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Evaluating Chlorine Isotope Effects from Isotope Ratios and Mass Spectra of Polychlorinated Molecules

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Compound-specific chlorine isotope analysis receives much interest to assess the fate of chlorinated hydrocarbons in contaminated environments. This paper provides a theoretical basis to calculate isotope ratios and quantify isotope fractionation from ion−current ratios of molecular- and fragment-ion multiplets. Because both ^{35}Cl and ^{37}Cl are of high abundance, polychlorinated hydrocarbons consist of molecules containing different numbers of ^{37}Cl denoted as isotopologues. We show that, during reactions, the changes in isotopologue ratios are proportional to changes in the isotope ratio assuming a nonselective isotope distribution in the initial compound. This proportionality extents even to fragments formed in the ion source of a mass spectrometer such as C_2Cl_2 (double dechlorinated fragment of perchloroethylene, PCE). Fractionation factors and kinetic isotope effects (KIE) may, therefore, be evaluated from isotope, isotopologue or even fragment ratios according to conventional simple equations. The proportionality is exact with symmetric molecules such as dichloroethylene (DCE) and PCE, whereas it is approximately true with molecules containing nonreactive positions such as trichloroethylene (TCE). If in the latter case isotope ratios are derived from dechlorinated fragments, e.g., C_2HCl_2, it is important that fragmentation in the ion source affect all molecular positions alike, as otherwise isotopic changes in reactive positions may be underrepresented.

Compound-specific stable isotope analysis of carbon, hydrogen and other elements is increasingly used to differentiate between different sources of the same environmental organic contaminant and to identify and possibly quantify contaminant transformation processes based on a characteristic isotope fractionation during the process.[1–4] Because chlorinated hydrocarbons are important environmental pollutants, much interest is directed toward using also chlorine isotope analysis.[5–14] Evaluation of chlorine isotope fractionation in previous studies, however, has been inconsistent. Whereas in some studies a simple Rayleigh model was applied,[8,14–16] others relied on more complicated relationships,[10] and others still did not derive fractionation factors from substrate data, but from isotopic branching in the products (e.g., between DDE and chloride in dehydrohalogenation of DDT).[11,13] Also, whereas previous studies involved time-consuming off-line sample preparation to obtain singly chlorinated chemical species such as CH_3Cl or CsCl for isotope analysis,[17–23] very recently, the first instrumental methods for online compound-specific chlorine isotope analysis have been presented. Mass spectrometers are online coupled to the effluent of a GC-column, either *via* an inductively coupled plasma[24] or without conversion by direct transfer.[25,26]

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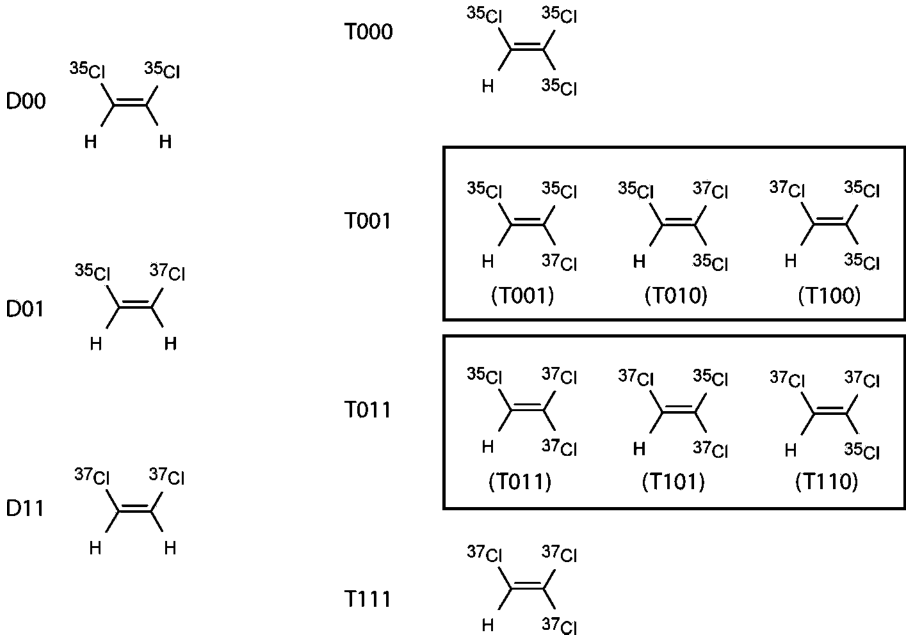

Figure 1. Isotopologues of *cis*-DCE and TCE, together with the short notation used in this paper. While *cis*-DCE contains only indistinguishable reactive positions, positions in TCE may be reactive or not. Isotopomers of the same TCE isotopologue are given in boxes, where brackets in the short notation indicate position-specificity.

How Determine Isotope Ratios from Isotopologue Data? In the latter case, organic target compounds like dichloroethylene (DCE), trichloroethylene (TCE) or perchloroethylene (PCE) are no longer converted into CH_3Cl prior to chlorine isotope analysis. Instead, ions with multiple chlorine atoms are analyzed directly such as $C_2H_2Cl_2$ (molecular ion of DCE), C_2HCl_2 (single dechlorinated fragment ion of TCE) or C_2Cl_2 (double dechlorinated fragment ion of PCE).[25,26] Such molecules or fragments that contain different numbers of heavy and light isotopes inside their structure are denoted as isotopologues (Figure 1). Specifically, if a compound has two or more chlorine substitutents, three or more possible isotopologues occur (Figure 1). To obtain accurate isotope ratios, ideally, the abundance of all isotopologues should be determined. In reported methods,[25,26] however, isotope ratios were not calculated from the total mass balance, but were inferred from only the most abundant isotopologue pair. In this case the isotopologue distribution pattern must be known for calculations. Sakaguchi-Söder et al. assumed a nonselective isotope distribution to derive an algorithm for calculation of $^{37}Cl/^{35}Cl$ ratios.[26] It is presently not well established whether this approach remains valid when isotope data is measured during a fractionating (bio)chemical process where the abundance of isotopologues relative to each other changes. Even more important, a theoretical basis is missing to infer such isotopologue ratios from fragment ratios generated in the ion source of a mass spectrometer.

How Evaluate Isotope Effects from Average Isotope Data? Another prevalent knowledge gap concerns the calculation of isotope fractionation factors and kinetic isotope effects (KIE) values from average chlorine isotope ratios. The term fractionation commonly describes an uneven distribution of isotopes in different fractions during a physicochemical process such as in substrate and product of a reaction. Isotopes in organic substances, however, do not occur isolated but are contained inside molecules. Competitive discrimination caused by physicochemical processes therefore does not occur between isotopes, but between such different molecules (i.e., isotopologues) which carry the isotopes along. Consequently, the term "fractionation" in the context of this paper is used to describe a fundamental competitive discrimination between any two species (e.g., atoms, isotopes, ions, molecules) for which the Rayleigh equation in its original form can be derived:[27]

$$\frac{\text{Isotopologue 1}}{\text{Isotopologue 1}_0} = \left(\frac{\text{Isotopologue 2}}{\text{Isotopologue 2}_0}\right)^{\alpha} \tag{1}$$

$$\frac{\text{Isotopologue 1}}{\text{Isotopologue 1}_0} \Big/ \frac{\text{Isotopologue 2}}{\text{Isotopologue 2}_0} = \left(\frac{\text{Isotopologue 2}}{\text{Isotopologue 2}_0}\right)^{\alpha-1} = \left(\frac{\text{Isotopologue 2}}{\text{Isotopologue 2}_0}\right)^{\varepsilon/1000} \tag{2}$$

where subscripts "0" indicate concentrations at the beginning of the fractionating process, α is the fractionation factor and ϵ is the enrichment factor in permil.

