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**STUDIES ON BEHAVIOR OF ORGANOTIN
COMPOUNDS IN AQUATIC ENVIRONMENT**

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1998

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ABBREVIATIONS

Ot	Organotin	Ksw	Sediment-water partition
Bt	Butyltin	Kpw	Plankton-water partition
Pt	Phenyltin	Kmw	Mussel-water partition
MBT	Monobutyltin	ND	Not detected
DBT	Dibutyltin	SS	Suspended solid
TBT	Tributyltin	WS	Water solubility
TeBT	Tetrabutyltin	TBP	Tributyl phosphate
TBTO	Bis (tri-n-butyltin) Oxide		
MPT	Monophenyltin		
DPT	Diphenyltin		
TPT	Triphenyltin		
PCB	Polychlorinated biphenyl		
GC	Gas chromatography		
FPD	Flame photometric detector		
ECD	Electron capture detector		
CFU	Colony forming unit		
BCF	Bioconcentration factor		
P_{ow}	Octanol-water partition		
AAS	Atomic Absorption spectrometry		
AES	Atomic emission spectrometry		
LC	Liquid chromatography		
ICP	Inductively coupled plasma		
MS	Mass spectrometry		
SFC	Supercritical fluid extraction		
GPC	Gel permeation chromatography		

PREFACE

Although human activity e.g. agriculture, energy production, manufacturing, recreation, and transportation, etc., intended to be beneficial, these often affect the physical environment in an adverse manner through the production and release of chemical compounds. Nevertheless, there are some 60,000 chemicals in current commercial production, and approximately 1000 chemicals are added every year. Of these, perhaps 500 are of environmental concern because of their presence in detectable quantities in various components of the environment. Further, the use of these chemical substances has increased both in kinds and amounts year by year.

Recently, organometallic compounds are applied to various manufactured products such as antifouling paint, pesticide, catalyst, medicine etc.. These compounds consist of a single central metal atom, covalently bound to one to several groups. Organomercury, organolead, organotin, organogermanium and organosilicon are representative organometallic compounds. These compounds can differ quite extreme in toxicity from their inorganic counterparts. In general, organometallic compounds have a higher toxicity than their inorganic analogs. Nevertheless many of organometallic compounds exist in aquatic environment. and their persistence in the environment poses long term threats to public health and indigenous wildlife. Furthermore, as these compounds have both hydrophobic and ionic properties, it is expected that the behavior of these compounds in environment to each compartment is different from those of metals and organic compounds. So, in order to remediate aquatic environment from contaminants by organometallic compounds and prevent aquatic organisms, the current status of contaminants must be investigated. From the current status of contaminants, many information such as the level, geological distribution, partition to each compartment and etc., can be obtained. Furthermore, temporal trend can be estimated from the continuance of this investigation and effects of regulation are appreciable. In order to conserve the aquatic organisms, it is important that the accumulation of organometallic compounds in

living organisms is clarified.

We focused on Ots which are most representative substances in organometallic compounds in this study, because Ots were directly inputted from vessels to aquatic environment and caused adverse effects such as imposex for aquatic organisms at levels of 1ng/l or less (Gibbs *et al.*, 1986, 1987). Preliminary, the levels and distribution of Ots made clear in aquatic environment and in order to elucidate distribution of Ots, the biodegradation of Ots by microorganisms in water, and from sediment were studied and discussed. Furthermore, the characterization of Ots behavior in environment was also investigated in comparison with that of organochlorine compounds such as PCBs.

SUMMARY

Chapter I reviewed the structure, properties, physico-chemical toxicology and the regulation concerning Ots were described. In summary, TBT and TPT has been used as active biocide of antifouling paints since 1960s. These compounds were noted as environmental pollutants, because these compounds caused the deformity of oyster and imposex of shellfish at concentration of 1ng/l or less. Accordingly, the regulation for these compounds set in various countries between 1982-1992.

In chapter II, optimization of the analytical method of these Ots including MBT, DBT, TBT, MPT, DPT and TPT in water, sediment and aquatic organisms were described. The average recoveries of Ots in water, sediment and tissues of fish are in the range of 73-100, 68-109 and 81-105%, respectively and the relative standard deviations of Ots were 12 % or less. On the other hand, the detection limits of Ots in water, sediment and aquatic organisms for a signal -to- noise ratio of three were 0.003 µg/l, 0.005 mg/kg dry and 0.005 mg/kg wet, respectively.

Chapter III showed distribution of Ots in aquatic environment. In section I the distribution of Ots described in Osaka Bay including the Port of Osaka. As the highest concentration of TBT in coastal area among Osaka Bay were observed. Therefore, the distribution of Ots in the Port of Osaka which located in coastal area of Osaka Bay were investigated. In section II, preliminary, the temporal trend of Ots in the Port of Osaka were described. In conclusion, TBT concentration in water and mussels decreased remarkably in marina and the mooring places in a zone of poor flushing during 1989-1996. Large variations of TBT concentrations in sediment and plankton were noticed, however, on the whole, the drastic changes of TBT in sediment and plankton were not observed in the period of investigation. On the other hand, detected frequencies of TPT in water, sediment, plankton and mussels were very low. Secondly, horizontal distribution of Ots in the Port of Osaka was described in 1996. The Port of Osaka were widely contaminated by Ots. In particular, TBT levels in water, sediment, plankton and mussels

were high in marina and the mooring places of small and medium hull vessels. TPT were not detected in water and were lower level than TBT in sediment, plankton and mussels. In section III, the current status of Ots was showed in Otsuchi Bay which has a long history of fisheries. The highest concentrations of TBT and TPT were detected near the shipyard and the ratios of triorganotin compounds to the total Ots were high in water, sediment and aquatic organisms. The levels of Ots in the Port of Osaka were compared to those in Otsuchi Bay. Levels of TBT in water from the Port of Osaka were lower than those from Otsuchi Bay, however these compounds in sediment, plankton and mussels from the Port of Osaka were higher than those from Otsuchi Bay. TPT in water scarcely detected in both of the bay. Levels of TPT in sediment, plankton and mussels from the Port of Osaka was lower than those from the Otsuchi Bay.

In chapter IV, degradations of Ots by microorganisms in water and sediment were studied in order to account for the distribution of Ots in aquatic environment. Susceptibility of bacterial populations to Ots was investigated as a preliminary experiment. CFU of microorganisms in river water was decreased markedly at 10 mg/l of TBT, DBT or MPT, however, the populations increased after 5 days. The decreasing of CFU was not observed at 10mg/l of MBT, DPT and TPT. Next, biodegradation of Ots was studied using river die-away method. When the initial concentrations of TBT were adjusted to 9.3 $\mu\text{g/l}$, half-life of TBT was 15 days in water and half-life of DBT was 10 days at the initial concentration of 8 $\mu\text{g/l}$, DBT. TPT was degraded scarcely during 60 days of culture. When TBT was adjusted to 7.5 mg/l, half-lives by microorganisms from sediment in the Port of Osaka ranged from 7 to 9 days.

Chapter V indicated the accumulation of Ots in marine organisms. Preliminary, Ots levels in fish were discussed. It is conclusion that Ots concentration in fish from sea areas were higher than those from rivers and TPT was also the same trend as TBT. Next, BCF of TBT was estimated in fish and shellfish. BCF of fish from sea areas, mussel and

scallop were 4.0, 4.1 and 4.1, respectively. BCF of fish from rivers was lower than those of fish from sea area and shellfish. Further, the distribution in organs and tissues of fish and scallop were investigated. In conclusion, TBT levels were highest in the liver of Japanese sea perch, white croaker and yellowtail. TPT concentration in liver, brain and muscle of white croaker and yellowtail were higher levels in comparison with the other organs and tissues. TBT concentrations in organs and tissues of scallop were high the order of muscle > liver > muntle > gill and Pts concentrations in organs and tissues of scallop were low.

In chapter VI, the characterization of behavior of Ots in aquatic environment were found by comparing with those of PCB. K_{sw} and K_{pw} of TBT were lower than those of PCBs and K_{mw} of TBT was the same as that of PCB. Though K_{sw} , K_{pw} and K_{mw} of PCBs could estimate from physico-chemical parameter, those of TBT could not estimate from physico-chemical parameter. While, K_{sw} , K_{pw} and K_{mw} of TBT in field was same as those in laboratory experiment as well as PCBs.

Chapter VI indicated conclusion in this study.

List of results of this study were shown in chapter VII.

CHAPTER I

GENERAL INTRODUCTION

Ots are clarified as R_3SnX , R_2SnX_2 and $RSnX_3$ in industrial importance. R is usually a methyl-, ethyl-, butyl-, phenyl, octyl-, or cyclohexyl- group, and X is a chloride, fluoride, oxide, hydroxide, carboxylate or thioate. $RSnX_3$ is used as stabilizers in poly(vinyl chloride) films and smaller quantities are used for glass coating (e.g. MBT). R_2SnX_2 is also used mainly as PVC stabilizers, which are dimethyltin, DBT and dioctyltin compounds. Furthermore, dioctyltin are used as additives for PVC food packaging products. Other important industrial use for R_2SnX_2 is as catalysts in the production of polyurethane foams, in the room temperature vulcanization of silicones and heat-transfer fluids. As R_3SnX such as TBT and TPT have biocidal properties, these

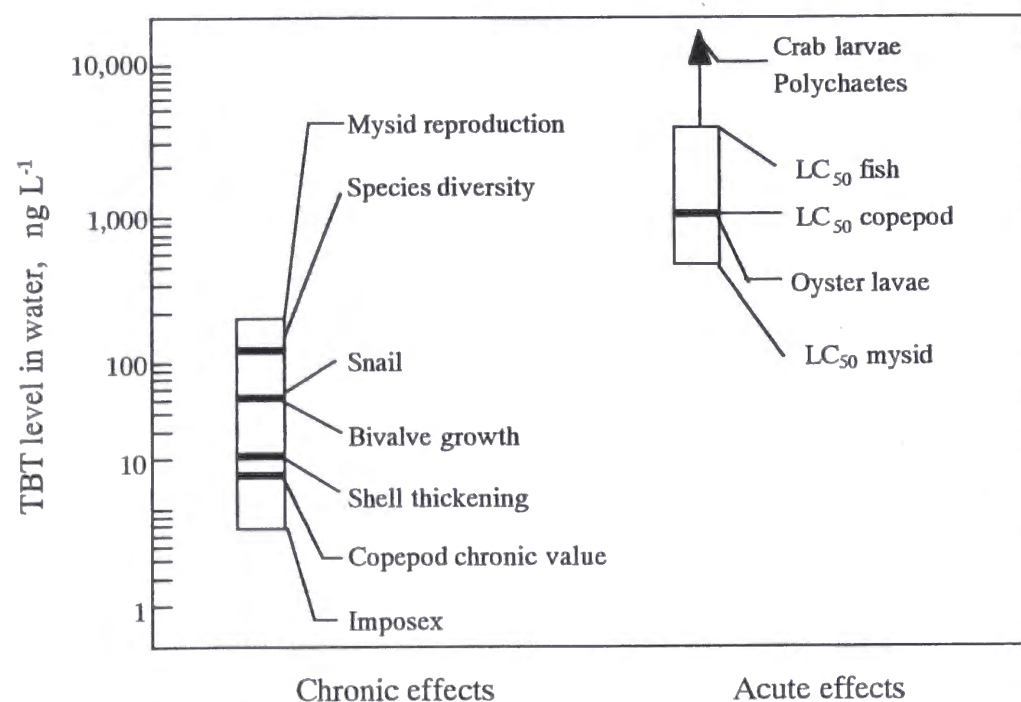


Fig.I-1. Summary of selected tributyltin toxicity data (Huggett *et al.*, 1992)

compounds have used as fungicides, bactericides, antihelminthics, miticides, herbicides, molluscicides, nematocides, ovicides, rodent repellents, antifoulants in boat paint and cooling systems of electric power generating.

Direct application of Ots to aquatic systems generally occurs through uses in antifouling paints, molluscicides and insecticides. Especially, TBT and TPT as substitutes for PCB and copper have been utilized for an active biocide in antifouling paints for ships, pleasure boats and docks since the early 1960s. The world-wide annual production of Ots is increased from about 30000 tons to approximately 50000 tons during 1982-1992 (Maguire, 1991; Mercier *et al.*, 1994). In the case of application of TBT to antifouling paint, the direct input of TBT into aquatic environment gave the impact for production of Pacific oyster (*Crassostrea gigas*) inhabiting Arcachon Bay on the Atlantic coast of France by poorly growth and the grossly malformation of shell valves (Waldock and Thain, 1983, Alzieu *et al.*, 1986b). After the event, Ots levels in environment and its toxicity were investigated everywhere (Laughlin and Linden, 1985, 1987). TBT toxicity data of aquatic organisms are shown in Fig.I-1 (Huggett *et al.*, 1992). Lenwood and Pinkney (1986) reported that acute toxicity (24 to 144-hr LC50s) was found to range from 0.0002 to 0.002 mg/l TBT for several zooplankton, acute mortality data (96-hr LC50s) for macroinvertebrates ranged from 0.0003 mg/l TBT for juvenile mysid shrimp (*Metamysidopsis elongata*) to 0.15 mg/l TBT for the pacific oyster, *Crassostrea gigas*, and ninety-six hour LC50s for adult estuarine and marine fish ranged from 0.003 mg/l TBT for sleepslead minnows to 0.019 mg/l TBT for the sole (Lenwood and Pinkney, 1986). Further, Lenwood and Pinkney (1986) reported that chronic effects were observed at less than 100ng/l of TBT and avoidance of estuarine fish species occurred at TBT concentrations ranging from 0.005 to 0.033 mg/l TBT. Histological changes were found to occur in striped bass after 20- to 40- min exposures to TBT concentrations of 0.02 to 0.03 mg/l (Lenwood and Pinkney, 1986). The following changes occurred : edema in gills, necrosis of the gill epithelium, swelling of the epithelial cells in the nasal pits.

Table I-1 shows the effect of TBT acetate on oyster as a typical aquatic organisms (Alzieu, 1986a). High mortality rate of *C. gigas* was observed after ten days at 0.03µg/l TBT acetate.

TBT is also of concern as the endocrine-disrupting chemicals (Colborn *et al.*, 1993). Namely TBT act like hormones and cause endocrine-disrupting effect such as imposex. Imposex means that female sexual character changes to male, because increase of penis size. Imposex was found to occur in the mud snail and sea snail at TBTO concentrations below the LC50 (Lenwood and pinkney, 1986, Horiguchi *et al.*, 1997). Gibbs *et al.* (1986, 1987) also reported that TBT was caused imposex of Dog-whelk (*Nucella lapillus*) even at TBT levels of 1ng/l or less.

Toxicity tests indicate that Ots are by far more toxic than inorganic tin and that the toxicity increases with progressive introduction of organic groups at the tin atom (Wong

et al., 1982). Therefore, MBT and DBT are lower toxic than TBT. For DBT, the mosquito larva (*Culex pipiens*) is the most sensitive freshwater organisms in acute toxicity tests. The 24-h LC₅₀ for this larva was 0.2 mg/l (Gras and Rioux, 1965). The most sensitive marine organism tested was the diatom, *Skeletonema costatum*. The 72-h EC₅₀ for growth for this diatom was 0.02 mg/l (Walsh *et al.*, 1985). Exposure of freshwater clams (*Anodonta anatina*) to 0.029 mg/l DBT for seven months caused decreases in weight (Holwerda and Herwig, 1986). The non-observed-effect-concentration (NOEC) for histopathological effects in guppies (*Poecilia reticulata*) was < 0.245 mg/l for exposure of three months (Wester and Canton, 1987). For MBT, the red killifish (*Oryzias latipes*) was the most sensitive freshwater organism in acute toxicity tests. The 48-h LC₅₀ for this fish was 24mg/l (Nagase *et al.*, 1991). No data were identified for the chronic toxicity of MBT aquatic organisms. TPTs are highly toxic for fish and other aquatic inhabitants. Several kinds of fish (*Gambusia affinis*, *Carassius auratus* and *Anguilla anguilla*) expire to 100% within 24hr at a concentration of only 0.4 mg/l of TPT acetate (Gras and Rioux, 1965). A 10% mortality for sunfish in ponds with TPT acetate concentration of 0.86mg/l, and high mortality for guppies, tadpoles, toads, and crayfish in fresh water at concentration of 4.3mg/l of active ingredient (Rudoff, 1981). For 6.9cm long carp (*Cyprinus carpio*) weighing 8.5g, median of tolerance limits were 0.521mg/l after 24hr and 0.32mg/l after 48 hr (Rudoff, 1981). No data were identified for the acute and chronic toxicity of DPT and MPT to aquatic organisms.

Physico-chemical values of representative Ots were shown in Table I-2 (Watanabe, 1994). Although informations for physico- chemical properties are a little, physico-chemical values are varied by each Ot. This indicates that it is difficult to analyze these compounds simultaneously.

Table I-1 Effect of TBT acetate on *C. gigas* embryogenesis and larvae development (Alzieu, 1986a)

TBT acetate (µg/l)	Effects on reproduction
100	inhibition of fecundity
50	inhibition of segmentation
25	partial reduction of segmentation
10	absence of the formation of trocophores
3-5	absence of veligers-malformation of trocophores
1	abnormal veligers-malformation of trocophores
0.5	numerous anomalies-total mortality in 8 days
0.2	perturbation in food assimilation-total mortality after 12 days
0.1	normal D-larval ; slow growth, almost total mortality after 12 days
0.05	slow growth ; high mortality rate after 10 days
0.02	no observed effect

Table-2 Physico-chemical property of Bts and Pts (Watanabe, 1994)

Compounds	M.W.	m.p.(°C)	b.p.(°C/mmHg)	W.S. (mg/l)	LogPow
MBTCI	282.2	—	—	Soluble	0.09, 2.2
DBTCI	303.8	41-42	102-103 / 12	92 (distilled water)	0.05, 1
TBTCI	325.5	—	152-156 / 14	17 (distilled water)	3.11
MPTCI	302.2	—	—	Soluble	0.22, 1.73
DPTCI	343.8	—	—	50 (distilled water)	0.11, 1.43
TPTCI	385.5	—	—	5.2 (sea water)	2.02, 2.11

In various countries, regulation and legislative risk management action for Ots adopted during 1982-1992. The contents of them are listed below.

France (Alzieu, 1991)

January 1982 : Prohibition of the use of protective paints containing over 3% Ots on the hull of boats with a gross registered tonnage under 25tons.

September 1982 : Ban of all Ots -based paints for boats under 25m long.

Application of the above provisions to the entire coastline

Introduction of a waiver for light-alloy boats

Ordinance defining labeling requirements

Enforcement term of the decree set for 2years and renewable for the same term.

December 1989 :Ban of the sale and use of Ots based antifouling paints on hulls of boats under 25m as well as on traps, floats, nets and all immersed equipment, apparatus or constructions.

Ots-based paints would only be sold to maintenance sites in containers of at least 20l.

In shellfish culture areas, local authorities would be entitled.

United Kingdom (Hugget *et al.*, 1992)

1985 : Restriction of the sale of TBT paints containing more than 7.5% total tin in copolymers are 2.5% total tin as free tin.

A "safe" water target concentration of 20ng/l as a goal.

February 1987 : Reduction of the target concentration to 2ng/l

Ban of the supply of antifouling paints containing TBT for use on small boats.

United States (Schrantz, 1990)

June 1988 :Set of "the Organotin Antifouling Paint Control Act"

Release rate of TBT is below 4mg/cm²/day.

Penalties for violating.

October 1988 :Ban of TBT on nonaluminum vessel under 82ft.

Japan

January 1990 : TBTO is designated as a Class 1 Specified Chemical Substance with the Law Concerning the Examination and Regulation of Manufacture etc. of Chemical Substances, the manufacture, import and use etc..

: Seven TPT species are designated as Class 2 Specified Chemical Substances.

September 1990 : Thirteen TBT species are designated as Class 2 Specified Chemical Substances

1992 : Environment Agency set guideline Ots concentration in the area within 100m from source on ones was under 0.1 µg/l and that in the other area was 0.01 µg/l.

In spite of these regulations, coastal areas found to be contaminated widely by Ots. For example, sediment samples in Hong Kong close to shipyards and marinas showed very high concentration of TBT and corresponding decay products and the maximum

concentration was 130mg/kg(Ko *et al.*, 1995). Hasan and Juma (1992) were reported that TBT concentration in seawater from Bahrain from 2.29 to 17.88 $\mu\text{g/l}$ with a mean of 5.97 $\mu\text{g/l}$ and in sediments it is ranges from 128-1930 $\mu\text{g/kg}$ and with a mean of 732 $\mu\text{g/kg}$. Further, TBT is degraded to DBT and MBT in aquatic environment. Dirkx *et al.* (1993) reported that concentration of DBT and MBT in water varied between 1.45 and 120; and 1.08 and 38 ng/l at various locations and sampling times in the harbour docks of Antwerp, France. TPT was detected at the concentration of 1 to 29 ng/l in most of the marinas and ports cote d'Azur coastline, France (Tolosa *et al.*, 1996)

The objective of this study is to be clear the behavior of Ots including MBT, DBT, TBT, MPT, DPT and TPT (Fig. I- 2) and find the characterization of distribution of Ots, and furthermore, propose the basic data for the behavior of organometallic compounds in aquatic environment.

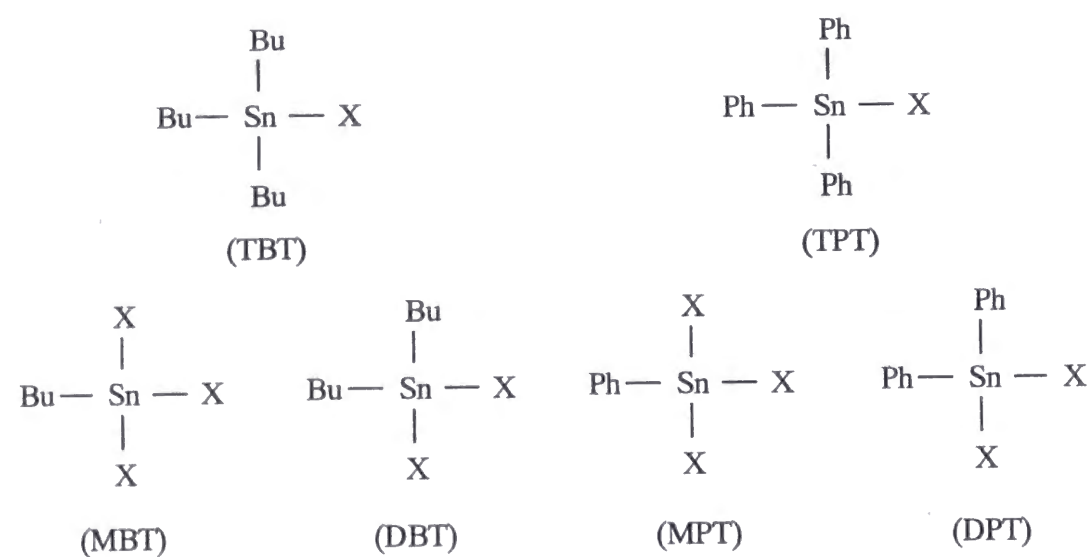


Fig.I-2 The structure of Bts and Pts

Bu : Butyl group, Ph : phenyl group, X : Inorganic or polar organic groups

CHAPTER II

ANALYTICAL METHOD OF ORGANOTIN COMPOUNDS IN AQUATIC ENVIRONMENT

SECTION I

DETERMINATION OF ORGANOTIN COMPOUNDS IN WATER SAMPLES

1. INTRODUCTION

As described in chapter I, TBT and TPT give harmful effects to aquatic organisms when released into the environment even at levels of 1 μl or less. Therefore, both TBT and TPT at ultra-trace levels must be precisely determined with their degradation products such as mono- and disubstituted analogues, in order to investigate their environmental behavior of these compounds. Analytical method of Ots have been developed since 1980s. Hitherto, Ots in water were determined by various methods (Table II-1). In general, it is proposed that analytes are derived after extraction with hydrophobic solvent such as benzene and determined by GC/FPD. Recently, MS or AAS., LC-ICP/MS (Inoue *et al.*, 1995), LC/MS and GC-AED (Gremm and Frimmel, 1992) were also applied to the analysis of Ots. However, these methods can not apply to the Ots including Bts (MBT, DBT, TBT) and Pts (MPT, DPT, TPT) simultaneously, because there is no solvent which can extract these compounds to organic layer from aquatic layer. In this section, simultaneous determination method of Bts and Pts in water by GC/FPD are described.

