# D-2 Studies on the Development of Monitoring and Evaluation Technology for Global-Scale Marine Pollution with Hazardous Chemicals

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## 1. Introduction

In recent years, numbers and amounts of hazardous chemical substances used for industrial and domestic purposes have rapidly increased. It is said that these substances are finally accumulated in seawater and sediment. However, the distribution and the temporal variation of the hazardous chemical substances in marine environment have not been clarified yet in a global scale due to the limited number of observation data.

We believe that more frequent spatial and temporal observations are required in order to understand the marine pollution mechanism.

## 2. Research objective

The aim of this project is to develop the monitoring systems for marine pollution with higher flexibility in sampling station and time by using merchant vessels and by developing the automated sampling system in order to clarify the spatial and temporal variation and chemodynamics of hazardous chemicals in seawater in a global scale.

## 3. Method

To know the occurrence and behavior of persistent organic pollutants (POPs), endocrine disrupting chemicals and heavy metals in marine environment, automated sampling systems were developed and installed in both a tanker transporting from the Persian Gulf to Japan and a coal carrier vessel from Australia to Japan. For the measurement of POPs and eastrogenic activity large amounts of seawater (100 L – 300 L) were passed through columns containing poly-urethane foam (PUF) and active carbon filter (ACF). POPs and endocrine disrupting chemicals were extracted by the Soxhlet's extraction with acetone and dichloromethane from these columns. POPs were determined by gas chromatography/mass spectrometry (GC/MS) and estrogenic activities were determined by chemiluminescent reporter gene assay. For the measurement of organotin compounds and heavy metals seawater (0.5 L) were collected in glass (for organotins) or polypropylene (for heavy metals) bottles. Main and trace elements including hazardous heavy metals were determined by chelate preconcentration/inductively coupled plasma mass spectrometry (ICP-MS). Organotin compounds were determined by GC/ICP-MS. These seawater samples were collected several times between January 2001 and December 2002. Water temperature, pH, chlorophyll-A and conductivity were also monitored. A photo of the automatic sampling systems is shown in Fig. 1. Typical sampling locations between Japan and the Persian Gulf and those between Japan and Australia were shown in Fig. 2 and 3, respectively.
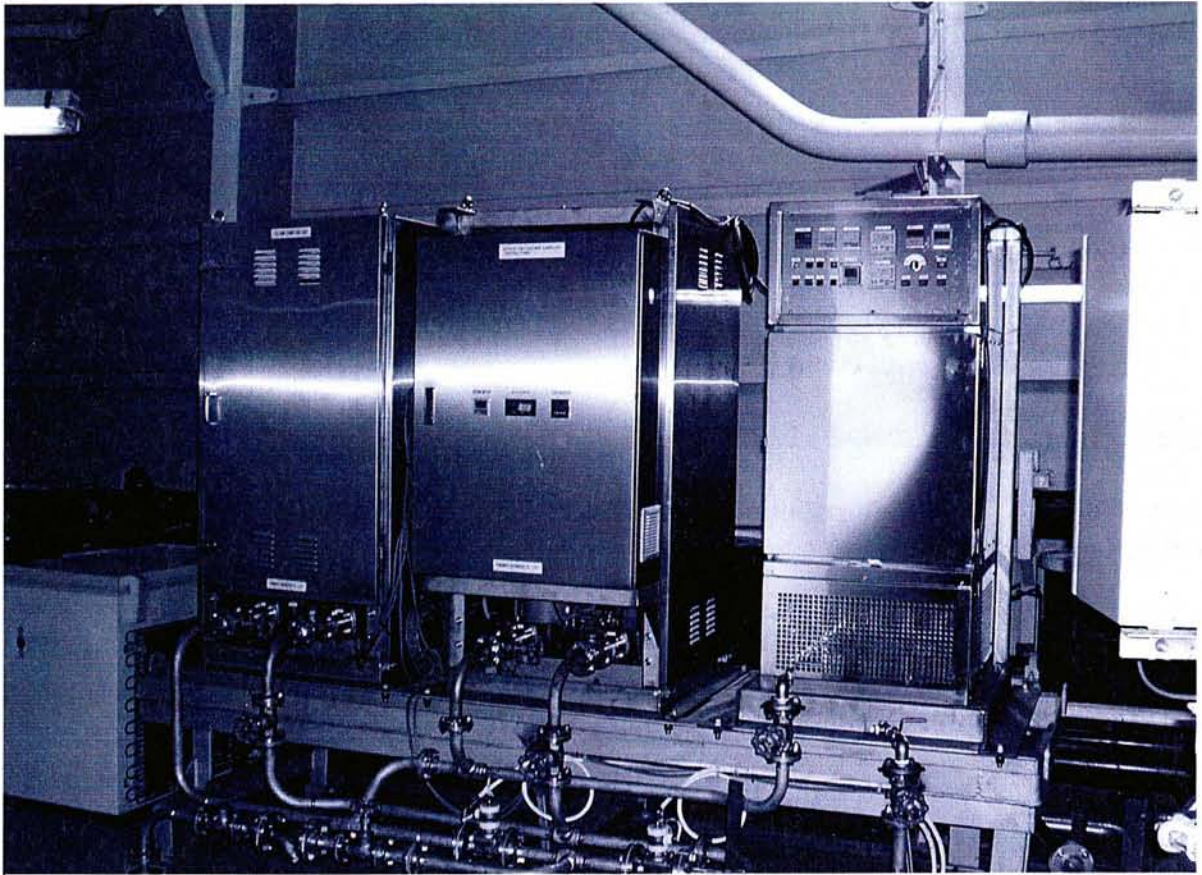
Figure 1 Photograph of marine pollution observation system onboard the coal carrier.
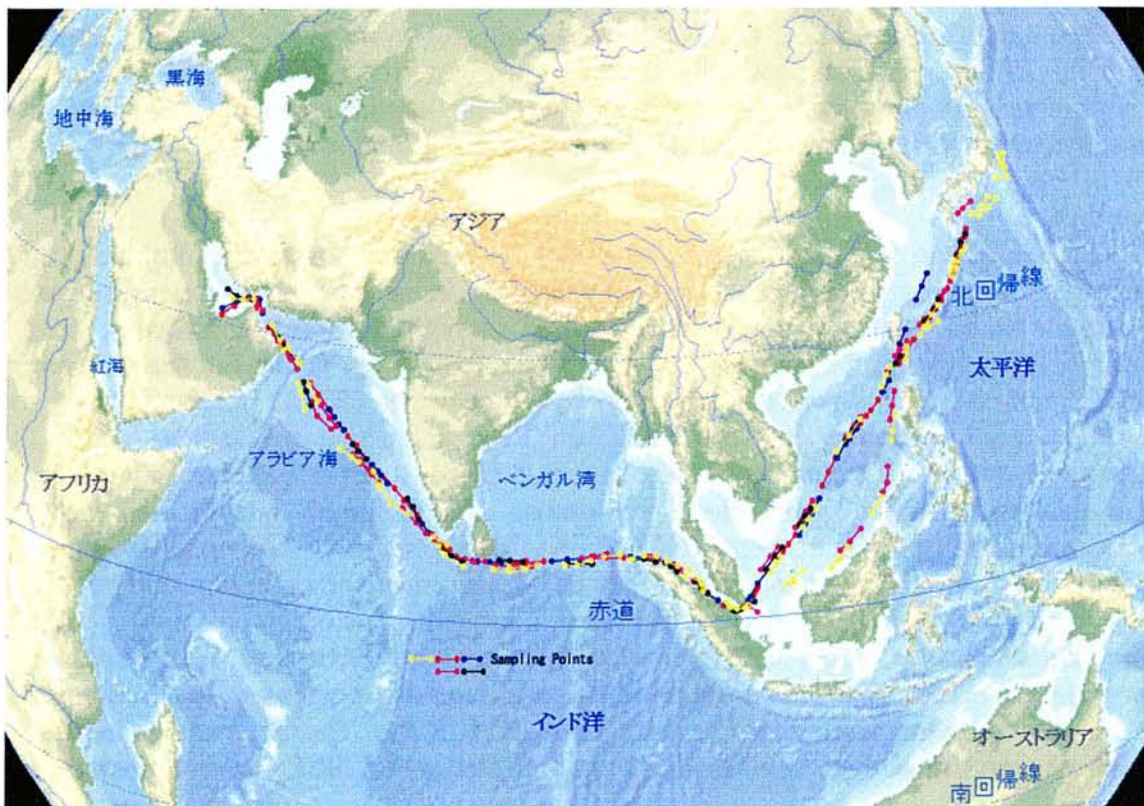


