carrying out isotopic analysis as part of their research programs can be expected to report sufficient information to ensure quality assurance and control. Notwithstanding the lack of established protocols, the growing body of work described in the first paragraph of this article is demonstrating its potential to yield information which is directly applicable to the contaminant and the processes which have affected it. In many cases CSIA allows direct information on the source and environmental processing of a compound which previously could only be inferred by other parameters. However, although isotopic analysis can be a powerful tool in environmental chemistry, it must be remembered that it is not a “silver bullet.” It cannot answer all questions at all field sites. What it can do is provide another tool, an additional, independent line of evidence, with which contaminant hydrogeologists and environmental chemists can better understand the complex sources and fates of organic compounds in the environment.

Source versus fate

The information that can be gained from CSIA is dependent on the isotopic behavior of the compound as it undergoes environmental processes (i.e., any process involved in the transport and fate of the contaminant in the environment, including dissolution, volatilization, sorption, and degradation). If the isotopic composition of a contaminant is conserved as it undergoes environmental processing, then isotopic analysis has the potential to differentiate contributions from sources with distinct isotopic compositions. Alternatively, if the isotopic composition of a
contaminant is fractionated during environmental processing, then isotopic analysis has the potential to yield information on what processes have affected the contaminant and to what extent these have occurred. Much of the initial work in the field of environmental isotopic analysis has been to characterize the isotopic effects of such environmental processes for different contaminants. Figures 2 and 3 illustrate the results of such studies using data from published studies isotopically characterizing environmental processes (Figure 2) and sources differences (Figure 3) for trichloroethene (TCE).

Figure 2 shows the results of several laboratory studies characterizing the isotopic effects of environmental processes acting on TCE as a representative VOC. The diamonds on this figure show the $\delta^{13}$C of TCE, which has undergone equilibrium sorption to both activated carbon and graphite as reported in Slater et al. (2000). The solid horizontal line represents the initial isotopic composition of the TCE ($\approx -30.8_{\text{‰}}$). The dashed lines on either side of this represent a range of 0.5‰ on either side of this data. The error bars on this data represent the reported precision of 0.5‰. This data shows that while there is some variability in the $\delta^{13}$C of the TCE during sorption, the range of variability does not exceed 0.5‰. The one data point which shows a variability of $>0.5_{\text{‰}}$ is still not significantly different relative to the initial $\delta^{13}$C of the TCE when a precision of 0.5‰ is applied to both values. These results are representative of results that have been reported for nondegradative processes affecting the VOCs including sorption, volatilization, and dissolution (Harrington et al., 1999; Poulson and Drever, 1999; Slater et al., 1999, 2000; Hunkeler et al., 2001b). Though small isotopic effects have been reported for volatilization of VOCs from pure phase (Harrington et al., 1999; Huang et al., 1999; Poulson and Drever, 1999), the energetics of volatilization from pure phase and aqueous solution are different such that it is arguable that these small isotopic effects will not be observed for volatilization from aqueous solution, as has been reported in the literature (Slater et al., 1999, 2000). However, even if these isotopic effects were observed to affect environmental samples, in the worst case it would require $>90\%$ volatilization of TCE to shift its $\delta^{13}$C by $>1\%$. Though the one data point which shows a variability of $>0.5_{\text{‰}}$ is still not significantly different relative to the initial $\delta^{13}$C of the TCE when a precision of 0.5‰ is applied to both values. These results are representative of results that have been reported for nondegradative processes affecting the VOCs including sorption, volatilization, and dissolution (Harrington et al., 1999; Poulson and Drever, 1999; Slater et al., 1999, 2000; Hunkeler et al., 2001b). Though small isotopic effects have been reported for volatilization of VOCs from pure phase (Harrington et al., 1999; Huang et al., 1999; Poulson and Drever, 1999), the energetics of volatilization from pure phase and aqueous solution are different such that it is arguable that these small isotopic effects will not be observed for volatilization from aqueous solution, as has been reported in the literature (Slater et al., 1999, 2000). However, even if these isotopic effects were observed to affect environmental samples, in the worst case it would require $>90\%$ volatilization of TCE to shift its $\delta^{13}$C by $>1\%$. Though

