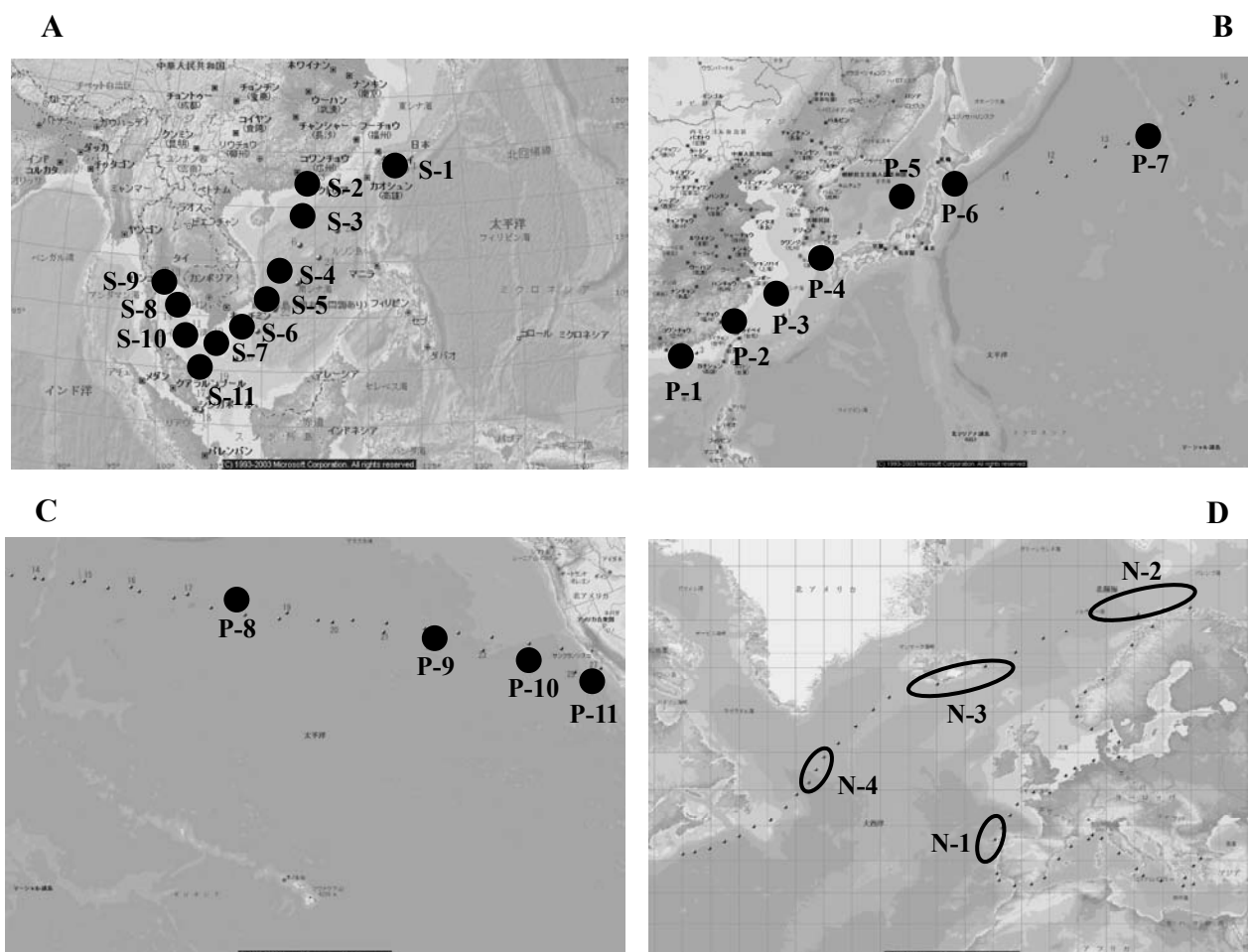


## Enantiomeric Excess of POPs in the Environment

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The enantiomeric excess (EE) of chiral persistent organic pollutants (POPs) in seawater and air samples have been investigated. The seawater samples were taken from South China Sea and Pacific Ocean, and the air samples were collected from North Atlantic Ocean. The enantiomeric composition of *alpha*-HCH, *trans*-chlordane and *cis*-chlordane was racemic (average EE = 2.1-6.2% in air, 5.8-6.9% in seawater). However, each EE value of heptachlor *exo*-epoxide and *o,p'*-DDD, which are metabolites of heptachlor and *o,p'*-DDT respectively, was significantly different from racemic (average EE = 31.3% in air, 22.8-50.8% in seawater). These results suggest that it is possible to distinguish between newly caused pollution and past preserved by monitoring EE.



**Figure 1.** Sampling site; (A) South China Sea, (B) Pacific Ocean (from South China Sea to middle Pacific Ocean), (C) Pacific Ocean (from middle Pacific Ocean to San Francisco coast), (D) North Atlantic Ocean

### Introduction

It is feared that long-range transport of POPs through air and water cause global environmental contamination. Since Stockholm Convention held in May 2001, international regulation have made on reducing emissions to the environment, and various investigation have been studied in the world.

Little is known about differences in biological effects between enantiomers of chlorinated pesticides. Toxicity studies about chlordane congeners revealed that *cis*-chlordane and *trans*-chlordane showed gender-specific enantiomer ratios<sup>1</sup>. Pollutants accumulate in the marine food web, and enantioselective decomposition of one enantiomer by