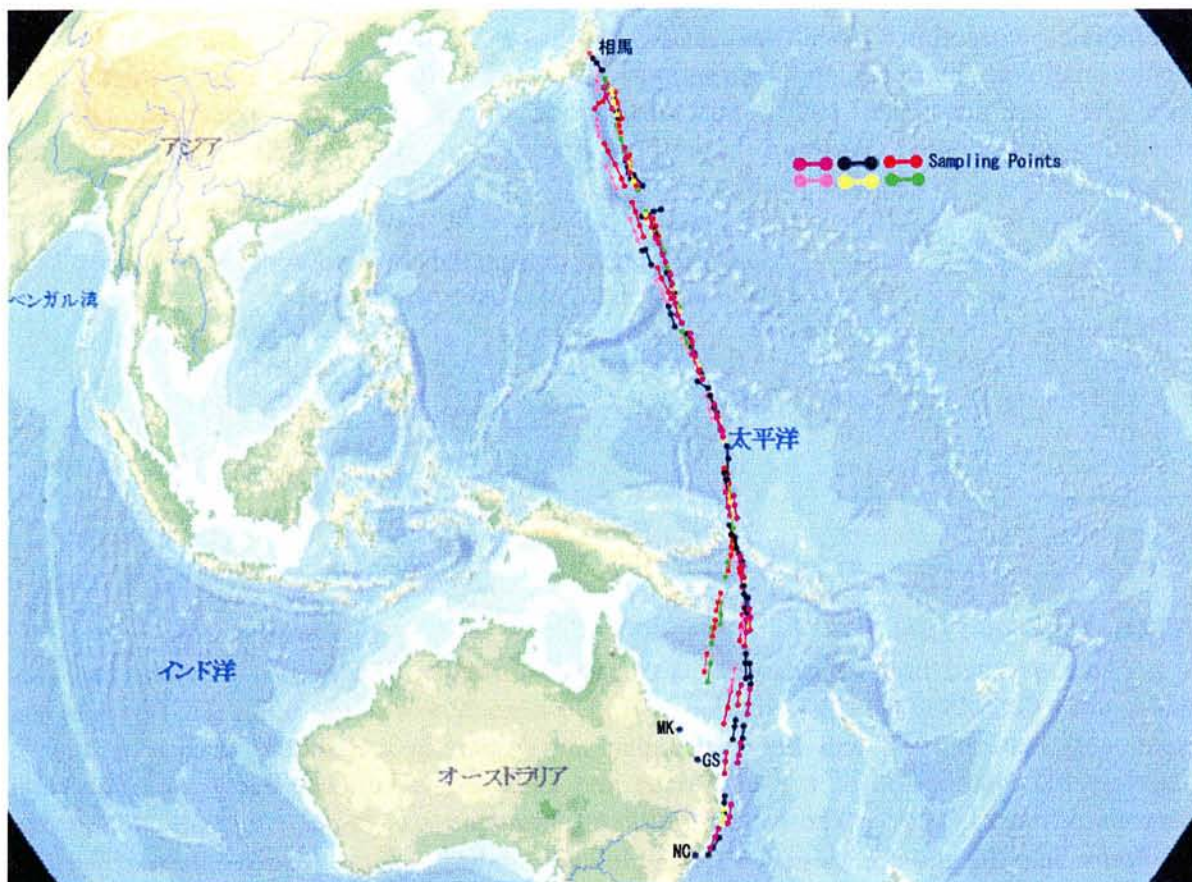Figure 2 Sampling positions between Japan and Persian Gulf.

