ENANTIOSELECTIVE ANALYSIS OF CHLORDANES IN SERUM

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Abstract
Enantioselective analysis of chlordanes was performed by gas chromatography-mass spectrometry using a chiral column (BGB-172; 30m by 0.25mm by 0.25 um; BGB Analytik), and the enantiomers of chlordenes, heptachlor, heptachlor-*exo*-epoxide, chlordanes, and oxychlordane were separated in serum sample. The enantiomeric excess (EE) of MC-5 and *trans*-chlordane were 71% and 24%, and the last eluting enantiomers of these were depleted compared with the first eluting enantiomers. The metabolite heptachlor-*exo*-epoxide and oxychlordane were enriched in the first eluting enantiomer, and EE values were 50% and 21%. Only *cis*-chlordane was depleted in the first eluting enantiomer, and EE value was 48% in serum sample.

Introduction
Technical chlordane, a ubiquitous and persistent pesticide, was the mixture of more than 140 compounds, and was used for killing lawn and garden pests, for termite control, and for pest control on crops in U.S. from 1945 until it was banned.[1, 2, 3] In Japan, this mixture was mainly used for termite control from 1950. In 1968, it was banned for agriculture, however used for other usage, such as termite control, until the Chemical Substances Control Law restricted it in 1986. This mixture is complex including 40% of octachlorinated isomers, 20% of nonachlorinated isomers, 4.8% of heptachlorinated isomers, and 7.3% of hexachlorinated isomers.
Other isomers except nonachlor and these metabolites are chiral.[2, 3] When manufactured and introduced into the environment, these compounds are racemic. When they transfer through the environmental components, biological degradation changes the enantiomer fractions (EF=(+)/[(+)+(-)]). Physical processes such as volatilization, photolysis, and OH radical reactions do not change EF. Clearly, by measuring EF, more knowledge can be obtained, i.e. the information about sources, fates, metabolism, and transport of these compounds.[4] If the elution order of enantiomer is unknown, the enantiomer excess (EE is absolute of 100X[Area-first – Area-last]/[Area-first + Area-last]) is useful to obtain the information.[2]
The determination of individual chiral chlordane components was carried out in a technical product, environmental samples, and human serum sample, using BGB-172 column.

Materials and Methods
Serum collection: The sample was obtained after receipt of written informed consent. After about 10ml of blood were centrifuged by 3000 rpm, serum samples were obtained and stored in -20°C until analysis.
Extraction and cleanup: About 2g of serum was added cleanup spike solution, 2.5ml of diethyl ether, and 5ml of ethanol, then extracted with 10ml of hexane twice. Hexane extracts were passed through 1g of florisil/1g of silica gel double layer column (Supelco Inc., U.S.), eluted with 15ml of 15% diethyl ether/hexane. Eluate was concentrated to 0.1ml under gentle stream of nitrogen, and then added syringe spike solution.
Analysis: Determination of enantiomeric composition was done with a high-resolution gas chromatography / mass spectrometry (GC/MS, HP 5890 series II / JMS-700, JEOL, Japan). Separations were carried out using a BGB-172 column (20% *tert*-butyldimethylsilylated *beta*-cyclodextrin in methylphenylcyanopropylpolysiloxane, 30m length X 0.25mmID, 0.25µm Film Thickness, BGB Analytik AG, Switzerland). Carrier gas was helium, and injector and

Table1 The enantiomeric excess (EE is absolute of 100 X [Area-first – Area-last]/[Area-first + Area-last]) of chlordane component in serum sample. (%)

chl6-2*	41
beta-chlordene	3.8
gamma-chlordene	21
heptachlor-*exo*-epoxide	50
MC-5	71
trans-chlordane	24
cis-chlordane	48
oxy-chlordane	21

