Origin and Fate of Organic Compounds in Water: Characterization by Compound-Specific Stable Isotope Analysis

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Abstract

Within the past 15 years, compound-specific stable isotope analysis has continued to increase in popularity in the area of contaminant hydrology of organic molecules. In particular, in cases where concentration data alone are insufficient to elucidate environmental processes unequivocally, the isotope signature can provide additional unique information. Specifically, it can help answer questions about contaminant source apportionment, quantification of biotic and abiotic processes, and identification of transformation reactions on a mechanistic level. We review advances in laboratory and field investigations and exemplary applications in contaminant hydrology via stable isotope analysis. We also highlight future directions in the field.

1. INTRODUCTION

CSIA: compound-specific stable isotope analysis

IRMS: isotope ratio mass spectrometry

Compound-specific stable isotope analysis (CSIA), performed through the coupling of gas chromatography (GC) and liquid chromatography (LC) with isotope ratio mass spectrometry (IRMS), has been developed into a mature analytical tool in different branches of science (1, 2). Authenticity testing of food ingredients (3, 4) and control of steroid doping in sports (5) are prominent examples. Over the past 15 years, CSIA has also found broad application in environmental sciences, especially in the hydrology of organic contaminants (6–8). Monitoring of organic water contaminants in terms of identification and quantification is a major application area of modern analytical chemistry. Hyphenated analytical tools such as GC–mass spectrometry (MS) and LC–tandem MS (LC-MS/MS) have been continuously improved and are standard for structural and quantitative compound determination (9, 10). However, such traditional approaches often fail when questions arise about origin and fate (11) in terms of (*a*) contaminant source differentiation and apportionment on a local and regional scale (7), (*b*) the identification and quantification of biotic and abiotic transformation reactions on scales ranging from batch experiments to contaminated field sites (8), and (*c*) the identification of such transformation reactions on a mechanistic level (9, 12, 13). In particular, the determination of natural attenuation processes in an aquifer is of great economic interest (11) because long-term, expensive technical remediation is often not necessary or even feasible (8). However, if only concentration data are available, it is difficult to differentiate between dilution and dispersion on the one hand and contaminant degradation by natural attenuation or abiotic transformation processes on the other hand (7, 11). In such cases, CSIA can provide additional information through the application of stable isotope ratios of elements such as carbon, hydrogen, nitrogen, oxygen, sulfur, and chlorine as natural intrinsic tracers. The bases of CSIA measurements are slight variations in the isotope ratios, which can be used as isotope fingerprints. These slight variations at the natural abundance level are small, and mass spectrometers that can provide a precision on the order of 10^{-2} to 10^{-4}% are generally necessary (14). Such high precision can be obtained with magnetic sector field instruments with Faraday cups that enable the simultaneous detection of ion currents from different mass-to-charge ratios for isotope ratio determination (15). The relative abundance of the heavy (^{h}E) and light (^{l}E) isotopes of a given element E in a molecule of a given compound is determined and expressed by the isotope ratio $R_{\mathrm{comp}} = (^{h}E/^{l}E)$. For the light elements considered here, the heavier isotope is less abundant and appears in the numerator, whereas the more abundant, lighter isotope appears in the denominator. **Table 1** lists the measured isotope ratios with their natural abundance. These very small differences between isotope ratios are commonly expressed by the so-called δ notation (16). Equation 1 defines the δ value of a compound $\delta(E)_{\mathrm{comp}}$ as the relative difference between the isotope ratio of a sample or compound R_{comp} and the isotope ratio of an internationally accepted primary reference material R_{std}:

$$\delta(E)_{\mathrm{comp}} = \left(\frac{R_{\mathrm{comp}} - R_{\mathrm{std}}}{R_{\mathrm{std}}}\right) = \left(\frac{R_{\mathrm{comp}}}{R_{\mathrm{std}}} - 1\right). \qquad (1)$$

The δ value is not part of the International System of Units, and such values are usually expressed in parts per thousand or per mill (with the symbol ‰) because they are always much smaller than one in natural abundance studies. Note that we have omitted the common multiplier 10^3 in this equation to be consistent with the latest International Union of Pure and Applied Chemistry recommendations, as recently emphasized by Elsner (9). The δ notation has the advantage that the values are expressed relative to a common international primary reference material, which is a prerequisite for data comparability in different laboratories on an international scale. Furthermore,

Table 1 Characteristics of the light stable isotopes utilized in compound-specific isotope analysis

Element	Stable isotopes[a]	Natural abundance (%)[b]		Relative mass difference	International primary reference material with accepted isotope ratio ($\times 10^6$)[c]
		^{h}E	^{l}E		
Hydrogen	$^{2}H/^{1}H$	0.0155	99.98	2.00	VSMOW, 155.75 ± 0.08
Carbon	$^{13}C/^{12}C$	1.1060	98.89	1.08	VPDB, 11,180.2 ± 2.8
Nitrogen	$^{15}N/^{14}N$	0.3663	99.63	1.08	AIR-N_2, 3,678.2 ± 1.5
Oxygen	$^{18}O/^{16}O$	0.2000	99.76	1.13	VPDB, 2,005.2 ± 0.45; VSMOW, 2,067.2
Chlorine	$^{37}Cl/^{35}Cl$	24.2200	75.78	1.06	SMOC, 319,627 ± 199[d]

[a] Measured isotope ratio, where the lower abundant heavy isotope is written in the nominator.

[b] Values adapted from Reference 101.

[c] Values adapted from Reference 17.

[d] Value adapted from Reference 102. Abbreviations: AIR-N_2, nitrogen from air; SMOC, standard mean ocean chloride; VPDB, Vienna Pee Dee Belemnite; VSMOW, Vienna standard mean ocean water.

a determination of relative ratios is easier and allows for correction for mass-discriminating effects in a single instrument (17).

The small variations in the isotope ratios mainly have their origin in mass-dependent isotope fractionation processes caused by (thermodynamic) equilibrium isotope effects (EIEs) or kinetic isotope effects (KIEs) (18, 19). Primary KIEs in which the isotope effect occurs directly in the reacting bond during, for example, a transformation process provide the main information, according to isotopic fractionation in such systems. The isotope fractionation between two compounds, such as a substrate and its degradation product, can be expressed either by the fractionation factor α or by the enrichment factor ε, according to Equation 2:

$$\alpha = \frac{R_{\text{product}}}{R_{\text{reactant}}} = \frac{\delta(E)_{\text{product}} + 1}{\delta(E)_{\text{reactant}} + 1} = \varepsilon + 1, \qquad (2)$$

where R_{reactant} and R_{product} are the ratios of the heavy isotope to the light isotope in the substrate and the degradation product, respectively, that appear in an infinitely short period of time (7).

For small molecules in which all isotopes are located in the same reactive position, α and the KIE are linked by KIE $= {}^{l}k/{}^{h}k = \alpha^{-1}$, where ${}^{h}k$ and ${}^{l}k$ are the rate constants of compounds containing heavy and light isotopes at the reactive position (9). If a specific bond is broken, the involved elements generally show a normal isotope effect (KIE > 1), so molecules containing the heavier isotope at the specific position react more slowly than those containing the lighter one. The heavy isotope is then accumulated in the reactant. Inverse KIEs (KIE < 1) are observed in cases in which stiffer bonds are formed in the rate-limiting step (12).

