

D-2.1.3 The Origin and Accumulation of Hazardous Substances in East-Asian Seas (Final Report)

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Total Budget for FY1998-FY1999 14,994,000 Yen (FY1999; 7,494,000 Yen)

Abstract The sensitivity of GC combined with ICP-MS (GC-ICP-MS) was improved more than 1000 fold by using PTV method and by operating a shield torch at normal plasma conditions. Consequently, 1) Occurrence of tributyltin (TBT) and triphenyltin (TPhT), below 1 ppt in the East China Sea and in the western parts of the Sea of Japan was clarified. 2) Pollution of octyltins, used as plastic stabilizer, was observed. 3) Butyltins and phenyltins (except for TPhT) were found to exist as dissolved form, but TPhT and octyltins were found to exist as particulate form. 4) Principal component analysis revealed the origin of pollution, i.e., whether it came from antifouling paint or plastic stabilizer. 5) Contamination of organotins is not restricted in local harbors and bays, but is spread over a large area, and therefore should be considered in a global scale. The accumulation of organochlorine compounds (OCs) in squid, *Todarodes pacificus*, was investigated. Since concentrations of OCs in liver displayed a significant linear correlation with the concentrations in surface seawater, it was suggested that it may be possible to estimate the concentrations of OCs in seawater from levels of α -HCH and PCB congener (Cl₅-Cl₉) in squid liver.

Key Words Marine pollution, Organotin, GC-ICP-MS, PCBs, Squid Watch

1. Introduction

The use of organotin compounds as antifouling paints has lead toxic effects to non-target aquatic species. They cause deleterious effects, such as shell anomalies in oysters and imposex in gastropods, even at concentrations below a few ng/L. In many countries, the use of TBT and TPhT compounds as antifouling paints for small boats is now restricted by law. However, they remain in use for large vessels, and about 69% of all large ships are reported to use them. In addition, large amounts of other organotin compounds, such as dibutyltin and dioctyltin, are used as stabilizers for plastics such as polyvinylchloride (PVC). The toxicity of these species depends on the chemical form, which therefore makes the speciation of organotin compounds necessary. The most popular current analytical method involves the use of gas chromatography, in conjunction with a flame photometric detector (GC-FPD). The detection limit is sufficient for the monitoring of organotin compounds in polluted areas, such as harbors and bays, but in order to clarify the occurrence and behavior of organotin compounds in the open ocean, more sensitive methods are required.

The Mussel Watch concept, based on the bioaccumulation of contaminants by natural blue mussels, is becoming a worldwide monitoring system of chemical contamination in the coastal environment. On the other hand, the worldwide distribution of squid has led researchers to propose that these annual animals be used for persistent organic chemical biomonitoring in the open ocean. However, their utility as a bioindicator species has not

been determined in detail.

2. Research Objective

The purposes of the present study are to develop a speciation method which enables the determination of ultra-trace levels of organotin compounds in open ocean seawater and to clarify the behavior of organotin compounds in East-Asian Sea. The other purposes are to clarify the characteristics of organochlorine compounds accumulation by squid under various conditions in the Sea of Japan, and to estimate the usefulness of the Squid Watch program for monitoring of organochlorine compounds in open ocean seawater.

3. Research Method

To improve the detection limit, a large volume injection method using programmed temperature vaporization (PTV) was investigated. Compared with LC, the injection volume into a capillary GC is generally limited to 1 μL , and this poses serious restrictions on the detection limit in terms of relative concentration. To overcome this problem, the PTV method using silanized quartz wool as a packing material was investigated. To select the most suitable derivatization method for PTV, three derivatizations, namely, ethylation using sodium tetraethylborate (NaBEt_4), propylation using propylmagnesium bromide (PrMgBr), and pentylation using pentylmagnesium bromide (PeMgBr), were examined and the efficiency of these reagents were compared. During the experiment, the authors found that the detection limit as absolute amounts was also improved by more than two orders of magnitude by operating a shield torch at normal plasma conditions. The optimum operating condition of the plasma with the shield torch was investigated in detail.