Figure 3 Sampling positions between Japan and Australia.

## 4. Results and Discussion

### 1) Method Development for POPs measurements

Two PUF columns connected in series and two (PUF+ACF) columns connected in series were tested for collecting POPs in tap water. Recoveries are shown in Table 1. The recoveries from the first PUF column exceeded 100% and those from the second PUF column also showed approximately 50 %. These facts means that there are some contaminations and that the recoveries by PUF column are not enough. On the other hand, the recoveries from the first (PUF+ACF) were approximately 100% and those from the second column were almost zero. Therefore, the (PUF+ACF) was selected as the adsorbent.

Table 1 Recovery tests of HCHs, aldrine, dirdrine, chlordane and nonachlor of PUF and PUF+ACF for solid-phase extractor(%)

| Solid-phase extractor | PUF | | PUF+ACF | |
|---|---|---|---|---|
| Column | 1st | 2nd | 1st | 2nd |
| α-HCH | 164 | 44 | 110 | 2 |
| β-HCH | 176 | 50 | 115 | 0 |
| γ-HCH | 223 | 70 | 111 | 0 |
| aldrine | 217 | 22 | 128 | 0 |
| dirdrine | 175 | 33 | 104 | 0 |
| t-chlordane | 67 | 53 | 113 | 29 |
| t-nonachlor | 78 | 56 | 121 | 30 |
| c-nonachlor | 110 | 59 | 101 | 35 |

Recoveries of sarogate compounds labeled with $^{13}$C tested using a real seawater in the field are shown in Table 2. The recoveries from the first (PUF+ACF) column were 30 to 80% and those from the second column were 0%. Since this seems to be due to the

incomplete extraction from the ACF, a further extraction from the ACF with dichloromethane by Soxhleh's extraction was conducted. Even by this extraction the recoveries did not reach 100%, but fairly good improvement was observed, and this extraction was adopted. Washing the PUF with acetone by Soxhlet's extraction before use was very effective to decrease the blank.

Table 2   Recovery tests of sarogate compounds labeled with $^{13}$C of PUF+ACF for solid-phase extractor(%) *in situ.*

| Sample No. | No. 3 | | No. 19 | |
|---|---|---|---|---|
| Column | 1st | 2nd | 1st | 2nd |
| α -HCH | 34 | 0 | 28 | 0 |
| β -HCH | 79 | 0 | 73 | 0 |
| γ -HCH | 42 | 0 | 46 | 0 |
| t-chlordane | 56 | | 34 | |
| t-nonachlor | 68 | | 47 | |
| c-nonachlor | 69 | | 64 | |

2) Distribution of POPs

One hundred liters of seawater was passed through (PUF+ACF) columns and was analyzed by GC/MS (SIM). Distribution of α, β, γ-hexachlorocyclohexanes (HCHs) between Japan and Persian Gulf and between Japan and Australia are shown in Fig. 4 and 5, respectively. Distribution of t, c-chlordane and t-nonachlor between Japan and Australia were also shown in Fig. 6. The concentration of chlordane was some pg/L level and became minimum near the equator. Between Japan and the Persian Gulf the concentration of β-HCH were high near Japan, at Strait of Malacca and in the Bengal Sea. Between Japan and Australia the concentration of β-HCH gradually decreased from Japan to the equator and was constantly low at pg/L level from the equator to the Australia. The emission source of β-HCH is suspected to be Asian countries, probably Japan and China. The distributions of α-HCH were similar to those of β-HCH but the concentrations ranged from 10 to 30 pg/L between Japan and the Persian Gulf and ranged below 10 pg/L between Japan and Australia, which were
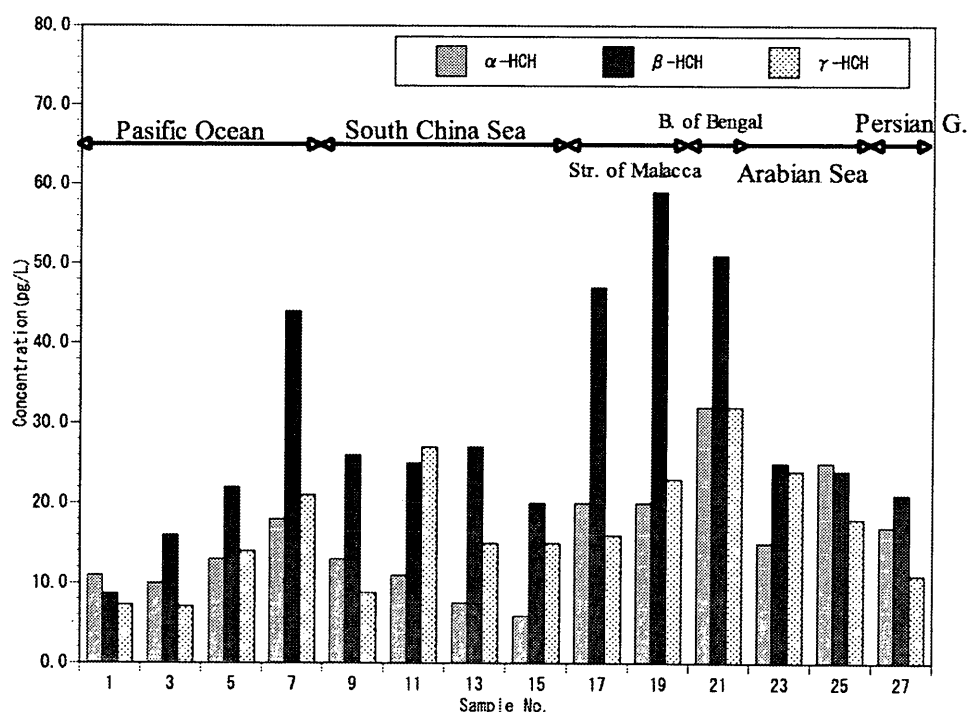