2. INSTRUMENTAL DEVELOPMENTS IN COMPOUND-SPECIFIC STABLE ISOTOPE ANALYSIS

CSIA in contaminant hydrology is currently carried out mainly by GC-IRMS (9). Coupling involves a three-step process: (*a*) separation of individual carbon-bearing compounds on a gas chromatograph, (*b*) quantitative conversion of each compound into a conversion gas, and (*c*) measurement of the isotope ratio in the isotope ratio mass spectrometer (**Figure 1**) (7). Depending on the measured element, two types of conversion are usually applied. The first type is a

KIE: kinetic isotope effect

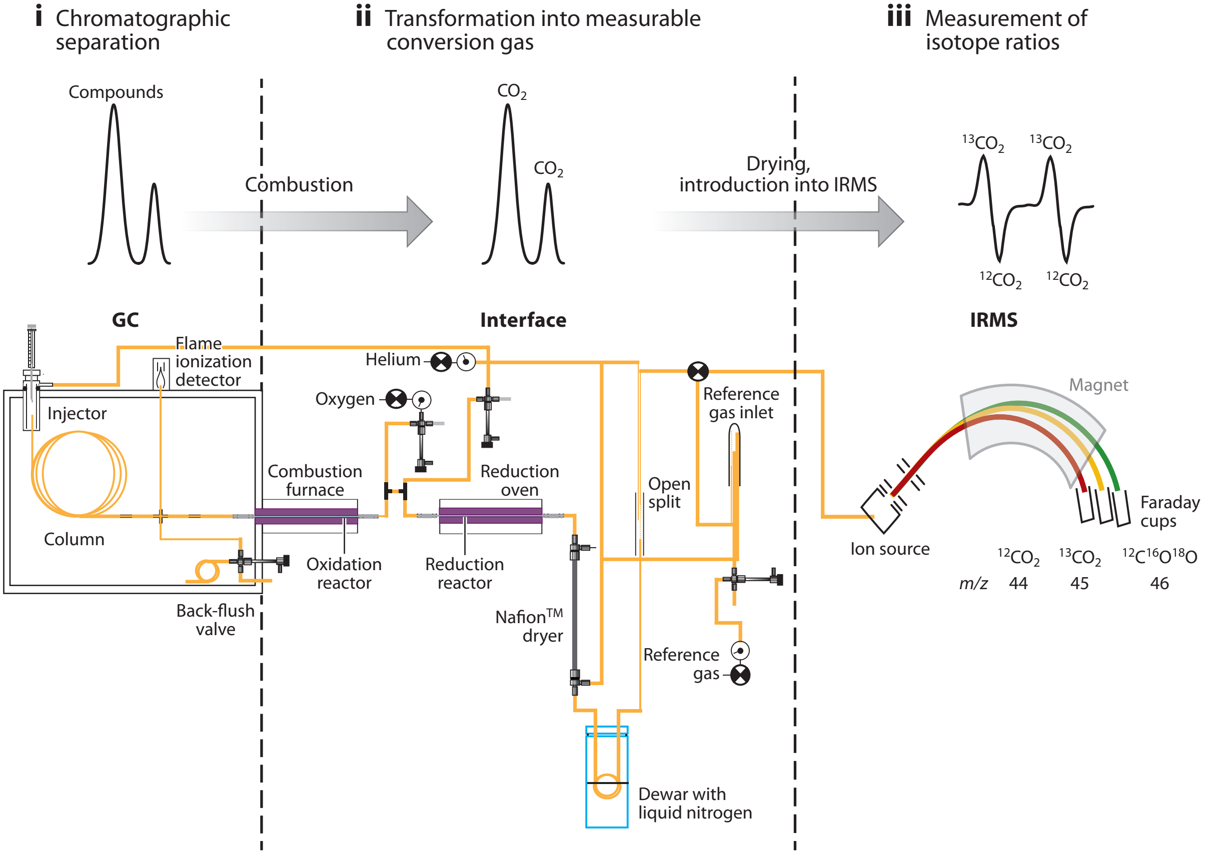

Figure 1

Schematic of the gas chromatography–isotope ratio mass spectrometry (GC-IRMS) instrument and the general procedure used in compound-specific stable isotope analysis of carbon. At bottom is a schematic view of the instrumentation; at top is the respective output from the different steps.

combustion or oxidation interface for the conversion of carbon and nitrogen in organic compounds to carbon dioxide and nitrogen/nitrogen oxides. In the case of nitrogen isotope analysis, following the oxidation process, the developed nitrogen oxides are converted to molecular nitrogen through an additional online reduction oven. The second type of conversion is referred to as a pyrolysis or high-temperature conversion interface for hydrogen and oxygen isotope ratio determination. In this case, an online reduction of organic compounds to hydrogen gas or carbon monoxide is used (20). Although dual isotope plots and the measurements of different isotopes for the determination of transformation mechanisms are very useful (see Section 3.2, below), so far, carbon isotope measurements are much more frequently utilized than the other CSIA-amenable elements (7). However, there have been increasing efforts to use the other elements (21–23).

LC-IRMS coupling has been commercially available since 2004 (24). However, so far the method has been restricted to aqueous solvents and, therefore, mainly to ion-exchange chromatographic separations. Attempts to overcome these restrictions with high-temperature LC are under investigation (15, 25). Thus, despite its great potential for non-GC-amenable compounds, LC-IRMS so far has not been used in contaminant hydrology studies.

Table 2 Characteristics of gas chromatography–isotope ratio mass spectrometry (GC-IRMS) measurements

Element	Gas[a]	Measured mass-to-charge ratios (m/z)[b]	Typical sensitivity (nmol)[c]	Typical precision (‰)[c]
Hydrogen	H_2	2,3	10–50	2–5
Carbon	CO_2	44, 45, 46	0.1–5	0.1–0.5
Nitrogen	N_2	28, 29, 30	1–10	0.3–0.7
Oxygen	CO	28, 29, 30	4–14	0.3–0.6
Chlorine	—	Molecular fragments	N/A	N/A

[a] Conversion gas measured in the gas ion source of the isotope ratio mass spectrometer. In the case of chlorine isotopes, ratios of molecular fragments can be measured with GC–quadrupole MS.

[b] Mass-to-charge ratios of isotopologues measured for isotope ratio determination.

[c] Values adapted from Reference 20.

Sample preparation steps such as extraction, cleanup, and preconcentration are important aspects of CSIA. For GC- and LC-IRMS, a very good separation of analytes from matrix components (unresolved complex matter) is a prerequisite and frequently requires cleanup because such interferences can affect the isotope ratio measurement (11, 26). Furthermore, the mass detector in IRMS instruments is inherently much less sensitive than that in organic mass spectrometers (**Table 2**). In the concentration domain, nanogram-per-liter levels of target compounds, even in complex matrices, can often be measured straightforwardly with modern MS-based equipment. Such concentration ranges are far below the lowest achievable levels in stable isotope analysis. Thus, investigators place a major emphasis on improving and validating available preconcentration techniques to supply a sufficient amount of a compound to the separation column for subsequent isotope analysis. In this context, enrichment techniques such as solid-phase microextraction (21, 27, 28) and purge and trap (28–30) have been validated and applied.

