Enantiomeric Ratios of Chlordane Compounds in Air near the Great Lakes

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Abstract

Enantiomeric ratios (ER) can provide new insights into the environmental fate of chemicals. In this study, cyclodextrin gas chromatographic columns were used to separate the enantiomers of chlordane. This GC separation was combined with electron capture, negative ionization, mass spectrometry (ECNI-MS) to provide the selectivity and sensitivity necessary for environmental measurements. Initial studies using racemic and enriched standards confirmed that this system gave reproducible and accurate measurements. In addition, peak fitting software proved to be a more reliable method of quantitation than an electronic integrator. Forty-eight air samples, taken from August 1994 through September 1995 at Sturgeon Point, New York, were analyzed to determine ER temperature and temporal trends. Samples from Eagle Harbor, Michigan, and Sleeping Bear, Michigan, were also analyzed to determine ER spatial trends. The ERs at Sturgeon Point did not vary significantly with time; the ERs at this location had yearly averages of 1.1 ± 0.3, 0.93 ± 0.1, and 0.54 ± 0.2 for α-chlordane, γ-chlordane, and heptachlor epoxide B, respectively. Ratios at the other two sites agreed well with those from Sturgeon Point. The ratios for α- and γ-chlordane are approximately 1.00, which indicate that these compounds have not been significantly metabolized in the environment. Heptachlor epoxide B can only enter the environment after heptachlor has been metabolized, a fact which accounts for the particularly low ER for this compound.

Introduction

Technical chlordane is a mixture of more than 140 different compounds, most of which are chiral.[1] The compounds of interest in this study are α- and γ-chlordane, and heptachlor epoxide B which is a metabolite of heptachlor. These compounds are all chiral, separable on cyclodextrin columns, and readily found in gas phase air samples.

Enantiomeric ratios can provide new insight into environmental processes. Since most chiral chemicals are introduced into the environment racemically, metabolism can be tracked. As organisms break down the chemicals, the chiral enzymes selectively degrade one enantiomer. The further the ER is from 1.00, the more the compound has been metabolized.[2] Depending on the organisms in different environmental compartments, the ER in the soil may be quite different from that in lake water. These values can then tell us something about the air in the surrounding area. In this way, new information can be learned about the source of the contamination.[3]

While enantiomers have identical boiling points, spectral properties, and vapor pressures, the toxicity, persistence, and biological activity are often quite different.[4] Different organs selectively accu-

mulate different enantiomers.[5] Like drugs, one enantiomer may possess the desired pesticidal properties, while the other is simply a hazard to the environment. We can use this knowledge to more effectively chose what is introduced into the environment.[6]

Concentrations of such chiral compounds only represent part of the picture. ER provides a different clue about the fate and transport of these ubiquitous chemicals. Future production and introduction of chemicals can be evaluated in a new light. Chiral gas chromatography combined with mass spectrometry is a powerful tool in gathering new information about enantiomers in the environment.

Experimental

Standards Analyzed: Racemic standards were purchased from Ultra Scientific (North Kingstown, RI). These were combined into a mixture of α- and γ-chlordane, heptachlor, heptachlor epoxide A and B; and they were diluted with hexane to a concentration of 1.0 ng/μL. The enantiomerically enriched standards were purchased from Axact Standards (Commack, NY). A mixture of the above mentioned compounds and oxychlordane was diluted with hexane to a concentration of 0.1 ng/μL.

Cyclodextrin Columns: Two GC columns were used in this study because of their different separation characteristics and for confirming results. An α- and γ-cyclodextrin column were purchased from Supelco (Bellefonte, PA). Both the Gamma-DEX and Alpha-DEX-120 columns were 30 m in length, 250 μm ID, and 0.25 μm film thickness.

Gas Chromatography Conditions: A temperature program was developed for each column. Both used a head pressure of 40 psi. A 1.0 μL sample was injected in the splitless mode onto a retention gap of methyl deactivated silica (Chrompack, Raritan, NJ). For the α-cyclodextrin column, the temperature program was 50 °C for 1 min., ramped to 130 °C at 5 °C/min., ramped to 155 °C at 0.2 °C/min., ramped to 230 °C at 20 °C/min. and held for an additional minute. The total run time was about 150 min., and a constant flow was used. The temperature program for the γ-cyclodextrin column also began with a 1 min. hold at 50 °C, ramped to 150 °C at 20 °C/min., ramped to 185 °C at 0.5 °C/min., ramped to 230 °C at 20 °C/min. and held for 1 min. The total run time was about 80 min., and constant pressure was used in this case.

ECNI-MS Analysis: The HP 5890 series II gas chromatograph (GC) was connected to a HP 5989A mass spectrometer. The carrier gas was helium (Gas Tech, Hillside, IL), and methane (Liquid Carbonic, Chicago) was used as the reagent gas. The reagent gas pressure was 0.60 Torr, source temperature was 125 °C, and the quadrupole was at 100 °C. To detect heptachlor epoxide A and B, ions at m/z 354, 386, 388, 390, and 392 were monitored. To detect α- and γ-chlordane, ions at m/z 374, 376, 406, 408, 410, and 412 were monitored. For the γ-cyclodextrin column, m/z 410 ion was not monitored, but the other ions remained the same.

