D-2 **Studies on Environdynamics and Forecast of Global-Scale Marine pollution with Hazardous Chemicals (Abstract of the Final Report)**

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1. Introduction

Anthropogenic hazardous chemicals, such as pesticides and polycyclic hydrocarbons (PAHs), are widely transported by mechanisms such as oceanic and atmospheric currents[1)] and detected even in remote areas far from their emission sources[2)]. These compounds are liable to accumulate through marine ecosystems. Furthermore, these compounds, for example PAHs, are transformed by ultraviolet irradiation or through the reaction with hydroxy radicals in the environment to various chemical forms (hydroxy-PAHs), some of which newly develop estrogenicity[3)]. Therefore, it is important to study these transport and transformation mechanisms in the ocean in order to better understand the nature and extent of marine pollution by hazardous chemicals. For this purpose, we developed a continuous-extraction, hazardous chemical sampling system that was installed on merchant vessels. The samples of hazardous chemicals in surface seawater were collected between Japan and Australia at January 2003 and August 2004, between Japan and United States of America at March and September 2005, Southern Pacific Ocean and Antarctic Ocean at January and February 2004, and Mediterranean Sea and Northern Atlantic Ocean at May and June 2005. The occurrence and behaviors of pesticides and hydroxy-PAHs (OH-PAHs) were investigated. Photodecomposition process of PAHs was investigated using a solar simulator. We evaluated the total estrogenicity and determined the contribution of individual OH-PAHs and other endocrine disrupting compounds by using both in vitro bioassay and chemical analysis of seawater. For clarification and forecast of behavior of chemical substances, an ocean general circulation model including a marine ecosystem model was developed.

2. Research Objective

The aims of this project are summarized as follows:

1) Development of the small size monitoring systems for marine pollution with higher flexibility in sampling station and time by using merchant vessels in order to clarify the spatial and temporal variation and chemodynamics of hazardous chemicals in seawater, especially in the Pacific Ocean.
2) Clarification of the concentrations of OH-PAHs in marine environment and the transformation process from PAHs. Evaluation of newly generated estrogenicity of OH-PAHs.
3) Clarification and forecast of behavior of hazardous chemicals emitted from coastal area in the North Pacific and the development of an ocean general circulation model including a marine ecosystem model.

3. Method

To know the occurrence and behavior of POPs and endocrine disrupting chemicals in marine environment, 2 types of automated sampling systems were developed and installed in the container ship between Japan and USA, and the cruising vessel around the world. A photo of the one of automatic sampling systems is shown in Fig. 1.