A remaining problem is normalization in CSIA. To obtain the highest possible accuracy, it is preferable to compare the sample and standard within each chromatogram, and they should be handled as similarly as possible according to the principle of identical treatment (17). The mere introduction of reference gas for normalization suffers from the fundamental drawback that it does not experience the same conditions as do analytes with respect to injection, separation, and chemical conversion into a gas; therefore, the principle of identical treatment cannot be completely fulfilled. Unfortunately, the lack of widely available GC- and LC-amenable organic standards of known isotope composition remains a significant problem for practitioners of CSIA because the development of suitable organic stable isotope reference standards for CSIA has not kept pace with the evolving needs of users (31). To overcome this lack, users have normalized working standards for GC-IRMS through off-line methods such as elemental analyzer IRMS or dual inlet IRMS. Further, the Schimmelmann group offers a set of useful standards (**http://mypage.iu.edu/~aschimme/hc.html**).

3. ISOTOPE FRACTIONATION IN ENVIRONMENTAL SYSTEMS

Isotope fractionation can be used to identify or even quantify processes such as diffusion and, in particular, abiotic and biotic transformation reactions in natural systems without the addition of reactive tracers. In contrast to source discrimination, a prerequisite is a measurable and constant isotope fractionation during the process of interest (e.g., degradation of a compound by microorganisms).

3.1. Physical Processes

Vapor-liquid isotope fractionation for both carbon and hydrogen (and presumably for other elements studied by CSIA) is typically rather small (32) and becomes relevant in the residual fraction at nonequilibrium only if a large percentage (i.e., 95% or more) of the initial compound has been removed by evaporation. Both normal and inverse isotope effects have been reported for evaporation, which makes predictions difficult. Isotope fractionation induced by evaporation may be relevant for volatile organic compounds in specific scenarios, such as shallow nonconfined aquifers and remediation by air sparging or soil bioventing (33, 34), and it may confound isotope evidence of degradation in such scenarios.

The few studies carried out to date on dissolution of an organic compound from its pure phase in water have indicated no discernible isotope effect in one-step equilibrium experiments (32). However, mass-transfer limitations, for example, from a non-aqueous-phase liquid to water, may mask the inherent KIE (9), as experimental data have demonstrated for microbial trichloroethylene (TCE) reduction to *cis*-dichloroethylene (35). For sorption from aqueous phases, many previous investigations revealed a nondiscernible isotope effect for carbon (33, 36, 37) and hydrogen (36). However, Kopinke et al. (38) induced significant sorption-related isotope fractionation in multistep sorption equilibrium or specifically designed column experiments at a mass loss exceeding 95%. Numerical modeling of sorption-based isotope fractionation has quantitatively confirmed this effect for non–steady state fronts (but not lateral fringes) of a contaminant plume (39, 40). However, to date, there is no clear evidence for sorption-related isotope fractionation at the field scale. In general, high-velocity flow in thin aquifers and heterogeneous flow systems tends to magnify fractionation. In a recent experimental study of transversal dispersion, Rolle et al. (41) demonstrated a rather strong fractionation of nondeuterated versus perdeuterated ethylbenzene. In a source-apportionment study, Hunkeler et al. (42) observed an isotope shift in TCE of up to 2.4‰ near the fringe zone to an underlying aquitard. The authors attributed this shift at least partially to diffusional isotope fractionation into the aquitard, but they could not exclude biodegradation as a responsible process in this fringe zone. In the vadose zone of a soil, a much clearer isotope effect due to gas-phase diffusion is observed as long as no steady state is achieved (43). Kuder et al. (44) recently emphasized the need to consider isotope effects for carbon and hydrogen during volatilization and subsequent gas-phase diffusion in the vadose zone; they studied these effects by using methyl *tert*-butyl ether (MTBE) as the probe compound in column systems.

In summary, for all phase-transfer processes involving (local) equilibrium between two phases, no substantial isotope effects have been observed. With the exception of gas-phase diffusion, there is no clear evidence for the relevance of phase-transfer and -transport processes on observed isotope fractionation in field studies, but such evidence cannot always be excluded a priori.

3.2. Transformation and Biodegradation Processes

CSIA has three major areas of applications for transformation processes: (*a*) proving the occurrence of transformation reactions in the environment; (*b*) quantifying the fraction of a compound transformed by applying previously determined isotope enrichment factors, typically in a Rayleigh-type exponential relationship between isotope composition and residual fraction; and (*c*) probing for prevalent reaction mechanisms, thereby allowing in-depth insights into natural (and engineered) systems. In application area *a*, CSIA has been extensively used in contaminant hydrology and site management either to meet requirements set for monitored natural attenuation at contaminated sites or to provide a qualitative indicator of efficacy of an active remediation (for a discussion of the latter, see Section 3.3, below). An observed shift in carbon isotope composition of 2‰ toward more positive values is deemed sufficient for such a qualitative assessment if no comingling of various sources is present (11). For many sites, this approach is the easiest and most cost-effective means of providing such evidence.

For other elements, so far there are insufficient data to yield similar threshold values, but similar to carbon, a significant trend of enrichment of the heavy isotopologue along a flow path must be observed. Several studies have pointed out that hydrogen may be a better qualitative indicator of contaminant degradation [as exemplified for BTEX (benzene, toluene, ethylbenzene, and xylenes) and MTBE] due to the much larger isotope fractionation (45–49). Furthermore, chlorine isotope analysis in organic compounds has recently been pioneered (9). Sakaguchi-Söder et al. (50) and Aeppli et al. (51) have determined isotope ratios for chlorine containing organic compounds such as TCE, perchloroethylene (PCE), *p,p′*-dichlorodiphenyltrichloroethane, and pentachlorophenol by GC–quadrupole MS with sufficient precision to characterize the degradation processes. Jin et al. (52) have investigated this method in terms of instrumental settings, and it has recently been validated in an interlaboratory comparison study (53).