TableII-1 Analytical method of Bts and Pts in water

Compounds	Method	Detection limit	Ref.
DBT, TBT, TBTO	0.05% tropolone benzene solution - methylation (methyl magnesium bromide) - GC/FPD	DBT 10 μ g/l, TBT 10 μ g/l, TBTO 10 μ g/l,	Mainema <i>et al.</i> , 1978
MBT, DBT, TBT	1% tropolone benzene solution - pentylation (pentyl magnesium bromide) - GC/FPD		Maguire <i>et al.</i> , 1981
TBT	n-Pentane - methylation (methyl magnesium bromide)- GC/FPD	TBT 0.001 μ g/l	Mueller <i>et al.</i> , 1984
DBT, TBT, TPT	Benzene- silica gel column - Hydrogenation (NaBH ₄)-GC/ECD	DBT 0.613 TBT 0.713 μ g/l, TPT 0.363 μ g/l	Hattori <i>et al.</i> , 1984
MBT, DBT, TBT DPT, TPT	0.2% tropolone Sep Pak C18- ethylation (ethyl magnesium bromide) - GC/FPD,GC/MS	MBT 0.001 μ g/l, DBT 0.001 μ g/l, TBT 0.001 μ g/l DPT 0.001 μ g/l, TPT 0.001 μ g/l	Muller <i>et al.</i> , 1987
MBT, DBT, TBT	0.2% tropolone CH ₂ Cl ₂ - Hexylation (hexyl magnesium bromide) - GC/ITD	MBT 0.005 μ g/l, DBT 0.005 μ g/l, TBT 0.005 μ g/l	Reader <i>et al.</i> , 1988
MBT, DBT, TBT DPT, TPT	C18 column- HPLC-AAS	MBT 0.03 μ g/l, DBT 0.03 μ g/l, TBT 0.03 μ g/l	Kadokami <i>et al.</i> , 1988
MBT, DBT, TBT	0.2% tropolone hexane solution - hexylation (hexyl magnesium bromide) - GC/FPD	MBT 10 μ g/l, DBT 10 μ g/l, TBT 10 μ g/l	Graves <i>et al.</i> , 1988
TBT	HPLC - ICP/MS	TBT 25 μ g/l	Branch <i>et al.</i> , 1989
DBT, TBT	Hydrogenation (NaBH ₄) - hexane - Silica Column - GC/FPD	DBT 0.025 μ g/l, TBT 0.020 μ g/l	Nagase <i>et al.</i> , 1990
TBT,TPT	Hexane- propylation (propyl magnesium bromide) - GC/FPD	TBT 0.009 μ g/l, TPT 0.027 μ g/l	Hattori <i>et al.</i> , 1991
MBT, DBT, TBT	NaDDTC, pentane - pentylation (pentyl magnesium bromide)- GC/AAS, GC/AED	MBT 0.050 μ g/l, DBT 0.050 μ g/l, TBT 0.050 μ g/l	Lobinski <i>et al.</i> , 1992
MBT, DBT, TBT	Ion exchange liquid chromatography - AAS	MBT 5.5 μ g/l, DBT 9.3 μ g/l, TBT 7.8 μ g/l	Schulze <i>et al.</i> , 1994
MBT, DBT, TBT MPT, DPT, TPT	10%DDTC pentane solution - methylation (methyl magnesium bromide) - basic alumina column - GC/ITD	MBT 0.001 μ g/l, DBT 0.001 μ g/l, TBT 0.001 μ g/l	Stab <i>et al.</i> , 1994

The unit was expressed as the cation of organotin compounds.

2. MATERIAL AND METHOD

2-1 Apparatus

A Hewlett-Packard Model 5890 GC equipped with a FPD with a 610 nm cut-off interference filter was used. The separation of Ots was carried out on a capillary column (30 m X 0.25 mm i.d.) coated with methylsilicone (0.25 μ m film thickness). The column temperature was held at 80 °C for the first 1 min, then increased to 160 °C at 15 °C/min and to 260 °C at 5 °C/min. Helium was used as the carrier gas at a flow-rate of 1.4 ml/min. The flow-rates of air and hydrogen for the detector at 290 °C were 120 and 160 ml/min, respectively. Splitless injection (2 μ l) was employed. Injection temperature was 290 °C.

TeBT and TPT chloride and n-propylmagnesium bromide were purchased from Tokyo Kasei and MPT trichloride, DPT dichloride and tropolone were purchased from Aldrich. Organic solvents were of pesticide residue-free grade. Special-grade sodium chloride from Nacalai Tesque and distilled water were employed. Sep-Pak disposable cartridges packed with Florisil were purchased from Waters.

2-2 Sampling description

Sea water used for the recovery test of Ots, was collected from station A1 in the Port of Osaka in 1991 (Fig.II-1). Salinity at station

A1 was ranged of 23.5 -

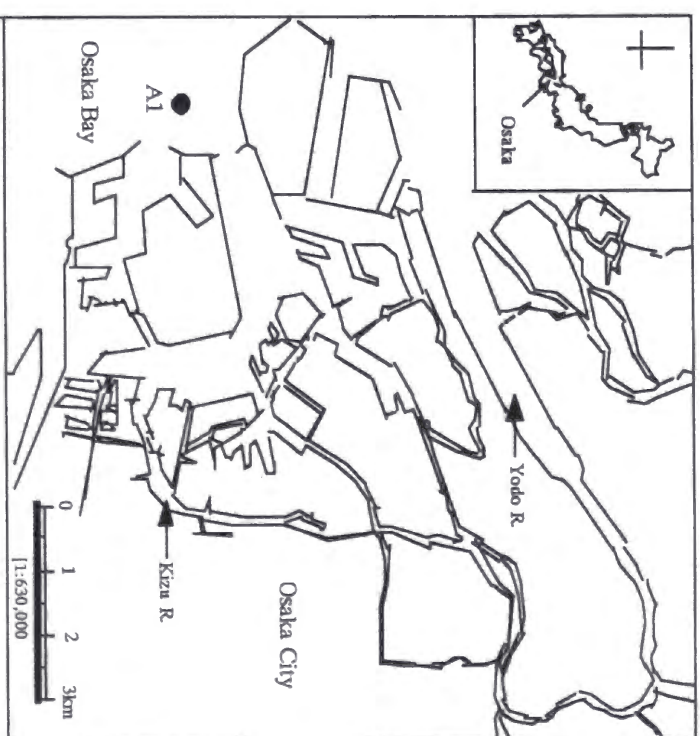


Fig.II-1 Sampling site

28.9 . Water samples without filtration were stored at 5 °C until chemical analysis were carried out.

2-3 Analytical method

In a separating funnel, 1l of water samples was mixed with 10 ml of 1N HCl, 20 ml of acetone and 250 g of NaCl. The mixture was extracted with two 50 ml portions of 0.1% tropolone-benzene solution. The combined organic layers were dried with anhydrous Na₂SO₄ and concentrated to 0.5 ml on a rotary evaporator at 40 °C.

Extract was made up to 5 ml with benzene, 1 ml of n-propylmagnesium bromide in THF (ca.2M) were added and the mixture was shaken gently for 10 ml at 40 °C in incubator. The organic phase was separated by mixing with 10 ml of 1N H₂SO₄ and 40 ml of water. The aqueous phase was then extracted twice with 10 ml of hexane/benzene (9/1). The combined organic layers were dried with anhydrous Na₂SO₄. After concentration up to 1ml with rotary evaporator, hexane was added to the extracts up to 5ml.

The Florisil Sep-Pak cartridge for clean-up was prewashed with 5 ml of hexane. The solution containing the propylated derivatives were passed through the prewashed cartridge. Moreover, Ots were eluted with 10ml of hexane/benzene (9/1) solution. These eluates concentrated to ca. 0.5 ml on a rotary evaporator and finally made up to 5 ml with hexane. The eluates were carefully evaporated with a stream of nitrogen to less than ca.0.5 ml, added the internal standard (TeBT) and made up to 0.5 ml. Analytes were detected with GC equipped with a FPD.

2-4 Standard solution

A stock standard solution containing 1.0 mg/l of each compound was prepared by diluting a acetone solution containing Bts and Pts (2000mg/l each) with benzene. This standard solution (0.5 - 2.5 ml) was propylated as described above. To the combined

extracts, 1.5 µg of TeBT were added and the mixture was concentrated under a stream of nitrogen and made up to 5 ml.

3. RESULTS AND DISCUSSION

3-1 Extraction

The results for the extraction of Bts and Pts from water are given in Table II-2. All the Ots are converted into their chlorides by reaction with HCl and extracted. In general, hexane (Langseth, 1984), dichloromethane , and benzene (Meinema *et al.* , 1978) are used as extraction solvents. When dichloromethane was used as the solvent, the recoveries were not good except for TBT. Benzene exhibited high recoveries (93-98%), except for MBT and MPT. Their low recoveries were greatly improved by addition of tropolone (0.1%) to benzene. In contrast, the recoveries of the other Ots decreased significantly. An increase in the amount of NaCl added to the aqueous layer from 100 to 250 g greatly enhanced the recoveries. However a recovery of 70 % for TPT is not satisfactory. The use of polar solvent such as acetone and tetrahydrofuran resulted in recoveries of more than 92 % for all the compounds tested.

Table II-2 Effect of solvent and NaCl on extraction of Bts and Pts from water (0.1mg of Bts and Pts in 1l of distilled water)

	Tropolone (%)	NaCl (g)	Recoveries (%)					
			MBT	DBT	TBT	MPT	DPT	TPT
Hexane	0	100	0	37	97	0	14	95
Dichlorometnane	0	100	28	73	91	53	42	42
Benzene	0	100	0	95	93	7	97	98
Benzene	0.1	100	98	36	37	76	79	40
Benzene	0.1	200	93	35	30	88	88	40
Benzene	0.1	250	100	80	85	90	95	70
Benzene-THF	0.1	250	94	99	98	97	99	92
Benzene-acetone	0.1	250	84	73	100	74	93	98

3-2 Propylation

The effect of reaction time on the propylation reaction of the Ots is shown in Fig.II-2. It is apparent that they immediately reacted with n-propylmagnesium bromide after its addition. Each peak height reached a maximum with a reaction time of 10 min. The reaction time was therefore fixed at 10 min.

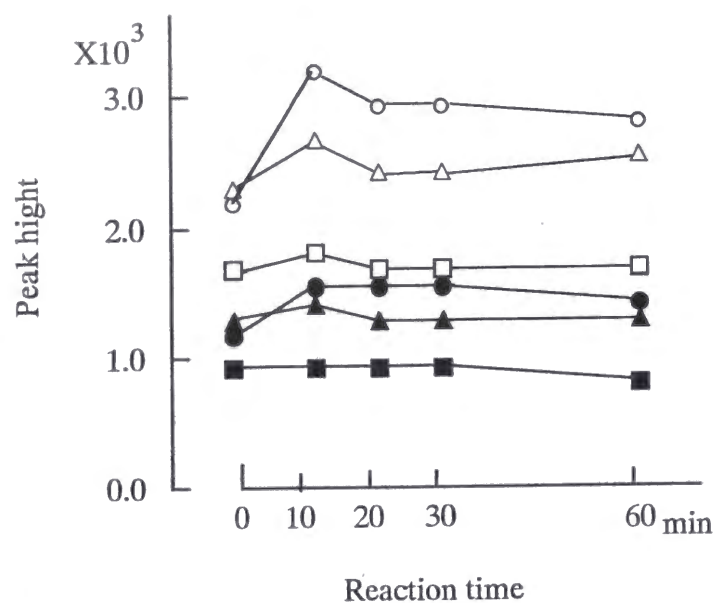


Fig.II-2 Propylation of organotin compounds.
(○) MBT; (△) DBT; (□) TBT; (●) MPT; (▲) DPT; (■) TPT

3-3 Gas chromatography

Relationships between injection temperature and sensitivity of Bts and Pts were investigated (Table II-3). In general, sensitivity was increased with increasing injection temperature. Especially, sensitivity of DPT and TPT was increased drastically. From these results, injection temperature was set at 290 °C.

The emissions of Ots in a small flame of a photometric detector were observed at the blue (360 - 490 nm) and red (maximum at 610nm) regions (Kapila and Vogt, 1980). The

TableII-3 Relationship between injection temperature and count of GC

Injection temperature	MBT	DBT	TBT	MPT	DPT	TPT
200 °C	14276	24018	18483	10772	3348	1572
230 °C	15634	25791	21234	15583	11095	8500
260 °C	15209	26316	20494	16045	15110	18169
290 °C	16132	26864	21869	17177	17642	25102

count

latter emission was used owing to its sharpness and intensity.

The detector response depended significantly on the air and hydrogen flow-rates; the results for TBT and TPT are shown in Fig.II-3. At a constant hydrogen flow-rate of 75 ml/min, the maximum peak heights were obtained at an air flow-rate of 110 ml/min. At this air flow - rate, a hydrogen flow-rate of 145 ml/min gave the maximum peak heights. The effect of both flow-rates on the detector response was examined keeping the hydrogen-to air flow-rate ratio constant at 1.3. It was found that the optimum flow-rates were 160 and 120 ml/min for hydrogen and air, respectively.

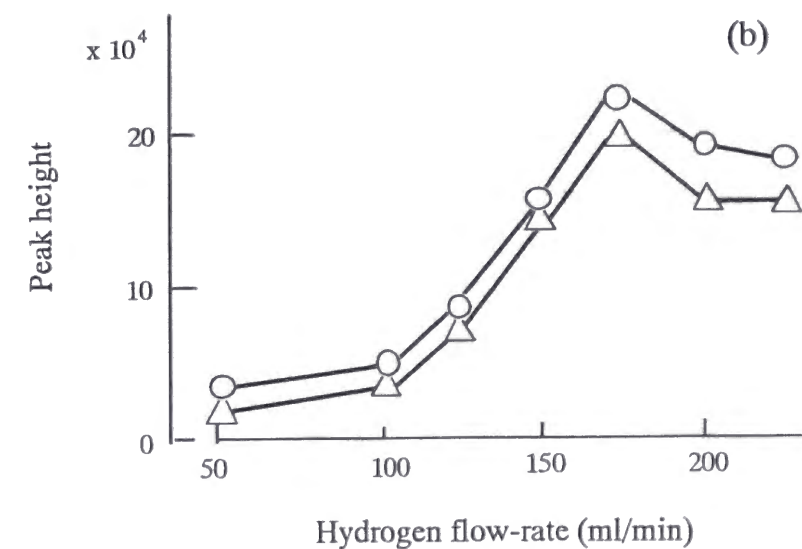
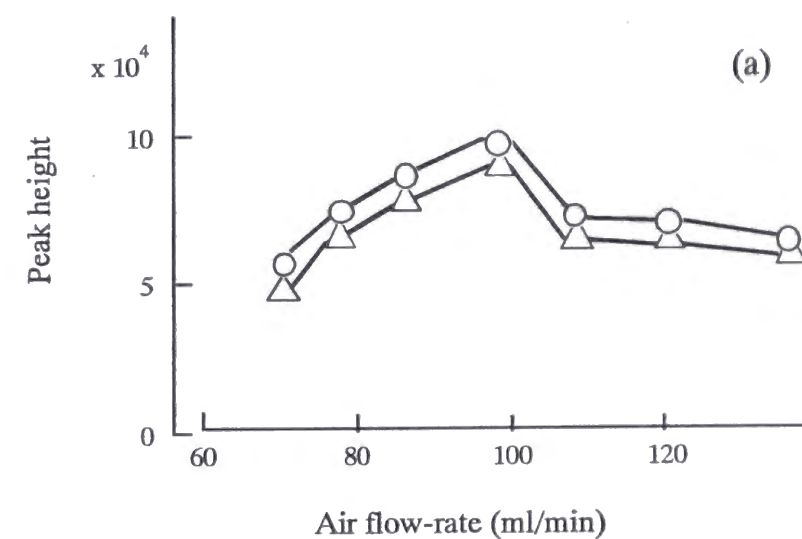


Fig.II-3 Effect of air and hydrogen flow-rates on detector response of (○) TBT and (△)TPT
(a) Hydrogen flow rate 75 ml/min (b) Air flow rate 110ml/min

3-4 Recoveries of organotin compounds

One mg of each Ots was spiked to 1 liter of water and Ots concentrations were determined through the whole procedure. The recoveries of Bts and Pts from water samples are given in Table II-4. The recovery of each Ot in water samples (0.1 mg in 1 l of water) was in the range 73-100% with relative standard deviations of less than 5.5 %.

Table II-4 Recoveries of Bts and Pts from water

	Amount (m l)	Addition amounts (μ g)	Recoveries (%)					
			MBT	DBT	TBT	MPT	DPT	TPT
Sea water	1000	0.1	84 (1.0) ^a	73 (2.7)	100 (1.2)	74 (5.5)	93 (2.1)	98 (1.6)

a : relative standard deviation

The detection limits of the Ots corresponding to a signal-to-noise ratio of three were 3 ng/l. Typical gas chromatograms for the separation of the Ots are shown in Fig.II-4. The peaks were sharp and well separated within 30 min.

4. SUMMARY

Findings which were obtained in this section were described below. 1)The extraction effects by benzene containing 0.1% tropolone and 25% NaCl were high, 2) The reaction time of propylation was fixed at 10 min, 3) the optimum flow-rates of FPD were 160 and 120 ml/min for hydrogen and air, respectively.

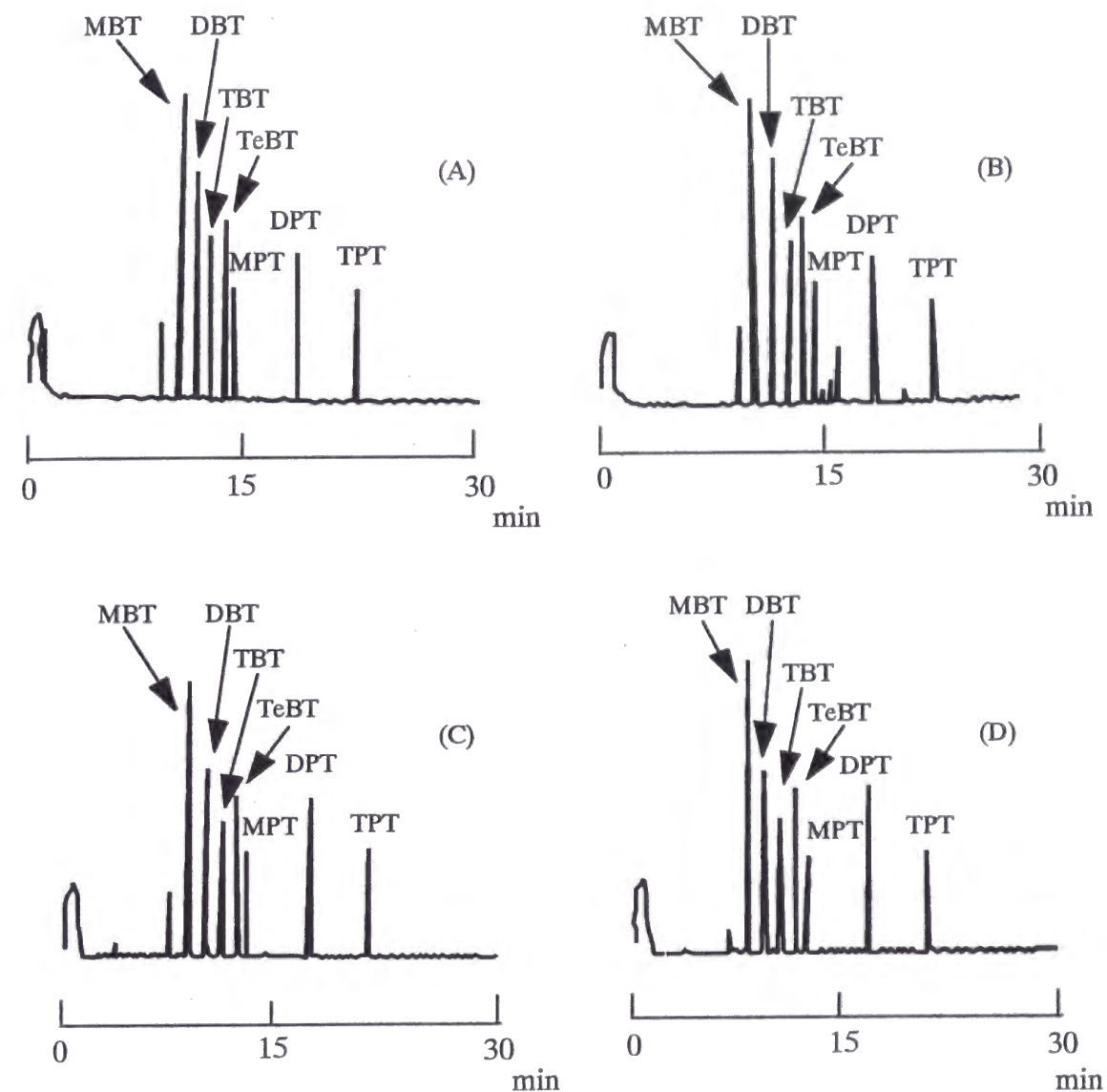


Fig.II-4 Typical gas chromatograms for separation of Ots in (A) standard solution, (B) sea water, (C) sediment and (D) aquatic organisms

SECTION II
DETERMINATION OF ORGANOTIN COMPOUNDS IN SEDIMENT
SAMPLES

1. INTRODUCTION

Hydrophobic pollutants such as PCB are absorbed to SS easily and deposit to sediment. As a result, these compounds concentrations in sediment are higher than those in water (Tanabe *et al.*, 1989). TBT and TPT are estimated to accumulate in sediment easily, judging from physico-chemical properties of these compounds. Accordingly, Ots in sediment are necessary to be measured precisely. There are many publications relate to analytical methods of Ots in sediment (Table II-5). However, there are two unreasonable problems in these methods. preliminary, most of these methods can not analyze both Bts and Pts in sediment. Secondly, as sulfur is existed in sediment, recoveries and reproducibility of analytes are varied by interfering with sulfur. In this section, analytical method of Ots in sediment samples were discussed.

