# Analytical techniques for assessing transformation processes of organic contaminants by compound-specific stable isotope analyses

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**Abstract**

The analysis of variations in stable isotope compositions is becoming an essential approach for evaluating enzymatic and abiotic reactions of organic contaminants in soils and aquatic systems. Different, sometimes complementary, analytical techniques are currently used and developed for the purpose of determining stable isotope ratios in individual organic compounds. Anticipating an increasing demand for compound-specific isotope analysis, this survey compiles information for choosing the most promising analytical approach to an isotope-related problem. To this end, we review the principles of instrumentation for compound-specific isotope analysis and show how they can be exploited to assess contaminant transformation processes. Using chlorinated solvents and triazine herbicides as illustrative examples, we discuss how the isotope-sensitive techniques impact the investigation of stable isotope fractionation in environmental chemistry and microbiology.

*Key words:* Compound-specific stable isotope analyses (CSIA), isotope-ratio mass spectrometry (IRMS), multicollector inductively coupled plasma mass spectrometry (MC-ICPMS), gas and liquid chromatography, cavity ring-down spectroscopy (CRDS), isotope fractionation, kinetic isotope effect (KIE), degradation pathways.

## 1. Introduction

The analysis of the stable isotope compositions in individual compounds is one of the key techniques for identifying the sources of organic soil and groundwater contaminants as well as for characterizing their transformation processes [1–3]. While conservative stable isotope ratios are exploited to infer precursor materials and/or synthesis routes and thus

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reveal the origin of contaminants, changes of isotopic composition, especially if multiple elements are considered simultaneously, are crucial to assess contaminant formation and degradation. The latter is takes advantage of the fact that stable isotope ratios measured in organic molecules (i.e., the ratio of heavy to light isotopologue concentrations, ${}^{h}\mathrm{E}/{}^{l}\mathrm{E}$, of an element E) vary systematically depending on the type of chemical bond(s) that are broken or formed. This process of stable isotope fractionation is due to kinetic or equilibrium isotope effects at the reacting bond(s) [4]. Isotope effects result from different reaction rates of isotopologues containing the light or heavy isotope, respectively, and are indicative for the reaction mechanism. Indeed, enzymatic or abiotic contaminant degradation, for example, via electron transfer, substitution, elimination, or photochemical reactions exhibit very different isotope effects [1, 2, 5, 6]. As the isotope fractionation generated by these reactions is often significantly larger than that induced by phase-transfer processes, variations of isotope ratios usually indicate that a concentration decrease observed for a contaminant is not just the consequence of dilution or sorption to the environmental matrix. Moreover, because the extent of isotope fractionation is proportional to the fractional conversion of the contaminant, systematic changes of isotope ratios have also been evaluated quantitatively, for example, to calculate the extent of *in-situ* (bio)degradation [7].

Compound-specific isotope analysis (CSIA) thus offers novel avenues to trace transformation processes of contaminants in complex environments because isotopic analyses can be used to identify the reactive atoms within an organic compound. This approach is also appealing for two other reasons. Isotope ratio variations in the reactant already bear information on the products that are or have been formed [1, 2]. In addition, the interpretation of isotope ratios can be carried out independent of the contaminants' concentrations. The latter alleviates the need for apportioning concentration decreases to transformation processes, a task that requires expensive and labor-intensive sampling networks and is often biased by non-degradative processes. There are, in principle, two prerequisites for the successful application of CSIA. First, analytical devices and measurement strategies are needed that allow for quantification of isotope ratios of the elements of interest. Most isotope-selective detectors usually have low resolving power in terms of ion mass and are not very sensitive but achieve high levels of accuracy and precision to resolve isotopologue concentrations [8]. The use of on- and offline pre-concentration procedures in CSIA is therefore common [9]. Second, meaningful data interpretation calls for some fundamental knowledge about isotope effects in (bio)chemical reactions and how they lead to isotope fractionation measured in the bulk molecule. The extent to which isotope ratios can change during a reaction depends

on the reaction kinetics as well as on features pertinent to the reaction mechanisms and the elements involved such as relative masses of the isotopes, bond strength, etc. [4]. Thus, some transformation pathways lead to substantial isotope fractionation and might therefore be easily detected with a given analytical uncertainty, while other (bio)degradation reactions are more difficult to quantify. An illustrative example for the interdependence of analytical and (bio)chemical boundary conditions for the application of CSIA is given in box 1.

Given the many advantages that stable isotope analysis offers for various scientific disciplines, different instrumental approaches to CSIA have emerged in parallel in recent years. Many of them will propel the use of stable isotope fractionation to trace contaminant transformation processes in the near future as more isotopic elements are becoming accessible and procedures for the application of CSIA to a wide range of organic compounds are being developed. In this review, we provide an account of the currently pursued instrumental techniques for the analysis of stable isotope ratios in individual organic compounds from an environmental chemistry perspective. We briefly discuss the principles of alternative instrumentations for CSIA. This section also conveys some basic knowledge that is required for making decisions as to which technique is the most promising one for approaching an isotope-related, analytical problem. For the fundamental aspects of the theory and instrumentation for stable isotope analysis as well as more comprehensive discussions of the materials illustrated here, we refer the reader to some of the excellent, more scholastic reviews and compilations [8–12]. Finally, we discuss two illustrative examples to elaborate how the various techniques (will) impact the investigation of isotope fractionating processes in environmental chemistry and microbiology.