![Figure 2](image-url) **Figure 2.** The isotopic composition ($\delta^{13}$C) of TCE versus fraction of TCE remaining in solution during sorption to graphite and activated carbon from Slater et al. (2000) (diamonds). The horizontal line represents the initial $\delta^{13}$C of the TCE. The dashed lines represent a range of 0.5‰ around this value. This data shows that during sorption variation of up to 0.5‰ can be induced. Similar variation has been observed for dissolution and volatilization. The triangles represent the isotopic composition of TCE undergoing microbial degradation by the Pinellas consortium as reported by Sherwood Lollar et al. (1999). The error bars represent the 0.5‰ field precision. The solid line represents the Rayleigh model which describes isotopic fractionation by this consortium. The outer dashed lines represent the range of enrichment factors which have been observed for anaerobic degradation of TCE (Bloom et al., 2000; Slater et al., 2001). Clearly, the isotopic fractionation during degradation can be resolved from the isotopic effects of nondegradative processes such as sorption.

![Figure 3](image-url) **Figure 3.** $\delta^{13}$C TCE from different sources as reported in van Warmerdam et al. (1995), Holt et al. (1997), Beneteau et al. (1999), and Jendrzejewski et al. (2001). Manufacturers’ identities are given in Table 1. Inner error bars represent reported off-line precisions. Outer error bars represent 0.5‰ “maximum field precision.” Hatched areas denote samples which cannot be isotopically distinguished within a “maximum field precision” of 0.5‰.

<table>
<thead>
<tr>
<th>Numerical key</th>
<th>Manufacturer</th>
<th>$\delta^{13}$C</th>
<th>Off-line precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dow</td>
<td>$-31.90$</td>
<td>0.05</td>
</tr>
<tr>
<td>1A</td>
<td>Dow</td>
<td>$-29.84$</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>ICI</td>
<td>$-31.32$</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>PPG</td>
<td>$-27.80$</td>
<td>0.01</td>
</tr>
<tr>
<td>3A</td>
<td>PPG</td>
<td>$-31.68$</td>
<td>0.17</td>
</tr>
<tr>
<td>4</td>
<td>Aldrich</td>
<td>$-33.49$</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>Holt et al. (1997)</td>
<td>$-27.18$</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>$-31.53$</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>$-27.90$</td>
<td>0.08</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>$-29.93$</td>
<td>0.18</td>
</tr>
</tbody>
</table>

As reported by van Warmerdam et al. (1995), Holt et al. (1997), Beneteau et al. (1999), and Jendrzejewski et al. (2001). Numerical key gives identity of manufacturers plotted in Figure 3. Samples 1 to 3 are from van Warmerdam et al. (1995). Data 1A and 3A are repeat analyses of samples 1 and 3 reported in Beneteau et al. (1999). Samples 4 and 6–8 are from Jendrzejewski et al. (2001) and sample 5 is from Holt et al. (1997). Identities of manufacturers reported by Holt et al. (1997) and Jendrzejewski et al. (2001) were not given, so it is uncertain whether these data represent new manufacturers or repeat analysis of manufacturers analyzed by van Warmerdam et al. (1995) and Beneteau et al. (1999).
this possibility cannot be discounted without considering the specifics of a given site, at the majority of sites such extreme extents of volatilization are unlikely, and thus nondegradative processes are very unlikely to result in significant isotopic fractionation of the VOCs with respect to a precision of 0.5‰.

In contrast to the results observed for nondegradative processes, degradative processes result in significant isotopic fractionation of some of the VOCs, the chlorinated ethenes in particular. The circles on Figure 1 show data for the dechlorination of TCE by the Pinellas microbial consortium as reported in Sherwood Lollar et al. (1999). The error bars on these data points represent the reported precision of 0.5‰. The solid line represents a Rayleigh model of this data using an enrichment factor of −7.1‰. The dashed lines represent Rayleigh model lines showing the range of enrichment factors which have been observed for anaerobic degradation of TCE from −2.5‰ to −13.8‰ (Bloom et al., 2000; Slater et al., 2001). This figure demonstrates one of the most powerful potentials of isotopic analysis which is that the isotopic effects of degradation of TCE can clearly be distinguished from the variations induced by nondegradative processes. Similar results have been found for degradation of other VOCs such as MTBE (Hunkeler et al., 2001b; Gray et al., 2002) and in some cases the BTEX compounds (Meckenstock et al., 1999). Isotopic evidence of degradation can be taken even further for the chlorinated ethenes because microbial dechlorination takes place via sequential removal of chlorine atoms, and each step is isotopically fractionating (Bloom et al., 2000; Slater et al., 2001). The isotopic fractionation of the chlorinated ethenes can thus be followed throughout the degradation series, allowing degradation products to be related to their parent compounds. However, because of the complexities which can occur at field sites such relationships can be difficult to establish. Thus observation of isotopic enrichment of the $\delta^{13}C$ of perchloroethylene (PCE) and TCE is the best evidence to demonstrate degradation of these compounds at a site.