2. MATERIAL AND METHOD

2-1 Apparatus

Apparatus was used as described in section I.

2-2 Sampling description

Sediment was used the recovery test of Ots , was collected from station A1 in the Port of Osaka in 1991 (Fig.II-1) and stored at -20 °C until chemical analysis was carried out. Ignition loss of sediment from station A1 was ranged from 10 to 13%.

TableII-5 Analytical method of Bts and Pts in sediment

Compounds	Method	Detection limit	Ref.
TBT	Diethyl ether - methylation (methyl magnesium bromide) - GC/FPD	TBT 500 µg/kg	Mueller <i>et al.</i> , 1984
DBT, TBT, TPT	Benzene- silica gel column - Hydrogenation (NaBH ₄) -GC/ ECD	DBT 0.03 µg/kg, TBT 0.04 µg/kg, TPT 0.02µg/kg	Hattori <i>et al.</i> , 1984
MBT, DBT, TBT MPT, DPT, TPT	Ethylacetate - hydrogenation (NaBH ₄) -Silica gel column - GC/ECD	MBT 6.2 µg/kg DBT 0.38 µg/kg TBT 0.89 µg/kg MPT 1.6 µg/kg DPT 0.79 µg/kg TPT 2.27 µg/kg	Tsuda <i>et al.</i> , 1987a
MBT, DBT, TBT	Methanol- hydride generation (NaBH ₄) - GC/AAS	MBT 0.30 µg/kg, DBT 0.20 µg/kg, TBT 1.08 µg/kg	Cai <i>et al.</i> , 1993
TBT	SFE - ethylation (ethyl magnesium bromide) - GC/FPD		Dachs <i>et al.</i> , 1994
MBT, DBT, TBT MPT, DPT, TPT	SFE (hexyl magnesium bromide) - GC/FPD		Cai <i>et al.</i> , 1994

The unit was expressed as the cation of organotin compounds.

2-3 Analytical method

In a flat-bottomed flask, 10 g of wet sediment were placed together with 25 ml of acetone and the mixture was extracted for 30 min in a mechanical shaker. After removal of the supernatant with centrifuge, the residue was re-extracted for 20 min with 25 ml of acetone and then the mixture was centrifuged. The organic layer was separated by shaking the combined supernatants with 500 ml of 25% NaCl solution and 50ml of 0.1% tropolone-benzene solution for 5 min. After removal of organic layer, analytes were extracted again with 25ml of 0.1% tropolone-benzene solution. The resulting aqueous layer was extracted with 50 ml of 0.1% tropolone-benzene solution. In order to remove co-extracted inorganic sulphur-containing species, the combined organic layers were washed with a mixture of 2 ml of 3.3% tetrabutylammonium hydrogensulphate and 40 ml of 16% sodium sulphide. Finally, the organic layer was concentrated to 0.5 ml after drying with anhydrous Na_2SO_4 .

Extract was made up to 5 ml with benzene, 3 ml of n-propylmagnesium bromide in THF (ca.2M) were added and the mixture was shaken gently for 10 ml at 40 °C in incubator. The organic phase was separated by mixing with 10 ml of 1N H_2SO_4 , 40ml of water. The aqueous phase was then extracted twice with 20 ml of hexane/benzene (9/1). The combined organic layers were dried with anhydrous Na_2SO_4 , then concentrated to ca. 0.5 ml on a rotary evaporator and finally made up to 5 ml with hexane.

The Florisil Sep-Pak cartridge for clean-up was prewashed with 5 ml of hexane. The hexane solution containing the propylated derivatives and then 10 ml of hexane/benzene (9/1) solution were passed through the prewashed cartridge. The eluates were carefully evaporated with a stream of nitrogen up to 5ml.

2-4 Standard solution

Standard solution was prepared as described in section I.

3. RESULTS AND DISCUSSION

3-1 Extraction

Considering from recovery of Ots from water, it is estimated that high recovery of Ots from sediment is obtained by extraction with acetone or THF as polar solvent. However, when analytes were re-extracted with 0.1% tropolone-benzene in the case of THF, H_2O with THF was contained in organic layer. Consequently, drying of organic layer was very difficult. If H_2O in organic layer is remained, grignard reagent is degraded with H_2O . Therefore, Ots are not reacted quantitatively with grignard reagent. Acetone instead of THF was used as a extraction solvent from sediment. Acetone was transferred to aquatic layer, when aquatic and organic layer were separated. Therefore, H_2O in the organic layer was not contained. Ots were reacted quantitatively with grignard reagent, as acetone was used as a solvent.

3-2 Removal of sulfur

In polluted area, sediments contain large amounts of inorganic sulphur-containing species, resulting in many unknown peaks on gas chromatogram. In order to remove co-extracted inorganic sulphur-containing species, the combined organic layers were washed with a mixture of 2 ml of 3.3% tetrabutylammonium hydrogensulphate and 40 ml of 16% sodium sulphide(Yamada, 1982). Total ion chromatograms of Ots by GC/MS were shown in Fig.II-5. Decrease of impurity

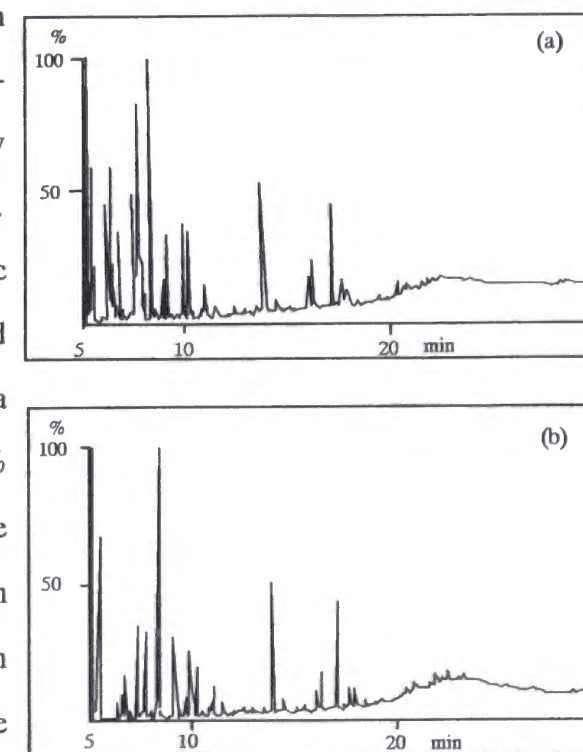


Fig.II-5 Total ion chromatograms of Ots
(a) none, (b) desulfur

peaks were observed in chromatograms of removal sulfur.

Thus, by introducing the washing step to remove the co-extracted inorganic sulphur-containing species, the gas chromatogram which was shown in Fig.II-4C was obtained.

3-3 Recoveries of organotin compounds

In order to examine the quality of data obtained by this analytical method, 0.1 µg of Bts and Pts were spiked to 10 g of sediment. The recoveries of Ots through the whole procedure are presented in Table II-6. The average recoveries of Ots in sediment are in the range of 68-109%. Though the recovery of MBT was lower than other Bts, MBT concentrations in sediment samples were not corrected. The relative standard deviations of each Ots except for MPT were less than 10 % and ones of MPT in sediment were 11%. On the other hand, the detection limit of each Ots in sediment samples for a signal -to-noise ratio of three was 0.005 mg/kg dry. Typical gas chromatogram for the separation of Ots in sediment are shown in Fig.II-4c. The units of Ots were expressed as organotin cations.

TableII-6 Recoveries of Bts and Pts from sediment

	Amount (g)	Addition amounts (µ g)	Recoveries (%)					
			MBT	DBT	TBT	MPT	DPT	TPT
Sediment	10	1	68 (2.9) ^a	98 (3.1)	93 (3.2)	98 (11)	90 (6.0)	109 (7.0)

a : relative standard deviation

4. SUMMARY

Acetone was used as a extraction solvent from sediment, because of higher reproducibility of derivatives. In order to remove co-extracted inorganic sulphur-

containing species, Mixtures of 2 ml of 3.3% tetrabutylammonium hydrogensulphate and 40 ml of 16% sodium sulphide were used. The recoveries of Ots in sediment ranged from 68 to 109% and the detection limits of Ots were 0.005mg/kg dry using this method.

SECTION III
DETERMINATION OF ORGANOTIN COMPOUNDS IN AQUATIC ORGANISMS

1. INTRODUCTION

Judging from physico-chemical properties, TBT and TPT are considered to concentrate in aquatic organisms easily as well as sediment. Aquatic organisms were used as a indicator of contaminants frequently (Soule and Kleppel, 1987). Further, as aquatic organisms are important human foods, contaminants of aquatic organisms are matter of concern. Table II-7 shows analytical methods were hitherto reported. The methods which analyzed Bts and Pts simultaneously were a little among analytical methods. So, it is desired to detect Ots concentrations in aquatic organisms. Analytical method of Ots in aquatic organisms was discussed in section III.

2. MATERIAL AND METHOD

2-1 Apparatus

Apparatus was used as described in section I.

2-2 Sampling description

Japanese sea perch (*Lateolabrax japonicus*) was captured from station A1 in 1991 (Fig. II-1) and stored at -20 ° C until chemical analysis was carried out. Total length and weight of sample were 60 cm and 1600 g, respectively. Muscle and liver of Japanese sea perch were used the recovery test.

2-3 Analytical method

Ten grams amount of muscle or one gram of liver of fish was homogenized for 5

TableII-7 Analytical method of Bts and Pts in aquatic organisms

Compounds	Method	Samples	Detection limit	Ref.
MBT, DBT, TBT MPT, DPT, TPT	Ethylacetate - hydrogenation (NaBH ₄)- Silicagel column - GC/ECD	Fish	MBT 0.006 mg/kg DBT 0.0004 mg/kg TBT 0.0009 mg/kg MPT 0.0016 mg/kg DPT 0.0008 mg/kg TPT 0.0023 mg/kg	Tsuda <i>et al.</i> , 1987a
TBTO	Hexane/ethylether (3/1) solution - GC/ECD	Yellow tail, Sea bream Sea bass, Short necked clam	TBT 0.0018mg/kg	Takeuchi <i>et al.</i> , 1987
TBT, TPT	Methanol/ethyl acetate (1/1) solution - anion and cation exchange resin - NaBH ₄ - GC/FPD	Fish	TBT 0.009mg/kg, TPT 0.018mg/kg	Takami <i>et al.</i> , 1988
MPT, DPT, TPT	Benzene- butylation (butyl magnesium bromide) -- GC/FPD	Rat	MPT 4 mg/kg, DPT 4 mg/kg TPT 4 mg/kg	Ohhira <i>et al.</i> , 1990
MBT, DBT, TBT	0.05%toropolon toluene solution - pentylation (pentyl magnesium bromide) - Florisil column - GC/FPD	Oyster, Shrimp, Minnow		Uhler <i>et al.</i> , 1991
MBT, DBT, TBT, TPT	0.05% toropolone methanol solution - CH ₂ Cl ₂ - HPLC-hydride generation - ICP-AES	Mussel	MBT 7ng, DBT 7ng, TBT 7ng	Rivaro <i>et al.</i> , 1995
DBT, TBT	Methanol - hexane - GPC - methylation (methyl magnesium bromide) - GC/FPD	Tuna, Yellow tail, Horse-mackerel, Pond smelt	DBT 0.015mg/kg, TBT 0.018mg/kg	Sasaki <i>et al.</i> , 1995

The unit was expressed as the cation of organotin compounds.

min with 50 ml of acetone. After removal of the supernatant, the residue was homogenized for 5 min with 50 ml of acetone. The organic layer was separated by shaking the combined supernatants with 500 ml of 25 % NaCl solution and 50 ml of 0.1% tropolone-benzene solution for 5 min. After removal of organic layer, analytes were extracted with 25 ml of 0.1% tropolone benzene solution. The resulting aqueous layer was extracted with 50 ml of 0.1% tropolone-benzene solution. Extract was made up to 5 ml with benzene, 3 ml of n-propylmagnesium bromide in THF (ca.2M) were added and the mixture was shaken gently for 10 ml at 40 °C in incubator. The organic phase was separated by mixing with 10 ml of 1N H₂SO₄, 40ml of water. The aqueous phase was then extracted twice with 20 ml of hexane. The combined organic layers were dried with anhydrous Na₂SO₄, then concentrated to ca. 0.5 ml on a rotary evaporator and finally made up to 5 ml with hexane.

The Florisil Sep-Pak cartridge for clean-up was prewashed with 5 ml of hexane. The hexane solution containing the propylated derivatives and then 10 ml of hexane/benzene (9/1) solution were passed through the prewashed cartridge. The eluates were carefully evaporated with a stream of nitrogen less than ca.0.5 ml, added the internal standard and made up to 5 ml.

2-4 Standard solution

Standard solution was prepared as described in section I.

3. RESULTS AND DISCUSSION

3-1 Extraction

Acetone was used as extraction solvent with the same reason as sediment.

3-2 Clean-up

When the mixture containing analytes was not cleaned with Sep-Pak Florisil, the each peak of Ots was not found. It is considered that quenching is encountered in GC, owing to contamination of fat in sample. The peak was sharp and good separated by passing throughout the Florisil column (Fig.II-4d).

3-3 Recoveries of organotin compounds

A hundred ng of each Ots was spiked to 10g of muscle and after homogenization, Ots concentrations were determined through the whole procedure. Ten ng of each Ots was spiked to 1 g of liver and Ots was measured in liver. The recoveries of the Bts and Pts from environmental samples are shown in Table II-8. The recovery of each Ots in muscle of aquatic organisms except for MBT were higher than 80%. The relative standard deviations ranged from 2.5 to 12%. The recoveries of Ots were in the range of 74-84 % and relative standard deviations ranged 7.2-9.0 %. The detection limits of Ots in muscle and liver corresponding to a signal-to-noise ratio of three were 0.5 µg/kg wet and 0.1

Table II-8 Recoveries of Bts and Pts in aquatic organisms

	Amount (g)	Addition amounts (µg)	Recoveries (%)					
			MBT	DBT	TBT	MPT	DPT	TPT
Muscle	10	1	85 (2.5) ^a	84 (3.6)	103 (5.9)	87 (4.4)	100 (8.3)	99 (12)
Liver	10	1	80 (8.4)	76 (8.1)	84 (7.2)	80 (8.5)	77 (8.0)	74 (9.0)

a : relative standard deviation

µg/kg wet, respectively. Typical gas chromatograms for the separation of the Ots in muscle of fish are shown in Fig.II-4d.

4. SUMMARY

Analytes were cleaned with florisil column. By this clean-up, the each peak of Ots was sharp and good separated. The recoveries of Ots in muscle of aquatic organisms were higher than 80 % and the detection limits of Bts and Pts were 0.005 mg/kg wet. The recoveries of Ots in liver ranged from 74 to 84 % and detection limit of Ots was 0.1 mg/kg wet.

SECTION IV

CONCLUSION

A method for the simultaneous determination of Bts and Pts in the aquatic environment was developed. The analytes in water, sediment and aquatic organisms were extracted into 0.1% tropolone-benzene solution after adding hydrochloric acid and acetone. With sediment samples, inorganic sulphur-containing species co-extracted with the analytes were removed with tetrabutylammonium hydrogen-sodiumsulphide, which brought about a decrease in many of the accompanying peaks. After propylation with n-propylmagnesium bromide, the analytes were cleaned with florisil column and were determined by GC.

In order to examine the quality of data obtained by this analytical method, 0.1 µg of Bts and Pts were spiked to 1 L of water and 1 µg of these Ots were spiked to 10 g of sediment 10 g of muscle of fish or 1 g of liver of fish. The average recoveries of Ots in water, sediment, muscle and liver of fish were in the range of 73-100, 68-109, 84-103%, and 74-84 % , respectively and the relative standard deviations of each Ots were less than 12 %. The detection limit of each Ots in water, sediment, muscle and liver of fish for a signal-to-noise ratio of three was 0.003 µg/l, 0.005 mg/kg dry and 0.005 mg/kg wet and 0.001 mg/kg wet , respectively.

The advantages of our developed methods can be summarized as follow. 1) Ots in water could be determined at the level of 0.001µg/l. 2) Ots including MBT, DBT, TBT, MPT, DPT and TPT were detected simultaneously. The behavior of Ots in aquatic environment became possible to investigate using this method.

CHAPTER III
DISTRIBUTION OF ORGANOTIN COMPOUNDS IN AQUATIC
ENVIRONMENT

SECTION I
CURRENT STATUS OF OSAKA BAY

1. INTRODUCTION

The present status of Ots in aquatic environment have been investigated in various countries. In spite of regulation for Ots, High concentration of TBT have been frequently found in water, sediment and aquatic organisms from the vicinity of marinas and shipyards, and TBT levels which cause imposex for shellfish were detected (Clark *et al.*, 1988, Dirx *et al.*, 1993). It was reported that TBT concentration in sediment from San Diego Bay were ranged from 0.002 to 1.1 mg/kg dry during 1988-1990 (Valkirs *et al.*, 1991). Stewart *et al.* (1994) reported that TBT was a widespread contaminant in the marine environment of south western British Columbia which is a heavy industrial area and has intensive shipping activity. Cocchieri *et al.* (1993) also reported that Ots concentrations exceeded level were toxic for certain sensitive marine species even in the relatively uncontaminated areas of the Gulf on Naples. Thus, there are many publications concerning contaminants of Ots in Europe and USA. Yonezawa *et al.* (1993) reported that the relatively higher concentrations of Bts were detected in the surface sediment from Ise bay, Japan in 1987. However, reports concerning the current status of Ots contamination in bay area of Japan are a little in comparison with papers from the other countries.

As TBT leached from the paint is extremely active, this compound is the adsorption to particulate material with subsequent sedimentation (Seligman *et al.*, 1989). It is well known that TBT concentration in sediment is a thousand times as higher as those in water (Fent and Hunn, 1991). In addition, Ots are stable in sediment (Dowson *et al.*, 1993a).

Therefore, it is important to clarify the vertical distribution of Bts in sediment in order to elucidate the history of Ots contaminants.

Osaka Bay is contaminated heavily by many kind of chemical substances such as PCBs (Tanabe *et al.*, 1991), because of industry area in the zone of poor flushing . The object of this research is to assess the extent of TBT contamination as well as the presence of its degradative product such as DBT and MBT in Osaka Bay, and to estimate temporal trends from vertical profiles of Ots in sediment.

2. MATERIAL AND METHOD

2-1 Sampling Description

Fig.III-1 shows the sampling sites. Osaka Bay is located in mid-western Japan and its average depth is about 30m. The peripheral area of Osaka Bay is an industrial zone with trading ports, industrial plants and installations. Many rivers flow into Osaka Bay and Yodo River is the largest among them and its discharge occupies about 80% of the total river discharge into the bay. The Port of Osaka situated in the north-east of the bay, is a prominent international trading port in Japan and about 80,000 ships enter the Port of Osaka in a year.

Both water and sediment samples in Osaka Bay were collected from 15 sites in November and December 1989, and 25 sites in May 1990. Sampling sites in Osaka Bay in 1989 were different from those in 1990. Water column and sediment core were taken at stations B1-2 and B3, respectively. Water samples from rivers (Yodo river basin) were taken from 10 sites in February 1989 and May 1990, and estuary water and sediment samples (the Port of Osaka) were taken from 4 sites in December 1989 and May 1990.

Surface water were collected with bucket and surface, medium and bottom water at station B1 (water depth 14 m) and B2 (water depth 20 m) were collected with Von Dorn water sampler. Surface sediment samples were taken with Eckman birge grab sampler and sediment were taken with gravity core sampler and were cut in 2 cm long slice. Water

samples without filtration were maintained at 5 °C and sediment samples were stored at -20 °C until chemical analysis.

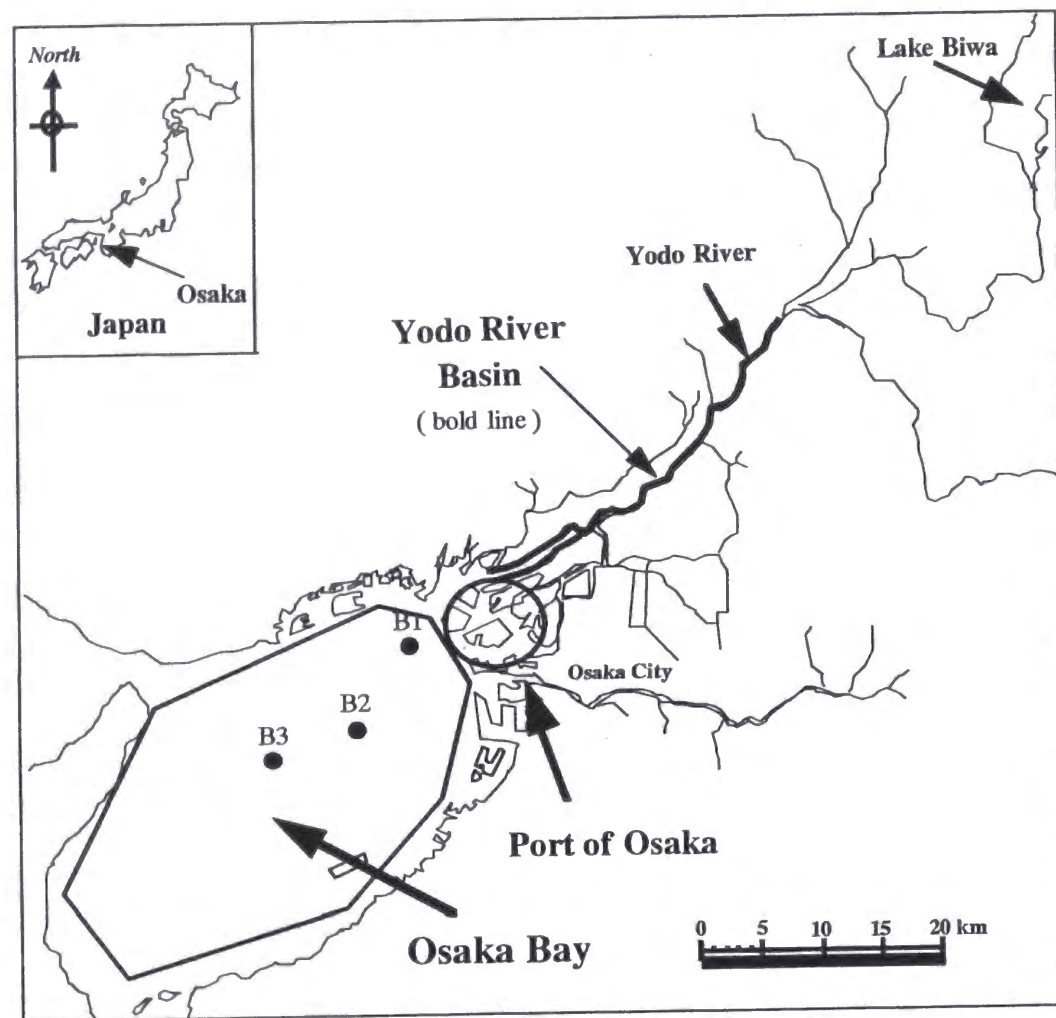


Fig.III-1 Sampling sites

2-2 Analytical method

Analytical procedure was described in chapter I.