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**Box 1 – Influence of analytical uncertainty on the assessment of toluene biodegradation based on compound-specific carbon isotope analysis**

Fuel constituents such as benzene, toluenes, or methyl-*tert*-butyl ether (MTBE) are frequently encountered groundwater pollutants [13]. Evaluating their natural attenuation via biodegradation by indigenous microbial communities is important for the risk assessment at contaminated sites. Quantitative estimates regarding the amount of biodegraded contaminants can be obtained from comparisons of stable isotope signatures of element E, $\delta^h$E, in which the concentrations of heavy ($^h$E) and light ($^l$E) isotopologues (e.g., $^{13}$C/$^{12}$C, $^2$H/$^1$H) of an analyte are reported relative to a reference material (eq. 1).

$$\delta^h\mathrm{E} = \left( \frac{{}^h\mathrm{E}/{}^l\mathrm{E}_{\,sample}}{{}^h\mathrm{E}/{}^l\mathrm{E}_{\,reference}} - 1 \right) \times 1000 \qquad (1)$$

Toluene, for example, is frequently analyzed for C and H isotope ratios in aqueous samples. Even at concentrations of only 0.1 $\mu$g $L^{-1}$, samples can be processed for accurate $\delta^{13}$C-measurements with a precision of $\pm$0.5‰ [14] corresponding to a change of $^{13}C/^{12}C$-toluene isotopologue ratio by $\pm$ 0.0000056. How does this analytical uncertainty impact the assessment of toluene biodegradation? In fact, oxidative toluene transformation can be initiated by reactions at two different positions within the molecule, that is via methyl group or aromatic ring oxidation to benzyl alcohol or methylcatechol, respectively, which are both further biodegraded (Figure 1a).

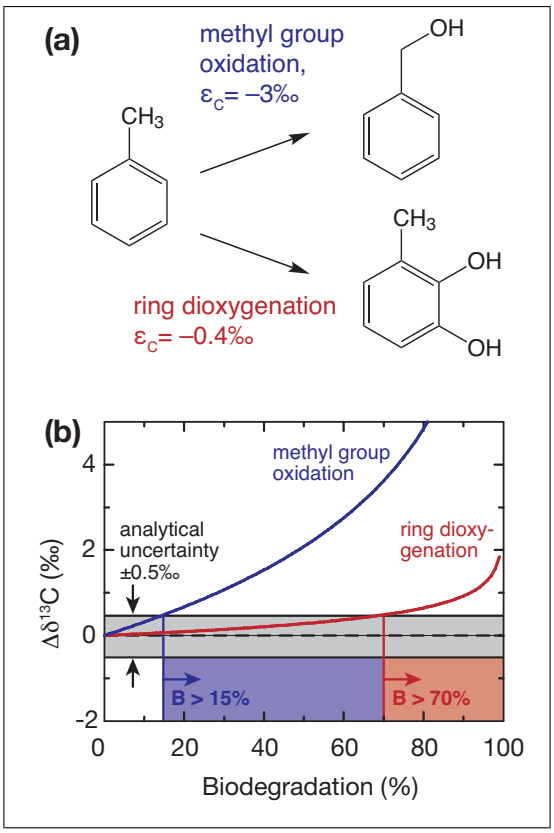


Figure 1: Consequences of analytical uncertainty for assessing oxidative toluene biodegradation pathways by $\delta^{13}$C-measurements (illustrative example): (a) Methyl group oxidation *vs.* aromatic ring dioxygenation exhibit different C isotope enrichment factors, $\epsilon_C$. (b) While toluene biodegradation via methyl group oxidation (blue line) can cause $\delta^{13}$C to shift beyond analytical uncertainty ($\pm$0.5‰, grey shaded area) once 15% of the contaminant has been biodegraded, it is more difficult to assess toluene ring dioxygenation (red line) precisely unless more than 70% are transformed (red area).

Owing to the variable number of reactive C atoms in toluene per pathway and distinct transition state structures, $^{13}$C-kinetic isotope effects ($^{13}$C-KIE) differ depending on the position and mechanism of initial oxidative attack. This behavior is reflected in distinct bulk

toluene $^{13}$C enrichment factors, $\epsilon_C$. While $\epsilon_C$-values of toluene for methyl group oxidations vary between –2‰ and –6‰ , they are much smaller for reactions at the aromatic ring (–0.4‰ to –1‰) [15, 16].

Thus, the enrichment of residual toluene in $^{13}$C during its oxidation is more substantial for the case of toluene oxidation at the methyl group compared to reactions that oxygenate the aromatic ring (compare blue and red lines in Figure 1b). The relationship between isotope fractionation and extent of (bio)transformation ($B$) follows in eq. 2, where $\delta^{13}$C and $\delta^{13}C_0$ represent measured and initial C isotope signatures of toluene at different locations or time points in a contaminated groundwater.

$$B = 1 - \left( \frac{\delta^{13}\mathrm{C} + 1000}{\delta^{13}\mathrm{C}_0 + 1000} \right)^{1000/\epsilon_C} \tag{2}$$

An analytical uncertainty of ±0.5‰ has different implications for the detection of toluene biodegradation along the two oxidation pathways. Biodegradation via methyl group oxidation will cause isotope fractionation beyond the analytical uncertainty (grey area in Figure 1) as soon as more than 15% of the substrate are consumed. In contrast, ring dioxygenation might only become obvious from changes in $\delta^{13}\mathrm{C} > 0.5$‰ at 70% toluene turnover. Thus, the analytical uncertainty of ±0.5‰ makes it quite difficult to assess toluene ring dioxygenation precisely because this pathway only gives rise to minor isotope fractionation. In contrast, analytical precision does not compromise the monitoring of toluene degradation via methyl group oxidation.