Variation in $\delta^{13}C$ of TCE at a field site may also be the result of multiple sources of TCE having distinct $\delta^{13}C$ signatures. Figure 3 shows the isotopic composition of free product TCE obtained from different manufacturers as reported by van Warmerdam et al. (1995), Beneteau et al. (1999), Holt et al. (1997), and Jendrzejewski et al. (2001). (The data for these studies are listed in Table 1.) The inner error bars on these data points are the precisions reported for the off-line analysis of these samples. Using this level of precision it can be seen from Figure 3 that many of these sources of TCE are isotopically distinct. However, this high level of precision reflects only the precision of the instrument and sample handling procedure. Because field samples will have been obtained from an environment where they likely have been affected by dissolution, sorption, and volatilization and will mostly likely have been analyzed by on-line techniques, the isotopic variations which can be induced by these processes must be assumed to have affected these samples. Figure 2 shows representative results of the up to 0.5‰ isotopic variation that is observed for these nondegradative environmental processes. Because any sample of TCE obtained in the field may have been affected by these processes to an unknown extent, it is presently impossible to isotopically differentiate between sources which differ by less than 1.0‰ (or ±0.5‰ precision on each $\delta^{13}C$).

This difficulty in reliable interpretation of small variations in isotopic composition is also supported by a confidence interval argument. In the field of GC-IRMS there is some controversy over what analytical precisions should be quoted for analysis of environmental samples. Generally, the standard deviation of multiple, independent analyses of a sample can be expected to be in the range of 0.1 to 0.3‰. This value is often what is quoted in papers and implied as the resolution of this technique. However, one standard deviation represents a confidence interval of only 68%. In order to delineate 95% confidence intervals for a sample two standard deviations are required, which corresponds to a range of 0.2 to 0.6‰. The implication of these two arguments to interpretation of isotopic data from field samples is that in order to reliably determine that two samples are isotopically distinct, taking into account our present understanding of the small isotopic variations due to nondegradative processes and the use of 95% confidence intervals, the $\delta^{13}C$ of the samples must differ by at least 1.0‰. Hopefully with advances in our understanding of the controls on the isotopic effects of nondegradative subsurface processes and advances in analytical techniques, isotopic variations of lesser magnitude can become interpretable, but presently isotopic differences of <1.0‰ cannot be reliably interpreted.

Implication to Application

Applying this argument to the $\delta^{13}C$ of TCE from the different sources in Figure 3 it can be seen that many of the samples which appeared isotopically distinct based on the off-line precisions quoted for their analysis, become indistinguishable. In fact, use of 0.5‰ precision results in only four isotopically distinct groups of TCE sources which are shown as the hatched areas on Figure 3. This implies that in a field situation where no degradation is occurring, it is indeed potentially possible to use isotopic analysis to differentiate between sources of TCE, provided they fall into isotopically distinct ranges analogous to those represented on this figure.

However, there is another factor which may confound source differentiation which is illustrated by Figure 3 and Table 1. That is the issue of temporal source variation. If a TCE source is the result of a single release of TCE to the environment, then its source $\delta^{13}C$ is the $\delta^{13}C$ of the TCE which entered the subsurface. However, if a site experiences repeated releases of TCE, then the $\delta^{13}C$ of the TCE in the source will be an isotopic mass balance of the total amount of TCE released to the source area. The potential for temporal variation of the $\delta^{13}C$ TCE of such a source is illustrated by the repeated analysis of TCE from DOW and PPG reported by Beneteau et al. (1999) (Figure 3, Table 1). TCE from these suppliers was originally analyzed by van Warmerdam et al. (1995), and the isotopic compositions are reported as samples 1A and 3A. It can be seen from
Figure 3 that these two sets of samples differ by a significantly greater amount than the 1‰ range shown by the outer error bars. This temporal isotopic variation of TCE from a single supplier is sufficient to result in variations as great as that observed between suppliers. Thus, if multiple or long-term releases of TCE have occurred in an environment, temporal variation of sources must be taken into account before source differentiation is possible. Temporal source differentiation may also have significance relative to demonstration of degradation of TCE in the field as will be discussed later in the context of field site TS-1.