2-3 Determination of sedimentation rate

Three grams of every sediment core which were cut in 2 cm long slice from station B3 in Osaka Bay were dried at 105 °C. The activities of radionuclides ^{238}U and ^{210}Pb were determined by using γ ray spectrometer with a planer type Ge (Li) detector from the areas

of the photopeaks due to 63.1-keV γ rays of ^{234}Th (daughter nuclide of ^{238}U), 67.4-keV γ -rays of ^{230}Th and 46.5-keV γ -rays of ^{210}Pb , respectively (Megumi and Mamuro, 1977).

In order to calculate the sedimentation rate, it is assumed that (1) the flux of excess ^{210}Pb to the sedimentation is constant, (2) the sedimentation rate is constant at all times, (3) there is no postdepositional migration of ^{210}Pb within the sediment, and (4) depth profiling of ^{210}Pb supported ^{238}U in the sediment is not uniform. The accumulation rate at the surface was calculated from the vertical distribution of excess ^{210}Pb (Matsumoto and Wong, 1977).

3. RESULTS AND DISCUSSION

3-1 Butyltin compounds in water

Fig. III-2 shows the distribution of Bts in surface water from Osaka Bay in 1989. TBT concentrations ranged from 0.023 $\mu\text{g/l}$ to 0.061 $\mu\text{g/l}$ and were distributed uniformly at 15 sites from Osaka Bay. This is due to TBT from sailing vessels. On the other hand, the ratio of TBT to the total Bts was generally high, suggesting recent input from ships, because the half life of TBT in water is in the range of 4-19 days (Seligman *et al.*, 1986a, b, Lee *et al.*, 1987).

Bts in water were also measured at 25 sites in Osaka Bay in 1990. The concentration of TBT in water from Osaka Bay ranged from ND to 0.059 $\mu\text{g/l}$ and was the same level as in 1989. The concentrations of TBT in port area located in estuary and Yodo River Basin were also investigated in 1989 and 1990 and compared with that in sea areas (Osaka Bay) (Fig.III-3). The concentrations of TBT in port area were highest among the three areas and was in the range of 0.040 - 0.067 $\mu\text{g/l}$. TBT was scarcely detected in rivers. However, MBT and DBT were present.

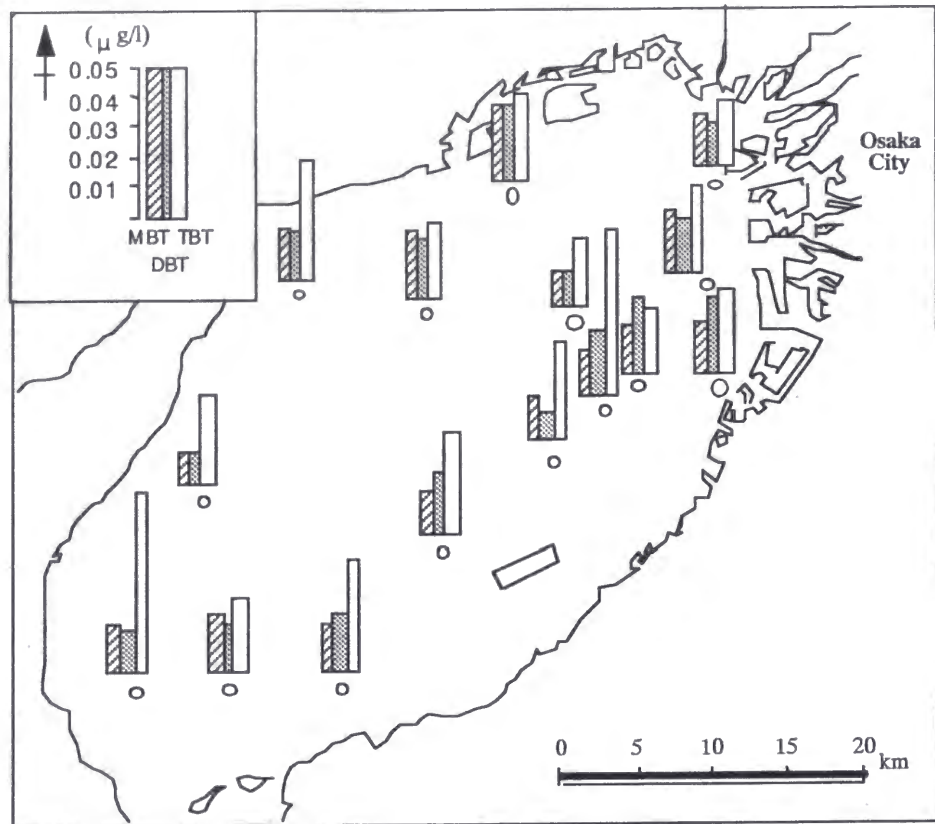


Fig.III-2 Horizontal distribution of Bts in water from Osaka Bay in 1989

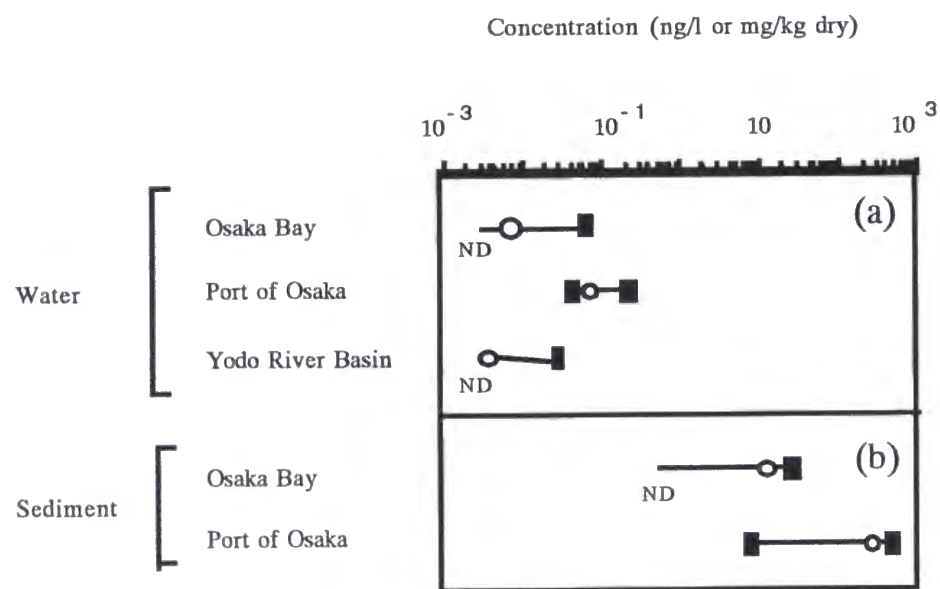


Fig.III-3 Levels of Bts in water and sediment from Osaka Bay, the Port of Osaka and the Yodo River basin in 1990
Solid lines and circles indicated ranges and medians, respectively.

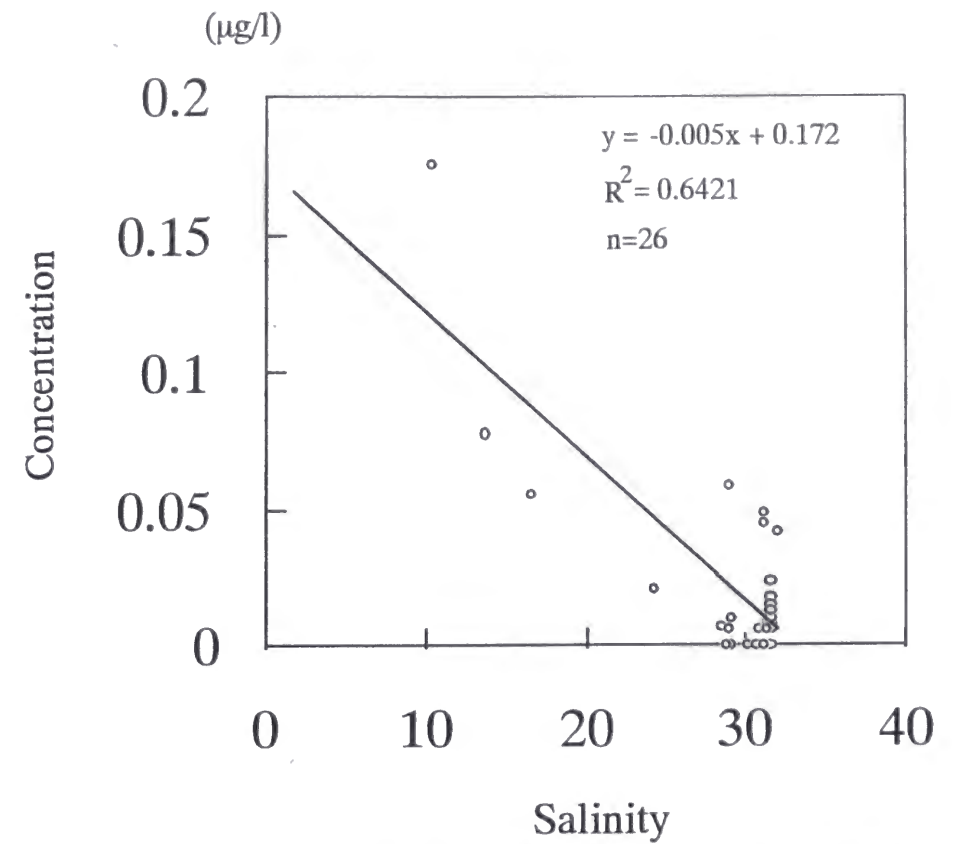


Fig.III-4 Correlation between TBT concentration and salinity in surface water from Osaka Bay and the Port of Osaka in 1990

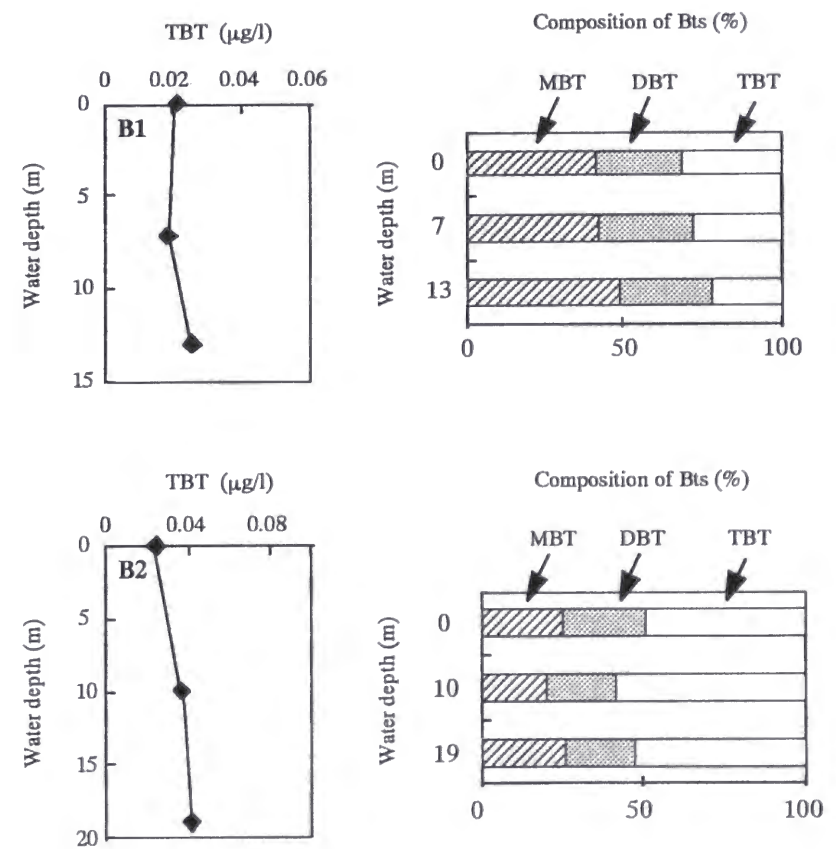


Fig.III-5 Vertical distribution of Bts in water columns from Osaka Bay

Fig.III-4 shows the correlation between TBT concentration and salinity in water from sea areas and port area in 1990. The graph showed a downward slope. This shows that the lower the salinity, the higher the TBT concentration in water, indicating that estuary is heavily contaminated with TBT and TBT from estuary to off-shore is diluted with seawater.

Bts in the surface, medium and bottom layers of water columns are shown in Fig.III-5. The marked difference of concentration of TBT in water column at station B1(water depth 14m) and B2 (water depth 20m) were not observed. The composition of Bts was also uniform in water column. Salinity in surface (B1:28.36, B2:32.34), medium (B1:30.89, B2:32.34) and bottom (B1:31.98, B2:32.34) layers was also almost the same, suggesting vertical mixtures.

3-2 Butyltin compounds in sediment

Fig.III-6 shows TBT concentration in surface layer of sediment from Osaka Bay and the Port of Osaka. The concentration of TBT in Osaka Bay ranged from ND to 0.023mg/kg dry and median is 0.011 mg/kg dry. Range and median of TBT concentration in the Port of Osaka were from 0.011 to 0.692mg/kg dry and 0.356mg/kg dry, respectively. It is considered that TBT level in the Port of Osaka are higher than those in Osaka Bay (Fig.III-3). This investigation was carried out before the regulation of TBT. Data of Ots is a few in other sites of Japan before the regulation. Yonezawa *et al.* (1993) reported that TBT levels in sediment from 44 sampling sites in Ise Bay collected in 1987. The utilization of Osaka Bay was similar to that of Ise Bay, because there are industrial area around the Ise Bay. Accordingly, TBT levels in sediment from Osaka Bay and the Port of Osaka were compared with those in Ise Bay. The concentration of TBT in Ise Bay ranges from ND to 0.38 mg/kg dry. TBT in the Port of Osaka was higher concentration than that in Ise Bay,

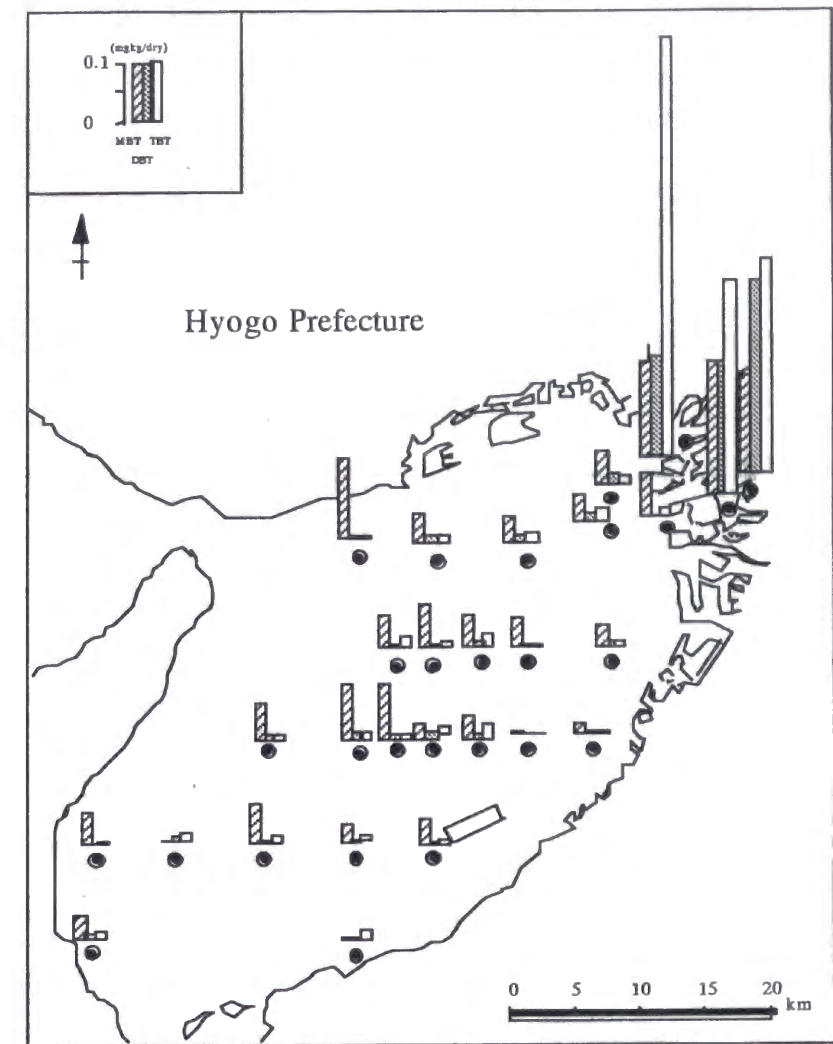


Fig.III-6 Concentration of Bts in surface layer of sediment from Osaka Bay in 1990

Table III-1 Composition of Bts in sediment from the Port of Osaka and Osaka Bay

Stations	MBT (%)	DBT (%)	TBT (%)
Osaka Bay	11 - 88 (64)	5 - 67 (13)	6 - 55 (21)
Port of Osaka	19 - 86 (24)	0- 38 (25)	13 - 57 (44)

suggesting that the Port of Osaka is a zone of poor flushing. TBT in Osaka Bay was lower than that of Ise Bay. The ratio of TBT to the total Bts was high in the Port of Osaka, whereas MBT which is degradation substance of TBT, was dominant among Bts in sediment from Osaka Bay (Table III-1). This means that the TBT load to sediment in the Port of Osaka is higher than those in Osaka Bay.

3-3 Vertical distribution of butyltin compounds in sediment cores

A sediment core was collected from Osaka Bay. Fig.III-7 shows the concentration of Bts in every 2cm segment of core from station B3 in Osaka Bay. In this sediment core, MBT concentration was generally high following by TBT and DBT.

It is reported that TBT persists for several years within sediments (Fent and Hunn, 1991). Accordingly, it is possible to estimate the times when release of TBT began in this area from the profile of TBT in the sediment core. The concentration of TBT in the top 10cm of the core from station B3 was higher than in the other lower part of the sediment layers. Concentration of TBT in core from below 10 cm ranged from ND to 0.007mg/kg dry. Fig.III-8 shows composition of Bts. Composition of Bts were uniformed in each layer. While, the deposition rate of the sediment at Station B3 was estimated to be 3.1mm/year. Consequently, it is estimated that TBT has been used since the 1960s. Our results was therefore agreed with the general assertion that TBT has been in use since the 1960s. Bts were detected in segment less than 10 cm (especially, sediment of depth during 26-28 cm), suggesting the bioturbation. It is also considered that MBT penetrated to ground, because of high water solubility of MBT. Total Bts concentration as Sn cation in each sediment layer roughly decreased with increasing the depth of sediment and these were detected in segment less than 10 cm (Fig.III-7). It can be assumed that the input of DBT used as stabilizer in industrial products accumulated to sediment.

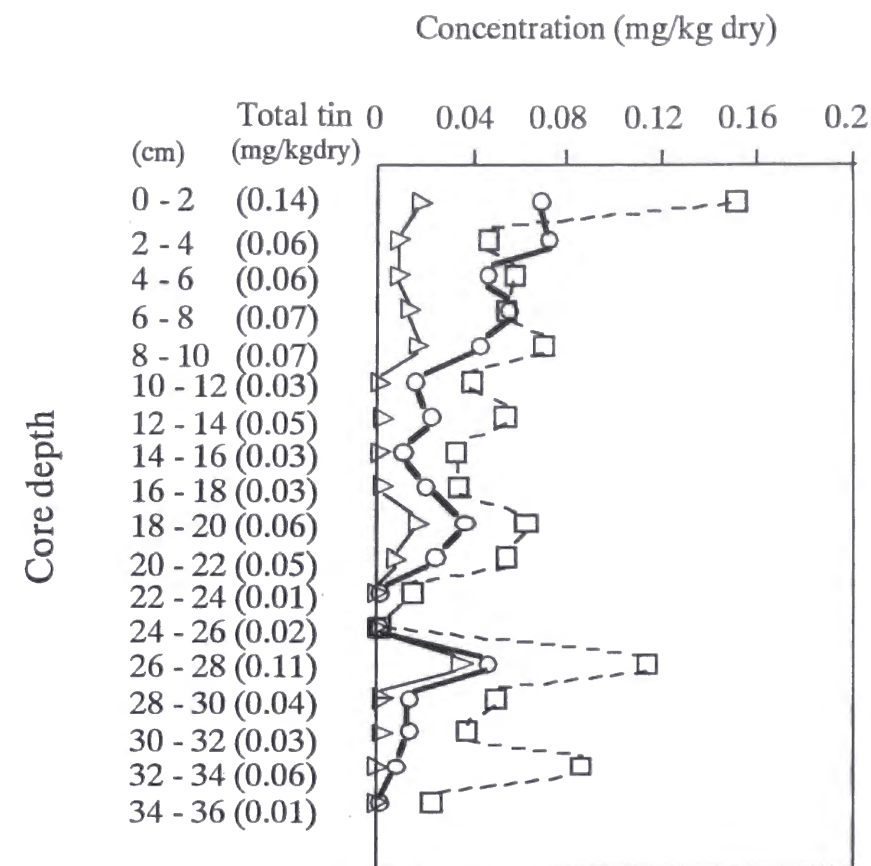


Fig.III-7 Vertical distribution of Bts in sediment core from station B3

○:TBT, △:DBT, □:MBT

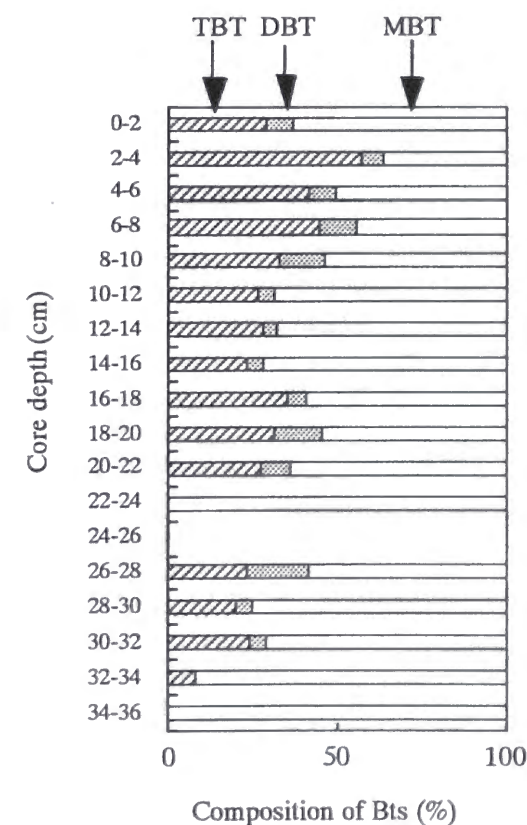


Fig.III-8 Composition of Bts in sediment core from station B3

4. SUMMARY

TBT concentration in surface water from Osaka Bay ranged from 0.023 to 0.061 $\mu\text{g/l}$ in 1989 and from ND to 0.059 $\mu\text{g/l}$ in 1990, respectively. TBT concentration in Osaka Bay were compared with that in the Port of Osaka and Yodo River basin. TBT levels were highest the Port of Osaka, followed by Osaka Bay and Yodo River basin. TBT concentration s were distributed uniformly in water samples from Osaka Bay. These results indicated that the main source of TBT is sailing vessels. To decrease TBT levels in the aquatic environment, the use of TBT on ships should therefore be reduced.