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## 2. Analytical techniques for measuring stable isotope ratios in individual organic compounds

### *2.1. Mass spectrometry for compound-specific isotope analysis*

To date, compound-specific isotope ratio measurements can, in principle, be carried out for most elements present in organic compounds at or near the natural isotope abundances (Table 1). Isotopic analyses of the elements C, H, and N are becoming a routine procedure for some typical pollutants such as fuel components, chlorinated solvents as well as some agrochemicals and explosives. In contrast, methods for measurement of O, S, Cl, and Br are more challenging and currently being developed for specific sets of compounds. From an analytical perspective, the reasons for this uneven "popularity" of the various isotope systems relates to instrumental difficulties of converting these elements online without iso-

tope fractionation into analyte gases and partly to the very different abundances of light and heavy isotopes for each element.

Almost every organic contaminant contains two or more isotopic elements. Thus, even small molecules consisting of just a few atoms and two stable isotopes per element will give rise to many different isotopologues, whose relative abundances follow from a combination of binomial distributions. However, resolution and precision of isotope ratio mass spectrometers (IRMS) were not designed to resolve and quantify all these isotopologues reliably, and, owing also to additional instrumental and methodological restrictions, measurements of isotope ratios are performed only in simple molecules consisting of few isotopologues such as $CO_2$ and $H_2$ for $^{13}C/^{12}C$ and $^{2}H/^{1}H$, respectively. As a consequence, interface systems have been developed for the on-line conversion of organic molecules into suitable target analytes. The approaches pursued, however, are quite different for the target elements (listed in Table 1) and so is the versatility of their application for different organic compounds.

Table 1: Stable isotope systems and natural abundance isotope ratios for typical elements in organic contaminants [a]

| Isotope system | Isotope ratio (%) |
|---|---|
| $^{2}H/^{1}H$ | 0.01558 |
| $^{13}C/^{12}C$ | 1.123 |
| $^{15}N/^{14}N$ | 0.3663 |
| $^{18}O/^{16}O$ | 0.2005 |
| $^{34}S/^{32}S$ | 4.416 |
| $^{37}Cl/^{35}Cl$ | 31.96 |
| $^{81}Br/^{79}Br$ | 97.27 |

[a] approximate values; see refs [17, 18] for a list of standards and reference materials.

In the following survey of instrumental approaches, we deliberately neglect the discussion of so-called "offline" procedures, which are carried out either without analyte separation by chromatographic means (e.g., coupling continuous flow elemental analysis (EA) to IRMS) or without continuous conversion of organic compounds into analyte gases (dual-inlet (DI) systems). EA- and DI-IRMS are superior in terms of analytical precision but they require larger sample size and do not facilitate online sample preparation, separation, and data acquisition for several compounds in complex mixtures. The use of methods such as EA-

or DI-IRMS is nevertheless essential in that they are required for accurate isotope reference data, with which compound-specific methods are developed and calibrated [19].

### *2.1.1. Gas chromatography coupled to isotope ratio mass spectrometry - the typical approach to CSIA*

Gas chromatography coupled to isotope ratio mass spectrometry (GC/IRMS, [8]) is currently the most widely used instrumental setup for compound-specific isotope analysis of organic contaminants. These systems usually consist of units for sample pre-concentration and injection, pollutant separation, conversion to analyte gases and the isotope selective detection ([8, 9], Table 2). Rather few compound classes have been made accessible for CSIA owing to the great structural diversity of micropollutants [13] and thus the need for developing and calibrating analytical procedures on a compound-by-compound basis [20]. In fact, compared to concentration measurements of pollutants by (high-resolution) mass spectrometry, which is routinely done even in the ng $L^{-1}$ range [21], CSIA requires efficient pre-concentration steps such as purge and trap, solid phase (micro)extraction, and vacuum extraction [22–26] to enable isotope analysis of contaminated soils or groundwaters at more than thousand-fold higher concentrations (i.e., 10–100 $\mu$g $L^{-1}$) for routine operations. The challenges of separating components of contaminant mixtures are similar to those in standard gas chromatography; however, GC/IRMS needs to deal with larger amounts of analytes and matrix effects.

In the interface system, organic compounds are reacted to $H_2$, $CO_2$, $N_2$, or CO for measuring H, C, N, or O isotopes, respectively, through optimized combustion, pyrolysis, or combustion coupled to reduction processes followed by the removal of reaction byproducts such as water or corrosive gases. Quantitative chemical conversion of organic molecules is achieved in narrow-bore reactor tubes, usually containing CuO, NiO, and/or Pt as catalysts, which are operated at high temperatures depending mostly on the isotope system investigated (see Table 2, [27]). Finally, the sample gas isotopologues, for example $^{14}N_2$, $^{14}N\,^{15}N$, and $^{15}N_2$ of $N_2$ for $^{15}N/^{14}N$-ratios, are analyzed in magnetic sector field mass spectrometers, which are specialized for maximizing ion beam currents and stability. The high precisions arises from the system of differential measurements of analyte and standard gases with known isotopic composition simultaneously for at least two masses using multiple detectors. The difference in ion-current ratios measured in the detectors is exactly proportional to the difference in isotope ratios even though the absolute isotopic abundances are poorly constrained [28] thus requiring isotope ratios to be reported relative to reference materials (i.e., in the "delta notation", eq. 1).

