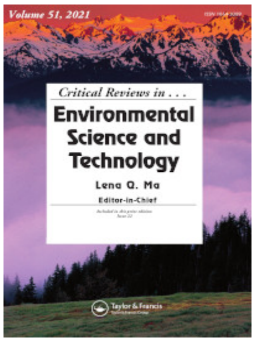

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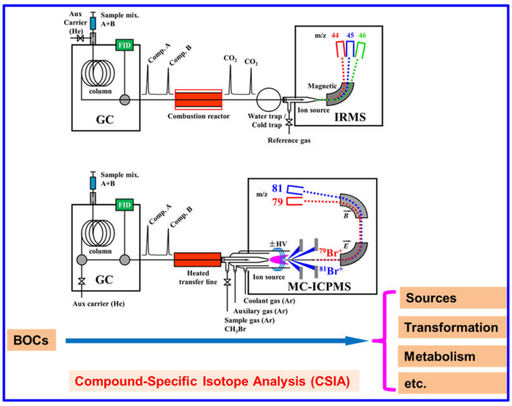
INVITED REVIEW

New advance in the application of compound-specific isotope analysis (CSIA) in identifying sources, transformation mechanisms and metabolism of brominated organic compounds

Jukun Xiong[a,b], Guiying Li[a,b], and Taicheng An[a,b]

[a]Guangdong Hong Kong-Macao Joint Laboratory for Contaminants Exposure and Health, Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Institute of Environmental Health and Pollution control, Guangdong University of Technology, Guangzhou, China; [b]Key Laboratory of City Cluster Environmental Safety and Green development (Department of Education, China), Guangzhou Key Laboratory of Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou, China

ABSTRACT



Brominated organic compounds (BOCs) originate from nature and anthropogenic activities, and are plentiful in the environment. Most BOCs are hazardous and pose significant risk to the environment and human health. Controlling these pollutants requires understanding their sources, behaviors, and final environmental fates. Compound-specific isotope analysis (CSIA), which has been a new important tool for scientific breakthrough in many research areas, such as environmental, ecological, and geochemical sciences, can help scientists elucidate the sources, transformation pathways and fates of BOCs. The appearance of gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) and gas chromatography multiple collector inductively coupled plasma mass spectrometry (GC/MC/ICPMS) in the past decades, has greatly developed the application of CSIA for the stable isotope analysis of BOCs. This review first describes the current analytical methods for measuring the carbon, hydrogen, oxygen and bromine stable isotopes of BOCs using mass spectrometry. Then, the review discusses the application for identifying sources, tracing transformation pathways and biological metabolisms. This includes a discussion of the kinetic isotope effects (KIEs), evaluated using (bio)chemical reaction, based on the reaction mechanism. Finally, the review highlights the future challenges and prospects regarding bromine isotope effects, multiple isotope enrichment, and position-specific isotope analysis. This review ends with a perspective on future activities that may benefit the development of the fast-growing field of CSIA.

KEYWORDS Brominated Organic Compounds (BOCs); Compound-Specific Isotope Analysis (CSIA); Transformation Mechanism

HANDLING EDITORS Albert Juhasz and Lena Ma

CONTACT Taicheng An ✉ antc99@gdut.edu.cn Guangdong Hong Kong-Macao Joint Laboratory for Contaminants Exposure and Health, Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Institute of Environmental Health and Pollution control, Guangdong University of Technology, Guangzhou 510006, China.

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1. Introduction

Brominated organic compounds (BOCs) are ubiquitous organic contaminants in different environmental matrices, and are therefore of wide interest in environmental science, atmospheric chemistry, marine chemistry, and geochemistry. BOCs have both natural and anthropogenic sources in the environment (Carrizo et al., 2011). More than 1600 kinds of BOCs are naturally produced, and may be generated through abiotic, photochemical, Fe-mediated, and enzymatic reactions (Carrizo et al., 2011; Gribble, 1999). For example, brominated phenols are excreted by marine organisms such as algae, polychaetes, and hemichordates (Teuten et al., 2006). Anthropogenic BOCs can be generated in large amounts and are broadly used as flame retardants, pesticides, germicides, disinfectants, gasoline additives and solvents (Alaee et al., 2003). Industrial BOCs are mostly produced by bromination of hydrocarbon precursors using bromine gas and by addition reactions of already brominated precursors. BOCs are usually discharged into environment in large amounts as agricultural, industrial and urban contaminants (Jones & de Voogt, 1999), and undergo various chemical and biochemical transformations through different mechanistic pathways in the environment. In these pathways, cleavage of the carbon-bromine specific bond is a significant step leading to the formation of debrominated product. High levels of BOCs are detected in blood and tissue of wildlife and human beings (Currier et al., 2020; Lima et al., 2005; Lin et al., 2020; Loseth et al., 2019). Unfortunately, many BOCs in the environment exhibit the characteristics of persistence, bioaccumulation, long-range transport, toxicity, and biomagnification through food webs (Currier et al., 2020; de Wit et al., 2006; Dietz et al., 2013; Leat et al., 2019; Lippold et al., 2019; Thanh et al., 2016). As such, many studies have focused on the sources, transports, transformations, ecotoxicology, and final fates of BOCs. Among these studies, compound-specific isotope analysis (CSIA), used to identify sources, clarify the transformation mechanisms, and trace the metabolisms of BOCs, has attracted widespread attention.

CSIA enables the molecular stable isotope of organic compounds to be analyzed to trace their origins and fates in various environmental matrices. Stable isotopes are naturally abundant; however, the degree to which these isotopes change in natural abundance is slight, usually in the level of one part in 10^4 for the major isotopes (Preston, 1992). The ability to precisely analyze the stable isotopes of different organic elements could provide significant information for researchers. However, the application of CSIA has been significantly limited due to poor precision or low sensitivity of conventional stable isotope ratio analysis (SIRA). Because the conventional analytical instruments require either large expensive doses of highly enriched labeled compounds or large samples, these limitations originate from the nature of sample throughput, sample sizes, and the difficulties of preparing gas samples manually with analytical instruments (Preston & Owens, 1983, 1985).

CSIA was mainly first used for elements carbon, nitrogen, and oxygen in geochemistry laboratories more than 70 years ago (Nier, 1947). A gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) was first commercialized in the past 40 years. It was introduced specifically to enable high precision and accuracy analysis of carbon, hydrogen, oxygen, nitrogen, and sulfur stable isotopes in the natural environment. The approach has small sample size requirement and high sample throughput, allowing this form of stable isotope analysis to be increasingly applied in geoscience and biogeoscience fields, including geology, ecology, oceanography, and environmental science (Ahad et al., 2021; Lopes et al., 2021; Sun et al., 2021; Zakon et al., 2021).

Generally, the isotopic compositions of organics vary due to the mass-dependent isotopic fractionation effects: light isotopes react faster compared to heavy isotopes (Kozell et al., 2015; Kuntze et al., 2016; Xiong et al., 2015). These isotopic fractionation effects may be expressed during sample production, postproduction, storage, and handling. Moreover, isotopic compositions are generally conserved during non-reaction processes. Therefore, CSIA can be applied to identify different sources of organic compounds (Ahad et al., 2021; Beneteau et al., 1999; Chen et al., 2017; Drenzek et al., 2002; Jendrzejewski et al., 2001).

In addition, CSIA can be applied to characterize transformation pathway and describe transformation mechanism of various organics (Horst et al., 2019; Hunkeler & Aravena, 2000; Keppler et al., 2000; Kuntze et al., 2016). (Bio)chemical reaction can change the relative abundance of stable isotopes within molecules. Heavier isotopes form stronger chemical bonds and are therefore less reactive than lighter ones, resulting in an enrichment of heavier isotopes in parent compounds and lighter isotopes in their products (Horst et al., 2019; Melander & Saunders, 1980). This change in stable isotopes of an element is called isotopic fractionation. Isotopic fractionation mainly originates from bonding changes, especially bond cleavage in the rate-limiting step. This fractionation leads to kinetic isotope effects (KIEs) for these reacting atoms. Their KIEs values provide important information for the (bio)chemical reaction. For example, isotopic fractionation measured in individual compounds during (bio)chemical reaction process can often be rationalized according to bonding change along with (bio)chemical reaction pathway. As such, they serve as a valuable proxy for characterizing reaction types and describing mechanistic pathway of reaction (Elsner et al., 2005; 2012; Kuntze et al., 2016; Ratti et al., 2015; Xiong et al., 2015; 2020). Previous studies have also shown the broad applications of CSIA in characterizing transformation reaction and describing transformation mechanism of organic compounds (Balaban et al., 2016; Elsner et al., 2005; Fischer et al., 2016; Kuntze et al., 2016; Nijenhuis & Richnow, 2016; Wang et al., 2015; Xiong et al., 2020).

Moreover, multielemental CSIA, which analyzes two or more elements, has emerged as a new powerful approach for investigating conversion pathway of (bio)chemical reaction. The isotope ratios of several elements are correlated to one another in (bio)chemical reaction. Therefore, isotope ratios can be used to reveal pathway-specific information. The specific correlation factor and corresponding bond cleavage for a given conversion pathway can be predicted when the isotopic fractionation changes of two elements are correlated (Zwank et al., 2005). Several studies have been conducted to show how multielemental CSIA can be applied to explain mechanistic pathways. For example, δ^{13}C-δ^{2}H analysis was used to study biotransformation of methyl tert-butyl ether (MTBE) (Kuder et al., 2005), and δ^{13}C-δ^{15}N analysis was used to describe mechanisms of atrazine degradation (Meyer & Elsner, 2013). Recently, a two-dimensional δ^{13}C-δ^{81}Br analysis was developed to evaluate transformation mechanisms of BOCs (Balaban et al., 2016; Kuntze et al., 2016; Woods et al., 2018; Zakon et al., 2013). Compared with one-element CSIA, the multielement CSIA generated more data related to isotopic fractionation during the degradation and transformation of organics, and better and more accurately revealed their mechanistic pathways.

Furthermore, the analysis of stable isotopes has been proposed as a powerful approach to investigate bioaccumulation and biotransformation of organic contaminants in biota, because the analysis can identify potential routes of organic contaminant movement in biota and environment (Luo et al., 2013). For example, in common carp (*Cyprinus carpio*), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) has been shown to mainly transform to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) (Stapleton et al., 2004), while debrominating to 2,2′,4,5′-tetrabromodiphenyl ether (BDE-49) in common sole (*Solea solea L.*) (Munschy et al., 2011). Further, field studies found a difference in the polybrominated diphenyl ether (PBDE) congener profiles in the same species (Zhang et al., 2011). It is hard to differentiate PBDE congeners that were accumulated through diet from congeners that represents the reductive debromination products of higher brominated congeners (Xiong et al., 2016; Yu et al., 2019). Stable carbon isotope signatures of PBDEs have been used to explore species-specific debromination and trophic dynamic process (Luo et al., 2013), and the stable carbon isotope ratios of individual congeners of PBDE have been used to study in-situ PBDE biodegradation in sediment cores (Huang et al., 2019). These examples show that CSIA has become an increasingly significant approach for tracing the source and metabolism of organics in biota (Luo et al., 2013; Zeng et al., 2013).

Table 1. The relative natural abundance, analyzed gas and the international standard of element.

Element	Natural abundance [%]	Analyzed gas	standard
H/D	99.9844/0.0156	H_2	Vienna Standard Mean Ocean Water (V-SMOW)
$^{12}C/^{13}C$	98.8890/1.1110	CO_2	Peedee Belemnite (PDB)
$^{14}N/^{15}N$	99.6340/0.3660	N_2	Air
$^{16}O/^{18}O$	99.7628/0.2000	CO	Vienna Standard Mean Ocean Water (V-SMOW)
$^{79}Br/^{81}Br$	50.7/49.3	–	Strontium (Sr) (Gelman & Halicz, 2010)

Note: More information on international reference materials is provided by the International Atomic Energy Agency (http://www.iaea.org).

This paper provides a detailed review of CSIA, to explore the potential application of CSIA for investigating ecotoxicology and health effects of BOCs, by identifying environmental sources, characterizing and describing transformation mechanisms, and distinguishing potential routes of movement.

2. Stable isotope analysis (analytical method)

Carbon, nitrogen, hydrogen, oxygen, and bromine are the main elements of BOCs. These elements have two or more stable isotopes that can be differentiated using mass spectrometry (Table 1). To eliminate systematic isotopic bias and accurately calibrate isotopic results, the ratio of stable isotopes has been standardized to international reference standards issued by the International Atomic Energy Agency (IAEA, Vienna, Austria). The ratio of the stable isotope is expressed in delta (δ) notation (Eq. 1) (McKinney et al., 1950):

$$\delta^{\mathrm{h}}E_{sample}(\text{inpermil}) = \left(\frac{R\left({}^{\mathrm{h}}E/{}^{\mathrm{l}}E\right)_{sample}}{R\left({}^{\mathrm{h}}E/{}^{\mathrm{l}}E\right)_{std}} - 1\right) \times 1000 \quad (1)$$

where, $\delta^{h}E_{\mathrm{sample}}$ represents isotope ratio of sample relative to the standard of element (E). $R(^{h}E/^{l}E)_{\mathrm{std}}$ and $R(^{h}E/^{l}E)_{\mathrm{sample}}$ represent the fractions of heavy (^{h}E) to light (^{l}E) isotopes in the standard and sample, respectively. The 1 represents subtracted from $R(^{h}E/^{l}E)_{\mathrm{sample/}}R(^{h}E/^{l}E)_{\mathrm{standard}}$ fraction; the result is that samples with a positive $\delta^{h}E_{\mathrm{sample}}$ value (per mil) are enriched in heavy isotopes (^{h}E) relative to the reference standard. In contrast, a negative $\delta^{h}E_{\mathrm{sample}}$ value (per mil) reflects a depletion of heavy isotopes (^{h}E) in the sample compared to the standard data. Usually, isotopic changes are in the per mil range; as such, they are multiplied by a factor of 1000 for the final result. The data of measured isotopic composition must be calibrated with the international reference scale for the accurate interlaboratory comparison of results. These standard calibration approaches include: single-point anchoring, linear shift between the measured and the true isotopic composition of two certified reference standards, two-point and multipoint linear normalization methods, referring to (Paul et al., 2007).