Given that fractionation takes place between isotopologues rather than isotopes, analytical methods targeting isotopologues are actually favorable to quantify isotope fractionation. However, most analytical methods measure average isotope ratios by converting the organic compounds into simple gases like CO_2 (for $^{13}C/^{12}C$ analysis), H_2 (for $^{2}H/^{1}H$ analysis) and CH_3Cl (for $^{37}Cl/^{35}Cl$ analysis) [17–19] or Cl^- ions.[21,23,24] This raises the question whether such average isotope data can still be evaluated according to the Rayleigh equation.

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For elements with a low abundance of the heavy isotope, such as for carbon and hydrogen, the mathematical treatment can be simplified because isotopologues with more than one heavy isotope are rare and therefore can be neglected. For this case, it was shown that even though fractionation according to eqs 1 and 2 takes place between isotopologues and not isotopes, the average isotope data can still in very good approximation be described by the Rayleigh equation.[4] Moreover, it was shown that even position specific kinetic isotope effects can be estimated provided that measured average ("bulk") isotope data is subjected to an adequate correction for the nonfractionating isotopomers (i.e, nonreacting positions) and that intramolecular competition between indistinguishable reactive positions is subsequently taken into account.[4] In the case of chlorine, however, the heavy isotope is present at a much higher abundance and consequently isotopologues with multiple heavy isotopes occur. Although the Rayleigh equation has repeatedly been applied to evaluate average chlorine isotope ratios,[8,11,15,16] its use is presently not based on a solid theoretical understanding and it is not clear how KIE values can be extracted.

In summary, the measurement of isotopologue ratios allows direct determination of Rayleigh fractionation factors, but may be difficult to convert into isotope ratios. Vice versa, traditional determinations of chlorine isotope ratios allow reliable interlaboratory comparisons, but necessitate the theoretical justification to apply the Rayleigh equation. Theoretical relationships are much needed that link isotope ratios, isotopologue ratios, their observable fractionation and position-specific isotope effects. It is the aim of this paper to present such a framework for evaluating chlorine isotope data obtained by different methods and to generally lay the basis for evaluation of compound-specific chlorine isotope data in future applications. The initial part discusses the behavior of different chlorinated isotopologues during fractionation processes and the relationship between isotopologue and isotope ratios. In a second part, based on these relationships, it is evaluated how isotope ratios, fractionation factors and position-specific isotope effects can be derived from measured data.

PART 1: EVOLUTION OF ISOTOPOLOGUE AND ISOTOPE RATIOS DURING REACTIVE PROCESSES

In analogy to the reaction of carbon and hydrogen atoms in organic molecules[4] two different types of chlorine atoms can be distinguished when considering the initial transformation of polychlorinated molecules: chlorine atoms in bonds that may be involved in the initial transformation step (denoted as reacting positions) and atoms in bonds that do not take part in the initial transformation (nonreacting positions). Chlorine atoms in symmetric molecules occur in identical reacting positions and are in intramolecular competition when they engage in reactions. Examples are dechlorination of PCE, DCE (Figure 1), 1,2-dichloroethane (1,2-DCA) or 1,1,1-trichloroethane (1,1,1-TCA). Nonsymmetric molecules like trichloroethylene, in contrast, have molecular positions that are not identical (Figure 1). If a certain transformation reaction involves only one of them, this position must be considered reactive, while the others are nonreactive. Consequently, for a given nonsymmetric molecule, the division into reacting and nonreacting positions varies depending on the reaction mechanism considered. In the following, the evolution of isotopologue and isotope ratios is first discussed for molecules with all chlorine atoms at reacting positions, starting with the simplest case DCE and moving on to PCE. Subsequently, TCE is considered as an example of a molecule with chlorine in nonreacting positions.

Chlorine at Reacting Positions. DCE is composed of three different isotopologues with respect to chlorine isotopes, those with two ^{35}Cl (denoted as D00), those with one ^{35}Cl and one ^{37}Cl (denoted as D01) and those with two ^{37}Cl (denoted as D11) (Figure 1). These isotopologues are expected to react independently and to follow pairwise a Rayleigh enrichment trend according to

$$\frac{\mathrm{D01}}{\mathrm{D01}_0} = \left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\alpha_1} \tag{3}$$

$$\frac{\mathrm{D11}}{\mathrm{D11}_0} = \left(\frac{\mathrm{D01}}{\mathrm{D01}_0}\right)^{\alpha_1} \tag{4}$$

or

$$\frac{\mathrm{D01}/\mathrm{D01}_0}{\mathrm{D00}/\mathrm{D00}_0} = \left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{(\alpha_1-1)} = \left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\varepsilon_1/1000} \tag{5}$$

$$\frac{\mathrm{D11}/\mathrm{D11}_0}{\mathrm{D01}/\mathrm{D01}_0} = \left(\frac{\mathrm{D01}}{\mathrm{D01}_0}\right)^{(\alpha_2-1)} = \left(\frac{\mathrm{D01}}{\mathrm{D01}_0}\right)^{\varepsilon_2/1000} \tag{6}$$

where α_1, α_2 and ϵ_1, ϵ_2 are fractionation and enrichment factor of the respective isotopologue pair. To illustrate the approach in a general manner, two reaction scenarios may be considered, a reaction involving initially only one of the bonds containing Cl (stepwise reaction) and a mechanism involving both bonds simultaneously (concerted reaction). Here, fractionation for a stepwise reaction is discussed, whereas the concerted case is treated in the Supporting Information.

Stepwise Reaction of Dichloroethene. The evolution of the different isotopologue pairs can be related if the fractionation factors are expressed in terms of the kinetic isotope effect KIE, the most fundamental parameter to characterize isotope fractionation. The KIE corresponds to the ratio of the reaction rates for molecules with ^{35}Cl and ^{37}Cl in the reacting bond, respectively.

$$\mathrm{KIE} = \frac{^{35}k}{^{37}k} \tag{7}$$

Sometimes, kinetic isotope effects may not be fully observable, because biotransformations involve slow nonfractionating steps (transport, binding to enzymes, etc.) so that smaller apparent kinetic isotope effects (AKIE values) are observed. For the clarity of presentation, such effects are not considered in the following discussions. A detailed treatment can be found in the Supporting Information of Elsner et al.[4]

For a stepwise reaction the relationship between fractionation factors, enrichment factors and the KIE is given by

$$\alpha_1 = \frac{(^{37}k + {}^{35}k)/2}{(^{35}k + {}^{35}k)/2} = \frac{1}{2}\cdot\left(\frac{^{37}k}{^{35}k} + 1\right) = \frac{1}{2}\cdot\left(\frac{1}{\mathrm{KIE}} + 1\right) = \frac{1}{2}\cdot\frac{1+\mathrm{KIE}}{\mathrm{KIE}} \tag{8}$$

$$\frac{\varepsilon_1}{1000} = \alpha_1 - 1 = \frac{1}{2}\cdot\left(\frac{1}{\mathrm{KIE}} + 1\right) - 1 = \frac{1}{2}\cdot\left(\frac{1}{\mathrm{KIE}} - 1\right) \tag{9}$$

$$\alpha_2 = \frac{({}^{37}k + {}^{37}k)/2}{({}^{37}k + {}^{35}k)/2} = \frac{2}{1 + \mathrm{KIE}} \tag{10}$$

$$\frac{\varepsilon_2}{1000} = \alpha_2 - 1 = \frac{2}{1 + \mathrm{KIE}} - 1 = \frac{2 - (1 + \mathrm{KIE})}{1 + \mathrm{KIE}} = \frac{1 - \mathrm{KIE}}{1 + \mathrm{KIE}} \tag{11}$$