To clarify the behavior of organotin compounds in East-Asian Sea, seawater samples were collected during the cruise of the R. V. Yoko-maru in October 1998 and during the cruise of Tokyo University of Fisheries in July 1999 from the East China Sea. Seawaters from the Seto Inland Sea were collected by using a ferry between Osaka and Beppu in January, May and September in 1999, respectively. Seawaters from Tsushima, Oki, Kumejima, Hateruma-jima, and Kochi were also collected. Depth profile was investigated at the offshore Amami-Oshima and Bohsoh-Peninsula.

To clarify the accumulation of organochlorine compounds by squid, the Japanese common squid, *Todarodes pacificus*, was sampled in the Sea of Japan. The concentrations of various organochlorine compounds in a variety of organs of squid were determined and the relationship with the concentrations in surface seawater was examined. Effect of maturation of the squid on the accumulation was also investigated.

4. Results and Discussion

4.1 Comparison of Derivatization Yields

The yields for the three derivatization methods were estimated by comparing the signal peak area for each derivative. Filtered seawater samples containing approximately 200 ng of each organotin species were processed. The results are shown in Fig. 1. The peak area depends on, not only the derivatization yield, but also the extraction efficiency of the derivatives from water and the sensitivity of the GC-ICP-MS. To calculate the derivatization yields, it was assumed that all organotin species gave the same extraction efficiencies after being fully derivatized. It was also assumed that all the organotin derivatives gave the same sensitivity with respect to tin in the GC-ICP-MS. Based on these assumptions, the derivatization yield could be obtained by normalizing the individual peak area with that of TeBT, which was originally fully derivatized. It can be seen from Fig. 1 that the ethylation yields for all the organotin species except for DPhT chloride were approximately 100%. The reason for the low yield for DPhT chloride is not clear. A comparison of the individual

derivatizations shows that the pentylation of bulkier molecules such as phenyltin species gave considerably lower yields. This is probably due to steric hindrance between the phenyl and pentyl groups. Lower yields were also observed for mono-substituted species such as MBT and MPhT, which might be explained by the fact that these species must react three times with the bulky pentyl group to give the final products. Similar phenomena were reported in the comparison of methylation and pentylation by Grignard reagents. The high value encountered for pentylation of TBT chloride was due to an impurity in the PeMgBr reagent. In terms of derivatization yield, ethylation by NaBEt_4 is preferred to propylation or pentylation by Grignard reagents.

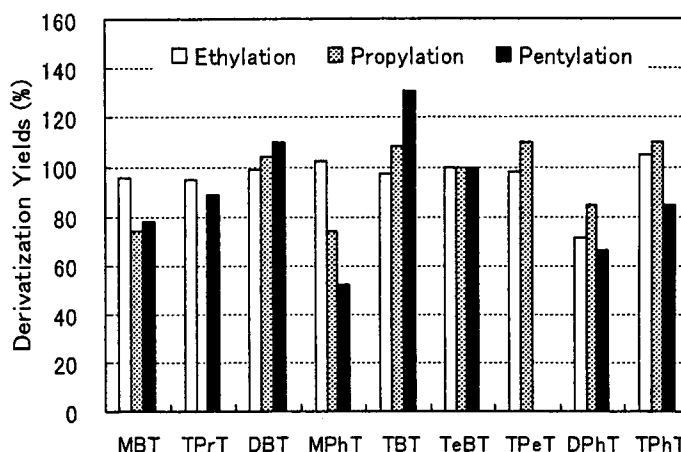


Fig.1 Comparison of the derivatization yields for several organotin species by Grignard reagents and NaBEt_4 . Neither the propylation of TPrT nor the pentylation of TPeT were examined.