TBT in surface sediment from Osaka Bay ranged from ND to 0.023 mg/kg dry. Relationship between deposition rate of suspended solid (SS) in sediment and depth profile of sediment, suggests that use of TBT in this area started in the 1960's.

SECTION II

TEMPORAL TREND AND CURRENT STATUS OF ORGANOTIN COMPOUNDS IN THE PORT OF OSAKA

1. INTRODUCTION

In previous section, it was reported that TBT concentrations in the Port of Osaka were highest among Osaka Bay in 1989 and 1990. Therefore, it is important to the investigate the current status of Ots in the Port of Osaka.

The concentration of Bts and Pts have been monitored seasonally in the harbour area of the Port of Osaka since 1989. In this section, the temporal trend of Ots is assessed base on these monitoring data. Furthermore, the levels of Bts and Pts are described in water, sediment, plankton and mussels from harbour area of the Port of Osaka in 1996 and the partition of Ots to sediment, plankton and mussels are also discussed.

2. MATERIAL AND METHOD

2-1 Sampling description

The Port of Osaka is situated in the west of Osaka City and is used every year by about 80,000 ships. A map of the sampling stations is shown in Fig.III-9. Station C1 is located at the mouth of Yodo river, which rises from Lake Biwa, and flows through the cities of Kyoto and Osaka to the Port of Osaka. Station C2 is located at a marina with berths for 121 yachts. Most of the vessels moored at station C3 are small and medium hull craft from southeast Asia. The vessels moored near station C4 are mainly trading boats. Station C5 is located in the canal of the Kizu River, poorly flushed. Station C6 is situated at the mouth of Kizu river and is near to many factories. Station C7 is in the ferry port. Station A1 is located in an inner harbor passage and is a zone of well flushing. Four sites in the Port of Osaka which described in previous section were C2, C3, C4 and A1.

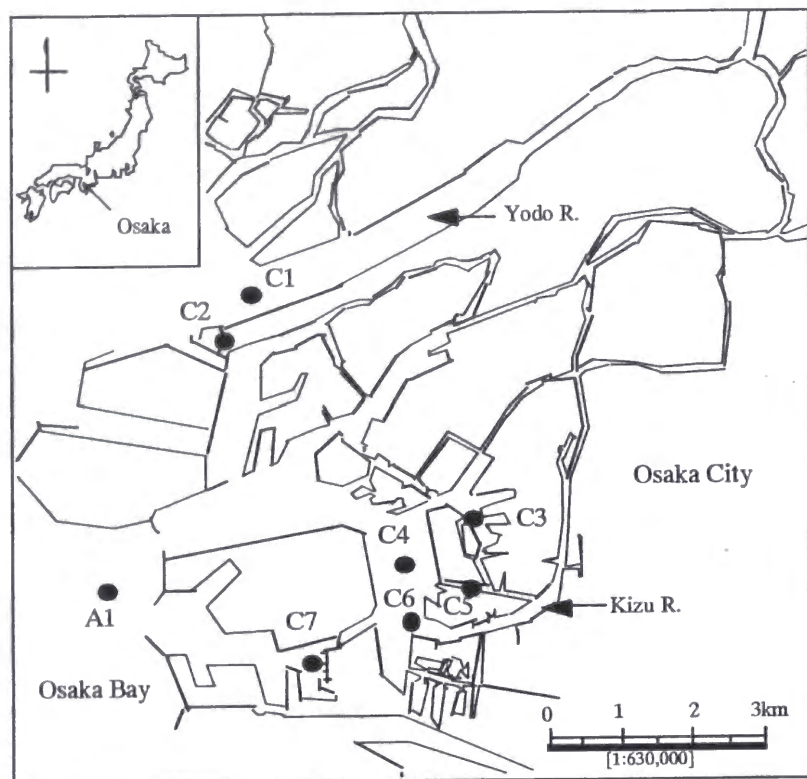


Fig.III-9 Sampling sites in the Port of Osaka

Water and sediment samples have been collected seasonally from stations C1-C7 and A1 with bucket and Eckman birge grab sampler since 1989. Plankton have been also taken seasonally with plankton net (NXX17, horizontal haul) at stations C1-C7 and A1 except for station C2 since 1989. In this study, plankters were not identified. However, Kawai *et al.* (1983) reported that plankton collecting with NXX17 net from station C5 during 1978-1981 was dominated by *skeletonema costatum*, a kind of diatom. Mussels (*Mytilus edulis*) have been taken from the wharf at stations C2, A1 and C7 since 1989. From these monitoring data, temporal trend of Ots was studied. Further, the current status in the port of Osaka were investigated from data of water, sediment, plankton and mussel samples in February, June, August and November 1996. Shell length of mussels from C2, A1 and C7 ranged from 1.7cm to 6.3cm, from 1.6 cm to 2.5 cm and from 2.5 cm to 5.6 cm, respectively. water samples were used without filtration and water samples were maintained at 5 °C and sediment and aquatic organisms were stored at -20 °C until

chemical analysis.

2-1 Analytical method

Analytical method was described in chapter II.

3. RESULTS AND DISCUSSION

3-1 Variation of Ots concentration during 1989-1996

Water

Surveys at eight stations were undertaken to investigate the temporal change of TBT level from 1989 to 1996. Three types of temporal distribution of TBT were apparent in water from the Port of Osaka. The first type was characterized by high concentration between 1989 and 1990 and drastic decrease in comparison with the levels of TBT in 1989-1991. This type was noticed at stations C2-C3. The temporal trend at station C2, typical of this type is shown in Fig.III-10a. TBT concentration decreased to the level of 1/4 - 1/10 during 1989 - 1996 at station C2. At station C3, the median of TBT concentration was 0.07 $\mu\text{g/l}$ in 1990 and decreased to 0.03 $\mu\text{g/l}$ in 1996. Further, yearly variation of TBT concentration decreased with time. In these stations, the ratio of TBT was the dominant among Bts in the period of 1989-1990. However the ratios of MBT and DBT were higher than those of TBT recently. These results show that input of TBT has decreased enormously, because antifouling paint on vessels and pleasure boats has been replaced by anti-organotin paint as a result of regulation. At these stations, it is concluded that the effect of registration is clearly reflected TBT concentration.

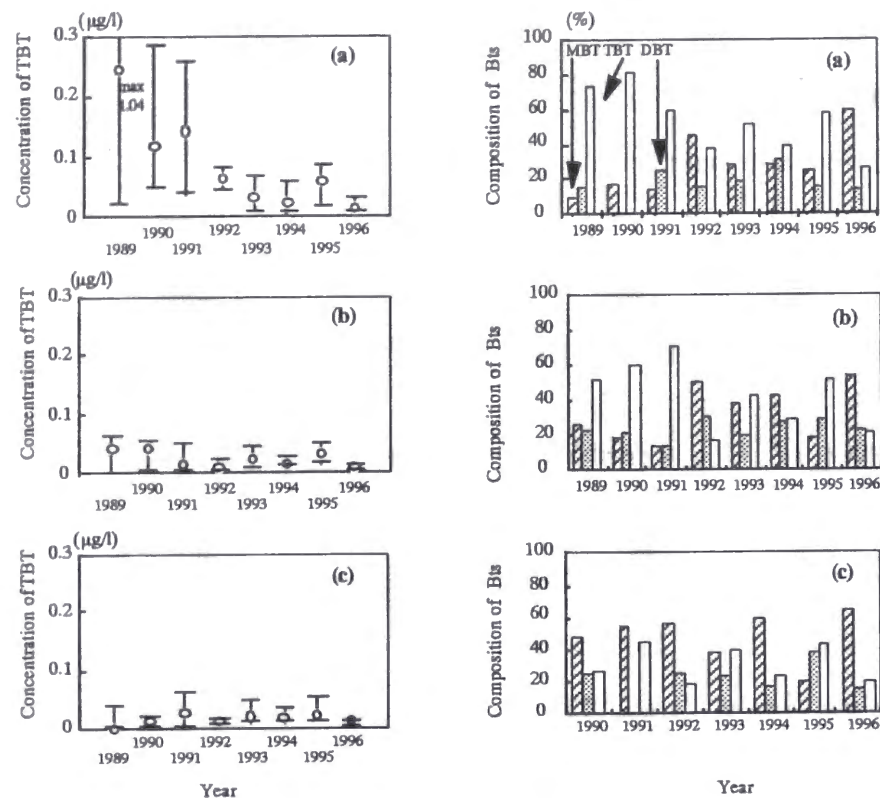


Fig.III-10 Representative changes of TBT concentrations and Bts compositions in water
a:station C2 b:station C4, c:station C7

Analytical values in four times by year were shown in this figure.

The composition of Bts was calculated from the medians of Bts concentration in each

The second type of trend is noted at C1, C4-C6 and A1 is a slight reduction of TBT concentration during the period of 1989-1996 and TBT levels at these stations were lower than those at stations C2 and C3 during 1989 and 1991. Fig.III-10b shows a representative temporal trend of the second type. Concerning TBT levels in 1989, TBT concentration at station C4 was lower than station C2, because of well flushing in comparison with station C2. TBT concentration decreased a little during 1989 - 1992. Although the ratio of TBT to the total Bts was high among stations of this type in 1989, combined ratios of MBT and DBT which are degradative products of TBT exceeded ratio of TBT between 1992 and 1996. A number of ships from countries with no legislation of Ots enter and are berthed at the international trading port of Osaka, where these stations are

located. Though input from Japanese-registered vessels has decreased, TBT has still been released by foreigner-registered vessels painted with Ots. Therefore, a slight reduction of TBT concentration is observed during the study period.

The final type is characterized by low concentration between 1989 and 1991 and unchanged levels of TBT between 1989 and 1996 (Fig.III-10c). Station C7 belongs to this type. The level of TBT in water from station C7 was lower than other area between 1989 and 1991 and was in the range of 0.003-0.01µg/l. The ratio of MBT to the total Bts was high among Bts between 1990 and 1996. The ships moored in station C7 were mainly Japanese ferry boats. This suggests that antifouling paint containing Ots was not used in ferry boats in 1989.

TPT concentration in water ranged from ND to 0.072µg/l during 1989-1990 and maximum concentration of 0.072 µg/l was observed at station C5. After then, TPT was not detected at any of the stations.

Sediment

Fig.III-11 shows the temporal trend of TBT concentration and Bts composition at representative sampling sites (stations C2, C4 and A1) in the study period. Remarkable change of TBT concentration at stations C1-C7 and A1 were observed between 1990 and 1996. It is reported that the half-life of TBT in sediment was 4-5 months (Maguire *et al.*, 1985). Dowson *et al.* (1993a) also reported that half-lives of TBT ranged from 360 to 775days in surface sediment. In spite of the slow degradation of TBT in sediment, TBT levels were not determined throughout study period. This is due to the equilibrium the degradation of TBT in sediment and continuous input of TBT.

TPT was detected in sediment from Stations C2-C4 and C5-C7 between 1989-1996. The temporal trend of TPT concentrations was not clear because levels varied widely in the range of ND - 0.426 mg/kg dry.

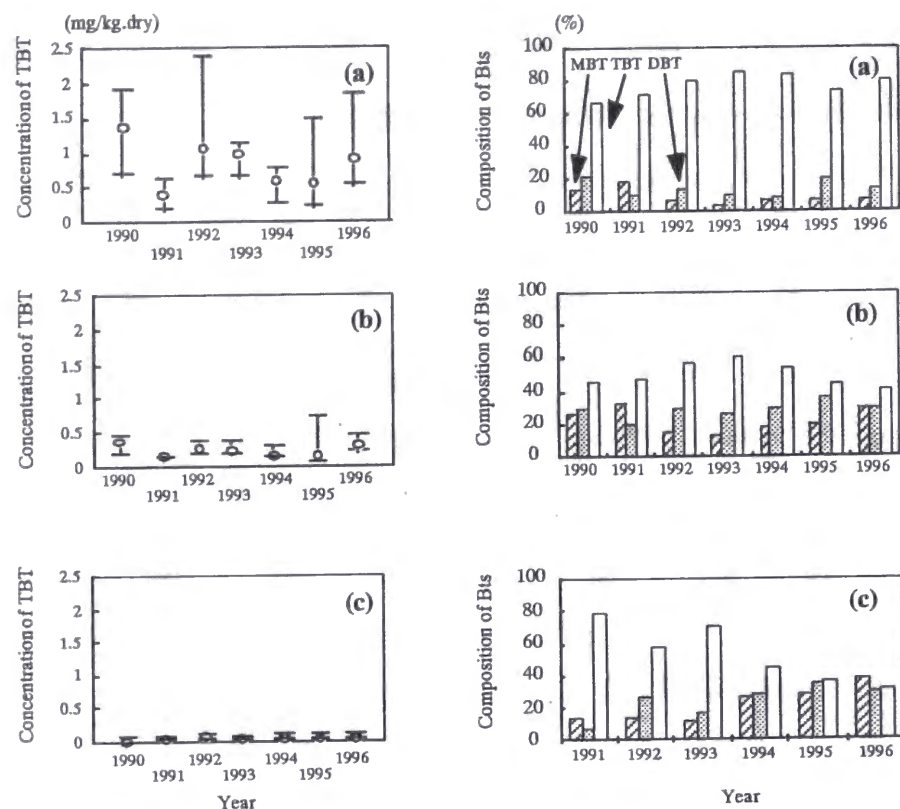


Fig.III-11 Representative changes of TBT concentrations and Bts composition in sediment
a:station C2, b:station C4, c:station A1
The composition of Bts was calculated from the median of Bts concentration in each year.

Plankton

TBT in plankton has been measured since 1992. The representative trend is shown in Fig.III-12. It is estimated that TBT levels were not decreased in the period of 1992-1996 and the range of concentrations varied widely in each year. The ratio of TBT was also dominant among Bts. TBT have highest hydrophobic property among Bts. Accordingly it is presumed that TBT is dominant species in plankton because of hydrophobic interaction between plankton and TBT.

TPT have been detected in plankton during study periods. As the concentrations of TPT in plankton varied widely as well as those in sediment, the temporal trend of TPT concentrations in plankton was not clear.

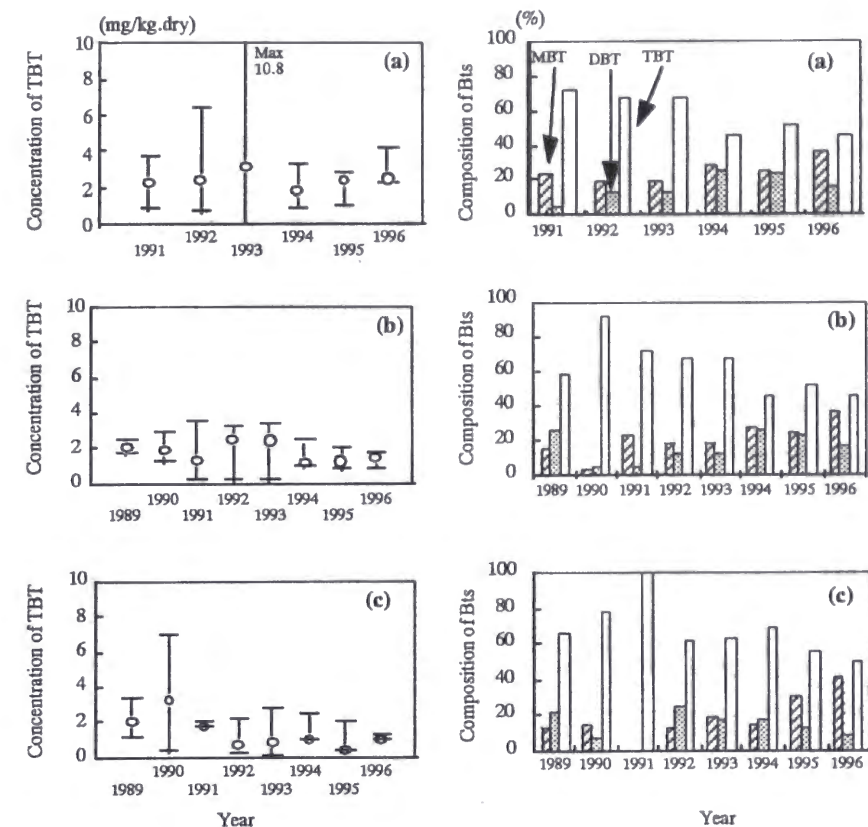


Fig.III-12 Representative changes of TBT concentrations and Bts compositions in plankton
a;station C3, b:station C4, c:station A1
The composition of Bts was calculated from the median of Bts concentration in each year.

Mussels

A remarkable decrease in TBT concentration in mussels was observed from station C2 during 1989-1991 and then the concentrations did not change. TBT at stations A1 and C7 also showed a decrease in comparison with 1990-1991 levels (Fig.III-13). it is considered that the trend of TBT levels in mussels is due to a reduction of TBT levels in water. TBT levels in mussels was reported to begin to reflect on those in water within 2 or 3 months (Short *et al.*, 1989). Change of the composition of Bts in mussels

was not observed between 1992-1996, in spite of change of composition of Bts in water. As this factor, it may be the difference of bioconcentration of MBT, DBT and TBT in mussels. Further, ratio of TBT to total Bts was dominant. This is explained that ratio of TBT in plankton which is diet of mussels, is highest among Bts and TBT is absorbed easily, in order to by hydrophobic chemical substance.

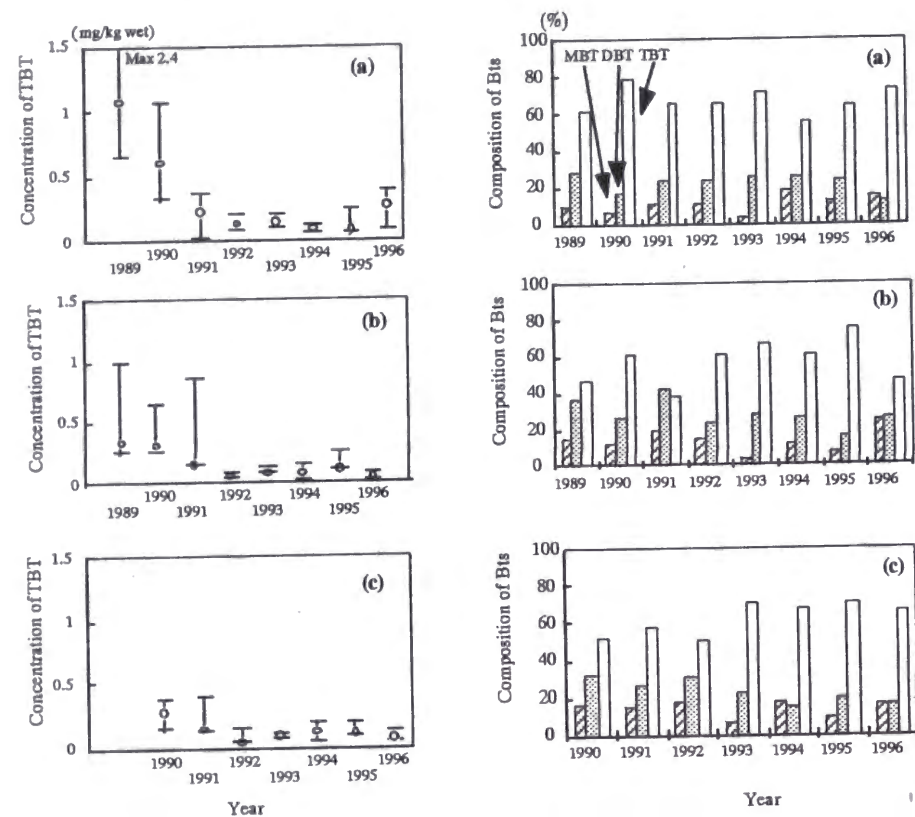


Fig.III-13 Representative changes of TBT concentrations and Bts compositions in mussels
a:station C2, b:station A1, c:station C7

The composition of Bts was calculated from the medians of Bts concentrations in each year.

TPT in mussels from stations C2 and A1 have been detected throughout the study period (Fig.III-14). The medians of TPT at stations C2 and A1 respectively were 1.2 and 1.5mg/kg wet in 1989 and fell to 0.005-0.282 mg/kg wet during 1990-1996. However, because the detection frequencies of TPT in mussels from station C7 were 14/30, the

temporal trend of TPT in mussel was not clear. TPT was scarcely detected in plankton which is diet of mussel. Nevertheless, ratio of TPT in mussel was dominant among Pts.