In these equations, the term $({}^{37}k + {}^{35}k)/2$ describes the overall reaction rate of the isotopologue D01, whereas $({}^{35}k + {}^{35}k)/2$ describes that of the isotopologue D00, etc. The terms are derived by considering that the isotopologue D01, for example, contains a C$-{}^{35}$Cl as well as a C$-{}^{37}$Cl bond. In a stepwise reaction, only one of the two bonds is involved in the initial step so that both bonds take turns in reacting (intramolecular competition) and the overall rate of D01 is composed of ${}^{37}k$ and ${}^{35}k$ to equal parts. In analogy, the reaction rate of D00 is $({}^{35}k + {}^{35}k)/2$, and that of D11, $({}^{37}k + {}^{37}k)/2$. Inserting eq 3 into eq 6 leads to

$$\frac{\mathrm{D11}/\mathrm{D11}_0}{\mathrm{D01}/\mathrm{D01}_0} = \left(\left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\alpha 1}\right)^{(\alpha_2 - 1)} = \left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\alpha 1\cdot(\alpha_2 - 1)} \tag{12}$$

with

$$\alpha_1\cdot(\alpha_2 - 1) = \frac{1}{2}\cdot\frac{1 + \mathrm{KIE}}{\mathrm{KIE}}\cdot\frac{1 - \mathrm{KIE}}{1 + \mathrm{KIE}} = \frac{1}{2}\cdot\frac{1 - \mathrm{KIE}}{\mathrm{KIE}} = \frac{1}{2}\cdot\left(\frac{1}{\mathrm{KIE}} - 1\right) = \alpha_1 - 1 = \frac{\varepsilon_1}{1000} \tag{13}$$

Proportionality of Isotopologue Fractionation. Inserting eq 13 into eq 12, one obtains

$$\frac{\mathrm{D11}/\mathrm{D11}_0}{\mathrm{D01}/\mathrm{D01}_0} = \left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\alpha_1 - 1} = \left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\varepsilon_1/1000} \tag{14}$$

Comparison with eq 5 gives

$$\frac{\mathrm{D01}/\mathrm{D01}_0}{\mathrm{D00}/\mathrm{D00}_0} = \frac{\mathrm{D11}/\mathrm{D11}_0}{\mathrm{D01}/\mathrm{D01}_0} \tag{15}$$

which transforms to

$$\frac{\mathrm{D01}/\mathrm{D00}}{\mathrm{D01}_0/\mathrm{D00}_0} = \frac{\mathrm{D11}/\mathrm{D01}}{\mathrm{D11}_0/\mathrm{D01}_0} \tag{16}$$

or

$$\frac{\mathrm{D01}/\mathrm{D00}}{\mathrm{D11}/\mathrm{D01}} = \frac{\mathrm{D01}_0/\mathrm{D00}_0}{\mathrm{D11}_0/\mathrm{D01}_0} \tag{17}$$

Hence, D11 is enriched relative to D01 in the same way as D01 is enriched relative to D00, a phenomenon that is illustrated in Figure 2 and may be described as proportionality of isotopologue fractionation. In general, all isotopologue pairs that differ by one heavy isotope show the same enrichment trend during a reaction and their ratios to each other remain constant although an overall enrichment of heavy isotopes in the remaining substrate occurs. If a nonselective distribution of isotopes among all positions is assumed, the isotopologue ratios can be related to the isotope ratio according to

$$\frac{\mathrm{D01}_0}{\mathrm{D00}_0} = \frac{2\cdot{}^{37}p_0\cdot{}^{35}p_0\cdot C_{\mathrm{tot},0}}{{}^{35}p_0{}^2\cdot C_{\mathrm{tot},0}} = \frac{2\cdot{}^{37}p_0}{{}^{35}p_0} = 2\cdot R_0 \tag{18}$$

$$\frac{\mathrm{D11}_0}{\mathrm{D01}_0} = \frac{{}^{37}p_0{}^2\cdot C_{\mathrm{tot},0}}{2\cdot{}^{35}p_0\cdot{}^{37}p_0\cdot C_{\mathrm{tot},0}} = \frac{{}^{37}p_0}{2\cdot{}^{35}p_0} = \frac{1}{2}\cdot R_0 \tag{19}$$

where $C_{0,\mathrm{tot}} = {}^{35}\mathrm{Cl}_0 + {}^{37}\mathrm{Cl}_0$ is the total concentration of chlorine atoms at the beginning of the reaction, ${}^{35}p_0$ and ${}^{37}p_0$ are the probabilities of encountering ^{35}Cl and ^{37}Cl, respectively in an arbitrary molecular position of an arbitrary isotopologue, and R_0 is the initial ^{37}Cl/^{35}Cl ratio. A combination of eqs 18 and 19 gives

$$R_0 = \frac{{}^{37}p_0}{{}^{35}p_0} = \frac{1}{2}\cdot\frac{\mathrm{D01}_0}{\mathrm{D00}_0} = 2\cdot\frac{\mathrm{D11}_0}{\mathrm{D01}_0} \tag{20}$$

which is equivalent to

$$\frac{\mathrm{D01}_0}{\mathrm{D00}_0}\Big/\frac{\mathrm{D11}_0}{\mathrm{D01}_0} = 4 \tag{21}$$

According to eq 17, this initial proportion of isotopologue ratios will stay constant throughout the reaction as illustrated for an example of strong isotope fractionation in Figure 2A:

$$\frac{\mathrm{D01}}{\mathrm{D00}}\Big/\frac{\mathrm{D11}}{\mathrm{D01}} = \frac{\mathrm{D01}_0}{\mathrm{D00}_0}\Big/\frac{\mathrm{D11}_0}{\mathrm{D01}_0} = 4 \tag{22}$$

Proportionality of Isotope and Isotopologue Fractionation. Due to the proportionality of isotopologue fractionation, a simple relationship between isotope ratio and isotopologue ratio occurs. Taking into account the number of different chlorine isotopes in the different isotopologues, the isotope ratios can be expressed in terms of isotopologue concentrations according to

$$\frac{{}^{37}\mathrm{Cl}}{{}^{35}\mathrm{Cl}} = \frac{\mathrm{D11}\cdot 2 + \mathrm{D01}}{\mathrm{D00}\cdot 2 + \mathrm{D01}} \tag{23}$$

Introducing the proportionality of isotopologue fractionation (eq 22) into eq 23 results in

$$\frac{{}^{37}\mathrm{Cl}}{{}^{35}\mathrm{Cl}} = \frac{\mathrm{D11}\cdot 2 + \mathrm{D01}}{\mathrm{D00}\cdot 2 + \mathrm{D01}} = \frac{2\cdot\frac{\mathrm{D11}}{\mathrm{D01}} + 1}{2\cdot\frac{\mathrm{D00}}{\mathrm{D01}} + 1} = \frac{2\cdot\frac{\mathrm{D01}}{4\cdot\mathrm{D00}} + 1}{\frac{2\cdot\mathrm{D00}}{\mathrm{D01}} + 1} = \frac{\frac{\mathrm{D01} + 2\cdot\mathrm{D00}}{2\cdot\mathrm{D00}}}{\frac{\mathrm{D01} + 2\cdot\mathrm{D00}}{\mathrm{D01}}} = \frac{\mathrm{D01}}{2\cdot\mathrm{D00}} \tag{24}$$

Hence, the relationship of eq 20 that was derived for the beginning of the reaction under the assumption of a nonselective distribution of isotopes over isotopologue positions is quite generally valid, irrespective of the extent of fractionation or the total net isotope ratio.

$$R = \frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}} = \frac{^{37}p}{^{35}p} = \frac{1}{2}\cdot\frac{\mathrm{D01}}{\mathrm{D00}} = 2\cdot\frac{\mathrm{D11}}{\mathrm{D01}} \tag{25}$$

Dividing this equation by eq 20

$$\frac{R}{R_0} = \frac{\mathrm{D01}}{\mathrm{D00}} \Big/ \frac{\mathrm{D01}_0}{\mathrm{D00}_0} = \frac{\mathrm{D11}}{\mathrm{D01}} \Big/ \frac{\mathrm{D11}_0}{\mathrm{D01}_0} \tag{26}$$

demonstrates that the isotopologue ratios are expected to show the same enrichment trend as the isotope ratio as illustrated in Figure 2A, which will be very useful when quantifying isotope fractionation based on different types of data (see Part 2).