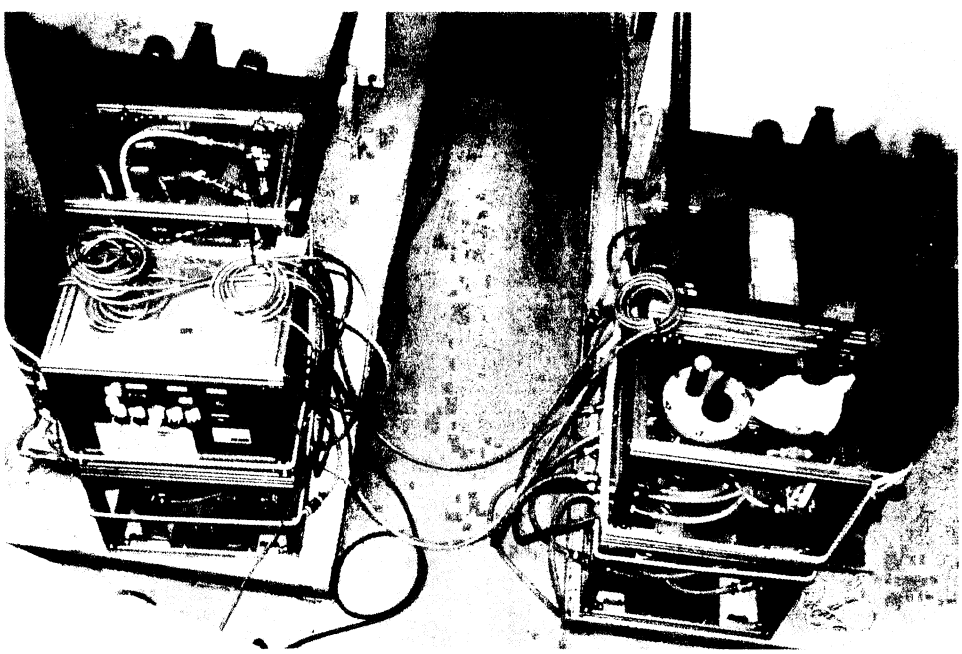

Figure 1 Photograph of marine pollution observation system on board the container ship

Collection of each water sample was conducted for 200 minutes at a time, a total of 100 L at a flow rate of about 0.5 L/min, by solid phase extraction method. Solid phase extraction columns were containing a poly-urethane foam (PUF) and active carbon fiber filters (ACF). And we spiked ^{13}C stable-isotope standard mixture sample into a sampling line when sample collection was started at all sampling periods. Collection samples were conserved in the freezer at -20℃. Hazardous chemicals were extracted by the Soxhlet extraction with acetone (24hr) and dichloromethane (24hr) from these columns. POPs were determined by high resolution gas chromatograph/high resolution mass spectrometry (HRGC/HRMS, JMS-700). Water temperature, pH, chlorophyll-A and conductivity were also monitored. We have been observing hazardous chemicals in sea water around the world from 2003 using these systems. All sampling positions of this work are shown in Fig. 2.

PAHs and OH-PAHs in coastal area were determined by thermal desorption gas chromatography/quadrupole mass spectrometry (TD-GC/MS) after acetylation and stir-bar sorptive extraction method, developed by us. For the determination of ultra-low levels of these compounds in open ocean area, we developed a new method based on solid phase extraction with TENAX column and the silylation - TD- GC/MS. Estrogenicity of PAHs and OH-PAHs was tested by the yeast two-hybrid system with or without rat liver S9 treatment. This treatment was intended to examine the estrogenicity of metabolites of OH-PAHs in organism. We also evaluated these compounds' toxicity to *Photobacterium phosphoreum* by measuring their inhibition of bacterial luminescence. Photodecomposition rates of PAHs were investigated using solar simulator with xenon lamp and the products were identified by GC/MS.

4. Results and Discussion

One hundred liters of seawater was passed through (PUF+ACF) columns and was analyzed by HRGC/HRMS (SIM). The distribution of α, β, and γ-hexachlorocyclohexane (HCHs) in the global ocean is shown in Fig. 3. 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. This result was similar observation result on January 2003 and August 2004. The concentration of β-HCH gradually increased from New Zealand to Tahiti Islands, but those of α-HCH and γ-HCH decreased. β-HCH was detected near Tahiti Islands and central part of the South Pacific, nevertheless α-HCH and γ-HCH were not detected or detected only at very low concentration. From east part of the South Pacific Ocean to the offing of Chile, α-HCH and γ-HCH were detected, but β-HCH could not be detected. From the Patagonia fiord to the Antarctic Ocean, α, β, γ-HCH were detected. Then, from the Atlantic Ocean side of the Drake Channel to the offing of Argentina, α-HCH gradually decreased from south to north. We think that these distributions of HCHs depend on the temperature of seawater and distance of the emission source and sink of hazardous chemicals. Between Japan and United State of America, the concentration of HCHs gradually increased from Japan to USA, especially a-HCH was shown most high concentration (320pg/L) on the coast of USA. On Mediterranean Sea and Northern Atlantic Ocean, results of HCHs are variety. On Mediterranean Sea, β-HCH was high concentration more than other isomers and gradually decreased from Aegean Sea to Str. of Gibraltar. On the coast of Europe, γ-HCH and α-HCH were similar concentration, and were high concentration more than β-HCH. And on Northern Atlantic Ocean, α-HCH was high concentration more than other isomers, and was shown most high concentration (630pg/L) on the coast of Canada.

