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Compound Specific Isotope Analysis in Hydrogeology

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Summary of Career Highlights

Professor Barbara Sherwood Lollar has had a remarkable research career. Her work has been recognized through numerous awards – the 2019 NSERC Gerhard Herzberg Gold Medal, the 2019 C.C. Patterson medal for Environmental Geochemistry, the 2016 John Polanyi Award, 2012 Eni Prize for Protection of the Environment, and recognition as an international Fellow of the U.S. Academy of Engineering, the U.S. Academy of Sciences, and a Fellow of the Royal Society of London, to name a few. Furthermore, she was President of the Geochemical Society from 2014-2015. Sherwood Lollar's impact includes pioneering work using compound specific isotope analysis (CSIA) in contaminant hydrogeology and investigating the deep terrestrial subsurface. However, one of the largest impacts Prof. Sherwood Lollar has had throughout her career is the mentorship and guidance she has provided for over 70 student undergraduate and graduate research projects and 40 postdoctoral fellows and research associates, which we have summarized in an online academic tree (https://academictree.org/chemistry/tree.php?pid=351447). Prof. Sherwood Lollar has led teams to novel discoveries and insights, balancing big picture thinking with a keen eye for detail. Prof. Sherwood Lollar has trained a next generation of scientists to value scientific rigor and produce data of the utmost quality for both fundamental and applied applications.

Highlights

- We review Prof. Sherwood Lollar's contributions to Compound Specific Isotope Analysis
- We address contributions to contaminant hydrogeology and environmental chemistry
- We discuss contributions to fundamental science and to the remediation industry

Abstract

Here, we review the contributions of Professor Barbara Sherwood Lollar to Compound Specific Isotope Analysis (CSIA) in contaminant hydrogeology and environmental chemistry. We first discuss the seminal work by Professor Sherwood Lollar's lab on establishing CSIA as a quantitative tool for contaminant (bio)transformation at contaminated groundwater sites. We describe the critical research by her lab in the development and validation of sample collection techniques, single- and multi-element CSIA, and isotope data evaluation for reliable interpretations. We highlight the contributions of Professor Sherwood Lollar's lab towards the development of best practices for the successful application of CSIA by industry to demonstrate the occurrence of (bio)transformation, identify (bio)transformation mechanisms, quantify the extent and rate of degradation and differentiate among potential contaminant sources. We then explore Professor Sherwood Lollar's applications of CSIA to hydrogeology of the deep subsurface and the fast widening of the field to new environments (e.g., sediments), contaminants (e.g., chlorofluorocarbons, pesticides), and systems (e.g., plant, enzymes).

1. Introduction

Legacy contaminants in groundwater are contaminants with a long history of industrial use that have been regulated or banned due to findings on their adverse effects on human and/or environmental health. However, these contaminants persist in the environment due to past improper storage, handling, or disposal practices. This includes classes of contaminants such as chlorinated solvents, which began to receive awareness in the 1970s. Tackling this problem not only requires strategies to remediate contaminated groundwater sites but reliable monitoring tools to make robust interpretations on the success of these strategies. Compound specific isotope analysis (CSIA) was developed as a result of this crucial need, leading to its evolution to an established tool for monitoring the fate of legacy contaminants in groundwater today.

Professor Barbara Sherwood Lollar's research team has pioneered the approach of CSIA to investigate contaminant fate and the success of remediation strategies at field sites. The research from Sherwood Lollar's Stable Isotope Laboratory has been crucial in establishing the principles of CSIA and its application for differentiating contaminant sources, detecting the occurrence of degradation, identifying the degradation mechanisms, and quantifying degradation efficiency (i.e., extents and rates) in the subsurface. In this review, we refer to both biotic and abiotic contaminant transformation as (bio)transformation. The path toward the development of CSIA began in the mid- to late- 1990s. At that time, there was great interest in the potential of the recently commercially available Gas Chromatography Isotope Ratio Mass Spectrometry (GC/IRMS) systems to elucidate the behavior of organic contaminants in groundwater systems using $\delta^{13}C$ analysis (Sessions, 2006). However, no publications had yet demonstrated that $\delta^{13}C$ analysis of dissolved groundwater contaminants by GC/IRMS could be achieved at concentrations relevant to field applications, nor that such analysis would yield insights into the fate and transport of these compounds. In this review, we summarize the current state of CSIA applied to contaminant hydrogeology, highlighting the contributions of Sherwood Lollar and her team and how they have shaped the field. We conclude by summarizing how we can continue to build on the foundation Sherwood Lollar has established by identifying important future areas of research in CSIA applied to contaminant hydrogeology.

2. Establishing CSIA as a potential quantitative tool for investigating contaminant fate and transport

Compound specific isotope analysis relies on the natural abundance of a light (l) and heavy (h) stable isotope of an element (E) in a compound, which is represented by the isotope signature or delta value, $\delta^{h}E$:

$$\delta^{h}E = (R_{sample}/R_{standard} - 1) \qquad \text{(Eq. 1)}$$

where R_{sample} and $R_{standard}$ are the $^{h}E/^{l}E$ ratios in a sample and an international standard, respectively. $\delta^{h}E$ is commonly expressed in permil (‰), which is equivalent to milliurey (mUr), or parts per thousand (Brand et al., 2014).

Today, CSIA is a widely accepted tool for *in situ* monitoring of natural and engineered attenuation of solvents and other organic contaminants in groundwater as illustrated by the EPA publication “A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)” that was co-authored by Sherwood Lollar (Hunkeler et al., 2008). Sherwood Lollar recognized that the first requirement to achieve the application of CSIA to groundwater systems was to develop sampling and sample preparation approaches that were demonstrated to preserve the isotopic signature of the dissolved organics of interest. Her group publications demonstrated that pentane extraction (Dempster et al., 1997) and headspace analysis (Slater et al., 1999) conserved the $\delta^{13}C$ signature of petroleum hydrocarbons such as toluene and chlorinated solvents such as trichloroethylene, (TCE) while achieving field-relevant concentrations. This laid the foundation for the broad application of CSIA to groundwater systems. Sherwood Lollar recognized that the next critical requirement was to determine whether isotopic fractionation, that is changes in the isotopic signature of a compound, occurred during subsurface processes. Her group's initial papers demonstrated that there was negligible carbon isotopic fractionation of chlorinated solvents/TCE and toluene/BTEX associated with dissolution (Dempster et al., 1997) and volatilization (Slater et al., 1999). Slater et al. (2000) followed this up demonstrating that the $\delta^{13}C$ isotope signatures of TCE and toluene were conserved during sorption. These observations on non-destructive processes have been borne out by numerous studies since. Except in the case of very low fraction remaining, carbon isotope fractionation by these physical processes is negligible within analytical uncertainty in most cases (Hunkeler et al., 2008). However, exceptions have been emphasizing the need for careful evaluations for specific scenarios. For example, in the case of pure product evaporation in open systems, inverse isotope fractionation i.e., enrichment of the heavy isotope in the vapour phase, hinted at in Slater et al. (1999), can result in isotopic fractionation during open system evaporation from free product phases e.g., for chlorofluorocarbons due to vapor pressure effects (Horst et al., 2016).

