Chapter 32
Utility of Stable Isotopes of Hydrogen and Carbon as Tracers of POPs and Related Polyhalogenated Compounds in the Environment

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Abstract Polyhalogenated persistent organic pollutants (POPs) belong to the most hazardous environmental pollutants of our days. The concentrations of compounds such as PCBs, PCDD/Fs, chloropesticides, and brominated flame retardants in the environment are well-documented. In order to determine the origins and fate of the POPs, however, the concentrations in the environment must be related to the processes that redistribute these pollutants. A suitable tool for such a purpose is stable isotope analysis. This chapter describes the techniques used for the determination of stable carbon and hydrogen isotopes of POPs. Element analyzer-isotope ratio mass spectrometry (EA-IRMS) was used for the determination of bulk δ^{13}C values and bulk δ^{2}H values. Linking of a gas chromatograph to IRMS enabled the compound-specific isotope analysis (CSIA) of δ^{13}C values. Moreover, theoretical aspects as to the intramolecular deuterium distribution can be explored by means of quantitative ^{2}H-NMR. The knowledge of published applications in stable isotope analysis of POP is presented. On several occasions IRMS data was suited to distinguish technical products from different producers, metabolites from native POPs, and to address kinetic isotope effects (KIE) during the transformation of POPs. Problems and limitations of the current state of the art in stable isotope analysis of POPs are presented as well.

32.1 Introduction

Polyhalogenated persistent organic pollutants (POPs) are a group of compound classes used for decades by mankind in diverse industrial and agriculture areas. Unintended leaching of POPs and related compounds into the environment since the 1930s has polluted the globe. The high persistency and their semi-volatile nature led to a widespread distribution and transport of particularly recalcitrant pollutants to remote areas. Cold condensation resulted in a transfer from the areas of application of POPs to the Arctic, the Antarctic, and also mountain areas. The given toxicity attributed to these compounds made them to a serious environmental problem and a hot topic for the consumers of food. To better understand the fate of these compounds national monitoring programs have been established worldwide and the distribution of POPs in different environmental compartments has been modelled. In this context, knowledge of the history of the POPs detected in the environment is of central interest. Some of the key questions include: Are POP residues in a given sample the result of long-range transport? Has biodegradation been involved and, if so, to what extent? Are some of these compounds metabolites of others? Is there one source or are there multiple ones? Some of these questions can be answered by means of stable isotope analysis.

Stable isotope analyses of POPs mainly take advantage of the ratio of the carbon isotopes (^{13}C, ^{12}C) and hydrogen (^{2}H, ^{1}H) isotopes in samples by means of isotope ratio mass spectrometry (IRMS). For instance, in food authentication it is possible by IRMS to determine the country in which the “sample” was harvested or produced, or if a natural product (e.g. a flavor compound) is authentic or synthetic. In food authentication such measurements are standard for about two decades. However, the application of IRMS in the field of research on POPs is a relatively young and scarcely used technique. The reason for this is that it appears that these measurements are rather complicated. There is a lack of suitable reference standards for a proper

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and straight forward isotope determination of POPs. The compounds are diverse in structure and concentration and the respective gas chromatograms of sample extracts are full of peaks which may interfere with each other. Moreover, any remainders of the sample matrix will also hamper the correct determination, and isotope fractionation during sample preparation may be a point of concern. The conversion of polyhalogenated organic material into CO_2 is accompanied with the formation of aggressive halogenated compounds which are detrimental to the system. The high masses of Cl and Br reduce the mass percentage of carbon in a POP. Moreover, kinetic isotope effects (KIE) may take place under the chemical POP synthesis. This in turn was suggested to be useful to identify the producer of a given POP by means of stable isotope analysis of both the standard and its residues in the environment. Like all compounds, POPs are degraded in the environment albeit at very low rate. These environmental processes are usually linked with KIE, and it has been suggested that stable isotope data of POPs might add valuable knowledge to the fate of the pollutants. The techniques useful for this approach are element analyzer-isotope ratio mass spectrometry (EA-IRMS), gas chromatography interfaced with isotope ratio mass spectrometry (GC-IRMS), and from a theoretical point of view, deuterium isotopomer analysis by nuclear magnetic resonance spectroscopy (NMR).

32.2 Materials and Methods

In contrast to conventional mass spectrometers that yield structural information by screening of a wide mass range for characteristic fragment ions, IRMS achieves highly accurate and precise measurements of the isotopic abundance of simple gases at the expense of the flexibility of scanning MS (Meier-Augenstein 1999, 2002). In IRMS, the polyhalogenated compound has to be converted into an element-specific gas (i.e. CO_2 in the case of carbon and H_2 for hydrogen isotopes) which is then analyzed in relation to a gas obtained from a standard compound of known isotope composition. The pre-requisite is that this conversion is quantitative and reproducible. However, this is much more complicated in the case of POPs (see below). A second problem is that any compound converted into the measured gas can falsify the result. Hence, only standards or isolated compounds can be analyzed by conventional element analyzer isotope mass spectrometry (EA-IRMS, Sect. 32.2.1). The scope can be broadened when the IRMS inlet is linked to a chromatographic system which is typically a gas chromatograph (Sect. 32.2.2). Due to the chromatographic peak resolution, the different sample compounds can be analyzed individually, and this technique is usually termed compound-specific isotope analysis (CSIA). Still the problem exists that co-eluting compounds will be measured together and the interference will falsify the result obtained. Therefore, different quality control measures have been introduced (see below).

In IRMS (Sect. 32.2.1) conversion of all carbon into CO_2 is a prerequisite for the measurements of carbon isotopes. In that, the isotope information on individual atoms (i.e. the isotopomer distribution) gets lost. The best strategy to obtain site-specific isotope data is currently achievable with NMR. Isotopomer distributions (i.e. the differences in the abundances on individual nuclei of the same type) can then be studied if all signals are resolved. In this field it is of particular disadvantage that ^{12}C has no nuclear spin. Thus, application of NMR is currently limited to determine $^{2}H/^{1}H$ distributions (Sect. 32.2.2). Noteworthy, Philp and Monaco (2011) deal with stable isotopes in hydrocarbon exploration in the present book. This chapter adds some valuable additional information the reader may take advantage of.

32.2.1 Isotope Ratio Mass Spectrometry

The standard IRMS method is based on the conversion of organic materials into gases like CO_2 and H_2. For this purpose any covalent bond in the molecule hosting the target element has to be cleaved. This is obtained in an oxidative furnace for carbon to produce CO_2 and in a reductive furnace to produce H_2 (for typical settings see below). Owing to instrumental limitations with regard to long-term stability and lab-to-lab comparability, the use of reference standards compared to which the analytes are measured was mandatory for the success story of IRMS. The worldwide accepted reference standard for carbon isotopes to give standard CO_2 is Pee Dee Belemnite, a carbonate which has received the name from its origin, i.e. the habitat of the Pee Dee American native tribe in South Carolina. Likewise standard mean ocean water is used for the generation of standard H_2 for hydrogen isotope measurements. Both references can be obtained from the International Atomic Energy Agency (IAEA) in Vienna (http://www.iaea.org). For this reason, these primary reference standards are labelled Vienna Pee-Dee Belemnite (V_{PDB}) and Vienna standard mean ocean water (V_{SMOW}). Current EA-IRMS configurations take advantage of a dual inlet system which allows for the alternating measurement of reference gas and sample while GC-IRMS is performed in continuous flow mode. The setup of a modern GC-IRMS instrument is shown in Fig. 32.1. Owing to the small differences, the result is multiplied by 1,000 and thus generally expressed in ‰. The resulting δ-value (in ‰) expresses the isotope measurements as ratio between sample and standard (32.1):

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) \times 1,000‰ \qquad (32.1)$$

The relation between sample and standard becomes more explicit in (32.2) where the term "1" was substituted with $^{13}C/^{12}C_{standard}/^{13}C/^{12}C_{standard}$.

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{standard}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} \right) \times 1,000‰ \qquad (32.2)$$

As can be gathered from (32.2), the richer a sample is in ^{12}C the smaller the $^{13}C/^{12}C$ fraction. Thus, a sample richer in ^{12}C (or depleted in ^{13}C) than the reference standard has a $\delta^{13}C$ value <0‰. The $\delta^{13}C$ value of the reference standard can also be depicted from (32.2). If the sample is substituted by the reference standard, the numerator in (32.2) is zero, and thus the $\delta^{13}C$ value is per definition 0‰. Noteworthy, the accepted $^{13}C/^{12}C$ ratio of the reference standard V_{PDB} according to Chang and Li is 0.0111802 (Werner and Brand 2001), and this would give the opportunity to calculate the exact $^{13}C/^{12}C$ ratio in samples (Fig. 32.2), but this is scarcely done. Usually, the $\delta^{13}C$ value is reported in papers dealing with IRMS determinations of stable isotopes. However, to give an impression of the quantities involved in isotope effects, a depletion of the $\delta^{13}C$ value by 1‰ will

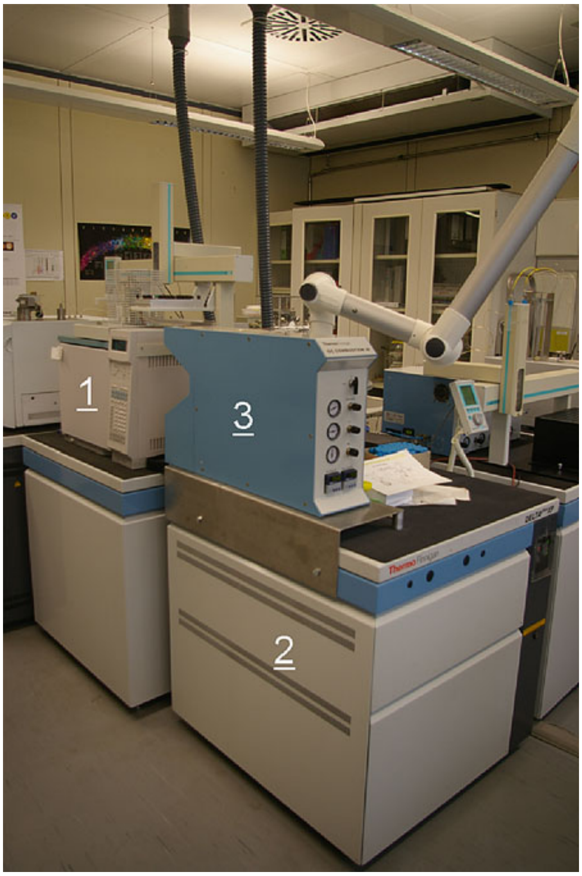

Fig. 32.1 Photo of a typical GC-IRMS system with (1) the gas chromatograph, (2) the mass spectrometer unit and (3) the combustion interface (photo by Natalie Rosenfelder)

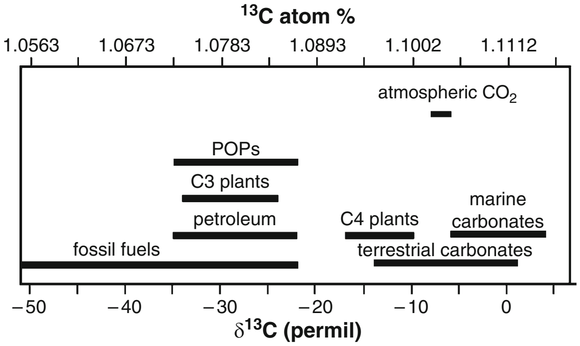

Fig. 32.2 Ranges of $\delta^{13}C$ values (vs. V_{PDB}) of relevant groups of compounds and the corresponding ^{13}C atom percentages (modified from Meier-Augenstein 1999)

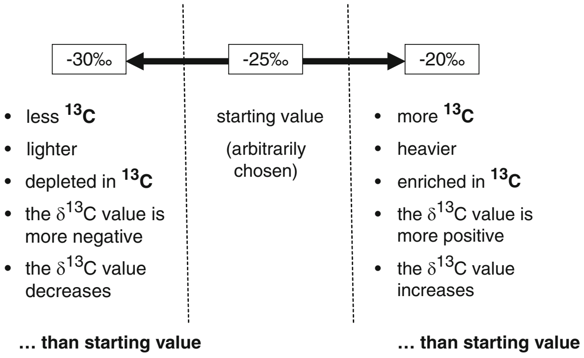

Fig. 32.3 Schematic presentation of changes in $\delta^{13}C$ values

decrease the ^{13}C weight percent by 0.0011% or 11 parts per million (Fig. 32.2).

