Enantiomer Fractions Are Preferred to Enantiomer Ratios for Describing Chiral Signatures in Environmental Analysis

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The enantiomer ratio (ER) is currently the standard descriptor of enantiomeric (chiral) signatures for environmental samples. In this paper, we argue for the adoption of the enantiomer fraction (EF) as the standard descriptor by showing drawbacks to the use of ER. The enantiomer fraction is superior because it provides a more meaningful representation of graphical data and is more easily employed in mathematical fate expressions. Several useful expressions are presented that allow EF to be used for tracking and apportioning chemical movement between environmental compartments and for investigating microbial degradation processes.

Introduction

Chiral analysis is becoming increasingly popular in the field of environmental science for investigating the transport and fate of chemicals in various media (*1*). This technique has recently been applied to environmental samples to yield information on air−water exchange of α-HCHs in oceans (*2*) and lakes (*3*), the revolatilization of pesticides from soils (*4*), and the importance of microbial degradation in controlling environmental lifetimes of persistent chemicals (*5*). Analysis of chiral compounds in various biological compartments may provide valuable insight to how chemicals are accumulated, degraded, and translocated within food chains (*6*).

The property of chirality is attributed to a compound if it can exist as two non-superimposable mirror image forms—similar to our left and right hands. These two forms are designated as (+) and (−) enantiomers based on their interaction with plane-polarized light. Chiral compounds of environmental significance include α-hexachlorocyclohexane (α-HCH), *cis*- and *trans*-chlordane, *o,p′*-DDT, heptachlor, and heptachlor-*exo*-epoxide (HEPX). Some PCBs and their metabolites (e.g., methylsulfone PCBs) exhibit axial chirality (atropisomerism) due to hindered rotation about the biphenyl σ-bond (*6*). Physical processes are not able to distinguish between the two enantiomeric forms of a compound. Consequently, chiral chemicals are almost always produced as a racemate in which 50% of the compound is the (+) form and 50% is the (−) form. In the environment, the racemic signature remains unchanged by physical removal mechanisms such as hydrolysis and photolysis reactions. However, the mechanisms of microbial degradation and biological metabolism may be enantioselective and thus alter the enantiomer signature. Enantioselective permeability through biological membranes has also been indicated. Totally selective transfer of (+)-α-HCH across the blood−brain barrier occurs in seals and rats, whereas the ER in blubber is between 1 and 2 (*7, 8*). This altered signature or "fingerprint" can be exploited to track a compound's movement and transformation.

Chromatography, using a chiral stationary phase, is able to separate the (+) and (−) enantiomers in environmental samples. Until now, the most popular way for describing this altered signature was to use the concept of enantiomer ratio (ER) where

$$ER = A_+/A_-$$

[A_+ and A_- correspond the peak areas of the (+) and (−) enantiomers; equal molar response factors are assumed].

The ER in the sample is often compared to the value in a standard that is typically racemic, i.e., ER = 1.0. However, there are several limitations to using ER. When used graphically, the ER results in misleading representation of data. Because of the way it is defined, the ER can range from 0 to infinity. The ER of α-HCH in seal brain is approximately infinity, and it is therefore not possible to represent it graphically (*7*). Therefore, a unit change in ER away from unity in the downward direction (i.e., <1) is not equivalent to the same unit change in the opposite direction. Complications may also arise when the ER is employed in mathematical expressions.

We propose that a better representation of the chiral signature is the enantiomer fraction (EF) where

$$EF = A_+/(A_+ + A_-) \text{ or } EF_x = A_1/(A_1 + A_2)$$

where A_1 and A_2 are the first and last eluting enantiomers on chiral column *x* when the identity of the (+) and (−) forms is not known. Dividing the numerator and denominator by A_2 gives

$$EF = (A_1/A_2)/[(A_1/A_2) + (A_2/A_2)] = ER/(ER + 1)$$

Now dividing both numerator and denominator by ER results in the simple relationship EF = 1/(1 + 1/ER).