2.1. Carbon

Approximately 98.8890% of all carbon on earth is composed of ^{12}C stable isotope; approximately 1.1110% is the ^{13}C stable isotope (Table 1). Stable $^{13}C/^{12}C$ isotope ratios can be determined using CO_2 gas during mass spectrometry, with masses of 44 for $^{12}CO_2$ and 45 for $^{13}CO_2$. The isotope ratios are reported using δ notation relative to an international reference standard, which is PeeDee Belemnite (PDB), a belemnite fossil from the cretaceous Peedee formation in South Carolina, US (Barrie et al., 1984).

The ^{13}C stable isotope ($\delta^{13}C$) analysis method has been well developed for various kinds of organics using isotope ratio mass spectrometry (IRMS) (Abe et al., 2009; Horst et al., 2019; Kuder et al., 2012; Vetter et al., 2008). In this method, a high-resolution capillary column is usually equipped in GC before an online combustion device, connected for online gas purification and IRMS analysis. Organics are first separated using GC column. The effluent from GC separation is then oxidized with catalyst to CO_2, N_2 and H_2O in the vacuum capillary combustion furnace; while H_2O was removed by passing the effluent from the combustion furnace through a water-permeable Nafion tube and the remaining gases moved to a vacuum sample tube for IRMS analysis (Xiong et al., 2015). GC separation makes it possible for instrument to separate organics from complex mixtures, and to evaluate their individual stable isotope ratios at natural abundances. The combustion interface is often composed of oxidized coppers and/or nickel metal wires threaded through a slim ceramic reactor tube. The ion source and fixed detector cups of IRMS cannot directly use organic molecules; instead, they depend on gas (CO_2) originated from organic molecule combustion for $^{13}C/^{12}C$ analysis. This method has a reproducibility of $0.2‰ - 0.5‰$ and provides outstanding precision ($\leq 0.2‰$) (Barrie et al., 1984; Meckenstock et al., 2004; Preston & Slater, 1994).

The stable ^{13}C analysis method for BOCs has been well developed for investigating substances e.g. PBDE (Vetter et al., 2008), brominated phenols (Xiong et al., 2015; Zakon et al., 2013), tetrabromobisphenol A (TBBPA) (Wang et al., 2015; Xiong et al., 2015), ethylene dibromide (EDB) (Kuntze et al., 2016), and brominated ethenes (BE) (Woods et al., 2018). Previous studies over the past decades have documented the development of GC/C/IRMS for ^{13}C analysis of BOCs. However, the methods have varied slightly for different BOCs. For volatile or less polar BOCs, GC/C/IRMS can include GC separation to directly determine $\delta^{13}C$ values for individual compounds. For example, Vetter *et al.* used GC/C/IRMS to directly determine $\delta^{13}C$ values of individual congeners in two technical PBDE products (Vetter et al., 2008). However, less volatile or more polar BOCs, containing hydroxyl, carboxyl, or amino groups, are not directly amenable to GC separation. Therefore, derivatization is needed to reduce the polarity of BOCs. This approach achieves an improved thermal stability of BOCs and improves chromatographic separation. Further, Xiong *et al.* developed a novel methodology to analyze $\delta^{13}C$ values in phenolic brominated flame retardants during biodegradation; *N*,*O*-bis(trimethylsilyl)trifluoroacetamide served as a derivative to reduce polarity of brominated phenolic flame retardants (Xiong et al., 2015).

2.2. Hydrogen

Among all Earth elements, hydrogen has the most naturally occurring variations in the ratio of its stable isotope. This leads to the frequent scientific need to analyze ^2H in organic compounds. Generally, hydrogen from organic compounds is released during pyrolysis at high temperature, thus analyzing hydrogen requires a separate combustion tube filled with chromium oxide instead of copper oxide used for carbon and nitrogen analysis. Hydrogen isotope ratios are determined using H_2 gas, with masses of 3 for ^2H-^1H and 2 for ^1H-^1H. The isotope ratios are reported in δ^2H notation relative to Vienna Standard Mean Ocean Water (V-SMOW), which is an international reference standard. However, the measurement of masses 2 and 3 from H_2 in the presence of He carrier gas ($He/H_2 \sim 500$) and incomplete conversion of analytes, make the analysis of hydrogen isotope ratios less sensitive than that of carbon isotopes. The resulting reproducibility ranges only from $2.0‰ - 5.0‰$ (Hilkert et al., 1999). In 1998, researchers first used commercial GC/C/IRMS to analyze hydrogen isotope ratios (Burgoyne & Hayes, 1998; Hilkert et al., 1999). Since then, few studies are known to have analyzed hydrogen stable isotopes for BOCs. This may be because low natural abundance of ^2H (only 0.0156% of all hydrogen) makes it hard to produce a sufficiently strong ^2H-containing ion current in the IRMS (Burgoyne & Hayes, 1998; Elsner et al., 2012; Hilkert et al., 1999).

2.3. Oxygen

Oxygen has three stable isotopes ^{16}O (99.762%), ^{17}O (0.038%) and ^{18}O (0.200%). The analyses of oxygen stable isotope ($^{18}O/^{16}O$) rely on pyrolysis and reduction of organic compounds over carbon to quantitatively yield CO as the analyzed species. The first commercial GC/IRMS instrument for oxygen stable isotope analyses of organic compounds has been available in 1996 (Sessions, 2006). Between GC and IRMS, the instrument introduced a pyrolysis reactor for the compound-specific conversion of molecularly bound oxygen into CO analyte gas and direct $^{18}O/^{16}O$ determination via IRMS (Hitzfeld et al., 2017; Tuthorn et al., 2014). Usually, the pyrolysis reactor systems were constructed of alumina and lined with Pt or Ni tubes (Sessions, 2006). The $\delta^{18}O$ measurements were carried out on CO by monitoring the ion currents at masses of 28 for $C^{16}O$, 29 for $C^{17}O$ and 30 for $C^{18}O$. The isotope ratios are reported in $\delta^{18}O$ notation relative to V-SMOW, which is an international reference standard.

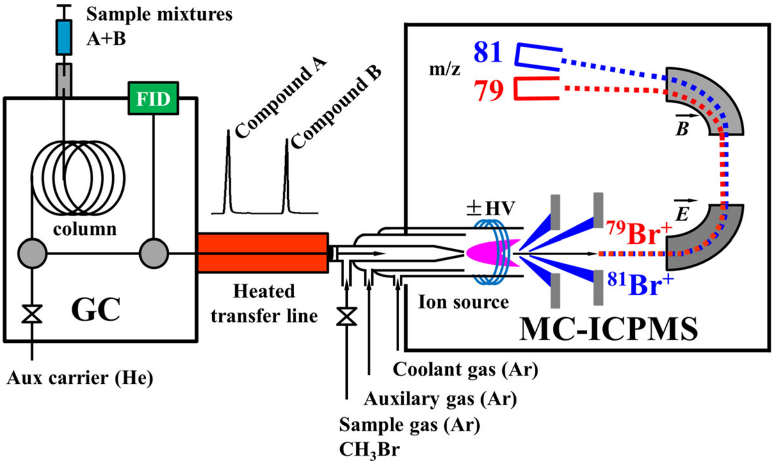
2.4. Bromine

Bromine has two stable isotopes, ^{79}Br and ^{81}Br, with natural abundances of approximately 50.7% and 49.3% (average $^{81}Br/^{79}Br = 0.97277$), respectively. Bromine isotope ratio in a sample is expressed in $\delta^{81}Br$ (in per mil) notation; the ratio $^{81}Br/^{79}Br$ in sample is relative to the corresponding ratio in the reference material (Eq. 1). Bromine isotopic composition in BOCs is a potentially effective indicator for the sources, degradation, transformation mechanism of BOCs (Xiong et al., 2020). There are relatively small differences in the relative mass of different bromine isotopes, because of their high mass. As such, they are expected to exhibit only slight isotope changes, highlighting the need for a more precise bromine stable isotope analysis of BOCs.

The bromine isotopes of organics have been analyzed in studies since 1978 (Willey & Taylor, 1978). In the study, dual inlet-isotope ratio mass spectrometry (DI/IRMS) to evaluate the bromine isotopic composition of methyl bromide was adapted; and this studies found that relative standard deviation of measured bromine isotope ratio for methyl bromide was 7×10^{-6} (Willey & Taylor, 1978). However, the analytical approach proposed was unable to analyze bromine isotopic composition of individual BOCs, because the method was unable to separate BOCs in complex mixtures.

Sylva *et al.* first introduced gas chromatography coupled to multiple collectors inductively coupled with plasma mass spectrometry (GC/MC/ICPMS) to analyze bromine isotopes in a mixture of three brominated benzenes (Sylva et al., 2007). Based on the on-line separation of three brominated benzenes by GC, followed by the introduction of individual brominated benzenes into MC/ICPMS, the analysis of bromine isotope was achieved using GC/MC/ICPMS system, without any preliminary chemical conversion of the sample. That study obtained a bromine isotope analysis precision of 0.3‰, when 0.3 nmol of bromine was injected for three brominated benzenes (Sylva et al., 2007). For sample containing bromine at a level more than 0.3 nmol, the precision of analysis was within 3 times of the shot-nose limits. During analysis, 1,4-dibromobenzene (DBB) has been generally used as a reference standard for $\delta^{81}Br$ ($\delta^{81}Br = 0$‰ for DBB was proposed in that study).

Later, Gelman and Halicz used strontium ($^{84}Sr/^{86}Sr$) as an external spike for instrumental mass bias correction to improve the precision of bromine isotope analysis using GC/MC/ICPMS (Gelman & Halicz, 2010). Using the proposed methodology, an analytical precision of approximately 0.1‰ (2SD) was achieved for a sample with less than 1 nmol of bromine. Holmstrand *et al.* utilized GC/MC/ICPMS to analyze bromine isotope ($\delta^{81}Br$) of brominated diaromatic compounds, by way of constructing heated transfer lines from GC to MC/ICPMS (Holmstrand et al., 2010). In that experiment, PBDEs in the technical flame-retardant mixture Bromkal 70-5DE, including mainly BDEs-47, −99 and −100, were used as test substances, using monobromobenzene (MBB) with a known $\delta^{81}Br$ of −0.39‰ as an internal standard. The analytical precision of $\delta^{81}Br$ for MBB and BDEs was found to be 0.4‰ (1SD, n = 18) and 1.4‰-1.8‰ (1SD, n = 10-12), respectively. The difference in the precision for MBB and BDEs may reflect different temperatures required to avoid condensation of the analytes in ICP torch assembly. Horst *et al.* also used GC/



Scheme 1. Instrumental setup of GC/MC/ICPMS system.

MC/ICPMS to determine ^{81}Br/^{79}Br isotope ratio in methyl bromide (Horst et al., 2011). Sample amounts larger than 40 ng were determined with a precision of 0.1‰ (1SD, n = 3). Using this analytical method, the measurement of bromine isotopic composition of atmospheric methyl bromide was achieved for the first time (Horst et al., 2013).

Due to significant improvements in the precision of the bromine isotope ratio analysis of BOCs by GC/MC/ICPMS, bromine stable isotope analysis using GC/MC/ICPMS has been successfully applied to analyze bromine isotopes in many BOCs, and the instrumental setup is given as Scheme 1. These include bromobenzene and bromophenol (Bernstein et al., 2013; Carrizo et al., 2011; Hitzfeld et al., 2011; Zakon et al., 2013), 3-bromo-2,2-bis(bromomethyl)propanol (TBNPA) (Balaban et al., 2016), ethylene dibromide (Kuntze et al., 2016), and brominated ethenes (Woods et al., 2018). The standard deviation is generally less than 0.2‰ (1SD).

3. Source identification

Certain anthropogenic BOCs have been shown to exhibit properties of environmental concern persistence and long-range transport. This highlights the importance of identifying their environmental sources and environmental dynamics. Information about the relative magnitude of natural and anthropogenic sources may be useful for reducing BOC sources. Understanding the isotopic compositions of BOCs may provide considerable information about sources and other fate processes of BOCs in the environment.