*2.1.2. Analytical procedures for CSIA of organic contaminants by GC/IRMS*

Establishing procedures for organic contaminant CSIA by GC/IRMS comes with a series of tests to ensure accuracy and precision. Given that many steps of the analytical procedure such as sample preparation, analyte enrichment, and conversion can give rise to isotope fractionation, referencing strategies for comparing the isotope signatures of known and unknown compounds on the GC/IRMS are key for accurate isotope ratio analysis [19, 29]. Such comparisons are based on standard compounds, whose isotope ratios have been measured independently by alternative techniques (EA- or DI-IRMS, see above), and, in most cases, they allow one to identify suitable operating conditions for GC/IRMS of organic contaminants. As the attainable precision of an isotope ratio measurement increases with the amount of isotopologue ions in mass spectrometry and with increasing abundance of the rare isotope [30], optimum concentration ranges for analysis need to be identified for every compound separately.

Instrument performance is, in principle, limited at low concentrations by intolerable loss of precision and, on the other hand, by the amount of analyte that can be loaded onto the chromatographic column and converted adequately in the interface system. However, acceptable limits of measurement uncertainty lack clear-cut definitions as they not only include the reproducibility of repeated measurements but also have to account for the rather narrow linear range of continuous flow IRMS (typically one order of magnitude in contaminant concentration) [14, 20, 31]. Deviations of isotope ratio measurements due to nonlinearity effects arise from too different signal sizes of standard and sample. The lack of accuracy from multiple sample measurements carried out over a range of signal sizes thus additionally reduces precision [31]. Current experience suggests that total instrumental uncertainties are $\pm 0.5‰$ for $\delta^{13}C$ and $\delta^{15}N$-values while they are $\pm 0.8‰$ and $\pm 5‰$ for $\delta^{18}O$ and $\delta^{2}H$, respectively [9]. Depending on the compound and the sample matrix, these limits can be surpassed by by variations imposed by sample preparation. Method detection limits for GC/IRMS are linked to these definitions of uncertainty in that they reflect the lowest concentration in a(n) (environmental) sample, for which the measured isotope signature does not deviate by more than the total instrumental uncertainty from the accurate value [14].

Finally, chromatographic resolution to baseline separation of the analyte is essential for unambiguous quantification of isotope ratios [32]. Even though algorithms are used in standard software solutions that can deconvolute isotopologue signals from partially co-eluting peaks [33], this step can be a source of error. Substantially improved separation can be

Table 2: Overview of instrumental setups for compound-specific analyses of stable isotope ratios in organic contaminants (abbreviations: GC = gas chromatography, IRMS = isotope ratio mass spectrometry, LC = liquid chromatography, qMS = quadrupol mass spectrometry, MC-ICPMS = multicollector inductively coupled plasma mass spectrometry, CRDS = cavity ring-down spectroscopy

| Instrumentation | Separation | Interface system | Analyte | Ionization | Mass analysis / ion detection | Isotope ratio |
|---|---|---|---|---|---|---|
| GC/IRMS | GC<br>GC×GC [c] | Comb [a]<br>Comb/Red [d]<br>Pyr [e]<br>Pyr | $CO_2$<br>$N_2$<br>$H_2$<br>CO | EI [b] | Magnetic sector / Faraday cups | $^{13}C/^{12}C$<br>$^{15}N/^{14}N$<br>$^{2}H/^{1}H$<br>$^{18}O/^{16}O$ |
| LC/IRMS | LC | Wet oxidation | $CO_2$ | EI | Magnetic sector / Faraday cups | $^{13}C/^{12}C$ |
| GC/IRMS [f] | GC | none | fragment ions | EI | Magnetic sector / Faraday cups | $^{37}Cl/^{35}Cl$<br>$^{81}Br/^{79}Br$ |
| GC/qMS | GC | none | molecular ion & fragment ions | EI | Quadrupole / Electron multiplier | $^{37}Cl/^{35}Cl$ |
| GC/MC-ICPMS | GC | ICP [g] | Cl<br>Br<br>S | ICP | Magnetic sector / Faraday cups | $^{37}Cl/^{35}Cl$<br>$^{81}Br/^{79}Br$<br>$^{34}S/^{32}S$ |
| GC/CRDS [h] | GC | Comb | $CO_2$ | none | Infrared spectroscopy | $^{13}C/^{12}C$ |

[a] Comb = combustion at 900–950 °C; [b] EI = electron ionization [c] applications reported exclusively for analysis of C isotopes; [d] Comb/Red = combustions followed by reduction at 600–650 °C; [e] Pyr = pyrolysis at 1200–1450 °C; [f] direct injection GC/IRMS, see text for details; [g] ICP = inductively coupled plasma; [h] also denoted as GC/C/CRDS owing to the use of a combustion interface.

achieved through the online coupling of comprehensive two-dimensional gas chromatography (GC×GC) to isotope ratio mass spectrometry. This approach, which is in an early stage of development, follows the general setup of GC/IRMS systems (Table 2) but requires complex instrumental modifications such as microreactors for analyte conversion and accelerated IRMS signal processing [34–36].

### *2.1.3. Instrumental approaches to expand the capabilities of mass spectrometry-based CSIA*

The instrumental approaches chosen for satisfying the need of analyzing isotope ratios in small isotopologue molecules apparently precludes a more comprehensive application of CSIA. Therefore, more recent developments address the analysis of non-volatile organic compounds and new approaches are being proposed to quantify sulfur and halogen isotopes in organic contaminants.