Moreover, rephrasing eq 25 in terms of D01/D00 and D11/D01 gives

$$\frac{\mathrm{D01}}{\mathrm{D00}} = \frac{2\cdot {}^{37}p}{^{35}p} = \frac{2\cdot {}^{37}p\cdot {}^{35}p\cdot C_{\mathrm{tot}}}{^{35}p^2\cdot C_{\mathrm{tot}}} \tag{27}$$

and

$$\frac{\mathrm{D11}}{\mathrm{D01}} = \frac{^{37}p}{2\cdot {}^{35}p} = \frac{^{37}p^2\cdot C_{\mathrm{tot}}}{2\cdot {}^{35}p\cdot {}^{37}p\cdot C_{\mathrm{tot}}} \tag{28}$$

demonstrating that the chlorine isotopes remain nonselectively distributed throughout the reaction even though the overall $^{37}\mathrm{Cl}/^{35}\mathrm{Cl}$ ratio steadily increases. Analogous equations apply also for other polychlorinated molecules with indistinguishable reactive positions as demonstrated for PCE in the Supporting Information. Quite generally, we may consider an isotopologue pair for a compound A containing *n* chlorine atoms. One of the isotopologues contains *k* heavy isotopes and, according to our notation, is written as $\mathrm{A}0_{(n-k)}1_{(k)}$. The other contains $(k-1)$ heavy isotopes and is $\mathrm{A}0_{(n-k+1)}1_{(k-1)}$. The relationship between the isotope ratio and the ratio of these isotopologues is then given by

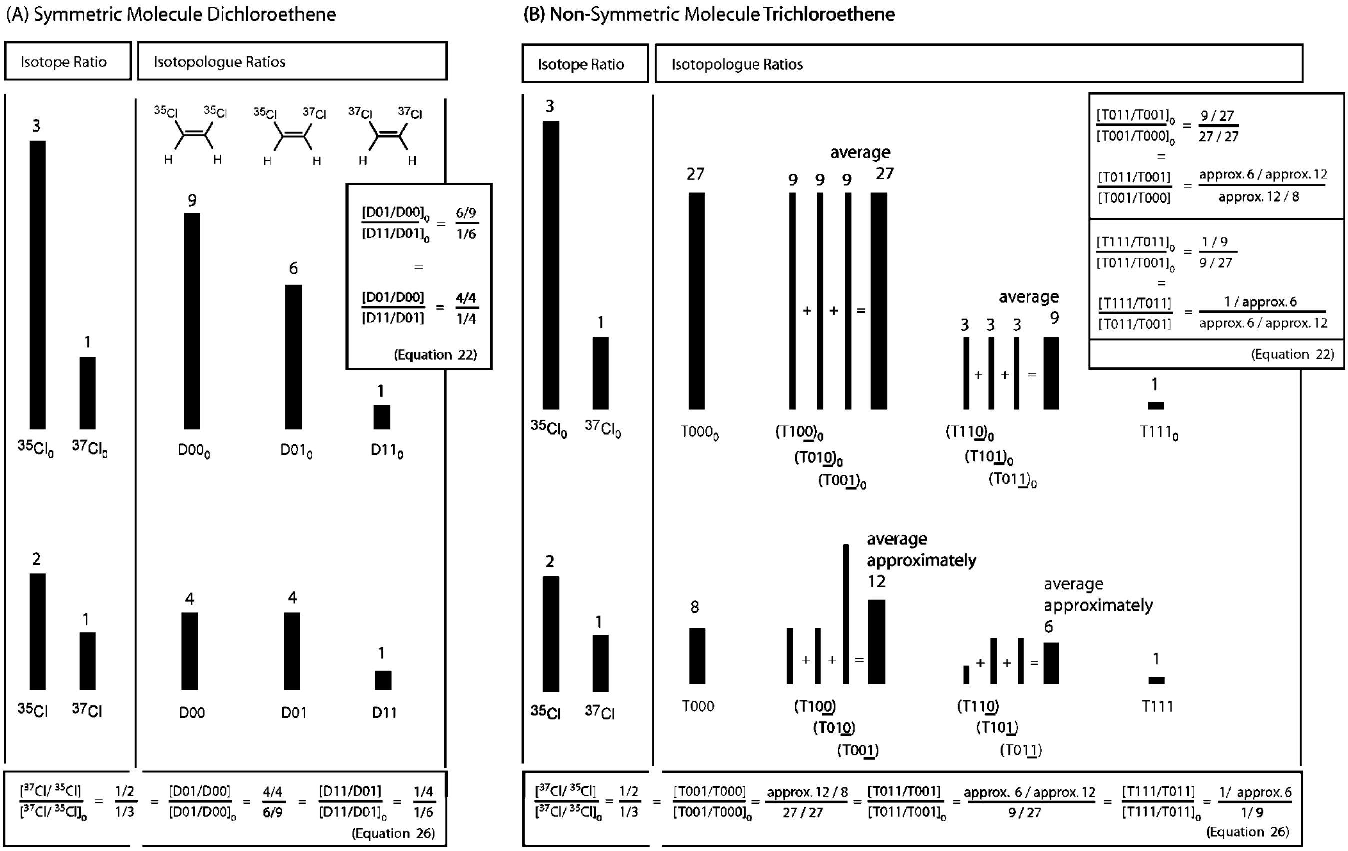

Figure 2. (A) Proportionality of isotopologue fractionation in symmetric molecules illustrated for dichloroethylene in the extreme, hypothetical case of a strongly fractionating reaction. The net isotope ratio of $(^{37}\mathrm{Cl}/^{35}\mathrm{Cl})_0 = 1/3$ (upper part) changes to $^{37}\mathrm{Cl}/^{35}\mathrm{Cl} = 1/2$ (lower part). Concomitantly, dichloroethylene isotopologue ratios change in a way that their proportion relative to each other remains constant. Construction of the figure is based on a nonselective isotope distribution and the isotopologue abundances are normalized to the abundance of the isotopologue with heavy isotopes only. With an initial $(^{37}\mathrm{Cl}/^{35}\mathrm{Cl})_0 = 1/3$, the initial isotopologue distribution corresponds to $\mathrm{D00}_0:\mathrm{D01}_0:\mathrm{D11}_0 = (3/4)^2:(2\cdot 3/4\cdot 1/4):(1/4)^2 = 9:6:1$ while the final distribution is given by $\mathrm{D00}:\mathrm{D01}:\mathrm{D11} = (1/2)^2:(2\cdot 1/2\cdot 1/2):(1/2)^2 = 4:4:1$ for $(^{37}\mathrm{Cl}/^{35}\mathrm{Cl}) = 1/2$. (B) Approximate proportionality of isotopologue fractionation in nonsymmetric molecules. Trichloroethene isotopologue ratios fractionate in a strongly position-specific way. Even though isotopes are therefore no longer nonselectively distributed among the different isotopomers, average isotopologue ratios still remain approximately proportional to each other as outlined in detail in the Supporting Information. The *average* initial and final isotopologue distribution was derived similarly as for DCE. The isotopomer distribution is sketched taking into account that isotopomers with a $^{37}\mathrm{Cl}$ at the underlined reacting position become enriched relative to those with a $^{35}\mathrm{Cl}$ in the underlined position.