In application area *b*, quantification of degradation on the basis of the evolution of the isotope composition of a compound over space and/or time is typically performed by the Rayleigh equation in its simplified form,

$$\frac{R_t}{R_0} = f^{(\alpha-1)}, \tag{3}$$

where R_t and R_0 are the ratios of the heavy isotope to the light isotope in the reactant at times t and $t = 0$, respectively; f is the remaining fraction of the reactant (c_t/c_0) at time t; and α is the fractionation factor. This form can be used in good approximation in studies at the low natural abundance level of the heavy isotopes and if the fractionation is small (7). In particular, the Rayleigh equation has been used to determine isotope fractionation factors for biodegradation in microcosm studies. **Figure 2** gives an example for the two important groundwater contaminants TCE and benzene (the original data are from References 54 and 55). The figure shows clearly that (*a*) the more of the compound is degraded, the larger is the isotope shift in the residual fraction; (*b*) isotope shifts and, therefore, enrichment factors may differ substantially among compounds (and also dominant reaction mechanisms); and (*c*) consequently, a significant isotope shift

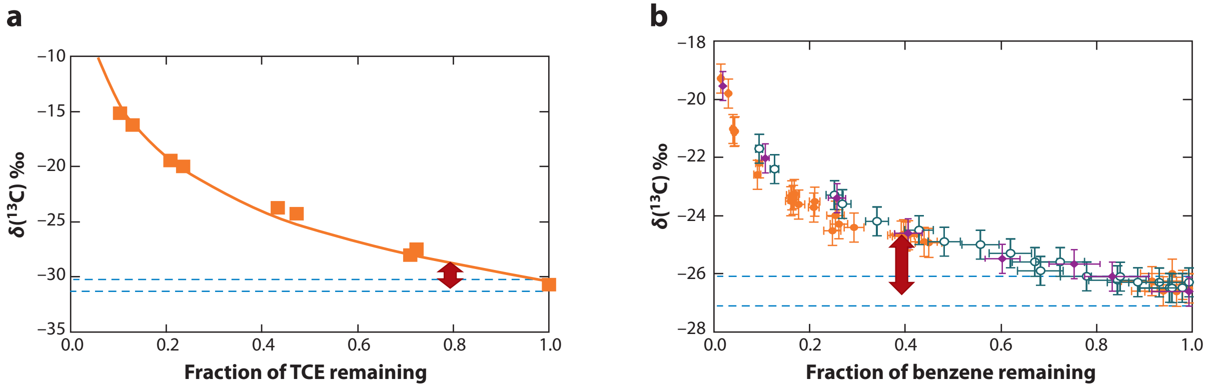

Figure 2

Degradation of (*a*) trichloroethylene (TCE) and (*b*) benzene by enrichment cultures. The carbon isotope ratios are plotted against the remaining fraction of the contaminant. The dotted lines represent ±0.5‰ around the initial $\delta(^{13}C)$ values of TCE and benzene. The red arrow represents the extent of fractionation required to observe an isotope shift of 2‰, which is necessary to identify transformation by isotope analysis. Modified with permission from Reference 11.

(i.e., larger than 2‰) can be observed at different degrees of transformation (20% for TCE and 60% for benzene for the example shown in **Figure 2**).

To determine the fractionation factor α or the enrichment factor ε from such data, one linearizes the Rayleigh equation (isotope ratios in δ notation):

$$\ln\left(\frac{\delta({}^{b}E_t)+1}{\delta({}^{b}E_0)+1}\right)=\ln\left(\frac{\delta({}^{b}E_0)+\Delta\delta({}^{b}E)+1}{\delta({}^{b}E_0)+1}\right)=\varepsilon\ln f=(\alpha-1)\ln f. \tag{4}$$

Isotope data may then be presented in a double logarithmic plot in which $\alpha - 1$ or ε is equivalent to the slope. It is not recommended to force the linear regression line through the origin (12).

Scott et al. (56) have thoroughly compared data-analysis methods for Rayleigh-type isotope data and have concluded that the best generally applicable method involves the use of Pitman estimators to combine data sets, in particular if the level of error varies among data sets. Use of the Rayleigh equation has allowed fractionation factors α or enrichment factors ε to be determined for many relevant organic contaminants under specified conditions. Lists of α or ε values have been compiled in several recent reviews (e.g., References 7 and 8), a U.S. Environmental Protection Agency guideline (11), and a book chapter (57). An inherent limitation of CSIA in this context is obvious from the following calculation: If we assume that one specific bond is affected in the rate-limiting step of transformation, as a first approximation, the same intrinsic enrichment factor ε_i should be obtained. However, if the number of atoms of the same element in the molecule rises, the measured bulk ε decreases. Such "dilution" arises from the fact that in the typical instrumental setup all atoms of an element within a molecule are converted to the measuring gas, regardless of their participation in an isotope fractionating transformation. In other words, if there is only 1 atom out of 10 in a molecule involved in the rate-limiting reaction, the measured change in isotope signature is 10-fold smaller. Thus, degradation of compounds larger than C_{10} to C_{15} typically does not yield a measurable carbon isotope fractionation (7, 8). In addition to the dilution of the isotope signal with the same element at nonreactive positions, rate-limiting transport steps that partially mask the observed isotope fractionation become more important for larger molecules. Some investigators have attempted to measure position-specific isotope signatures in environmental contaminants (58), but this process is rather tedious and specialized and has not yet been adopted for routine measurements. The decrease in measurable isotope fractionation has been illustrated by studies on chloroalkanes of 2 to 4 carbon atoms (59) and *n*-alkanes of 3 to 10 carbon atoms (43). In the latter case, the authors reported a log-linear relationship between the number of carbon atoms and the isotope enrichment factor. Reference 9 provides a detailed discussion of this topic.

Under the assumption of the applicability of the Rayleigh equation at a site, the extent of degradation B of a compound of interest may be calculated without the need to incorporate concentration data (8):

$$B=1-\left(\frac{\delta({}^{b}E_t)+1}{\delta({}^{b}E_0)+1}\right)^{\frac{1}{\alpha-1}}. \tag{5}$$

Typically, the estimates of the extent of degradation, based on CSIA data and laboratory-derived fractionation factors, are conservative; in other words, they tend to underestimate the true extent.

The Rayleigh equation applies in principle only to closed systems. Nevertheless, it has been extensively applied to inherently open environmental systems. Abe & Hunkeler (60) have studied in detail the conditions under which this approach may nevertheless be useful, in particular with regard to physical heterogeneity. By applying an advection-dispersion transport model, they found that the Rayleigh equation always leads to an underestimation of enrichment factors and degradation rates. Thus, derived estimates of contaminant loss are conservative. However, the contribution of bias from application of the Rayleigh model in these estimates generally does not exceed that from uncertainties of other input parameters, such as concentration data and travel times. Furthermore, methods that do not involve isotope data to quantify contaminant loss often yield much higher errors (60). Applicability of the Rayleigh equation for quantification of (bio)degradation in the field has been verified in a tracer test at a contaminated site through the injection of toluene-d_8 and toluene-d_5 as reactive tracers and bromide as a conservative tracer (61).

AKIE: apparent kinetic isotope effect

In a study comparing various methods for the quantification of degradation of *o*-xylene at a contaminated site, the derived extent of degradation by integral pumping tests, carbon isotope analysis (quantification of degradation based on the Rayleigh equation), and a combination of both methods gave consistent results. This result confirmed the applicability of the Rayleigh equation at the site (62). Recently, van Breukelen (63) extended the simple Rayleigh model by a dilution term. He substantiated this approach with an application to a benzene plume and showed that it allowed for a more reliable prediction of degradation.