Concurrently with establishing that non-destructive processes typically result in negligible isotope fractionation, Sherwood Lollar et al. (1999) was one of the handful of publications that initially demonstrated the most exciting potential of CSIA; that transformation of organic solvent groundwater contaminants resulted in large carbon isotope fractionation of the residual contaminants, a critical observation that has led to the ongoing application of CSIA in groundwater systems. While some initial papers had used field and microcosm studies to demonstrate that stable isotope fractionation was associated with dechlorination of PCE and its metabolites (Hunkeler et al., 1999) and with biodegradation of hydrocarbons (Stehmeier et al., 1999), Sherwood Lollar et al. (1999) provided the first quantification in the literature of isotope fractionation effects involved in biotransformation of chlorinated ethenes (e.g., TCE) and BTEX (e.g., toluene) using models from previous work involving nitrogen isotopes (Mariotti et al., 1981). Specifically, Sherwood Lollar et al. (1999) demonstrated that, the anaerobic biodegradation of TCE resulted in large isotope fractionations and further that these fractionations could be modelled using the Rayleigh model. The Rayleigh model is a fractional distillation model that relates the fraction of remaining compound (*f*) to the isotopic ratio at any time t (*R*) and its initial value (R_0) via a constant fractionation factor (α).

$$R/R_0 = f^{(\alpha - 1)} \qquad \text{(Eq. 2)}$$

The existence of a consistent fractionation factor (α), also sometimes expressed as an enrichment factor ($\varepsilon = \alpha - 1$, in ‰), indicated that the fractionation was characteristic of the (bio)transformation process. Indeed, later research confirmed that the values of α and ε are related to the kinetic isotope effect (KIE) defined as ${}^{L}k/{}^{H}k$, where $\alpha = 1/\text{KIE}$ (e.g., Elsner et al., 2005). This was a truly exciting observation as it demonstrated that stable isotope fractionation not only could be used to identify the occurrence of contaminant (in this case, TCE) (bio)transformation independent of non-degradative mechanisms of mass loss, but also that there was a quantifiable relationship between the extent of isotope fractionation and the extent of (bio)transformation that had occurred using process-specific α and ε , resulting in the potential to use these parameters to distinguish different (bio)transformation processes.

This was a starting point in creating a database of α and ε values for various compounds and (bio)transformation reaction pathways to which the Sherwood Lollar group contributed, e.g., aerobic and anaerobic biotransformation of toluene (Sherwood Lollar et al., 1999; Ahad et al., 2000), perchloroethylene (PCE) (Slater et al., 2001), TCE (Slater et al., 2001), cis-dichloroethylene (c-DCE) (Slater et al., 2001), vinyl chloride (VC) (Slater et al., 2001; Chartrand et al., 2005), 1,2-dichloroethane (1,2-DCA) (Hirschorn et al., 2004; Hirschorn et al., 2007), chloroform (CF) (Chan et al., 2012), and mono-, di-, and tri-chlorobenzenes (MCB, DCB, and TCB) (Liang et al., 2011, 2014).

In some cases, minimal ^{13}C fractionation effects were observed, such as during the aerobic biotransformation of toluene in Sherwood Lollar et al. (1999), thus precluding the use of δ^{13}C-CSIA to identify (bio)transformation in this case. However, an important implication of this early work (e.g., Ahad et al., 2000; Sherwood Lollar et al., 1999), is that δ^{13}C-CSIA has the potential to be used with isotopically-conservative processes for source differentiation.

The next step was to expand the isotope systems being investigated. Exploring elements other than carbon, initially hydrogen (δ^2H), demonstrated that other characteristic isotope effects may be occurring even when there was negligible fractionation of δ^{13}C. For example, anaerobic toluene biotransformation resulted in significant hydrogen isotope effects (> 5‰ analytical uncertainty), which were otherwise small for carbon (Ahad et al., 2000; Ward et al., 2000). These early developments of multi-element CSIA demonstrated the potential to combine δ^{13}C- and δ^2H-CSIA to identify (bio)transformation of groundwater contaminants, e.g., methyl tert-butyl ether (MTBE) (Gray et al., 2002; McKelvie et al., 2009) and benzene (Mancini et al., 2008; Mancini et al., 2003) and possibly distinguish between different reaction mechanisms (Mancini et al., 2008; McKelvie et al., 2009), facilitating the emergence of dual-isotope analysis discussed in more detail in Sections 5 and 6.

Beyond natural attenuation, engineered attenuation, e.g., via the introduction of reductants such as zero-valent iron (ZVI), was proven to be an effective means to drive reductive dechlorination of chlorinated solvents. Sherwood Lollar's group showed that, as was the case for biological transformation, the extent of abiotic remediation can be monitored via CSIA applying the Rayleigh model, e.g., for TCE dechlorination by ZVI reaction (Slater et al., 2002; VanStone et al., 2004) and for 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA) and 1,1,1-trichloroethane (1,1,1-TCA) transformation by Fe and Cu/Fe metals (Elsner et al., 2007). These laboratory studies by Sherwood Lollar and her group made foundational contributions to the establishment of key principles for the use of CSIA as a powerful tool to either identify contaminant sources or identify and quantify natural or engineered attenuation of groundwater contaminants.

3. Demonstrating the feasibility of applying CSIA to investigate (bio)transformation at contaminated sites

One of the main advantages of CSIA in contaminant hydrogeology is its application to distinguish between non-destructive (e.g., sorption, dilution, dispersion, and volatilization) and (bio)transformation processes affecting contaminants at field sites, without the need to identify (bio)transformation products. This is because non-destructive processes typically cause minimal isotope fractionation compared to (bio)transformation processes, especially for carbon (as discussed in Section 2). Sherwood Lollar and her group were leaders in the demonstration of CSIA at field sites to investigate whether (bio)transformation is occurring, and if at rates and extents such that contaminants will be sufficiently reduced to meet regulatory guidelines. Research from Sherwood Lollar and her team has applied CSIA to sites investigating a variety of remediation strategies (detailed in Table 1), including both natural processes, e.g., by relying on the ability of intrinsic microbes to degrade contaminants (intrinsic biotransformation), and engineered processes, e.g., by adding chemicals (e.g., using ZVI for abiotic transformation, electron donors for biotransformation (i.e., biostimulation) or adding microbes to enhance biotransformation (i.e., bioaugmentation). Early contributions also include developing criteria for the successful application of CSIA to demonstrate the occurrence of (bio)transformation in the field in Sherwood Lollar et al. (1999):

1. Systematic changes in δ^hE values, or fractionation, must occur during (bio)transformation.
2. The extent of fractionation must be greater than analytical uncertainty.
3. Under a given set of conditions, isotope fractionation must be reproducible.
4. The effects of isotope fractionation during a given (bio)transformation pathway must be readily discernable from isotopic effects associated with other subsurface processes of mass attenuation such as volatilization, dissolution, and sorption.

These criteria were adopted in the CSIA Field Guidance (Hunkeler et al., 2008). Sherwood Lollar's research has demonstrated that these criteria are met for certain compounds and conditions but not for others (e.g., fractionation observed for anaerobic transformation of TCE but not for aerobic (bio)transformation of toluene by sand from a contaminated aquifer; Sherwood Lollar et al., 1999, although further work showed aerobic toluene biotransformation can produce significant carbon isotope effects; Morasch et al., 2002 and Vogt et al., 2008). These early findings emphasized the importance of determining compound-specific and condition-specific enrichment factors, shaping further research and application of CSIA to investigation of (bio)transformation at field sites.

Table 1. Summary of studies from Sherwood Lollar's Stable Isotope Lab applying CSIA to investigate (bio)transformation at field sites. The '✓' symbol shows studies that used CSIA for the application indicated in each column. The 'x' symbol shows where CSIA was used to show biotransformation was not a major process at the investigated field site, in itself also an essential groundtruth required for remediation strategies.