The implication of our present understanding of isotopic behavior of VOCs in the subsurface to the demonstration of degradation in the field is primarily to define the extent of degradation required for a resolvable signal of degradation. In order for degradation to be reliably resolved from nondegradative subsurface processes, a VOC must have been fractionated by more than 1.0‰. For the range of enrichment factors measured to date for TCE this may occur after as little as 7% degradation for a system with an enrichment factor of $\delta^{13}C = 13.8$‰ but could require as much as 33% degradation in a system with an enrichment factor of $\delta^{13}C = 2.5$‰. However, as will be discussed in reference to TS-1, the potential for temporal variability at most sites requires much greater extents of fractionation for reliable demonstration of degradation.

**Application to Field Sites**

A set of criteria to demonstrate the occurrence of biodegradation using isotopic analysis have been outlined by Sherwood Lollar et al. (1999). These criteria can be worded in a more general way so that they can be applied to both identification of degradation and source differentiation.

**Criteria**

1. *A clear difference or distinction in isotopic values must exist between the pools of a compound that are to be resolved.* In the case of source differentiation, the pools that are being resolved are the two source pools of the contaminant. In the case of degradation, the two pools are the degraded and undegraded contaminant.

2. *This isotopic difference must be greater than the precision at which the isotopic compositions of the compounds of interest can be known.* As discussed above, the isotopic distinction must be greater than the small isotopic variations/imprecision induced by environmental processes and instrumental precision.

3. *Isotopic behavior of the compound of interest must be predictable.* The isotopic effects of environmental processes on the compounds of interest must be known.

Once a basis for interpretation has been generated in the laboratory, such as the results illustrated in Figures 2 and 3 which fulfill criteria 2 and 3 for TCE, then isotopic analysis can be applied to field situations. However, when isotopic analysis moves from the controlled conditions of the laboratory to the field there are several issues that arise which need to be understood in order to make reliable interpretations of field data. These issues can be illustrated by looking at data from a field site.

**Case Study**

Figure 4 shows TS-1, a TCE-contaminated field site where isotopic analysis of the TCE was carried out and reported in Slater (2001). This field site consisted of an anaerobic, unconfined aquifer located in glacial till deposits that extend down 6.5 m into fractured upper layers of the underlying shale bedrock. The water table is 0.3 to 1.0 m below ground surface. Groundwater flow direction is indicated on Figure 4. There are two source zones at the site, related to TCE transfer and storage points in the facility and indicated on Figure 4 as Area 1 and Area 2. Area 1 was associated with TCE storage and has concentrations of TCE which approach saturation. Area 2 was associated with TCE transfer in the facility and has lower concentrations of TCE. Concentrations decrease down-gradient from these areas to reach levels of $<50 \mu g/L$ at the periphery of the site. Two transects of wells were sampled at the site in order to profile each of the source zones along the primary groundwater flow direction. Transect 1 profiled Area 1 and consisted of wells BH-1, BH-2, BH-3, BH-4, BH-5, and BH-6. Transect 2 profiled Area 2 and consisted of wells BH-7, BH-8, and BH-9. (Figure 4) The isotopic composition of the TCE along these two transects is shown in Figures 5a and 5b for Transect 1 and 2 respectively. Large, progressive isotopic enrichments of 10‰ and 4‰ are observed in Transect 1 and Transect 2 respectively. These enrichment trends are coincident with concentration decreases of 3 orders of magnitude in Transect 1 and one order of magnitude in Transect 2.
The data from this field site illustrates several important points about the application of isotopic analysis in the field. First, these results show clear evidence of the degradation of TCE at TS-1. The isotopic enrichments observed are orders of magnitude greater than the reported precision of 0.5‰. This demonstrates the capability of isotopic analysis to indicate the occurrence of degradation of TCE in the field. However, this site can also be used to demonstrate some of the challenges that arise when isotopic analysis is applied in the field.