4.2 Increase of Sensitivity in Absolute Amounts by Using the Shield Torch

The shield torch has been typically used with cool plasma conditions to decrease polyatomic ion interference such as ArO^+ and Ar_2^+ . However, in the present study, the shield torch is used with normal (hot) plasma conditions. The sensitivity was drastically improved with this condition. Contours of Xe signal intensities, obtained with and without the shield torch as a function of sampling depth and plasma power, could be used for the optimization of the ICP-MS parameters instead of a transient Sn signal. Both contours were totally different. The Xe signal intensity obtained without using the shield torch increased with decreasing power, reaching a maximum at a power of 0.7 kW and at a depth of 12 mm. On the contrary, the Xe signal intensity increased with increasing power and with decreasing depth when using the shield torch, and it was much higher than that without the shield torch. The sensitivity for tin with the shield torch was approximately 100 times higher than that without the shield torch, while the background signals were nearly the same. The reason for this improvement of the sensitivity is not clear at this time, but it might be because a secondary discharge at the interface region is diminished by using the shield torch, which would decrease the dispersion of ion energy and consequently increase ion transmission to the mass spectrometer.

4.3 Detection Limits and Repeatability

The instrumental detection limit for each species is defined as the amount which would give three times the standard deviation of the integral values of the baseline noise around the respective retention time when injecting 100 μL of hexane. The method detection limit is defined

Table 1 Comparison of absolute detection limits reported by various hyphenated techniques for the determination of butyltin species

technique	detection limit* (pg)	ref
GC-FPD	0.2	1
GC-AAS	25	2
GC-MS	0.5 - 1	3
GC-MIP-AES	0.4	4
GC-MIP-AES	1	5
GC-MIP-AES	0.05**	6
GC-MIP-AES	0.15	7
GC-ICP-MS	0.3 - 0.8	8
GC-ICP-MS	0.052 - 0.17	9
GC-ICP-MS	0.015 - 0.021**	10
GC-ICP-MS	0.05	11
GC-ICP-MS	0.0007 - 0.0016**	this method
GC-ICP-MS	0.0038-0.17	this method
LC-ICP-MS	8 - 9	12
LC-ICP-MS	20 - 40	13

* method detection limit

** instrumental detection limit

as the amount which would give three times the standard deviation of the peak areas for 6 replicates of the blank. Table 1 compares the absolute detection limits reported for butyl-tin species. It can be seen that the detection limit obtained in this work is the lowest obtained thus far and is approximately two orders of magnitude lower than the values obtained with a similar GC-ICP-MS due, primarily, to the operation of the shield torch at normal plasma conditions. Repeatability was evaluated from 5 replicates of 1 L of seawater spiked with 100 μ L of a mixed standard containing ca. 5 μ g/L of each species. The relative standard deviations of the peak area with and without the internal standardization by TPrT chloride ranged 1.1-5.4% and 6.0-9.3%, respectively. The repeatability was improved by the internal standardization.