Owing to the extraordinary high proportion of ^{13}C in Pee Dee Belemite, more or less all other compounds and all the POPs dealt in this chapter have negative $\delta^{13}C$ values. The more negative a $\delta^{13}C$ value is, the less ^{13}C the compound contains (or the "more depleted in ^{13}C" or the "lighter" it is) compared to the reference standard V_{PDB}. Especially for scientists new in the field, these relationships cumbersome to remember are summarized in Fig. 32.3.

Major differences in the $\delta^{13}C$ values of organic compounds originate from the biosynthesis pathways of organic materials, namely in the fixation of CO_2 in photosynthesis. Atmospheric CO_2 has currently a $\delta^{13}C$ value of -8‰ (Whiticar 1996). Over the past decades, this $\delta^{13}C$ value has gradually shifted towards lighter carbon (~1‰) due to the human impact via fossil biofuel and biomass (Whiticar 1996). C_3 plants pass through the Calvin circle. In Rubisco, ^{13}C is particularly discriminated so that all C_3 plants have $\delta^{13}C$ values between -30 and -20‰. The discrimination of ^{13}C relative to atmospheric CO_2 is more moderate in C_4 plants which typically have $\delta^{13}C$ values between -20 and -10‰. Petroleum is the primary source of industrial organic chemistry and thus the production of polyhalogenated compounds. The $\delta^{13}C$ value of the carbon source of crude oil aligns with that of C_3 plants (Whiticar 1996).

The hydrogen isotope signature of a molecule is also expressed via the δ-scale with Vienna standard mean ocean water (V_{SMOW}) being the reference standard. Owing to the relationship given above, samples depleted in ^{2}H relative to the reference standard have negative $\delta^{2}H$ values. The effect between ^{1}H and ^{2}H in a molecule is much more pronounced compared to ^{12}C and ^{13}C due to percentage mass difference.

The "weight" of hydrogen in a compound and sample is mainly governed by climatic factors, especially in the form of evaporation and condensation effects in the natural water cycle. Evaporation of water supports the release of water lighter in hydrogen, while condensation favours the precipitation of water enriched in ^{2}H. Thus, the farther water vapour is transported from the ocean over the landmass, the lighter it gets in hydrogen (and oxygen). Due to the enormous ocean water reservoir, the permanent evaporation of water does not change the $\delta^{2}H$ value within the lifespan of isotope research. Thus, the reference standard (standard mean) ocean water is among the most heavy natural compounds with regard to the hydrogen content so that virtually all compounds are depleted in ^{2}H and thus have negative $\delta^{2}H$ value. C_3 plants and petroleum have $\delta^{2}H$ values in the range -175 to -75‰ (Whiticar 1996). As can be seen from these numbers, the variations and effects of hydrogen isotope abundances are more pronounced compared to carbon isotopes. The origins are primarily due to variations in the $\delta^{2}H$ value of the groundwater used in plant growth (Whiticar 1996).

However, the conversion of the analyte into CO_2 or H_2 might not be as straight forward as that of the reference standard which is only available for limited series of measurements. Therefore, the use of a secondary reference standard (laboratory standard) of known isotopic composition is the rule in virtually all laboratories (Werner and Brand 2001). The laboratory standard is first calibrated against the IAEA reference standard. Thereafter, the laboratory standard is used to determine the samples and the measured $\delta^{13}C$ value is corrected by the difference between laboratory and reference standard to obtain values on the δ-scale.

Difficulties also exist in the conversion of the laboratory standard into CO_2. Consequently, the laboratory standard should be as similar as possible to the analyte (s). That is where the key-problem arises with POPs. Currently, there are no suitable laboratory standards available that are suited for the referencing of semi-volatile polyhalogenated compounds. Individual researchers are pursued to choose their own laboratory standard. Typically, the laboratory standard should be structurally and, for CSIA, chromatographically related to the analytes. Application of a structurally-related laboratory standard provides a good precision of the measurements because systematic errors by insufficient combustion efficiency are minimized. With a proper laboratory standard, variations and drift of $\delta^{13}C$ values may be limited to ~0.1‰ (Drenzek et al. 2001). While the precision obtainable by using a suitable laboratory standard can be optimized it does not necessarily mean that the $\delta^{13}C$ values are correct. Consequently, $\delta^{13}C$ value determined in different laboratories for a given POP may not be identical (see below). Accuracy would require a laboratory POP standard with known $\delta^{13}C$ value. A further key-problem with polyhalogenated compounds is that they include carbon–halogen bonds from which the reactive halogens are released upon combustion. Two problems are linked to this, i.e. that the oxidation/reduction unit might get destroyed and that there is no standard reference material available which is similar to the properties of the analytes. Thus, specific precautions have to be undertaken in order to produce valuable data. Unfortunately, the problems associated with the isotope measurements of polyhalogenated compounds are not well documented. It might seem odd that the measurement technique for POPs is not more frequently used. It should be said at this point that reliable and reproducible stable isotope measurements are far more difficult in the case of polyhalogenated compounds compared to other organic compounds. Those starting in the field should be aware of such problems and it might take some time to obtain reliable and satisfactory results in the laboratory.

In the case of CO_2, three different isotopomers (i.e. isomers having the same number of each isotopic atom but differing in their positions, http://www.iupac.org/goldbook/I03352.pdf) – $^{12}C^{16}O_2$ (m/z 44), $^{13}C^{16}O_2$ (m/z 45) and $^{12}C^{16}O^{18}O$ (m/z 46) – are formed and measured when organic material is converted into CO_2. In addition, the instrument software performs the so-called Craig-correction by 7% for eliminating the impact of $^{12}C^{16}O^{17}O$ on m/z 45 (Werner and Brand 2001). The three m/z values are registered simultaneously by three Faraday cups. The resulting ion currents are continuously monitored, and leveled to obtain a more proportional visualization. The relative abundances – also known as sensitivity factors – (in parentheses) at which these ions are displayed in standard mode are m/z 44 (1), m/z 45 (100), and m/z 46 (300) (Werner and Brand 2001). The corresponding peak areas are quantified and isotope ratios are calculated. Because ^{13}C is only of low abundance in natural materials, the IRMS system has to be very sensitive for low amounts of ^{13}C. It is thus essential for the measurements that no isotope-labelled internal standards are added to the sample. Thus, isotope dilution method taking advantage of ^{13}C marked internal standards is not possible in the measurement of native POPs.

Finally, it should be noted that “CSIA” and “bulk δ-values” are terms which have non-uniformly be used in different IRMS applications. In this article, “bulk $\delta^{13}C$ values” and “bulk $\delta^{2}H$ values” is used when the measurement was performed in combination with EA-IRMS, even when reliable POP standards or isolated POPs were measured, while CSIA is exclusively used when δ-values were determined using GC-IRMS.

32.2.1.1 Bulk Carbon Isotope Ratios by EA-IRMS

As mentioned above, the determination of polyhalogenated compounds is more difficult compared to other compounds. Consequently, it is more complicated to generate and verify stable (precise) and correct (accurate) δ-values. A large part of the problem is related to the movement of the halogens upon CO_2 generation. Jarman et al. (1998) predicted that the lifetime of the furnace used for PCB oxidation would be reduced due to the Cl buildup. Although no degradation of the reactor was observed, a re-oxidation was performed once a day for ~15 min (Jarman et al. 1998). Such measures are highly recommended in practice although they have not been distilled into general rules, yet.

$\delta^{13}C$ value evaluations cannot be based on single measurements. Despite the use of a dual inlet system for calibration, individual measurements are very sensitive to changes. Therefore, samples need to be analyzed repeatedly in order to eliminate outliers. A suitable measurement scheme as reported by Werner and Brand (2001) may serve as a blueprint for the setup within individual laboratories. For bulk $\delta^{13}C$ values, the temperature and time of the combustion process were typically set at 850°C and 4 or 5 h (Jarman et al. 1998; Drenzek et al. 2002). A higher temperature of 940–1,020°C was used by Vetter et al. (2005, 2006a, b). High quality crystalline standards (0.5–2 mg) were weighed in tin capsules (Vetter et al. 2005, 2006a) or sample solutions (in the volatile pentane) were weighed in pre-combusted quartz tubes at amounts to yield 5–30 μmol carbon (Drenzek et al. 2002). Jarman et al. (1998) evaporated PCB solutions in isooctane in sealed tubes and added silver foil for chlorine uptake.

32.2.1.2 GC-IRMS for Carbon Isotopes

The coupling of GC with IRMS is not as straight forward as with conventional mass spectrometers. Any analyte leaving the GC column has to be converted on line into the measured gas (e.g. CO_2) in order to provide analytes amenable to high-precision isotopic analysis (Ricci et al. 1994). Thus, the GC cannot be directly connected with the IRMS but is connected through the oxidation/reduction platform. GC-IRMS was introduced in the 1970s (Sano et al. 1976; Matthews and Hayes 1978) and further developed in the 1980s (Barrie et al. 1984; Freedman et al. 1988) for routine use (Ricci et al. 1994). The GC effluent is interfaced to the combustion reactor filled with CuO/Pt or CuO/NiO/Pt. Water vapour generated during combustion is separated by means of a water trap because it would protonate CO_2 and thus falsify the measurements (Meier-Augenstein 1999).

In GC-IRMS we must additionally take into account that compounds labeled with ^{13}C or ^{2}H elute prior to the native compounds. This so-called gas chromatographic isotope effect or inverse isotope effect is due to the higher volatility of isotopically heavier organic compounds. Due to the chromatographic isotope effect, isotope distribution within a peak is not symmetrical, and in the combustion unit, the m/z 45 signal ($^{13}CO_2$) precedes the m/z 44 signal ($^{12}CO_2$) by 150 ms on average (Meier-Augenstein 1999). As a consequence the sensitivity-corrected ratio of m/z 45 to m/z 44 is not a constant parallel line but a curve starting positive (relatively higher abundance of the earlier eluting $^{13}CO_2$) and gets negative in the end of the peak (relatively higher proportion of $^{12}CO_2$) (Werner and Brand 2001). For this reason, it is essential that the entire GC peak is converted into CO_2. Incomplete analysis of a partially collected and integrated peak will severely compromise the quality of the isotope ratio measurement by GC-IRMS. In addition, the analyte has to be baseline separated from other compounds in the sample because traces of an interfering peak eluting in close proximity to the analyte will make accurate peak integration virtually impossible. These circumstances must be born in mind when complex mixtures and environmental sample extracts are analyzed. In GC-IRMS, a true baseline separation for adjacent peaks is of paramount importance for high-precision CSIA (Meier-Augenstein 1999). Problems due to co-elutions can be overcome by multidimensional- or heart-cut 2D-GC (MDGC) in which (only) the analyte is trapped from one column onto the second column on which it is analyzed free from interferences. This technique has been used by Horii et al. (2005, 2008) for the analysis of PCB mixtures and octachlorodibenzo-*p*-dioxin (OCDD). These authors also received a better sensitivity when MDGC was used as a prelude to isotope analysis.

In polyhalogenated compounds, carbon typically contributes <50% to the molecular weight. The carbon produced from the analyte should be in the range of 25 ng in order to produce signals that can be precisely integrated and reproduced. This would require injections of solutions containing 50 ng or more of an organic substance onto the column. Transferred to a molar level it is the low or even sub-nM range. For instance, the molecular weight of the hexachlorobiphenyl PCB 153 is 360.9 g/mol, the carbon content is ~40% (144.1 g/mol) so that 1 nM will provide ~144 ng carbon. To increase confidence levels, ~0.33 nM PCB 153 should be injected onto the GC column. However, the amount available for a study should be higher because replicate analyses are required to determine and verify the required precision. Since the injection medium is a solution which can hardly be reduced to <30 μL, a good starting point for POPs would be 30–100 nM. Using MDGC the amount of carbon required for precise measurements could be reduced to 24 ng OCDD or ~7.6 ng carbon (Horii et al. 2008).