* unknown isomer on chlordane
Area-first : area of first eluting enantiomer
Area-last : area of last eluting enantiomer

transfer line temperature were 230°C and 245°C. 1µl of samples were injected splitless (split opened after 1.5 min) at an initial temperature of 120°C, and constant carrier gas flow was used. The oven of a gas-chromatograph was ramped at 4°C/min to 180°C, ramped at 1°C/min to 230°C, and held for 15minute. The ion source was operated in the electron-impact mode (EI, 38eV, 250°C). Target compounds were quantified by ^{13}C isotope dilution operating in the selected ion monitoring mode (SIM). Target compounds were Chlordenes (*alpha-*, *beta-*, *gamma-*, *m/z* 302.8883 and 304.8853), oxychlordene (*m/z* 318.8832 and 320.8803), heptachlor (*m/z* 271.8102 and 273.8072), heptachlor-epoxide (*exo-*, *endo-*, *m/z* 352.8442 and 354.8413), oxychlordane (*m/z* 386.8052 and 388.8023), chlordanes (MC-5, *trans-*, *cis-*, MC-4, *m/z* 372.826 and 374.8231), nonachlors (MC-6, *trans-*, *cis-*, *m/z* 406.787 and 408.784). A lock mass of *m/z* 292.9824 from perfluoro-kerosene was used.

Technical chlordane: A technical chlordane was obtained from Kemiholz Co. Ltd., Kyoto, Japan. Solution in hexane were prepared and used for analysis.

POPs standard solutions: *alpha-* and *beta*-chlordene, heptachlor, heptachlor-*exo*-epoxide, heptachlor-*endo*-epoxide, *trans-* and *cis*-chlordane, oxychlordane, *trans-* and *cis*-nonachlor were obtained from CIL Inc. (U.S.) $^{13}C_{10}$ Labeled chlordene, $^{13}C_{10}$ labeled heptachlor, $^{13}C_{10}$ labeled heptachlor -*exo*-epoxide, $^{13}C_{10}$ labeled *trans*-chlordane, $^{13}C_{10}$ labeled oxychlordane, $^{13}C_{10}$ labeled *trans-* and *cis*-nonachlor were from CIL Inc. (U.S.), used for clean-up spike. $^{13}C_{12}$ labeled 2,3',4',5-tetrachlorobiphenyl (PCB#70) and $^{13}C_{12}$ labeled 2,2',4,5,5'- pentachlorobiphenyl (PCB#101) were obtained from Wellington Laboratory Inc. (Canada), used for syringe spike.