We developed a marine pollution observation system, suitable for mounting on a merchant vessel. By mounting these systems in bulk carriers, a container ship and a cruising vessel, navigated around the world. We observed the concentration of hazardous chemicals

such as persistent organic pollutans, endocrine disrupting chemicals, organotin compounds, poly-aromatic hydrocarbons, and heavy metals in seawater a total of 10 tomes and confirmed the usefulness of the system. We detected a-HCH, b-HCH, g-HCH, clordanes, PCBs 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

Determination of OH-PAHs in natural water in urban area and in coastal seawater was performed by stir bar sorptive extraction (SBSE) with thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS). The concentrations of some of the OH-PAHs, such as 2-OH-naphthalene (NP) and 9-OH-phenanthrrene (PT), ranged from 1 μg/L at urban area to 0.01 μg/L at coastal seawater. However, the detection limit of this method was not low enough to determine the concentration in open ocean. Then, we developed the solid phase extraction with TENAX/silylation/TD-GC/MS method. The detection limit was ca. 0.01 ng/L, which was improved almost 1000 times, compared with the previous method. It becomes possible to determine low level of OH-PAHs even in the Antarctic Ocean. Several OH-PAHs, such as 1-OHNP, 2-OHNP, 9-OHPT, 1-OHAQ (anthraquinone), 1,8-diOHAQ, 2,6-diOHAQ, and so on, were detected between 0.03 and 0.45 ng/L.