The EF can only range from 0 to 1.0 with EF = 0.5 representing a racemic mixture. Each unit of deviation from the racemic value (0.5), both in the upward and downward direction, is equivalent. Because it is a proper fraction, the EF can also be applied more naturally in mathematical fate expressions. The EF of α-HCH in seal brain is ∼1 (*7*), which is easy to represent graphically.

In this paper, two examples are presented that highlight the advantages of EF versus ER. We also consider several useful mathematical fate expressions from the literature that employ ERs and rewrite these equations using EF format. Ultimately, we hope to make clear that the enantiomer fraction (EF) is the preferred descriptor of chiral signatures in environmental samples and should be adopted when presenting results in the literature.

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10.1021/es9906958 CCC: $19.00
Published on Web 12/29/1999

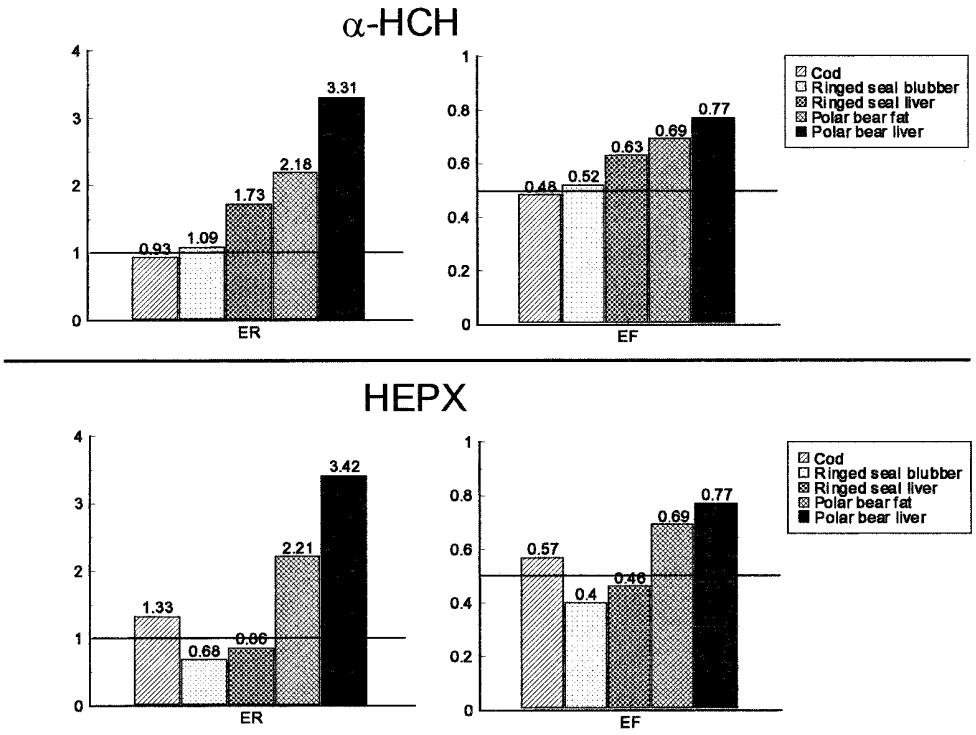

FIGURE 1. Plots of ER and EF for α-HCH and HEPX for different members of an arctic food chain.

Example 1: Chiral Signatures in an Arctic Food Chain—Graphical Representation of Data and Enantiomer Mass Balances

Figure 1 shows ER and EF plots for two chiral compounds [α-HCH and heptachlor-*exo*-epoxide (HEPX)] that are accumulated in arctic biota. These results are from a study by Wiberg et al. (*9*) where chiral analysis by GC−MS was performed on samples of three members of the arctic food chain—cod, ringed seal (fat and liver), and polar bear (blubber and liver).