Another limitation of the simplified Rayleigh model is that it can be applied only to parent compounds—that is, compounds that are not formed as intermediates during transformation—because both formation and further transformation of the intermediate can be accompanied by isotope fractionation. However, more sophisticated models that allow one to follow isotope shifts in sequential transformation reactions have recently been developed for the important sequential dechlorination of polychlorinated ethenes; these methods have been successfully used to describe real-world data (63, 64). Finally, in a complex environment, several competing pathways for transformation may be present simultaneously; these pathways cannot be captured by the simple Rayleigh model but require a model extension (65) or an application of an appropriate reactive transport model (66).

In summary, the simple Rayleigh equation may often be sufficient for a quantification of transformation, both in laboratory experiments and in field sites, so its applicability should be tested in a first step (11). However, keep in mind the potential limitations discussed in detail above. By taking them into account, one can extract useful quantitative information on contaminant behavior, even if the Rayleigh equation initially appears to fail.

Application area *c* has seen major developments over the past few years. An early example was provided by Hirschorn et al. (67), who investigated carbon isotope fractionation during aerobic degradation of 1,2-dichloroethane. Unexpectedly, the authors found a bimodal distribution of derived isotope enrichment factors for various microcosms, enrichment, and pure cultures that was centered around $-3.9‰$ and $-29.2‰$. Recalculation in terms of KIEs gave values of 1.01 and 1.06, which are typical for oxidation and substitution reactions, respectively. These different initial reaction steps among microorganisms were subsequently confirmed with dedicated pure strains of well-known enzymatic degradation pathways. This example shows how powerful CSIA results can be in unraveling primary reaction mechanisms.

On the basis of such results from environmental CSIA studies over the past decade, and taking into account previous knowledge about isotope effects in chemistry and biochemistry (68), Elsner and others developed a conceptual framework of isotope fractionation to discern transformation processes in the environment; this framework is described in detail in recent landmark papers (9, 12). A major development put forward in this framework is the bridging from observed isotope fractionation factors α to apparent kinetic isotope effects (AKIEs), which have already been used in (bio)chemistry for several decades (18). Here, the word apparent indicates that KIEs are derived from measured data and that the true or intrinsic KIEs might be partially masked, as is discussed briefly below and much more extensively in the cited papers.

Partial or total masking of KIEs may occur if the bond conversion process that induces isotope fractionation is preceded by a process that affects the whole molecule and is not accompanied by isotope fractionation. Such processes include transport to reactive sites, surface adsorption, or substrate-enzyme complex formation (12). If the reverse process is very slow, essentially all the molecules are converted in the second step, thereby leaving no isotope imprint on the residual substrate fraction. As an example, smaller isotope fractionation for both hydrogen and carbon has been observed in anaerobic toluene oxidation by *Geobacter metallireducens* with mineral suspensions, in comparison with homogeneous solutions of Fe(III) as terminal electron acceptor. This result was attributed to transport limitations to sessile microorganisms in the former case (69). Note, however, that as long as there remains a measurable AKIE, a Rayleigh-type model still links concentration and isotope data.

Elsner (9) has summarized six important conclusions with regard to mechanistic information:

1. Isotope fractionation provides insights that cannot be obtained from product analysis.
2. Evidence from isotope fractionation may elucidate degradation pathways even if no products are detected.
3. Isotope fractionation is mechanism specific.
4. Compound-specific isotope fractionation must be evaluated in mechanistic scenarios.
5. Observable isotope fractionation decreases for larger molecules.
6. There is a need for KIE reference data.

Rather than discuss each point in detail, we refer the reader to Elsner's excellent, in-depth overview (9). The work described therein provides the basis for going beyond identifying transformation with a yes-or-no decision or quantifying degradation on the basis of enrichment factors; it shows how, and under what conditions, isotope information can be linked with an elucidation of reaction mechanisms without the addition of artificial tracers and directly in natural systems. These opportunities make CSIA a unique and exciting approach for researchers working in environmental contaminants studies. It is unsurprising, therefore, that the derivation of AKIEs has quickly become nearly routine in environmental studies (13).

Elsner et al. (12) and Hofstetter et al. (13) have recently discussed the derivation of AKIEs; because a discussion and derivation would go beyond the scope of this review, refer to these papers for details. Primary KIEs for hydrogen can be orders of magnitude higher than both secondary hydrogen and primary carbon KIEs. For carbon, highest KIEs are observed for S_N2 reactions that, therefore, can often be well distinguished from other reaction mechanisms. Although KIEs for several important chemical reactions are available, there is still a lack of data for many others. Therefore, there is a wide-open field of research that includes the need to determine AKIEs for elements other than carbon and hydrogen. For example, Hofstetter et al. (22, 70) have reported KIEs for nitrogen (and carbon) in the transformation of nitrobenzene. Such work is extremely useful in expanding the reference database for KIEs in environmental transformation reactions. Finally, in the future, computational chemistry will help to better constrain or even predict KIEs in transformation reactions, as several examples have already shown (71). The power of two-dimensional (or dual) isotope approaches has recently been recognized not only for better resolution of sources of target compounds but also for better characterization of transformation reactions. Plotting isotope enrichment in one element versus another allows transformation mechanisms to be better characterized by the resulting slope, given that influences on residual concentrations (e.g., by dilution) and masking effect (e.g., by commitment to catalysis) that affect the apparent fractionation in each element should be eliminated (9, 12). This advantage was recently exemplified by Tobler et al. (69), who found that the extent of isotope fractionation differed according to the nature of Fe(III) as the terminal electron acceptor. However, these authors found almost identical slopes in a dual isotope plot of $\Delta\delta(^{2}H)$ versus $\Delta\delta(^{13}C)$, which supports the assumption that there are identical reaction mechanisms and partial masking of the KIE.