Photochemical decomposition of PAHs under the solar simulator

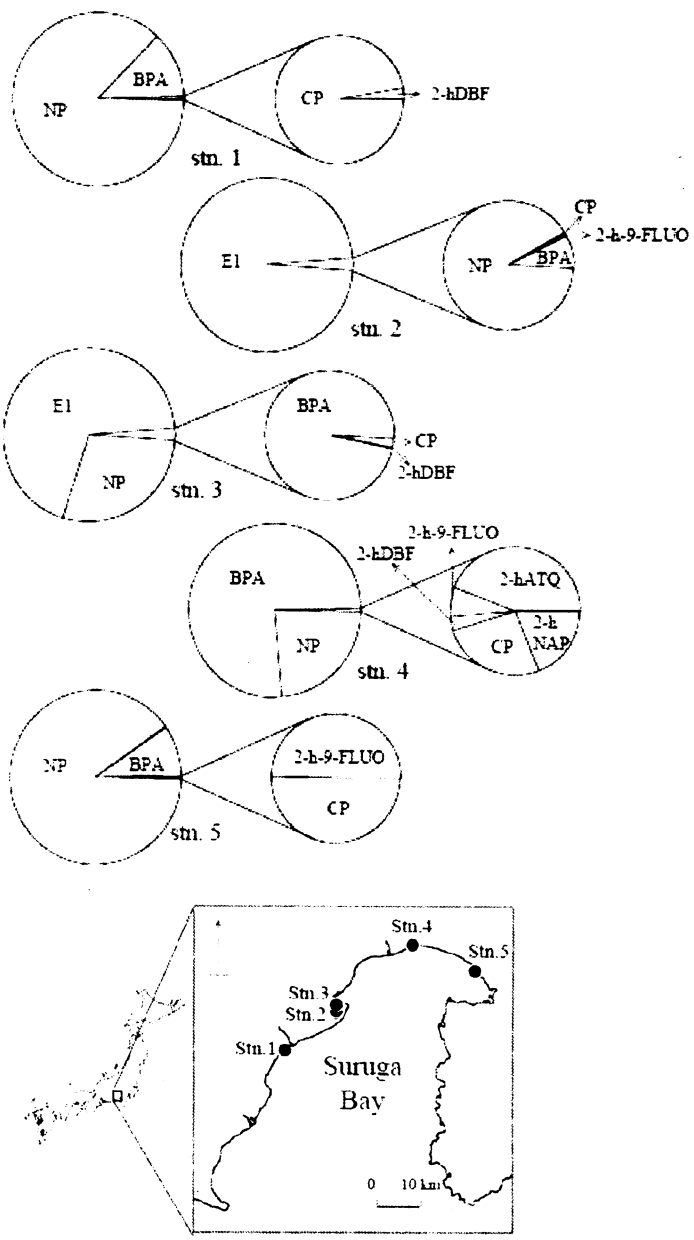

Fig. 4 Sampling points at Suruga Bay and contribution of individual compound to estrogenicity

NP = nonylphenol; BPA = bisphenol A; CP = Cumylphenol; 2-hDBF = 2-hydroxydibenzofuran; E1 = Estrone; 2-h-9-FLUO = 2-hydroxy-9-fluorenone; 2-hATQ = 2-hydroxyanthraquinone; 2-hNAP = 2-Naphthol.

was investigated, and half lifetime of anthracene (74 min), pyrene (120 min), chrysene (11.8 min), fluorancene (159.6 min) were determined. As the photochemical products of PAHs, keton and hydroxyl derivatives were observed. In the case of anthracene, central ring was the first target for photochemical reaction, and anthraquinone and hydroxyanthraquinone were produced.

In the first year, estrogenicity of anthraquinone (AQ), 8 kinds of hydoxy-anthraquinones (OH-AQs), was examined by the yeast two-hybrid system, and some of them showed both estrogenic activity and toxicity to bacteria. They are known to be photoreaction products of AQ upon illumination with simulated solar radiation. Therefore, AQ can be transformed into toxic compounds in the environment when it is irradiated by natural sunlight. In the second year, estrogenicity of naphthalene (NP) and 10 kinds of hydroxy-NPs (OHNPs) was examined, and some of the OH-NPs showed estrogenic activities: the compound (6-bromo-2-OH-NP) showing the highest estrogenic activity like 2-hydroxyanthraquinone. In the final year, we evaluated the metabolic activation of estrogenicity, measured by using both in vitro bioassay and chemical analysis of seawater from Suruga Bay, Japan. Nonylphenol, bisphenol A, estrone, 17b-estradiol and hydroxy-polycyclic aromatic hydrocarbons, some of which show estrogenic activity, were selected as the target compounds. The yeast two-hybrid system was used to evaluate the estrogenic activities of seawater. Concentrations of estrogenic compounds in seawater were measured by chemical analysis using GC/MS. The main estrogenic compounds in seawater were estrone ($\leq$9.2 ng/L), bisphenol A ($\leq$1070 ng/L) and nonylphenol ($\leq$276 ng/L). The highest estrogenic activities in seawater were observed near a sewage treatment plant, but the estrogenic activity in seawater after S9 treatment could not be explained from the calculated activity based on the presence of the measured chemicals. Our results reveal that metabolic activation has the potential to induce estrogenicity that disturbs populations of wild organisms such as fish living locally. These results indicated that when regulations on wastewater from industrial or sewage plants in the marine environment are considered to limit contamination, we should take into account the fact that estrogenic metabolites are generated in biological reactions that occur in ecosystems.

For clarification and forecast of behavior of chemical substances emitted from coastal area in the North Pacific, an ocean general circulation model including a marine ecosystem model was developed. The marine ecosystem model was developed for simulating the production and decomposition of organic carbon by considering the phytoplankton, zooplankton, bacteria, detritus and nutrients. The ecosystem model is designed to include adsorption-desorption processes of chemical substances to detritus in the ocean. Several cases of numerical simulation of behavior of chemical substances assuming the source area are performed.