Figure 4 Concentration of α, β, γ-HCH in the seawater between Japan to Persian Gulf.

lower than those of β-HCH. On the contrary, the concentrations of γ-HCH were constantly low between Japan and the equator but the concentrations increased gradually from the equator to Australia. This reflects the fact that not BHC (containing α, β, γ, δ-HCH isomers) but linden (γ-HCH) was used in Australia in the past. The concentrations of α- and γ-HCH were often below the detection limit between the Persian Gulf and Japan in January 2001. This is probably because these compounds with lower boiling points than that of β-HCH had been already vaporized from seawater, or probably because of the farmers' slack season in Asian countries. The concentrations of the same compounds collected at November 2002 ranged from 10 to 30 pg/L. From these observations, it is speculated that the occurrence and distribution of HCHs in these areas quickly changes.

3) Distribution of Bisphenol A and Alkylphenols (C4-C9)

Bisphenol A and alkylphenols (C4-C9) were selected as the subject compounds because these compounds are one of endocrine disrupters (EDs) and their quantity of production are large compared with other so-called environmental hormones. Samples were collected along the route from Japan to the Persian Gulf in January 2001. The recovery was checked by connected column in series.

The adsorbents were extracted by Soxhleh's extraction using acetone as eluate (24 hr). Then solutions were concentrated by N₂ gas purge. $^{13}C_6$-HCB and fluoranthene-$d_{10}$ were added to samples as internal standard. The acetone extracts were re-extracted with hexane. After concentration by N₂ gas purge, the solutions were made up to 100 μL with hexane solvent. Phenanthrene-$d_{10}$ was added to the samples as another internal standard. Subject compounds were derived by BSTFA, and then measured
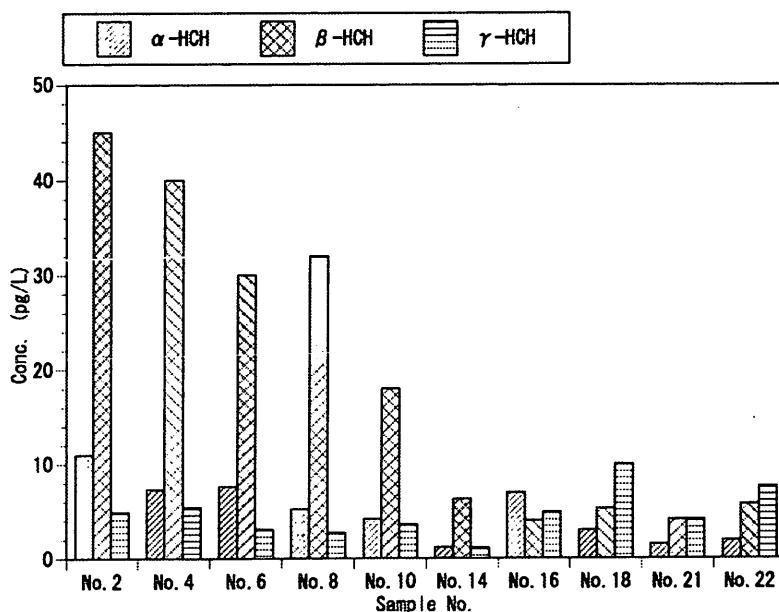


Figure 5 Concentration of α, β, γ-HCH in the seawater between Japan to Australia.
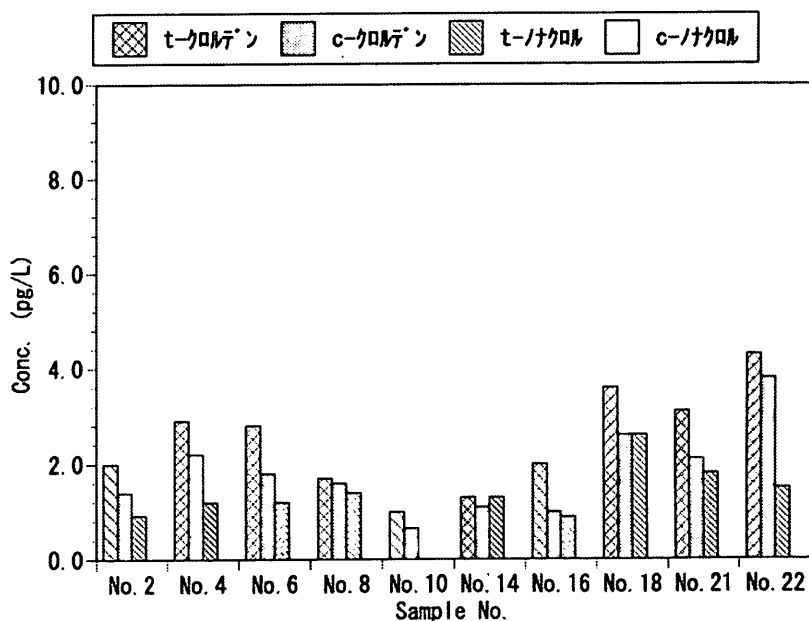