BOCs are commonly detected in different matrices, including environment and living organisms, and have both natural and anthropogenic sources. Natural BOCs can be produced through photochemical (Pelizzetti & Calza, 2002), abiotic, and enzymatic reactions (Theiler et al., 1978). The isotopic compositions of these natural BOCs formed *in-situ* are consistent in natural environments, and their temporal and spatial abundances correlate with the abundances of organisms that generate these metabolites (Fielman et al., 1999). Anthropogenic BOCs are generated through additional reactions of brominated precursors, and/or bromination reactions of hydrocarbon precursors, using bromine gas (Gelman & Dybala-Defratyka, 2020). The isotopic compositions of these anthropogenic BOCs change based on the characteristics of their precursor compounds and synthesis. During these processes, lighter isotopes react faster than heavier isotopes. This results in enriching lighter isotopes in products and heavier isotopes in reactants; these are called kinetic isotope effects (KIEs) (Melander & Saunders, 1980). Stable isotope ratios are generally conservative for non-reaction processes. Thus, BOCs provided by different sources may display various stable isotopic compositions because of differences in the isotopic compositions of their precursors and their different synthetic processes. Hence, isotopic compositions can be used to identify BOC sources (Horst et al., 2019; Huang et al., 2019; Xiong et al., 2020).

CSIA can provide vital information that clarifies the sources of BOCs in environment. Vetter *et al.* used a GC interfaced with a IRMS to analyze individual PBDE congeners in two technical PBDE products: DE-71 (pentabromo diphenyl ether mixture) and DE-79 (octabromo diphenyl ether mixture) (Vetter et al., 2008). In both products, a higher degree of bromination in products was associated with more negative δ^{13}C values. The δ^{13}C values of the three hexa-BDEs were more depleted in δ13C in more highly brominated technical octabromo diphenyl ether DE-79. This demonstrated that a carbon stable isotope analysis may support research about source and synthesis process associated with PBDE.

As another example, Holmstrand *et al.* used GC/ICPMS to determine δ^{81}Br values of BDE-47 and methoxy-BDE-47 in environmental samples (Holmstrand et al., 2010). In their study, samples were extracted from the blubber of a whale. The average difference in bromine isotopic composition (δ^{81}Br), using BDE-47 component as the isotope reference, was $-0.3‰ \pm 0.7‰$ (1SD, $n = 6$). The negligible difference between δ^{81}Br values of BDE-47 and methoxy-BDE-47 indicates that formation mechanisms of the two compounds imprint very similar bromine isotope features. Thus, either these BOCs have similar sources, or if those sources differ, the differences in isotope effects are smaller than that can be analytically resolved. Carrizo *et al.* used GC/MC/ICPMS to analyze the bromine stable isotope composition (δ^{81}Br) of six industrially synthesized BOCs and one natural BOC (Carrizo et al., 2011). The measured δ^{81}Br values for the six industrial BOCs ranged from $-4.3‰$ to $-0.4‰$. In contrast, average δ^{81}Br value of natural 2,4-dibromophenol was $0.2‰ \pm 1.6‰$ (1SD), and average value for industrial 2,4-dibromophenol was $-1.1‰ \pm 0.9‰$ (1SD). That is, δ^{81}Br values of four out of the six industrial BOCs significantly differed from the natural sample.

These results verified that the variability of bromine isotopes can be established among industrially produced BOCs when compared to a natural sample. Therefore, bromine stable isotope analysis can be used to distinguish the sources of BOCs. For example, bromine isotope analyses have also been applied to identify sources of BOCs in atmosphere. Horst *et al.* analyzed bromine isotope composition of atmospheric methyl bromide (CH_3Br) using GC/MC/ICPMS, using standard mean ocean bromide (SMOB) as a reference standard (Horst et al., 2013). This was the first study to determine δ^{81}Br values of atmospheric CH_3Br, and showed that δ^{81}Br values of different atmospheric source regions vary from $-0.47‰$ to $+1.75‰$ SMOB. By comparing relationship between δ^{81}Br value and concentration, the researchers described the mixing trend and isotopic fractionation processes that create a reservoir for atmospheric CH_3Br.

In addition to using one-element CSIA to trace BOC sources, multielement CSIA has been increasingly used for the same purpose. Chen *et al.* applied a two-dimensional bromine and carbon isotopic fractionation to identify PBDE congener sources (Chen et al., 2017). They measured δ^{13}C and δ^{81}Br values of two different PBDE congeners (BDE-47 and BDE-209) from different suppliers, and found that the δ^{13}C values for BDE-47 from three different suppliers were $-26.40 \pm 0.37‰$, $-26.41 \pm 0.21‰$, and not detected, respectively, the corresponding δ^{81}Br values were $-0.34 \pm 0.13‰$, $-0.33 \pm 0.16‰$, and $-0.26 \pm 0.09‰$; while the δ13C values for corresponding BDE-209 from three different suppliers were $-28.26 \pm 0.18‰$, $-25.81 \pm 0.24‰$, and $-27.17 \pm 0.48‰$, respectively, the δ^{81}Br values were $-0.50 \pm 0.24‰$, $-0.43 \pm 0.15‰$, and not detected. This indicates that individual PBDE congeners have distinct isotope characteristics, because of the different precursor compounds and production processes. This illustrates that determining isotopic compositions is a significant emerging method for identifying sources of PBDE congeners. The researchers completing that study noted that caution is needed to mitigate problems like indistinguishable differences of organic compounds from different sources in isotopic compositions.

4. Description of the transformation pathway

BOCs have different transformation processes; as such, their distribution in environment is influenced by (bio)chemical reaction and physical processes like dilution, adsorption, and mass-transfer. Therefore, their environmental fates are difficult to monitor and explain. Nevertheless, using CSIA to determine the isotopic fractionation of hydrogen, carbon, and bromine stable isotopes of BOCs may serve as a basis to describe their reaction pathways. According to an experimental comparison of the apparent kinetic isotope effect (AKIE) with the theoretical known kinetic isotope effect (KIE), the reaction pathways and products (intermediates) may be identified without analyzing and determining the products.

For example, element E (carbon, hydrogen, and other elements) related to a bond cleavage reaction generally exhibits a conventional isotope effect. In other words, molecules with a lighter isotope at specific cleavage location react faster compared to those with a heavier isotope. The different reaction speeds of heavier and lighter atom are quantified using (pseudo)-first order rate constants ${}^{h}k$ and ${}^{1}k$, respectively. Therefore, an intrinsic kinetic isotope effect $\mathrm{KIE_E}$ is expressed as (Elsner et al., 2005):

$$\mathrm{KIE_E} = \frac{{}^{1}k}{{}^{\mathrm{h}}k} \tag{2}$$

Usually, (bio)chemical reactions exhibit a normal isotope effect (KIEE > 1); however, when formation or strengthening of bonds is the rate-limiting step, an inverse isotope effect ($\mathrm{KIE_E} < 1$) may occur (Marlier, 2001; Paneth & O'Leary, 1991; Zakon et al., 2013). A heavy isotope may be located at positions that are not involved in a reaction; in contrast, a molecule may have two or more reacting positions, but only one of the positions reacts. As such, a simplified mathematical formula was developed to relate fractionation factor (α) to KIEs, where α is the reciprocal of KIE value, i.e., $\alpha = \mathrm{KIE_E}^{-1}$ (Elsner et al., 2005).

The experimentally determined isotope fractionation and isotope enrichment factor (ε) resulting from the prioritized cleavage of bonds involving lighter isotope is generated according to the Rayleigh equation (Meckenstock et al., 2004):

$$ln\frac{R_t}{R_0} = (\alpha - 1) \cdot lnf = \frac{\varepsilon_{bulk}}{1000} \cdot lnf \tag{3}$$

where, α represents fractionation factor; f represents the fraction of remaining compound; $\varepsilon_{\mathrm{bulk}}$ represents experimental bulk isotope enrichment factor ($\varepsilon_{\mathrm{bulk}} = 1000\cdot(\alpha\text{-}1)$ [‰]); R_0 and R_t represent the isotopic composition of compound at times t and 0, respectively.

The isotopes of interest in a compound may be in several positions, with only some participating in reaction. As a result, the changes in $\varepsilon_{\mathrm{bulk}}$ may be smaller because of "dilution" effect from non-reactive isotopes. Thus, a position-specific isotope enrichment factor is calculated by converting bulk isotope enrichment factor to a position-specific isotope enrichment factor (Elsner et al., 2005):

$$\varepsilon_{reactive\ \ position} = \frac{n}{x} \cdot \varepsilon_{bulk} \tag{4}$$

where, $\varepsilon_{\mathrm{reactive\ \ position}}$ represents the reactive position-specific isotope enrichment factor; n represents number of atoms in the molecule; x represents the number of atoms in a reactive position. Considering the uncertainty of $\varepsilon_{\mathrm{bulk}}$, a 95% confidence interval from isotope measurements at different time points is established. The mean of individual uncertainties is used for final calculation. AKIE is also estimated based on previous studies (Elsner et al., 2005; Vanstone et al., 2008):

$$\mathrm{AKIE_E} = \frac{1}{1 + z \cdot \frac{n}{x} \cdot \varepsilon_{bulk}/1000} \tag{5}$$

where, n represents the number of atoms in molecule; x represents the number of atoms located at the reactive site; z represents the number of chemically equivalent reactive positions that compete for reaction.

The transformation of the determined $\varepsilon_{\mathrm{bulk}}$ into a reactive position-specific AKIE provides two advantages when explaining the transformation pathways of BOCs in environment. One is that when the (bio)chemical reactions (e.g., reduction of a C-Br bond) are the same, reactive position-specific AKIE can be compared between different compounds or between structurally dissimilar compounds, regardless of molecular size. The other is that common trends with respect to isotope fractionation can be established and used to infer nonfractionating step, even when the reactive position-specific AKIE and theoretical KIE significantly differ. These advantages have been widely used to describe multiple BOC transformation pathways (Kuntze et al., 2016; Woods et al., 2018; Zakon et al., 2013).

Next, this review discusses the observed stable isotope KIEs for different reaction mechanisms, to describe the application of stable isotope analysis in environment. The discussion describes stable isotope effect related to transformation pathway of BOCs for the reactions of nucleophilic substitution (S_N2), radical oxidation, and reductive dissociation of C-Br bond.

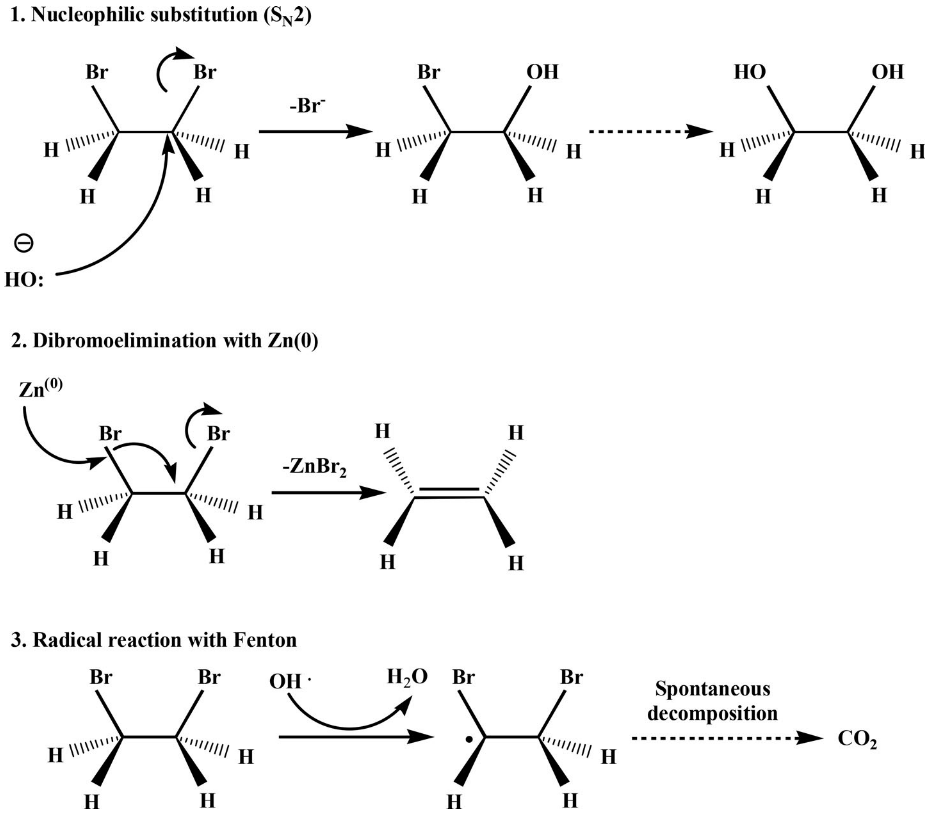
4.1. 1,2-Dibromoethane (termed as ethylene dibromide, EDB)

Ethylene dibromide (EDB) is a typical BOC, and has been widely used as an agriculture fumigant and lead scavenger in gasoline over the past few decades (Falta et al., 2005; Pignatello & Cohen, 1990). It is transformed through chemical and biological pathways, e.g. hydrolysis, dibromoelimination, oxidative transformation, and aerobic and anaerobic biotransformation (Scheme 2) (Kuntze et al., 2016). The carbon and bromine kinetic isotope effect reflect chemical bond cleavage in particular reaction type.