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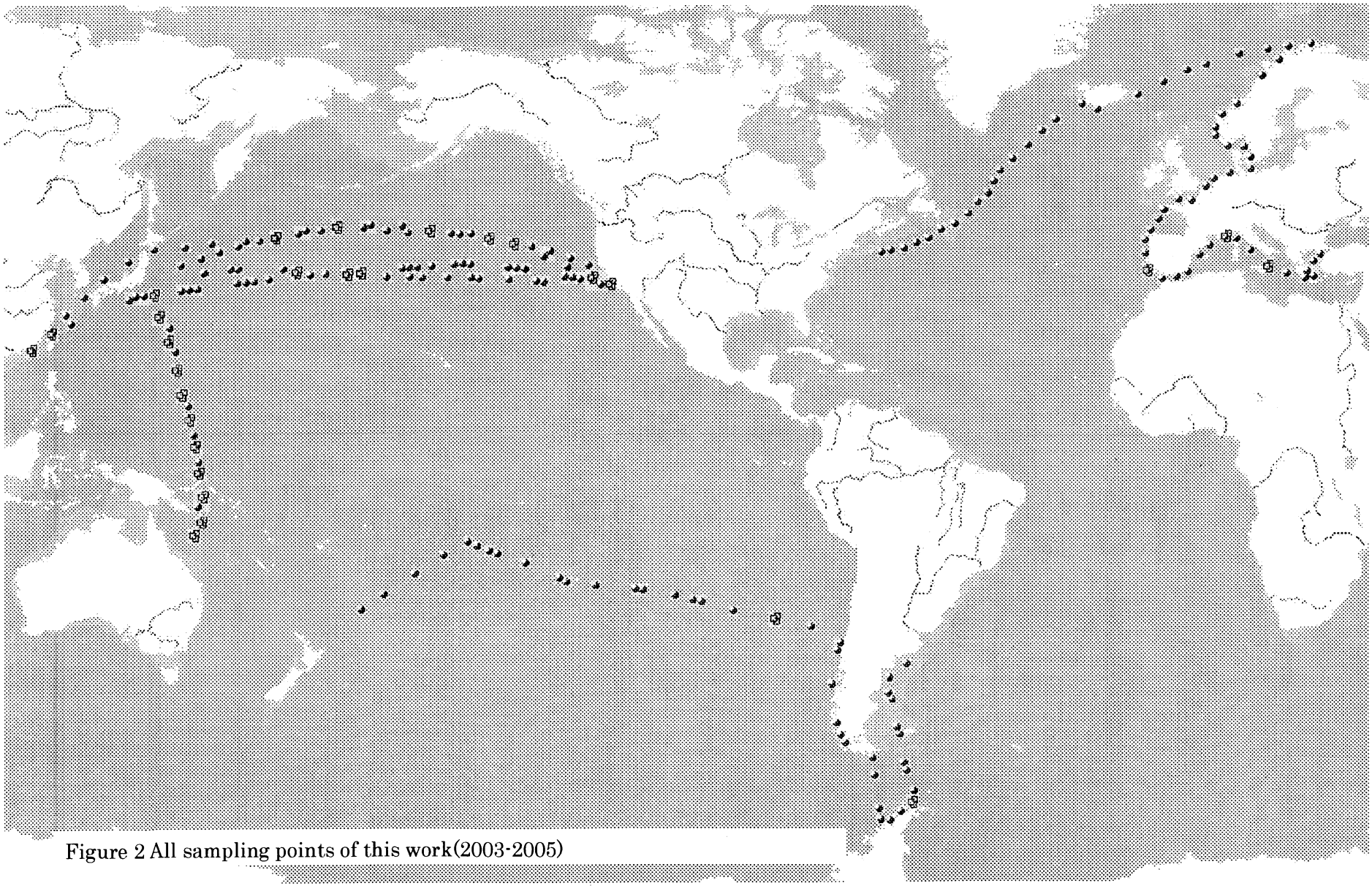

Figure 2 All sampling points of this work(2003-2005)