enzymatic processes in marine biota<sup>2</sup>. They are transported by oceanic and atmospheric currents to all over the world. The atmospheric behavior and distribution of organic pollutants depend on their physical properties and reactivity, the dynamics of the atmosphere, and location of sources<sup>3</sup>.

It is particularly important to identify the behavior of the high persistent pollutants in the environment, such as POPs. In this study, EE of the chiral POPs in seawater and air were investigated. In the technical product, the chiral POPs are present as racemates (EE=0%). These chemicals transfer into the environments, and undergo physicochemical changes such as volatilization and adsorption. During changes in physicochemical status, the EE value does not change. However, the metabolites showed enantioselective degradation of (-) or (+) enantiomer. Remarking on this fact, the chiral signatures have been investigated as a parameter for monitoring chiral POPs in the environment.

### Materials and Methods

**Samples:** Seawater samples were taken at various points from South China Sea in June and July 2006 and from Pacific Ocean in August and September 2005. Air samples were collected from North Atlantic Ocean in May and June 2005. The sampling sites were shown in Figure 1. Each sample was collected using the marine pollution observation system developed by Kunugi et al<sup>4</sup>. The automatic seawater sampling system is shown in Figure 2, which was mounted on container-ship. The column unit was equipped with a polyurethane foam (PUF) and five sheets of active carbon fiber filters (ACF) into a glass holder. Each sample was collected by solid phase extraction method. Sample volume of seawater and air were 100L and 400m<sup>3</sup>, respectively.