**Source Zone δ¹³C**

The first challenge is in determining source zone δ¹³C. At this site the δ¹³C of the TCE in the source areas is the most depleted observed, at the site at −31.9‰ and −29.3‰ for Areas 1 and 2, respectively. This is what is expected based on the results of laboratory studies which indicate that degradation should enrich the δ¹³C of the TCE from its source isotopic composition. But the difference in isotopic composition between these source zones (2.6‰) might be taken to indicate that the isotopic composition of these two sources is different. However, the TCE at this site came from one storage and transfer system using the same TCE which suggests that the δ¹³C of the TCE in the two source zone should be the same. A more likely reason for the observed variability between these two source zones is that the TCE sampled in Area 2 has been isotopically enriched due to degradation. The concentrations of TCE in BH-2 (Area 1) approach TCE solubility, and this well has yielded free product TCE in the past. These high concentrations likely inhibit any bacterial degradation of TCE, and thus the δ¹³C at this well is likely representative of the source. In contrast, BH-7 has TCE concentrations well below solubility (the highest concentration measured here was 8000 μg/L), thus the TCE in this well may have been degraded and isotopically enriched from its original value. Since it is unusual to have a well that samples a source directly (as was the case at BH-2) at a site where VOC degradation may be occurring, it is almost impossible to differentiate isotopic differences due to source differences from those induced by degradation. Only at a site where there is no degradation and thus no isotopic fractionation of the VOCs can isotopic analysis be used to differentiate between sources.

**Temporal Variability**

A second potential cause of variability in the δ¹³C of contaminants in field applications of isotopic analysis is the possibility of temporal source zone variability. Since the isotopic composition of VOCs has been shown to vary (Figure 3, Table 1) it is possible that isotopic variation between sources or downgradient at a site is a result of temporal variation of the isotopic composition of the source zone due to releases of chemicals obtained from different sources. Temporal variation in the δ¹³C of TCE released to a source zone will result in variation in the δ¹³C of the source, the extent of which will be determined by an isotopic mass balance between the old and new TCE in the source zone. This mixing will moderate the extent of isotopic variation induced by source zone variation. In the worst case, however, the potential for temporal variability means that reliable interpretation of degradation of TCE can only be achieved in cases where degradation has enriched the δ¹³C of the TCE to values outside the potential range of source variation. In the case of field site TS-1, the reported range of isotopic compositions of TCE is from −27‰ to −32‰. Thus, temporal variability is another possible explanation of the observed difference in δ¹³C between the two source areas. With respect to degradation, the potential range of δ¹³C temporal variability means that only the last three data points in Transect 1 and the last point in Transect 2 give strong evidence of degradation, as they are isotopically
enriched beyond the range of $\delta^{13}C$ which can be explained due to temporal variability.

Data Density

This demonstrates the importance of sampling density, and preferably sampling transects, at a site. If only two or three samples had been taken at this site, it is possible that no data outside of the range of potential source variation would have been obtained. The fact that strong evidence of degradation is seen for the latter data points at this site, and that the extent of fractionation is correlated to decreases in concentration is strong evidence that this observed isotopic enrichment is indeed due to degradation. Furthermore, the progressive nature of the isotopic enrichment observed at this site suggests that degradation is responsible for the isotopic enrichment of all the samples. That is, the fact that degradation is responsible for the latter portion of the observed trend can be used as evidence that degradation is in fact responsible for the entire isotopic enrichment trend at the site. It is unlikely that source zone variability has resulted in isotopic enrichment of the early data points, which is coincidentally consistent with the latter degraded data points. In addition, it can be argued that source zone variability should not produce a consistent enrichment trend, but that it should result in a step function that may vary in either direction. That is, the addition of a new source of TCE with a $\delta^{13}C$, which is enriched relative to the original source, should result in dissolution of TCE to the plume, which has an isotopic composition determined by the isotopic mass balance between the two sources. This variation should occur relatively rapidly as the sources intermingle. After this initial variation, the $\delta^{13}C$ of TCE in the plume should stabilize at the new, mixed source $\delta^{13}C$. This variation would be expected to be observed as a consistent $\delta^{13}C$ for part of the plume, followed by an enrichment, or depletion trend, which then stabilizes at a new $\delta^{13}C$. Since it is equally likely that a new source of TCE will be isotopically depleted relative to the previous TCE as that it would be isotopically enriched, successive temporal source zone variation is more likely to produce a trend of enrichment and depletion rather than consistent enrichment. Measuring the $\delta^{13}C$ of TCE along a transect of a plume allows assessment of the potential contribution of source zone variation, and thus a more reliable interpretation of the data.