Study	Compound(s)	Isotopes	Source Differentiation	Identify	Quantify Extent	Rates
Source Differentiation						
Mancini et al., 2008	Chlorobenzenes and benzene	δ^{13}C, δ^{2}H	✓			
Intrinsic Biotransformation						
Sherwood Lollar et al., 2001	PCE, TCE	δ^{13}C		✓	✓	
Mancini et al., 2002	Benzene, ethylbenzene	δ^{13}C, δ^{2}H		✓	✓	
McKelvie et al., 2007a	MTBE, TCE	δ^{13}C, δ^{2}H		x		
Gilevska et al., 2019	MCB, Benzene	δ^{13}C		✓	✓	✓
Phillips et al., 2020	CFCs	δ^{13}C	✓	✓	✓	

Gilevska et al., 2021	MCB, Benzene	$\delta^{13}C$, $\delta^{37}Cl$, $\delta^{2}H$		✓	✓	✓
Biostimulation						
Hirschorn et al., 2007	1,2-DCA and TCE	$\delta^{13}C$		✓	✓	
Bioaugmentation						
Chartrand et al., 2005	TCE	$\delta^{13}C$		✓	✓	
Morrill et al., 2005	*cis*-DCE	$\delta^{13}C$		✓	✓	✓
Mundle et al., 2012	Chlorinated ethenes	$\delta^{13}C$		✓	✓	
Combination of Intrinsic Biotransformation + Biostimulation +/- Bioaugmentation						
McKelvie et al., 2007b	MTBE	$\delta^{13}C$		✓	✓	✓
Morrill et al., 2009	PCE	$\delta^{13}C$		✓		
Abiotic Transformation						
Vanstone et al., 2005	TCE	$\delta^{13}C$		✓		
Elsner et al., 2010	TCE and 1,1,1-TCA	$\delta^{13}C$		✓	✓	

Nunez Garcia et al., 2020	Broad range of chlorinated VOCs[a]	δ^{13}C		✓		

[a] VOC: volatile organic compounds

3.1 Source Differentiation. The initial isotopic composition of a compound depends on two primary variables: the isotopic composition of the source material (e.g., petroleum feedstocks for chlorinated solvent production) and any isotopic fractionation that occurs during production (Hunkeler et al., 2008 and references therein). Thus, variations in either of these two variables between manufacturers or production batches will be reflected by different initial isotopic signatures. These variations can be used with CSIA to determine if multiple source zones of contaminants exist at a site. Successful application of CSIA for source differentiation benefits from knowledge of the isotopic compositions of various pure-phase undegraded material to confirm the potential for source differentiation and establishing ranges of known pure-phase values to compare to field data (Hunkeler et al., 2008). Professor Sherwood Lollar's laboratory has confirmed the potential for CSIA as a means for source differentiation in the field for priority contaminants including BTEX (Dempster et al., 1997), hexachlorocyclohexane (HCH; Chartrand et al., 2015), chlorofluorocarbons (CFCs; Phillips et al., 2020), and 1,2-dichloroethane (1,2-DCA; Chartrand et al., 2007). Furthermore, carbon and hydrogen CSIA was used in conjunction with hydrogeology, contaminant concentrations, and site history to identify sources of benzene and chlorobenzene contamination at a complex field site (Mancini et al., 2008). In this work, homogenous $\delta^{13}C$ and $\delta^{2}H$ signatures of chlorobenzene indicated a single chlorobenzene source, while variable isotope signatures of benzene that were inconsistent with the effects of biotransformation indicated two distinct benzene source areas (Mancini et al., 2008).

3.2 Identifying and Evaluating the Extent of (Bio)transformation. The Rayleigh equation is used to estimate the extent of contaminant (bio)transformation (Equation 2; Mariotti et al., 1981). The first step in assessing whether the Rayleigh model is appropriate for the investigated field site is to plot a Rayleigh correlation, where contaminant isotope compositions are plotted against the natural logarithm of concentrations (Hunkeler et al., 2008). If these data are well described by a linear relationship and vary in a manner consistent with (bio)transformation, then the application of the Rayleigh model is appropriate and contaminant (bio)transformation is a significant process controlling contaminant concentrations (Hunkeler et al., 2008). Variation consistent with (bio)transformation includes when concentrations decrease with isotopic compositions increasingly enriched in the heavy isotope (i.e., normal isotope effect) or enriched in the light isotope (inverse isotope effect, observed in rare cases, e.g., Heckel et al., (2019)). A poor correlation to the Rayleigh model may indicate strong influence of physical processes on concentration, however this does not automatically disqualify a site for applying CSIA to understand contaminant (bio)transformation (Hunkeler et al., 2008).

Despite the multiplicity of both chemical (e.g., (bio)transformation) and physical (non-destructive, e.g., volatilization, sorption, dispersion, etc.) processes affecting contaminant concentrations in the subsurface, Sherwood Lollar's team verified early on that the Rayleigh model was appropriate at various sites (Sherwood Lollar et al., 2001; Chartrand et al., 2005; Morrill et al., 2005; VanStone et al., 2005), demonstrating the validity of this approach. CSIA can also indicate when intrinsic (bio)transformation is not occurring, which can be particularly valuable when potential daughter products of (bio)transformation are present as primary contaminants (McKelvie et al., 2007a). An early warning that intrinsic (bio)transformation is not occurring (e.g., Morrill et al 2005; McKelvie et al., 2007a) is critical in contaminant remediation planning and can contribute to substantial cost and time savings. In scenarios with evidence that (bio)transformation is occurring, the extent of (bio)transformation can be estimated using the Rayleigh model. For this, enrichment factor(s) (ε) must be known for the most likely transformation process(es) acting on the contaminant of interest. Equation 3, i.e., the linearized form of the Rayleigh equation can then be applied to calculate the fraction of original contaminant remaining (*f*):

$$\ln[(\delta^{h}E + 1)/(\delta^{h}E_{o} + 1) = \varepsilon \times \ln f \qquad \text{Eq. 3}$$

where $\delta^{h}E$ is the isotopic composition of element E at a given location, and $\delta^{h}E_{o}$ is the isotopic composition of the contaminant in the source zone, which can be estimated using source values from the literature, using the most negative value at the site, or from a source well. Alternative approaches are described in Hunkeler et al. (2008). The extent of (bio)transformation (EoT) can then be calculated using Equation 4:

$$\text{EoT} = 1 - f \qquad \text{Eq. 4}$$

This approach allows practitioners to estimate whether contaminant (bio)transformation is occurring at sufficient levels to bring contaminant concentrations within regulatory limits. Sherwood Lollar's research has successfully demonstrated this approach to estimate the extent of intrinsic biotransformation (Sherwood Lollar et al., 2001; Morrill et al., 2009; Gilevska et al., 2019; Phillips et al., 2020), to evaluate the success of biostimulation (Hirschorn et al., 2007; McKelvie et al., 2007b) and bioaugmentation (Chartrand et al., 2005; Morrill et al., 2005).

3.3 Calculating Rates. A powerful application of CSIA is to estimate contaminant (bio)transformation rates. (Bio)transformation rates are key parameters needed for design of remediation approaches, however accurate rates are often difficult to determine using conventional data collection (i.e., composition only) as contaminant concentrations are affected by a variety of processes, as discussed previously. CSIA provides a unique advantage in calculating (bio)transformation-specific rates. First-order rate constants ($\lambda_{(\text{bio})\text{transformation}}$) can be approximated according to Eq. 5:

$$\lambda_{(\text{bio})\text{transformation}} = -\frac{\Delta\delta^{h}E}{\varepsilon * T} \qquad \text{Eq. 5}$$

where T is the average travel time calculated between two sampling ports (1 and 2) with a difference in measured contaminant isotopic signatures $\Delta\delta^{h}E$ (Hunkeler et al., 2008). Sherwood Lollar's research has demonstrated the utility of using CSIA to estimate field-derived first-order rate constants for compounds such as *cis*-DCE (Morrill et al., 2005), MTBE (McKelvie, Mackay, et al., 2007), chlorinated benzenes, and benzene (Gilevska et al., 2019). Importantly, all isotopically-derived rate constant estimates from Morrill et al. (2005) and Gilevska et al. (2019) agreed with concentration-derived estimates within an order of magnitude. However, estimates calculated using isotope data are typically lower than those calculated solely using decreases in concentrations along a flow path. This is because concentration decreases can result from various non-destructive processes (e.g., diffusion, dispersion, adsorption, volatilization) as well as (bio)transformation, while isotope data provide more conservative estimates that reflect (bio)transformation rates only, highlighting the utility of CSIA to calculate more accurate (bio)transformation rates.