$$R=\frac{^{37}p}{^{35}p}=\frac{k}{(n-k+1)}\cdot\frac{\mathrm{A0}_{(n-k)}1_{(k)}}{\mathrm{A0}_{(n-k+1)}1_{(k-1)}} \tag{29}$$

Chlorine at Nonreacting Positions. As discussed above, nonsymmetric molecules may contain isotopes in reactive and nonreactive positions. Using TCE as an example, the isotopologues T000, T001, T011 and T111 can be distinguished (Figure 1). Whereas T000 and T111 are unique, T001 and T011 may contain ^{35}Cl and ^{37}Cl in different molecular positions giving rise to different isotopomers of the same isotopologue. Initially, a nonselective isotope distribution may be assumed (for associated errors see Supporting Information of Elsner et al.[4]). During a reaction, isotope ratios remain constant in the nonreactive positions while heavy isotopes become enriched in the underlined reacting positions. Consequently, if secondary chlorine isotope effects are neglected, (T10$\underline{0}$), (T01$\underline{0}$) and even (T11$\underline{0}$) react at the same rate as T000 (i.e., ^{35}k), whereas (T01$\underline{1}$), (T10$\underline{1}$) and even (T00$\underline{1}$) react at the same rate as T111 (i.e., ^{37}k). Such isotopomers, however, cannot be detected by mass spectrometric methods which measure only average isotopologue ratios (Figure 2B). In the Supporting Information a detailed treatment shows that these measurable average isotopologue ratios follow in good approximation the same proportionality of isotopologue fractionation that is observed for symmetric molecules, even though different isotopomers become selectively enriched or depleted within the same isotopologue group:

$$\frac{\mathrm{T001}/\mathrm{T001}_0}{\mathrm{T000}/\mathrm{T000}_0}=\frac{\mathrm{T011}/\mathrm{T011}_0}{\mathrm{T001}/\mathrm{T001}_0}=\frac{\mathrm{T111}/\mathrm{T111}_0}{\mathrm{T011}/\mathrm{T011}_0} \tag{30}$$

$$\frac{\mathrm{T001}/\mathrm{T000}}{\mathrm{T001}_0/\mathrm{T000}_0}=\frac{\mathrm{T011}/\mathrm{T001}}{\mathrm{T011}_0/\mathrm{T001}_0}=\frac{\mathrm{T111}/\mathrm{T011}}{\mathrm{T111}_0/\mathrm{T011}_0} \tag{31}$$

Furthermore, similarly as for cDCE, the isotopologue ratios are related to the isotope ratio by

$$R=\frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}}=\frac{^{37}p}{^{35}p}=\frac{1}{3}\cdot\frac{\mathrm{T001}}{\mathrm{T000}}=\frac{\mathrm{T011}}{\mathrm{T001}}=3\cdot\frac{\mathrm{T111}}{\mathrm{T011}} \tag{32}$$

In conclusion, our derivations in Part 1 have demonstrated several important principles of chlorine isotope fractionation in polychlorinated hydrocarbons:

(1) Proportionality of Isotopologue Fractionation. If polychlorinated isotopologues react, the relative abundance of heavy isotopologues increases and clearly leads to changes in isotopologue ratios as illustrated in Figure 2A (e.g., from [D01$_0$/D00$_0$] = [6/9] to [D01/D00] = [4/4]). However, these changes occur in a way that the proportion of these ratios remains constant (e.g., [D01$_0$/D00$_0$]/[D11$_0$/D01$_0$] = [6/9]/[1/6] = 4 and [D01/D00]/[D11/D01] = [4/4]/[1/4] = 4). This conclusion is in good approximation valid also for compounds with nonreacting positions as long as the average isotopologue ratios are considered (Figures 2B and S1a) and independent of the initial distribution of isotopes among isotopologues.

(2) Nonselective Distribution of Isotopes among Isotopologues. If symmetric polychlorinated hydrocarbons react and isotope fractionation occurs, isotopes remain nonselectively distributed over the different isotopologues if they are nonselectively distributed at the beginning. Also in the case of nonsymmetric molecules containing nonreactive positions the average isotopologue ratios will seem to follow the same trend, even though isotopes are no longer nonselectively distributed on an isotopomer basis (Figure 2B).

(3) Proportionality of Isotopologue Fractionation and Isotope Fractionation. The nonselective distribution of isotopes implies that also the isotope ratio changes in a constant proportion relative to the changes of the isotopologue ratios. Consequently, isotope fractionation factors and isotope ratios may be derived from any of the isotopologue pairs according to eqs 3 to 6 or eq 25 and 29, even without the need to measure all of them or to determine actual isotope ratios. This aspect is taken up in the Part 2 when discussing new experimental methods of chlorine isotope analysis.

PART 2: QUANTIFICATION OF ISOTOPE FRACTIONATION FACTORS, AKIE VALUES AND ISOTOPE RATIOS

Evaluation Based on Isotope Ratios. As outlined in the introduction, the Rayleigh equation strictly applies to isotopologue rather than isotope ratios. In contrast, traditional analyses transform the compound of interest into methyl chloride followed by its measurement in a dual inlet or continuous flow isotope ratio mass spectrometer relative to a methyl chloride reference gas.[17–19] The question arises whether the measured isotope ratios can be expected to follow a Rayleigh enrichment trend in the same way as it was found for the fractionation of DCE isotopologue pairs:

$$\frac{\mathrm{D01}/\mathrm{D01}_0}{\mathrm{D00}/\mathrm{D00}_0}=\left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{(\alpha_1-1)}=\left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\varepsilon_1/1000} \tag{5}$$

Using the proportionality of isotopologue and isotope fractionation again (eq 26) isotopologue ratios can be substituted by isotope ratios

$$\frac{^{37}\mathrm{Cl}/^{35}\mathrm{Cl}}{^{37}\mathrm{Cl}_0/^{35}\mathrm{Cl}_0}=\left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{(\alpha_1-1)}=\left(\frac{\mathrm{D00}}{\mathrm{D00}_0}\right)^{\varepsilon_1/1000} \tag{33}$$

The total concentration of DCE is given as

$$C_{\mathrm{DCE}}=\mathrm{D00}+\mathrm{D01}+\mathrm{D11}=\mathrm{D00}\cdot\left(1+\frac{\mathrm{D01}}{\mathrm{D00}}+\frac{\mathrm{D01}}{\mathrm{D00}}\cdot\frac{\mathrm{D11}}{\mathrm{D01}}\right) \tag{34}$$

and, with eq 25

$$\mathrm{C}_{\mathrm{DCE}}=\mathrm{D00}+\mathrm{D01}+\mathrm{D11}=\mathrm{D00}\cdot(1+2\cdot R+R^2) \tag{35}$$

The fraction of remaining substrate is, therefore, given by the equation

$$f=\frac{C_{\mathrm{DCE}}}{C_{0,\mathrm{DCE}}}=\frac{\mathrm{D00}\cdot(1+2\cdot R+R^2)}{\mathrm{D00}_0\cdot(1+2\cdot R_0+R_0^{\,2})}\approx\frac{\mathrm{D00}}{\mathrm{D00}_0} \tag{36}$$