This comparison is a good example of the disadvantage of using ERs. Recall that for a racemic compound the ER = 1 and the EF = 0.5. In the ER plot, the enrichment of the (+) enantiomer is exaggerated by the height of the bar—the larger the ER, the less meaningful its value becomes as a measure of true change. In the case of the EF plots however, the integrity of change is preserved. Each unit of deviation above and below the "0.5 line" has the same meaning and is easy to interpret. For instance, if we consider the enrichment of (+)-HEPX as we go from ringed seal blubber to polar bear liver. The corresponding ER values are 0.68 and 3.42, respectively. Graphically, the change in ER appears to be quite significant—almost a factor of 5 increase! But what does this really mean? In the case of EF, the corresponding values are 0.40 and 0.77 and can be correctly interpreted to mean that the proportion of the (+) enantiomer has increased from 40% to 77%—almost a factor of 2!

The other advantage of EFs, especially when considering different organs in a biological sample is that it allows us to easily calculate a mass balance for chiral compounds (an "enantiomer balance"). In other words to determine the weighted total EF (EF_Σ) for several organs in a sample. If the enantiomer signature was measured for all organs and tissues and concentrations were also known, the EF_Σ for the organism would be

$$EF_\Sigma = \sum_{i=1}^{n}(EF_i C_i M_i)/\sum_{i=1}^{n}(C_i M_i)$$

where C_i is the achiral concentration [determined on an achiral column; mass/mass fresh (or wet) weight] of the compound in organ i and M_i is the mass of that organ. [Note: The numerator of this expression represents the total mass of the (+) enantiomer. The total mass of the (−) enantiomer is determined by subtracting the mass of the (+) enantiomer from the total mass (i.e., the denominator of the expression)].

In the case of α-HCH in polar bear fat and liver (see Figure 1), the EF_Σ for these two organs would be (using hypothetical concentrations of 10 pg kg^{-1} and 200 kg for fat and 15 pg kg^{-1} and 10 kg for liver)

$$EF_\Sigma = (0.69)(10)(200) + (0.77)(15)(10)/[(10)(200) + (15)(10)]$$

$$EF_\Sigma = 0.70$$

This is a useful value, especially when trying to interpret to what extent a compound is selectively metabolized or partitioned within an organism. In the food chain, predators often consume whole prey. Thus, the difference in EF_Σ between predator and prey may serve as a marker of trophic status or indicate an eating preference.

Example 2: Two-Source Apportionment Model

In many cases in environmental analysis, the enantiomer composition of a sample is predominantly a result of contributions from two sources. Apportioning the contribution of each source has been previously investigated for water−air and soil−air systems (*3*, *4*). Bidleman and Falconer (*10*) have recently described an ER-based two-source apportionment model (see below).

To demonstrate the development of a simpler and more straightforward EF-based two-source apportionment equation, let us consider the example of a soil−air system where a chiral chemical in the soil has been subject to significant enantioselective microbial degradation. In this instance, we are sampling air above soil (the "mixed" layer) and are interested in determining how much of the chemical in this

sample originated from the soil. The chiral signature in air above soil (EF_{MIX}) will be a weighted fraction of the signature in the soil (EF_{SOIL}) and background air (EF_{AIR}). The weighting depends on the contribution from each source. We can therefore express the signature in this mixed layer (EF_{MIX}) as

$$EF_{MIX} = f_{SOIL}(EF_{SOIL}) + f_{AIR}(EF_{AIR})$$

$$= f_{SOIL}(EF_{SOIL}) + (1 - f_{SOIL})(EF_{AIR})$$

where f_{SOIL} and f_{AIR} represent the fraction of compound that originated from soil and air, respectively (i.e., $f_{SOIL} + f_{AIR} = 1$). This can be rearranged as follows to isolate the fraction contributed from soil:

$$EF_{MIX} = f_{SOIL}(EF_{SOIL}) + EF_{AIR} - f_{SOIL}(EF_{AIR})$$

$$f_{SOIL} = (EF_{MIX} - EF_{AIR})/(EF_{SOIL} - EF_{AIR})$$

For illustrative purposes lets assume that the EF values for background air and soil are 0.49 and 0.30 and that the value measured in the air above the soil is 0.42. Using the above expression, we can calculate the fraction contributed from soil to be 0.368 or 36.8%, i.e.

$$f_{SOIL} = (0.42 - 0.49)/(0.30 - 0.49) = 0.368$$

The model can be expressed in general terms as

$$f_1 = (EF_{MIX} - EF_2)/(EF_1 - EF_2)$$

where the subscripts 1 and 2 correspond to the two sources.