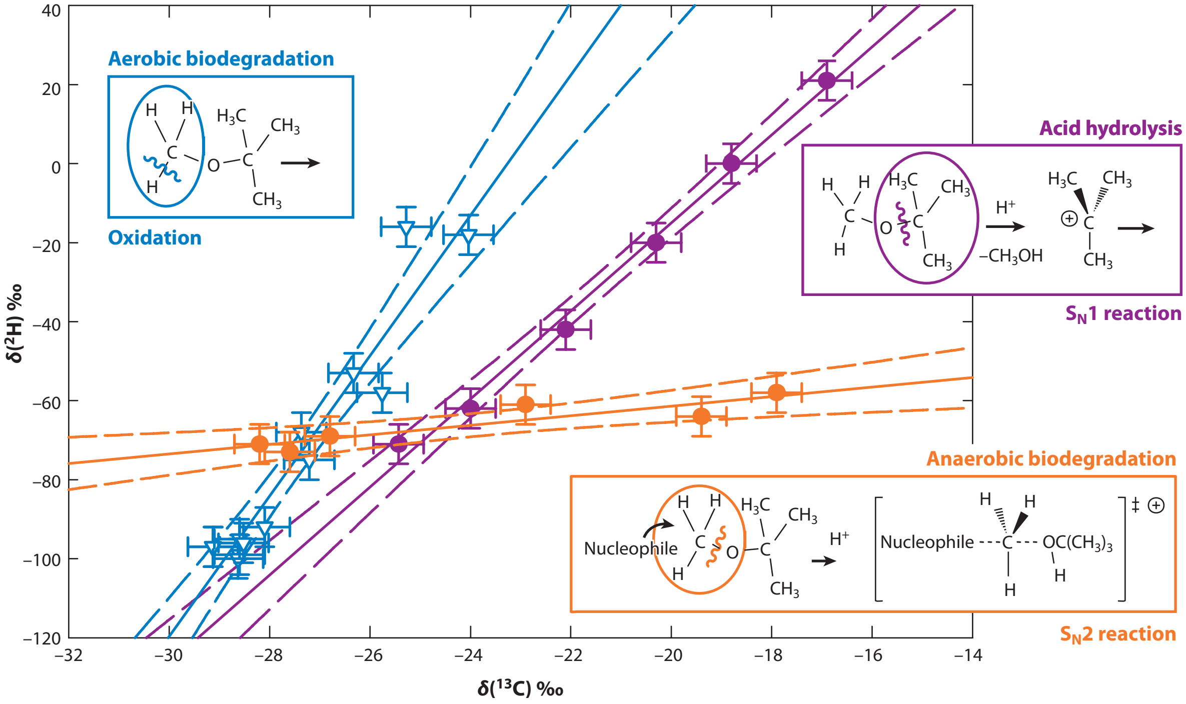

Figure 3

Dual isotope plot of $\delta(^2H)$ versus $\delta(^{13}C)$ for methyl *tert*-butyl ether degraded by three different reaction mechanisms. Modified with permission from Reference 9.

To date, one of the best-investigated organic compounds is the fuel oxygenate MTBE. As a result of multiple studies, the isotope fractionation in both hydrogen and carbon has been well constrained for three different primary reaction mechanisms. **Figure 3** shows a dual isotope plot of hydrogen versus carbon. Evidently, one can readily distinguish the three reaction mechanisms if the extent of transformation [and, accordingly, the corresponding isotope shifts $\Delta\delta(^2H)$ and $\Delta\delta(^{13}C)$] is sufficiently large. During aerobic degradation, cleavage of the carbon-hydrogen bond in the methyl group is the primary step. This process is accompanied by a large primary KIE for hydrogen but a small primary KIE for carbon. In contrast, in the S_N2 reaction, one observes a weak secondary hydrogen KIE that represents the primary step in anaerobic biodegradation, whereas carbon isotope fractionation in that step is very strong. Abiotic hydrolysis via a S_N1 reaction is intermediate in both KIEs.

3.3. Elucidation of Environmental Technical Processes

In addition to confirming natural attenuation processes, CSIA can also be used to obtain deeper insight into environmental technical processes, including active attenuation measures for groundwater at contaminated sites, drinking water purification, and wastewater treatment. In the first area, several studies have been carried out, whereas in the last two areas, few applications have been documented despite the great potential of CSIA.

By using carbon isotope analysis, McKelvie et al. (72) successfully demonstrated a stimulated biodegradation on the basis of ethanol addition to an aquifer contaminated with MTBE. The authors directly compared two lanes at the site, one without amendment as a field control and one with addition of ethanol. By $\Delta\delta(^{13}C)$ analysis, McKelvie et al. obtained an unequivocal proof of MTBE biodegradation in the ethanol-added lane after a relatively short period of time. In estimating the overall efficacy of ethanol amendment for enhanced fuel-oil spill remediation, however, one needs to take into account that the presence of ethanol retards the degradation of often co-occurring BTEX compounds in such contaminated aquifers (73).

In another biostimulation study, an emulsion of soybean oil in water and lactate was injected into an aquifer with a mixed contamination plume of 1,2-dichloroethane and TCE. Supply of these additional electron donors stimulated microbial growth through consumption of residual oxygen, which eventually led to anoxic conditions that were favorable for contaminant transformation by dehalogenation (74). By performing carbon isotope analysis over a monitoring period of 183 days in a single monitoring well, the authors obtained a clearer picture of the relevant in situ processes. The dichloroelimination of 1,2-dichloroethane formed ethene, whereas stepwise reductive dehalogenation of TCE formed vinyl chloride (VC), which was not further degraded. Transformation was observed within the first 90 days of monitoring. Afterward, the return of isotope signatures to their original values indicated both depletion of the electron donors (this finding was corroborated through analysis of volatile fatty acids and sum parameters) and inflow of nondegraded or previously sorbed contaminants. Such information was key to the successful upscaling of the pilot test to a full-site treatment. Similar conclusions were drawn elsewhere through the active biostimulation of TCE degradation in groundwater by addition of lactate (75) and bioaugmentation of TCE degradation by injection of KB-1® cultures at two sites (76, 77).

For groundwater contaminated with chlorinated hydrocarbons, over the past two decades investigators have implemented active remediation efforts at the field scale on the basis of dehalogenation induced by zero-valent iron (ZVI), either in permeable reactive barriers or via slurry injection of ZVI particles in the nanometer-to-micrometer range. As early as 1999, a large carbon isotope fractionation that occurred during dehalogenation of various chlorinated ethenes was reported. Also, CSIA has been used in the monitoring of remediation efficacy (78).

To predict the formation of harmful intermediates such as VC, one should be able to distinguish biotic reduction that, in the case of TCE or PCE transformation, may lead to the accumulation of VC and abiotic reduction that typically does not involve VC formation (79). To this end, a more detailed investigation of isotope signature evolution in the degradation intermediates should be conducted to allow distinction between abiotic and biotic transformation (**Figure 4**). It is possible to achieve such a distinction even in the field (9, 79). On the basis of several studies using ZVI and other metals, VanStone et al. (80) concluded that for the reduction of chlorinated ethenes and ethanes, the prevalent reaction mechanism governs the observed isotope fractionation rather than the type of reductant. In contrast, Zwank et al. (81) reported a bimodal distribution of isotope enrichment factors among different Fe(II) minerals but concluded that, in the case of the lower observed enrichment, a partial masking of the intrinsic fractionation occurred.

Several studies (e.g., Reference 82) have demonstrated the usefulness of carbon isotope data in in situ investigations of the success of permeable reactive barriers as an active remediation approach. The advantages of employing isotope data in addition to concentration data include not only evidence of compound transformation but also a better understanding of the process.