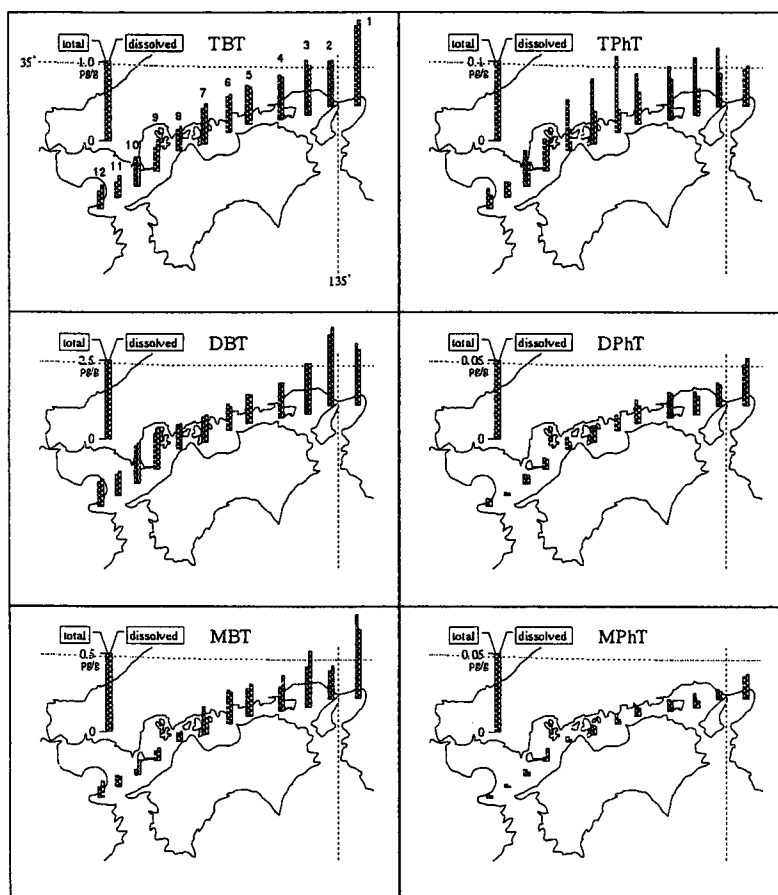


Fig. 2 Distribution of butyltin and phenyltin species in seawater of the Seto Inland Sea.

4.4 Distribution of Organotin Compounds

Distributions of 9 organotin species below 1 ng Sn/L in the Seto Inland Sea were clarified for the first time as shown in Fig. 2. The concentrations of TBT and TPhT were in the range of 0.21-1.02 ng Sn/L and 0.021-0.096 ng Sn/L, respectively. Three unknown species among them were identified as mono-, di-, and tri-octyltin species, which were used as plastic stabilizers. Butyltin and phenyltin species except for TPhT were found to occur as dissolved form, but di- and tri-octyltin species were found to occur as particulate form. Principal component analysis revealed the origin of pollution at each sampling station, i.e., whether it came from antifouling

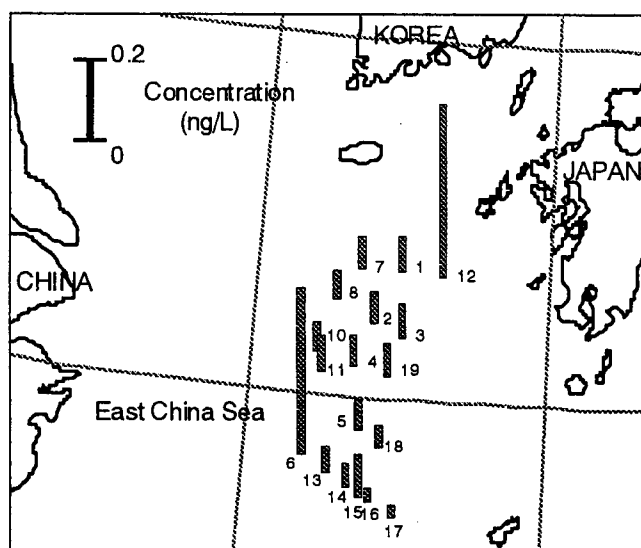


Fig.3 Occurrence of TBT in surface seawater in the East China Sea in October 1998.

paints or plastic stabilizers.

Analytical results of seawater from the East China Sea are given in Fig. 3. The concentrations were determined against the internal standard of TPPrT chloride. TPPrT chloride was preferred to TPeT chloride at this low concentration level because unidentified peaks were sometimes observed at the same retention time as TPeT chloride. Taking a closer look at the data, it is interesting to note that the concentrations of butyltin and phenyltin species increase with decreasing distance from the coast of China and Japan. Contamination of organotins is not restricted in local harbors and bays, but is spread over a large area, and therefore should be considered in a global scale.

