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Chiral Signatures of α-Hexachlorocyclohexane in Surface Water, Porewater, and Sediment System

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One of the most widely detected organochlorine pesticides in the environment is hexachlorocyclohexane (HCH). Although several properties of HCH isomer, such as isomer ratios of α/γ -HCH, can be used to infer chemical sources and distinguish fate processes (Falconer et al. 1995), the different physical property of the isomers limited using the ratio to monitor the fate processes. In most case α-HCH is typically predominant in ambient air, sediment and water. Although the fact that α-HCH chiral and existence as pairs of enantiomers were already realized in 1949, it was not considered during the production climax between 1950 and 1980. After König et al succeeded in the enantiomer separation of α-HCH by GC and trace α-HCH chiral separation became possible, it was recognized that the enantiomer separation of this compound may be used to study the enantioselective degradation of α-HCH in the environment (König et al. 1989). As a synthetic product, technical HCH contains the enantiomers of α-HCH in the racemic ratio 1:1. Only enzymic catalysis metabolism can result in enantioselective degradation (Leone et al. 2001). Thus, if degradation of α-HCH in the environment is dominated by biological systems, this will probably produce nonracemic residues. These signatures may be useful for tracking transformations of α-HCH in the environment. Numerous reports have shown that selective biological degradation of α-HCH by microorganisms does occur and can result in nonracemic signatures of α-HCH in soil (Leone et al. 2001; Müller et al. 1992), water (Zhang et al. 1998; Faller et al. 1991), air (Falconer et al. 1995) and biological samples (Müller et al. 1992; Pfaffenberger et al. 1992). However, the behavior of the chiral pollutants, including biotransformation and biodegradation, in surface water, porewater and sediments system was unclear and rarely studied.

The major objective of this study is to provide evidences on enantioselective breakdown of α-HCH in surface water, porewater and sediment. To our knowledge, it is the first report on the enantioselective degradation of α-hexachlorocyclohexane in porewater. The relationship among ERs of surface water, porewater and the corresponding sediment was also discussed.

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MATERIALS AND METHODS

A standard solution of racemic α-HCH was obtained from ChemService, Inc. (West Chester. PA, USA) at a concentration of 100 mg L^{-1} and further diluted with isooctane to obtain the desired concentration. Anhydrous sodium sulfate (analytical grade) was baked at 600 °C for 6 h to destroy organic contamination. All solvents, including acetone, petroleum ether, hexane and isooctane, were of analytical grade and redistilled in all-glass system to remove impurities prior to use. Sulfuric acid silica (50% concentrated sulfuric acid, w/w) was prepared by dropwise addition of concentrated H_2SO_4 (analytical grade) to dry silica (100-200 mesh, Desiccation Reagents Factory of Qingdao Haihua) under continuous stirring by electromagnetic stirrer.

The study sites (A, B, C, D and E) are on the agricultural regions on the outskirts of Beijing City. A, B, C and D are ponds with a depth of 30-50 cm water. A and B are located in the northeast of the city, and C and D in the north of it. In A and B Jiaobai *(Zizania laquatica)* grows. Similarly, in C and D lotus *(Nelumbo nucifera)* grows. The sampling site E is a channel with a depth of 50-70 cm water, which is located in the northwest of the city. There is a lot of floating grass growing in it. Surface water and sediments were collected from the five sampling sites at the same day (13th September 2003). Surface water samples were collected into glass bottles and sediments into stainless cans from about 2-5 cm below the surface （These sample containers were cleaned with solvent prior to use） . After the sediments were shipped to laboratory, they were immediately centrifuged at 2000 rpm for 10 min to obtain porewater. Then the sediments were air-dried at room temperature.

About 5 g sediment was accurately weighed and ultrasonically extracted with 30 ml acetone/petroleum ether (1:1) for 5 minutes. The residue and extract were separated by centrifugation and the extraction process was repeated three times. The extracts were combined. For surface water or porewater samples, liquid-liquid extraction was performed by separation funnel with 30 ml dichloromethane three times and the combined extracts were dried by a column packed with about 10 cm anhydrous sodium sulfate.

The extracts from sediment or water were then concentrated to 1-2 ml by rotary evaporator. An amount of active copper (grain copper pretreated with 10 % nitric acid prior to use) was added to the extract and shaken on a Super-Mixer (USA) to remove sulfur. The sample was then loaded on a 5g sulfuric acid silica column with about 1 cm anhydrous sodium sulfate on the top. The column was eluted with 20 ml petroleum ether. The eluent was concentrated by K. D. evaporative apparatus. The fractionation was performed by a LC-Si column (Sorbent mass 500 mg, Volume 3 ml) (Supelco Park Bellefonate, PA 16823-0048 USA). After the column was conditioned with hexane the sample was loaded on the column and then the column was eluted with hexane. The first 3 ml eluant was discarded and the second fraction (4 ml hexane) was collected, which contained α-HCH. The solvent was evaporated and then the solution volume was reduced to 0.2 ml by a gentle steam of nitrogen.