3-2 Comparison of temporal trend of organotin compounds in the other areas

There are many researches concerning the effects of Ots regulation. In 1989, the UK government introduced legislation to control the sale of paint containing TBT. Dowson *et al.* (1993a)

monitored the concentration of Bts in water and sediment from 22 sites in six estuarine systems in coastal area of North Sea, including the Rivers Alde, Deben, Urwell, Blackwater, Roach and Walton, Backwaters, U.K. between 1990 and 1992. Since 1991, concentration in water from has declined and most data were below detection limits ($>0.003\mu\text{g/l}$) in 1992. TBT concentration in sediment has generally decreased in the three years since legislation. Waites *et al.* (1991) undertook monitoring between 1986 and 1989 in water and sediment from Blackwater, Crouch, Southampton water, Beaulieu River, Teign, Dart, Kingsbridge and Dlymouth in coastal area of southern England. In 1989, the concentrations of TBT in water, oysters and mussels were generally only one-third to one-quarter of those observed in 1986. On the other hand, changes in concentrations of TBT in sediment showed no clear decreasing trend from 1986 to 1989. Valkirs *et al.* (1991) also

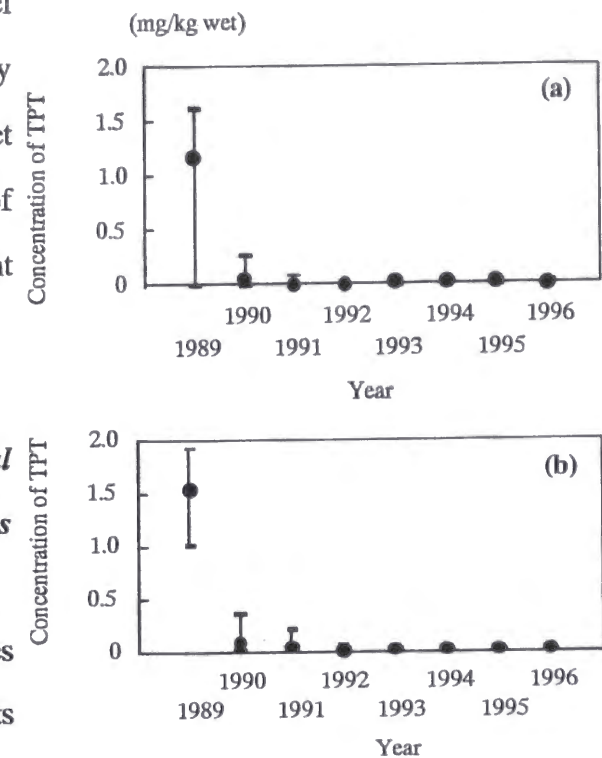


Fig.III-14 Representative figures from TPT levels in mussels a:station C2, b:station C5

evaluated the legislative restrictions on TBT antifouling paint use enacted in January 1988. Concentrations in three of four regions of surface water in San Diego Bay California were below the water quality criteria concentration (0.006 $\mu\text{g/l}$) adopted by the state of California for the protection of marine species in 1990. TBT concentrations in mussels generally decreased as those in water decreased. However, TBT concentrations in sediment from San Diego Bay did not reflect the decrease in water column and were variable among stations over time. Chau *et al.* (1997) surveyed TBT concentrations in surface water and sediment from 131 locations across Canada in 1993-1994 to assess the effectiveness of the 1989 regulation of antifouling use of TBT under the Canadian Pest Control Products Act. The main conclusion is that the 1989 regulation has only been partially effective. It has had some effect in the reduction of TBT concentrations in fresh water, but not in seawater, and it has had less effect in the reduction of TBT concentrations in sediment.

Our results concerning the temporal trend in the Port of Osaka agreed approximately with the other reports. Although TBT concentrations in water and mussels have been reduced, TBT has still been detected in water, sediment, plankton and mussels of the levels of 0.01 $\mu\text{g/l}$, 0.5mg/kg dry, 1.5mg/kg dry and 0.1mg/kg wet respectively, in spite of four years since regulation. This level may cause imposex and deformation in shellfish (Gibbs *et al.*, 1986, 1987). Meanwhile, TBT was detected at low levels in water, sediment, plankton and mussels. To our knowledge, this is the first documentation of the temporal trend of TBT for these 7 years. It is therefore impossible to compare variations of TBT levels in our study area with those in other areas.

3-3 Butyltin and phenyltin compounds in the harbour area of the Port of Osaka

Fig.III-15 shows the levels of TBT of 4 times measurement at the 8 stations of the Port of Osaka in 1996. The detection frequencies of TBT in water were 30/32 and the concentrations of TBT in water were in the range of 0.004-0.036 $\mu\text{g/l}$. TBT concentrations from stations C2 and C3 were higher than the other sites, and the variations of TBT concentration at these stations were greater. This considered that the leaching of TBT from vessels into water has continued at stations C2 and C3, which were situated in a marina and a mooring for small- and medium-hull vessels or TBT elutes from sediment to water column. The differences of TBT concentrations among other station were small in the range of 0.004 - 0.010 $\mu\text{g/l}$.

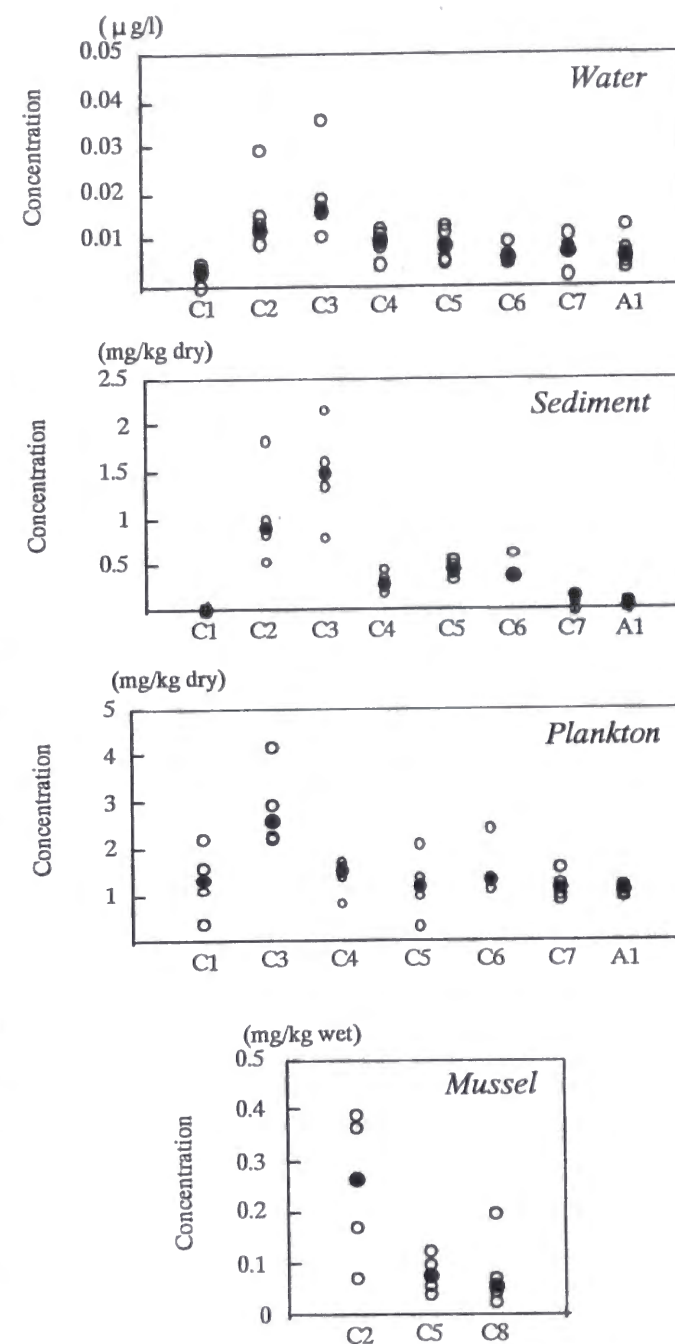


Fig.III-15 Horizontal distribution of TBT at stations C1-C7 and A1 in 1996 Black circles indicate medians

The concentration of TBT in sediment was also high at stations C2 and C3, followed in the order by stations C5, C6, C4, C7, C5 and A1. It was reported that half-lives of TBT in the sediment by microorganisms were in the range of 360-775 days (Dowson *et al.*, 1993b). Seligman *et al.* (1986a,b, 1989) and Maguire *et al.* (1985) also reported that the half-lives of TBT in water by microorganisms were 6-19 days and those of sediment were several months. These findings show that TBT in sediment is biodegraded more slowly than in water. It is therefore concluded that large amounts of TBT will be remained in sediment over a long period at stations C2 and C3.

Horizontal distribution of TBT in plankton from stations C1-C7 and A1 is shown in Fig. III-15. TBT concentration in plankton ranged from 0.324 to 4.17 mg/kg dry. TBT levels in plankton from station C3 is highest than other stations and the differences of TBT levels in stations except for station C3 were not observed. The horizontal distribution of TBT in plankton was agreed with that in water. Levels of TBT in mussels from station C2 were higher than from other stations.

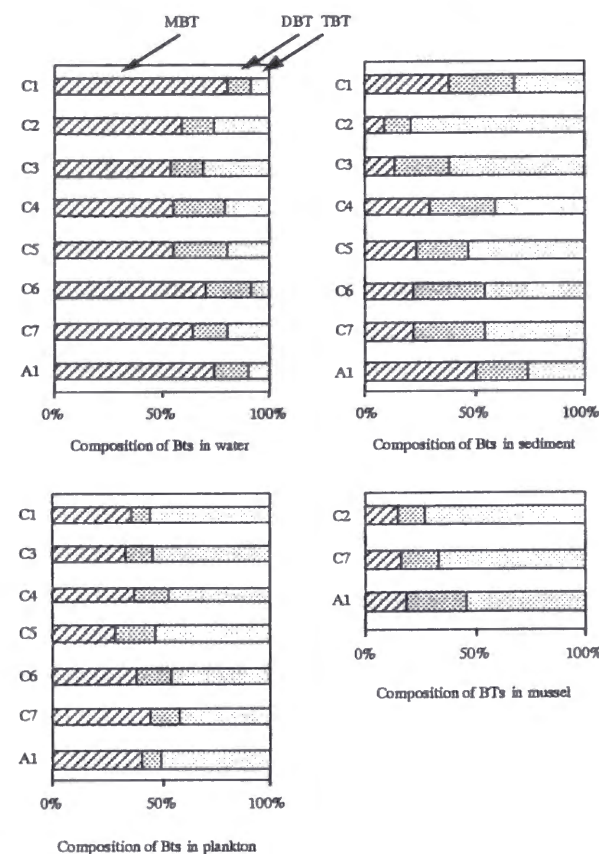


Fig.III-16 Composition of Bts in water, sediment, plankton and mussels

So, the levels of TBT were apparently higher in the marina and the mooring places of medium and small hull vessels.

The compositions of Bts in water, sediment, plankton and mussels from stations C1-C8 are shown in Fig.III-16. The compositions of Bts were calculated from the median of the concentration detected at each station. The ratio of MBT to total Bts was above 50% in

water from stations C1-C7 and A1, indicating that the degradation rate of TBT is greater than the input of TBT into water. In sediment from the Port of Osaka, except for stations C1 and A1, the ratio of TBT was dominant among Bts. This is due to its stability in sediment. The combined ratios of MBT and DBT which were degradative products of TBT, was about 75% in sediment from stations C1 and A1. As ignition loss (5-6%) of sediment from station C1 was lower than that of other stations, partition coefficient of TBT to sediment from station C1 in lower in comparison with other stations. The bottom of station A1 is dredged frequently, because this station is located in a route of ship. At these station, because of low concentration of TBT, it is considered that ratio of degradation products is high. The ratio of TBT was about 50% in plankton from stations C1-C7 and A1. The ratio of TBT to total Bt in mussel was occupied more than 50%. The composition of Bts in mussels were in descending order TBT, DBT and MBT. This indicates that TBT is more hydrophobic than DBT and MBT.

Table III-1 TPT concentration in sediment from the Port of Osaka

	C1	C2	C3	C4	C5	C6	C7	A1
March	ND	0.01	0.05	ND	0.04	0.02	0.01	ND
June	ND	ND	0.05	ND	0.03	0.02	0.01	ND
Sep.	ND	ND	0.02	ND	0.05	0.01	ND	ND
Nov.	ND	ND	0.01	ND	0.10	0.01	ND	ND

(mg/kg dry)

TPT was not detected in water from the Port of Osaka. TPT in sediment, plankton and mussel was detected in 1996 (Table III-1, III-2 and III-3). The median values for TPT in sediment, plankton and mussels were ND, 0.05 mg/kg dry and ND, respectively. The levels of TPT in sediment, plankton and mussels were low in comparison with those of TBT. The detection frequencies in sediment, plankton and mussels were 9/32, 17/28 and

6/12, respectively. Thus, both concentration and detection frequency of TPT were low in the Port of Osaka. The ratios of MPT and DPT among Bts in sediment showed higher than that of TPT, but the ratio of TPT in plankton and mussels was high.

Table III-2 TPT concentration in plankton from the Port of Osaka

	C1	C3	C4	C5	C6	C7	A1
March	ND	0.21	ND	0.03	0.07	ND	ND
June	ND	0.07	ND	0.01	ND	ND	ND
Sep.	ND	0.01	0.16	ND	0.09	0.07	ND
Nov.	0.22	0.13	ND	0.06	ND	ND	ND

(mg/kg dry)

Table III-3 TPT concentration in mussel from the Port of Osaka

	C2	C7	A1
March	0.01	0.01	ND
June	ND	0.01	ND
Sep.	ND	ND	ND
Nov.	ND	ND	ND

(mg/kg wet)

3-3 Partition of organotin compounds to sediment and aquatic organisms

K_{sw} , K_{pw} and K_{mw} were calculated by dividing the Bts concentration in the sediment (mg/kg dry), plankton (mg/kg dry) and mussels (mg/kg wet) by the concentration of water ($\mu\text{g/l}$) and the distribution of Bts between water and sediment was then assessed (Fig.III-17). The partition of Pts could not be calculated, because Pts were not detected in water. Mean of K_{sw} of TBT was 3.8×10^4 . Fent and Hunn (1991) calculated sediment-water distribution ratios at three sites in marina of lake Lucerne in Switzerland and these values were in the range from 4.0×10^2 to 1.72×10^3 . K_{sw} values were high in order of TBT, DBT and MBT. Organic substances in sediment from the port of Osaka are rich, because of 11-13 % of ignition loss. The hydrophobic property also increases in the order of TBT > DBT > MBT. The partition of Bts to sediment may therefore depend on the hydrophilic property. K_{pw} of Bts was higher than K_{sw} of Bts. It can be assumed that

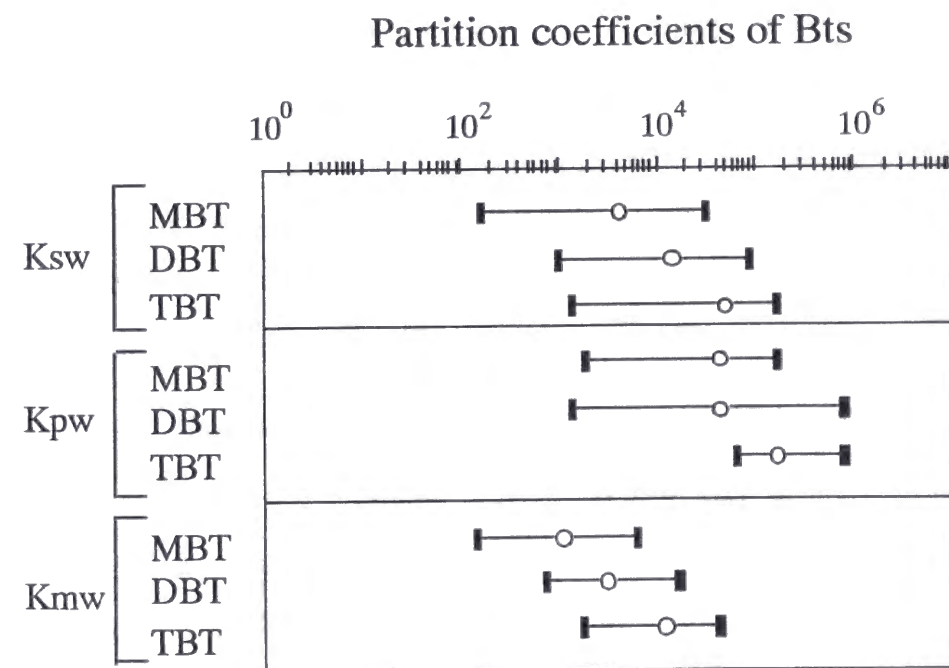


Fig.III-17 Partition coefficients of Bts in the Port of Osaka

Solid lines indicate ranges and open circles indicate medians

accumulation of Bts in plankton depends on adsorption on the plankton surface and uptake in plankton and the accumulation of Bts in sediment surface is depends only on adsorption. This explains why K_{sw} is lower than K_{pw} K_{mw} was also highest in the order of TBT, DBT and MBT.

4. SUMMARY

Between 1989-1996, Bts and Pts were monitored in water, sediment, plankton and mussels from the Port of Osaka. The concentration of TBT in water and mussels have decreased to levels of 0.01 μ g/l and 0.1mg/kg wet, respectively. In particular, a marked reduction of TBT levels was observed in water from a marina and a mooring place in a zone of poor flushing during the study period, and dominant compound to the total Bts changed from TBT to MBT. Although TBT concentration also decreased in mussels, the ratio of TBT to the total Bts remained high during 1989 - 1996. On the whole, the temporal trend of TBT in sediment and plankton was estimated to be uniform in the period of investigation. On the other hand, a low level of TPT was detected in water between 1989 and 1991. After that, TPT levels decreased and have not been detected recently. Detection frequencies of TPT in sediment, plankton and mussels were very low.

Bts and Pts were measured in the harbour area of the Port of Osaka in 1996. The levels of TBT in water, sediment, plankton and mussels were in the range of 0.004-0.036 μ g/l, 0.01-2.1 mg/kg dry, 0.32-4.2 mg/kg dry and 0.02-0.39 mg/kg wet, respectively. The highest concentration of TBT was observed at stations C2 and C3, which were located respectively in a marina and a mooring place of small- and medium-hull vessels. The dominant compounds among total Bts were MBT in water and TBT in sediment, plankton and mussels. TPT was not detected in water. TPT levels in sediment, plankton and mussels were very low. The dominant compounds among total Pts in sediment and aquatic organisms were MPT and DPT, which were degradative metabolites of TPT. The partitions of Bts to sediment, plankton and mussels were studied. TBT was accumulated

in sediment and aquatic organisms at much higher levels than DBT or MBT.

SECTION III

CURRENT STATUS OF SANRIKU COASTAL AREA AND COMPARISON OF ORGANOTIN COMPOUNDS LEVELS BETWEEN THE PORT OF OSAKA AND OTSUCHI BAY

1. INTRODUCTION

Sanriku coastal area has many fishing ports and scallop farms and fishery activity such as fix shore net fishing, gill net and dragnet are conducted in this bay. Many private fishing boats are cruised and moored in this area. Thus, there are many point which can be postulate as source of Ots in this area. It is important to evaluate the present status of contamination for water, sediment, plankton and mussel by Ots in Otsuchi Bay located in the Sanriku coastal area.

In general, the port are classified into two types by the different of utilization (trading port and fishing port). The port of Osaka as describe section II, is a trading port. The level of organic pollution in such port is high, because a trading port is situated in the industrial area. Additionally, Japanese registered and foreigner registered vessels cruise and are moored in this area. The levels of Ots in Osaka Bay are compared to those in Otsuchi Bay of fishing port and it is found what the extend the activity in the port reflect the levels of Ots.

2. MATERIAL AND METHOD

2-1 Sample description

Figs.III-17 and 18 show sampling sites. Otsuchi Bay is located on the Pacific coast of northern Japan. It has a fishing port and several scallop farms and Otsuchi River flows to Otsuchi Bay. In order to compare the Ots levels in Otsuchi Bay with adjacent bays, Miyako, Yamada and Kamaishi Bay were also selected for this study. Miyako Bay and Yamada Bay are situated in the northern site of Otsuchi Bay and Kamaishi Bay is in the southern part of

Otsuchi Bay. These bays are also fishing port. Water, sediment, plankton, mussels (*Mytilus edulis*), scallops (*Pecten caurinus*) and fish (*Physiculus maximowiczi*) were collected from Otsuchi Bay in July, 1996. The mussels from Otsuchi, Yamada, Kamaishi and Miyako Bay were also collected in August, 1995.

Water, sediment and plankton samples were collected from stations D1-D7 in Fig.III-18. Seawater and sediment samples were obtained by using a bucket and a Smith-McIntyre grab sampler, respectively. Ignition loss of sediment ranged from 6.2

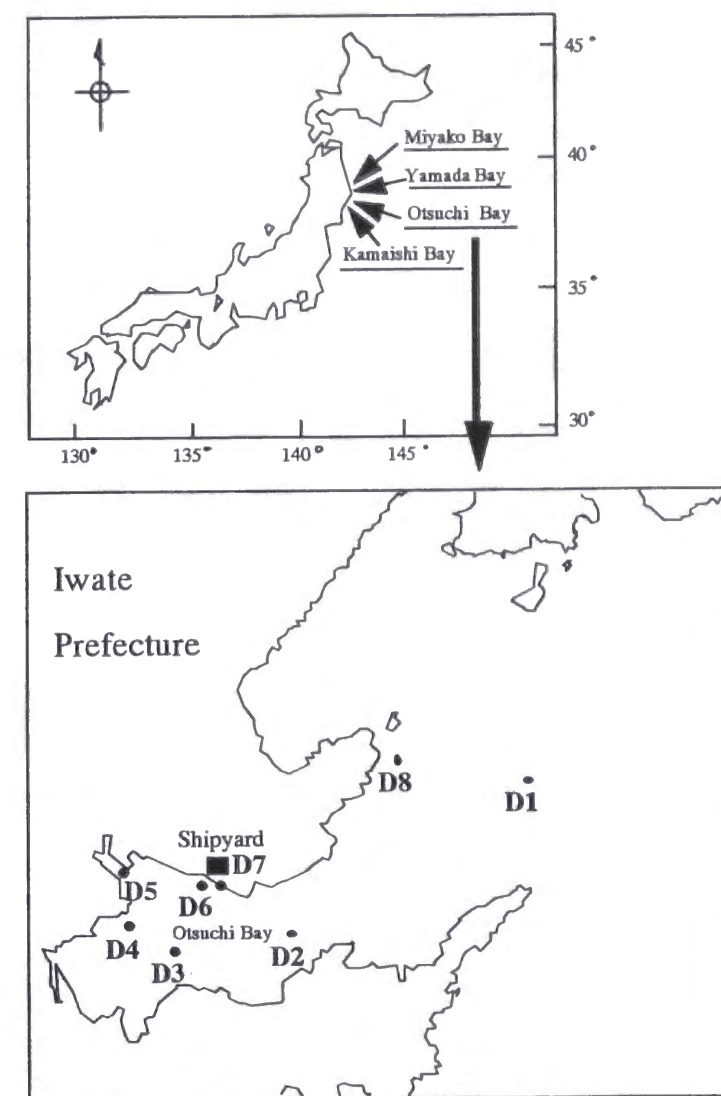


Fig.III-18 Location of Otsuchi Bay and sampling positions of water, sediment and plankton

to 13.5%. Plankton samples were taken by a plankton net, (25 μm in pore size, horizontal haul). Mussel samples which were attached at the median of intertidal zone, were taken from the wharf at stations E1-E8 in Fig.III-19. Shell length of these mussel from Otsuchi Bay in 1996 ranged from 5.3 cm to 9.2 cm and shell length of mussel from Otsuchi Bay, Yamada Bay, Miyako Bay and Kamaishi Bay in 1995 ranged from 2.9 cm to 6.5 cm, from 3.5 cm to 5.0 cm, from 2.5 cm to 5.1 cm and from 3.0 cm to 5.9 cm, respectively. Scallop samples were

taken from a farm near stations E2 and E5 in Fig.III-18. Shell length of scallops in the range of 8.3 cm - 12 cm. Fish were collected from stations D4 and D8 (Fig.III-17). Total length of fish ranged from 14 cm to 22 cm and body weight ranged from 24-101g. Five grams of the soft parts of mussels which mixed 5 individuals scallops, and muscle tissue in

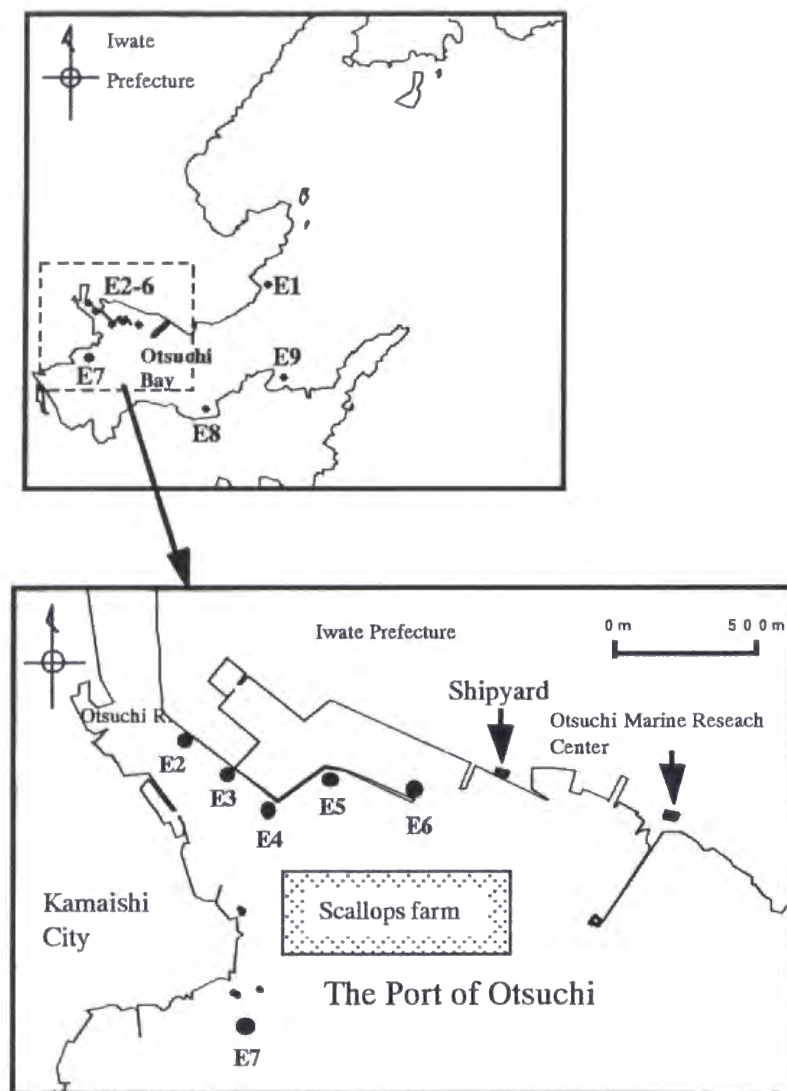


Fig.III-19 Sampling positions of mussels in Otsuchi Bay

fish were analyzed. Water samples were maintained 5 °C without filtration and other samples stored at -20 °C until chemical analysis was carried out.