4.1.1. Nucleophilic substitution

Under alkaline hydrolysis, one study found that EDB transformation occurred through the nucleophilic substitution (S_N2) mechanism, resulting in ^{13}C-AKIE $= 1.0620 \pm 0.0052$ and ^{81}Br-AKIE $= 1.0020 \pm 0.0002$ (Kuntze et al., 2016). The resulting carbon isotope effect was consistent with the following values: ^{13}C-AKIE $= 1.0678 \pm 0.0070$ (S_N2 reaction of EDB with hydrogen sulfide) (Kuder et al., 2012) and ^{13}C-AKIE $= 1.0520 \pm 0.0080$ (S_N2 of tribromoneopentyl (TBNPA)) (Kozell et al., 2015) (Table S1). Further, the observed bromine isotope effect approximate to the values ^{81}Br-AKIE $= 1.0017 \pm 0.0000$ (S_N2 reaction of *n*-butyl bromide) (Willey & Taylor, 1980), ^{81}Br-AKIE $= 1.0012 \pm 0.0003$ (S_N2 reaction of TBNPA) (Kozell et al., 2015) and ^{81}Br-AKIE $= 1.0012 \pm 0.0004$ (S_N2 reaction of methyl bromide) (Horst et al., 2019). Therefore, by comparing the observed apparent bromine and carbon kinetic isotope effect during EDB transformation, with the known and determined carbon or bromine kinetic isotope effect, researchers inferred the reaction type of chemical bond cleavage of EDB and clarified the transformation pathway.

To study aerobic biotransformation of EDB by *Ancylobacter aquaticus*, a study assumed a S_N2 nucleophilic substitution pathway (Kuntze et al., 2016). Previous studies found that the chlorinated analogue 1,2-dichloroethane (1,2-DCA) was catalyzed by analogue dehalogenases and followed a S_N2 reaction route with the ^{13}C-AKIE $= 1.06 - 1.07$ and ^{37}Cl-AKIE $= 1.009$ (Hunkeler & Aravena, 2000; Lewandowicz et al., 2001; Palau et al., 2014a). The carbon isotope effect of 1,2-DCA was consistent with the value obtained for EDB reaction under alkaline conditions (^{13}C-AKIE $= 1.062 \pm 0.0052$ and ^{81}Br-AKIE $= 1.0020 \pm 0.0002$). Therefore, the reaction of 1,2-DCA catalyzed by analogue haloalkane dehalogenases followed a S_N2 mechanism. However, the



Scheme 2. Transformation pathways of BDE: 1. Nucleophilic substitution (SN2); 2. Dibromoelimination with Zn (0); 3. Radical reaction with Fenton (Kuntze et al., 2016).

observed carbon isotope effect (^{13}C-AKIE = 1.0140 ± 0.0008) and bromine isotope effect (^{81}Br-AKIE = 1.0012 ± 0.0002) for aerobic biodegradation of EDB significantly differed from the values of EDB generated in alkaline conditions (^{13}C-AKIE = 1.0620 ± 0.0052 and ^{81}Br-AKIE = 1.0020 ± 0.0002) (Kuntze et al., 2016); they also differed from the value of 1,2-DCA (^{13}C-AKIE = 1.06 – 1.07) obtained from reaction catalyzed by analogue haloalkane dehalogenases (Poma et al., 2014). This highlighted the presence of a different reaction mechanism. The equilibrium isotope effect led to a different isotope effect between the two analogues, because of the nature of substrate's binding to enzyme.

4.1.2. Dibromoelimination

With Zn (0)/corrinoids (*Norpseudovitamin* B$_{12}$ and *Cyanocobalamin*), the transformation of EDB involved in dibromoelimination results in both carbon and bromine isotope effects. Dibromoelimination of EDB, either by Zn (0) or corrinoids, leads to a lower carbon isotope fractionation value, as follows: ^{13}C-KIE (1.0223 ± 0.0022 – 1.0350 ± 0.0018) (stepwise, $z = 2$) and ^{13}C-KIE (1.0110 ± 0.0011 – 1.0172 ± 0.0009) (concerted, $z = 1$). However, it leads to higher values for ^{81}Br-KIE (1.0042 ± 0.0006 – 1.0079 ± 0.0008) (stepwise, $z = 2$) and ^{81}Br-KIE (1.0021 ± 0.0003 – 1.0039 ± 0.0004) (concerted, $z = 1$), compared to the isotope fractionation of EDB (^{13}C-AKIE = 1.062 ± 0.0052 and ^{81}Br-AKIE = 1.0020 ± 0.0002) under alkaline hydrolysis, respectively (Kuntze et al., 2016). These data were hard to interpret, so researchers recommended more experimental and theoretical work to explain the mechanism associated with this reaction.

For anaerobic biotic transformation of EDB with crude extract of *Sulfurospirillum multivorans* (*S. multivorans*), the carbon isotope enrichment factor was $\varepsilon_{\text{reactive position}} = -5.3 \pm 0.5‰$. Ethene was the sole detected intermediate. A previous study had hypothesized that an anaerobic enrichment culture of EDB with a $\varepsilon_{\text{reactive position}}$ of approximately $-5‰$ was likely a dibromoelimination reaction (Henderson et al., 2008). Furthermore, the bromine isotope effects of EDB with ^{81}Br-AKIE $= 1.0023 \pm 0.0002$ (concerted mechanism, $z = 1$) and 1.0046 ± 0.0004 (stepwise, $z = 2$) were consistent with the values from dibromoelimination with Zn (0) and corrinoids: ^{81}Br-AKIE $= 1.0021 \pm 0.0003 - 1.0079 \pm 0.0008$), respectively (Kuntze et al., 2016). Therefore, the reaction of EDB by *S. multivorans* was assumed as dibromoelimination reaction.

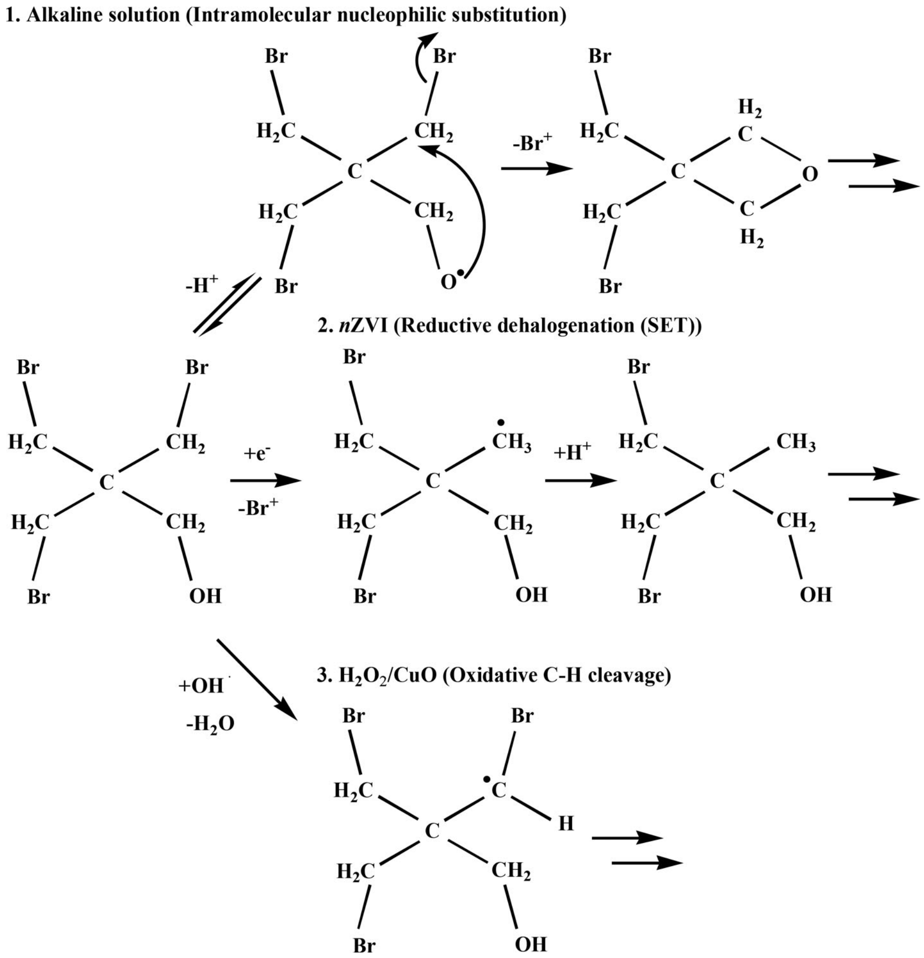
4.1.3. Fenton oxidation

A carbon isotope effect (^{13}C-AKIE $= 1.0086 \pm 0.0006$) was found during the oxidative transformation of EDB using Fenton reagent (Kuntze et al., 2016). This was within the scale of the typical ^{13}C-AKIE ($1.01 - 1.03$) characteristics for an oxidative C-H bond cleavage mechanism (Elsner et al., 2005). In this case, H abstraction from C-H bond limited transformation rate, and all C-H bonds equally competed for the transformation. The carbon isotope effect value of EDB using Fenton oxidation (^{13}C-AKIE $= 1.0086 \pm 0.0006$) was well aligned with the carbon isotope effect value of TBNPA for a similar mechanistic reaction (1.012 ± 0.0015) (Kozell et al., 2015). It was also well aligned with the value observed for oxidative transformation of 1,1,1-trichloroethane using heat activated persulfate (^{13}C-AKIE $= 1.008$) (Palau et al., 2014b). The oxidative C-H bond cleavage mechanism of these two similar compounds and their carbon isotope effects for corresponding mechanism were understood; as such, this supported the conclusion that oxidative C-H bond cleavage mechanism of EDB by Fenton reagent was generated by the carbon isotope effect.

4.2. Tribromoneopentyl alcohol (TBNPA)

The carbon and bromine isotope effects of TBNPA were analyzed during three different chemical reactions: reductive dehalogenation by Fe (0) nanoparticles in anoxic condition (*n*ZVI); a conversion reaction in alkaline solution (pH $= 8$); and oxidative degradation by H_2O_2 in the presence of CuO nanoparticles (*n*CuO) (Kozell et al., 2015). In alkaline solution (pH $= 8$), researchers found a carbon isotope effect ($\varepsilon_C = -10.4 \pm 1.6‰$) and a bromine isotope effect ($\varepsilon_C = -1.9 \pm 0.2‰$) (Table S1). To relate these obtained isotope enrichment factors to the specific transformation pathways, the carbon and bromine isotope enrichment factors were converted to AKIE, with values of ^{13}C-AKIE $= 1.052 \pm 0.008$ and ^{81}Br-AKIE $= 1.0012 \pm 0.0003$, respectively. These values aligned well with the values for S_N2 reaction of EDB (^{13}C-AKIE $= 1.062 \pm 0.0052$) (Kuntze et al., 2016) and *n*-butyl bromide (*n*-BuBr) (^{81}Br-AKIE $= 1.00169 \pm 0.00003$) (Table S1) (Willey & Taylor, 1980). Therefore, researchers hypothesized the presence of S_N2 reaction pathway, based on the bromine and carbon isotope effects for TBNPA transformation in alkaline solution (pH $= 8$). In alkaline solution, for TBNPA molecule, all C-Br bonds equally competed for the reaction, and the nucleophilic substitution of intramolecular C-Br bond cleavage was a rate-determining step (Scheme 3).

With *n*ZVI in anoxic conditions, researchers observed values of ^{13}C-AKIE $= 1.038 \pm 0.0035$ and ^{81}Br-AKIE $= 1.0057 \pm 0.0003$ for a TBNPA transformation (Kozell et al., 2015), indicating that the cleavage of C-Br bond was a rate-determining step. The ^{81}Br-AKIE value (1.0057 ± 0.0003) was greatly higher than the value (≈ 1.0008) measured for reductive debromination through stepwise reductive debromination of brominated phenols (Bernstein et al., 2013). The value was consistent with the semiclassical Streitwieser limit of maximum KIE $= 1.002$. Based on the AKIE values and the relatively high ^{81}Br-AKIEs, researchers hypothesized that pathway involved simultaneous reductive debromination at several C-Br positions (Scheme 3).



Scheme 3. Transformation pathways of TBNPA: 1. Alkaline solution (Intramolecular nucleophilic substitution); 2. nZVI (Reductive dehalogenation (SET)); 3. H2O2/CuO (Oxidative C-H cleavage) (Kozell et al., 2015).

With H_2O_2/nCuO process, TBNPA degradation led to a carbon isotope fractionation, where $\varepsilon_C = -2.4 \pm 0.3‰$ (^{13}C-AKIE $= 1.012 \pm 0.0015$); there was no statistically significant detection of bromine isotope fractionation (Kozell et al., 2015). This indicated that C-H bond cleavage was the rate-determining step. The ^{13}C-AKIE $= 1.012 \pm 0.0015$ value aligned well with the scale of the typical ^{13}C-AKIE $(1.01 - 1.03)$ for oxidative C-H bond cleavage pathway (Elsner et al., 2005). Thus, the C-H bond cleavage pathway of TBNPA during oxidative degradation by H_2O_2/nCuO was proposed (Scheme 3).

In summary, different isotope effects accompany different chemical processes of TBNPA, and specific transformation mechanism is clarified by information about the element's isotope effect values.