Peak Fitting: Peak fitting was necessary for environmental samples with a low S/N ratio which made the electronic integrator ineffective. The software package used for this purpose, Peak Fit v4.0, was purchased from Jandel Scientific (San Rafael, CA). Peak areas, standard errors, R^2 values, peak centers, and resolution results were then copied to spreadsheets in Microsoft Excel v5.0 or 97. The fast Fourier transform smoothing function was chosen to remove high frequency oscillations in the data. The linear progressive baseline was used in most cases. Occasionally, the presence of another compound required that the linear or constant baseline be used instead. The "vary widths" op-

tion was also enabled to allow for two peaks of different width. The peak form chosen to model the chromatographic peaks was the Exponentially Modified Gaussian-Half-Gaussian Modified Gaussian. This form of the Gaussian models intracolumn effects such as dispersive effects, mass transfer resistances, axial diffusion, and slow kinetics of adsorption and desorption which tend to broaden peaks.[7] The program was set to run and restart as necessary until the R^2 value was no longer changing, suggesting the best fit had been obtained.

Environmental Samples: The samples analyzed were gas phase air samples collected for 24 hours through a high volume air sampler (Graesby General Metal Works, Smyrna, GA). The organochlorines were collected on Amberlite XAD-2 resin (Sigma, St. Louis, MO) and Soxhlet extracted with 1:1 acetone: hexane (EM Science, Gibbstown, NJ) for 24 hours. The extract was concentrated and exchanged into hexane for fractionation on Silica gel (Aldrich Chemical, Milwaukee, WI). The chlordane components eluted in the 1:1 hexane: methylene chloride fraction. The fraction was again concentrated before injection. These samples were collected as part of the Integrated Atmospheric Deposition Network (IADN) project, and were previously analyzed for concentrations. Since that time they were stored at -20 °C.

Results and Discussion

Standards: To ensure that reliable results could be obtained, a series of standards was analyzed. Racemic standards should give an ER of 1.00, but another calibration point is also desirable. Prof. König of the University of Hamburg has prepared enantiomerically enriched standards which are commercially available. Unfortunately, the method of quantitation for these standards is the same method used here. Results from these studies are presented inTable 1. ER here is presented as area of the (+) enantiomer divided by area of the (-) enantiomer. Elution order was determined using the enriched standards. Even though five standards were run in all cases, since the number of ions monitored changed, the value of N also changed; this is given in the table.

Table 1. Enantiomeric Ratios and standard deviations for peak fit standards

Compound	Racemic Standards		Enriched Standards		Reported
	α-cyclodextrin	γ-cyclodextrin	α-cyclodextrin	γ-cyclodextrin	
α-chlordane	1.00 ± 0.02	1.03 ± 0.01	0.181 ± 0.003	0.170 ± 0.005	0.178
γ-chlordane	1.042 ± 0.005	0.98 ± 0.01	16.8 ± 1.7	17.9 ± 1.5	26.03
heptachlor epoxide A	1.030 ± 0.005	DNS	0.12 ± 0.01	DNS	0.0917
heptachlor epoxide B	1.1 ± 0.1	1.035 ± 0.007	DNS	51.0 ± 3.9	36.04
N=	45, 45, 55, 55	25 for all	45, 45, 55	25 for all	

DNS -- did not separate

Note the racemic ratios are within 4% of the actual value. The enriched values do not agree as well, but in cases where the compound can be separated on both cyclodextrin columns, the two values agree well. If ER is switched into units of percentage, then the experimental values agree within 2% of the reported value. This seems to be very reasonable considering that both the reported and experimental values may be off by ± 1%.

Environmental Samples: Once the method of analysis was proven to be reliable and reproducible, analyzing environmental samples was the next step. Since all of the components could not be separated by one column, both were used. This strategy also provides duplicate analysis to ensure correct results. Both α- and γ-chlordane separate on both columns, but it was discovered that endosulfan I coelutes with one of the α-chlordane peaks on the γ-cyclodextrin column.

The data from Sturgeon Point, NY, are presented in Figure 1. Notice that there is a considerable amount of scatter, but no particular temporal or temperature trends can be found. A majority of the γ-chlordane points from the two different columns agree within the margin of error. This confirms again that this analysis is producing reliable results. Generally, the α- and γ-chlordane ER is close to 1.00, suggesting they have not been metabolized or undergone any chiral processes in the atmosphere. In contrast, the ER of heptachlor epoxide B is much lower.

Since heptachlor epoxide B is a metabolite of heptachlor, this compound should have a different ER. The epoxide is not anthropogenically introduced to the environment; it only appears after undergoing some processing. The most likely explanation is processing by chiral enzymes in organisms. The chiral reaction rates are certainly different, and thus the ER is not 1.00.