Since the IRMS signal is a direct response to CO_2 any co-eluting peak cannot be distinguished from the signal. Thus, an effluent split leading to ~10% of the gas flow to a conventional ion trap mass spectrometer are partly in use for quality control (for instance in the BGC-Jena-lab, Vetter and Gleixner 2006; Vetter et al. 2006b). However, such systems are not standard in IRMS laboratories, so that other quality controls should be established. Suitable techniques could be previous analysis with the same chromatographic setup. Vetter et al. (2007) used derivative chromatography for monitoring peak purity.

All essential details on the method were not reported in all studies. In the following, typical

GC-IRMS settings are listed: Solutions with POPs were usually injected in splitless mode into hot injector ports (280°C) (Jarman et al. 1998). In virtually all studies with POPs a capillary column coated with 95% methyl 5% phenyl polysiloxane was used. The column dimensions were between 30 and 60 m length, 0.25 or 0.32 mm i.d. and a film thickness of 0.17–0.25 μm. Longer columns were especially used for the analysis of multicomponent mixtures (Jarman et al. 1998; Drenzek et al. 2001; Yanik et al. 2003) which is also recommended at this point. When shorter columns were used, this was done in combination with additional features such as an effluent split to a conventional MS (see above) or with MDGC (Horii et al. 2005). The setup for MDGC took advantage of a GC combination of 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB5 followed by an RTX-200 (25 m × 0.32 mm i.d. × 0.25 μm d_f) column. Details on the reference gas were not reported in all earlier studies. Jarman et al. (1998) used reference gas with a δ^{13}C value of −34.42‰ vs. V_{PDB} whereas Vetter and Gleixner (2006) used NBS 19 and V_{PDB} as isotope standards.

The oxidation reactor/combustion furnace usually consisted of CuO and was heated at 850°C (Yanik et al. 2003), 940°C (Horii et al. 2005; Vetter and Gleixner 2006) or 960°C (Jarman et al. 1998). The reduction reactor was typically set at 650°C (Jarman et al. 1998).

Water was removed by an ethanol/CO_2 cryogenic trap (−80°C; Yanik et al. 2003) or via a Nafion tube (Horii et al. 2005). The later is the typical design used in most instruments. Nafion is a fluorinated polymer that acts as a semi-permeable membrane through which water passes freely while all the other combustion products are retained in the carrier gas stream (Meier-Augenstein 1999).

For POPs, CSIA (for PCBs) precision was reported to be ~0.5‰ (Jarman et al. 1998). This is the typical precision reported for GC-IRMS of POPs and agrees with values reported for volatile halogenated compounds (Sherwood Lollar et al. 2001). Using an internal standard eluting between two PCBs studied, Drenzek et al. (2001) noted that the δ^{13}C values of individual PCBs (in CSIA) were determined relative to a co-injected PCB standard rather than the reference gas. They stated that this type of measurement minimized any bias associated with GC inlet or combustion unit. The drift of internal standard and reference gas was 0.10‰. This method is however limited to scenarios with sufficient separation in the gas chromatogram between the individual analytes. This is difficult to obtain in the case of multicomponent mixtures or environmental samples.

32.2.1.3 IRMS Determination of Hydrogen Isotopes

Clearly, the determination of δ^2H values would provide additional and valuable insights into the fate of POPs (see also the paper by Philp and Monaco (2011)). While GC-IRMS was used to determine δ^2H value of other nonhalogenated pollutants such as benzene (Mancini et al. 2003), in the case of polyhalogenated compounds, only δ^{13}C values can currently be obtained with standard instruments equipped with a high-temperature pyrolysis (HTP) unit. HTP of polyhalogenated compounds fails to deliver significant signals for H_2 due to the formation of HCl (Armbruster et al. 2006). Only 5–20% of the theoretical hydrogen content in the polyhalogenated compounds was picked up by the detector. The resulting hydrogen peaks generated were very small and non-reproducible (Armbruster et al. 2006). This drawback was overcome in a two-step method by transformation of hydrogen from organohalogen compounds first into H_2O followed by reduction to H_2 with Zn at 500°C (Poulsen and Drever 1999). However, this two-step method cannot be used in the continuous flow mode (Chartrand et al. 2007). Morrison et al. (2001) developed a chromium reactor for the on-line determination of δ^2H value of nL-water samples size. A similar chromium-loaded reactor was used by Armbruster et al. (2006) to determine bulk δ^2H value of POPs. This system consisted of a self-made chromium reduction reactor (1,000°C) in the form of a quartz tube filled with 10 cm quartz chips and 6 cm Cr reagent and quartz chips (4:1, w/w). Problems associated with this technique have been reported by Armbruster et al. (2006). This approach was simple but restricted to analyze bulk δ^2H values of POPs (Armbruster et al. 2006; Vetter et al. 2006a). Although a similar design could be used in combination with a GC interface to generate CSIA, this has not been achieved, yet.

Chartrand et al. (2007) presented the first working system for GC-IRMS method for the determination of δ^2H values of halogenated volatile organic compounds

(VOCs), and this system seems to be appropriate for POPs as well. They added a trap filled with liquid N_2 for the elimination of HCl from gas flow after the pyrolysis oven. This system was used to determine the δ^2H values of 1,2-dichloromethane from different producers. Application of this technique would be an excellent starting point for the generation of δ^2H values of POPs in environmental samples. Thus, it could be interesting to follow these trends in future.

32.2.2 Deuterium Isotopomer Determination by Quantitative ^{2}H-NMR

Most molecules exhibit nonstatistical hydrogen isotope distribution, and the true mole fraction of the monodeuterated isotopomers can be directly determined by quantitative ^{2}H-NMR (Martin et al. 2008). This technique is also referred to as site-specific natural isotope fractionation (SNIF) NMR (maybe intramolecular isotope fractionation would have been a better term). These isotope abundances in non-symmetrical molecules depend on physical and (bio)chemical isotope fractionations (Augusti et al. 2009). A prerequisite for such measurements is that the hydrogen is fixed in the molecule and does not equilibrate with its surrounding. For this reason, hydrogen in hydroxyl or carboxyl groups cannot be evaluated because they rather reflect the medium in which they were produced or stored. Further drawbacks of SNIF-NMR are the high amounts required for the measurements: ~50 mg of good quality compounds are required making it difficult to apply this technique to environmental samples. Moreover, only simple molecules in which the NMR resonances are resolved can be studied. Excluded from measurements are POPs which do not form stable conformations. For instance, hexachlorocyclohexanes (HCHs) cannot be determined by ^{2}H-NMR because of the ring flipping which permanently changes between chair and boat conformations. Despite these drawbacks, ^{2}H-NMR may add valuable information to isotope distribution of POPs and three studies on SNIF-NMR experiments with POPs have been published to date (Nikiforov et al. 2005; Vetter et al. 2006a; Augusti et al. 2009).

The SNIF-NMR spectrum of 2,4-dibromoanisole as determined by the group of J. Schleucher is shown in Fig. 32.4 (Vetter et al. 2006a). The abundances of H_B and H_C slightly exceeded H_A. In turn, the three aromatic hydrogens exceeded the intensity of the methoxy-hydrogens (whose signal area must be divided by three due to the three individual H contributing to this moiety). Deuterium isotope data of individual isotopomers is usually described relative to the average deuterium abundance of the whole molecule set to 1.00 or 100%. In the example given in Fig. 32.4, the area of proton signals H_A, H_B, H_C, and CH_3 is summed up and set to 600%. Thus, each signal in the spectrum has an average intensity of 100% (the three equivalent methyl protons count three). Consequently, individual signals with abundances >100% are obtained for a deuterium content above average while signals (<100%) are depleted in deuterium relative to the mean deuterium content of the molecule. In the example of Fig. 32.4, signals H_A, H_B and H_C are 1.8, 9.7, and 8.5%, respectively, richer in deuterium (together by 20%) than the average molecule while the methyl group is poorer in deuterium by 6.7% (with three equivalent protons this group is 20% lighter in deuterium than the average molecule). Interestingly, the results of the SNIF-NMR measurements can be related to the bulk δ^2H value determined with EA-IRMS (Vetter et al. 2006a; Augusti et al. 2009). If the bulk δ^2H value (in ‰) is known, the individual isotopomer distributions as determined with SNIF-NMR will directly lead to the isotope abundances of the individual nuclei.

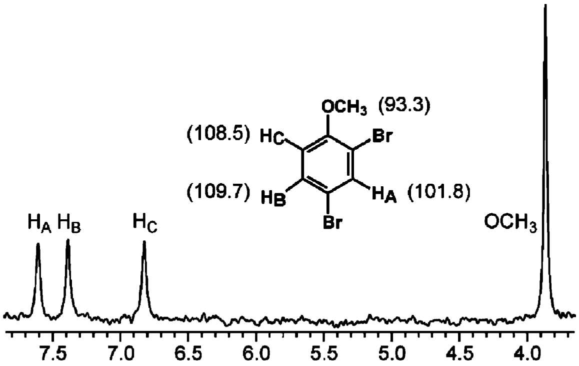

Fig. 32.4 Deuterium NMR spectrum of 2,4-dibromoanisole (Vetter et al. 2006a). Integrals of the signals are proportional to ^{2}H isotopomer abundance (values relative to the average deuterium abundance of the whole molecule, which was set to 100)

32.2.3 Carbon Isotopomer Distributions, Kinetic Isotope Effects (KIE), and the Rayleigh Model

While deuterium isotopomer distributions can be determined by SNIF-^{2}H-NMR, it is difficult to obtain isotopomer-specific data for carbons. However, carbon isotope abundances are also not symmetrically distributed in the molecule. The origins for these intramolecular nonstatistical isotope distributions are governed by statistical mechanics (Schmidt 2003). As reported above, in IRMS all carbons are converted into CO_2 and information on the isotope composition on individual atoms – the so-called isotopomer distribution – is lost. Still the determination of $\delta^{13}C$ values by IRMS is a valuable tool to study the underlying principles and mechanisms of isotope fractionation processes.

Many studies have unequivocally shown that kinetic isotope effects (KIE) determine the isotopic abundance in many defined molecule positions (Schmidt 2003). Most of these studies were based on biosynthesis of organic material (Hayes 2001). However, KIE must essentially contribute to the formation of intramolecular isotope distributions in irreversible or undirectional reactions (Schmidt 2003). In biosynthesis, the isotope discriminating effect at particular positions can be severe. For instance, C-1 of natural glycerol from plant fat may be depleted by up to −14‰ relative to the corresponding C-3 and C-4 positions of its precursor glucose (Weber et al. 1997).

An impact of these effects can be assessed by a look at the $\delta^{13}C$ value of individual carbons of fructose with a $\delta^{13}C$ value of −25.3‰. However, selective step-by-step elimination of carbons (Rossmann et al. 1990) allowed one to determine that the $\delta^{13}C$ values (in parenthesis) at C-1 (−26.5‰), C-2 (−26.1‰), C-3 (−23.4‰), C-4 (−18.8‰), C-5 (−26.3‰), and C-6 (−30.4‰) differed significantly (Weber et al. 1997; Schmidt 2003). Results from measurements on individual carbons are very difficult and complicated to obtained. In order to better understand the meaning of carbon isotopomer distributions, let us consider a single polyhalogenated compound (reactant or substrate) that is converted into metabolite with one halogen less by reductive dehalogenation:

$$\text{R-Hal} \rightarrow \text{R-H}$$

According to the data discussed for glycerol and fructose, an effect of −14‰ on one individual carbon can be categorized as a severe event. We must assume that the effect is frequently lower. However, PCBs, for instance, contain 12 carbons, and the reductive dechlorination will almost exclusively affect the carbon from which the halogen is removed. When the PCB is combusted to CO_2, the overall effect would be $-14‰/12 \approx -1.2‰$. Thus, a process which changes the $\delta^{13}C$ value of POPs by ~1‰ must be taken for one accompanied with a severe isotope effect. Still changes in the $\delta^{13}C$ value in the range of 0.5‰ may weigh high in the case of POPs. This is where the problem arises with POPs, because the typical precision of $\delta^{13}C$ values for polyhalogenated compounds is 0.5‰ and is in the same range as large isotope effects. Therefore, caution has to be undertaken when $\delta^{13}C$ value in reactions are going to be interpreted. This makes POPs completely different than VOC (such as $CHCl_3$ and CCl_4). In these light molecules all carbons are involved in the bond that is dechlorinated and the full effect can be measured without dilution by non-involved carbons.