4.5 Squid Watch

4.5.1 PCBs Concentrations in Each Organs of Squid

Fig. 4 shows the total PCBs concentrations in six organs, i.e., liver, oviductus, ovarium, gill, mantle and nidamental gland of the squid collected from the Sea of Japan. Accumulation of PCBs was primary in the liver, and the concentrations did not vary with stage of maturation of the animals. This suggests that an apparent equilibrium of PCBs between liver and seawater may occur in relatively short time.

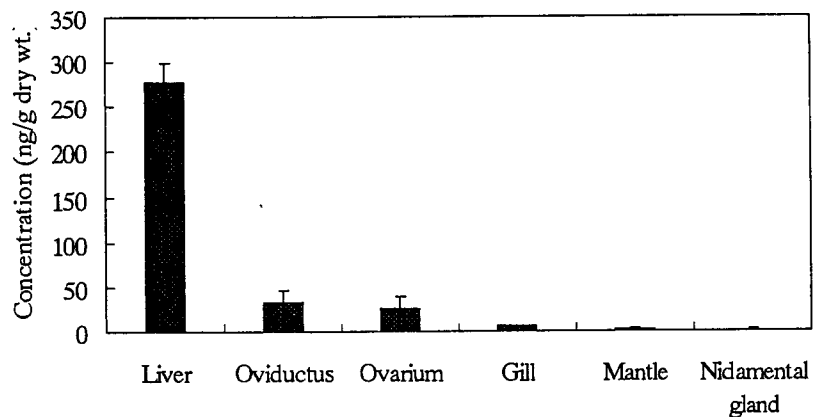


Fig.4 Total PCBs concentrations in six organs (liver, oviductus, ovarium, gill, mantle and nidamental gland).

4.5.2 Relationship in Organochlorine Compounds (OCs) Concentrations between Squid Livers and Surface Seawaters

Fig. 5 shows the relationships in total PCBs and α -HCH concentrations between surface seawaters and squid livers. The correlation coefficients (r^2 , $P < 0.05$) for total PCBs and α -HCH were 0.5052 and 0.8643, respectively.

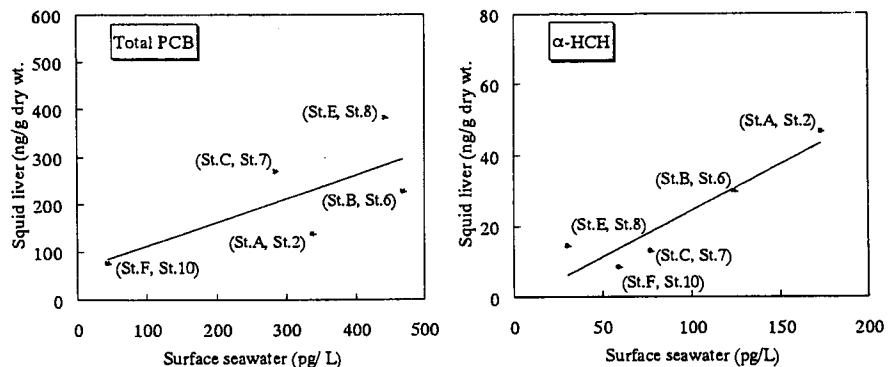


Fig. 5 Relationships in total PCBs and α -HCH concentration between surface seawaters and squid livers.