However, consistent progressive isotopic enrichment trends are not always observed. Sherwood Lollar et al. (2001) observed a more chaotic pattern of $\delta^{13}C$ of TCE at Dover AFB. In particular, their Transect II showed a trend of isotopic enrichment of TCE in the first two wells down gradient of the source zone that was followed by depletion of the $\delta^{13}C$ TCE in the next well and a second enrichment trend. The observation of such a complex pattern again illustrates the need to analyze transects of samples and the need to know the history and potential sources at a site. There are several possible causes of the $\delta^{13}C$ pattern observed at Dover AFB. The observation of down-gradient TCE that is isotopically depleted ($\sim-27.2‰$) relative to the TCE in the source zone ($\sim-26.0‰$) may indicate temporal variability of the TCE source. The observed variation is quite small and is well within the observed variation of TCE sources. This variation may also indicate the presence of another source of TCE at the site. This may be an unknown source zone at the site, in which case isotopic analysis has provided information that was not obtained in the extensive application of more traditional groundwater methods. It is also possible that the isotopically depleted TCE is being produced by the degradation of PCE at the site. Degradation of PCE will produce TCE, which is isotopically depleted relative to the PCE. The $\delta^{13}C$ of PCE in this area is isotopically depleted ($\sim-31.7‰$) relative to the TCE in the well, and even assuming that no fractionation took place during the degradation of the PCE it would only require that 3.8% of the TCE in this well be the result of degradation of PCE in order to generate the 1.2‰ isotopic difference between the $\delta^{13}C$ TCE of the source and the $\delta^{13}C$ TCE in this well. Though differentiating between these possible causes of this observed isotopic depletion is very difficult, by sampling a transect of data convincing isotopic evidence of degradation at this site was obtained, notwithstanding this one data point. A more limited data set may not have provided reliable evidence of degradation at this site.

A further reason to obtain transects of samples at as high density as possible is the delineation of zones of degradation. This was again observed by Sherwood Lollar et al. (2001), where highly isotopically enriched TCE ($\sim-18.0‰$) was observed in one well. This well was also where the highest concentration of degradation products (cis-dichloroethene, cDCE; vinyl chloride, VC; ethene) was observed. Thus, a densely sampled transect can allow areas of high degradation to be identified as well. This may be particularly important for compounds which do not have easily measurable degradation products, such as MTBE and the BTEX compounds.

Quantification

In laboratory studies the Rayleigh model has been shown to describe the relationship of isotopic fractionation to the extent of degradation for some compounds. This suggests that isotopic analysis can be used to quantify the extent of degradation of these compounds. As shown in Figure 1, the enrichment factors ($\epsilon$) obtained from laboratory studies can be used to predict the isotopic composition of the residual reactant pool using the following equation

$$\delta^{13}C_r = \delta^{13}C_o + \epsilon \ln f$$

(4)

where $r$ is the reactant, $o$ is the initial reactant, and $f$ is the fraction of reactant remaining. It can be seen from this equation that if the initial and degraded isotopic compositions of a contaminant are known, then they can be combined with the enrichment factor to determine the fraction remaining, $f$. Accurate estimation of the total extent of degradation of a compound depends on knowing the initial $\delta^{13}C$. However, in cases where this is not possible, this equation can be used to predict the extent of degradation between selected data points. The most crucial factor in
the accuracy of these estimations is knowing the enrichment factor, which is applicable at a site.

As previously stated, an enrichment factor can be determined by plotting the isotopic composition of a compound versus fraction remaining. The linear nature of the change in $\delta^{13}$C TCE versus concentration seen in Figure 5 at TS-1 suggests that such an approach could be used to generate a fractionation factor for this site. Indeed, when the data from Transect 1 is regressed using Equation (3), an enrichment factor of $\varepsilon = -1.3\%$ is obtained with an $r^2$ of 0.99. This result illustrates a potential danger in using isotopic enrichment to quantify degradation at a site. Though the results at TS-1 indicate that there is a constant relationship between reduction of dissolved concentrations of TCE and the isotopic composition of the TCE, it is highly unlikely that this enrichment factor is describing exclusively isotopic fractionation during degradation. This is primarily due to the fact that the site is not at steady state. Therefore, concentration changes over the plume will be due to groundwater transport as well as degradation, and therefore the enrichment factor will reflect the effects of both of these processes. The fact that a nonlinear relationship is observed in Transect 2 demonstrates that the relationship observed for Transect 1 is not valid over the whole site. These factors demonstrate that careful consideration must be taken when interpreting isotopic data from contaminated field sites. A mathematical relationship between data is not sufficient to assume that a fractionation factor is accurate. An understanding of the hydrogeological and geochemical factors at the site is essential to making valid interpretations of the data.