The isotopic compositions of organic compounds differ because of mass-dependent isotopic fractionation effects where one isotope may react faster or be incorporated preferentially (Melander and Saunders 1980). Isotope fractionation may occur during production, post-production, handling and storage, and hence have the potential to be used in identifying different sources as was shown for VOCs (van Warmerdam et al. 1995; Beneteau et al. 1999).

Although IRMS measurements provide only bulk $\delta^{13}C$ values and the isotope information on the individual nuclei gets lost, the evaluation of $\delta^{13}C$ values of POPs still can provide valuable insights into kinetics, mechanisms and even the history of pollution. Partitioning of isotopes between two compounds containing the same element (or between two phases) with different isotopic ratios is called isotope fractionation (Mariotti et al. 1981). In a simple reactant-product (two component) scheme, the kinetic isotope fractionation factor α can be dealt using the mathematical description developed by Rayleigh (1896) for the fractional distillation of mixed liquids. This classical relationship originally derived for fractionation in the diffusion of gases was later used to describe kinetic isotope fractionation in different disciplines of natural science

(Elsner et al. 2005). For isotope analysis, the Rayleigh equation was transferred by taking advantage of the δ notation (Mariotti et al. 1981). Initially developed for nitrogen isotopes, this model has been used to determine the isotopic fractionation and the amount of transformation of contaminants in single step unidirectional reactions (32.3):

$$\ln R_t/R_0 = \ln[(1{,}000 + \delta^{13}C_0 + \Delta\,\delta^{13}C)/(1{,}000 + \delta^{13}C_t)] = (\alpha - 1)\ln f = \varepsilon\,10^{-3}\ln f \tag{32.3}$$

with

R_0 and R_t being the $^{13}C/^{12}C$ ratios at start (0) and after the transformation (t)

α: fractionation factor of the reaction

f: fraction of parent compound remaining

$\delta^{13}C_0$: initial isotopic signature of the parent compound

$\delta^{13}C_t$: isotopic signature of the compound after transformation

$\Delta\delta^{13}C$: difference in the isotopic signature between after transformation (C_t) and before transformation (C_0)

ε: is the enrichment factor in ‰ with $\varepsilon = 1{,}000\ (\alpha - 1)$

Using $\delta^{13}C_0$, $\delta^{13}C_t$ and α, the extent of transformation can be calculated (Hirschorn et al. 2007). Non-degradative processes such as volatilization, dissolution and sorption were found to be relatively non-fractionating in terms of isotope distributions while degradation processes usually produce significant isotopic enrichments in (volatile) chlorinated aliphatics (Hirschorn et al. 2007). Quantitative estimates of the extent of biotransformation using the Rayleigh model provide conservative estimates of the extent of biotransformation (Hirschorn et al. 2007). In a wider, more general scope, it is a chemical reaction with cleavage of covalent bonds that has the highest contribution to isotope fractionation (Elsner et al. 2005). Significant isotope fractionation can only be expected at positions where covalent bonds (and to a lesser degree adjacent positions with impact on the nucleus studied) are broken or formed during the rate-limiting step(s) of a given process. Processes that act on the compound as a whole such as advective-dispersive transport, volatilization, sorption/desorption or binding to an enzyme cause much smaller changes in the overall isotope composition, which are often not detectable within the precision of the analytical method (Elsner et al. 2005).

As pointed out, the heavier isotopes will react more slowly than the lighter normal isotope effect. This results in the known normal kinetic isotope effect (KIE).

$$\text{KIE} = \text{rate constant of light isotope}/\text{rate constant of heavy isotope}\ (k_L/k_H)$$

Inverse isotope effects are scarce. All isotope effects are mass-dependent. The mass is related to vibrational and not electronic energies (i.e. the determining factors for activation energies in reactions). Therefore, KIEs are not directly correlated with reaction rates. Degradation of POPs is mostly dependent on the cleavage of C–Hal bonds, and this can be assumed to have the following major consequences. Briefly, the KIE will be higher if:

1. The relative mass difference is higher (so that it is higher for H than C isotopes)
2. The mass of the bonded partner is higher (for instance Br > Cl)
3. The bond strength is changed between ground and transition state of a reaction

The type of reaction (S_N1 or S_N2) has an effect, too (Elsner et al. 2005). An in-depth evaluation of this topic is beyond the scope of this review, and readers are recommended to refer to the original textbooks or the review of Elsner et al. (2005) as valuable introductions to this topic. Typical KIE for reactions involving cleavage of C–Cl bonds are in the range KIE = 1.00−1.03 (S_N1) and KIE = 1.03−1.09 (S_N2) (Elsner et al. 2005). The effect will be greater for C–Br bonds (see (2), above). Oxidative processes such as epoxidation are in the range 1.01–1.02. The values and ranges might appear small especially for POPs where the dilution effect mentioned above overlay the measurements due to the multiple carbons that remain unaltered.

Elsner et al. (2005) pointed out that the current use of the Rayleigh model has some limitations. Its merits are undisputed for simple situation (one compound transferred into another) but it is unclear if it will be valid for more complex situations which are found when POPs and other pollutants are investigated in natural systems. For instance let a compound be transformed to another with a change in the $\delta^{13}C$ value. Elsner et al. (2005) pointed out that under environmental conditions, permanent input of a reactant into the system being studied will dilute the isotope effect which may become hard to measure (due to small changes in the $\delta^{13}C$ value) and to interpret (if transformation effect on $\delta^{13}C$ value is not exactly known). Thus, the measured KIE usually referred to as the apparent KIE (AKIE) and may not be identical with the intrinsic KIE.

A procedure on how to use experimental IRMS data to determine the KIE, was shown by Elsner et al. (2005) for CCl_4 dehalogenation. Linear regression of plots of ε over ln*f* according to (32.3) results in a straight line with the slope (here: −0.0262) being the $\delta^{13}C$ value of −26.2‰ (Fig. 32.5). Insertion of the $\delta^{13}C$ value (in form of −0.0262) gives the fractionation factor $\alpha = 1.000 - 0.0262 = 0.9738$. The KIE is the reciprocal value of this measurement, i.e. ~1.027 (Elsner et al. 2005). This means that over the whole reaction time, $^{12}CCl_4$ molecules reacted 1.027-fold more than $^{13}CCl_4$ molecules. As a consequence, the remaining reactant CCl_4 (S_t) at a given time will be relatively enriched in ^{13}C (i.e. a less negative $\delta^{13}C$ value compared to S_0).

However, this relation is only valid for the conversion of one reactant S into one product P (which does not undergo degradation). This situation is hardly found for POPs because the P with one halogen less will be transformed into product P′ with two halogens less (and so on). Therefore, the transformation/dehalogenation rate of such intermediate products cannot be simply determined by the Rayleigh model (Hirschhorn et al. 2007). In such scenarios the real amount of degradation will likely be higher than those calculated by means of the Rayleigh model (compare AKIE with KIE). Different models have been developed for calculations in such situations (Elsner et al. 2005; van Breukelen 2007). However, the database for POPs is currently too small to warrant adoption of these models to the compounds dealt in this chapter.

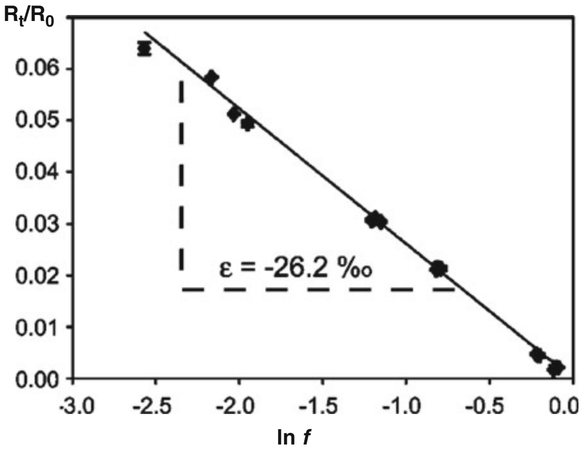

Fig. 32.5 Rayleigh plot of the reductive dechlorination of CCl_4 by a reduced porphyrin (Elsner et al. 2005), with $R_t/R_0 = [(1{,}000 + \delta^{13}C_0 + \Delta\,\delta^{13}C)/(1{,}000 + \delta^{13}C_t)]$ and $\ln f = \ln[(CCl_4)_t/(CCl_4)_0]$ according to (32.3)

32.3 Applications of Stable Isotope Analyses of POPS

32.3.1 Bulk $\delta^{13}C$ Values of Polyhalogenated POPs

Most POPs and VOCs are synthesized from raw materials from the petroleum industry. Petroleum $\delta^{13}C$ values (typical starting materials from crude oil/coal: −33 to −19‰) (Whiticar 1996) usually match those of POPs (Drenzek et al. 2002) but some halogenated VOCs (e.g. chloroform and carbon tetrachloride) had $\delta^{13}C$ values of ~ −38 and −45‰, respectively, and were isotopically lighter in carbon (Zwank et al. 2003). Consequently, the carbon signature of the raw materials is occasionally retained by the product during the synthesis of POPs in contrast to some VOCs. Because of the given wide range of $\delta^{13}C$ values for petroleum, and hence petrochemicals, the isotope signature of the starting materials might constitute the key factor for the bulk $\delta^{13}C$ values of POPs. This would mean that the same polyhalogenated compound or mixture produced by different companies could be distinguished by their $\delta^{13}C$ values if they were made from starting materials with different $\delta^{13}C$ values, and to a lesser degree because of the reaction pathways and/or post-reaction procedures such as precipitation/crystallization, separation/purification, or any other fractionation step. If the same product of a multicomponent mixture with different degrees of halogenation such as PCBs, is produced from the same quality of raw material from the same source at a given period, then, the reactant (e.g. biphenyl in the case of PCBs), is expected to have constant $\delta^{13}C$ values within the range of petrochemicals. If we further assume that no carbon is lost during the synthesis and that the biphenyl quantitatively reacted to a technical PCB mixture, it is likely that the $\delta^{13}C$ value of the product will match that of the reactant. Moreover, PCB products of the same manufacturer halogenated to different degrees (processed from the same raw material) would have the same $\delta^{13}C$ value. This is the situation described for closed two-component systems (Mariotti et al. 1981):

$$P_t + S_t = S_0 \tag{32.4}$$

with

S_0 = isotope composition of the reactant at start

S_t = isotope composition of the reactant at any point t of the reaction

P_t = isotope composition of the product at any point t of the reaction

Expressed for a closed multicomponent system we obtain (32.5):

$$\sum P_t + S_t = S_0 \tag{32.5}$$

In (32.5), all reaction products are summed up by taking into account the molar proportions of the individual compounds that contribute to the reaction mixture. In a closed system, the bulk $\delta^{13}C$ value before and after a reaction will be unaltered. The pre-requisite is that no reactant (or product) is lost (so that all products are determined with the bulk value), and this is independent of the amount of reactant consumed. Likewise, the number of products will not play a role but it is essential that all products produced are registered. A reaction in which all reactant is converted into one or more products, can be expressed as follows:

$$\sum P_t = S_0 \tag{32.6}$$

with $S_t = 0$; S_0 = isotope composition of the reactant at start and P_t = isotope composition of the product at anytime t of the reaction.