4.3. Tribromoethene (TBE)

Biotransformation of TBE to vinyl bromide (VB) and ethene enzyme-catalyzed by *S. multivorans* and *Desulfitobacterium hafniense* PCE-S (*D. hafniense* PCE-S) was studied and stable isotope analysis was utilized to monitor its fate (Woods et al., 2018). The ^{13}C-AKIE values for the debromination of trans1,2-dibromoethene (trans1,2-DBE) and cis-1,2-dibromoethene (cis-1,2-DBE) by *S.*

multivorans were 1.035 ± 0.0036 and 1.044 ± 0.0032, respectively, and of TBE, trans1,2-DBE and cis-1,2-DBE by *D. hafniense* PCE-S were 1.016 ± 0.0030, 1.019 ± 0.0024, and 1.030 ± 0.0007, respectively (Table S1). All these values were close (in the same order of magnitude) to the calculated KIE value (^{13}C-AKIE = 1.043) for a common C-Br bond cleavage (Zakon et al., 2013). However, ^{13}C-AKIE (1.002 ± 0.0004) value for the debromination of TBE by *S. multivorans* was one order of magnitude smaller than the calculated KIE value (^{13}C-AKIE = 1.043) for a common C-Br bond cleavage. That the hydrophobicity of these compounds may cause rate-limiting step at the active site of the enzyme, resulting in masking of intrinsic isotope effects. It is expected that this is an enzyme-specific effect, related to physico-chemical properties of the reductive dehalogenase. Thus, the results indicated that either there was a rate-limiting step before C-Br bond cleavage, which leads to covering intrinsic isotope effect, or the reaction pathways differed.

The bromine isotope fractionations were also determined during biotransformation of TBE, trans1,2-DBE, and cis-1,2-DBE by *S. multivorans* and *D. hafniense* PCE-S. The ^{81}Br-AKIE values for debromination of trans1,2-DBE, cis-1,2-DBE and TBE by *S. multivorans* were 1.001 ± 0.0003, 1.002 ± 0.0005, and 1.004 ± 0.0004, respectively; the values for cis-1,2-DBE, trans1,2-DBE, and TBE by *D. hafniense* PCE-S were 1.002 ± 0.0006, 1.003 ± 0.0006, and 1.003 ± 0.0011, respectively (Table S1). All measured ^{81}Br-AKIE values were close to the semiclassical Streitwieser limit of KIE = 1.002 (Hunkeler & Elsner, 2009). Of note, some ^{81}Br-AKIE values of trans1,2-DBE and TBE biotransformation by *S. multivorans* and *D. hafniense* PCE-S exceeded the semiclassical Streitwieser limit. However, all these ^{81}Br-AKIE values were within the same order of magnitude. This similarity indicated that biotransformation of TBE, trans1,2-DBE, and cis-1,2-DBE by *S. multivorans* and *D. hafniense* PCE-S have the same debromination reaction mechanism. Therefore, the reductive debromination reaction mechanism can be applied (Scheme S1).

4.4. Brominated phenols (BP)

The photodegradation of 2-bromophenol (2-BP), 3-bromophenol (3-BP), and 4-bromophenol (4-BP) was investigated in aqueous and ethanolic solution under UV light irradiation, allowing researchers to study their possible reaction pathways (Scheme S2-1) (Zakon et al., 2013). All photodegradation reactions involved the C-Br bond cleavage of these brominated phenols; however, their primary detected intermediates varied between research groups. Furthermore, Pinhey and Rigby reported that phenol was the primary intermediate in light-induced reaction of different halophenols in ethanol/isopropanol solution; they hypothesized that a radical reaction mechanism was caused by C-Br bond homolysis (Pinhey & Rigby, 1969). Lipczynska-Kochany reported that 3-BP reacted via ionic mechanism, accompanied by heterolysis of C-Br bond. In contrast, 4-BP photodegraded via radical pathway that included homolytic C-Br bond cleavage (Scheme S2-2) (Lipczynska-Kochany, 1992). Finally, Durand *et al.* proposed that 4-BP experienced photodegradation via ionic pathway that includes heterolytic C-Br bond cleavage (Durand et al., 1998).

Many researchers have studied the photodegradation intermediates and pathways of brominated phenols, however, details related to the photodegradation pathways remained unclear using traditional methods. As such, researchers used CSIA to determine the carbon and bromine isotope effects to better describe the pathway details. This stable isotope analysis has served as a powerful tool for identifying the photodegradation mechanism of brominated phenols.

For example, Zakon *et al.* investigated the carbon and bromine isotope effects of 4-BP, 3-BP, and 2-BP during UV-irradiation in water and ethanol (Zakon et al., 2013). Except for 2-BP in water, inverse high bromine isotope effects (^{81}Br-AKIE < 1) were observed for the other brominated phenols (Table S1). Three brominated phenols in ethanol, and 4-BP in water, became enriched with ^{13}C; however, the carbon isotope composition of 2-BP and 3-BP showed no fractionation in water. Therefore, according to the observed carbon and bromine isotope effect, a superposition of mass-independent magnetic isotope effect (MIE) and mass-dependent kinetic isotope effect (KIE) for phototransformation was proposed to illustrate bromine isotope effects (Eq. 6).

$$\mathrm{Br-AKIE = Br-KIE + Br-MIE} \tag{6}$$

This allowed for the development of a hypothesis related to photodegradation pathways of brominated phenols (Scheme S3). For three brominated phenols in ethanol and 4-BP in water, there was both a normal carbon isotope effect (^{13}C-AKIE > 1) and an anomalous inverse bromine isotope effect (^{81}Br-AKIE < 1) (Table S1). This indicated that photodegradation was related to an analogous radical mechanism induced by homolytic C-Br bond cleavage in the rate-determining step (Zakon et al., 2013). In this context of homolytic C-Br bond cleavage, the bromine isotope effect is a synergistic contribution of normal KIE caused by a bond cleavage between the lighter isotopes, and MIE resulting from different rates of intersystem-crossing between singlet-triplet states. For 2-BP in water, there were no carbon and bromine isotope effects (^{13}C-AKIE $=$ 1, ^{81}Br-AKIE $= 1$) (Zakon et al., 2013). This demonstrated that C-Br bond cleavage was not the rate-determining step, and therefore there was no KIE. The absence of MIE shows that photodegradation reaction of 2-BP in aqueous solution underwent an ionic mechanism. In contrast, for 3-BP in aqueous solution, an inverse bromine isotope effect (^{81}Br-AKIE < 1) and no carbon isotope effect (^{13}C-AKIE $= 1$) were obtained.

In these cases, the bromine isotope effect may result entirely from MIE related to triplet-singlet conversion. In contrast, for carbon isotope effect, it may be that C-Br bond cleavage has no rate-limiting step for conversion, and therefore does not have a significant mass-dependent KIE. This indicates that 3-BP photodegradation in water proceeds through an ionic pathway. The measured carbon and bromine isotope effects can effectively describe the photodegradation pathway details of these brominated phenols; however, Zakon *et al.* found that further experiments combined with theoretical calculations are important for verifying the correlation between the observed isotope fractionation and possible reaction mechanism (Zakon et al., 2013).

In addition, TBBPA was degraded under LED white light ($\lambda > 400\,\mathrm{nm}$) illumination and the isotope fractionation of carbon and bromine was used to describe the cleavage of C- Br and C-C bonds (Xiong et al., 2020). Furthermore, TBBPA photodegradation in water under simulated solar light illumination was investigated and the carbon isotope effects during photodegradation were measured (Wang et al., 2015). Inverse isotope effects were also observed for TBBPA photolysis with rose bengal as the $^{1}O_2$ photosensitizer (^{13}C-AKIE $= 0.9821 \pm 0.0009$) or without rose bengal in air (^{13}C-AKIE $= 0.9678 \pm 0.0030$) and N_2 atmosphere (^{13}C-AKIE $= 0.9525 \pm 0.0032$) (Table S1). Thus, a MIE-hydrolysis mechanism has also been identified as a main pathway.

5. Tracing metabolic activity

CSIA is also a promising tool for studying biodegradation of in-situ organics in environment, and for improving descriptions of organics during in-situ biotransformation. During biodegradation, light isotopes are more favorable for transformation than heavy isotopes, leading to enriching heavy isotopes in the parent compounds of the remaining substrate (Elsner et al., 2005). CSIA has been extensively applied to evaluate the in-situ biodegradation of chlorinated ethenes (Lollar et al., 2001), halogenated benzene (Sohn et al., 2018), and chlorpyrifos (Tang et al., 2017). However, few studies have applied CISA to analyze BOC biodegradation.

Recently, a stable carbon isotope analysis was conducted to explore the in-situ biodegradation of PBDE congeners at an e-waste recycling site (Huang et al., 2019). The δ^{13}C values of five PBDE congeners (2,4,4′-tribromodiphenyl ether (BDE-28), BDE-47, BDE-49, BDE-99, and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153)) were measured in sediment at different depths. The heavy isotope (^{13}C) was enriched with increasing core depth. Compared with δ^{13}C values in

PBDE commercial mixtures, $\delta^{13}C$ values of PBDEs demonstrated the potential for tracing the fate of PBDEs through in-situ microbial degradation.

Multi-elemental CSIA has also been used to trace the fate of organic contaminants. Balaban *et al.* used dual carbon and bromine stable isotope analysis to investigate biotransformation of TBNPA (brominated flame retardant) using indigenous aerobic microorganisms enrichment culture (Balaban et al., 2016). In laboratory experiment, bromine and carbon isotope effects were determined throughout TBNPA biotransformation. Under aerobic condition, biotransformation was rapid and full debromination occurred within a few days. Significant carbon isotope enrichment occurred, with $\varepsilon_{C\ bulk} = -8.8 \pm 1.5‰$ but no variation in the bromine isotope composition. Under anaerobic condition, no biodegradation was observed. This indicates that the initial step of TBNPA biotransformation was H abstraction from C-H bond under aerobic conditions. The C-H bond cleavage led to generating less stable intermediates, which were quickly debrominated (Balaban et al., 2016). In the in-situ experiment, carbon, and bromine isotope analysis of TBNPA in the polluted groundwater was also performed, to provide information about the in-situ biodegradation of TBNPA. Result showed that both bromine and carbon had the same isotope composition as the isotope composition of TBNPA in the production line. Therefore, when bromine and carbon isotope values of TBNPA in in-situ contaminated groundwater were compared with TBNPA values in the production line, and when they were combined with TBNPA oxidative pathway created by aerobic biodegradation in a groundwater enrichment culture, the bromine and carbon isotope values measured in TBNPA in-situ contaminated groundwater indicated a lack of TBNPA in-situ biodegradation.

Most BOCs possess greatly persistent, bioaccumulative, and ecotoxic properties. This means they are potentially harmful to ecosystems and humans; and verifying their metabolism and trophic dynamics in biota is critical for effective scientific risk assessments. CSIA has been recently used to describe biological metabolism and trophic dynamics of BOCs. For example, Luo *et al.* applied CSIA to track debromination and trophic dynamics of PBDE congeners in fish (Luo et al., 2013). Stable carbon isotope compositions of five PBDE congeners (BDE-28, BDE-47, BDE-49, 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), and BDE-99) in three kinds of fish were accurately measured. Results indicated that $\delta^{13}C$ values of BDE-100 did not significantly differ among PBDE commercial mixture DE71 (-27.78 ± 0.15‰), tiger barb (-27.90 ± 0.11‰ and −27.88 ± 0.25‰), oscar fish (-27.83 ± 0.23‰ and −27.78 ± 0.22‰), and redtail catfish (-27.82 ± 0.12‰ and −27.83 ± 0.19‰).

Based on the structure-activity relationship in fish with respect to debrominating PBDE congeners (Roberts et al., 2011), and the same isotope signature of BDE-100 in fish and in diet. It can be concluded that fish were directly exposed to BDE-100 through their diet. The $\delta^{13}C$ values of BDE-99 in tiger barb were a little higher compared to DE71, and the discriminations were smaller than the total analytical uncertainty of 0.5‰. This demonstrates that no obvious isotope fractionation took place. This is because of the masking effect created by large amount of BDE-99 in tiger barb, which did not debrominate or dilute the other 11 carbons that did not participate in the reaction. However, $\delta^{13}C$ values of BDE-99 decreased as trophic level increased (from −26.25‰ in DE71 to −27.76‰ in oscar fish and to −27.33‰ in tiger barb). Carbon isotope fractionation of BDE-47 in these fish species was a result of the debromination of highly brominated congeners, e.g., BDE-99 and 2,2′,3,4,4′-pentabromodiphenyl ether (BDE-85). A similar variation in $\delta^{13}C$ values was also seen for BDE-28. The $\delta^{13}C$ values of BDE-49 experienced no significant differences among DE71 within three fish species. Compared with $\delta^{13}C$ values of PBDE congeners, the conservation of $\delta^{13}C$ values of BDE-49 among fish species indicated that BDE-49 was not the intermediate of highly PBDE congeners.