Though precise quantification of degradation cannot be achieved at this site, an estimate of degradation extent can be made using the isotopic enrichment factors known for this reaction. Studies of isotopic fractionation during biodegradation of the chlorinated ethenes have shown there is significant variability in $\varepsilon$ for the TCE, from $-2.5\%$ to $-13.8\%$ (Bloom et al., 2000; Slater et al., 2001). The factors which control this range of fractionation are not known at this time. Therefore, which of these $\varepsilon$ is applicable to a field site cannot be determined. But by using the range of $\varepsilon$ known for a particular process an estimate of the extent of degradation which has occurred can be generated based on isotopic analysis. Applying the range of known $\varepsilon$ to the 10% enrichment observed over Transect 1 at TS-1 from BH-2 to BH-6 results in an estimate of 98% to 52% degradation for enrichment factors of $-2.5\%$ and $-13.8\%$, respectively. Because the initial $\delta^{13}$C TCE at BH-2 is likely representative of the $\delta^{13}$C of the source, this estimate represents an estimate of the total degradation of TCE over this transect. If the same range of $\varepsilon$ ($-2.5\%$ and $-13.8\%$) is applied to the data from Transect 2, an estimate of 88% to 24% degradation is obtained. Because the initial $\delta^{13}$C for this transect is not known, this represents an estimate of the amount of degradation between BH-7 and BH-9. If the initial $\delta^{13}$C TCE for Transect 2 was assumed to be the same as Transect 1, the estimate of degradation would become 92% to 37%. The implication of this estimate is that degradation is responsible for a maximum of one order of magnitude degradation of TCE over Transect 1. This means that the other two orders of magnitude concentration decrease observed over Transect 1 must be due to groundwater transport effects. This isotopic estimate of the extent of degradation of a compound is independent of observed mass loss due to processes such as groundwater transport effects and other nondegradative processes at the site. This type of information is very useful for estimating degradation rates to be input into flow models at field sites which is a necessary part of understanding the longevity and transport of contaminants at a site. This type of estimate may be even more important for compounds such as MTBE where no easily measurable degradation products exist to use in estimating the extent of degradation.

**Conclusions**

The results presently in the literature demonstrate that isotopic analysis can give strong evidence of the degradation of some environmental contaminants, such as the chlorinated ethenes, MTBE, and in some cases the BTEX compounds. This evidence of degradation is independent of mass losses due to nondegradative, nonfractionating environmental processes such as dissolution, volatilization, and sorption. Further, for compounds where the range of isotopic enrichment factors has been characterized, such as the chlorinated ethenes, isotopic analysis can be used to generate an estimate of the extent of degradation which has occurred between two samples. This type of application is presently most reliable for stable carbon isotopic analysis of the chlorinated ethenes because the knowledge base for interpretation of field data for these compounds is the most developed. However, present research on other compounds, other isotopes such as $^{37}$Cl and $^2$H, and other processes is building this knowledge base for many other potential applications, including analysis of multiple isotopes on the same sample to increase our ability to characterize its behavior.

Generation of this knowledge base is essential for the interpretation of field data, as demonstrated by this paper. The isotopic variability which will be induced by environmental processes must be characterized so that reliable interpretation of field data is possible. This includes further refinement of our understanding of the controls on processes which result in small isotopic variations must be achieved if the information contained in these small variations is to be accessed. The potential isotopic variability in contaminant sources must be characterized in order to validly interpret isotopic differences as the results of degradation and not temporal variation. The isotopic enrichment factors for degradative processes must be known such that the extent of degradation of a compound can be estimated from the isotopic shifts observed in the field. And the reproducibility of these enrichment factors for different microbial/environmental systems must be known so that situations in which isotopic fractionation is expected to occur can be defined. Furthermore, valid application and interpretation of isotopic analysis in the field requires sufficient data density and coverage. Sampling of transects of groundwater plumes and relating isotopic data to other
groundwater parameters is an important part of valid interpretation of isotopic field data.

The development of sample preparation and analysis techniques has brought the timeframes, interpretability, and costs of isotopic analysis much closer to that for traditional concentration analysis. Thus, isotopic analysis is a powerful tool for investigation and monitoring of the sources and fates of environmental contaminants, provided it is applied in situations where the basis of knowledge is sufficient for isotopic forensics to work.

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