ENANTIOMERIC SIGNATURE OF CHIRAL POLYCHLORINATED BIPHENYL ATROPISOMERS IN A BENTHIC FOOD CHAIN

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Abstract
Sediment, polychaeta and flatfish were collected from Masan Bay, Korea in December 2004 and May 2005. Muscle, liver and stomach content of flatfish, polychaeta, and sediment were analyzed for polychlorinated biphenyls (PCBs) concentrations and the enantiomeric fractions (EFs) of six chiral PCB congeners (CBs 91, 95, 136, 149, 174, and 176) to quantify enantiomer-specific accumulation of PCBs in the benthic environment of the bay. Stomach content and stable isotope of carbon and nitrogen were analyzed in samples and used to confirm the prey and predator relationship between them. The total PCB concentrations in the samples clearly showed that PCBs are biomagnified in higher trophic level organisms. The congener pattern of chiral PCBs are similar to between the benthic food chain members, however, they had different chiral signatures, indicating that PCBs were accumulated enantioselectively through the food chain. The change of chiral signatures of chiral PCB atropisomers in the benthic food chain of the bay can be summarized as follows: CB 91, E2 to racemic; CB95, racemic to E1; CB 136, racemic to (-)-enantiomer; CB 149, (+)-enantiomer to racemic; CB 174, racemic to racemic.

Introduction
Chiral environmental chemistry is a research area that can provide enhanced insight into environmental fate processes. The enantiomers of chiral compounds may exhibit different biological and toxicological properties from each other and from the racemic mixture, which are released to the environment as a racemic and sequentially changed their chirality with biological processes. Therefore, understanding the environmental behavior of the enantiomers is important for determining the hazards they pose. Of the 209 PCB congeners, 78 exhibit axial chirality in their non-planar conformations. Nineteen of these atropisomers with three or four *ortho* chlorines remain stable in the environment due to restricted rotation around the central biphenyl bond[1]. Several PCB congeners have been found in nonracemic concentrations in sediment[2] and biota[3], indicating that a biotransformation and enantioselective assimilation had occurred because abiotic reactions are not enantioselective.

In this study, we collected sediment and benthic organisms from Masan Bay of Korea in order to investigate the enantiomeric composition of chiral PCB atropisomers in a benthic food chain of the bay. The prey and predator relationship in the food chain were confirmed by stomach content analysis and stable isotope analysis.

Materials and methods
Sampling site and sampling strategy
Masan Bay is a semi-enclosed embayment located in the southern part of Korea (Figure 1). This bay has been the focus of many previous studies on pollution monitoring, as it has been designated as a 'special management coastal area' since 1983. This bay is surrounded by heavily populated cities and industrial complexes. Some of these pollutants are introduced into the coastal waters from rivers or streams via discharges of industrial waste, municipal sewage, and urban and agricultural runoff. The semi-enclosed nature of the

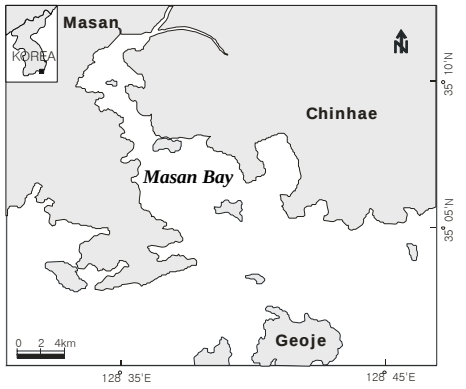

Figure 1. Map of Masan Bay, Korea

bay leads to accumulation of pollutants in the bay. In summer time, red tide events frequently occur, which lead to anoxic condition of bottom sediment.

Sediment and benthic organisms including polychaeta and flatfish (marbled sole, *Limanda yokohamae*) were collected from the Masan Bay of Korea in December 2004 and May 2005 (Figure 1). Surface sediment and polychaeta samples were collected using a van Veen grab sampler. For sediment samples, approximately 2 cm of top sediment was taken from each site for chemical analysis. Polychaeta was depurated overnight in seawater from the sampling site to evacuate gut contents before further analysis. Flatfish was gill-netted, and liver, muscle and stomach content were taken. All samples were stored at -20°C until chemical analysis.

Stomach content and stable isotope analysis

To reveal the prey and predator relationship between each matrix, stomach content and stable isotope analyses were conducted. The gut content of flatfish was collected, chemically fixed with 10% formalin, and classified. Isotopic signature of nitrogen and carbon was analyzed at Iso-Trace New Zealand Ltd. To perform isotopic analysis for biota samples, tissues were lipid extracted with a mixture of chloroform and methanol (87:13 v/v). After lipid extraction, samples were dried and homogenized. To remove inorganic carbon from sediment and polychaeta samples, sample was dried and treated with 10% (v/v) HCl. Then, the samples were analyzed using a nitrogen/carbon analyzer interfaced to a mass spectrometer.