In this context a lab-experiment was carried out with camphene (i.e. the starting material of toxaphene) by Vetter et al. (2006a). The reactant camphene had a bulk $\delta^{13}C$ value of −30.5‰, and low, medium, and high chlorinated toxaphene produced by the three chlorination products of this camphene gave bulk $\delta^{13}C$ values of −30.5, −30.5, and −30.1‰, respectively. It was assumed that the slight difference observed for the high-chlorinated toxaphene was due to analytical problems rather than the course of the chemical reaction (Vetter et al. 2006a). For instance the uncertainty in the $\delta^{13}C$ value determination of volatile polyhalogenated compounds (i.e. the precision) usually reported is ~0.5‰ (Hirschorn et al. 2007). We must also take into account that the higher the degree of chlorination of a compound or mixture, the less the carbon contributes to its mass. A PCB (mixture) with three chlorine substituents (on average) has a carbon content of ~56% while the perchlorinated PCB 209 has a carbon content of ~29% only. Consequently, determination of the $\delta^{13}C$ value will be more difficult the higher the degree of halogenation is. Typically, the deviations in the $\delta^{13}C$ values increase with increasing degree of halogenation. The synthesis experiment with camphene confirmed predictions made by Drenzek et al. (2001) who analyzed different PCB mixtures produced in the same plant and found rather narrow bulk $\delta^{13}C$ values (−26.17 ± 0.37‰, $n = 10$). The authors reported an excellent precision of 0.10‰ for the measurements (but it is still difficult to conclude that the accuracy was on the same level for all products, although there are no concerns). Changes in the $\delta^{13}C$ value by 0.4‰ during the reductive dechlorination of a PCB congener were not considered to be an isotopic effect. Based on these evaluations the variations observed in technical PCBs by Drenzek et al. (2001) and that in the chlorination of camphene (Vetter et al. 2006a) represent no isotope effect.

In their ground-breaking paper, Jarman et al. (1998) studied bulk $\delta^{13}C$ values of technical PCB mixtures from four manufacturers (for more information on PCBs see Sect. 32.3.1.1). They reported an increased depletion in ^{13}C with increasing average chlorine content of the technical PCBs. However, this statement was not fully justified by the data shown in the paper: the Clophen products did not consistently follow this trend (Table 32.1) and five Kanechlors showed only slightly more negative $\delta^{13}C$ values. Finally, only the heaviest Phenoclor mixture was depleted in ^{13}C compared to the other Phenoclors. Furthermore, the $\delta^{13}C$ value reported by Drenzek et al. (2002) for the same type of (heavy) product was ~2‰ less depleted in ^{13}C

Table 32.1 Bulk $\delta^{13}C$ values (‰) of different commercial PCB products (data from Jarman et al. 1998, except where noted)

Technical PCB product (number of products analyzed)	$\delta^{13}C$ values (‰)
Clophen ($n = 3$)	−26.95/−25.46/−26.26[a]
Kanechlor ($n = 5$)	−25.00/−25.31/−25.69/−25.39/−25.67[a]
Aroclor ($n = 1$)($n = 12$)[b]	−26.13/−26.17[b]
Phenoclor ($n = 4$)($n = 1$)[b]	−22.34/−22.67/−22.50/−25.63[c] or −23.55[b]

[a] Products listed with increasing chlorine content

[b] Data from Drenzek et al. (2002)

[c] Potential outlier

and closer to the $\delta^{13}C$ value observed for other Phenoclors (Table 32.1). If we re-evaluate the bulk $\delta^{13}C$ of PCBs, one may come to the conclusion that the $\delta^{13}C$ values of products from the same producer were rather constant. In this context it will be valuable to study bulk $\delta^{13}C$ values of other polyhalogenated compounds.

The relationship between the reactants and products discussed above may be used to get access to secondary standards of POPs with known $\delta^{13}C$ values. Suitable laboratory standards of polyhalogenated compounds could be obtained by the halogenation of a non-halogenated reactant with known isotope composition. In a closed system reaction where only one product is obtained, the bulk $\delta^{13}C$ value of reactant and product will be the same (see (32.6) with only one product formed). Thus, the bulk $\delta^{13}C$ value of the unhalogenated reactant may be used to verify the accuracy of the halogenated product.

32.3.1.1 Bulk $\delta^{13}C$ Values of Polychlorinated Biphenyls (PCBs)

The commercial production of PCBs started in 1929 (Tanabe 1988). Technical PCB mixtures with different chlorine content were produced worldwide for use in a wide field of industrial applications (DFG 1988). Major producing countries/products were USA/Aroclor (61.4%), Germany/Clophen (12.4%), France/Phenoclor (9.6%), UK (6.3%), Japan/Kanechlor (5.6%), Spain (2.4%) and Italy (2.2%) while detailed data from Eastern Europe (e.g. former CSSR/Delor and USSR/Sovol) is not available (Kannan et al. 1991). In 1966, PCB residues were discovered (Jensen 1966) and since then have been found in every environmental compartment throughout the world. Although the application range has been stepwise reduced since the 1970s and finally a global ban was declared in the Stockholm convention. PCB residues are still a major environmental problem and PCBs research is still very active after more than 40 years. In theory, 209 PCB congeners can exist and about 130 of them have been identified in different technical products (Frame 1996a, b). Individual PCB mixtures still contain 60–70 different PCB congeners which are difficult to separate by GC. In fact, a range of co-elutions have been reported irrespective of the column type and conditions used (Frame 1996a, b).

As mentioned above, it is likely that different products produced within one plant at a given time have more or less identical bulk $\delta^{13}C$ values. In the following, published bulk $\delta^{13}C$ values of such products were rounded to the mean after elimination of potential outliers. Based on this data treatment, different Clophens ($\delta^{13}C$ value −26.3‰), Kanechlors ($\delta^{13}C$ value −25.4‰), Phenoclors ($\delta^{13}C$ value −22.5‰) and one Aroclor ($\delta^{13}C$ value −26.1‰) showed some distinct differences (Jarman et al. 1998). Similar results can also be extracted from data provided by Drenzek et al. (2002) who studied 12 Aroclor samples (according the data available all Aroclor was generated in the only PCB-producing plant in North America located in Alabama) and determined the $\delta^{13}C$ value to be −26.17 ± 0.37‰ (Table 32.1). This value is in excellent agreement with the sample studied by Jarman et al. (1998). These analyses also covered the same (labeled) Aroclor products which spanned over 0.4 and 0.6‰. Since the same technique was used this range could represent different periods or more generally batches. It appears that Phenoclors have distinct bulk $\delta^{13}C$ value in that they were heavier in carbon than the other PCB products.

32.3.1.2 Bulk $\delta^{13}C$ Values of Chloropesticides

DDT was the most frequently used pesticide in the 1950s and is one of the most controversial chemicals ever synthesized. On one hand, its use as an insecticide in the control of Malaria saved the life of millions of humans. On the other hand, the massive use in agriculture led to severe environmental pollution and the detection of DDT in animals and its investigation may be seen as the starting point of environmental chemistry. DDT is synthesized by the condensation of chloral hydrate ($Cl_3C–CH(OH)_2$) with chlorobenzene. The process not only yielded the desired *p,p′*-DDT but also *o,p′*-DDT along with many other minor products (WHO 1989). Technical DDT (Aldrich, technical grade) and a sample of neat *p,p′*-DDT (Ultra Scientific) had bulk $\delta^{13}C$ values of −28.3‰ (Drenzek et al. 2002). Less depleted in ^{13}C were several DDT-related standards from other sources ($\delta^{13}C$ values −27.5 to −22.9‰, Vetter et al. 2006a). Some of these standard compounds were of analytical and some of technical grade, and thus difficult to compare because the procedures for purification may alter the isotope composition (Vetter et al. 2006a). The range of $\delta^{13}C$ values was comparable with the PCBs despite the very different routes of production. PCBs are obtained by the chlorination of biphenyl and – as discussed above – should resemble the $\delta^{13}C$ value of the starting material. In the case of DDT, already the technical production of the volatile chloral could have been accompanied with a stronger depletion of ^{13}C (as found e.g. for $CHCl_3$, see above), the starting material and the reaction, and finally, the purification in order to obtain standards could have added some isotope fractionation effects, too.

Toxaphene is special in that it is produced from young carbon (and not from petrochemicals) by chlorination of waste from tree production (Saleh 1991). The multicomponent mixture consists of ~1,000 compounds (Korytár et al. 2003). However, only a few of the compounds of technical toxaphene are recalcitrant and thus accumulate in environmental samples (Vetter and Oehme 2000). Toxaphene was mainly produced in the USA along with lower amounts in Eastern Germany and Russia (Saleh 1991). Drenzek et al. (2002) combusted three samples of toxaphene and found bulk $\delta^{13}C$ values of −27.9, −28.7, and −28.2‰, respectively. An authentic Hercules toxaphene (USA) had a bulk $\delta^{13}C$ value of −28.4‰ whereas the East-German product Melipax had a bulk $\delta^{13}C$ value of −27.6‰ and thus was slightly less depleted in ^{13}C (Vetter et al. 2006a).

Hexachlorocyclohexanes (HCHs) were produced for the first time in 1825 by M. Faraday. In 1912 van der Linden succeeded in the isolation of α-, β-, γ- and δ-HCH from the reaction mixture. Technical HCH was used since the early 1940s as an insecticide. Later it was shown that γ-HCH (lindane) is the only active HCH isomer. Since 1950, technical HCH was gradually substituted with lindane (i.e. a product consisting of >99% γ-HCH, DFG 1983). Lindane is not specifically synthesized but has to be obtained from technical HCH by crystallization and/or extraction. This is important to note because this specific production procedure includes different steps that could be associated with isotope fractionation. However, the bulk $\delta^{13}C$ values of three samples of lindane (−26.3, −25.6, and −25.9‰) were in a narrow range (Drenzek et al. 2002; Vetter et al. 2006a). Compared to lindane, other HCH isomers were lighter, with α-HCH being most depleted in ^{13}C (bulk $\delta^{13}C$ value −31.7‰, Vetter et al. 2006a).

Technical grade chlordane and heptachlor are classic (hexachloro)cyclodiene chloropesticides. Two products differed by almost 9‰ in their bulk $\delta^{13}C$ values (Drenzek et al. 2002). This was surprising given the fact that the principal compounds in both products are identical except for one hydrogen on heptachlor substituted with chlorine in the case of chlordane. The bulk $\delta^{13}C$ values of two further cyclodiene pesticides (Aldrin and Dieldrin) were similar to heptachlor, and these three products were from the same source (Drenzek et al. 2002). Hence, it is almost impossible that the chlordane was produced with the same reactant. Further research would be useful if this difference can be generalized and if the origin of the residues of selected cyclodiene pesticides in environmental samples can be identified.

Two synthesized standards of pentachlorophenol (PCP) had bulk $\delta^{13}C$ values of −31.7 ± 0.1 and −30.9 ± 0.7‰ (Horii et al. 2008).

32.3.1.3 Bulk $\delta^{13}C$ Values of Further POPs and Related Compounds

Brominated flame retardants (BFR) are a group of novel POPs. BFRs are routinely added to industrial products for several decades to reduce fire-related injury and property damage. The major compound classes with POP-like properties are polybrominated diphenyl ethers (PBDEs, see Sect. 32.3.2.3), polybrominated biphenyls (PBBs), and hexabromocyclododecane (HBCD).

PBDEs were produced with an average content of five, eight, and ten bromine substituents. The average carbon content in these products is only ~15–25%. A sample of technical pentabromodiphenyl ether (Bromkal 70-5DE, produced in Germany) had a bulk δ^{13}C value of −26.7‰ (Reddy et al. 2002a) and a sample of technical octabromodiphenyl ether from North America (DE-79) had a bulk δ^{13}C value of −27.9‰ (Rosenfelder et al. 2010). A sample of HBCD had a bulk δ^{13}C value of −25.9‰ (Vetter et al. 2006a). The BFR dibromopropyltribromophenyl ether (DPTE) is synthesized from bromoallyltribromophenyl ether (ATE) by addition of bromine (von der Recke and Vetter 2007). Since the bromination reaction of ATE can be performed quantitatively, the bulk δ^{13}C value should be unaltered, and this was in fact observed in terms of bulk δ^{13}C values of −28.70‰ (DPTE) (Rosenfelder et al. 2010) and −28.67‰ (ATE) (Rosenfelder et al. 2010, unpublished results).

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are a group of toxic POPs that occur as (unintended) by-products in various processes. The synthesized perchlorinated congeners octachlorodibenzo-*p*-dioxin (OCDD) and octachlorodibenzofuran (OCDF) were analyzed by Horii et al. (2008). The bulk δ^{13}C value of OCDD ($n = 4$) ranged from −30.7 to −28.6‰ and was lighter than that of OCDF (bulk δ^{13}C values: −25.8 to −24.1‰) (Horii et al. 2008).