Finally, another active groundwater remediation option utilizes injections of permanganate to chemically oxidize contaminants. Researchers have used CSIA to demonstrate the efficacy of this approach at a field site contaminated by TCE. In accompanying laboratory studies, large enrichment factors of −25‰ to −27‰ were found for this oxidation; following the injection of permanganate, strong isotope shifts in TCE were observed. However, these shifts were only

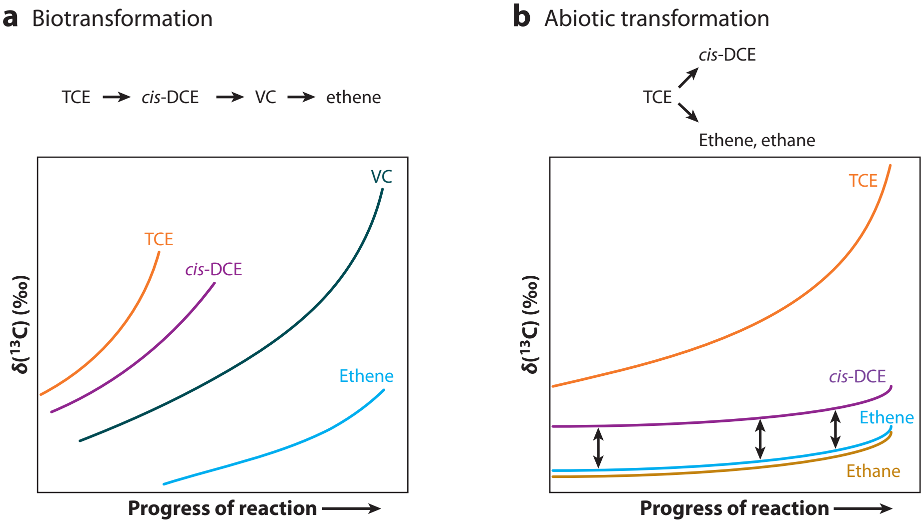

Figure 4

Evolution of $\delta(^{13}C)$ during the degradation of trichloroethylene (TCE) and its subsequent transformation products. (*a*) Biodegradation leading to sequential reductive dechlorination. (*b*) Abiotic transformation by zero-valent iron leading to several products. Abbreviations: DCE, dichloroethylene; VC, vinyl chloride. Modified from Reference 9.

transient and the original isotope composition was restored, which was explained by the presence of a dense non-aqueous-phase liquid (DNAPL) and the dissolution of TCE from this pool (83).

In summary, a major advantage of the use of CSIA as a control measure in active site remediation aiming at the degradation of contaminants is its potential to recognize early success or failure of the remediation strategy. In the latter case, results from isotope analysis may help to avoid useless expenditures. In contrast, mere concentration analyses are often inconclusive (11).

3.4. Contaminant Source Apportionment

The isotope composition of organic compounds may differ according to the isotope fractionation processes that occur during industrial synthesis, photosynthesis, and other biogenetic processes. In some cases, these differences may be sufficiently large to allow for the differentiation of sources in environmental systems. A further requirement for source-apportionment studies is that the isotope compositions of the compounds of interest remain stable over time or change only insignificantly. In organic contaminant hydrology, source-apportionment studies are important because they may provide direct evidence for the origin of contaminants. We discuss such studies below in terms of two exemplary compound classes, namely polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons.

PAHs are ubiquitous environmental contaminants; the fact that they arise from both anthropogenic and natural sources, as well as their great toxicological relevance, makes them one of the most thoroughly investigated compound classes in environmental chemistry. Dominant PAH sources in the environment are both petrogenic (i.e., they arise from the slow maturation of organic matter over long timescales and suitable temperature and pressure conditions) and pyrogenic (i.e., they arise from the incomplete combustion of organic matter) (84).

Pyrogenic PAHs are typically less depleted in ^{13}C than their petrogenic counterparts, which may be used to differentiate sources, mostly in combination with other indicators (such as molecular ratios of alkylated homologs to parent PAHs, other molecular indices, ratios of low–molecular weight to high–molecular weight compounds) and chemometric data analysis (84–88). For example, investigators at Lake Erie distinguished three areas with different histories of contamination. Furthermore, the authors found that the main emission pathway for PAHs was fluvial input (86). In further studies, investigators have characterized various sources of PAHs in sediments along highly industrialized rivers and estuaries (84, 87, 88). In these studies, the authors found that (*a*) very high $\delta(^{13}\mathrm{C})$ values for three-ring PAHs originated from aluminum smelting in an area along the St. Lawrence River (87); (*b*) coal transport and use had contributed considerably to PAH contamination, as had former wood-treatment facilities (84); and (*c*) pyrogenic rather than petrogenic sources of PAHs were dominant in harbor sediments (85). Finally, in one study, the authors even resolved changes in contributions of pyrogenic versus petrogenic sources of PAHs over time in sediment records (88).

Mazeas & Budzinski (89) have used isotope information on *n*-alkanes and PAHs to investigate sources of crude oil from a tanker wreck near the French Atlantic coast. With strong support from these data, the authors showed that tar balls stranded at several beaches along the coast did not originate from the tanker oil spill but rather from other sources. Furthermore, they concluded that the isotope composition of individual compounds is hardly affected over time and, therefore, allows for source discrimination even if the molecular distribution has already been considerably altered by weathering processes. In addition to the isotope profiles of phenanthrenes, the profiles of *n*-alkanes larger than C_{18} agreed well among spilled crude oil, samples retrieved from the northern Atlantic Ocean, and oil-coated bird feathers. However, the isotope profiles of tar balls from various beaches differed significantly from those of the *n*-alkanes, which provided clear evidence for one or more additional crude oil sources. Mansuy et al. (90) had previously demonstrated the potential of such isotope correlations in *n*-alkanes of spilled oil and oil-coated bird feathers.

Blessing (91) used information about the expected isotope ranges for PAHs from petrogenic and pyrogenic sources to distinguish major sources of PAHs at a contaminated field site of a former mineral oil–processing facility. Possible sources of contamination at the site included heavy fuel oils, waste oil, creosote, and petroleum fuel oils. The results showed $\delta(^{13}\mathrm{C})$ values that implicated creosote as the most likely contamination source for the soil samples at the site. The presence of isotopically extremely light PAHs [$\delta(^{13}\mathrm{C}) = -31‰$ to $-62‰$] in lagoon sediments at a site near Ravenna, Italy, led to the conclusion that emissions came predominantly from a former plant that used biogenic methane [$\delta(^{13}\mathrm{C}) = -69‰$ to $-73‰$] as feedstock, rather than from currently operating plants that used petrogenic feedstocks of much higher ^{13}C content (92, 93). However, neither the wide range of observed PAH isotope composition nor the enrichment (compared with the source values) could be explained, given that both superimposition from a secondary source and biodegradation were considered insufficient to induce the observed shifts (92).

Release of chlorinated hydrocarbons from industrial activities such as metal degreasing and dry cleaning is among the greatest sources of anthropogenic contaminations on a local scale. Considering the extensive work performed over the past 15 years on natural attenuation and isotope fractionation during transformation of such compounds, it is astonishing that publications on source allocations of potential release sources at industrial sites are sparse. Hunkeler et al. (42) investigated PCE release at a dry cleaning site; the authors assumed there were additional release sources, given the width of the plume and various concentration hot spots. Hunkeler et al. confirmed the existence of at least three sources for PCE on the basis of distinct differences in