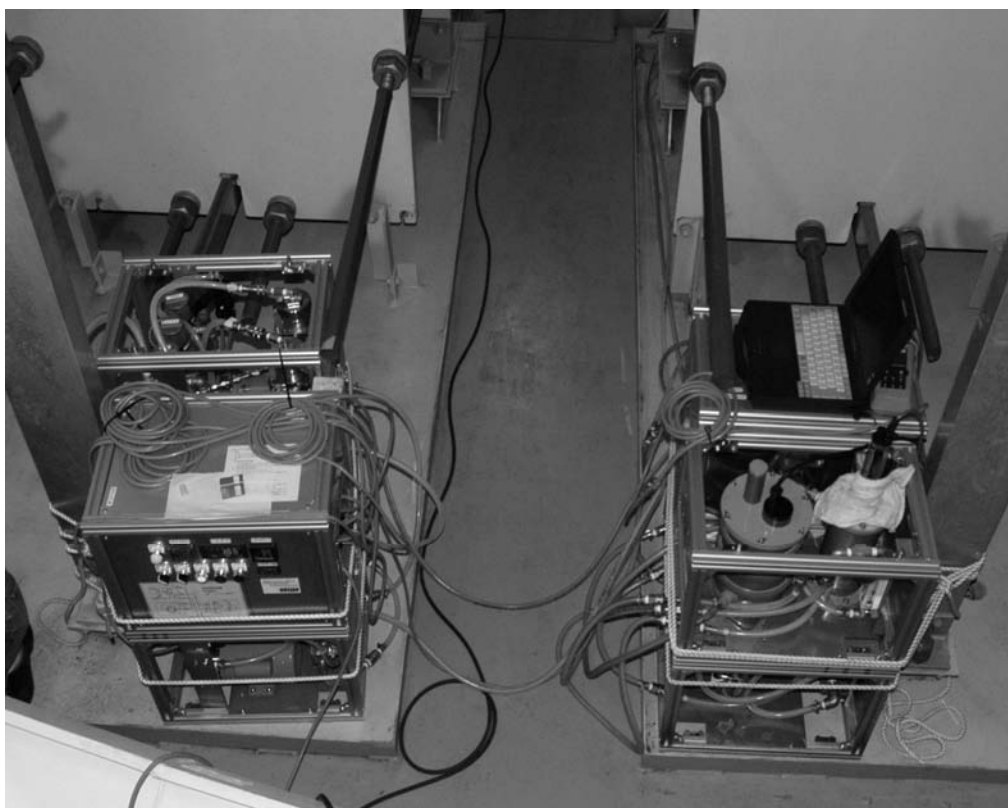
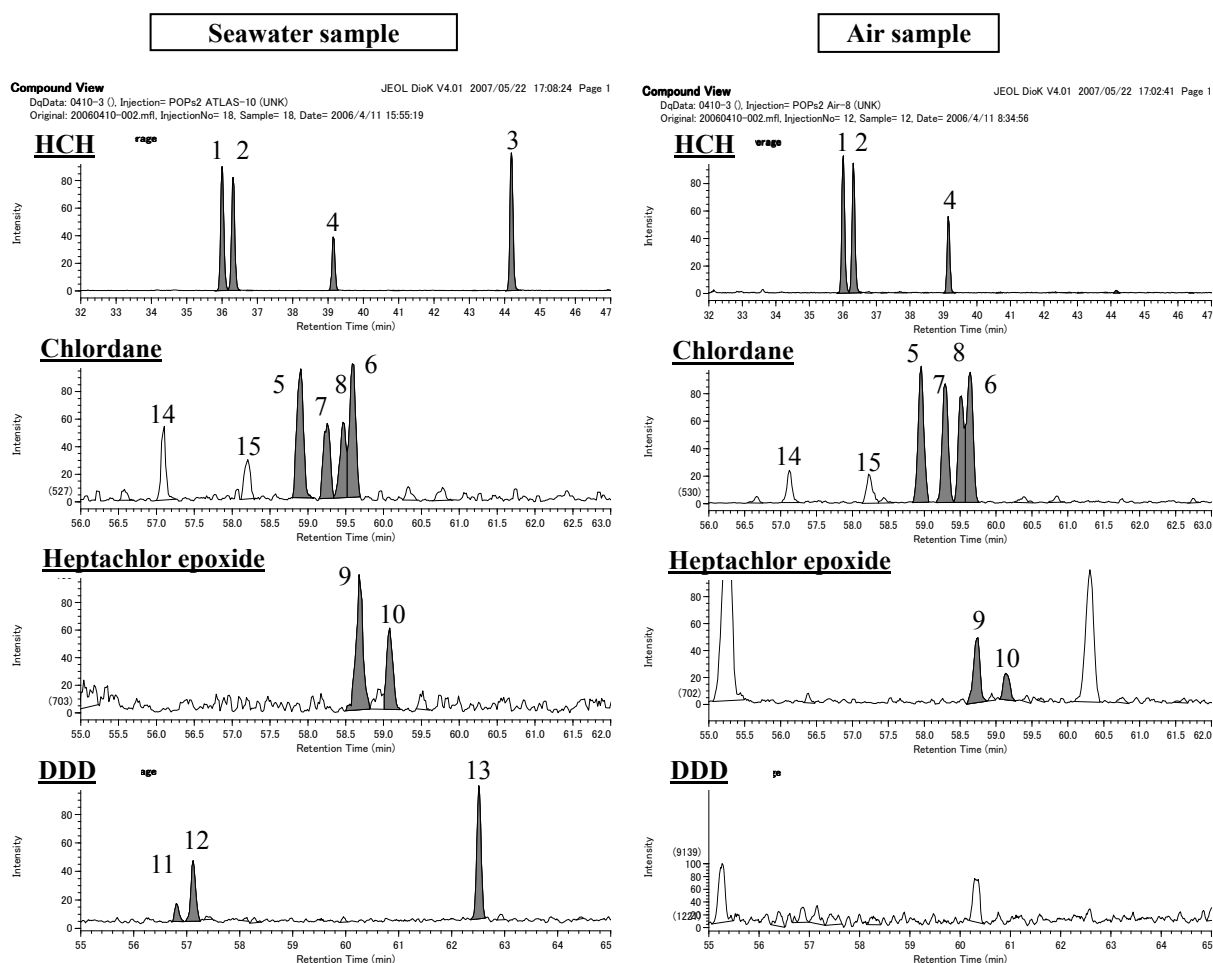