2-2 Analytical Method

Analytical method was conducted as described in chapter II.

3. RESULTS AND DISCUSSION

3-1 Horizontal distribution of organotin compounds in water, sediment and plankton from Otsuchi Bay

Fig.III-20 shows the horizontal distribution of Bts in water, sediment. TBT concentration in water ranged from 0.021 to 0.074 ng/l and plankton in Otsuchi Bay.

The highest concentration of TBT in water was detected at station D6 near the shipyard and the ratio of TBT to Bts was 61%. The ratio of TBT among Bts was low at the station D2 which TBT concentration was low (27%). TBT concentration in sediment ranged from 0.007 to 0.640 mg/kg dry. The concentrations of TBT in sediment were also high at stations D6-D7 near the shipyard and TBT

was dominant among Bts. The ratio of TBT among Bts was low at the station which TBT concentration was low.

TBT concentration in plankton ranged from 0.238 to 9.8 mg/kg dry. TBT in plankton showed similar distribution to that in water and

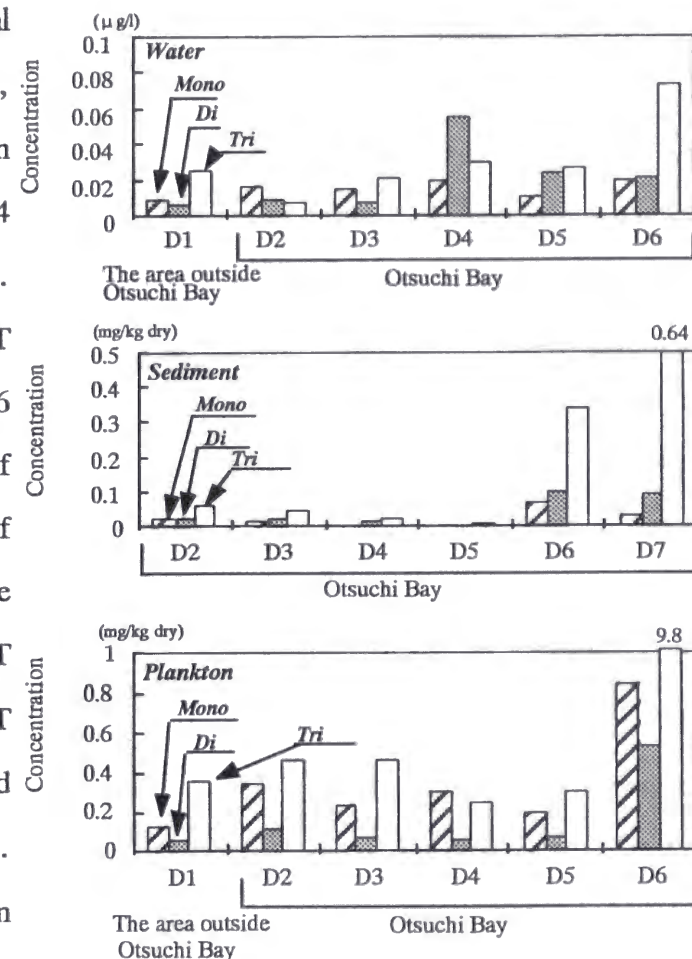


Fig.III-20 Horizontal distribution of Bts in water, sediment and plankton from Otsuchi Bay

sediment, and the ratio of TBT to the total Bts was also high at station D6. The ratios of TBT among Bts in plankton were higher than those in water at each stations. It is concluded that shipyard is the point-source of TBT. The levels of TBT in water and plankton were uniform at stations D1-D5. The distributions of TBT in sediment from stations D2-D5 were the same as those in water and plankton. These results show that the input of TBT to water environments appear to be responsibility of not only shipyard but also fishing boats. The high ratio of TBT to the total Bts in sediment and plankton at most of stations were observed.

Pts were detected only in water from station D6 and the concentrations of MPT and TPT were 0.006 and 0.009 $\mu\text{g/l}$, respectively.

DPT was not detected at station D6. Pts in sediment and plankton at each station are shown in Fig.III-21. TPT concentration in sediment ranged from ND

to 0.039 mg/kg dry and those in plankton ranged from 0.019 to 0.670

mg/kg dry. The same trend in the distribution for TPT of TBT was noticed in sediment and plankton. Namely, the concentrations of TPT at stations D6-D7 were higher than those of other sites and TPT was dominant in the Pts. These results indicate that TPT as well as TBT seem to originate from the shipyard. Though it can be assumed that there are fishing equipment which TPT was only used at station D2, the reason for higher TPT in sediment

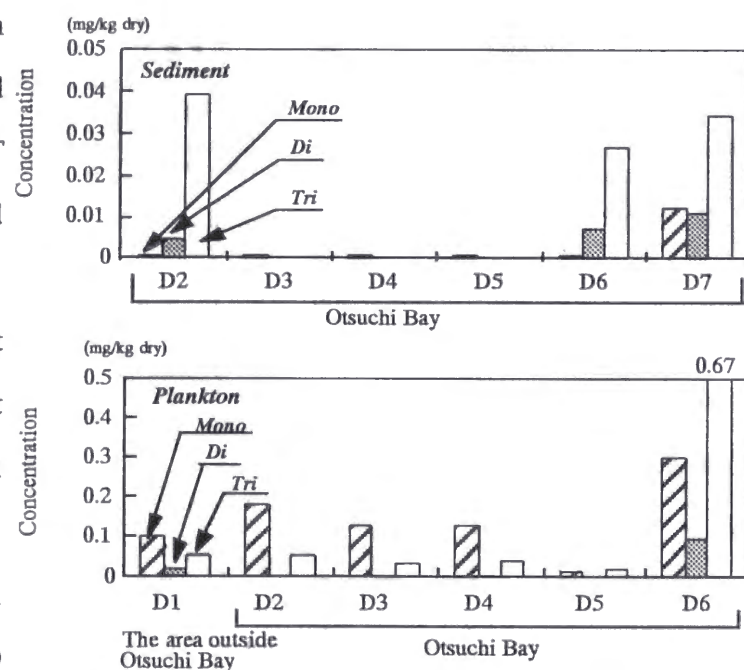


Fig.III-21 Horizontal distribution of Pts in sediment and plankton from Otsuchi Bay

from this is not clear. The ratio of MPT in Pts was high in plankton from stations D1-D5. It is assumed that the degradation rate of TPT is superior to the input rate of that, as the application of TPT is now decreasing.

3-2 Horizontal and vertical distribution of organotin compounds in mussel samples

The concentration of Bts and Pts in mussels are shown in Fig.III-22. Mussels at stations E2-E6 were collected along the break-water. TBT concentration in mussels ranged from 0.044 to 0.182 mg/kg wet. Page and Widdows (1991) reported that the threshold value of the effects on scope for growth of mussels was 2 mg/kg dry. TBT in mussels from these areas were lower than this threshold value. The concentration of TBT at station E6, near the

shipyard, was highest among the stations. The concentrations of TBT decreased from high to low levels with an increase in distance from the shipyard and the ratio of TBT in the total Bts also decreased from 68 to 52%. TPT concentration in mussels ranged from ND to 0.043 mg/kg dry. The levels of TPT at station E6 as well as TBT were

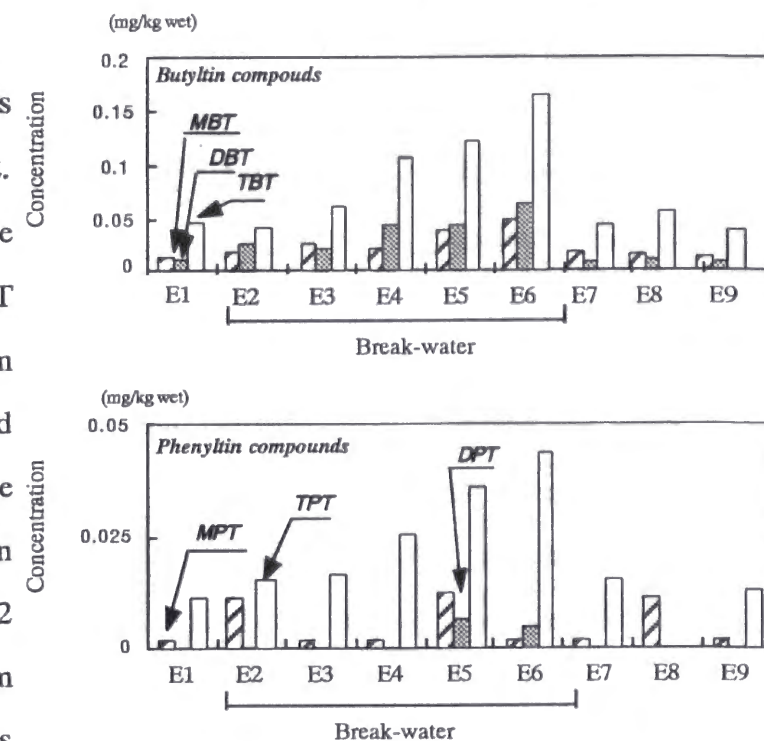


Fig. III-22 Horizontal distribution of Bts and Pts in mussels from Otsuchi Bay

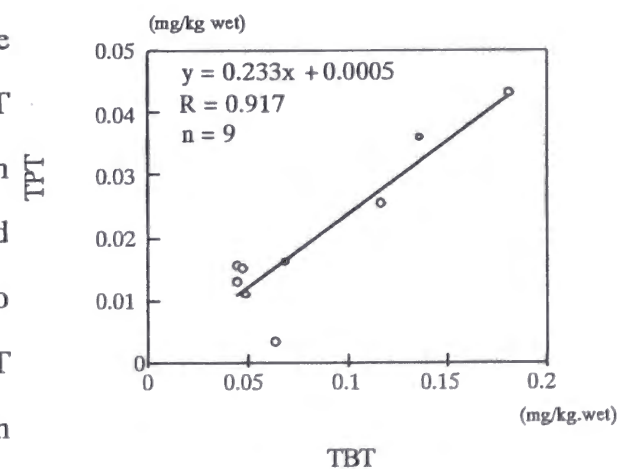


Fig.III-23 Correlation between TBT and TPT in mussels from Otsuchi Bay

also the highest among all stations and decreased with an increase in distance from the shipyard. These indicate that the source of contamination of TBT and TPT is probably discharged from the shipyard. TBT and TPT were dominant in Bts and Pts, respectively in mussel at most of the stations.

Correlation between the concentrations of TBT and TPT was calculated in mussels (Fig.III-23). Positive correlation ($R=0.917$) was found between TBT and TPT. This result shows that antifouling paint containing both TBT and TPT have been used in the shipyard and are diluted in seawater.

Bts and Pts in mussel from the different zone of intertidal zone were studied (Fig.III-24). Mussels were collected from three parts of the zone, namely below the water level, 40-60cm and 80-100cm above from low tide. The median shell length of mussels from the zone under the water level, 40-60cm and 80-100cm above water level were 43, 34 and 28 mm, respectively. The concentrations of TBT and TPT in mussels from 80-100cm above were the highest in those in intertidal zone and were decreasing toward the water level. Stab *et al.* (1996) reported that TBT concentrations decreased with the increasing of size of zebra

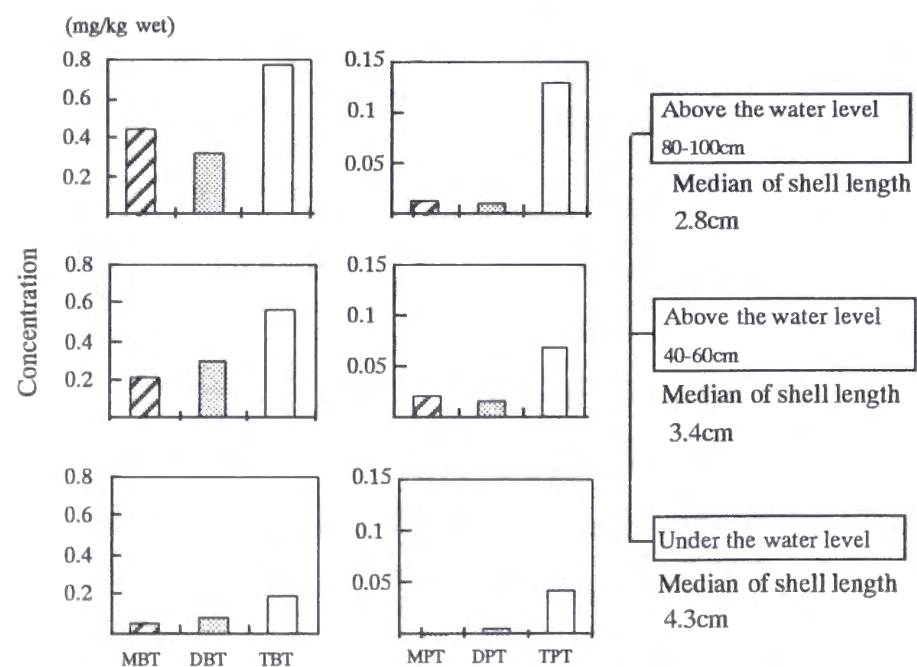


Fig.III-24 Vertical distribution of Ots in mussels from intertidal zone

mussels. It is not clear which Ots concentrations in mussel attribute to the attached zone or size of mussel. Furthermore, these results show that the zone which mussels were taken and size of mussel are important, when mussel is used as a bioindicator.

3-3 The levels of TBT and TPT in Otsuchi Bay and its adjacent bay

TBT was detected in the range of 0.008 - 0.074 $\mu\text{g/l}$ from the water. Laboratory studies showed that concentrations as low as 0.02 $\mu\text{g/l}$ would produce imposex for dock whelk, *Nucella lapillus* (Bryan *et al.*, 1986). Most of the TBT from Otsuchi Bay exceed the levels by which caused imposex. The concentrations of TBT (0.01-0.64 mg/kg dry) in sediment were about thousand times that of water. Yonezawa *et al.* (1993) reported that TBT ranged from ND to 0.27mg/kg dry in surface sediment from Ise Bay, Japan in 1987.

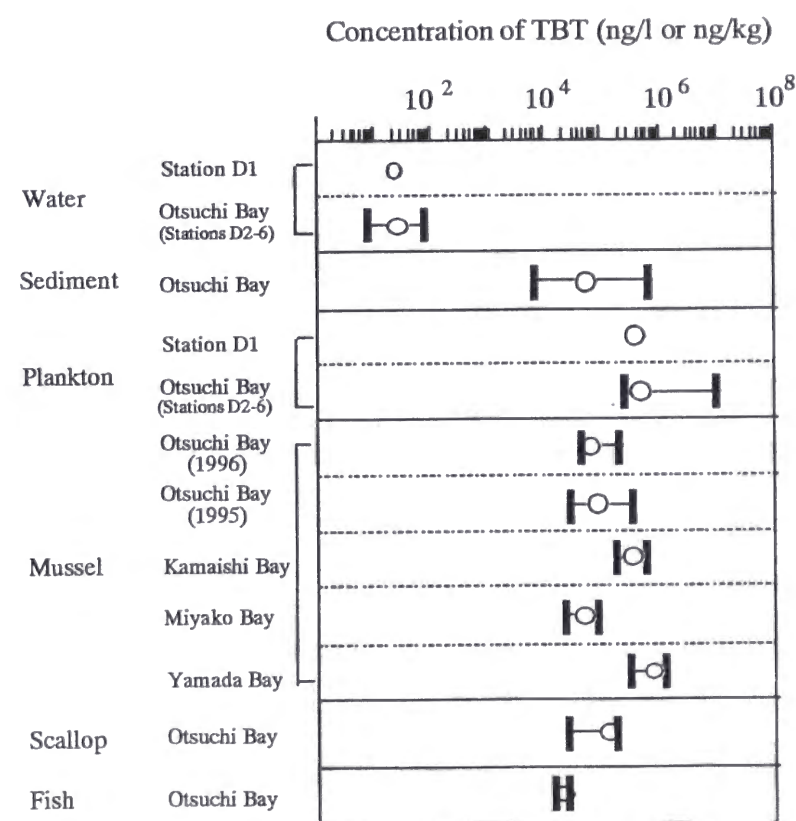


Fig.III-25 Levels of TBT in water, sediment, plankton, mussels, scallops and fish in Otsuchi Bay
Solid lines indicate ranges and open circles indicate medians.

TBT was detected in the range of 0.008-0.027 mg/kg dry in Boston Harbour sediment from England (Wuertz *et al.*, 1991). TBT in sediment of Otsuchi Bay in 1996 is considered to be of higher concentrations in comparison with that of the sediment of the above areas. TBT (0.24-9.8 mg/kg dry) in plankton was higher than that of sediment. Mussels were collected from Otsuchi Bay in 1995 and 1996. The level of TBT in 1996 was the same as those in 1995, and the median of TBT concentration in 1995 and 1996 from Otsuchi Bay were 0.074 and 0.063 mg/kg wet respectively. Mussels were also taken from Kamaishi, Miyako and Yamada Bay which were located in adjacent bay of Otsuchi Bay. TBT concentration in mussels from each Bay showed a large regional difference. The levels of TBT in mussels from Kamaishi, Miyako and Yamada Bay ranged from 0.177 to 0.574 mg/kg wet, from 0.023 to 0.080 mg/kg wet and from 0.306 to 1.10 mg/kg wet, respectively. The levels of TBT from Otsuchi Bay were lower than those from Kamaishi and Yamada Bay, and were higher than those from Miyako Bay. TBT in scallops were detected in the range of 0.10-0.13 mg/kg wet and were the same level as mussels. Though TBT was also detected in fish (0.01-0.02 mg/kg wet), the levels were the lowest among all the aquatic organisms in this study. The median and range of TBT concentration in water, sediment, plankton, mussels, scallops and fish from Otsuchi Bay, and mussels from adjacent bay are summarized in Fig.III-25.

TPT of 0.009 µg/l was only detected in the water from station D6. TPT was detected in the range of 0.03-0.04 mg/kg dry in half of the total sediment samples. TPT in plankton from Otsuchi Bay ranged from 0.02 to 0.67 mg/kg dry and TPT from Otsuchi Bay was the same level as that of the outer part of Otsuchi Bay. TPT in mussels was detected in the range of ND - 0.04 mg/kg dry and 0.01-0.05 mg/kg wet in 1996 and 1995, respectively. The level of TPT in mussels did not change between 1995 and 1996. In comparison to the concentration of TPT in mussels from Otsuchi Bay, those from Miyako Bay were lower,

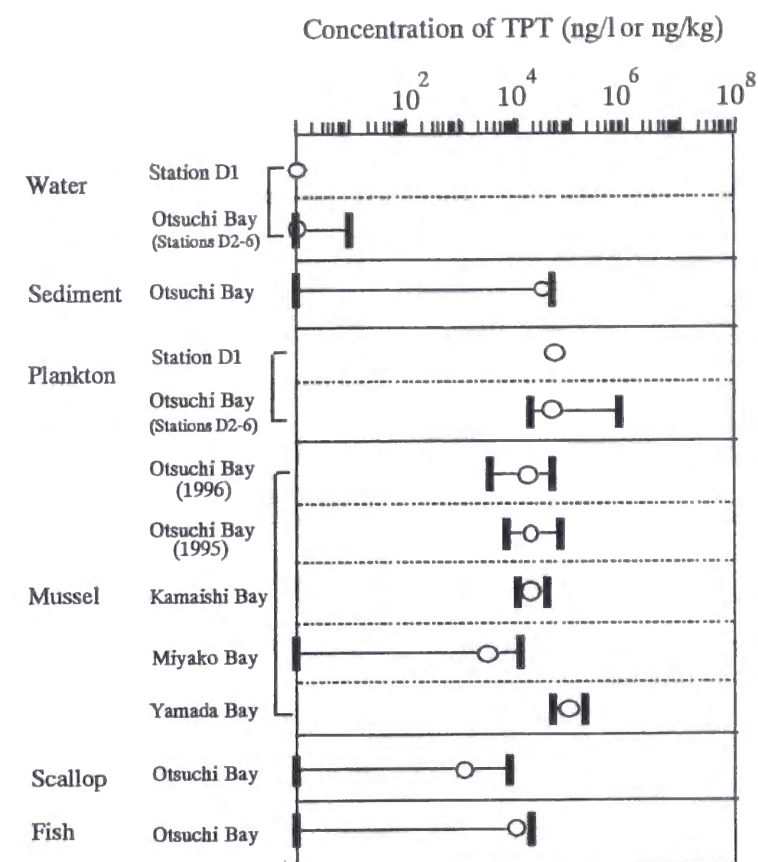


Fig.III-26 Levels of TPT in water, sediment, plankton, scallops and fish
Solid lines indicate ranges and open circles indicate medians.

however those from Kamaishi and Yamada Bay were higher. Environmental Agency Japan (1996) report that TPT was not detected in at 5 sites (Miura Peninsula, Noto Peninsula, Shimane Peninsula, Ise Bay and Naruto) from Japan in 1994. TPT concentrations in mussels from Otsuchi, Kamaishi, Miyako and Yamada Bay are higher than other areas from Japan.