While the bulk δ^{13}C values of all POPs shown so far fell into the range of petroleum values (Fig. 32.2), that of different brominated phenols, anisoles, and a guaiacol were frequently much lighter in carbon (Vetter et al. 2006a). Some of these compounds are also used in the synthesis of BFRs. 2,4,6-Tribromoanisole and a sample of pentachloroanisole were most depleted in ^{13}C with bulk δ^{13}C values of −38.2 and −36.1‰, respectively (Vetter et al. 2006a).

Halogenated natural products (HNPs) are a further class of polyhalogenated compounds detected in the environment. Over 4,500 structural varieties are produced by algae, sponges and bacteria (Gribble 1998), some of these HNPs have been detected in organisms that were not the producers but received them in a similar way as anthropogenic POPs (Vetter 2006). A synthesized sample of the HNP 1′-methyl-2,3,3′, 4,4′,5,5′-heptachloro-1,2′-bipyrrole (Q1) had a bulk δ^{13}C value of −34.78‰ (Vetter and Gleixner 2006).

The only bulk δ^{13}C value of a non-commercially synthesized polyhalogenated compound that did not originate from the laboratory but was isolated by natural products chemists from a biological sample is that reported for the HNP 3,5-dibromo-2-(2′,4′-dibromo) phenoxyanisole (BC-3). BC-3 isolated from its natural producer, the sponge *Phyllospongia foliascens*, was strongly depleted in ^{13}C and had a bulk δ^{13}C value of −35.9‰ (Drenzek et al. 2002). This value was unexpected because marine higher plants and organisms tend to be heavier than crude oil which resembles the range of cellulose (Whiticar 1996). Likewise marine plankton is isotopically heavier in carbon than freshwater plankton. Thus, the authors concluded that isotope fractionation might have occurred during isolation of the target compound from the sponge (Teuten et al. 2005). This was further evidenced by CSIA measurements of the δ^{13}C value of this compound and a further isomer isolated from whale blubber which was in the range −22‰ (Teuten et al. 2005). This example underscores the difficulties and limitations of EA-IRMS and some advantages in using GC-IRMS.

32.3.2 Compound-Specific Isotope Analysis (CSIA) of POPs by GC-IRMS

A reliable standard compound should give the same δ^{13}C value when analyzed by EA-IRMS and GC-IRMS (CSIA). However, this is not always the case with POPs. For instance, the δ^{13}C value of the heptahalogenated natural product Q1 differed by ~0.6‰ between EA-IRMS and CSIA (Vetter and Gleixner 2006). Similar results were also obtained in our laboratory for some other POP standards (unpublished results). This problem has been identified in the literature. For instance, bulk δ-values of PCBs presented by Jarman et al. (1998) were significantly heavier than mean value of the PCB congeners determined with CSIA. There are many possible sources of error in the CSIA system (e.g. caused by the GC part) so it might be plausible that δ^{13}C values determined with EA-IRMS are more accurate although this cannot be verified unequivocally at this point. However, since all isotope measurements are relative to a reference, the *difference* between individual isotope data obtained within a study ($\Delta\delta^{13}$C values) can be evaluated and easily transferred to other studies. Difficulties may arise with regard to the reported numbers (i.e. the $\delta^{13}C$ values measured in different labs) which may not be absolutely the same.

32.3.2.1 CSIA of Polychlorinated Biphenyls (PCBs)

While the bulk $\delta^{13}C$ values of technical PCB mixtures seem to represent the $\delta^{13}C$ value of the reactant biphenyl, this does not mean that the individual PCB congeners in these mixtures have identical $\delta^{13}C$ values. In fact, it was already shown in the first CSIA study by Jarman et al. (1998) that individual PCB congeners become more depleted in ^{13}C with higher chlorine content is. This is due to the fact that chemical bonds between heavy isotopes are less easily formed. Thus, halogens preferably add to the lighter isotope, ^{12}C. Considering the closed system already introduced in Sect. 32.3.1, the following scheme can be explained when the overlaying processes are separated: in the beginning of the reaction the reactant biphenyl (S_0, $\delta^{13}C$ value $\equiv$ 0‰) is chlorinated. Owing to the reactivity, addition of one Cl will result in a monochlorobiphenyl (P_t) which is lighter in carbon (negative $\delta^{13}C$ value relative to $S_0 \equiv$ 0‰) while the remaining biphenyl (S_t) would become heavier (positive $\delta^{13}C$ value relative to S_0) in order to fulfill (32.4).

The same scheme can be applied again when the monochlorobiphenyl is converted into a dichlorobiphenyl (and a dichlorobiphenyl in a trichlorobiphenyl a.s.o). However, in this step the monochlorobiphenyl is the reactant which gets enriched in ^{13}C in this reaction. As a consequence, intermediate products will not permanently become depleted in ^{13}C, but eventually may drop back in direction $\delta^{13}C$ value of S_0 and even below. Consequently, the individual congeners in a mixture will be more depleted in carbon the higher chlorinated they are, and all $\delta^{13}C$ values of individual compounds will be spread around the bulk $\delta^{13}C$ value of the product, irrespective of the degree of halogenation. Indeed, such a gradient from low to high chlorinated can be tentatively extracted from the data compiled in Table 32.2. Within one product, low chlorinated congeners (those appearing at the top of the columns in Table 32.2) are heavier in carbon than the high-chlorinated congeners listed at the bottom of the respective column.

The data in Table 32.2 points us to another problem associated with CSIA of complex samples. Partly, the same technical PCB products were studied by different groups and it is likely that the isotope composition was very similar if not identical on all occasions. However, the individual $\delta^{13}C$ values varied widely. For instance, the $\delta^{13}C$ value of PCB 18 in Aroclor 1242 was reported to be −25.7, −27.5, and −26.9‰ and clearly extended beyond the accepted uncertainty of 0.5‰ associated with $\delta^{13}C$ value determinations of POPs. Different co-elutions were noted for PCB congeners (Frame 1996a, b) and it is likely that at least some of the deviations in these measurements have their origins in GC separation problems and for this purpose an in-depth evaluation of $\delta^{13}C$ values of individual PCB congeners needs to be carried out.

32.3.2.2 CSIA of Polychlorinated Naphthalenes (PCNs)

Technical PCN products with varied weight% of chlorine were produced mainly in the 1930s–1950s for various industrial applications (Jakobsson and Asplund 2000). In theory, 75 congeners may exist and each technical product contains various PCN congeners. Horii et al. (2005) determined $\delta^{13}C$ values of individual congeners in six technical PCN products by application of MDGC. The weight percentage of chlorine in the products ranged from 23 (~2 Cl atoms/molecule on average) to 60% (~5 Cl atoms/molecule on average). The $\delta^{13}C$ values of individual PCN congeners spanned from −26.3 to −21.7‰. Average $\delta^{13}C$ values presented by Horii et al. (2005) were a little misleading because they did not take into account the quantities of individual PCNs but just calculated the mean value of the individual $\delta^{13}C$ values. Nevertheless, the results are in clear support of the principles demonstrated above with technical PCB mixtures. The congeners within a given technical product became depleted in ^{13}C with increasing Cl content. The technical PCN products (Halowax) were from the same source and it was apparent that the bulk $\delta^{13}C$ values were similar. The results also show that PCN congeners present in technical mixtures with different Cl content were heaviest in the highly-chlorinated product (Horii et al. 2005).

Table 32.2 CSIA analysis of $\delta^{13}C$ values (‰) of PCB congeners in different Aroclor mixtures

	PCB/PCB	Aroclor 1242			Aroclor 1254			Aroclor 1260	Aroclor 1262
		Yanik et al. (2003)	Jarman et al. (1998)	Horii et al. (2005)	Yanik et al. (2003)	Jarman et al. (1998)	Horii et al. (2005)	Yanik et al. (2003)	Yanik et al. (2003)
2,2′-di	4/10		−26.2	−25.9					
2,4′-di	8/5		−26.8	−26.2					
2,5-di	9	−25.6			−21.8				
2,2′,5-tri	18	−25.7	−27.5	−26.9	−25.3				−22.4
2,2′,6-tri	19								
2,3,3′-tri	20		−27.6						
2,4,4′-tri	28/31		−27.6	−26.7					
2,4′,6-tri	32/16			−26.7					
2,3′,4′-tri	33/20/53	−26.0		−26.3					−27.1
2,2′,3,5′-tetra	44		−27.5	−27.3			−25.3		
2,2′,4,4′-tetra	47/75/48			−27.2					
2,2′,4,5′-tetra	49/43	−22.3		−27.0	−16.2		−25.0		−18.1
2,2′,5,5′-tetra	52/73	−26.7	−28.3	−27.7	−25.1	−23.3	−26.3		−26.3
2,3,3′,6-tetra	59/42/37		−27.7	−27.6					
2,3,4,4′-tetra	60/56		−27.9						
2,3,4′,6-tetra	64	−26.9			−22.9				−22.4
2,3′,4,4′-tetra	66/95			−27.0		−26.6	−26.5		
2,3′,4′,5-tetra	70/76	−23.9		−26.7		−22.4	−24.0		−22.9
2,4,4′,5-tetra	74/61			−26.7					
2,2′,3,4,4′-penta	85	−23.5			−24.4				−17.9
2,2′,3,5,5′-penta	92	−21.8						−21.7	−14.5
2,2′,4,5,5′-penta	101/89/90	−25.0			−21.1	−26.3	−27.4	−21.6	−21.3
2,3,3′,4,4′-penta	105						−27.1		
2,3,3′,4′,6-penta	110	−26.6			−24.7	−26.6	−27.3	−21.6	−23.5
2,3′,4,4′,5-penta	118/106						−26.4		
2,2′,3,3′,4,4′-hexa	128				−25.4			−16.5	−24.9
2,2′,3,3′,6,6′-hexa	136				−18.9			−25.8	−20.9
2,2′,3,4,4′,5′-hexa	138/163/164				−27.7	−28.2	−27.5	−24.5	−24.8
2,2′,3,4,5′,6-hexa	144				−27.7			−25.6	−23.6
2,2′,3,4′,5,5′-hexa	146				−23.9			−24.1	−25.3
2,2′,3,4′,5′,6-hexa	149/118/139					−27.1	−27.8		
2,2′,4,4′,5,5′-hexa	153/132/168	−29.0			−27.0	−27.2	−27.2	−23.4	−23.8
2,2′,3,3′,4,4′,5-hepta	170/190				−33.1			−29.6	−31.0
2,2′,3,3′,4,5,6′-hepta	174				−29.3			−29.3	−25.0
2,2′,3,3′,4,5′,6′-hepta	177				−29.3			−23.7	−24.4
2,2′,3,3′,5,5′,6-hepta	178							−26.0	−30.1
2,2′,3,4,4′,5,5′-hepta	180				−27.6			−26.5	−24.8
2,2′,3,4,4′,5′,6-hepta	183				−26.3			−23.0	−24.2
2,2′,3,4′,5,5′,6-hepta	187/182				−28.2			−28.3	−25.0
2,2′,3,3′,4,4′,5,5′-octa	194							−28.2	−27.1
2,2′,3,3′,4,4′,5,6-octa	195							−27.8	−26.8
2,2′,3,3′,4,4′,5,6′-octa	196/203							−25.9	−26.6
2,2′,3,3′,4,5′,6,6′-octa	201							−26.5	−26.7

32.3.2.3 CSIA of Polybrominated Diphenyl Ethers (PBDEs)

From an environmental point of view, (poly)brominated diphenyl ethers (PBDEs) are the most relevant BFR. As with PCBs, 209 PBDE congeners can exist and the IUPAC numbers correspond with the PCB congeners. Technical PBDEs were available as penta-, octa-, and decabromo mixtures which all contain only a few congeners. The major congeners of technical PentaBDE are BDE 47 (ca. 38%), BDE 99 (ca. 49%), and BDE 100 (ca. 13%). Technical OctaBDE mainly consists of BDE 197 (ca. 22%) and BDE 183 (ca. 42%), while technical DecaBDE predominantly consists of BDE 209 (>90%) (La Guardia et al. 2006). While technical DecaBDE was used in the highest amounts, the main residues in the environment originate from BDE 47, BDE 99, and BDE 100, i.e. the major congeners of PentaBDE (although this product was used the least). Reductive debromination is thought to be a key-process in explaining the high amounts of low-brominated diphenyl ethers in environmental samples.