Sherwood Lollar proposed to apply the concept of representative elementary volume (REV) zones for the calculation of (bio)transformation rates at the field scale (Gilevska et al., 2019). The REV is the volume of sampling and measurement at which there is no change in the average value for a given parameter (averaged over the entire REV). This concept has been applied in hydrogeology to parameters such as porosity and hydraulic conductivity (Freeze and Cherry, 1979). In sediments, the rates of chemical and biological reactions can vary on very small scales due to variations in microbial communities, and the presence of nutrients and electron donors (Gilevska et al., 2019 and references therein). Gilevska et al. (2019) calculated rate constants using CSIA data in sediments within different depth profiles. The authors used statistical evaluation of CSIA data to differentiate between REV zones with different rate constants of (bio)transformation in sediment profiles. This approach demonstrated the presence of critical zones with maximum biotransformation rates in sediment profiles, responsible for sediment detoxification.

3.4 Combining CSIA with Numerical Modelling. As discussed in previous sections, CSIA provides an advantage over concentration data alone in investigating biotransformation studies. However, as noted previously, assessing whether (bio)transformation is a main process controlling contaminant concentrations must first be assessed using a Rayleigh correlation. Even if non-destructive processes are occurring, isotope data can still be useful for estimating (bio)transformation rates and extents provided these non-destructive processes are isotopically conservative. However, under complex field conditions, applications of the Rayleigh model as discussed so far may not be possible due to the occurrence of multiple non-destructive and (bio)transformation processes contributing to the overall isotope change, such as the case of dense non-aqueous phase liquid (DNAPL) dissolution (Hunkeler et al., 2004; Chartrand et al., 2005; Morrill et al., 2009), volatilization (Thullner et al., 2012), and sorption (Wanner et al., 2017; Halloran et al., 2021). These situations require more process-descriptive methods for interpreting CSIA data. Sherwood Lollar’s Stable Isotope Laboratory has developed and applied nuanced mathematical models for understanding how non-destructive but isotopically sensitive processes affect CSIA data interpretation in complex field conditions. For example, Xu et al., (2016, 2017) demonstrated that under certain spatial and temporal field sampling schemes, diffusion-related carbon isotope fractionation is often negligible, providing guides for sampling design when using CSIA to evaluate (bio)transformation. Morrill et al. (2009) presented an analytical model to understand how DNAPL dissolution overprints the signature of (bio)transformation and demonstrated its implications for CSIA interpretation. Another limitation of the Rayleigh model is that when interpreting CSIA data in a (bio)transformation chain when one or more contaminants are simultaneously produced and transformed, the rate constants for these processes need to be known (Morrill et al., 2006; Höhener and Atteia, 2014). Morrill et al. (2006) proposed an improved method to estimate ε for intermediate compounds in a (bio)transformation chain. More recent work from the Sherwood Lollar Laboratory couples modeling with CSIA to focus on combining field-collected concentration and CSIA data for investigating the fate and transport of MCB and benzene in contaminated sediments (Gilevska et al., 2019).

4. Sample collection, preservation, and analysis for CSIA

An essential step in CSIA is the development of sample collection, preservation, preparation, and analysis protocols that preserve the original isotope signatures of the target compounds. Research from the Sherwood Lollar's Stable Isotope Laboratory has led the path to the application of CSIA to unexplored environments for this technique such as contaminated sediments. This research showed that passive equilibrium pore water samplers, i.e., peepers, are compatible with CSIA (Passeport et al., 2014) and can be used to identify (bio)transformation of benzene and chlorinated benzenes (Passeport et al., 2016), and even estimate rates of natural attenuation (Gilevska et al., 2019).

In experimental studies, Sherwood Lollar and her team recognized the potential for increased uncertainty in calculating ε using traditional methods (headspace analysis for both composition and isotope ratios) for reactions where pressure increases occur over the course of a reaction due to daughter product formation. In response to this, a method was developed to monitor the extent of a reaction based on increases in headspace pressure (due to the production of CO_2 in a decarboxylation reaction) to calculate more accurate estimates of ε using the

Rayleigh model in reactions with non-isobaric pressure in closed systems (Mundle et al., 2013). Using the technique proposed by Mundle et al. (2013), ε values can be calculated with higher correlation coefficients and lower confidence intervals for laboratory experiments with non-isobaric pressure.

Applications of CSIA to contaminated sites first focused on volatile organic compounds including BTEX, chlorinated ethenes, and chlorinated ethanes. By letting such compounds equilibrate between the liquid and gaseous phases of a closed sample vial according to their Henry's constants, sufficient amounts of the compound relative to analytical detection limits can be found in the gas phase, with negligible differences in stable carbon isotope composition between the liquid and vapour phases (Slater et al., 1999). This has provided a clean (i.e., minimal sample matrix) and easy way to inject volatile compounds into a GC/IRMS for CSIA, even for multi-contaminant sample mixtures and complex matrices. Lower detection limits can be obtained through simple sample freezing (Elsner et al., 2006) and by further optimizing headspace extraction, e.g., in the dynamic headspace method in which a larger portion of the headspace volume was collected and cryogenically trapped before thermal desorption in the GC/IRMS (Morrill et al., 2004). Solvent extraction methods, from single-step liquid-liquid extraction (Dempster et al., 1997) to multiple-step procedures (Chartrand et al., 2015) were developed and carefully validated for specific compounds, e.g., BTEX, and for semi-volatile organic compounds such as HCHs to confirm that method-induced isotope fractionation for δ^{13}C-CSIA was less than analytical uncertainty (i.e. <0.5‰). Novel methods were later developed for new classes of compounds exhibiting their own analytical challenges. For example, the broad GC peaks observed for CFC-11 and CFC-113 in traditional purge-and-trap extraction prevented δ^{13}C-CSIA at field-relevant concentrations of these two chlorofluorocarbons. This challenge was overcome using an isotopically-conservative peak cutting and cryo-refocusing approach that allowed δ^{13}C-CSIA of dissolved CFCs down to 190 (CFC-11) and 270 µg/L (CFC-113) (Horst et al., 2015). The new δ^{13}C-CSIA method was applied at a contaminated groundwater site and provided evidence for *in situ* biotransformation of CFC-11 and the lack of natural attenuation but the presence of multiple source zones for CFC-113 (Phillips et al., 2020). Sherwood Lollar's Stable Isotope Laboratory has contributed to the development of δ^{2}H-CSIA for chlorinated hydrocarbons at typical field concentrations, showing that a liquid N_2 trap can efficiently remove undesirable HCl and result in reproducible and accurate δ^{2}H measurements of 1,2-dichloroethane (Chartrand et al., 2007). Sherwood Lollar and her team and colleagues at the Helmholtz UFZ later established criteria for chromium reactors for δ^{2}H measurements of chlorinated volatile organic compounds (VOCs) (Gilevska et al., 2020). Specifically, Gilevska et al. (2020) demonstrated that the Cr particle size range, Cr reactor position (vertical vs. horizontal), oven temperature, and Cr packing length can influence the trapping efficiency of undesirable HCl by-products during the conversion of chlorinated VOCs. This method was applied successfully to contaminated sediment porewaters where δ^{13}C-, chlorine (δ^{37}Cl-), and δ^{2}H-CSIA together identified the dominant pathways affecting the fate of chlorobenzene and benzene (Gilevska et al., 2021). These analytical advances contributed to the widespread application of multi-element CSIA to chlorinated compounds, which is now a mainstream technique, helping to constrain source zones and contaminant (bio)transformation pathways and reaction mechanisms.