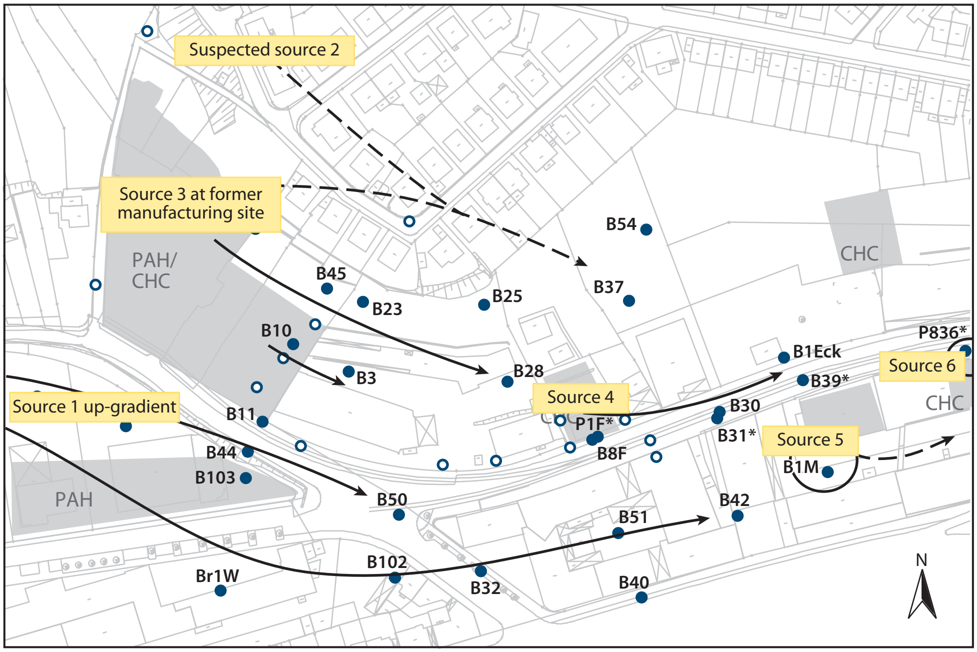

Figure 5

Delineation of at least six different sources of PCE (perchloroethylene) at a multiple-use industrial site with complex hydrogeology and geochemistry. Abbreviations: CHC, chlorinated hydrocarbons; PAH, polycyclic aromatic hydrocarbons. Modified from Reference 91.

PCE carbon isotope signatures. Due to the aerobic conditions in the aquifer, the PCE did not degrade, and the source signatures were preserved over a flow distance of more than 200 m (42). At another site, Eberts et al. (94) demonstrated that sources of TCE in groundwater at a former manufacturing site can be distinguished despite partial degradation. Carbon isotope data of TCE and its primary degradation products have been utilized, in conjunction with many other lines of evidence, to correct a previously proposed site scenario that implicated a unique source of TCE and the subsequent transport of a pure phase compound such as a DNAPL into various sections of the affected aquifer. An isotope mass balance of all products allowed the authors to identify PCE as the most likely source of pollution in one section of the aquifer and industrial TCE in another section. Recently, Blessing et al. (95) studied an even more complex field site that contained PCE contamination from previous multiple industrial uses, water flow in several fractured bedrock aquifers with hydraulic connections via vertical faults, and differing geochemical conditions. Despite the degradation of PCE in anoxic parts of the aquifer (best represented by manganese-dissolved concentration isolines), Blessing et al. delineated at least six different sources of PCE contamination, some of which had previously been unsuspected. As with the previously discussed field site (94), conclusions on source delineation could be drawn only through the combination of historical, hydraulic, geochemical, and concentration data and stable isotope data of PCE and its degradation products. **Figure 5** depicts the final site scenario.

Dual isotope approaches combining carbon and hydrogen, carbon and chlorine, or carbon and nitrogen isotope analysis are becoming more widely used in degradation studies but so far have been infrequently employed in source-apportionment studies. Coffin et al. (96) provided the first example of the use of combined carbon and nitrogen isotope analysis to distinguish the explosive trinitrotoluene in groundwater at a military site, although their study employed a preliminary data set as a proof of concept. Wang et al. (97) were the first group to combine carbon and hydrogen isotope analysis of *n*-alkanes and PAHs in a contaminated river to derive suggestions of different contaminant sources. More recently, Mancini et al. (98) used combined carbon and hydrogen isotope analysis to clearly differentiate two distinct sources of benzene at a contaminated site. By using a dual isotope plot of hydrogen versus carbon, Mudge et al. (99) classified fatty alcohol detergents in wastewater treatment plants and in receiving compartments in a comprehensive evaluation of synthetic and natural surfactants as potential release sources.

Finally, further statistical treatment of data will certainly increase in importance, given that the multitude of data in field studies often can no longer be evaluated by intuition or experience alone. For example, Boyd et al. (100) have developed a step-by-step protocol utilizing a sample-wise principal components analysis (in this case, of *n*-alkane carbon isotope data) followed by (*a*) a multivariate analysis of variance and (*b*) a second principal components analysis and/or hierarchical cluster analysis that helped unravel the probable source of a fuel-oil spill in Elizabeth River, Virginia. Such approaches require considerable additional expertise in chemometrics, but they will be invaluable in future field studies.

4. CONCLUSIONS

The instrumental and methodological developments discussed herein, along with an increasing number of publications, show the broad applicability of CSIA. In combination with traditional hydrochemical parameters, additional information about contamination sources, biotic and abiotic attenuation processes, and their mechanisms can be obtained.

SUMMARY POINTS

1. CSIA is a mature analytical tool that is routinely used to provide unique information about source apportionment, to elucidate transformation mechanisms, and to quantify natural or technical remediation processes.
2. CSIA can provide an important line of evidence in many application areas. However, with a few exceptions, CSIA alone is insufficient to unequivocally resolve a given question or problem. Thus, the full power of CSIA can be exploited only in combination with the use of other suitable methods, including structural and concentration analysis of target analytes and diagnostic metabolites (also known as signature metabolites or metabolic biomarkers), historical evidence, and relevant geochemical parameters.
3. For all phase-transfer processes involving (local) equilibrium between two phases, no substantial isotope effects have been observed. With the exception of gas-phase diffusion, no clear evidence for the relevance of phase-transfer and -transport processes on observed isotope fractionation in field studies has been provided, but such evidence cannot always be excluded a priori.
4. A simple Rayleigh-type equation may often be sufficient for a quantification of transformation both in laboratory experiments and in field sites; thus, its applicability should be tested in a first step. However, there are potential limitations (discussed in detail above). When they are taken into account, it may be possible to obtain useful quantitative information about contaminant behavior even if the Rayleigh equation initially appears to fail.

5. Apparent KIEs can be utilized to give insights in chemical processes in laboratory studies and at contaminated sites.
6. Dual (or even multiple) isotope approaches are a particularly powerful tool for better characterization of transformation reactions and, so far to a limited extent, for better resolution of sources of target compounds.

FUTURE ISSUES

1. The use of LC-IRMS can greatly enhance the areas of research in which CSIA is routinely used for nonvolatile compounds. So far, only carbon isotope analysis can be performed with LC-IRMS, and the development of interfaces for the measurement of other elements would be beneficial.
2. Additional international standard materials for the normalization of isotope data that can be utilized in CSIA have to be developed, validated, and provided to the CSIA community.
3. There is a need for KIE reference data for (bio)chemical transformation mechanisms in natural systems.
4. Source-apportionment studies will benefit from the intensive use of chemometric methods.
5. Few studies have been performed on water-treatment processes, although there seems to be great potential for further characterization of elimination processes for micropollutants and formation of unwanted by-products.

DISCLOSURE STATEMENT

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