*Liquid-chromatography coupled to IRMS.* To date, liquid-chromatography (LC) is coupled to isotope ratio mass spectrometers exclusively for compound-specific analysis of $^{13}C/^{12}C$-ratios [37]. In commercialized LC/IRMS-interfaces, a wet oxidation of organic compounds to $CO_2$ is carried out in a heated reactor by peroxodisulfate followed by a quantitative, membrane-based extraction of $CO_2$ under acidic conditions into a counter flow of helium [38]. This approach has enabled CSIA of many additional compound classes, despite constraints regarding mobile phase composition (buffers, organic modifiers), which compromise the use of reverse-phase LC. Alternative strategies for chromatographic separation include temperature-programmed LC, as used in gas chromatography, coupled to wet-oxidation IRMS (e.g., for organic acids [39]). In a less widespread approach, CSIA of liquid sample can be carried out with moving-wire devices after preparative separation of analytes [40].

*Chlorine isotope analysis.* Even though polychlorinated organic compounds belong to the most widespread anthropogenic contaminants [13], compound-specific methods for the analysis of chlorine isotopes are not fully established because the typical analytes for mass spectrometric analysis, that is, $CH_3Cl$, CsCl, and AgCl [41–43], cannot be prepared in a continuous flow mode. Three complementary approaches are currently pursued to overcome this issue (Table 2).

(1) The direct-injection GC/IRMS approach [44] has been developed for $^{37}Cl/^{35}Cl$-ratio measurements in tetrachloroethene (PCE), trichloroethene (TCE), and dichloroethene isomers (DCE). This approach is, in principle, similar to GC/IRMS described above except for the absence of a chemical conversion interface (Table 2). Instead of converting organic compounds to a few, small isotopologues, fragment ions of PCE, TCE, and DCEs are generated

in the ion source and only a subset thereof, typically two isotopologues per compound, is quantified in a specific Faraday cup alignment of the IRMS. In currently available instruments, the manual detector adjustment for the expected fragment ions requires dedicated instrumentation for a relatively small number of organic contaminants. While limits of quantification and analytical precisions of this instrumental setup are very promising and the approach allows for efficient handling of environmental samples, direct injection GC/IRMS also requires the separate preparation of reference gases of known isotopic composition.

(2) Connecting gas chromatography to multicollector inductively coupled plasma mass spectrometry (GC/MC-ICPMS, Table 2) provides another, more versatile avenue to measuring Cl isotope ratios even though it has been shown primarily for tetra- and trichloroethene [45]. In this setup, the ICP functions as conversion and ionization unit to ionize and filter off the carbon skeleton of organic compounds and ionize Cl isotopes for detection in multiple collectors thus simplifying sample preparation procedures. The high mass resolution of the MC-ICPMS is essential to separate Cl isotopologue signals from interferences of the Ar plasma. However, Cl ionization potentials are higher than those of the heavy elements, whose isotope ratios are typically analyzed by MC-ICPMS [46–48] and Cl ionization yields are therefore low. The ensuing low degree of ionization reduces the signal intensity of Cl isotopes and can make the operation of GC/MC-ICPMS more challenging to obtain accurate and precise results. Together with the need for independently calibrated standard materials and the high costs of instrumentation, these obstacles currently limit a more widespread application of GC/MC-ICPMS for polychlorinated organic contaminants.

(3) Even though the precision, with which benchtop quadrupole mass spectrometers (qMS) can measure chlorine isotopologues, is inferior to that of the multi-collector devices, the GC/qMS setup likely has the greatest potential to propel chlorine isotope analysis. This approach is favored by the large relative abundance of heavy halogen isotopes (Table 1), which enables quantification of both isotopes at lower concentrations without specialized mass spectrometers. Isotope ratios are obtained from the abundance of Cl isotopologues measured in the molecular ion and in (dechlorination) fragment ions after electron ionization [49, 50]. However, to obtain accurate and precise $\delta^{37}$Cl-values, a series of procedural measures have been proposed. Currently, they include an extensive bracketing of samples with standards containing the target analyte of known $^{37}Cl/^{35}Cl$ ratios in identical concentration as well as optimization of peak integration parameters [49]. Thus, while the GC/qMS approach can, in principle, be implemented with standard analytical equipment, it still requires rather large amounts of standard materials that need to be analyzed by conventional

isotope ratio mass spectrometers.

*Bromine isotope analysis.* Both of obstacles and solutions for compound-specific bromine isotope analysis in organic compounds resemble those of chlorine isotopes. To date, direct injection GC/IRMS [51] as well as GC/MC-ICPMS [52, 53] approaches have been reported, for example for polybrominated contaminants that are of similar persistence than their chlorinated analogs.

*Compound-specific sulfur isotope analysis.* Despite the abundance of sulphur atoms in many reactive functional groups of micropollutants, $^{34}S/^{32}S$-ratios have not yet been analyzed in individual contaminants. The only known approach takes advantage of the GC/MC-ICPMS setup [54, 55]. In contrast to MC-ICPMS-analysis of Cl and Br, accurate and precise results can be obtained with lower extraction voltages and medium mass resolution.