5. Establishing best practices for adoption of CSIA in hydrogeology

Community consensus on best practices is a central tenet in stable isotope geochemistry (e.g., Brand and Coplen, 2012, 2001; Hunkeler et al., 2008). Isotope ratios of international standards are established, distributed and regulated by the International Atomic Energy Agency (IAEA) in Vienna, Austria. As a result, all isotope laboratories are calibrated to the same set of international standards, allowing for a direct comparison of the results from any isotope laboratory worldwide. Accordingly, within the stable isotope community there is a strong emphasis on collaborative efforts to establish best practices within the discipline, including instrument calibration, estimating total uncertainties, and calculations to interpret stable isotope data. Work from the Sherwood Lollar‘s Stable Isotope Laboratory has set a standard for adopting best practices in CSIA applied to contaminant hydrogeology and produced significant contributions to establish best practices in the field.

5.1 Assessing Total Uncertainty

In contaminant hydrogeology, isotope data can be used to identify if (bio)transformation of a contaminant is occurring. However, variations in isotope signatures can be small relative to the analytical uncertainty of isotope ratio mass spectrometers. Thus, variations in isotope signatures must be considered in the context of well-constrained estimates of analytical uncertainty that span the entire range of instrumental parameters above detection limits. Notably, the uncertainty should be assessed for the compound analyzed and the sample introduction technique (e.g., purge & trap) to incorporate all uncertainty for the process (e.g., Kuder and Philp, 2013). Isotope data is then used to calculate parameters such as ε, applied to estimate rates and extents of (bio)transformation, and Λ, applied to identify relevant (bio)transformation mechanisms at field sites (parameters ε and Λ are discussed in more detail in Sections 2 and 6, respectively). However, uncertainty estimates for ε and Λ must also be carefully considered – including how to incorporate analytical uncertainties and any uncertainty that is introduced with the calculation (referred to here as mathematical bias). Some of Sherwood Lollar‘s most prominent contributions to the field include investigations into analytical uncertainty (Sherwood Lollar et al., 2007; Hunkeler et al., 2008) and mathematical bias (Mundle et al., 2013; Ojeda et al., 2019; Ojeda et al., 2021), resulting in best practice recommendations to reliably interpret isotope data.

5.1.1 Analytical Uncertainty

Total analytical uncertainty incorporates both accuracy (how close a measured value is to the “true“ value) and reproducibility (or precision, which is the ability to obtain the same value for repeated sample or standard measurements over the entire linear range of the instrument) (Sherwood Lollar et al., 2007; Hunkeler et al., 2008). The concentration below which accuracy and reproducibility are compromised beyond an acceptable limit (depending on what the data is used for and the target compound) is defined as the operational detection limit (Hunkeler et al., 2008). Sherwood Lollar‘s work includes a detailed description of how to assess total analytical uncertainty within the linear range of an instrument for certain elements and provides a detailed analysis for δ^{13}C-CSIA (Sherwood Lollar et al., 2007). Based on this analysis, total analytical uncertainty, incorporating both accuracy and reproducibility, was estimated to be ± 0.5‰ for carbon (Sherwood Lollar et al., 2007) and ± 5‰ for hydrogen (Gray et al., 2002). This approach has been adopted in best practice guidelines and the estimate of total uncertainty for carbon isotope analysis has been used as the basis for the criterion for carbon isotope fractionation ($\Delta\delta^{13}$C = 2‰) to positively identify contaminant (bio)transformation in the field (Hunkeler et al., 2008). This criterion has been used throughout the literature applying CSIA to contaminant hydrogeology, enabling more accurate interpretations of CSIA data.

5.1.2 Mathematical Bias

Mathematical bias refers to uncertainty that is introduced into the estimates of parameters as an artefact of calculations that propagate the analytical uncertainty. Mathematical bias is an important factor in using estimates of parameters calculated using isotope data to reliably interpret rates, extents, and relevant mechanisms of (bio)transformation, yet mathematical bias has received less attention in the literature than analytical uncertainty. Sherwood Lollar‘s research has identified areas where consensus is lacking in commonly calculated and reported parameters, ε and Λ, for more reliable interpretation of isotope data. This includes the critical finding that calculations of ε are highly sensitive to errors in fraction remaining (*f*) values (obtained from contaminant concentrations), with increasing errors when concentrations decrease (Mundle et al., 2013). Based on these results, Mundle et al. (2013) recommended that ε values be calculated using data points varying from 100% to 20% of remaining compound, but not at <20%, a recommendation that has been adopted in the CSIA literature. More recently, dual-isotope analysis, where the isotopic fractionation of two elements within a molecule undergoing a single (bio)transformation process are plotted against each other to gain insight into the (bio)transformation mechanism (described in more detail in Section 6), has become standard practice in CSIA applied to contaminant hydrogeology. Dual-isotope plots typically produce linear relationships, with a slope, Λ, that can be used to interpret the relevant reaction mechanisms. To avoid non-linear behavior that arises when one element fractionates much more than the other, Höhener and Imfeld (2021) recommend plotting the natural logarithm (ln) of each element in a dual-isotope plot. Practitioners using dual-isotope analysis have traditionally used ordinary-least squares regression to calculate the slope in dual-isotope plots. However, it is well-reported in other disciplines such as marine biology (Laws and Archie, 1981), clinical chemistry (Linnet, 1999), anthropology (Smith, 2009), and within the geological sciences (Wehr and

Saleska, 2017) that ordinary-least squares introduces uncertainty (mathematical bias) when significant errors are present in the x- and y-variables. Sherwood Lollar‘s work demonstrated that the York regression method (York, 1966, 1969; York et al., 2004) is a more appropriate regression method for dual-isotope plots – producing more accurate estimates of Λ and its uncertainty for more reliable interpretations of (bio)transformation reaction mechanisms (Ojeda et al., 2019; Ojeda et al., 2021).