Figure 6 Concentration of t, c-chlordane and t, c-nonachlor in the seawater between Japan to Australia.

by GC/MS (SIM). Results of measurement were shown in Fig. 7. The numbers of left side are sampling location, and the concentration (ng/L) was shown as the length of the bars. The upper axis means the concentration of subject compounds except nonylphenol, and the lower axis means the concentration of nonylphenol. Several alkylphenols were detected, but most of the measured values were near the detection limit, except nonylphenol. It might be said that the concentration was extremely low in the ocean, so even the large sampling volume of 50 – 150 L gave results under the detection limits. The values of nonylphenol were high at all station, although there is a fear that the contamination through the preparation might occur. Further experiments are necessary to assess this problem.
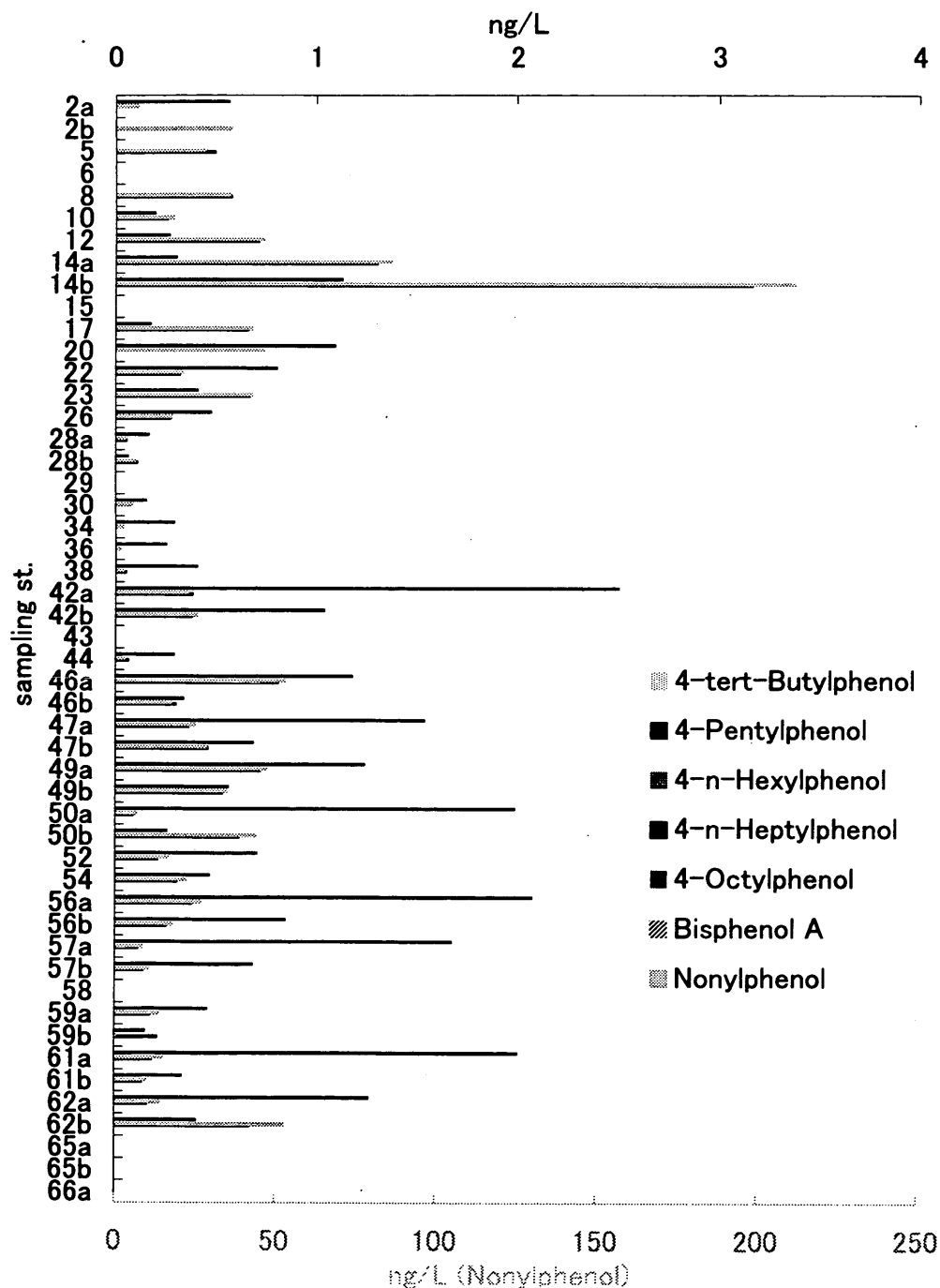


Figure 7 Concentration of bisphnols and alkylphenols in the seawater between Japan to Persian Gulf.