An Agilent 6890 gas chromatograph equipped with ^{63}Ni electron-capture detection system (Hewlett-Packard, Palo Alto, CA, USA) was used for quantitative analysis of α-HCH. The analytical column was HP-5 (30 m×0.32 mm×1.0 μm) (Hewlett-Packard, Palo Alto, CA, USA). The gas chromatography (GC) conditions were as follows: injector and detector temperature were 280 and 300 °C, respectively. Initial oven temperature was 60 °C, hold for 2 min, increased to 150 °C at a rate of 10 °C min^{-1}, hold at 150 °C for 10 min; then to 250 °C at 5 °C min^{-1}, hold for 2 min. The inlet was operated in splitless mode and nitrogen of high purity was used as carrier gas. External standard method was used for quantitative analysis.

Determination of α-HCH enantiomers was done with a Varian 3700 gas chromatograph equipped with ^{63}Ni electron capture detection on CP-Chirasil-Dex-CB column (25m×0.25mm×0.25μ m). A 1 μL aliquot sample was injected in splitless mode, and split valve was opened after 1 min. Oven temperature was programmed from 80 °C (hold for 2 min) to 180 °C at 4 °C/min and hold for 20min. Nitrogen was used as carrier gas and anode gas. The injector temperature was 200 °C and detector temperature was 250 °C. A WDL-95 workstation (Dalian Inst. Chem. Phys, CAS, China) was utilized to control the system and for acquisition of analytical data.

RESULTS AND DISCUSSION

A solvent blank sample and deionized (Milli-Q) water samples followed the sample to be analyzed through the entire analysis procedure. The limits of detection (LODs), estimated as a signal-to-noise ratio (S/N) of 3, were 0.08 ng g^{-1} and 0.4 ng L^{-1} α-HCH for sediment and water samples, respectively. Recovery experiments were done by spiking 40 ng α-HCH into samples, with recovery ranging from 82.3 to 115.23% (n=5).

The enantiomeric ratio (ER) is defined as A+/A-, where A+ and A- correspond to the peak areas of the (+) and (-) enantiomers. The two enantiomers can achieve baseline separation on the CP-Chirasil-Dex-CB column. The elution order of α-HCH enantiomers on CP-Chirasil-Dex-CB column is confirmed by injecting standard solutions of (+)-α-HCH and (-)-α-HCH (optical grade), respectively. The elution order is (+)-α-HCH eluting first on this column. α-HCH occurs in the technical mixture as a racemic mixture of two enantiomers. If no metabolism occurs the ER should be 1.00. Seven successive injections of 100pg racemic α-HCH were done to check the individual enantiomer response. Depending on the measurements, the average ratio of (+)α-HCH/(-)α-HCH was 1.00±0.005, which is in excellent agreement with the theoretical ER value. This reproducibility allows determination of enantiomer ratio changes of even a few percent with sufficient significance.

The concentrations of α-HCH in surface water, porewater and sediments are presented in Table 1. To understand the current status of α-HCH contamination in the studied areas, other investigation results in surface water, porewater and sediments from different places in China and others countries are also listed in Table 2. It seems that the contamination levels of α-HCH in the water and sediment samples are lower than those of earlier reported data. Therefore, we can conclude that the present contamination levels of α-HCH in the areas are less serious than those in other lakes, seas and rivers.

Table 1. Concentrations of α-HCH in surface water, porewater and sediments.

Sampling sites	A	B	C	D	E
Surface water (ng L^{-1})	1.26	1.72	0.994	1.54	0.909
Porewater (ng L^{-1})	1.25	——	1.63	0.995	0.997
Sediment (ng g^{-1})	0.391	0.224	0.105	0.319	0.159

Table 2. Comparison of α-HCH levels in surface water, porewater (ng L^{-1}) and sediments (ng g^{-1}) with those in other places.

Locality	Medium	Survey year	α-HCH	References
Liaohe River	Water Sediment	1998	24.5-63.8 0.4-1.2	Zhang et al. 2000
Xingang Harbour HaiheRiver Estuary	Water	1997	90-129 626-716	Zhang et al. 1998
North Sea	Seawater	1994-1995	0.3-4.6	Bethan et al. 2001
Macao Estuary	Sediment	1997	0.71	Kang et al. 2001
Jiulong River Estuary	Surface water Porewater	1999	0.17-23.5 0.30-471	Zhang et al. 2001
The Gulf of Bothnia	Sediment	1991	3.5-4.5	Strandberg et al. 2000
Ponds and channel in Beijing	Surface water Porewater Sediment	2003	0.909-1.72 0.995-1.63 0.105-0.391	Present study