### *2.2. Spectroscopic approaches*

Cavity ring-down spectrometry (CRDS) offers an alternative to mass spectrometric detection of isotopologues. CRDS is carried out by manipulating a laser beam that is used to detect the highly characteristic rotational-vibrational transitions of different isotopologues in the mid and near infrared spectrum [56, 57]. The sensitivity of this technique is based on absorption path length of several kilometers achieved via high reflectivity mirrors that keep the laser beam inside a cavity for a large number of reflections. The exponential decay of laser beam energy after discontinued light input with and without gaseous samples in the cavity is referred to as the "ring down" rate and provides information for quantifying isotopologues. This emerging technique currently enables the isotopic analysis of different gaseous molecules for various isotopes such as water ($^{2}H/^{1}H$, $^{18}O/^{16}O$), $CO_2$ and $CH_4$ ($^{13}C/^{12}C$), or $N_2O$ ($^{15}N/^{14}N$, $^{18}O/^{16}O$). CRDS systems are used at predefined wavelengths and cannot be modified for analysis of other gases than the preselected ones by standard users. This loss of versatility compared to (isotope ratio) mass spectrometers, however, is compensated for by significantly lower costs, especially for maintenance and operation, and the less bulky instrumentation.

Compound-specific analysis of isotope ratios by CRDS is in the early development stage. On the one hand, isotopologue detection by laser spectroscopy is inherently compound-specific. Analytes of interest do not necessarily need to be isolated if their relative abundance in a sample is sufficiently large and the molecule interest small (e.g., $^{13}C/^{12}C$-analysis of $CH_4$ in biogas samples [58]). This situation, however, does not apply for the most environmental micropollutants that are typically investigated by CSIA. On the other hand, using a

GC with a combustion interface identical to the GC/IRMS setup (Table 2), $^{13}C/^{12}C$-ratios can be measured in $CO_2$ after combustion of hydrocarbons [59]. However, accuracy and precision of CRDS in the continuous flow mode do not yet match GC/IRMS and detection limits are orders of magnitude higher. Nevertheless, depending on the evolution of CRDS instrumentation towards increased sensitivity, analyses of transient signals, and detectors for alternative sample gases (e.g., CO), one can envision new compound-specific setups for organic compound conversion and detection by laser spectroscopy.

## 3. New perspectives for assessing transformation processes of organic pollutants

### *3.1. Chlorine isotope analysis of polyhalogenated organic contaminants*

Polychlorinated and -brominated hydrocarbons represent one class of traditional and very widespread soil and water contaminants, which, owing to their persistence and toxicity, pose a significant risk for human and environmental health [13]. Even if (bio)transformation of such compounds happens, it usually occurs over time scales of decades (and more) and often only in the absence of oxygen. Quantifying such processes on the basis of contaminant concentration measurements is very challenging and costly. To this end, alternative approaches, such as the analysis of stable isotope fractionation, need to be pursued. Reductive dechlorination of solvent spills consisting, for example, of polychlorinated aliphatic and olefinic hydrocarbons in the subsurface is one of the most frequent applications of CSIA. Fractionation of C isotopes in chloroethenes and -ethanes not only enabled one to distinguish contaminant sources and to identify transformation pathways (e.g., [60–63]), but also to quantify biodegradation half-lives in the order of decades [7]. These interpretations rely on the accuracy of $^{13}C$ enrichment factors, $\epsilon_C$, and thus ultimately on the understanding of $^{13}C$-kinetic isotope effects at the chemical bond being broken during biodegradation. However, the intrinsic KIEs of many (bio)degradation reactions are often not known (see box 2) and, due to the kinetic complexity of enzymatic processes [64], observable isotope fraction can be modulated by other rate-limiting processes (e.g., dissolution of non-aqueous phases, bioavailability etc. [65–67]).

Two- or multidimensional analysis of isotope ratios allows one to deal with the kinetic effects that modulate the observable isotope fractionation. As illustrated for the four potentially competing degradation pathways of 1,1,2,2-tetrachloroethane in Figure 2, different C and Cl atoms participate in these reactions. All reactions are initiated by a cleavage of at least one C–Cl bond and thus some of the $^{13}C$- and $^{37}Cl$-AKIEs overlap [5]. However, the number of reactive C and Cl atoms is distinctly different in each transformation mech-

anism. This should be reflected in the bonding changes at C and Cl atoms in the reactant and thus in the evolution $\delta^{13}$C vs. $\delta^{37}$Cl during (bio)transformation. Therefore, trends in multi-dimensional isotope fractionation analysis will be invaluable to disentangle these processes.

For the comparison of $\delta^{13}$C-trends of polychlorinated compounds with those of $\delta^{37}$Cl or $\delta^{2}$H, the corresponding analytical procedures still need to be established. Unfortunately, H isotope ratio measurements by GC/IRMS are challenging and hardly reported because of the interferences of Cl chemistry during pyrolytic $H_2$ generation. In contrast, analytical approaches for CSIA of Cl isotopes are emerging (see previous discussion and Table 2) and strategies for interpreting competitive isotope effects among the various Cl isotopologues have proposed for adequate data evaluation [68, 69]. It is conceivable that, once fully established, the combined C and Cl isotope analysis of polychlorinated organic contaminants will contribute to a more reliable assessment of their transformation processes.