5.2 Technology Transfer for Industry Adoption

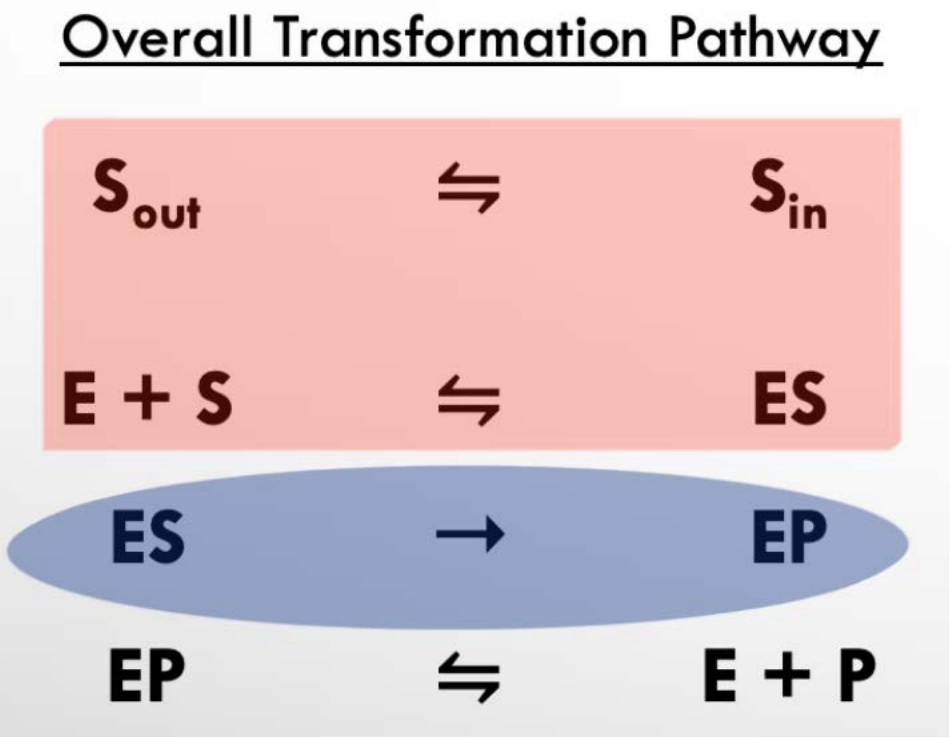
Since the earliest applications of CSIA in the late-1990s, industry interest and adoption of the technique has grown, requiring best practice recommendations for industry to be established and communicated. To meet this need, the IAEA and U.S. Environmental Protection Agency (EPA) jointly funded and managed the development of "A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants using Compound Specific Isotope Analysis (CSIA)" (Hunkeler et al., 2008). Sherwood Lollar was a lead author on this guide, which includes best practices for using CSIA in contaminant hydrogeology including sampling, calibration, isotope standards, and data interpretation. Sherwood Lollar has also contributed to best practices on using CSIA for environmental forensics (Mancini et al., 2017), where CSIA is used as a tool, usually to provide evidence to distinguish between groundwater contaminant sources, in litigation matters. Mancini et al. (2017) discusses the principles of CSIA, what CSIA can contribute to investigations and what its limitations are within a legal context (Federal Rule of Evidence 702). Sherwood Lollar has been a driving force in industry adoption of CSIA through contributions to best practices within the scientific literature and documents that bridge the gap between its technical and logistical aspects.

6. Using CSIA to Examine Reaction Mechanisms and Kinetics

Robust interpretations using the Rayleigh model require accurate estimates of ε values for a given contaminant and reaction pathway, including an understanding of what can cause variations in the ε values. If (bio)transformation is indeed occurring, it is also important to determine the relevant transformation pathway, the overall sequence of reactions from substrate to product(s), to assess whether the (bio)transformation process is producing less harmful daughter products than the original parent molecule itself, all of which are required to demonstrate remediation. For example, reductive dechlorination of TCE can result in accumulation of VC, which is more toxic than TCE. In these instances, (bio)transformation of TCE is occurring, but it is not considered remediation as it is producing more harmful products if VC is not further degraded to non-toxic ethene. Insight into (bio)transformation pathways can also have important implications for optimizing remediation in the field, for instance if aerobic (bio)transformation is identified, biosparging can be used to enhance it (Reddy et al., 1995), but this would inhibit anaerobic (bio)transformation processes. Pathway identification using daughter products alone can be limited if compounds are present as primary contaminants as well as products of transformation reactions. Since the early 2000s, CSIA has been applied to investigate (bio)transformation pathways and rate-limiting elementary reaction steps within an overall pathway. These studies provide insights into what can cause ε values and, in dual-isotope studies, Λ values to vary for the same (bio)transformation reaction. This information can be used to determine the ε and Λ values (or range of values) to use at field sites (Hunkeler et al., 2008 and references therein). The Sherwood Lollar laboratory has contributed substantially to the literature to provide reference ε and Λ values for (bio)transformation reactions and to explain the primary controls on these parameters.

6.1 Variability in enrichment factors, ε

An enrichment factor, ε, for a single reaction can vary for two primary reasons: differences in reaction mechanisms, or differences in the kinetics of elementary steps within an overall reaction pathway (an example simplified enzymatic reaction pathway, comprised of multiple elementary steps, is shown in Scheme 1). The latter is referred to as a "masking effect", which occurs if a step preceding the transformation step in a net reaction pathway is rate-limiting or partially rate-limiting (e.g., steps shaded in red in Scheme 1).



Scheme 1. Simplified overall transformation pathway for enzymatic transformation, shown with four elementary steps. S_{out} = substrate outside of the cell, S_{in} = substrate inside of the cell, E = free enzyme, S = free substrate, ES = enzyme-substrate complex, P = product, EP = enzyme-product complex. Double arrows indicate equilibrium steps, and the single arrow indicates a unidirectional step. Steps shaded in the red box indicate steps that, if rate-limiting,

could cause a masking effect. The step shaded in blue represents the transformation step, which can cause a difference in ε values from different reaction mechanisms.

Evaluating how the ε value changes for different (bio)transformation pathways and constraining the range of ε values for a single pathway, accounting for masking effects, is important for accurately interpreting CSIA data at a contaminated field site. For sound interpretations of (bio)transformation using the Rayleigh model, the range of ε values for a given (bio)transformation reaction should be well-constrained in the literature, and *f* estimates should be calculated using the minimum value, maximum value, and average value within the range as per best practice recommendations (Hunkeler et al., 2008). This approach relies on robust estimates of ε values and understanding their sources of variation, and whether masking effects are known to arise for a given compound and (bio)transformation reaction. Research from the Sherwood Lollar's Stable Isotope Laboratory includes key insights into ε value variations due to different reaction mechanisms and their links to (bio)transformation pathways (Hirschorn et al., 2007; Elsner et al., 2008; Vanstone et al., 2008; Liang et al., 2011, 2014; Mundle et al., 2017) and masking effects (Mancini et al., 2006; Sherwood Lollar et al., 2010). Findings from these studies are directly relevant to applying CSIA to interpret (bio)transformation in the field. For example, Hirschorn et al. (2004) found distinct ranges of ε values for 1,2-DCA biotransformation, with one mean centered at -3.9 ± 0.6‰ corresponding to enzymatic aerobic oxidation, and the other centered at -29.2 ± 1.9‰ corresponding to enzymatic aerobic hydrolytic dehalogenation. The distinct ranges of ε values for aerobic biotransformation mean that careful consideration of geochemical and biological parameters (e.g., microbes present and known biochemical pathways) must be considered to select the most appropriate estimate of ε value to use in the field. Similarly, research from the Sherwood Lollar's Stable Isotope Laboratory has shown distinct carbon ε values for anaerobic vs. aerobic biotransformation of 1,2,4-TCB and MCB (Liang et al., 2011), different anaerobic pathways for DCB isomers (Liang et al., 2014), and different anaerobic pathways for chlorinated alkane transformation by zero-valent zinc (Vanstone et al., 2008) and zero-valent iron (Elsner et al., 2008).

Masking effects can also cause variations in ε values for the same compound and (bio)transformation pathway. For a given substrate and (bio)transformation pathway, the potential for masking effects, their impact on the ε value, and any variables or conditions that can influence the presence of masking effects should be considered to calculate accurate estimates of extents and rates of (bio)transformation using the Rayleigh equation. Sherwood Lollar and her team investigated the presence of masking effects and their influence on observed ε values for anaerobic biotransformation of chlorinated ethanes (Sherwood Lollar et al., 2010) and aerobic biotransformation of toluene (Mancini et al., 2006). Furthermore, Mancini et al. (2006) assessed how external conditions such as trace element limitation influenced the presence of masking effects.