Technical PBDE mixtures composed of about ten PBDEs and a good GC separation of mixtures is much easier to achieve compared to PCBs (see above). On the other hand, the mass contribution of Br is higher than that of Cl and this reduces the amount of carbon in the molecule. For instance, carbon accounts for 44.0% of the molecular weight to DiBDEs, 25.5% to PentaBDEs (e.g. BDE 99) and only 15.0% to DecaBDE (BDE 209).

The δ^{13}C values of individual PBDE congeners in the technical PentaBDE product DE-71 followed the trend shown for PCBs (see above), i.e. they became depleted in ^{13}C with increasing degree of bromination, and the same tendency was also observed for the technical OctaBDE product DE-79 (Vetter et al. 2008; Rosenfelder et al. 2010). A closer look at the BDEs in both products shows that the three hexaBDEs (BDE 153, BDE 154, and BDE 138) present in both products had significantly different δ^{13}C values in both products. DE-71 was the highest brominated and thus were depleted in ^{13}C. The opposite was valid for the congeners in DE-79 where they represent low degrees of brominating and thus belong to the heaviest congeners (Vetter et al. 2008). Altogether, the δ^{13}C values of the three hexaBDEs differed by 3–6‰ in the two products (Vetter et al. 2008).

32.3.3 *Stable Isotope Analysis of POPs in Environmental Samples and Lab Reactions (Case Studies)*

A number of studies with POPs in environmental samples or in reactions that simulate environmental processes (application of standards to anaerobic solutions, UV-irradiation) have been evaluated by taking advantage of isotope data. Most of these studies focused on the transformation of individual POPs by dehalogenation reactions. A brief summary of issues associated with the δ^{13}C values is presented here. Isotopomer fractionation of 14‰ was considered severe (see Sect. 32.2.3). The POPs discussed below have 6–12 carbons and the non-reacting carbons will thus dilute the result because they are combusted as well. Thus, effects measured in such reactions with POPs can change the δ^{13}C value by 1‰ and are considered to be quite significant. Noteworthy, this is the range of isotope fractionation observed in the chemical reaction leading to the POP mixtures. Still changes in the range of 0.3–0.5‰ can be interpreted as relevant isotope effects, and this is within the range of the precision of δ^{13}C value measurements. Therefore, it is difficult to unequivocally identify isotope effects with POPs. The following studies are also examined in this regard.

32.3.3.1 Anaerobic Transformation of a PCB Congener

Drenzek et al. (2001) studied the transformation of 2,3,4,5-tetrachlorobiphenyl (PCB 61) by dehalogenating bacteria. PCB 61 is not present in any technical PCB mixture, and errors (e.g. from lab background) can be excluded. After 90 days, approximately 90% of the PCB 61 was converted into 2,3,5-trichlorobiphenyl (PCB 23) with no other products being detected (Drenzek et al. 2001). The δ^{13}C value of reactant and product were relatively constant throughout the reaction. For the reactant PCB 61 a δ^{13}C value of −28.2‰ was observed while the product PCB 23 had a δ^{13}C value of −27.8‰ (Drenzek et al. 2001). The average difference in the δ^{13}C value of reactant and product was 0.3‰ and significant at 95% but not at 98% confidence level (Drenzek et al. 2001). As pointed out by Drenzek et al. (2001), the δ^{13}C value was in the range of the uncertainty of δ^{13}C value determinations of 0.5‰ for polyhalogenated compounds. This assessment by the authors is further underscored by the direction of the potential development of the δ^{13}C value. In reactions of the type shown in (32.4), the δ^{13}C value of the reactant should become heavier than that of the product due to the higher strength of the bonds with heavy isotopes.

32.3.3.2 Light-Induced Transformation of Polybrominated Diphenyl Ethers (PBDEs)

Rosenfelder et al. (2010) irradiated BDE 47 and technical DE-79 by means of a sunlight simulator. In either case, remarkable isotope effects were observed for both the reactants and the products. BDE 47 was irradiated for 110 and 160 min, respectively, and the reactant (BDE 47) and the predominant products (BDE 28, BDE 15) were monitored:

$$\begin{aligned}&\text{BDE}\,47\,(2,2',4,4'\text{ - TetraBDE})\\&\quad\rightarrow \text{BDE}\,28\,(2,4,4'\text{ - TriBDE})\\&\quad\rightarrow \text{BDE}\,15\,(4,4'\text{ - DiBDE})\end{aligned}$$

After 110 and 160 min of irradiation, BDE 47 became enriched in ^{13}C by 1.8 and 3.1‰, respectively. After 110 min, BDE 28 and BDE 15 became depleted in ^{13}C by 0.1 and 2.2‰, respectively, compared to the initial value for BDE 47. After 160 min, BDE 28 became heavier by 0.3‰ and BDE 15 by 0.5‰ (Rosenfelder et al. 2010). This course of the reaction can be explained as follows: The average δ^{13}C value (i.e. the bulk δ^{13}C value) has to be retained in this closed system. Because of the stronger bonds between heavier isotopes, BDE 47 must become permanently heavier, and this was observed. At the start, when only BDE 28 was formed, this product (BDE 28) compensated for this by becoming lighter. However, BDE 28 was not only formed from BDE 47 but also converted into BDE 15. In turn, the heavier isotope will react less, so that BDE 28 became heavier in carbon while the resulting BDE 15 became lighter. Between 110 and 160 min, BDE 15 became heavier in carbon (but still was much lighter than BDE 47 at start) which may look odd. However, we must take into account that BDE 15 is much more persistent than BDE 28 (and BDE 47). Thus, comparably more BDE 28 will be converted into BDE 15, and BDE 28 became heavier between 110 and 160 min (Rosenfelder et al. 2010). The strong isotope effect observed in both reactant and products is remarkable because it completely differed from the anaerobic transformation of PCBs (see Sect. 32.3.3.1). Between start and 110 min of irradiation, BDE 47 became heavier in carbon by 1.8‰. This effect must solely go back to changes on C-2 from which the bromine is removed. Due to the symmetrical substitution of BDE 47, this carbon represents 1/6 of the molecule carbon. Thus, the isotope effect at C-2 would be 1.8 × 6 = 10.6‰, by which this isotopomer became heavier compared to its starting value. This can be considered as a severe isotope effect (see above). This was mostly at the cost of BDE 15, whose C-2 carbon(s) became depleted in ^{13}C to the same extend.

More complex interactions were observed when DE-79 was UV-irradiated for 80 min (Table 32.3) (Rosenfelder et al. 2010). During UV/vis-irradiation with simulated sunlight, the δ^{13}C values of the major compounds BDE 183 and BDE 197 became enriched in ^{13}C. By contrast, the newly formed Tri- to PentaBDEs became depleted in ^{13}C. As a consequence, in the solution at start (i.e. the technical product), the

Table 32.3 δ^{13}C (‰) values of different BDE congeners (mean and standard deviation) in the technical products DE-71and DE-79 as well as during the UV/vis-irradiation of DE-79 (Rosenfelder et al. 2010)

	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 197
DE-71	n.d.	−27.1 (±0.3) (*n* = 8)	−28.9 (±0.3) (*n* = 9)	−29.8 (±0.2) (*n* = 8)	−30.4 (±1.1) (*n* = 9)	−32.4 (±1.1) (*n* = 2)	n.d.	–
DE-79	–	–	–	–	−26.8 (±0.4) (*n* = 2)	−26.6 (±0.1) (*n* = 6)	−29.8 (±0.2) (*n* = 6)	−30.8 (±0.1) (*n* = 4)
DE-79 (UV/vis, 80 min)	−30.0 (±0.1) (*n* = 4)	−28.6 (±0.5) (*n* = 4)	−29.0 (±0.1) (*n* = 3)	−27.6 (±0.6) (*n* = 4)	−28.1 (±1.0) (*n* = 4)	−25.5 (±0.3) (*n* = 4)	−25.6 (±0.4) (*n* = 4)	n.d.

n Repetitive injections (Nalimov outliers were eliminated); *n.d.* not detected

$\delta^{13}C$ values *decreased* with increasing degree of bromination from −26.8 to −30.8‰ while after the irradiation they *increased* with increasing degree of bromination from −30.0 to −25.6‰ (Table 32.3). If the decisive reactions of the reductive debromination of high-brominated BDE congeners are accompanied with similar isotope effects, the $\delta^{13}C$ values of BDE 47, BDE 99, and BDE 153 in environmental samples can be used to elucidate their origins: if these $\delta^{13}C$ values become lighter in this order, the BDE residues would originate mainly from DE-71, if they become heavier in this order, they would originate from the transformation of higher brominated BDE congeners (Rosenfelder et al. 2010).

32.3.3.3 Isotope Analysis of Polychlorinated Dibenzo-*p*-Dioxins (PCDDs)

Ewald et al. (2007) studied the microbial degradation of two PCDD standards (1,2,4-TriCDD and 1,2,3-TriCDD) with *Dehalococcoides*. In both occasions chlorine was removed at the position flanked by the most electronegative substituents (i.e. position 1 in 1,2,4-TriCDD and position 2 in 1,2,3-TriCDD). For both reactants, the product is 1,3-DiCDD. The same principle led to the next dehalogenation product in form of 2-MonoCDD. The latter, 2-MonoCDD, was trapped by headspace SPME (Ewald et al. 2007). This process is not quantitative but simplified the sample clean-up (Ewald et al. 2007). Tests with standard compounds verified that the headspace SPME procedure had only a minor effect on the $\delta^{13}C$ value. The sterile control showed no alteration in concentration or $\delta^{13}C$ value. In addition, 1,2,3-TriCDD was enriched in $\delta^{13}C$ by 1.6‰. In the case of 1,2,3-TriCDD the alteration must go back to C-2, which is only one out of eight carbons. Consequently, the C-2 carbon would have become heavier by 12.8‰ (see discussion above on dilution effects). This has to be considered as a severe isotope effect at this position. This interpretation differs from that given by Ewald et al. (2007). No isotope effect could be identified for 1,2,4-TriCDD. However, the end product 2-MCDD was lighter in carbon once it was formed but became gradually enriched in ^{13}C throughout the reaction. This would point towards a much higher stability compared to the TriCDDs and 1,3-DiCDD.

The Rayleigh model (Sect. 32.2.3) was applied to 1,2,3-TriCDD. The fractionation factor α (see (32.3), Sect. 32.2.3) of the reaction was 1.00092 and thus one order of magnitude lower than in the case of TCE (Nijenhuis et al. 2005). However, when the dilution effect (Ewald et al. 2007) is eliminated, the actual effect on the carbon involved was almost comparable to that observed in the transformation of TCE (Nijenhuis et al. 2005). In fact, the authors concluded that isotope data will be a suitable parameter to study the PCDD degradation in field (Ewald et al. 2007). The approach was further expanded by transformation experiments with 1,2,3,4-tetrachlorodibenzo-*p*-dioxin which was converted into 1,2,4-TrCDD and 1,3-DCDD (Liu et al. 2010). The results confirmed carbon isotope fractionation during sequential reductive dechlorination of PCDDs (Liu et al. 2010).