When expressed using ERs, the two-source apportionment model and its derivation are more complex (*10*):

$$f_1 = (ER_{MIX} - ER_2)(ER_1 + 1)/(ER_1 - ER_2)(ER_{MIX} + 1)$$

Useful Equations Converted to EF Format

First-Order Microbial Degradation. Buser and Müller (*11*) have shown that when pseudo-first-order kinetics are assumed, i.e., $C = C_o \exp -kt$, the ER of a degraded sample can be expressed as

$$ER = \exp -(k_1 - k_2)t$$

To derive an analogous EF-based expression, we start with the definition

$$EF = C_{(+)}/C_{total}$$

where $C_{(+)}$ is the concentration of the (+) enantiomer and C_{total} is the sum of the concentration of the (+) and (−) enantiomers after some time, t [at $t = 0$, we assume a racemic signature and hence $C_{(+)} = C_{(-)} = C_o/2$]

$$C_{(+)} = C_o/2(\exp -k_{(+)}t);\ C_{(-)} = C_o/2(\exp -k_{(-)}t);\ \text{and}\ C_{total} = C_o/2[\exp -k_{(+)}t + \exp -k_{(-)}t]$$

So

$$EF = C_o/2(\exp -k_{(+)}t)/\{C_o/2[\exp -k_{(+)}t + \exp -k_{(-)}t]\}$$

which simplifies to

$$EF = \exp -k_{(+)}t/[\exp -k_{(+)}t + \exp -k_{(-)}t]$$

This equation simply states that the EF is equal to the rate of microbial degradation of the (+) enantiomer divided by the overall degradation rate for both enantiomers combined.

Degradation of α-HCH in the Eastern Arctic Ocean. Harner et al. (*5*) recently showed that microbial degradation is the dominant process removing α-HCH from the Arctic Ocean. Microbial degradation rates are calculated based on depth profiles of ER and concentration and an estimation of the "ventilation age" of the deep water masses, i.e., the time since the water mass was last at the surface and able to exchange gases with the atmosphere. This corresponds to the time, t, in the rate expression. Removal rates by hydrolysis are also required and can be easily determined from temperature and pH. The final expression for the microbial degradation rate constant for the (+) enantiomer of α-HCH is

$$k_{m+} = (1/t)\{\ln [1 + 1/ER] - \ln [C_\alpha/C^o_\alpha] - k_{h\alpha}t - 0.693\}$$

The simplest way to convert this expression to EF format is to use the relationship between EF and ER, i.e., $EF = 1/(1 + 1/ER)$. The simplified expression is

$$k_{m+} = (1/t)\{\ln [1/EF] - \ln [C_\alpha/C^o_\alpha] - k_{h\alpha}t - 0.693\}$$

In conclusion, the enantiomer fraction is the preferred descriptor of chiral signatures. Graphically, plots that employ EF present a more meaningful depiction of the results and make it possible to visually assess the relative magnitude of enantiomer depletion or enrichment. This integrity is lost when ER is used. Also, because it is a proper fraction, the EF can be incorporated into mass balance expressions—especially applicable in biological systems. Several useful expressions are presented that allow EF to be used for tracking and apportioning chemical movement between environmental compartments and for investigating microbial degradation processes.

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Received for review June 21, 1999. Revised manuscript received August 31, 1999. Accepted September 23, 1999.

ES9906958