CSIA has also been used to track transfer and biological metabolism of PBDE congeners in fish (Zeng et al., 2013). The $\delta^{13}C$ values were determined for PBDE congeners, including BDE-47, −99, and −100 in sediments, and BDE-47, −49, and −100 in northern snakehead and mud carp. The δ^{13}C values of BDE-47, −99, and −100 (-23.91 ± 0.21‰, −27.54 ± 0.76‰, and −29.97 ± 1.80‰, respectively) in sediments are consistent with values of PBDE commercial mixtures (Vetter et al., 2008). These results showed that BDE-47, −99, and −100 cannot degrade and transform in sediments, or if degradation and transformation occur, these processes do not change the stable isotope composition.

However, δ^{13}C values of BDE-47 were significantly higher in sediments compared to in mud carp (-26.59 ± 0.21‰) (Zeng et al., 2013). This is because debrominating highly brominated congeners may cause the pool of BDE-47 in mud carp to exhibit lower δ^{13}C values compared to the point of origination. As such, decreasing δ^{13}C values of BDE-47 demonstrates metabolic debromination of PBDE in mud carp. Furthermore, δ^{13}C values of BDE-47 in northern snakehead (-26.84 ± 0.47‰ – −26.65 ± 0.08‰) approximated to those in mud carp; they were expected to have a lower δ^{13}C value compared to prey mud carp. This result may be because BDE-47 was superimposed from metabolic debromination of highly brominated congeners by northern snakehead and BDE-47 from other prey species. However, δ^{13}C values of BDE-49 in northern snakehead (-25.25 ± 0.08‰ – −24.49 ± 0.47‰) were significantly higher compared to mud carp, resulting from hydroxylation of BDE-49 in northern snakehead and/or metabolism from other prey species. In contrast, for BDE-100, there were no significant differences in δ^{13}C values among mud carp, northern snakehead, and sediment, indicating that BDE-100 cannot undergo metabolic debromination in the two fishes. This example illustrates that CSIA can effectively trace the metabolic transformation of BOCs in organisms, and is an effective tool for tracking metabolic transformation of organic contaminants in organisms.

6. Future work and challenges

This paper has reviewed major aspects related to the recent application of CSIA for studying BOCs. The associated stable isotope analysis provides information on the source, abiotic and microbial transformation mechanism, and biological metabolism, helping scientists better understand the fate of BOCs in environment. CSIA has developed quickly and expanded to enable precise measurement of different elements (e.g., nitrogen, sulfur, chlorine, and bromine) (Elsner & Imfeld, 2016) and many organic compounds (e.g., trichloroethene, tetrachloroethene, and BOCs) (Elsner & Imfeld, 2016; Gelman & Halicz, 2010); it is also applied to study some areas, like biotransformation and biomagnification of toxic compounds (e.g., PBDEs) via food chain (Luo et al., 2013; Zeng et al., 2013). The advantages of CSIA are clear with respect to identifying sources, assessing transformation pathways, and tracing BOC metabolism in environment. However, the technology is still new and great benefits are likely to come from further development. Research in the following four areas would significantly advance the application of CSIA in the environmental and geochemical sciences.

1. Developing new analytical methods to analyze bromine stable isotope of BOCs: Over the past decades, CISA has been further developed to determine bromine stable isotope, and significant advances have been made in understanding bromine isotope effect for various BOCs in environmental/geochemical processes. GC/MC/ICPMS and GC/quadrupole mass spectrometry have been used to analyze the bromine isotope composition of individual compounds, and GC/MC/ICPMS analysis has been considered as the most promising technique for determining bromine isotope composition of BOCs. However, new methods face challenges. To compare bromine isotope data generated by different operators, equipment, and laboratories, there is a significant need for international standard materials with defined bromine isotope composition. For example, Holmstrand *et al.* used MBB with a known δ^{81}Br of -0.39‰ as a standard reference material to determine PBDEs (Holmstrand et al., 2010), while another research group applied strontium as a standard reference material to measure 3-

bromotoluene, tribromobenzene, tribromophenol, and bromochlorobenzene (Gelman & Halicz, 2010). Many BOCs contain hydroxyl, carboxyl, alcohol, or amino groups, which may reduce their volatility and increase their polarity. These are challenges those current methods do not address well, because BOCs tend to decompose when injected on a GC column, or adsorbed in the chromatography column. They are not easy to desorb. Even without these problems, the BOCs may adhere onto the interface between GC and MC/ICPMS. Therefore, the new high-precision bromine isotope analysis methods still face challenges when analyzing BOCs in different environments.

2. Deciphering the mechanisms involved in isotope fractionation during chemical reactions: When isotope fractionation occurs in a reaction, the KIEs obtained for specific elements provide significant information about the reaction to the pathway. However, CSIA mainly describes isotope characteristic of individual compounds. Therefore, isotope fractionation may be masked by non-reacting atoms and the isotope fractionation effects may be smaller than expected. Even though isotope enrichment factors of multiple elements may sometimes be effective, the CSIA technique still cannot meet the needs of mechanistic studies. In this situation, position-specific isotope analysis may be useful to investigate the pathway, and position-specific enrichment factor values can provide more important information about the mechanism. There is significant potential for advancing approaches to multiple isotope enrichment analyses and position-specific isotope analyses of BOCs; however, challenges remain with respect to developing the tools for better elaborating transformation mechanism of BOCs.
3. Distinguishing different degradation pathways of BOCs. Combining both experimental and computational approaches are an ideal way to clarify the transformation of BOCs. Nevertheless, only four studies have conducted in-depth analyses to characterize the bromine isotope effect using both theoretical predicted and experimentally determined methods. In one study, Willey and Taylor reported that the Br KIE of *tert*-butyl and *n*-butyl bromides during solvolysis could be significantly attributed to S_N1 and S_N2 transformation pathways (Willey & Taylor, 1980). The other three studies were conducted by researchers in Israel. They focused on bromine isotope effect during Grignard reaction in view of metal insertion mechanism (Szatkowski et al., 2013) and Finkelstein reaction based on S_N2 pathway (Żaczek et al., 2017); they also observed the carbon isotope effect and bromine isotope effect during dehydrobromination and nucleophilic substitution reaction of BOCs under alkaline conditions (Manna et al., 2018). These studies show that computational methods are very promising to further describe the transformation pathways of BOCs and the isotope effect.
4. Applying CSIA to study bioaccumulation and biotransformation of BOCs: Different applications of CSIA have matured to trace the biotransformation of a few organic pollutants in biota (Yanik et al., 2003). However, we have identified only two studies that applied CSIA to track the bio-metabolism of BOCs (PBDEs) in environmental biota and to identify the isotope composition of PBDE congeners in food web (Luo et al., 2013; Zeng et al., 2013). Currently, the isotope fractionation of BOCs with trophic transfers has not been well recorded, and the species-specific metabolism increases the challenges of tracing trophic transfer of BOCs using stable isotope analysis. In addition, CSIA is also an innovative method to distinguish intrinsic biotransformation of BOCs. Therefore, many challenges lie ahead in advancing CSIA in environmental applications, and we recommend that CSIA should be applied in more in this field to obtain more AKIE for biotransformation, in order to provide reference AKIE data and methods.

In summary, recent advances in GC/C/IRMS and GC/MC/ICPMS have opened the door to the development of CSIA investigations and application. This has led to significant applications of this technology across a range of new areas in environmental science, environmental ecology, and geochemical science. It is important to further develop and apply CSIA with more organic compounds and to improve the method's accuracy and precision. In particular, attempts to use CSIA to trace the metabolic transformation of organic contamination in organisms remain limited, but hold great potential for future scientific discoveries.

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ORCID

Guiying Li http://orcid.org/0000-0002-6777-4786
Taicheng An http://orcid.org/0000-0001-6918-8070

References

Abe, Y., Aravena, R., Zopfi, J., Shouakar-Stash, O., Cox, E., Roberts, J. D., & Hunkeler, D. (2009). Carbon and chlorine isotope fractionation during aerobic oxidation and reductive dechlorination of vinyl chloride and cis-1,2-dichloroethene. *Environmental Science & Technology*, *43*(1), 101–107. https://doi.org/10.1021/es801759k

Ahad, J. M. E., Pakdel, H., Labarre, T., Cooke, C. A., Gammon, P. R., & Savard, M. M. (2021). Isotopic analyses fingerprint sources of polycyclic aromatic compound-bearing dust in Athabasca oil sands region snowpack. *Environmental Science & Technology*, *55*(9), 5887–5897. https://doi.org/10.1021/acs.est.0c08339

Alaee, M., Arias, P., Sjodin, A., & Bergman, A. (2003). An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environment International*, *29*(6), 683–689. https://doi.org/10.1016/S0160-4120(03)00121-1

Balaban, N., Bernstein, A., Gelman, F., & Ronen, Z. (2016). Microbial degradation of the brominated flame retardant TBNPA by groundwater bacteria: Laboratory and field study. *Chemosphere*, *156*, 367–373. https://doi.org/10.1016/j.chemosphere.2016.04.127

Barrie, A., Bricout, J., & Koziet, J. (1984). Gas chromatography-stable isotope ratio analysis at natural abundance levels. *Biological Mass Spectrometry*, *11*(11), 583–588. https://doi.org/10.1002/bms.1200111106

Beneteau, K. M., Aravena, R., & Frape, S. K. (1999). Isotopic characterization of chlorinated solvents-laboratory and field results. *Organic Geochemistry*, *30*(8), 739–753. https://doi.org/10.1016/S0146-6380(99)00057-1

Bernstein, A., Ronen, Z., Levin, E., Halicz, L., & Gelman, F. (2013). Kinetic bromine isotope effect: Example from the microbial debromination of brominated phenols. *Analytical and Bioanalytical Chemistry*, *405*(9), 2923–2929. https://doi.org/10.1007/s00216-012-6446-0

Burgoyne, T. W., & Hayes, J. M. (1998). Quantitative production of H-2 by pyrolysis of gas chromatographic effluents. *Analytical Chemistry*, *70*(24), 5136–5141. https://doi.org/10.1021/ac980248v

Carrizo, D., Unger, M., Holmstrand, H., Andersson, P., Gustafsson, O., Sylva, S. P., & Reddy, C. M. (2011). Compound-specific bromine isotope compositions of one natural and six industrially synthesised organobromine substances. *Environmental Chemistry*, *8*(2), 127–132. https://doi.org/10.1071/EN10090

Chen, L., Shouakar-Stash, O., Ma, T., Wang, C., & Liu, L. (2017). Significance of stable carbon and bromine isotopes in the source identification of PBDEs. *Chemosphere*, *186*, 160–166. https://doi.org/10.1016/j.chemosphere.2017.07.109

Currier, H. A., Fremlin, K. M., Elliott, J. E., Drouillard, K. G., & Williams, T. D. (2020). Bioaccumulation and biomagnification of PBDEs in a terrestrial food chain at an urban landfill. *Chemosphere*, *238*, 124577. https://doi.org/10.1016/j.chemosphere.2019.124577

de Wit, C. A., Alaee, M., & Muir, D. C. G. (2006). Levels and trends of brominated flame retardants in the Arctic. *Chemosphere*, *64*(2), 209–233. https://doi.org/10.1016/j.chemosphere.2005.12.029

Dietz, R., Riget, F. F., Sonne, C., Born, E. W., Bechshoft, T., McKinney, M. A., Drimmie, R. J., Muir, D. C. G., & Letcher, R. J. (2013). Three decades (1983-2010) of contaminant trends in east greenland polar bears (Ursus maritimus). Part 2: Brominated flame retardants. *Environment International*, *59*, 494–500. https://doi.org/10.1016/j.envint.2012.09.008

Drenzek, N. J., Tarr, C. H., Eglinton, T. I., Heraty, L. J., Sturchio, N. C., Shiner, V. J., & Reddy, C. M. (2002). Stable chlorine and carbon isotopic compositions of selected semi-volatile organochlorine compounds. *Organic Geochemistry*, *33*(4), 437–444. https://doi.org/10.1016/S0146-6380(02)00004-9

Durand, A. P., Brown, R. G., Worrall, D., & Wilkinson, F. (1998). Study of the aqueous photochemistry of 4-fluorophenol, 4-bromophenol and 4-iodophenol by steady state and nanosecond laser flash photolysis. *Journal of the Chemical Society, Perkin Transactions*, *2*(2), 365–370. https://doi.org/10.1039/a705287a

Elsner, M., & Imfeld, G. (2016). Compound-specific isotope analysis (CSIA) of micropollutants in the environment - current developments and future challenges. *Current Opinion in Biotechnology*, *41*, 60–72. https://doi.org/10.1016/j.copbio.2016.04.014

Elsner, M., Jochmann, M. A., Hofstetter, T. B., Hunkeler, D., Bernstein, A., Schmidt, T. C., & Schimmelmann, A. (2012). Current challenges in compound-specific stable isotope analysis of environmental organic contaminants. *Analytical and Bioanalytical Chemistry*, *403*(9), 2471–2491. https://doi.org/10.1007/s00216-011-5683-y

Elsner, M., Zwank, L., Hunkeler, D., & Schwarzenbach, R. P. (2005). A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. *Environmental Science & Technology*, *39*(18), 6896–6916. https://doi.org/10.1021/es0504587

Falta, R. W., Bulsara, N., Henderson, J. K., & Mayer, R. A. (2005). Leaded-gasoline additives still contaminate groundwater. *Environmental Science & Technology*, *39*(18), 378A–384A. https://doi.org/10.1021/es053352k