4) Distribution and Photochemical Behavior of Poly-aromatic Hydrocarbons (PAHs)

In this study, we focused on the PAHs for their photochemical fate and distribution in the Pacific Ocean. The major anthropogenic source of PAHs is the combustion of fossil fuels and their distributions are spread out on the surface of the earth. After PAHs are discharged to the natural environment, their distribution and fate are almost unknown. Therefore, it is important to elucidate the decomposition of PAHs in natural water and their concentrations in the remote ocean area. This report is composed of the two parts: Photochemical decomposition of PAH and their measurement in the central Pacific.

In the first part, we investigated the photochemical decomposition of PAHs. A solar simulator irradiated to the solution of PAHs. The decomposition rates of PAHs in hexane solution are as follows: _Benzo[ghi]perylene_ (52 % remained after 4 hour irradiation) > 9-Methylanthracene > 2,3-Benzofluorene > Coronene > **Indeno[1,2,3-cd]pyrene** > 7-Methylbenzo[a]pyrene > Perylene > **Benzo[a]pyrene** > Anthracene > _Pyrene_ > 4H-Cyclopenta[def]phenanthren > **Benzo[a]anthracene** > Retene > 1-Methylphenanthren > _Fluoranthene_ > **Chrysene** > Phenanthrene > _Benzo[e]pyrene_ > **Benzo[b]Fluoranthene** > 1-Methylnaphthalene > 1,5-Dimethylnaphthalene > 2-Methylnaphthalene > Naphthalene (100% stable after 4 hours irradiation) (Bold: carcinogenic, Italic: carcinogenic promoter). Pyrene was dissolved in the solutions of various mixture of acetone and seawater. The solution of mixing rate of acetone (20 %) and seawater (80 %) is the most stable and used hereafter. Four PAHs was dissolved in the above solution. The decomposition rates are: 9-Methylanthracene (fast) > Anthracene > Benzo[a]pyrene > Benzo[e]pyrene (solw). The decomposition rate in the seawater is faster than in the distilled water, as shown in Fig. 8. The decomposition speeds in 3 % NaCl solution and distilled water are not different. The obtained results show the decomposition rate is related to the structure of PAHs, i.e., the addition of methyl group or the disposition of aromatic ring makes change in the decomposition rate. The dissolved organic compounds (DOC) in seawater can participate in the decomposition of PAHs.

In the second part, PAHs in the surface water of the middle of the Pacific Ocean were discussed. One hundred liter of seawater was passed through two PUF cartridges. Since the recoveries were below 50% (which depend on the species of PAHs), the obtained result shows the under-estimated value. At seven points of the central Pacific, 100-318 pg/L of PAHs were measured as shown in Fig. 9. The precise estimation of the collection recovery to the cartridge will be
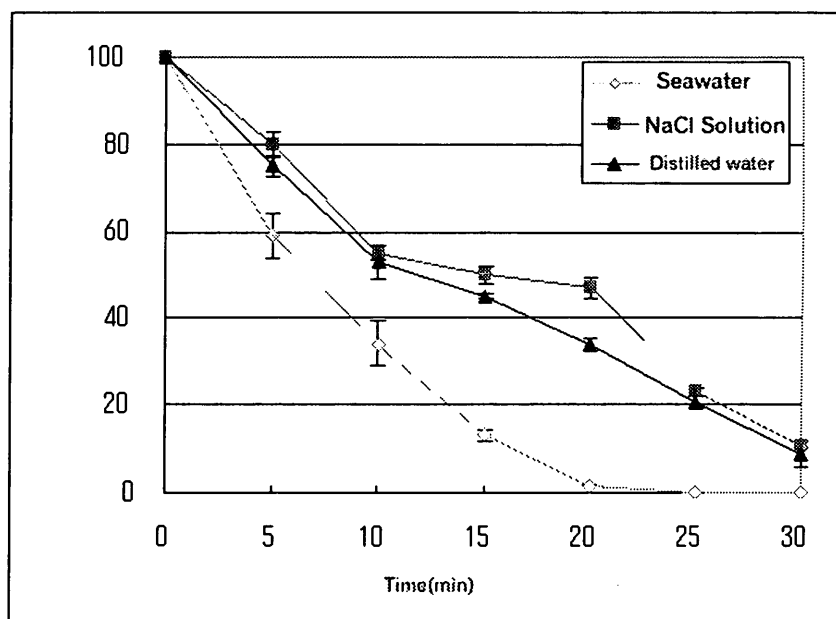


Figure 8 Deconposition rate of Pyrene in the seawater, Nacl solution and distilled water under UV radiation.

required for the farther measurements in the concentration of PAHs in the seawater.
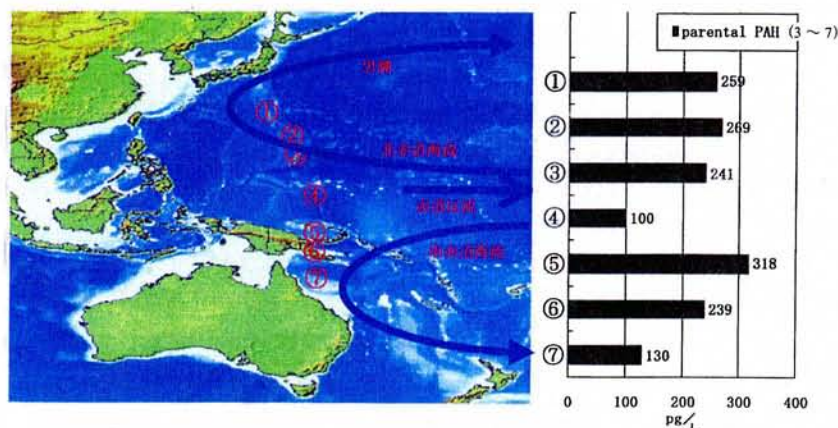


Figure 9 Sampling positions and concentration of PAHs in surface seawater between Japan and Astralia.