The second approximation can be justified by the relatively small shift in R during transformation processes. Using this approximation, eq 33 transforms to

$$\frac{^{37}\mathrm{Cl}/^{35}\mathrm{Cl}}{^{37}\mathrm{Cl}_0/^{35}\mathrm{Cl}_0} = f^{\,(\alpha_1-1)} = f^{\,\varepsilon_1/1000} \qquad (37)$$

Comparison of eqs 5 and 33 illustrates that the isotope ratio evolves in the same way as the ratio between isotopologues with one and no heavy isotopes. Hence, the fractionation factor derived from chlorine isotope data contains the same information as fractionation factors derived from carbon or hydrogen isotope data where only isotopologues with one heavy isotope play a role due to the low abundance of the heavy isotopes. Accordingly the same equations can be used to calculate (A)KIE values from the fractionation factor.[4] For nonsymmetric molecules, this gives the known approximate correction for nonreactive positions

$$\varepsilon_{\text{reacting-position}} \approx \frac{n}{x}\cdot\varepsilon_1 \qquad (38)$$

with n being the total number of atoms inside a molecule of which x are located in reactive positions. Kinetic isotope effects KIE, or apparent kinetic isotope effects AKIE in the case of preceding slow steps,[4] are subsequently obtained according to

$$\mathrm{AKIE} = \frac{1}{1 + z\cdot\varepsilon_{\text{reacting-position}}/1000} \qquad (39)$$

where z is the number of atoms in intramolecular competition. A combination of eqs 38 and 39 gives

$$\mathrm{AKIE} = \frac{1}{1 + z\cdot\frac{n}{x}\cdot\varepsilon_1/1000} \qquad (40)$$

which simplifies if isotope effects are primary and reactions are not concerted, $z = x$, so that eq 40 becomes

$$\mathrm{AKIE} = \frac{1}{1 + n\cdot\varepsilon_1/1000} \qquad (41)$$

Hence, kinetic isotope effects are the more "diluted" in the average enrichment factor the more chlorine substituents a compound has, in the same way as known previously from carbon and hydrogen.[4,28]

Evaluations Based on Isotopologue Ratios. As offline conversion into methyl chloride,[17–19] AgCl[21] or CsCl[23] is time-consuming, very recently methods have been proposed that transfer the chlorinated target molecules after GC separation directly into the ion source of the IRMS or a quadrupole MS.[25,26] The methods do not measure isotope ratios, but, instead, compare isotopologue or isotopologue-fragment ratios.

The GC–IRMS method has the advantage that it measures ion ratios at very high precision. However, the mass range and number of masses that can be measured is limited to the cup configuration of the instrument. Therefore, proposed methods analyze the molecular ion for cDCE ($m/z = 96, 98$) while for TCE and PCE the fragment ion that has lost one or two chlorines is measured (m/z = 95, 97 and m/z = 94, 96, respectively).[25]

The GC–quadrupole MS method is not as precise, but has the advantage that a greater number of masses can be analyzed. To compensate in part for the lower precision of the measurement, it was proposed that, after multiplication by appropriate factors (such as those in eq 29), data from both molecular and fragment multiplets could be used in the calculation of fractionation.[26] This approach relies on the silent assumption of a proportionality of isotopologue fractionation, of which the theoretical justification has been delivered in this paper.

Most critically, however, both methods rely on analysis of isotopologue fragments, and they consider only the fragments with just light chlorine isotopes and with one heavy chlorine isotope neglecting the rest of the isotopologue ion multiplet. These new procedures raise the question how "classical" isotope ratios and fractionation factors can be derived from such truncated multiplet data. In the following, data evaluation is discussed first for molecular ions and then for fragment ions.

Analysis of Molecular Ions. Using *cis*-DCE as an example, the ions with light isotopes only and with one heavy chlorine isotope (i.e., D00 and D01) are considered in the published methods. In case of GC–IRMS, the measurement is carried out relative to a reference gas of the same compound to take into account possible mass discrimination effects during sample treatment and ionization in the ion source of the mass spectrometer. If the isotopologue ratio of the reference gas is known, the isotopologue ratio of the sample can be calculated as follows:

$$^{\text{true}}(\mathrm{D01/D00})_{\text{sample}} = \frac{^{\text{meas}}(\mathrm{D01/D00})_{\text{sample}}}{^{\text{meas}}(\mathrm{D01/D00})_{\text{std}}}\cdot {}^{\text{true}}(\mathrm{D01/D00})_{\text{std}} \qquad (42)$$

The isotope ratio can then be calculated from the obtained isotopologue ion ratios using eq 25, and fractionation factors can be quantified either using eq 37 or taking directly the raw isotopologue ion ratios measured by IRMS or other methods of mass spectrometry yielding sufficiently precise data.

$$\frac{\mathrm{D01/D00}}{\mathrm{D01_0/D00_0}} = \frac{^{\text{true}}(\mathrm{D01/D00})_{\text{sample}}}{^{\text{true}}(\mathrm{D01/D00})_{\text{sample},0}} = \frac{^{\text{meas}}(\mathrm{D01/D00})_{\text{sample}}/^{\text{meas}}(\mathrm{D01/D00})_{\text{std}}}{^{\text{meas}}(\mathrm{D01/D00})_{\text{sample},0}/^{\text{meas}}(\mathrm{D01/D00})_{\text{std}}} = \left(\frac{\mathrm{D00}}{\mathrm{D00_0}}\right)^{(\alpha_1-1)} \qquad (43)$$

The same approach of measuring the molecular ion will work for larger compounds including molecules with nonreacting positions like TCE. A prerequisite, however, is that fragmentation in the ion source of the mass spectrometer affects all three molecular positions of TCE to the same extent, as discussed below for the case of isotopologue fragment ions.

Analysis of Fragment Ions. The following treatment considers first fragments from molecules that contain chlorine at reacting positions only such as PCE. Then examples of nonreacting positions such as in TCE are considered as well. To quantify isotope fractionation, it needs to be known how the measured fragment ion ratios are related to the isotopologue ratio that appears in the Rayleigh equation. Complications may arise mainly from two aspects: (i) During fragmentation, part of the chlorine

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(28) Morasch, B.; Richnow, H. H.; Vieth, A.; Schink, B.; Meckenstock, R. U. *Appl. Environ. Microbiol.* **2004**, *70*, 2935–2940.

atoms are eliminated from the parent compound so that the resulting fragments may not represent the original isotope and isotopologue ratios any more. (ii) In addition to this simple net loss of chlorine atoms, the fragmentation process itself may be associated with isotope fractionation, similarly as during natural reductive dechlorination reactions.