Figure 2. Photograph of marine pollution observation system mounted on container-ship

**Sample Preparation:** The samples were extracted by Soxhlet extraction for 3hr with acetone and then for 24hr with dichloromethane. Both extracts were mixed and concentrated, and then exchanged to hexane. The solution was fractionated on a silica gel (LC-Si, Supelco) column, eluting with 60mL of hexane. The samples were concentrated to 50 $\mu$ L under nitrogen stream.

**Enantiomer Selective Separation:** Analysis was carried out on high resolution gas chromatograph / high resolution mass spectrometry (HRGC/HRMS, JMS-800D) equipped with a BGB-172 column (30m, 0.25mm i.d., 0.25 $\mu$ m film thickness; 20% *tert*-butyldimethylsilyl- $\beta$ -cyclodextrin dissolved in 15% diphenyl-polysiloxane and 85% dimethylpolysiloxane, BGB Analytik). The HRGC/HRMS was operated under the following conditions: injector temperature, 230 $^{\circ}$ C; ion source temperature, 260 $^{\circ}$ C; temperature program, 120 $^{\circ}$ C for 2min, 2 $^{\circ}$ C /min to 250 $^{\circ}$ C, held for 3min. Target analytes were *alpha*-HCH, *o,p'*-DDD, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane and oxychlordane.



**Figure 3. Mass Chromatograms of seawater and air sample; 1&2: *alpha*-HCH, 3: *beta*-HCH, 4: *gamma*-HCH, 5&6: *trans*-chlordane, 7&8: *cis*-chlordane, 9&10: heptachlor *exo*-epoxide, 11&12: *o,p'*-DDD, 13: *p,p'*-DDD, 14&15: MC5**

**Table 1. The EE values for chiral POPs of each sample**

Air from North Atlantic Ocean					
	N-1	N-2	N-3	N-4	average
<i>alpha</i> -HCH	0.7	0.1	4.5	3.0	2.1
<i>o,p'</i> -DDD	—	—	—	—	—
heptachlor <i>exo</i> -epoxide	33.6	7.6	40.2	43.8	31.3
heptachlor <i>endo</i> -epoxide	—	—	—	—	—
<i>trans</i> -chlordane	4.7	15.9	0.4	3.8	6.2
<i>cis</i> -chlordane	2.0	2.2	2.2	7.1	3.4
oxychlordane	1.4	—	5.7	—	3.5