## 5) Distribution of Main and Trace Elements

From the analysis of seawater collected between Japan and the Persian Gulf, no severe marine pollutions by heavy metals were not observed, but there were characteristic concentration pattern of heavy metals in each marine area. Concentration distributions of Pb and Cd were shown in Fig. 10. The concentrations of Cd in the Persian Gulf were approximately 30-70 ng/l and about 5-10 times higher than those in other areas. The concentrations of Mo and Co were also higher than those in other areas. On the other hand, the concentrations of Pb were higher in East China Sea and in the neighboring area of Japan.
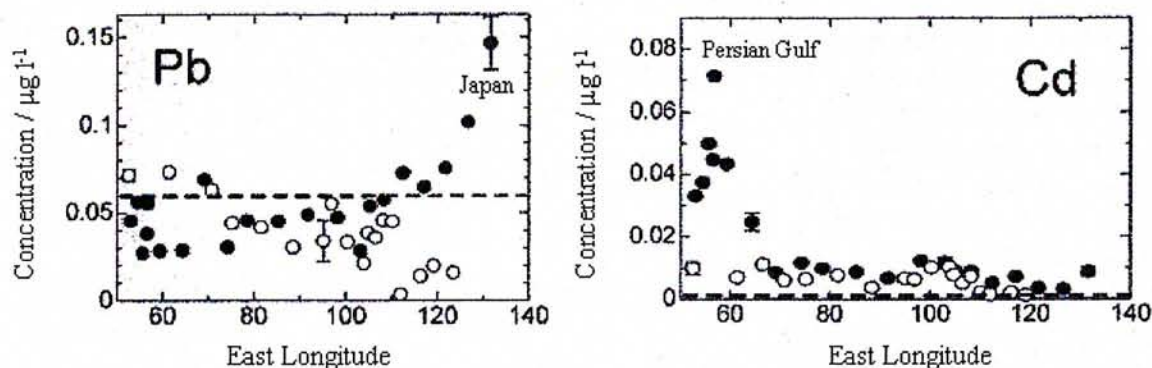


Figure 10 Concentration distributions of Pb and Cd in surface seawater between Japan and the Persian Gulf.

It was also found that the concentrations of trace elements of Zn, Cu and rare earth elements, classified as nutrient-type elements, were higher in the Bengal Sea, which may reflect the fact that the deep seawater in the Bengal Sea was transported to the surface, as suggested by the marine circulation model. Average concentrations of trace elements in seawater collected from northern hemisphere and southern hemisphere along the route between Japan and Australia are given in Table 3. Average values of Zn, Cu, Pb and Cd in the northern hemisphere are higher than those in the southern hemisphere. This may reflects the fact that anthropogenic emissions in the northern hemisphere are much higher than those in the southern hemisphere.

## 6) Distribution of Organotin Compounds

As for organotin compounds, tributyltin (TBT) was detected at all the sampling points between Japan and the Persian Gulf. The most severe pollution at about 1 ng/L was detected at the Strait of Malacca and the pollution was spread to the Bengal Sea. However, the concentrations of TBT were not high in the Persian Gulf in spite of the busy transportation of many tankers and its closed nature. On the contrary, the concentrations of monobutyltin (MBT) and inorganic tin were very high in the Persian Gulf. From these results, it is speculated that TBT released from the bottom of the ship was easily decomposed to MBT and inorganic tin by the strong sunshine in that area. Distribution of TBT between Japan and Australia is shown in Fig. 11. Again, TBT was detected at all the sampling points, along with dibutyltin (DBT) and monooctyltin (MOT). According to the thus-obtained results, it was concluded that the contamination by organotins was not restricted in local harbors and bays, but was spread to open ocean in a global scale.

Table 3 Average Concentrations of Trace Elements between Japan and Australia (unit: $\mu g\ l^{-1}$).

| Element | Hemisphere | | | Reference[a] |
|---|---|---|---|---|
| | Northern | Southern | Average | East-China Sea |
| Mo | 13.3 | 13.8 | 13.5 | 10.2 |
| Ni | 13.6 | 30.1 | 19.3 | 2.9 |
| U | 5.54 | 5.57 | 5.55 | 3.37 |
| Zn | 2.42 | 1.30 | 2.03 | 8.4 |
| Mn | 2.36 | 4.52 | 3.11 | 0.34 |
| Al | 1.68 | 1.32 | 1.55 | 2.17 |
| V | 1.06 | 1.02 | 1.05 | 1.59 |
| Cu | 0.620 | 0.509 | 0.582 | 0.97 |
| W | 0.024 | 0.030 | 0.026 | 0.012 |
| Pb | 0.0085 | 0.0066 | 0.0077 | 0.063 |
| Cd | 0.0058 | 0.0028 | 0.0047 | 0.0052 |
| Y | 0.0035 | 0.0022 | 0.0031 | 0.011 |
| La | 0.00101 | 0.00033 | 0.00077 | 0.0010 |
| Sm | 0.00093 | 0.00025 | 0.00068 | 0.00010 |
| Yb | 0.00014 | 0.00007 | 0.00012 | 0.00028 |