Fielman, K. T., Woodin, S. A., Walla, M. D., & Lincoln, D. E. (1999). Widespread occurrence of natural halogenated organics among temperate marine infauna. *Marine Ecology Progress Series*, *181*, 1–12. https://doi.org/10.3354/meps181001

Fischer, A., Manefield, M., & Bombach, P. (2016). Application of stable isotope tools for evaluating natural and stimulated biodegradation of organic pollutants in field studies. *Current Opinion in Biotechnology*, *41*, 99–107. https://doi.org/10.1016/j.copbio.2016.04.026

Gelman, F., & Dybala-Defratyka, A. (2020). Bromine isotope effects: Predictions and measurements. *Chemosphere*, *246*, 125746–125746. https://doi.org/10.1016/j.chemosphere.2019.125746

Gelman, F., & Halicz, L. (2010). High precision determination of bromine isotope ratio by GC-MC-ICPMS. *International Journal of Mass Spectrometry*, *289*(2–3), 167–169. https://doi.org/10.1016/j.ijms.2009.10.004

Gribble, G. W. (1999). The diversity of naturally occurring organobromine compounds. *Chemical Society Reviews*, *28*(5), 335–346. https://doi.org/10.1039/a900201d

Henderson, J. K., Freedman, D. L., Falta, R. W., Kuder, T., & Wilson, J. T. (2008). Anaerobic biodegradation of ethylene dibromide and 1,2-dichloroethane in the presence of fuel hydrocarbons. *Environmental Science & Technology*, *42*(3), 864–870. https://doi.org/10.1021/es0712773

Hilkert, A. W., Douthitt, C. B., Schluter, H. J., & Brand, W. A. (1999). Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry*, *13*, 1226–1230. https://doi.org/10.1002/(SICI)1097-0231(19990715)13:13≤1226::AID-RCM575≥3.0.CO;2-9

Hitzfeld, K. L., Gehre, M., & Richnow, H.-H. (2011). A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine and sulphur. *Rapid Communications in Mass Spectrometry : RCM*, *25*(20), 3114–3122. https://doi.org/10.1002/rcm.5203

Hitzfeld, K. L., Gehre, M., & Richnow, H.-H. (2017). Evaluation of the performance of high temperature conversion reactors for compound-specific oxygen stable isotope analysis. *Isotopes in Environmental and Health Studies*, *53*(2), 116–133. https://doi.org/10.1080/10256016.2016.1215983

Holmstrand, H., Unger, M., Carrizo, D., Andersson, P., & Gustafsson, O. (2010). Compound-specific bromine isotope analysis of brominated diphenyl ethers using gas chromatography multiple collector/inductively coupled plasma mass spectrometry. *Rapid Communications in Mass Spectrometry : RCM*, *24*(14), 2135–2142. https://doi.org/10.1002/rcm.4629

Horst, A., Bonifacie, M., Bardoux, G., & Richnow, H. H. (2019). Isotopic characterization (2H, 13C, 37Cl, 81Br) of Abiotic Degradation of Methyl Bromide and Methyl Chloride in Water and Implications for Future Studies. *Environmental Science & Technology*, *53*(15), 8813–8822. https://doi.org/10.1021/acs.est.9b02165

Horst, A., Holmstrand, H., Andersson, P., Andersson, A., Carrizo, D., Thornton, B. F., & Gustafsson, O. (2011). Compound-specific bromine isotope analysis of methyl bromide using gas chromatography hyphenated with inductively coupled plasma multiple-collector mass spectrometry. *Rapid Communications in Mass Spectrometry: RCM*, *25*(17), 2425–2432. https://doi.org/10.1002/rcm.5144

Horst, A., Thornton, B. F., Holmstrand, H., Andersson, P., Crill, P. M., & Gustafsson, O. (2013). Stable bromine isotopic composition of atmospheric CH_3Br. *Tellus B: Chemical and Physical Meteorology*, *65*(1), 21040. https://doi.org/10.3402/tellusb.v65i0.21040

Huang, C., Zeng, Y., Luo, X., Ren, Z., Tang, B., Lu, Q., Gao, S., Wang, S., & Mai, B. (2019). In situ microbial degradation of PBDEs in sediments from an e-waste site as revealed by positive matrix factorization and compound-specific stable carbon isotope analysis. *Environmental Science & Technology*, *53*(4), 1928–1936. https://doi.org/10.1021/acs.est.8b06110

Hunkeler, D., & Aravena, R. (2000). Evidence of substantial carbon isotope fractionation among substrate, inorganic carbon, and biomass during aerobic mineralization of 1, 2-dichloroethane by Xanthobacter autotrophicus. *Applied and Environmental Microbiology*, *66*(11), 4870–4876. https://doi.org/10.1128/AEM.66.11.4870-4876.2000

Hunkeler, D., & Elsner, M. (2009). Principles and mechanisms of isotope fractionation. In C. M. Aelion (Ed.), *Environmental isotopes in biodegradation and bioremediation*. CRC.

Jendrzejewski, N., Eggenkamp, H. G. M., & Coleman, M. L. (2001). Characterisation of chlorinated hydrocarbons from chlorine and carbon isotopic compositions: Scope of application to environmental problems. *Applied Geochemistry*, *16*(9–10), 1021–1031. https://doi.org/10.1016/S0883-2927(00)00083-4

Jones, K. C., & de Voogt, P. (1999). Persistent organic pollutants (POPs): State of the science. *Environmental Pollution*, *100*(1–3), 209–221. https://doi.org/10.1016/S0269-7491(99)00098-6

Keppler, F., Eiden, R., Niedan, V., Pracht, J., & Scholer, H. F. (2000). Halocarbons produced by natural oxidation processes during degradation of organic matter. *Nature*, *403*(6767), 298–301. https://doi.org/10.1038/35002055

Kozell, A., Yecheskel, Y., Balaban, N., Dror, I., Halicz, L., Ronen, Z., & Gelman, F. (2015). Application of dual carbon-bromine isotope analysis for investigating abiotic transformations of tribromoneopentyl alcohol (TBNPA). *Environmental Science & Technology*, *49*(7), 4433–4440. https://doi.org/10.1021/es504887d

Kuder, T., Wilson, J. T., Kaiser, P., Kolhatkar, R., Philp, P., & Allen, J. (2005). Enrichment of stable carbon and hydrogen isotopes during anaerobic biodegradation of MTBE: Microcosm and field evidence. *Environmental Science & Technology*, *39*(1), 213–220. https://doi.org/10.1021/es040420e

Kuder, T., Wilson, J. T., Philp, P., & He, Y. T. (2012). Carbon isotope fractionation in reactions of 1,2-dibromoethane with FeS and hydrogen sulfide. *Environmental Science & Technology*, *46*(14), 7495–7502. https://doi.org/10.1021/es300850x

Kuntze, K., Kozell, A., Richnow, H. H., Halicz, L., Nijenhuis, I., & Gelman, F. (2016). Dual carbon-bromine stable isotope analysis allows distinguishing transformation pathways of ethylene dibromide. *Environmental Science & Technology*, *50*(18), 9855–9863. https://doi.org/10.1021/acs.est.6b01692

Leat, E. H. K., Bourgeon, S., Hanssen, S. A., Petersen, A., Strom, H., Bjorn, T. H., Gabrielsen, G. W., Bustnes, J. O., Furness, R. W., Haarr, A., & Borga, K. (2019). The effect of long-range transport, trophic position and diet specialization on legacy contaminant occurrence in great skuas, Stercorarius skua, breeding across the Northeast Atlantic. *Environmental Pollution (Barking, Essex: 1987)*, *244*, 55–65. https://doi.org/10.1016/j.envpol.2018.10.005

Lewandowicz, A., Rudziński, J., Tronstad, L., Widersten, M., Ryberg, P., Matsson, O., & Paneth, P. (2001). Chlorine kinetic isotope effects on the haloalkane dehalogenase reaction. *Journal of the American Chemical Society*, *123*(19), 4550–4555. https://doi.org/10.1021/ja003503d

Lima, A. L. C., Farrington, J. W., & Reddy, C. M. (2005). Combustion-derived polycyclic aromatic hydrocarbons in the environment - A review. *Environmental Forensics*, *6*(2), 109–131. https://doi.org/10.1080/15275920590952739

Lin, M., Ma, S., Yu, Y., Li, G., Mai, B., & An, T. (2020). Simultaneous determination of multiple classes of phenolic compounds in human urine: Insight into metabolic biomarkers of occupational exposure to e-waste. *Environmental Science & Technology Letters*, *7*(5), 323–329. https://doi.org/10.1021/acs.estlett.0c00187

Lipczynska-Kochany, E. (1992). Direct photolysis of 4-bromophenol and 3-bromophenol as studied by a flash/HPLC technique. *Chemosphere*, *24*(7), 911–918. https://doi.org/10.1016/0045-6535(92)90009-G

Lippold, A., Bourgeon, S., Aars, J., Andersen, M., Polder, A., Lyche, J. L., Bytingsvik, J., Jenssen, B. M., Derocher, A. E., Welker, J. M., & Routti, H. (2019). Temporal trends of persistent organic pollutants in barents sea polar bears (Ursus maritimus) in Relation to Changes in Feeding Habits and Body Condition. *Environmental Science & Technology*, *53*(2), 984–995. https://doi.org/10.1021/acs.est.8b05416

Lollar, B. S., Slater, G. F., Sleep, B., Witt, M., Klecka, G. M., Harkness, M., & Spivack, J. (2001). Stable carbon isotope evidence for intrinsic bioremediation of tetrachloroethene and trichloroethene at area 6, Dover Air Force Base. *Environmental Science & Technology*, *35*(2), 261–269. https://doi.org/10.1021/es001227x

Lopes, A. A., Pereira, V. B., Amora-Nogueira, L., Marotta, H., Moreira, L. S., Cordeiro, R. C., Vanini, G., & Azevedo, D. A. (2021). Hydrocarbon sedimentary organic matter composition from different water-type floodplain lakes in the Brazilian Amazon. *Organic Geochemistry*, *159*, 104287. https://doi.org/10.1016/j.orggeochem.2021.104287

Loseth, M. E., Briels, N., Eulaers, I., Nygard, T., Malarvannan, G., Poma, G., Covaci, A., Herzke, D., Bustnes, J. O., Lepoint, G., Jenssen, B. M., & Jaspers, V. L. B. (2019). Plasma concentrations of organohalogenated contaminants in white-tailed eagle nestlings - The role of age and diet. *Environmental Pollution (Barking, Essex: 1987)*, *246*, 527–534. https://doi.org/10.1016/j.envpol.2018.12.028

Luo, X. J., Zeng, Y. H., Chen, H. S., Wu, J. P., Chen, S. J., & Mai, B. X. (2013). Application of compound-specific stable carbon isotope analysis for the biotransformation and trophic dynamics of PBDEs in a feeding study with fish. *Environmental Pollution (Barking, Essex: 1987)*, *176*, 36–41. https://doi.org/10.1016/j.envpol.2013.01.025

Manna, R. N., Grzybkowska, A., Gelman, F., & Dybala-Defratyka, A. (2018). Carbon-bromine bond cleavage - A perspective from bromine and carbon kinetic isotope effects on model debromination reactions. *Chemosphere*, *193*, 17–23. https://doi.org/10.1016/j.chemosphere.2017.10.153

Marlier, J. F. (2001). Multiple isotope effects on the acyl group transfer reactions of amides and esters. *Accounts of Chemical Research*, *34*(4), 283–290. https://doi.org/10.1021/ar000054d

McKinney, C. R., McCrea, J. M., Epstein, S., Allen, H. A., & Urey, H. C. (1950). Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. *The Review of Scientific Instruments*, *21*(8), 724–730. https://doi.org/10.1063/1.1745698

Meckenstock, R. U., Morasch, B., Griebler, C., & Richnow, H. H. (2004). Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated acquifers. *Journal of Contaminant Hydrology*, *75*(3–4), 215–255. https://doi.org/10.1016/j.jconhyd.2004.06.003

Melander, L., & Saunders, W. H. J. (1980). *Reaction rates of isotopic molecules*. John Wiley & Sons; Wiley Interscience Publication.