ERs of α-HCH in the surface water, porewater and sediments are listed in Table 3 and a typical chromatogram of α-HCH in porewater is shown in Figure 1 (b). For most of samples, ERs are below 1.00. These samples show a 10-20% depletion of (+)-α-HCH relative to (-)-α-HCH. Zhang et al reported the enantioselective breakdown of α-HCH in Xingang Harbour marine water and Haihe River estuary water of Tianjin, China (Zhang et al. 1998). The ERs of α-HCH in the marine water varied from 0.91 to 0.80 in early spring and early summer, indicating that the marine microbial degradation of α-HCH had enantioselectivity and

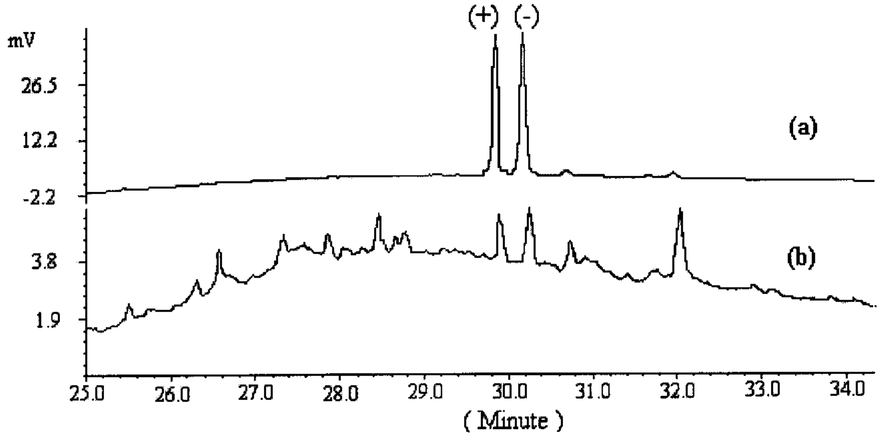

Figure 1. Chromatograms of α-HCH
(a) Standard chromatogram of α-HCH
(b) Typical chromatogram of α-HCH in porewater sample

(+)-α-HCH was degraded preferentially. However, depletion of the (-)-enantiomer was seen in marine mammals and birds (Müller et al. 1992; Pfaffenberger et al. 1992) as well as some soils(Müller et al. 1992). Samples from the North Sea showed degradation of the (+)-enantiomer in some regions and of the (-)-enantiomer in others (Faller et al. 1991). Amituk Lake is the first freshwater system where chiral degradation has been investigated. Differences in ERs up to 35% found in the lake system were much great. At the same time, enantioselective breakdown of α-HCH has been also reported in seawater (Pfaffenberger et al. 1992).

Table 3. ERs of α-HCH in surface water, porewater and sediments.

Sampling sites	A	B	C	D	E
Surface water	0.79	0.76	0.93	0.79	1.00
Porewater	0.79	——	0.74	0.79	0.88
Sediment	0.77	0.76	0.65	0.78	0.62

For A, B and D, ERs of α-HCH range from 0.76 to 0.79, but there is no significant difference among different media of surface water, porewater and the corresponding sediment. However, for C and E a significant difference of ERs value is found among surface water, porewater and the corresponding sediment. An obvious trend can be observed in the media. The enantioselective preference shows the trend that ER(sediment) < ER(porewater) < ER(surface water). The difference of ERs is even up to 38% between surface water and corresponding sediment in sample site E. This result can be explained as follows.

First, this ER trend might indicate that the microbes in sediments and porewater have greater capability of enantioselectively degradation of α-HCH than those in surface water, or in surface water the photodegradation might be the main pathway of decomposition of α-HCH, while in sediment and porewater biotic-degradation plays more important role. The second possible explanation for the observed phenomena is that the biota enantioselective degradation occurs in different situation. In another word, aerobic degradation mainly occurs in surface water, while anaerobic condition in porewater and sediment results in enantioselectively degradation of α-HCH. Buser et al found in sewage sludge under anaerobic conditions a 2-3 fold difference among the two enantiomers of α-HCH with (+)-α-HCH degraded faster (Buser and Müller, 1995). Jantunen and Bidleman reported chiral degradation of α-HCH increases with depth in Arctic Ocean to the point where the (+) enantiomers is almost absent in deep samples. (Jantunen and Bidleman, 1996). For the most northern stations in the Chukchi, the ERs were 1.07 at 1-40m, 0.85 at 53-106m, and 0.76 at 133-298m. In the north of Spitsbergen, the ER value fell from 0.82 at 47m to 0.14 at 753m. It goes without saying that with the water depth increasing, the concentration of O_2 should be very low. Their results also support the same conclusion that the microbes mainly responsible for enantioselectively degrading (+)-α-HCH should be anaerobic types. Little is known about the mechanisms of enantioselective degradation by different microbes by now. Further investigation and more laboratory experiments should be done to understand the enantioselective degradation by microbes.

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