Seawater from Pacific Ocean												
	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9	P-10	P-11	average
<i>alpha</i> -HCH	5.4	9.0	4.3	13.3	13.0	5.4	2.2	10.0	10.0	6.3	4.3	7.6
<i>o,p'</i> -DDD	20.7	58.9	—	64.0	62.0	57.9	—	—	—	—	—	52.7
heptachlor <i>exo</i> -epoxide	—	28.8	6.5	—	9.9	30.1	21.2	20.1	28.2	19.8	24.1	21.0
heptachlor <i>endo</i> -epoxide	—	—	—	—	—	—	—	—	—	—	—	—
<i>trans</i> -chlordane	0.6	4.8	4.8	38.4	3.0	2.6	3.3	9.0	3.9	4.1	8.7	7.6
<i>cis</i> -chlordane	7.3	6.8	1.6	9.9	8.7	7.7	4.9	18.9	1.0	1.5	3.3	6.5
oxychlordane	—	—	—	—	—	—	—	—	—	—	—	—

Seawater from South China Sea												
	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	average
<i>alpha</i> -HCH	3.8	15.0	5.9	7.6	6.6	1.3	7.0	2.5	5.9	8.9	5.0	6.3
<i>o,p'</i> -DDD	—	59.5	64.4	34.3	64.0	49.9	46.6	44.0	51.3	34.1	50.8	49.9
heptachlor <i>exo</i> -epoxide	—	—	—	—	—	—	—	—	—	—	38.8	38.8
heptachlor <i>endo</i> -epoxide	—	—	—	—	—	—	—	—	—	—	—	—
<i>trans</i> -chlordane	3.3	4.1	3.3	5.0	6.1	3.8	4.2	4.3	4.2	9.0	0.9	4.4
<i>cis</i> -chlordane	7.2	3.8	0.5	4.8	4.4	5.1	0.9	13.8	4.6	4.1	7.2	5.1
oxychlordane	—	—	—	—	—	—	—	—	—	—	—	—

## Results and Discussion

The EE value was defined as the difference between the peak ratio of the larger and the smaller enantiomer. It can be described following equation

$$EE(\%) = \frac{E_L - E_S}{E_L + E_S} \cdot 100$$

Where  $E_L$  is the amount of larger enantiomer,  $E_S$  is the amount of smaller enantiomer. EE value for heptachlor *endo*-epoxide of all samples, for oxychlordane of seawater samples and for *o,p'*-DDD of air samples were not obtained because the concentration was too low.

Figure 3 shows the mass chromatogram of chiral POPs in seawater and air samples. The EE values of *alpha*-HCH, *trans*-chlordane and *cis*-chlordane in air samples range from 0.1 to 4.5%, from 0.4 to 15.9% and from 2.0 to 7.1%, respectively, as shown in Table 1. Those in seawater samples were from 1.3 to 15.0%, from 0.6 to 38.4% and 0.5 to 18.9%, respectively. It was observed those chiral compounds in air sample were near racemic. The EE values in seawater samples were generally larger than air samples. However a few seawater samples were observed nonracemic, most seawater samples were close to racemic. The EE values of metabolites heptachlor *exo*-epoxide in air samples and in seawater samples range from 7.6 to 43.8 and from 6.5 to 38.8, respectively. Except only one seawater sample, enrichment of second eluting enantiomer was found in all other samples. The range of EE value of *o,p'*-DDD, which is the metabolite of *o,p'*-DDT, in seawater samples were from 20.7 to 64.4. In all samples, it was observed that the enantiomeric composition of *o,p'*-DDD was clearly nonracemic.

Further studies about global scale observations of chiral signatures are necessary to identify the environmental behavior of pollutants and to distinguish between the newly pollutant and the past preserved.

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