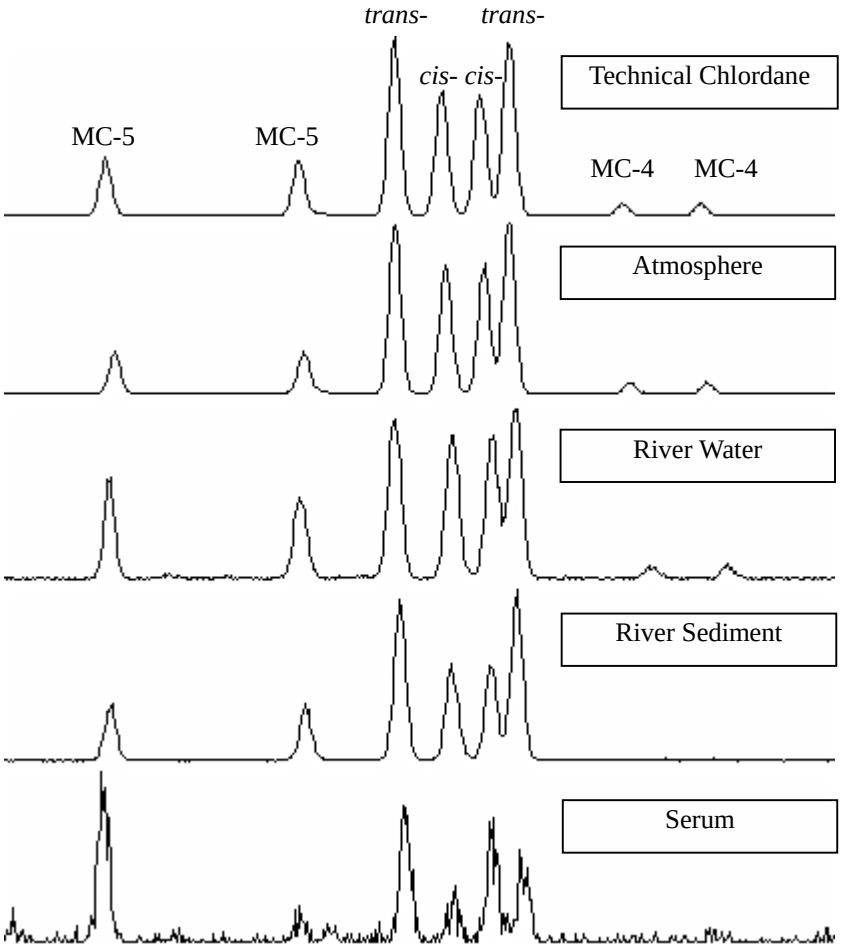

Figure1 GC/MS-SIM chromatogram showing elution of chlordane on BGB-172 HRGC column. (average of *m/z* 372.826 and 374.8231)

Results and Discussion

Figure1 is GC/MS-SIM chromatogram showing elution of chlordane on BGB-172 HRGC column (Average of *m/z* 372.826 and 374.8231). In technical chlordane, isomers (MC-5, *trans*-chlordane, *cis*-chlordane, and MC-4) were racemic mixture. In environmental samples, they were almost racemic mixture (EE were approximately 0%). The enantiomeric composition of chlordane isomers in serum sample was distinctly nonracemic. Figure2 is GC/MS-SIM chromatogram showing elution of chlordene on BGB-172 HRGC column (Average of *m/z* 302.8883 and 304.8853). The important isomer marked as "chl6-2" was detected with *alpha*-, *beta*-, and *gamma*-chlordene. In the atmosphere, these isomers were racemic mixture as well as technical chlordane. In the river water and sediment, these isomers were also racemic mixture (EE were approximately 0%) though the isomer distribution was different from technical chlordane. In the serum sample, the isomer distribution was different from technical chlordane, and chl6-2 was the predominant isomer. The enantiomeric composition of chl6-2 isomer in serum sample was distinctly nonracemic. Figure3 is GC/MS-SIM chromatogram showing elution of heptachlor-epoxide (average of *m/z* 352.8442 and 354.8413) and oxychlordane (average of *m/z* 386.8052 and 388.8023) on BGB-172 HRGC column in serum sample. These isomers are metabolites of chlordane and heptachlor, and not included in technical chlordane. These metabolites were detected in environmental samples and were nonracemic. In the serum sample, oxychlordane and heptachlor-*exo*-epoxide