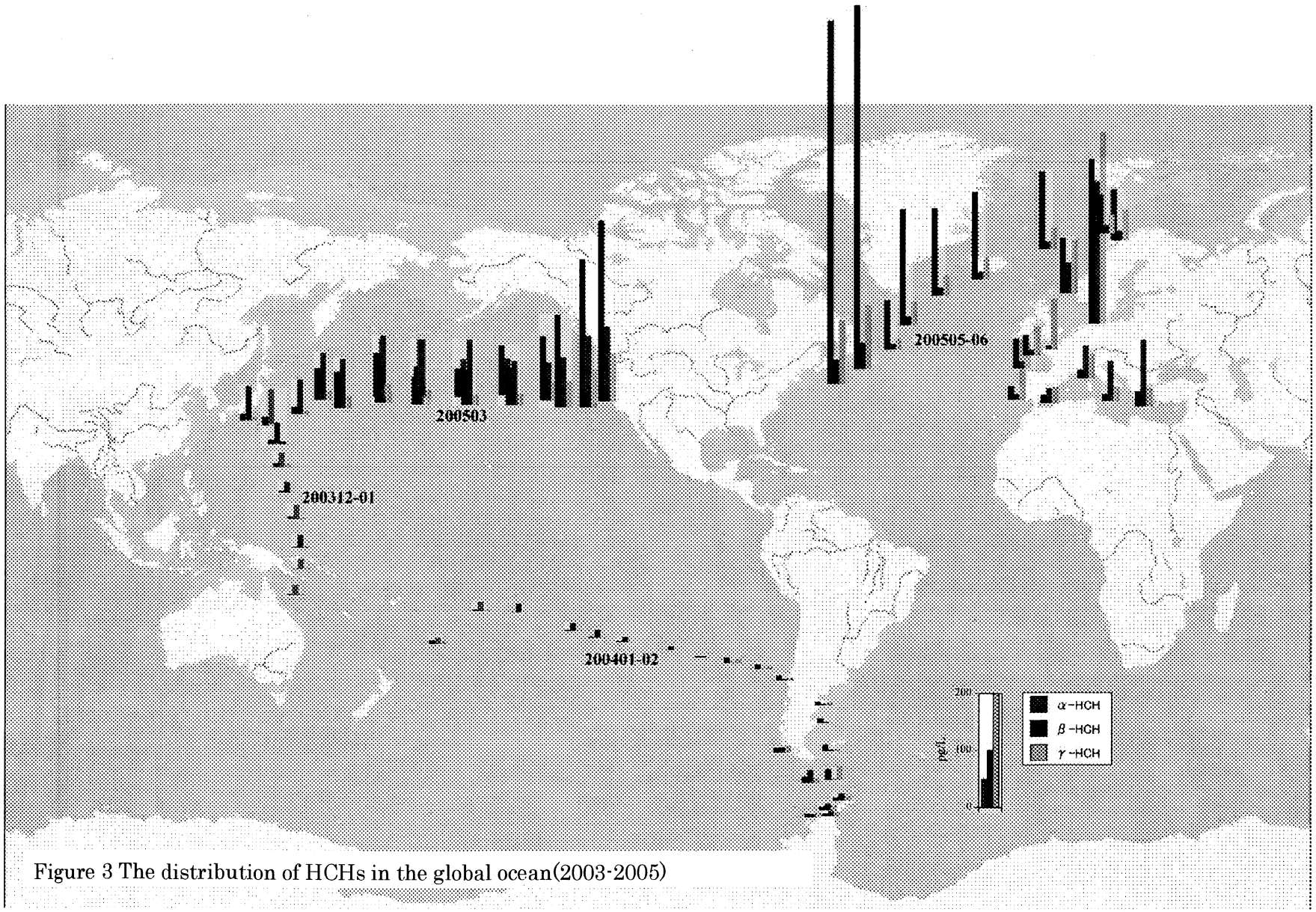

Figure 3 The distribution of HCHs in the global ocean(2003-2005)