Figure 1. Enantiomeric ratios for α- and γ-chlordane and heptachlor epoxide B at Sturgeon Point, New York. ACD and GCD are abbreviations for α- and γ-cyclodextrin. Yearly averages (in legend order) are 1.14 ± 0.3, 0.92 ± 0.1, 0.95 ± 0.1, and 0.54 ± 0.2.
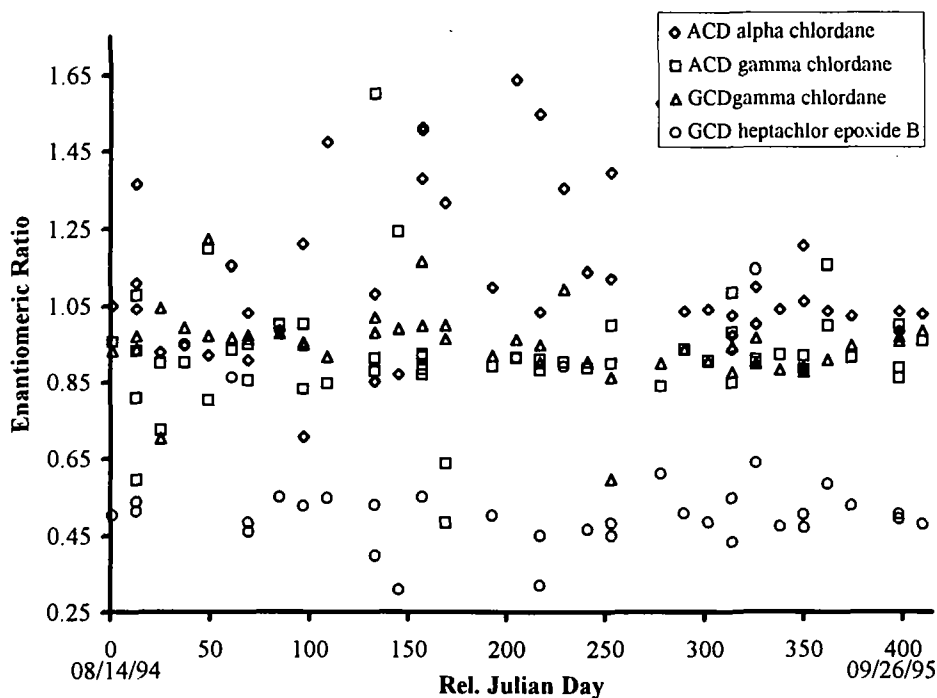
Figure 2. Enantiomeric ratios at three IADN sites for chlordane compounds
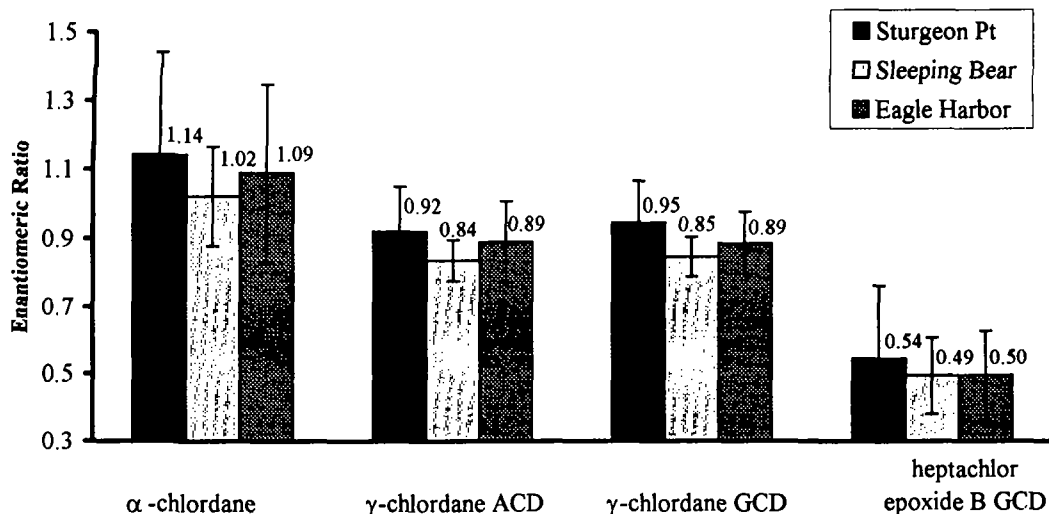
To investigate the effect of location, two other sites in Michigan were selected. The data from these two sites agree well with those found at Sturgeon Point. The averages of these data are presented in Figure 2.

Again notice the values of γ-chlordane agree well between the two columns. It appears the five γ-chlordane samples from Sleeping Bear, MI have a slightly lower ER than the other two sites. The large error bars on α-chlordane are most likely because the two enantiomers are not well resolved on the α-cyclodextrin column. It is very apparent here that heptachlor epoxide B has a significantly lower ER at all three sites, which further supports the arguments above.

From this study we have learned that chiral GC-MS is an accurate and reproducible method of calculating ER. However, long temperature programs on two separate columns are required to obtain duplicate measurements and separation of the compounds of interest. In air samples, the ER for α- and γ-chlordane is approximately 1.00, indicating that little metabolism has occurred. The ER for a metabolite is expected to be different from 1.00. Heptachlor epoxide B has an ER of 0.50 which indicates the (-) enantiomer is not degraded as quickly.

Acknowledgments

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