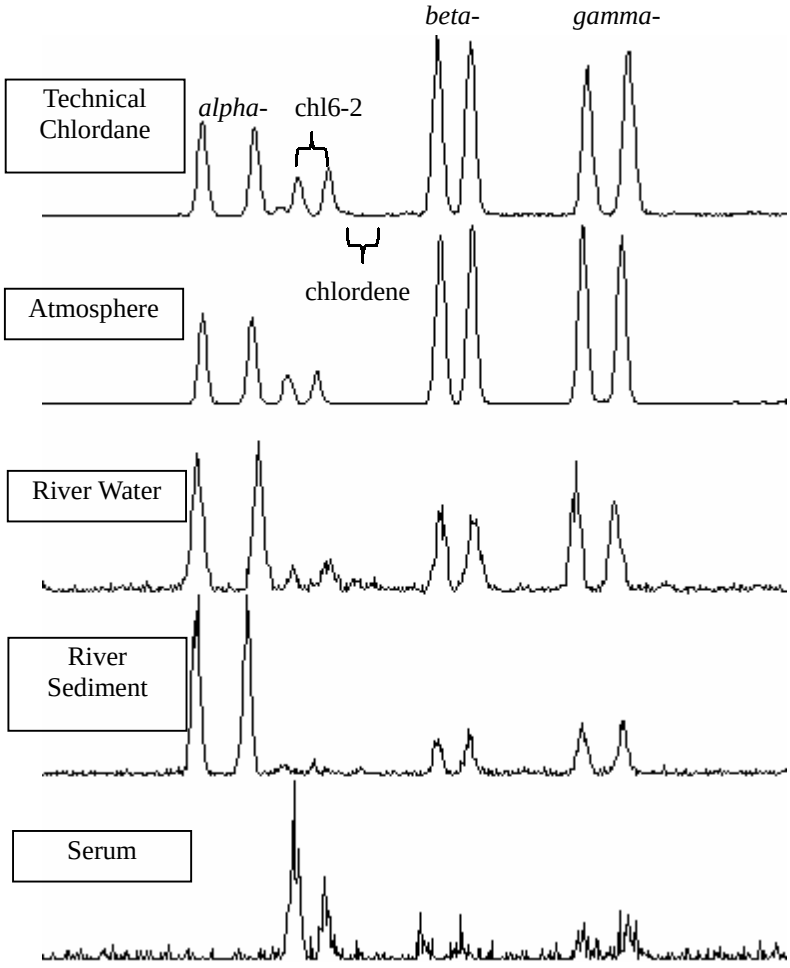

Figure2 GC/MS-SIM chromatogram showing elution of chlordene on BGB-172 HRGC column. (average of *m/z* 302.8883 and 304.8853)

were detected and were also nonracemic. In Table1, EE values of chlordane component in the serum sample were shown. EE of MC-5 and *trans*-chlordane were 71% and 24%, and indicated significant depletion of the last eluting enantiomer. On the other hand, EE of *cis*-chlordane was 48%, and indicated significant depletion of the first eluting enantiomer. Aigner et al.[5] found the (+) enantiomer of *trans*-chlordane was preferentially degraded in all soil samples, and the (-) enantiomer of *cis*-chlordane was degraded in most soil samples. In this study, last eluting enantiomer of *trans*-chlordane and first eluting enantiomer of *cis*-chlordane were degraded in serum sample, but the elution order was not known. The EE values of heptachlor-*exo*-epoxide and oxychlordane were 50% and 21%, and indicated significant depletion of the last eluting enantiomer. Bidleman et al.[6] found a heptachlor-*exo*-epoxide was nonracemic in air and soil samples, and an excess of the (+) enantiomer. In this oven programable temperature on BGB-172 column, there is a possibility that the first eluting enantiomer of heptachlor-*exo*-epoxide is (+) enantiomer. The isomer chl6-2 was gradually degraded compared with predominant isomers of chlordene (*alpha*-, *beta*-, and *gamma*-) in serum. In chlordane, isomers MC-5 was also gradually degraded compared with predominant isomers of chlordane (*trans*- and *cis*-) in serum.

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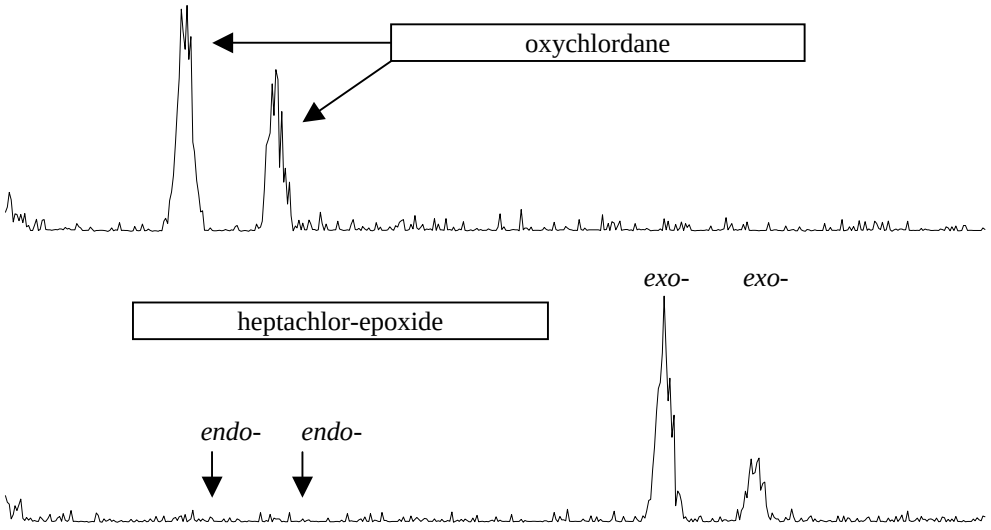

Figure3 GC/MS-SIM chromatogram showing elution of heptachlor-epoxide (average of m/z 352.8442 and 354.8413) and oxychlordane (average of m/z 386.8052 and 388.8023) on BGB-172 HRGC column in serum sample.