Variability in observed ε values for a substrate undergoing (bio)transformation can occur if multiple processes (i.e., multiple reactions) with different ε values are simultaneously affecting the same substrate, as was observed for biotransformation of *cis*-DCE and 1,2-TCA by the same enzyme (cytochrome P450) (Mundle et al. 2017). For substrates and conditions where simultaneous pathways occur, all pathways must be considered and, where possible, the dominant pathway should be assessed (e.g., with daughter product characterization, microbial genomics/proteomics) to prevent limiting the effectiveness of CSIA, as cautioned by Mundle et al. (2017). Isotopic compositions of daughter products may also be used to interpret simultaneous pathways. For simultaneous hydrogenolysis and dichloroelimination of chlorinated ethenes via abiotic transformation using iron nanoparticles, the isotopic composition of the daughter products shows distinct isotopic discrimination correlated to each pathway (Elsner et al., 2008). These studies from the Sherwood Lollar's Stable Isotope Laboratory illustrate the value of using CSIA to identify the occurrence of simultaneous (bio)transformation pathways at field sites.

6.2 Multi-Element Isotope Analysis

In the last twenty years, multi-element CSIA has rapidly developed to assess transformation pathways of organic contaminants. It consists of generating dual-isotope plots to calculate Λ (discussed in Section 5). These dual-isotope plots have two major applications. First, they help overcome factors that complicate CSIA interpretations, such as masking effects, as any masking present should affect both elements equally (as long as the masking effect does not cause isotopic fractionation itself). Second, they give valuable information for the given reaction, and in some cases, the (bio)transformation pathway. Dual-isotope analysis can be used in the field by comparing to reference Λ values from laboratory experiments to identify the relevant (bio)transformation pathways, giving valuable insight into how contaminants are being transformed, with implications to potentially optimize these reactions in the field (e.g., air sparging for aerobic biotransformation). Along with others in the field (e.g., Hunkeler et al., 2001), Sherwood Lollar's research team contributed some of the earliest studies combining CSIA data from two elements within the same molecule, such as $\delta^{13}C$ and $\delta^{2}H$ in MTBE (Gray et al., 2002) and benzene (Mancini et al., 2003) to interpret biotransformation reaction mechanisms based on both carbon and hydrogen ε. Further work by Sherwood Lollar's team and others in the field later combined carbon and hydrogen CSIA data into a dual-isotope plot, including for aerobic toluene biotransformation (Mancini et al., 2006), anaerobic benzene biotransformation

(Mancini et al., 2008), abiotic transformation of MTBE (Elsner et al., 2007b), and aerobic MTBE biotransformation (McKelvie et al., 2009). Early dual-isotope studies lead to the observation that Λ values could be correlated to reaction mechanisms and pathways, a crucial finding that catalyzed the evolution of CSIA in contaminant hydrogeology to where it is today. Later analytical developments in chlorine isotope analysis (recently reviewed by Zimmermann et al., 2020) allowed for further dual-isotope studies including $\delta^{13}C$ and $\delta^{37}Cl$ within the same molecule. A recent review from Sherwood Lollar and her team on the use, utility, and limitations of using multi-element isotope analysis (Ojeda et al., 2020), includes a compilation of literature Λ values and known causes for variation in Λ values. These studies investigate reaction mechanisms and pathways involved in the biotransformation of chlorinated organic compounds (Phillips et al., 2022; Chen et al., 2018; Lihl et al., 2019).

7. CSIA of the deep subsurface

In addition to shallow subsurface systems affected by anthropogenic contaminants, Sherwood Lollar's group has also extensively applied CSIA in novel ways to document and decipher the range and rates of biogeochemical processes at work in deeper groundwater systems in the crust. A significant focus of Sherwood Lollar's research has concentrated on Precambrian crystalline rock settings and has recently demonstrated this to be a hitherto undocumented, yet significant, reservoir containing as much as 30% of the planet's groundwater (Warr et al., 2018; Ferguson et al., 2021). In this global groundwater reservoir, (the 'hidden hydrogeosphere'), Sherwood Lollar and international collaborators have applied isotopic analyses of noble gases to reveal that these groundwaters can reside over extended timescales globally, ranging ka-Ga (i.e., from thousands to billions of years) (e.g., Heard et al., 2018; Holland et al., 2013; Lippmann-Pipke et al., 2011;

Warr et al., 2018). Research from Sherwood Lollar's Stable Isotope Laboratory has demonstrated how combining CSIA, compositional and modelling-based approaches can identify and quantify low temperature geochemical and water-rock abiotic reactions in these hydrogeologic reservoirs. These reactions can significantly modify the isotopic and elemental composition of these groundwaters (e.g., Li et al., 2016; Warr et al., 2021a), and generate significant quantities of methane, higher hydrocarbons, hydrogen, as well as helium and other potential economic and energy resources (e.g., Sherwood Lollar et al., 1993, 2002, 2008; Lin et al., 2005; Warr et al., 2019, 2022). This isotopic and elemental-driven approach has also been applied by Sherwood Lollar and collaborators to investigate cryptic long-term radiolytic and water-rock-driven (bio)geochemical cycling of electron acceptors and donors involving compounds of hydrogen, carbon, sulfur, and nitrogen, with the potential for sustaining a deep biosphere in isolation of the surface photosphere (e.g., Li et al., 2021; Lin et al., 2006; Lollar et al., 2019; Sheik et al., 2021; Sherwood Lollar et al., 2021, 2006, 1993; Silver et al., 2012). Sherwood Lollar's active and ongoing research in these deep hydrogeological systems and the processes operating therein continues to provide unique insights towards long-term, large scale, biogeochemical cycles and novel habitability models on Earth and analogue settings beyond (NASEM, 2019; Onstott et al., 2006; Sherwood Lollar et al., 2007; Smith et al., 2021; Tarnas et al., 2021, 2018).

8. Looking ahead

Research from Professor Sherwood Lollar and her team has contributed to the development of CSIA to contaminant hydrogeology across many aspects, as summarized in Figure 1. The seminal work by Sherwood Lollar and her team on establishing CSIA as a quantitative tool to investigate contaminant fate and transport (Section 2) has paved the path for its application at contaminated field sites for common groundwater contaminants (Section 3). Following these steps, the stable isotope community has been rapidly producing ε and Λ values for various compounds and experimental conditions, though much more data is needed to cover the range of priority groundwater pollutants (Ojeda et al., 2020). This growing database of key isotope parameters is critical to better characterize contaminant sources, calculate biotransformation rates and extents, and identify reaction mechanisms at field sites.