Chemical analysis

The sediment and biota samples were prepared for PCB analysis in accordance with previously reported method[4] that includes silica - alumina column chromatography and size-exclusion high pressure liquid chromatography (HPLC) for clean-up and chemical separation. PCB concentration was determined using a gas chromatograph (Hewlett–Packard 6890) with a micro electron capture detector (μECD with DB-5 capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness). Quality Assurance / Quality Control procedures (i.e., analysis of blanks and certified materials) were included within every batch of ten to twelve samples.

Enantiomers of six chiral PCBs were quantified by enantioselective capillary gas chromatograph / mass spectrometry (GC/MS) on a Shimadzu GC-2010/QP-2010 mass selective detector in the electron impact mode with a suite of modified cyclodextrin columns: PCBs 91, 95 and 136 on Cyclosil-B (30m x 0.25mm i.d. x 0.25 μm film thickness, Agilent Technologies); PCBs 136, 149, 174, and 176 on Chirasil-Dex (30m x 0.25mm i.d. x 0.25 μm film thickness, Varian).

Enantiomeric fractions (EFs) for the chiral PCB atropisomers were calculated as follows:

$$EF = \frac{ER}{1+ER} = \frac{1}{1+\frac{1}{ER}}$$

The enantiomeric ratios (ERs) were quantified as ER= (+)/(-) concentration ratios when enantiomer elution order was known on the chiral GC columns used (PCB-136, 149, 174, and 176) and as the areas of the first-eluting to the second-eluting enantiomers on Chirasil-Dex (for PCB-91, 95).[2,3,5]

Results and discussion

The result of stomach content analysis, polychaeta was found as the major food item of flatfish inhabiting the bay, which comprise above 80% and followed by crustaceans (the data is not shown). Moreover, stable isotope signatures in the samples also indicate that sediment, polychaeta and flatfish are members of a benthic food chain in the bay (Figure 2). Interestingly, the values of $\delta^{15}N$ and $\delta^{13}C$ found in stomach content of flatfish are

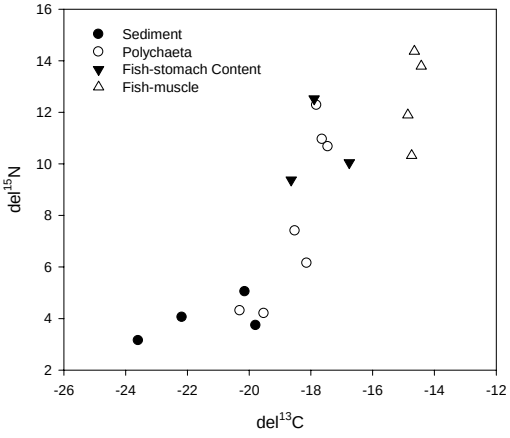

Figure 2. Carbon and nitrogen isotope values of the benthic food web members

partially overlapped with those of polychaeta, which is consistent with the result of stomach content analysis.

The total PCB concentrations in the samples from the bay clearly showed that PCBs are biomagnified ascending trophic level as follows (unit: ng/g dry weight): sediment 35.6±19.7 < polychaeta 610±77< Flatfish [liver 1140±200, stomach content 298±15, muscle 66.3±8.7]. The concentrations of chiral PCBs in the samples are presented in Figure 3. The congener patterns of chiral PCBs in sediment, polychaeta, and three organs of flatfish are very similar to each other. On the other hand, the patterns of zoo- and phytoplankton taken from the surface water column are a little different from those of the benthic species and sediment. The result implies that the PCB pattern in each food source (sediment for polychaeta and polychaeta for flatfish, respectively) is reflected into its consumer.