A significant linear correlation was observed for α -HCH but not for total PCBs. To investigate the relationship for each PCB congener, a detailed comparison was made. Fig. 6 shows the relationships in each PCB congener concentration between surface seawater and squid livers. The correlation coefficients for each congener were 0.1467 (Cl_2), 0.0057 (Cl_3), 0.2162 (Cl_4), 0.8216 (Cl_5), 0.6455 (Cl_6), 0.7697 (Cl_7), 0.3785 (Cl_8), and 0.3385 (Cl_9),

respectively. This result shows that there are relatively strong relationship for the PCB congeners whose chloride numbers are above 5. The lower values for Cl₈ and Cl₉ congeners were probably due to the difficulty of determination at very low concentrations. Since concentrations of these compounds in liver displayed a significant linear correlation with the concentrations in surface seawater, it was suggested that it may be possible to estimate the concentrations of OCs in seawater from levels of α -HCH and PCB congener (Cl₅-Cl₉) in squid liver. These results indicate that the Squid Watch program using *T. pacificus* can be a useful biomonitoring method to estimate OCs levels in surface seawater of the open ocean.

5. References

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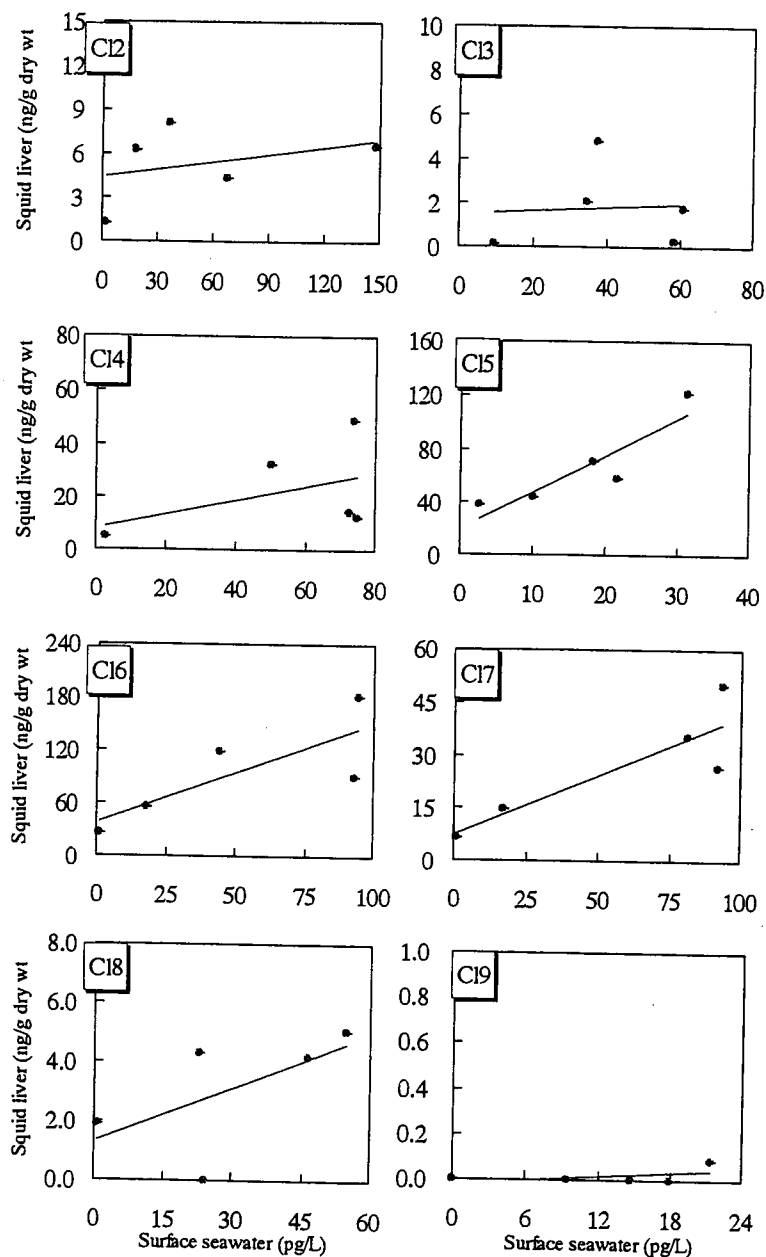


Fig.7 Relationships in each PCB congener concentration between surface seawater and squid livers.