Chlorine at Reacting Positions Only. To investigate how isotopologue ratios are represented by the fragments that have lost one or several chlorine isotopes in the ion source of the mass spectrometer, their fragmentation paths can be tracked back from the original perchloroethylene molecules as illustrated in Figure 3. Figure 3A shows that the measured fragment 00 can be formed by dechlorination of the triple chlorinated fragments 001 or 000 according to

$$\frac{\mathrm{d}00}{\mathrm{d}t} = 1 \cdot {}^{37}k_2 \cdot 001 + 3 \cdot {}^{35}k_2 \cdot 000 \qquad (44)$$

where ${}^{35}k_2$ and ${}^{37}k_2$ are rate constants per reacting bond associated with cleavage of C$-{}^{35}$Cl and C$-{}^{37}$Cl during the second fragmentation step in the mass spectrometer. The statistical factors 1 and 3 take into account that only one bond of the fragment 001 reacts to 00, whereas in 000 reaction of all three bonds leads to the same product. Similarly, fragment ion 01 can be formed from 011 or 001 according to

$$\frac{\mathrm{d}01}{\mathrm{d}t} = 2 \cdot {}^{37}k_2 \cdot 011 + 2 \cdot {}^{35}k_2 \cdot 001 \qquad (45)$$

Combining the equations and assuming for the time being that the formed ions leave the ion source, i.e. that the measured ions represent the instantaneous product, the following expression for the measured fragment ion ratio is obtained:

$$\frac{\mathrm{d}01}{\mathrm{d}00} = \frac{01}{00} = \frac{2 \cdot {}^{37}k_2 \cdot 011 + 2 \cdot {}^{35}k_2 \cdot 001}{1 \cdot {}^{37}k_2 \cdot 001 + 3 \cdot {}^{35}k_2 \cdot 000} = \frac{2 \cdot 011/001 + 2 \cdot \mathrm{KIE}_2}{1 + 3 \cdot \mathrm{KIE}_2 \cdot 000/001} \qquad (46)$$

where $\mathrm{KIE}_2 = {}^{35}k_2/{}^{37}k_2$ is the kinetic isotope effect of the second fragmentation step. In analogy, formation of the parent fragment is given by

$$\frac{001}{000} = \frac{2 \cdot {}^{37}k_1 \cdot \mathrm{P0011} + 3 \cdot {}^{35}k_1 \cdot \mathrm{P0001}}{{}^{37}k_1 \cdot \mathrm{P0001} + 4 \cdot {}^{35}k_1 \cdot \mathrm{P0000}} = \frac{2 \cdot \mathrm{P0011}/\mathrm{P0001} + 3 \cdot \mathrm{KIE}_1}{1 + 4 \cdot \mathrm{KIE}_1 \cdot \mathrm{P0000}/\mathrm{P0001}} \qquad (47)$$

$$\frac{011}{001} = \frac{3 \cdot {}^{37}k_1 \cdot \mathrm{P0111} + 2 \cdot {}^{35}k_1 \cdot \mathrm{P0011}}{2 \cdot {}^{37}k_1 \cdot \mathrm{P0011} + 3 \cdot {}^{35}k_1 \cdot \mathrm{P0001}} = \frac{3 \cdot \mathrm{P0111}/\mathrm{P0011} + 2 \cdot \mathrm{KIE}_1}{2 + 3 \cdot \mathrm{KIE}_1 \cdot \mathrm{P0001}/\mathrm{P0011}} \qquad (48)$$

where $\mathrm{KIE}_1 = {}^{35}k_1/{}^{37}k_1$ is the kinetic isotope effect of the first fragmentation step. For these equations, it is assumed that the ratios 001/000 and 011/001 are relatively constant and are not significantly influenced by further fragmentation or acceleration toward the flight tube of the mass spectrometer. A derivation including these processes under the assumption of a steady state is provided in the Supporting Information. Using the proportionality of isotopologue and isotope fractionation according to eq 29

$$R = \frac{{}^{37}p}{{}^{35}p} = \frac{1}{4} \cdot \frac{\mathrm{P0001}}{\mathrm{P0000}} = \frac{2}{3} \cdot \frac{\mathrm{P0011}}{\mathrm{P0001}} = \frac{3}{2} \cdot \frac{\mathrm{P0111}}{\mathrm{P0011}} = 4 \cdot \frac{\mathrm{P1111}}{\mathrm{P0111}} \qquad (49)$$

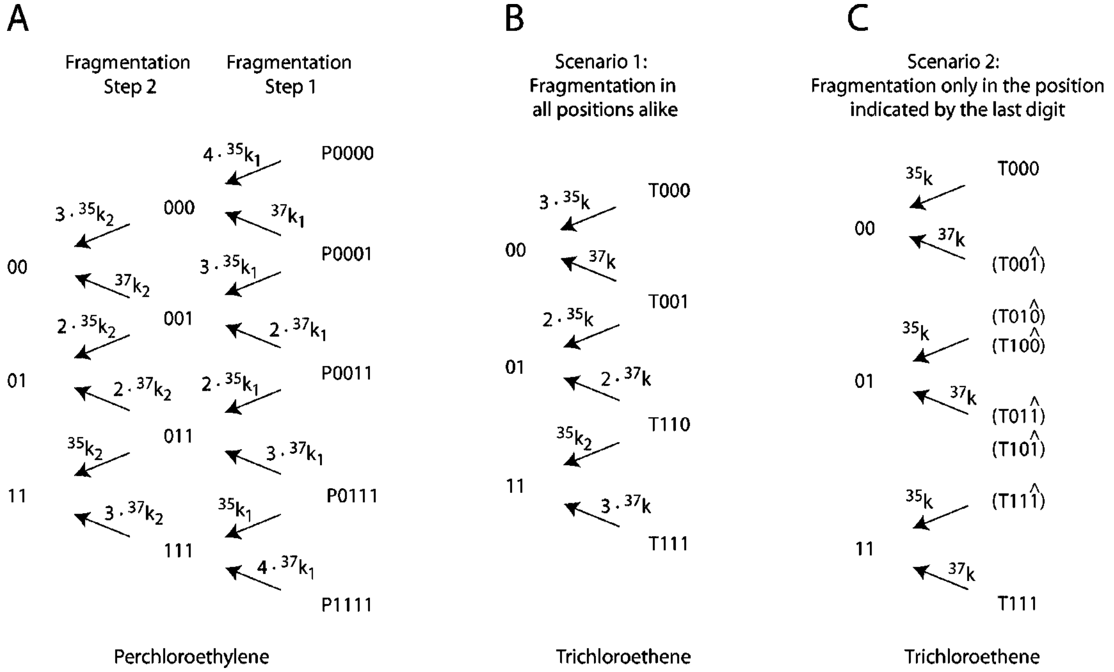

Figure 3. Fragmentation paths leading to fragment formation in the ion source of a mass spectrometer. (A) PCE, (B) TCE assuming that fragmentation occurs in all positions with the same probability, (C) TCE assuming that fragmentation occurs in one position only.

makes it possible to express the equations in terms of the isotope ratio R:

$$\frac{001}{000} = \frac{2 \cdot 3/2 \cdot R + 3 \cdot \mathrm{KIE}_1}{1 + 4 \cdot \mathrm{KIE}_1/(4 \cdot R)} = \frac{3 \cdot R + 3 \cdot \mathrm{KIE}_1}{1 + \mathrm{KIE}_1/R} = 3 \cdot R \cdot \frac{1 + \mathrm{KIE}_1/R}{1 + \mathrm{KIE}_1/R} = 3 \cdot R \qquad (50)$$

$$\frac{011}{001} = \frac{3 \cdot 2/3 \cdot R + 2 \cdot \mathrm{KIE}_1}{2 + 3 \cdot \mathrm{KIE}_1 \cdot 2/(3 \cdot R)} = \frac{2 \cdot R + 2 \cdot \mathrm{KIE}_1}{2 + 2 \cdot \mathrm{KIE}_1/R} = R \qquad (51)$$

Subsequent substitution into eq 46 gives

$$\frac{01}{00} = \frac{2 \cdot 011/001 + 2 \cdot \mathrm{KIE}_2}{1 + 3 \cdot \mathrm{KIE}_2 \cdot 000/001} = \frac{2 \cdot R + 2 \cdot \mathrm{KIE}_2}{1 + 3 \cdot \mathrm{KIE}_2/(3 \cdot R)} = 2 \cdot R \cdot \frac{R + \mathrm{KIE}_2}{R + \mathrm{KIE}_2} = 2 \cdot R \qquad (52)$$

Hence, similarly as when measuring the molecular ion, the fragment ion ratio can be directly transformed into the chlorine isotope ratio. Further, when substituting the isotopologue ratio in the Rayleigh equation by the measured fragment ion ratio, isotope fractionation factors can be quantified. The same result is given by a more stringent treatment in the Supporting Information which includes also mass discrimination caused by the extraction of ions toward the flight tube of the mass spectrometer. Although kinetic isotope effects of the fragmentation do not disappear completely like in the present treatment, they cancel out for differential comparisons relative to a standard.