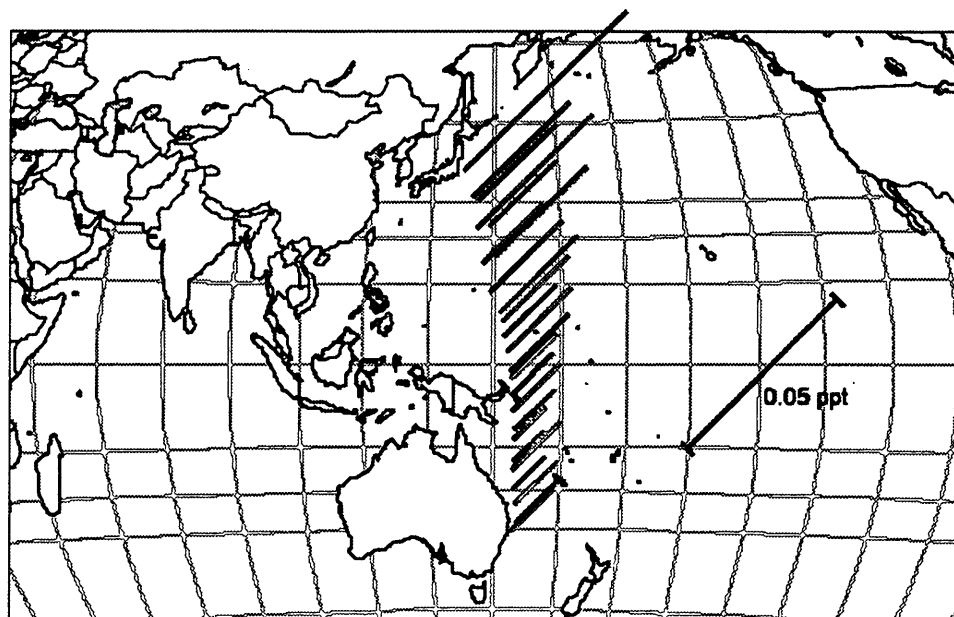a) [T. Yabutani et al, *Anal. Sci.*, **17**, 399(2001).



Fig. 11   Distribution of TBT between Japan and Australia.

7) Distribution of Estrogenic Activity

Evaluation of estrogenic activity of seawater from the open ocean was done using the yeast two-hybrid system. Water samples were collected from Pacific Ocean between Japan and Australia. The water samples (300 L) were extracted by solid phase (PUF+ACF). The solid phase was eluted with an organic solution by Soxhlet's extraction. The organic solution was concentrated under a nitrogen stream. A 96-well plate culture and a chemiluminescent reporter gene assay were used to measure the estrogenic activity. We also carried out –S9 and +S9 tests. The –S9 test is the usual test for evaluating the estrogenicity of original compounds. The +S9 test is a metabolic activation test based on rat liver S9. It shows the estrogenic activity of metabolic compounds in the liver. The positive controls were 17β-estradiol for the –S9 test and trans-stilbene for the +S9 test, respectively. The 20,000 times concentrated samples did not show any estrogen agonist activity. However, three out of eight samples show estrogen antagonist activity, as shown in Fig. 12. The ratio of chemiluminescent signals of these samples to that of a blank control were about 2-4. This result shows that the metabolite of compounds included in open ocean seawater samples might have some estrogen antagonist activity.
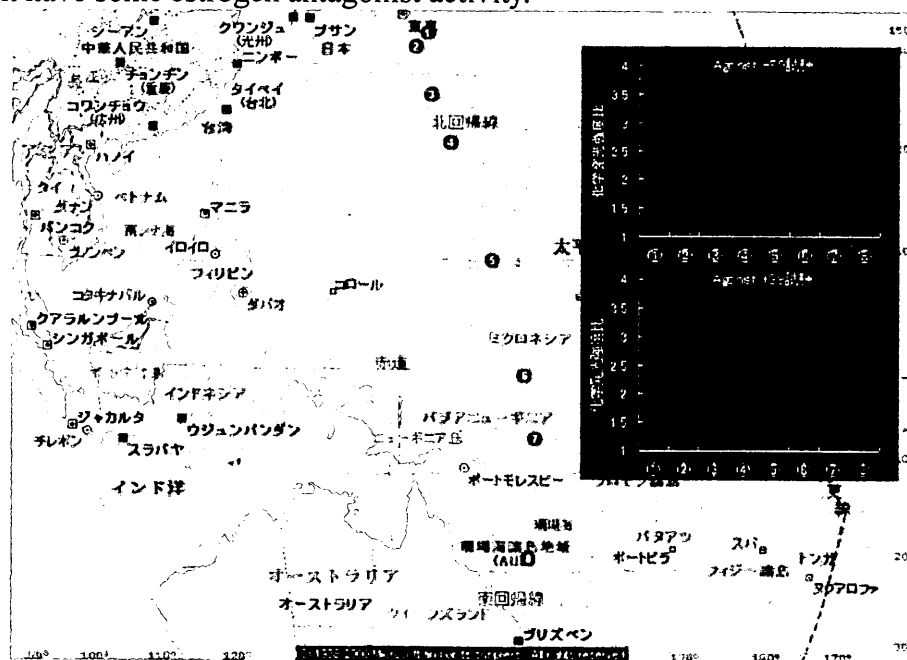


Fig. 12 Distribution of Estrogenic Activity between Japan and Australia.

5.Conclusion

We developed a marine pollution observation system, suitable for mounting on a merchant vessel. By mounting these systems in bulk carriers, which navigated between Japan and Persian Gulf, and also Japan and Australia. We observed the concentration of hazardouschemicals such as persistent organic pollutans, endocrine disrupting chemicals, organotin compounds, poly-aromatic hydrocarbons, and heavy metalsin seawater a total of 10 tomes and confirmed the usefulness of the system. We detected a-HCH, b-HCH, g-HCH TBT, nonylphenol, parental PAHs in seawater in almost all observations.

From these results, it is clear that global scale observations are indispensable to gain a grasp of the dynamics of marine pollution by hazardous chemicals.

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