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**Box 2 – Interpreting isotope fractionation**

Changes of isotope ratios in organic compounds are interpreted in terms of bulk compound isotope enrichment factors, $\epsilon_E$, which quantify the extent of isotope fractionation per incremental amount of reacted substrate (eq. 2). The origins of isotope fractionation are bonding changes at element E during the rate-limiting step of the reaction. Largest isotope fractionation typically occurs during the cleavage or formation of one or several chemical bonds owing to primary *intrinsic* kinetic isotope effects ($KIE_Es$) significantly different from unity [4]. Such $KIE_E$s reflect the isotopic activations energies in the elementary rate constants, $k$, pertinent to the reaction of light and heavy isotopologues (eq. 3) in a given mechanism. Notice that secondary isotope effects at atoms that are not localized at the reactive sites are often neglected.

$$KIE_E = \frac{^{l}k}{^{h}k} \tag{3}$$

To interpret observable $\epsilon_E$-values in terms of the underlying reaction mechanisms, for example through a comparison of isotope fractionation among different compounds reacting along the same pathway, *apparent* kinetic isotope effect ($AKIE_E$) are used. The conversion of $\epsilon_E$ into $AKIE_E$ requires *a priori* knowledge (or assumptions) of the number of reactive atoms and reactive sites as well as effects of intramolecular isotopic competition (simplified as $\lambda$ in eq. 4, see Elsner et al. [2] for details).

$$\mathrm{AKIE}_E = \frac{1}{1 + \lambda \times \epsilon_E / 1000} \quad (4)$$

If the KIE is not masked by less or not isotope-sensitive reaction steps or other kinetic phenomena, its value is approached by the $\mathrm{AKIE}_E$. Obtaining evidence for intrinsic and apparent isotope effects, e.g., through comparisons of experimental and computational studies [70, 71], is essential to derive typical $\epsilon_E$-values for transformation assessment.

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### *3.2. Multi-element isotope analysis of aquatic micropollutants*

Transformation processes of many typical micropollutants such as agrochemicals, personal care products, pharmaceuticals have not yet been investigated by CSIA in the environment. Low pollutant concentrations ($\mu$g to ng $L^{-1}$) require extensive compound-specific pre-concentration of large sample volumes. However, many of these micropollutants are inherently more polar and less volatile than compounds investigated by CSIA so far (e.g., chlorohydrocarbons, fuel components). These properties complicate both enrichment and measurement by GC/IRMS, while LC/IRMS is restricted to analysis of $^{13}C/^{12}C$-ratios. In addition, isotope fractionation, especially of C and H, is likely diluted by the increasing number of atoms thus requiring CSIA to resolve even smaller changes in isotope ratios (corresponding to a few ‰ in $\delta^h$E-value) very precisely. Therefore, CSIA of such compounds should include the elements at the reactive functional groups such as N, O, and S because they are potentially subject to larger and less diluted isotope fractionation.

Laboratory and computational studies on the multidimensional isotope fractionation associated with enzymatic and photochemical reactions of the herbicides atrazine and isoproturon highlight the potential of CSIA to distinguish between competing transformation processes [6, 72–74]. Even though the magnitude of observable isotope fractionation is smaller for C, N, and H than for contaminants like BTEX or chloroethenes due to dilution by nonreactive atoms, multi-dimensional trends are fundamentally different for alternative degradation pathways [75, 76]. As shown in an illustrative example (Figure 3 and 4), enzymatic hydrolysis, direct photolysis, and photo-catalyzed oxidations of atrazine act on different structural entities of the molecule (e.g., *N*-alkyl side chains, triazine ring etc.) via mechanisms that give rise to distinct isotope enrichment factors ($\epsilon_E$, Figure 3). Consequently, C and N isotope fractionation pertinent to each pathway can evolve along very different trajectories ($\Delta\delta^{15}N/\Delta\delta^{13}C$; see colored areas in Figure 4) despite sometimes identical reaction products. While a radical reaction pathway of direct photolysis leads to a

depletion of heavy C and N isotopologues, oxidative processes develop in the opposite trend, and enzyme-catalyzed hydrolysis exhibits normal C and inverse N isotope fractionation (Figure 4, based on data in refs [6, 26, 72]). Note that this type of evaluation is possible even if the reaction mechanisms and KIEs of the reaction are not fully understood. Such trends in multi-element isotope fractionation thus allow one to obtain information on a specific transformation process from the exclusive analysis of the reactant. This example illustrates some important conclusions that could be achieved for CSIA of micropollutant samples in the field once isotope selective detectors have become more sensitive and enrichment procedures more efficient.

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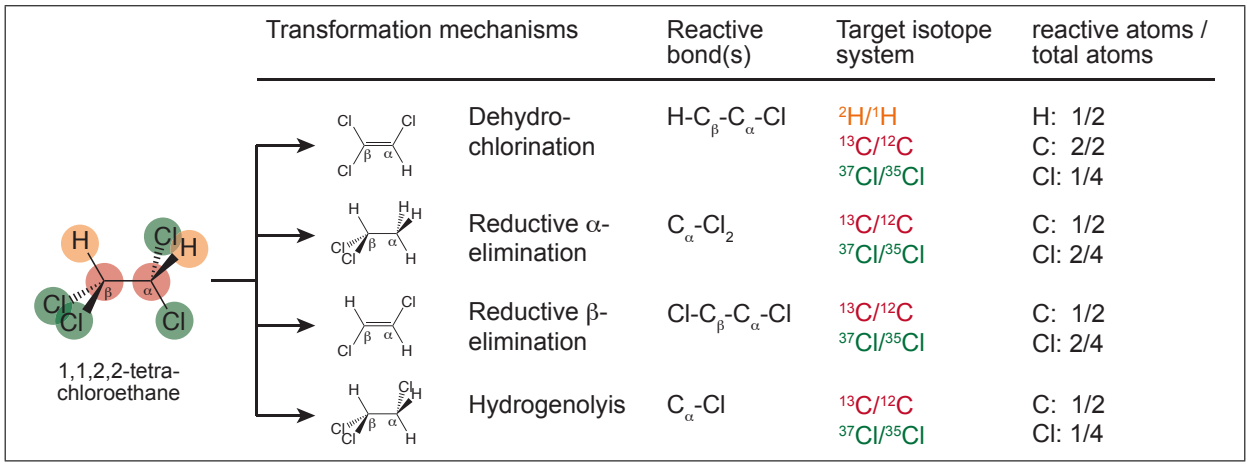