Meyer, A. H., & Elsner, M. (2013). 13C/12C and 15N/14N isotope analysis to characterize degradation of atrazine: Evidence from parent and daughter compound values. *Environmental Science & Technology*, *47*(13), 6884–6891. https://doi.org/10.1021/es305242q

Munschy, C., Heas-Moisan, K., Tixier, C., Olivier, N., Gastineau, O., Le Bayon, N., & Buchet, V. (2011). Dietary exposure of juvenile common sole (Solea solea L.) to polybrominated diphenyl ethers (PBDEs): Part 1. Bioaccumulation and elimination kinetics of individual congeners and their debrominated metabolites. *Environmental Pollution (Barking, Essex: 1987)*, *159*(1), 229–237. https://doi.org/10.1016/j.envpol.2010.09.001

Nier, A. O. (1947). A mass spectrometer for isotope and gas analysis. *The Review of Scientific Instruments*, *18*(6), 398–411. https://doi.org/10.1063/1.1740961

Nijenhuis, I., & Richnow, H. H. (2016). Stable isotope fractionation concepts for characterizing biotransformation of organohalides. *Current Opinion in Biotechnology*, *41*, 108–113. https://doi.org/10.1016/j.copbio.2016.06.002

Palau, J., Cretnik, S., Shouakar-Stash, O., Höche, M., Elsner, M., & Hunkeler, D. (2014a). C and Cl isotope fractionation of 1,2-dichloroethane displays unique $\delta^{13}C/\delta^{337}Cl$ patterns for pathway identification and reveals surprising C-Cl bond involvement in microbial oxidation. *Environmental Science & Technology*, *48*(16), 9430–9437. https://doi.org/10.1021/es5031917

Palau, J., Shouakar-Stash, O., & Hunkeler, D. (2014b). Carbon and chlorine isotope analysis to identify abiotic degradation pathways of 1,1,1-trichloroethane. *Environmental Science & Technology*, *48*(24), 14400–14408. https://doi.org/10.1021/es504252z

Paneth, P., & O'Leary, M. H. (1991). Nitrogen and deuterium-isotope effects on quaternization of N,N-dimethyl-para-toluidine. *Journal of the American Chemical Society*, *113*(5), 1691–1693. https://doi.org/10.1021/ja00005a037

Paul, D., Skrzypek, G., & Forizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales-a review. *Rapid Communications in Mass Spectrometry: RCM*, *21*(18), 3006–3014. https://doi.org/10.1002/rcm.3185

Pelizzetti, E., & Calza, P. (2002). *Chemistry of Marine Water and Sediment.* (Springer.

Pignatello, J. J., & Cohen, S. Z. (1990). Environmental chemistry of ethylene dibromide in soil and ground water. *Reviews of Environmental Contamination and Toxicology*, *112*, 1–47. https://doi.org/10.1007/978-1-4612-3342-8_1

Pinhey, J. T., & Rigby, R. D. G. (1969). Photo-reduction of chloro- and bromo-aromatic compounds. *Tetrahedron Letters*, *10*(16), 1267–1270. https://doi.org/10.1016/S0040-4039(01)87859-X

Poma, G., Volta, P., Roscioli, C., Bettinetti, R., & Guzzella, L. (2014). Concentrations and trophic interactions of novel brominated flame retardants, HBCD, and PBDEs in zooplankton and fish from Lake Maggiore (Northern Italy). *The Science of the Total Environment*, *481*, 401–408. https://doi.org/10.1016/j.scitotenv.2014.02.063

Preston, T. (1992). The measurement of stable isotope natural abundance variations. *Plant, Cell and Environment*, *15*(9), 1091–1097. https://doi.org/10.1111/j.1365-3040.1992.tb01659.x

Preston, T., & Owens, N. J. P. (1983). Interfacing an automatic elemental analyser with an isotope ratio mass sepectrometer: The potential for fully automated total nitrogen and nitrogen-15 analysis. *The Analyst*, *108*(1289), 971–977. https://doi.org/10.1039/an9830800971

Preston, T., & Owens, N. J. P. (1985). Preliminary ^{13}C measurements using a gas chromatograph interfaced to an isotope ratio mass spectrometer. *Biological Mass Spectrometry*, *12*(9), 510–513. https://doi.org/10.1002/bms.1200120913

Preston, T., & Slater, C. (1994). Mass spectrometric analysis of stable-isotope-labelled amino acid tracers. *The Proceedings of the Nutrition Society*, *53*(2), 363–372. https://doi.org/10.1079/pns19940042

Ratti, M., Canonica, S., McNeill, K., Erickson, P. R., Bolotin, J., & Hofstetter, T. B. (2015). Isotope fractionation associated with the direct photolysis of 4-chloroaniline. *Environmental Science & Technology*, *49*(7), 4263–4273. https://doi.org/10.1021/es505784a

Roberts, S. C., Noyes, P. D., Gallagher, E. P., & Stapleton, H. M. (2011). Species-specific differences and structure-activity relationships in the debromination of PBDE congeners in three fish species. *Environmental Science & Technology*, *45*(5), 1999–2005. https://doi.org/10.1021/es103934x

Sessions, A. L. (2006). Isotope-ratio detection for gas chromatography. *Journal of Separation Science*, *29*(12), 1946–1961. https://doi.org/10.1002/jssc.200600002

Sohn, S. Y., Kuntze, K., Nijenhuis, I., & Häggblom, M. M. (2018). Evaluation of carbon isotope fractionation during anaerobic reductive dehalogenation of chlorinated and brominated benzenes. *Chemosphere*, *193*, 785–792. https://doi.org/10.1016/j.chemosphere.2017.11.089

Stapleton, H. M., Letcher, R. J., & Baker, J. E. (2004). Debromination of polybrominated diphenyl ether congeners BDE 99 and BDE 183 in the intestinal tract of the common carp (Cyprinus carpio). *Environmental Science & Technology*, *38*(4), 1054–1061. https://doi.org/10.1021/es0348804

Sun, F., Mellage, A., Gharasoo, M., Melsbach, A., Cao, X., Zimmermann, R., Griebler, C., Thullner, M., Cirpka, O. A., & Elsner, M. (2021). Mass-transfer-limited biodegradation at low concentrations-evidence from reactive transport modeling of isotope profiles in a bench-scale aquifer. *Environmental Science & Technology*, *55*(11), 7386–7397. https://doi.org/10.1021/acs.est.0c08566

Sylva, S. P., Ball, L., Nelson, R. K., & Reddy, C. M. (2007). Compound-specific 81Br/79Br analysis by capillary gas chromatography/multicollector inductively coupled plasma mass spectrometry. *Rapid Communications in Mass Spectrometry: RCM*, *21*(20), 3301–3305. https://doi.org/10.1002/rcm.3211

Szatkowski, L., Dybala-Defratyka, A., Batarseh, C., Blum, J., Halicz, L., & Gelman, F. (2013). Bromine kinetic isotope effects: Insight into Grignard reagent formation. *New Journal of Chemistry*, *37*(8), 2241–2244. https://doi.org/10.1039/c3nj00197k

Tang, X., Yang, Y., Huang, W., McBride, M. B., Guo, J., Tao, R., & Dai, Y. (2017). Transformation of chlorpyrifos in integrated recirculating constructed wetlands (IRCWs) as revealed by compound-specific stable isotope (CSIA) and microbial community structure analysis. *Bioresource Technology*, *233*, 264–270. https://doi.org/10.1016/j.biortech.2017.02.077

Teuten, E. L., King, G. M., & Reddy, C. M. (2006). Natural C-14 in Saccoglossus bromophenolosus compared to C-14 in surrounding sediments. *Marine Ecology Progress Series*, *324*, 167–172. https://doi.org/10.3354/meps324167

Thanh, W., Yu, J., Wang, P., & Zhang, Q. (2016). Levels and distribution of polybrominated diphenyl ethers in the aquatic and terrestrial environment around a wastewater treatment plant. *Environmental Science and Pollution Research International*, *23*(16), 16440–16447. https://doi.org/10.1007/s11356-016-6828-5

Theiler, R., Cook, J. C., Hager, L. P., & Siuda, J. F. (1978). Halohydrocarbon synthesis by bromoperoxidase. *Science (New York, NY)*, *202*(4372), 1094–1096. https://doi.org/10.1126/science.202.4372.1094

Tuthorn, M., Zech, M., Ruppenthal, M., Oelmann, Y., Kahmen, A., Francisco del Valle, H., Wilcke, W., & Glaser, B. (2014). Oxygen isotope ratios (O-18/O-16) of hemicellulose-derived sugar biomarkers in plants, soils and sediments as paleoclimate proxy II: Insight from a climate transect study. *Geochimica Et Cosmochimica Acta*, *126*, 624–634. https://doi.org/10.1016/j.gca.2013.11.002

Vanstone, N., Elsner, M., Lacrampe-Couloume, G., Mabury, S., & Lollar, B. S. (2008). Potential for identifying abiotic chloroalkane degradation mechanisms using carbon isotopic fractionation. *Environmental Science & Technology*, *42*(1), 126–132. https://doi.org/10.1021/es0711819

Vetter, W., Gaul, S., & Armbruster, W. (2008). Stable carbon isotope ratios of POPS - A tracer that can lead to the origins of pollution. *Environment International*, *34*(3), 357–362. https://doi.org/10.1016/j.envint.2007.03.010

Wang, X., Hu, X., Zhang, H., Chang, F., & Luo, Y. (2015). Photolysis kinetics, mechanisms, and pathways of tetrabromobisphenol A in water under simulated solar light irradiation. *Environmental Science & Technology*, *49*(11), 6683–6690. https://doi.org/10.1021/acs.est.5b00382

Willey, J. F., & Taylor, J. W. (1978). Capacitive integration to produce high precision isotope ratio measurements on methyl chloride and methyl bromide samples. *Analytical Chemistry*, *50*(13), 1930–1933. https://doi.org/10.1021/ac50035a050

Willey, J. F., & Taylor, J. W. (1980). Temperature dependence of bromine kinetic isotope effects for reactions of n-butyl and tert-butyl bromides. *Journal of the American Chemical Society*, *102*(7), 2387–2391. https://doi.org/10.1021/ja00527a042

Woods, A., Kuntze, K., Gelman, F., Halicz, L., & Nijenhuis, I. (2018). Variable dual carbon-bromine stable isotope fractionation during enzyme-catalyzed reductive dehalogenation of brominated ethenes. *Chemosphere*, *190*, 211–217. https://doi.org/10.1016/j.chemosphere.2017.09.128

Xiong, J., Li, G., & An, T. (2015). Development of methodology for the determination of carbon isotope ratios using gas chromatography/combustion/isotope ratio mass spectrometry and applications in the biodegradation of phenolic brominated flame retardants and their degradation products. *Rapid Communications in Mass Spectrometry: RCM*, *29*(1), 54–60. https://doi.org/10.1002/rcm.7072

Xiong, J., Li, G., An, T., Zhang, C., & Wei, C. (2016). Emission patterns and risk assessment of polybrominated diphenyl ethers and bromophenols in water and sediments from the Beijiang River, South China. *Environmental Pollution*, *219*, 596–603. https://doi.org/10.1016/j.envpol.2016.06.021

Xiong, J., Li, G., Peng, P. A., Gelman, F., Ronen, Z., & An, T. (2020). Mechanism investigation and stable isotope change during photochemical degradation of tetrabromobisphenol A (TBBPA) in water under LED white light irradiation. *Chemosphere*, *258*, 127378. https://doi.org/10.1016/j.chemosphere.2020.127378

Yanik, P. J., O'Donnell, T. H., Macko, S. A., Qian, Y., & Kennicutt, M. C. (2003). Source apportionment of polychlorinated biphenyls using compound specific isotope analysis. *Organic Geochemistry*, *34*(2), 239–251. https://doi.org/10.1016/S0146-6380(02)00163-8

Yu, Y., Lou, S., Wang, X., Lu, S., Ma, S., Li, G., Feng, Y., Zhang, X., & An, T. (2019). Relationships between the bioavailability of polybrominated diphenyl ethers in soils measured with female C57BL/6 mice and the bioaccessibility determined using five in vitro methods. Environ Int, *123*, 337–344. https://doi.org/10.1016/j.envint.2018.12.022

Żaczek, S., Gelman, F., & Dybala-Defratyka, A. (2017). A benchmark study of kinetic isotope effects and barrier heights for the finkelstein reaction. *The Journal of Physical Chemistry. A*, *121*(12), 2311–2321. https://doi.org/10.1021/acs.jpca.7b00230

Zakon, Y., Halicz, L., & Gelman, F. (2013). Bromine and carbon isotope effects during photolysis of brominated phenols. *Environmental Science & Technology*, *47*(24), 14147–14153. https://doi.org/10.1021/es403545r

Zakon, Y., Halicz, L., & Gelman, F. (2021). Delta C-13 compound-specific isotope analysis in organic compounds by GC/MC-ICPMS. *Journal of Analytical Atomic Spectrometry*, *36*(9), 1884–1888. https://doi.org/10.1039/D1JA00096A

Zeng, Y. H., Luo, X. J., Yu, L. H., Chen, H. S., Wu, J. P., Chen, S. J., & Mai, B. X. (2013). Using compound-specific stable carbon isotope analysis to trace metabolism and trophic transfer of PCBs and PBDEs in fish from an e-waste site, South China. *Environmental Science & Technology*, *47*(9), 4062–4068. https://doi.org/10.1021/es304558y

Zhang, X. L., Luo, X. J., Liu, H. Y., Yu, L. H., Chen, S. J., & Mai, B. X. (2011). Bioaccumulation of several brominated flame retardants and dechlorane plus in waterbirds from an e-waste recycling region in South China: Associated with trophic level and diet sources. *Environmental Science & Technology*, *45*(2), 400–405. https://doi.org/10.1021/es102251s

Zwank, L., Berg, M., Elsner, M., Schmidt, T. C., Schwarzenbach, R. P., & Haderlein, S. B. (2005). New evaluation scheme for two-dimensional isotope analysis to decipher biodegradation processes: Application to groundwater contamination by MTBE. *Environmental Science & Technology*, *39*(4), 1018–1029. https://doi.org/10.1021/es049650j