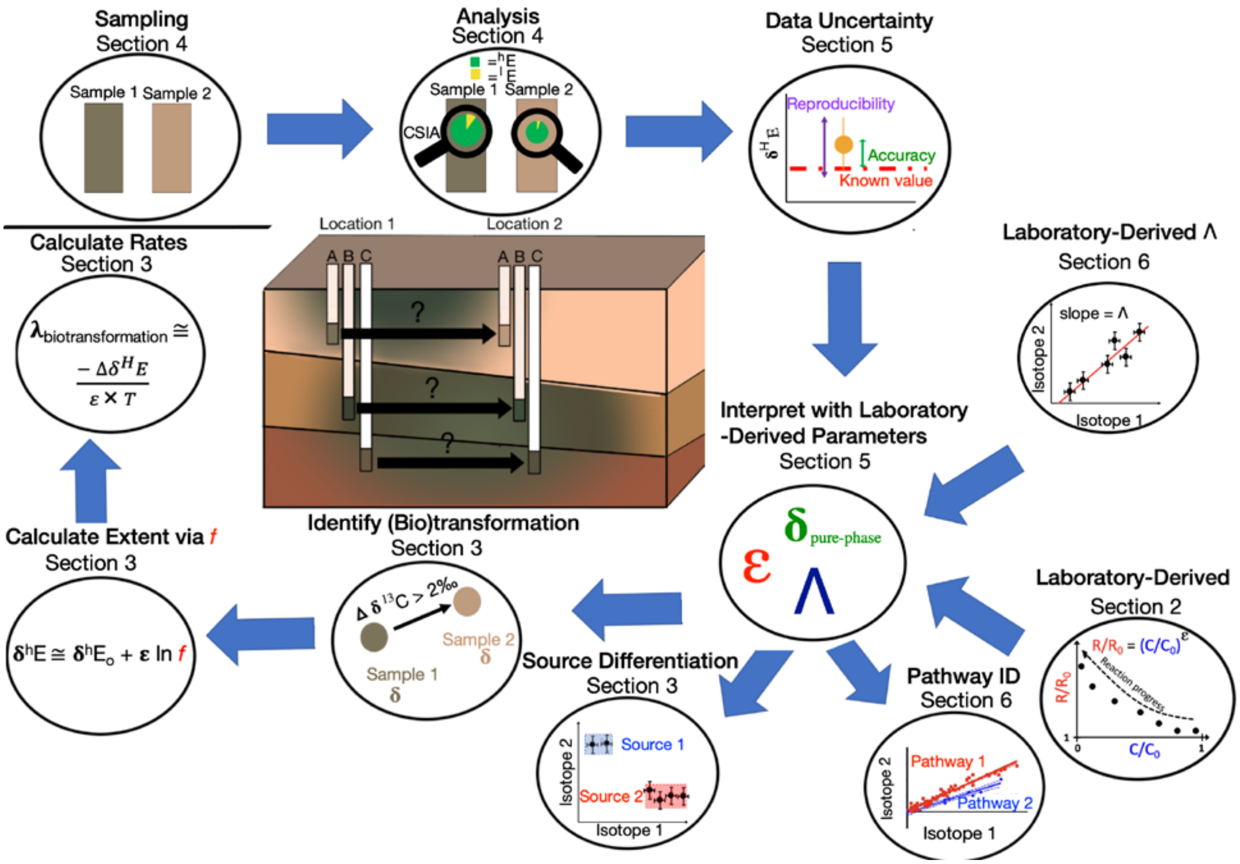


Figure 1. Summary of the different contributions of Sherwood Lollar and her team to CSIA in hydrogeology, and how they fit together in applying CSIA at contaminated field sites. For more detail on each aspect shown in the figure, the section is given below where more detail can be found in the text.

Sherwood Lollar's efforts to share best practices for accurate analysis of lab-derived isotope data and reliable interpretation of field-derived isotope data (Section 5) have helped maintain high quality standards in the field of environmental isotope chemistry. These efforts have been key to support wider adoption of this tool by industry (Bouchard et al., 2018; Mundle et al., 2012; Ottosen et al., 2021; Palau et al., 2016; Phillips et al., 2020). More field case studies would certainly help support an even wider adoption of CSIA as one of the advanced tools used for monitoring natural and engineered attenuation in the field (ITRC, 2013).

Research from Sherwood Lollar's Stable Isotope Laboratory on the development of new CSIA-compatible sampling and analysis methods (Section 4) has contributed to opening the field to new applications with respect to contaminants (e.g., CFC) and environments (e.g., contaminated sediments). Using innovative sample collection (Suchana et al., 2022) and preconcentration methods (Mogusu et al., 2015; Bakkour et al., 2018; Melsbach et al., 2019; Torrentó et al., 2019), CSIA is now being applied to an increasing number of larger, less volatile and more polar compounds such as pesticides (e.g., atrazine, isoproturon, HCH, S-metolachlor), corrosion-inhibitors (benzotriazole), and pharmaceuticals and personal care products (e.g., triclosan, ibuprofen) (Elsner and Imfeld, 2016 and references therein). CSIA is also being used to track surface water-relevant processes such as phototransformation (Ratti et al., 2015; Passeport et al., 2018; Drouin et al., 2021; Wu et al., 2021) and surface-water biotransformation (Alvarez-Zaldívar et al., 2018; Droz et al., 2021). With some of the first papers on CSIA application to elements other than carbon, Sherwood Lollar's work contributed to the development of multi-element CSIA (see reviews by Nijenhuis et al., 2016; Ojeda et al., 2020; Vogt et al., 2016), which is now the standard to positively identify and characterize (bio)transformation processes in the field (Section 6). Many priority pollutants include Cl atoms at reactive positions. These are usually in lower number than C or H atoms, resulting in less isotope dilution and therefore more potential for Cl-CSIA to identify (bio)transformation. Analytical methods for Cl-CSIA are being developed with various instruments such as GC-qMS, GC/IRMS, and (GC)-ICP-MS (Zimmermann et al., 2020). With such a rapidly growing field, and in line with Sherwood Lollar's major efforts to maintain the production of high-quality data, we urge the community to be cautious in the standardization of Cl-CSIA data, recently emphasized by Lihl et al. (2019), and in the estimation of fractionation factors. One of the underlying assumptions in the Rayleigh model is that the element considered (here Cl) has a dominant isotope. This allows the simplification of the equations where f is approximated by the isotopic ratio of the most abundant element (Mariotti et al., 1981), which leads to the final simple Rayleigh model (Equation 2). However, with natural abundances of 75.8% for ^{35}Cl and 24.2% for ^{37}Cl, this assumption may no longer be valid. Although this aspect of chlorine isotope analysis has been recognized and justified (Elsner and Hunkeler, 2008) it is important to note that this approximation is true only for conditions where the difference between measured isotope ratios R and R_0 is small. As recently reviewed by Zimmermann et al. (2020), models other than the simple Rayleigh equation exist to estimate contaminant (bio)transformation from Cl-CSIA incorporating the complexities specific to Cl isotopes, and those must be used to keep producing quality and reliable isotope data.

We are at an exciting stage in the field of CSIA, with many new areas of application to explore, in part building on the early work described here, including adopting recent analytical advances in position-specific isotope analysis (Eiler et al., 2017; Cesar et al., 2019; Gilbert et al., 2019), which build on early work from Sherwood Lollar and her team that was integral in showing the potential of position-specific isotope analysis using nuclear magnetic resonance

(NMR) spectroscopy (McKelvie et al., 2010); further investigation to identify enzymatic mechanisms and their relationship to protein structure (Phillips et al., 2022); evaluating pesticide transfer and (bio)transformation in agricultural watersheds (reviewed by Imfeld et al., 2021); characterizing contaminant fate in soil-plant systems (Liu et al., 2020); and potentially distinguishing between sorption and biodegradation of taste and odour products in drinking water treatment plants (Yuan et al., 2022). Likewise, recent CSIA application in deeper hydrogeologic settings continue to reveal much on the long-term global (bio)geochemical processes shaping the history, habitability, and evolution of Earth and beyond (NASEM 2019; Sherwood LOllar et al., 2021; Warr et al., 2021b; 2022). As we celebrate the scientific achievements of Professor Barbara Sherwood Lollar, we also recognize the impact she has made on the scientists she mentored and collaborated with, and industry. Her excellent communication skills allowed the principles of isotopic fractionation to be widely understood and enabled the uptake of CSIA for industrial applications. We are grateful for this opportunity to pay tribute to a remarkable woman whose vision and leadership paved the path for this work and instilled a high standard for this research carried out via the rewarding research collaborations we advanced under her stewardship. We extend our thanks to Professor Barbara Sherwood Lollar for laying the path, the high standards that she instilled in her research group, the mentoring she provided during our time in her laboratory and beyond, and the training and inspiration she continues to bestow on the next generation of researchers learning about CSIA to all fields of hydrogeology.

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