Horii et al. (2008) measured OCDD from different sources and found discrete ranges in the $\delta^{13}C$ value (Fig. 32.6). Compared to the standards analyzed ($\delta^{13}C$ value ~ −30‰), OCDD was enriched in ^{13}C in fly ash and ball clay samples while it was lighter in OCDD from PCP. The $\delta^{13}C$ values of OCDD in sediment and soil spanned over a wider range than the standards measured (Fig. 32.6). The difference between the classic OCDD sources (PCP and fly ash) was about 10‰. The sample size was not suited to generalize these values in a global content, and, as stated by Horii et al. (2008), further OCDD sources have to be taken into account in future. However, the observed

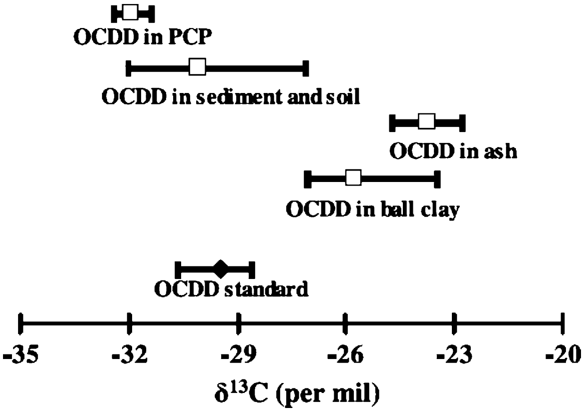

Fig. 32.6 Mean and range of $\delta^{13}C$ values of OCDD in ball clay and anthropogenic source materials, along with OCDD standards. The $\delta^{13}C$ value of OCDD in each matrix were 25.8‰ for clay, 30.1‰ for soil and sediment, 23.7‰ for fly ash, 31.9‰ for PCP, and 29.5‰ for OCDD standard (Horii et al. 2008)

differences in the δ^{13}C value are very promising especially also with regard to different modes new formation and degradation reactions which permanently may change or dilute isotope effects. Again it may be re-called that transformation of the reactant OCDD would lead to a permanent enrichment of ^{13}C (e.g. in soil or sediment). Consequently, the authors also suggested that there is a chance to distinguish naturally produced OCDD from anthropogenic OCDD (Horii et al. 2008). Natural formation of OCDD in kaolin clay has been suggested by Rappe et al. (2001) and Gaus et al. (2002).

32.3.3.4 Isotope Analysis of the Bacterial Degradation of Lindane

Badea et al. (2009) studied the degradation of lindane by sulphate-reducing bacteria (*Desulfococcus multivorans* and *Desulfovibrio gigas*) using the Rayleigh model approach in combination with stable carbon isotope analysis. The bacteria were found to transform lindane as follows: lindane → 3,4,5,6-tetrachlorocyclohexene → 5,6-dichlorocyclohexa-1,3-diene → benzene/chlorobenzene (Badea et al. 2009). In all experiments with living cells the δ^{13}C value of lindane was changed during the dehalogenation (Badea et al. 2009). With *D. gigas*, lindane became enriched in ^{13}C with time (from −27.6‰ at start to −16.4‰). In this period, 95% of the lindane was consumed. After 6 days, chlorobenzene was detected with an initial δ^{13}C value of −29.6‰. At the end of the experiment the chlorobenzene became heavier and it ended with a δ^{13}C value of −26‰ which is slightly heavier than the starting material. The results obtained with *D. multivorans* were similar (Badea et al. 2009). Unfortunately, further intermediate or dead-end metabolites could not be studied, so that a mass balance could not be established. However, the data was used to calculate the isotope enrichment factor ε (Fig. 32.7).

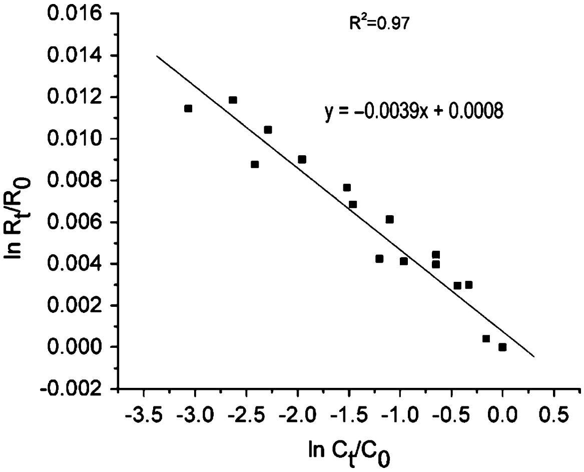

Fig. 32.7 Quantitative assessment of isotope fractionation of lindane in dechlorination experiments with *D. gigas* using the Rayleigh model using the bulk enrichment factor ε = −3.9 (Badea et al. 2009)

32.3.3.5 CSIA of Toxaphene Congeners in Technical Mixtures and Antarctic Seals

The multicomponent mixture toxaphene (Sect. 32.3.1.2) cannot be separated even by MDGC. Therefore, the δ^{13}C value of individual compounds in the technical product cannot be determined by GC-IRMS but have to be isolated and enriched. Vetter et al. (2006b) used HPLC to isolate the main toxaphene pollutants 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10, 10-octachlorobornane (B8-1413) and 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane (B9-1679) from two technical products and an extract of Antarctic Weddell seal (*Leptonychotes wedelli*). The δ^{13}C value of B8-1413 isolate from Melipax varied by 2‰ in the replicates, and only one isolate from Toxaphene could be analyzed, the uncertainty was rather high. The latter was lighter and this agreed with determinations of the bulk value (see above). The value of B8-1413 in the Weddell seal sample fell between the two technical products (Vetter et al. 2006b). Still, the δ^{13}C value of the compounds in the seal was in proximity to the technical products. This is interesting because the toxaphene congeners were likely long-range transported to Antarctica (which could be associated with isotope fractionation due to different volatilities and with UV transformation). In addition, they were bioaccumulated in the food chain and potentially transformed in these bodies since Weddell seals are top predators mainly feeding on fish. Although it is possible that these effects averaged out each other (distribution due to volatilization should result in depletion of ^{13}C), the rather unaltered δ^{13}C value points to rather little changes in the δ^{13}C values of the persistent toxaphene congeners in the environment (Vetter et al. 2006b). The absence of measureable isotope effects was also reported for PCBs (Reddy et al. 2002a) whereas lindane (Badea et al. 2009) and BDEs (Rosenfelder et al. 2010) showed such an isotope effect.

32.3.4 Hydrogen Isotope Analysis of POPs

Armbruster et al. (2006) and Vetter et al. (2006a) published bulk δ^2H values of about 50 POPs. The bulk δ^2H values ranged from −230 to +75‰. These values clearly exceeded the range reported for petrol compounds at both ends. The positive δ^2H values were particularly surprising. The chlorination of camphene gave first indications as to how such values are generated. Starting from camphene (δ^2H value −325‰), ^{2}H was enriched with the increase in the product chlorination (bulk δ^2H values −233‰ for low-chlorinated camphene → −184‰ for medium-chlorinated camphene → −96‰ for high-chlorinated camphene) (Vetter et al. 2006a). In agreement with the theory, the heavier deuterium reacted slower and more ^{1}H was substituted with chlorine. SNIF-NMR was used to study the synthesis of toxaphene in the laboratory (Nikiforov et al. 2005). Starting from α-pinene, the different positions involved in the rearrangement to bornylchloride and the subsequent halogenation was studied. KIE of two were found during the chlorination accompanied with an enrichment of deuterium in the products (Nikiforov et al. 2005).

The bulk δ^2H values of the North American and the East German toxaphenes differed by 80‰. This has most likely its origin in the monoterpene(s) used for the chlorination. An explanation could be that the US toxaphene plant is closer to the ocean and this would acquire a higher deuterium content in the reactant from this site (Armbruster et al. 2006). Aside from these samples mainly analytical standards were determined so far and it is difficult to evaluate these samples results because lack of experimental background data. However, two lindane samples showed bulk δ^2H values of −70 and −110‰ while the δ^{13}C values were almost identical (Armbruster et al. 2006). From these results it may be concluded that the combination of carbon and hydrogen isotope data will be more effective in tracking sources and pathways of POPs.

The δ^2H values 2,4-dibromophenol and 2,4-dibromoanisole are difficult to compare, since the phenolic H is arbitrarily in the molecule. Since this OH is also determined by IRMS, the bulk δ^2H value of both compounds cannot be compared. By contrast, using SNIF-NMR the proportions of isotopomers can be determined (Vetter et al. 2006a). Evaluation of only the aromatic hydrogens demonstrated that deuterium of H-6 was enriched relative to H-5 and H-3 in 2,4-dibromophenol but not in 2,4-dibromoanisole (Fig. 32.4) (Vetter et al. 2006a). The isotopomer abundances can be linked to δ^2H values (only for the aromatic system), the δ^2H values of the individual isotopomers in 2,4-dibromophenol ranged from −122 to +220‰ while they ranged from −46 to +17‰ in 2,4-dibromoanisole (Augusti et al. 2009). These ratios helped to verify that the anisole was not formed from the present phenol. Furthermore, they clearly show how much the δ^2H value of a reactant can be changed by the substitution of just one hydrogen atom with chlorine.

32.4 Current Limitations and Future Perspectives

It is the concentrations of POPs in the environment that are of major concern since the days of Paracelsus, as higher concentrations hazardous to humans and the environment. For understanding the fate of pollutants (transport, metabolism, accumulation etc.), a second, independent parameter has to be evaluated as well. Suitable options for this purpose are physico-chemical data and models that take advantage of them. Other options are enantioselective data if the pollutant is chiral. Another, particularly promising tool in the field of POPs is stable isotope analysis. Several interesting findings have been elaborated so far but the benefits IRMS and SNIF-NMR may add to the field have not been exhaustively exploited. The major reason for this is that applications in POP research are still far from being a routine method. A range of instrumental and theoretical unknowns should be explored in order to bring also IRMS full to fruition in POP research.

Based on existing equipment, a better lab-to-lab intercalibration of the protocols for EA-IRMS to CSIA-IRMS for the measurements of δ^{13}C values of POPs would be a large step forward for the usefulness of this technique. The generation of a widely available standard reference material (SRM) for polyhalogenated compounds would be useful in order to verify inter-comparability of $\delta^{13}C$ values. Currently, only a few groups are involved in stable isotope research of POPs, and a better precision would attract more researchers into the field.

It is very likely that the combination of multiple isotopic data will improve the quality of the assessment of POPs. Clearly, bulk isotope data were important in the beginning of IRMS research on POPs and still may add important aspects to the field. However, real progress can only be made when IRMS is coupled with a chromatographic separation system. In POP research, the choice will be GC given the complexity of the samples. In addition to $\delta^{13}C$ values it would be required to study the $\delta^{2}H$ values of POPs by GC-IRMS. A step forward in this direction was made by Chartrand et al. (2007) in the field of VOCs. Generally, the state of the art in the use of stable isotope analysis of water contaminants (VOCs) is much more developed compared to POPs and the articles published in this field (see Sherwood Lollar et al. 2001; Schmidt et al. 2004; Elsner et al. 2005) should be reviewed as well by those who wish to switch to IRMS in POP research. Moreover, it would be valuable to have $\delta^{37}Cl$ values and $\delta^{81}Br$ values at hand. Very promising bulk $\delta^{37}Cl$ value analyses (see the paper of Sturchio et al. (2011) for general aspects of chlorine isotopes) of technical PCB mixtures, PCB extracted from sediment, and of DDT transformation experiments have been carried out by Reddy et al. (2000, 2002b). These methods cannot be linked with a GC interface but, very recently, Aeppli et al. (2010) presented a method for the direct determination of $\delta^{37}Cl$ values of organochlorines by using benchtop gas chromatography-quadrupole mass spectrometry instruments. Moreover, Holmstrand et al. (2010) used GC interfaced to inductively coupled plasma mass spectrometry (GC/ICP-MS) for the compound-specific determination of $\delta^{81}Br$ values which was used for the analysis of PBDEs. Stable halogen isotope analysis will for certain add valuable facets to the field. Likewise, it would be important to generate more data on isotopomer distributions. Currently, only quantitative ^{2}H NMR is suited for such investigations with POPs. However, it would be desirable that quantitative isotopomer analysis ^{13}C NMR would become available as well. There is a chance that suitable methods will be introduced within this decade, and it is evident that they will be suited to bring the theoretical understanding of carbon isotopomer distributions of POPs and many other compounds on a higher level. This will surely improve the theoretical understanding with regard to the interpretation of KIE of POPs. Even without isotopomer distributions, IRMS data can be used to model the transformation rates of pollutants.

Finally, the *polluter pays* principle is getting more and more relevant in the field of POPs. A producer will faster reconsider the usefulness of products when analysts are able to trace back residues in an environmental or food sample to a specific product manufactured in a specific plant. Again, IRMS will be the most suited technique to provide such proof. The future has begun.

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