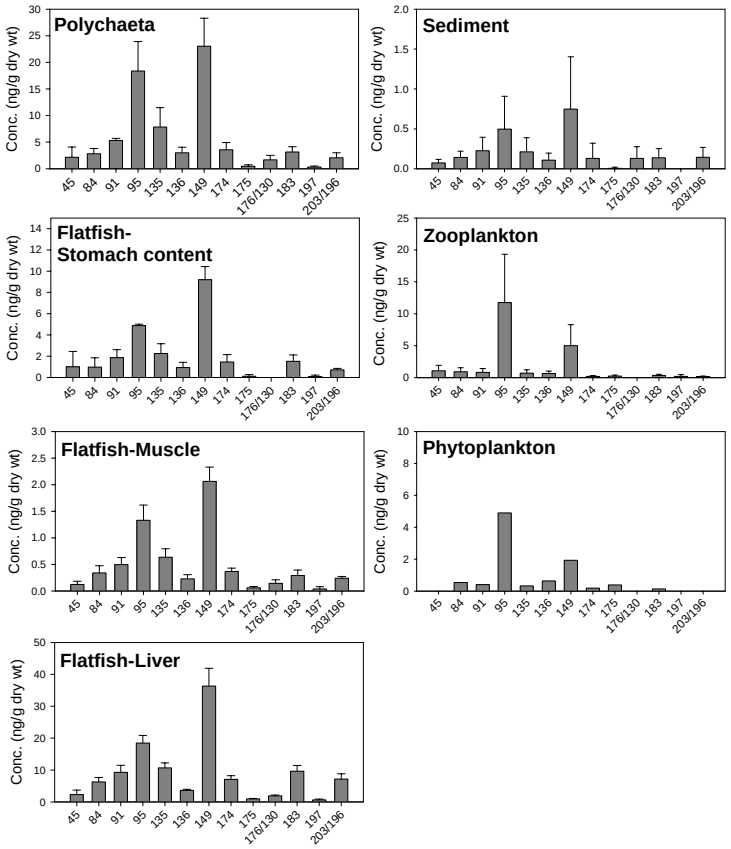

Figure 3. Congener pattern of chiral PCBs in sediment, plankton, polychaeta, and flatfish

Figure 4 shows the enantiomeric fraction of six chiral PCB atropisomers in sediment, polychaeta, and three organs of flatfish. Nonracemic amounts of chiral PCBs were found in the benthic food chain members. Atropisomeric PCBs were released into the environment as racemates. Therefore, the nonracemic presence of these congeners is strong evidence of bioprocessing. Although the congener pattern of chiral PCBs are similar to between the benthic food chain members, the chiral signatures between them are different. Except for CB 91, congeners in sediment were racemic or near racemic, indicating that less enantioselective bio-processing in the sediment than higher trophic organisms. Wong et al. (2001)[3] also reported that the chiral composition of PCBs in fish and bivalves was more nonracemic than in sediments. In general, the EF values in stomach content of flatfish closely approximated the values in polychaeta, which is strong evidence that polychaeta is the major prey of flatfish in this bay. The EF values in liver and muscle of flatfish were relatively similar to each other. The

change of chiral signatures of chiral PCB atropisomers in the benthic food chain (sediment-polychaeta-flatfish) of Masan Bay can be summarized as follows: CB 91, E2 to racemic; CB95, racemic to E1; CB 136, Racemic to (-)-enantiomer; CB 149, (+)-enantiomer to racemic; CB 174, racemic to racemic.

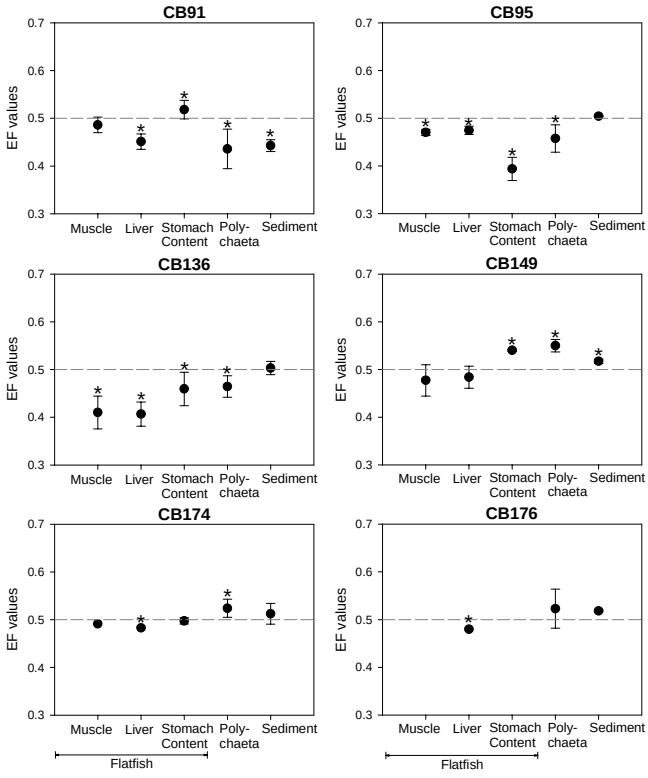

Figure 4. The change of enantiomeric fractions (EFs) of six PCB atropisomers in a benthic food chain in Masan Bay, Korea. Dash lines represent a racemic signature (EF=0.5). *: significantly different than EF of standard (t-test, $P < 0.05$).

Acknowledgments

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