Chlorine at Reacting and Nonreacting Positions. As discussed above, isotope fractionation during transformation only takes place at reacting positions. During the fragmentation process, however, chlorine ions can be removed from either reacting or nonreacting positions, possibly associated with kinetic isotope effects. As illustrated in Figures 3B and 3C, two extreme scenarios may be considered.

Scenario 1. Fragmentation Occurs in All Positions to the Same Extent. If all three positions of TCE give off their Cl substituent with the same likelihood, Figure 3B illustrates that the treatment is analogous to the case of the triple chlorinated fragment of PCE discussed above. In analogy to eq 46 the expression

$$\frac{\mathrm{d}01}{\mathrm{d}00} = \frac{01}{00} = \frac{2 \cdot {}^{37}k \cdot \mathrm{T011} + 2 \cdot {}^{35}k \cdot \mathrm{T001}}{1 \cdot {}^{37}k \cdot \mathrm{T001} + 3 \cdot {}^{35}k \cdot \mathrm{T000}} = \frac{2 \cdot \mathrm{T011/T001} + 2 \cdot \mathrm{KIE}}{1 + 3 \cdot \mathrm{KIE} \cdot \mathrm{T000/T001}} \qquad (53)$$

can be derived, where KIE is the kinetic isotope effect of the fragmentation. Subsequently, the approximate proportionality of isotopologue and isotope fractionation

$$R = \frac{{}^{37}\mathrm{Cl}}{{}^{35}\mathrm{Cl}} = \frac{{}^{37}p}{{}^{35}p} = \frac{1}{3} \cdot \frac{\mathrm{T001}}{\mathrm{T000}} = \frac{\mathrm{T011}}{\mathrm{T001}} = 3 \cdot \frac{\mathrm{T111}}{\mathrm{T011}} \qquad (54)$$

is used to give

$$\frac{01}{00} = \frac{2 \cdot \mathrm{T011/T001} + 2 \cdot \mathrm{KIE}}{1 + 3 \cdot \mathrm{KIE} \cdot \mathrm{T000/T001}} = \frac{2 \cdot R + 2 \cdot \mathrm{KIE}}{1 + 3 \cdot \mathrm{KIE}/(3 \cdot R)} = 2 \cdot R \cdot \frac{(R + \mathrm{KIE})}{(R + \mathrm{KIE})} = 2 \cdot R \qquad (55)$$

so that the same simple result as for PCE is obtained. In the case of scenario 1, isotope fractionation can, therefore, be directly evaluated from fragment ratios.

Scenario 2. Fragmentation Occurs in Only One Molecular Position. If fragmentation occurs only in one molecular position, clearly all fragments that can be detected will have lost their isotopic information from this position selectively and completely. This intuitive conclusion is confirmed by a fundamental mathematical treatment provided in the Supporting Information.

Consequences for the Evaluation of Isotope Fractionation in the Parent Compound. Such a possible selective elimination of isotopomer information has important consequences for the evaluation of isotope fractionation associated with transformation of parent compounds. Specifically, if also biodegradation of TCE involves the position that is preferentially cleaved off during fragmentation, such fractionation will not be observable in the fragment ion ratios at all! Vice versa, if a reaction takes place in a different position, the same fractionation may be over proportionally represented, as there is one nonreacting position less that dilutes the isotopic changes. However, ionization in a mass spectrometer is expected to be much less selective than an enzymatic process and hence the introduced bias may be small. This is confirmed by a recent study on reductive dechlorination by zerovalent iron where chlorine isotope enrichment factors in the expected range were obtained using GC−quadrupole MS analysis.[26] To resolve to what extent uneven ionization influences the calculated isotope ratios, it would be useful to compare isotope ratios derived from the direct ionization method with the classical method where all chlorine is transformed to methyl chloride. Such a comparison was already made with solvents from different manufacturers.[25] However, it is necessary to compare also samples from transformation experiments for processes with nonreacting positions where heavy isotopes become selectively enriched at the reacting positions.

CONCLUSIONS

Our study provides fundamental insight into the way chlorine isotopes are distributed among polychlorinated isotopologues and how this distribution evolves if the compound undergoes fractionation. To our knowledge, we thereby provide for the first time a solid theoretical basis that justifies evaluation of isotope fractionation in polysubstituted compounds according to the Rayleigh equation, without the need of more complicated treatments.[10] At the same time, our considerations allow a straightforward interpretation of enrichment factors in terms of position-specific kinetic isotope effects, in the same way as known from carbon or hydrogen isotope fractionation.[4] On a different matter, our treatment justifies the novel experimental approach of determining isotope ratios not only from single chlorinated species such as CH_3Cl, AgCl or CsCl but also from polysubstituted isotopologues such as $C_2H_2Cl_2$. A theoretical treatment of isotopologue fragmentation in the ion source of a mass spectrometer demonstrates that even fragment ions reflect the original isotope ratio, largely unaffected by kinetic isotope effects associated with fragmentation in the ion source. We thereby provide a theoretical justification for two recently published innovative experimental methods.[25,26] This paper also identifies possible prospects and limitations associated with these methods. On the one hand, more research needs to be done to address the possibility of position-specific fragmentation of nonsymmetric molecules in the ion source of a mass spectrometer, as this may lead to an over- or under-representation of isotope fractionation in certain molecular positions. On the other hand, the proportionality of isotopologue and isotope fractionation suggests that the precision of the methods may be further improved, if not only the most abundant, but all isotopologue and fragment masses are included in the calculation.

Finally, we add a warning brought to our attention by a helpful reviewer. The approach of measuring molecular ions or their fragments is not easily extended to isotopes that differ by only one mass unit such as H, C and N. The reason is hydrogen transfer processes in the ion source which create ions with an additional mass unit that would be incorrectly interpreted as deriving from ^{2}H, ^{13}C or ^{15}N substitution. This H-transfer, in turn, is sensitive to ion-source conditions so that stable measurements relative to a standard are difficult to achieve. The situation is different with Cl or Br for which isotopes are two masses apart. Here, artifacts can only arise if ^{2}H-, ^{13}C- or ^{15}N-substituted molecules receive in addition a hydrogen atom so that ions with two additional mass units are created. The probability that both events occur (i.e., substitution by a seldom isotope and H-transfer) is very small compared to the high natural abundance of ^{37}Cl. The fact that isotope analysis is routinely conducted with CH_3Cl and no critical interference from hydrogen transfer has been reported indicates indeed that the effect can be neglected.

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SUPPORTING INFORMATION AVAILABLE

S1, fractionation of dichloroethene isotopomers in a concerted reaction; S2, derivation of the proportionality of isotopologue and isotope fractionation for perchloroethylene (PCE); S3, derivation of proportionality of isotopologue and isotope fractionation for molecules with chlorine at nonreacting positions; S4, fragmentation of PCE isotopologues under steady state assumption; S5, position-specific fragmentation of TCE. This material is available free of charge via the Internet at http://pubs.acs.org.