Figure 2: Illustrative example of environmental transformation pathways of chlorohydrocarbons: Dechlorination mechanisms of 1,1,2,2-tetrachloroethane proposed for abiotic and enzymatic reactions, stable isotope systems suited for CSIA, as well as reactive bonds and atoms involved in each transformation pathway

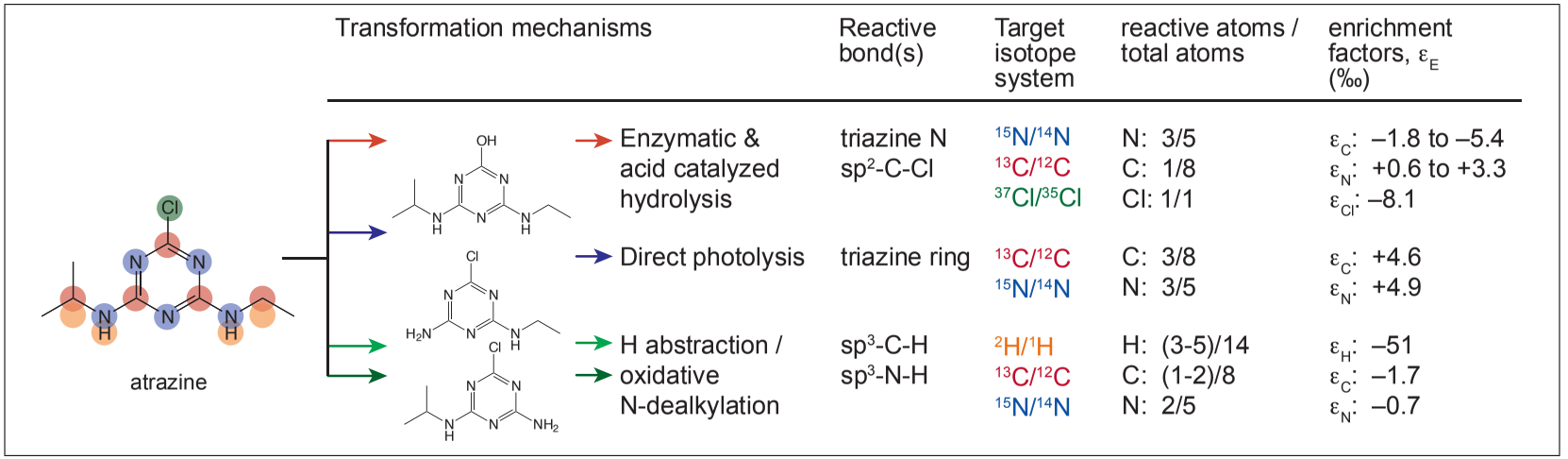


Figure 3: Atrazine transformation mechanisms, reactive bond(s), stable isotope systems suited for analysis of degradation processes, as well as the hypothesized number of reactive atoms in the molecule. The last column lists some typical bulk enrichment factors, $\epsilon_E$ (details regarding the range of values are discussed in refs [6, 26, 72, 73].)

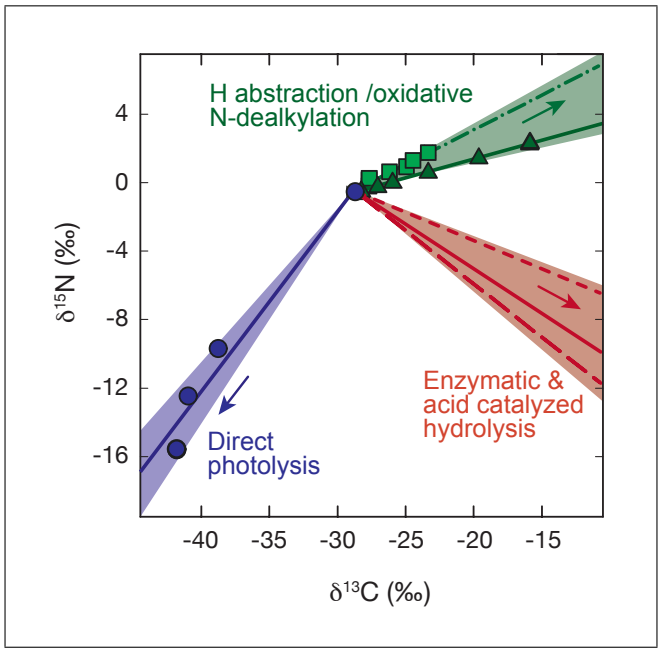


Figure 4: Two-dimensional isotope fractionation analysis, $\delta^{15}$N *vs.* $\delta^{13}$C, for different atrazine transformation mechanism. Lines, shaded areas and arrows illustrate the trends of isotope fractionation pertinent to each reaction pathway starting at the initial isotope signatures $\delta^{15}\mathrm{N} = -0.5‰$, $\delta^{13}\mathrm{C} = -28‰$. The slope of the lines ($\Delta\delta^{15}\mathrm{N}/\Delta\delta^{13}\mathrm{C}$) correspond approximately to the ratios $\epsilon_N/\epsilon_C$. Measured data from ref [6], $\epsilon_N/\epsilon_C$ from refs [6, 26, 72].