TPT in scallops were ND and 0.01 mg/kg wet. TPT concentrations in scallops were lower than those of mussels. TPT in fish ranged from ND to 0.02 mg/kg wet. Thus, the levels of TPT were low in all mediums from Otsuchi Bay. Fig.III-26 shows the levels of TPT in Otsuchi Bay and the adjacent bay.

3-4 Comparison of organotin compounds concentrations between the Port of Osaka and Otsuchi Bay

Levels of TBT in water, sediment, plankton, and mussel from the Port of Osaka and Otsuchi Bay in 1996 are shown in Fig. III-27. TBT levels in water from the Port of Osaka were lower level than those in water from Otsuchi Bay. As to horizontal distribution, the difference of TBT concentration among stations was small in water from the Port of Osaka. In contrast, TBT at the station near the shipyard in Otsuchi Bay was three times higher than that at stations in distance of 2 km from shipyard. The concentration of TBT in Otsuchi Bay thus showed highly localization. This trend shows that the source of TBT in the Port of Osaka is cruising vessels while TBT from the shipyard is significant in Otsuchi Bay. Fig. III-28 shows the composition of Bts calculated from median of the concentration in each area. The ratio of MBT was high in the Port of Osaka. On the other hand, TBT was dominant in water from Otsuchi Bay. In Otsuchi Bay, input of TBT exceeded the degradation of TBT in comparison with the Port of Osaka.

TBT concentration in sediment from the Port of Osaka was higher than in sediment from

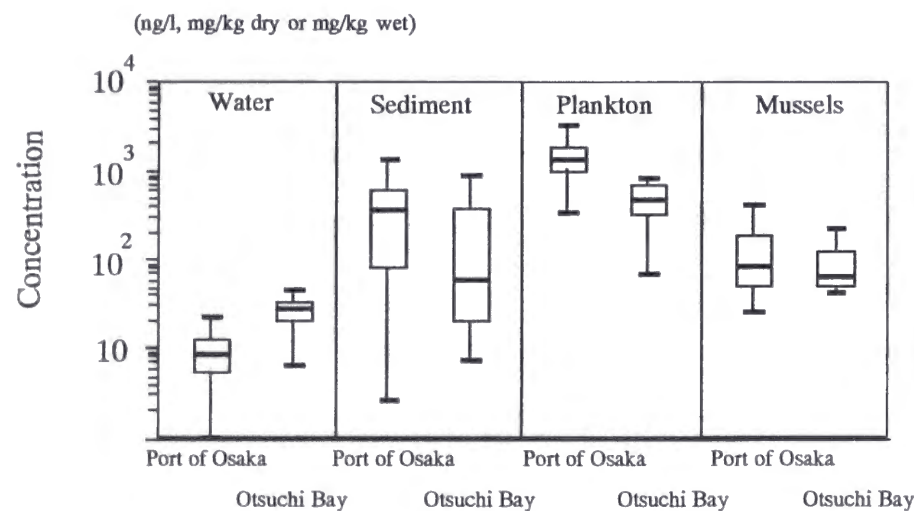


Fig.III-27 Box- and whisker displays of TBT concentrations between the Port of Osaka and Otsuchi Bay in 1995

Medians with quartiles in boxes, 90% confidence limits as bars.

Otsuchi Bay. Figures of MBT, DBT and TBT as a proportion of total Bts in sediment were the same in the Port of Osaka and Otsuchi Bay at about 20, 23 and 57%, respectively. Maguire *et al.* (1985) reports that the half-lives of TBT in sediment is several months. TBT in sediment is thus found to be stable, and represents the history of TBT contamination. It can therefore be concluded that contamination with of TBT has continued for a long time in these sea areas. Although composition of Bts in the Port of Osaka was different from those in Otsuchi Bay, each ratio of Bts in the Port of Osaka was the same as that in Otsuchi Bay. This reason is to be due to K_{sw} of each Bts.

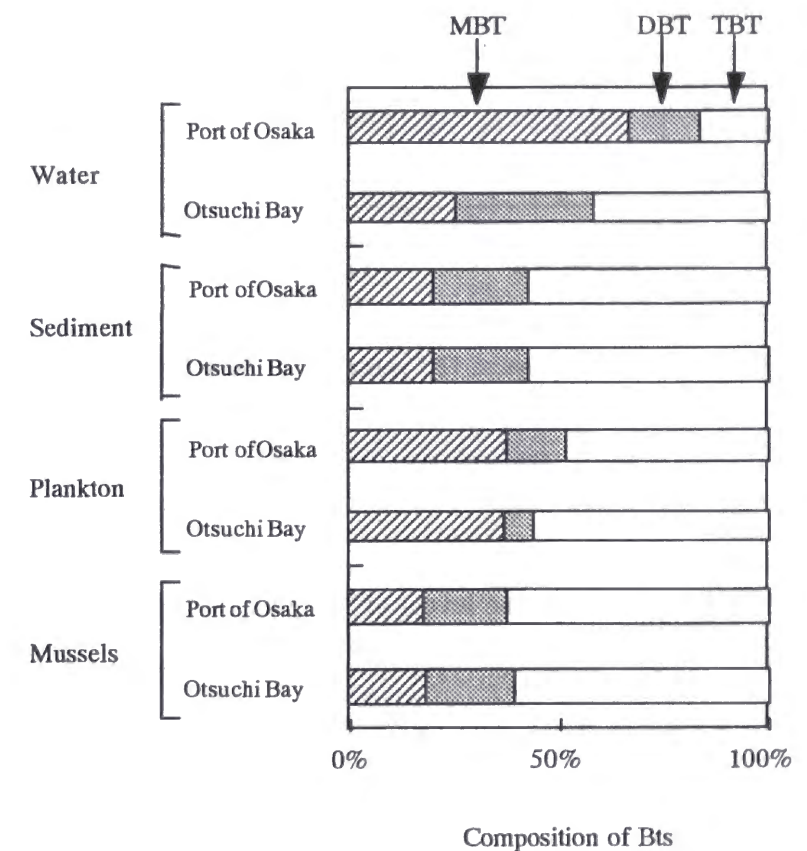


Fig.III-28 Composition of Bts in the Port of Osaka and Otsuchi Bay

TBT in plankton from the Port of Osaka was high level in comparison with that from Otsuchi Bay similarly to that in sediment. Source of TBT in Otsuchi Bay were shipyard. Therefore, although higher concentration of TBT was near the shipyard was observed. TBT concentration was low with increasing of distance of shipyard. Therefore, TBT

concentrations at every sites from Otsuchi Bay were localized. In the Port of Osaka, source of TBT is cruising vessels. Accordingly, high concentrations of TBT were observed uniformly. This trend was remarkable in sediment and plankton. It is considered that TBT levels in the Port of Osaka is higher than those in Otsuchi Bay.

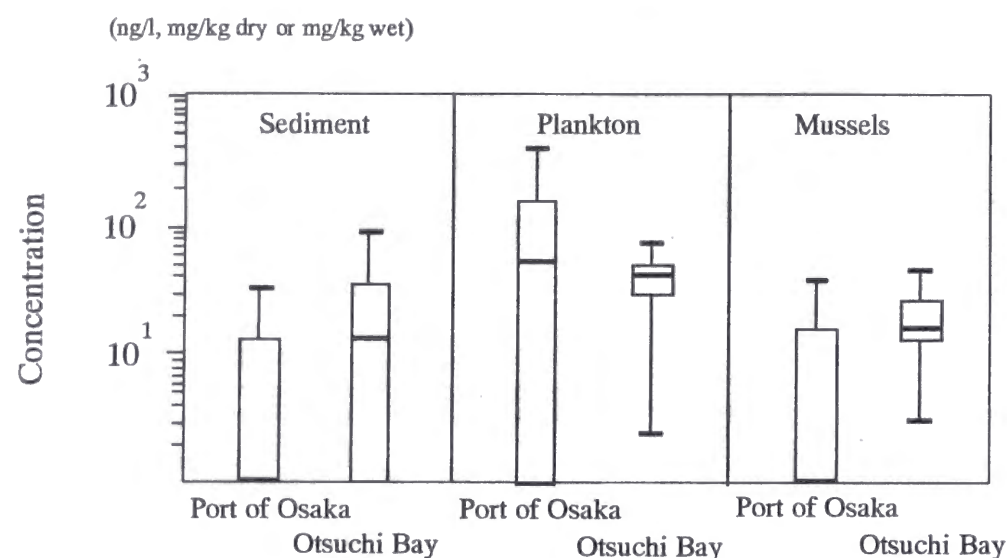


Fig. III-29 Box- and whisker displays of TPT concentration between the Port of Osaka and Otsuchi Bay
Means with quartiles in boxes, 90% confidence limits as bars.

Short *et al.* (1989) reported that the levels of TBT in mussels began to reflect those in waters within 2 or 3 months. However, TBT levels in water from the Port of Osaka were lower than those from Otsuchi Bay, while TBT in mussels were the same levels. As mussel samples in the Port of Osaka and Otsuchi Bay were taken from median of intertidal zone, the effect for TBT concentration by zone which mussels were taken, is not necessary to be considered. Stab *et al.* (1996) reported that differences of TBT concentration in mussels depended on shell length. Namely, TBT concentrations in small size was higher than those in large size mussel. The shell length of mussels from the Port of Osaka and Otsuchi Bay were in the range of 9-37 mm and 31-41 mm, respectively. This difference of shell length

may thus be the reason that TBT concentration in water is not reflected in mussels. The ratio of TBT in total Bts was high in both the Port of Osaka and Otsuchi Bay.

TPT was not detected in water from the Port of Osaka or Otsuchi Bay. TPT levels were therefore compared between sediment, plankton and mussels (Fig. III-29). TPT levels in sediment and mussels from Otsuchi Bay were higher than in those from the Port of Osaka. Although as a biocide, TPT is of shorter effect than TBT, it is cheaper and can therefore be bought individually. It is assumed that TPT used much more in Otsuchi Bay than in the Port of Osaka, because of the many fishing boats in Otsuchi Bay, most of which are private. TPT levels in plankton from the Port of Osaka were the same as in plankton from Otsuchi Bay. Recently, it is considered that TPT is not used on vessels. However, as many of vessels were cruised in the Port of Osaka, some of foreign flag vessels considered to use paint containing TPT. TPT is loaded to water from these vessels. Therefore, range of TPT concentration is wide in the Port of Osaka. Median of TPT in the Port of Osaka was agreed with that in Otsuchi Bay, because maximum in the Port of Osaka is considerably higher than that in Otsuchi Bay.

K_{sw} , K_{pw} and K_{mw} were compared with those in Otsuchi Bay (Fig. III-30). K_{sw} , K_{pw} and K_{mw} were calculated by dividing the concentration in sediment, plankton and mussel by that in water. K_{sw} calculated from the Port of Osaka were higher than from Otsuchi Bay. Bts in sediment represents the history of Bts contamination. This shows that the Port of Osaka was contaminated more heavily with Bts in past years than Otsuchi Bay, due to the many factories such as ironworks, a power station, incineration plant and coastal land reclamation in the area. K_{pw} and K_{mw} in the Port of Osaka were also higher than in Otsuchi Bay. K_{mw} in the Port of Osaka was also higher than in Otsuchi Bay. The size of analyzed mussels in the Port of Osaka was smaller than in Otsuchi Bay, which may account for this result.

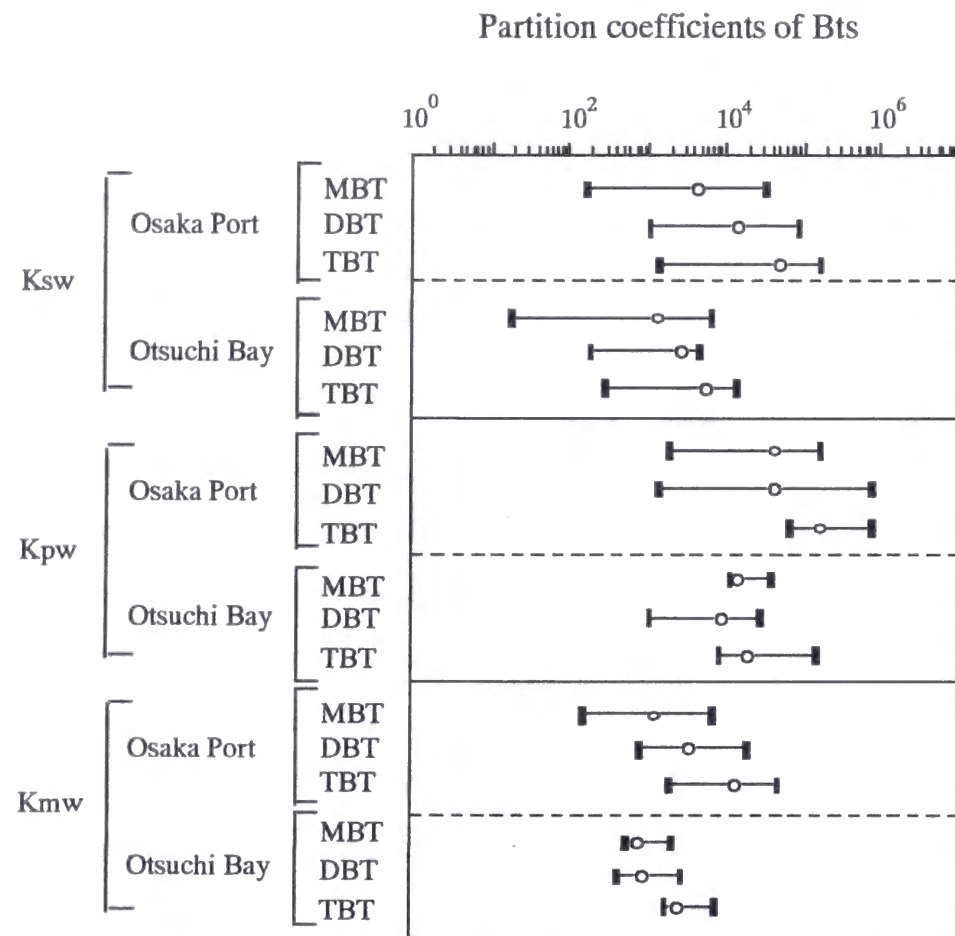


Fig. III-30 Accumulation in sediment, plankton and mussels in the Port of Osaka and Otsuchi Bay
Solid lines and circles indicate ranges and medians, respectively.

4. SUMMARY

The distribution of Bts and Pts were investigated in Otsuchi Bay. The highest concentration of TBT and TPT were detected near the shipyard and the ratio of triorganotin compounds to the total organotins were high in water, sediment and aquatic organisms. Positive correlation was found between the concentration of TBT and TPT in mussels. Vertical distribution in mussels from the intertidal zone was also studied. The concentrations of TBT and TPT in mussels decreased toward the water level.

Comparison of the level of Ots in water, sediment, plankton, mussels, scallops and fish from coastal area in Otsuchi Bay were made. TBT concentrations in sediment were about a thousand times more than those of water and TBT levels in plankton were higher than those

in sediment. TBT in mussels were the same levels as those in scallops and TBT levels in fish were the lowest among all aquatic organisms. TPT was only detected in water from station D6, and detection frequency and level of TPT in the other samples were lower than those of TBT.

Bts and Pts in water, sediment, plankton and mussels from the Port of Osaka were compared with those from Otsuchi Bay. The concentrations of TBT in water from the Port of Osaka were lower than in water from Otsuchi Bay, however TBT in sediment, plankton and mussels from the Port of Osaka were higher than in sediment from Otsuchi Bay. TPT was not found in water from either location. TBT in the Port of Osaka was accumulated in sediment and aquatic organisms at much higher than levels in Otsuchi Bay. On the other hand, levels of TPT were higher in Otsuchi Bay than in the Port of Osaka.

SECTION IV CONCLUSION

In chapter III, the distribution of Ots in Osaka bay, the Port of Osaka and Sanriku coastal area were discussed. The relative higher concentration of TBT was observed in water and sediment from Osaka Bay and particularly TBT in estuary showed high level. Therefore, the current status of Ots in the port of Osaka which were located in estuary was investigated. In the Port of Osaka, the highest concentration of TBT was observed at stations which located at marina and the mooring places of small and medium hull vessels. These findings indicates that the source of Ots is mainly vessels. The dominant compounds among total Bts were MBT in water and TBT in sediment, plankton and mussels. The level of TPT was low in these samples.

The temporal trend in the Port of Osaka was discussed during 1989-1996. TBT concentrations in water and mussels have decreased, however, TBT have been still detected at the concentration which poses an ecotoxicological threat. The levels of TBT in sediment were a thousand times higher than those in water. The levels of TBT in sediment and plankton were uniformed during the study periods.

Ots in Otsuchi Bay were investigated. TBT concentration in sediment was about a thousand times more than that of water and TBT levels in mussels were the same levels as those in scallops and TBT in fish were the lowest among all aquatic organisms.

The levels of Ots from the Port of Osaka were compared to those from the Otsuchi Bay. The concentration of TBT in water from the Port of Osaka were lower than those from Otsuchi Bay, however TBT in sediment, plankton, mussels from the Port of Osaka were higher than those from Otsuchi Bay. Thus it found that the pattern of Ots was various by the difference of the utilization of the port and that the Port of Osaka was contaminated more heavily with Bts in past years than Otsuchi Bay, due to the many factories such as ironworks, a power station, incineration plant and coastal land reclamation in this area.

CHAPTER IV BIODEGRADATION OF ORGANOTIN COMPOUNDS BY MICROORGANISMS

SECTION I TOLERANCE OF BACTERIA IN WATER FOR ORGANOTIN COMPOUNDS

1. INTRODUCTION

Ots toxicity data for 4 phytoplankton species, 5 zooplankton species, 19 species microinvertebrates and 11 fish species are reviewed (Lenwood and Pinkney, 1986). Zooplankton are the most sensitive groups among tested aquatic organisms. However, it is little known about the susceptibility of aquatic bacteria. It is known that the bacteria in rivers and estuary degraded TBT (Hattori *et al.*, 1988, Dowson *et al.*, 1993b). In order to study the degradation of Ots by microorganisms, it is essential to find what extent microorganisms in aquatic environment can be tolerant for Ots. TBT concentrations were in the range of ND-0.064 $\mu\text{g/l}$ in water from the lake Biwa and Yodo river basin in 1990 (Chapter III, Section I). Thus, contaminations of TBT were widely not only in sea area but also in rivers. In this contaminated area by TBT, it is of concern about the effect of Ots for microorganisms.

In this section, susceptibility of bacterial populations for Bts and Pts has been described and was compared to that of some type strains of bacteria.

2. MATERIAL AND METHOD

2-1 Sampling description

Fig. IV-1 shows the sampling area. Yodo River originates from the Lake Biwa and flows into the Port of Osaka. Stations F1-F2 are situated in Lake Biwa and stations F3-F4 are Yodo River basin. Water samples collected with bucket at stations F1-F4. Water samples were in May 1991 were used for tolerance test of bacteria within one day.

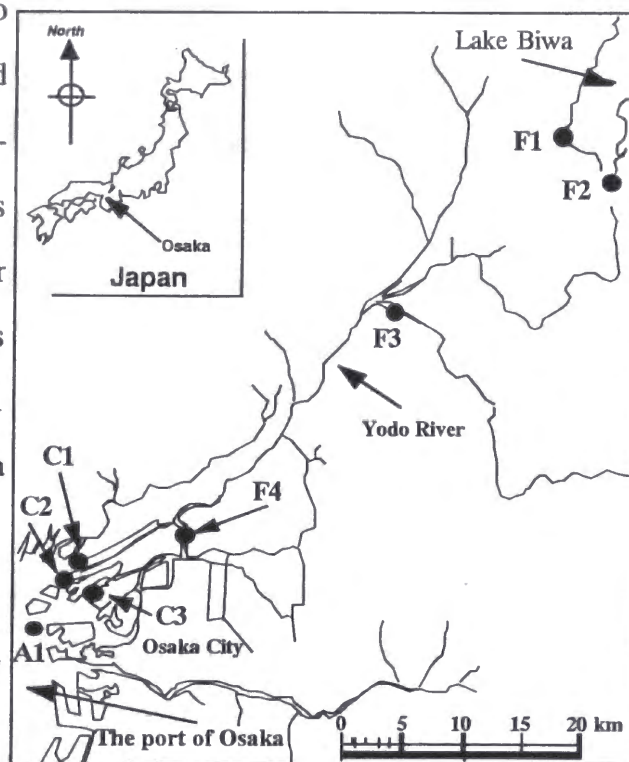


Fig.IV-1 Study area

2-2 Tolerance test of bacterial populations to organotin compounds

Bts and Pts were dissolved in DMSO and 1000 or 10000 mg/l of stock Ots solution was prepared. By adding 0.5 ml of these solution to 500 ml of water sample, concentration of each Ots in water was adjusted to 1 or 10 mg/l. Samples were incubated with shaking at 30°C in darkness. After 0, 1, 5, 12, 19 and 28 days, bacterial numbers were measured using agar plates purchased from Nissui Co. containing meat extract 5g, peptone 10g, sodium chloride 5g and agar 15g in one liter of distilled water.

Bacterial populations of *Escherichia coli* (C600), *Bacillus subtilis* (IFO 13719), *Alcaligenes faecalis* (IFO 13111) and *Pseudomonas dominute* which was isolated from river water by Kawai *et al.* (1983) were used to test of susceptibility. These bacteria were cultured using nutrient medium at 30 °C.

3. RESULTS AND DISCUSSION

3-1 Susceptibility of bacterial populations to organotin compounds

Initial log CFU in water samples of stations F1-F4 ranged from 4.1 to 5.2. The number of bacteria was dramatically declined after one day at the initial TBT concentration of 10 mg/l. However log CFU increased moderately after 5 days of incubation (Fig.IV-2). Only TBT-tolerant bacteria seem to increase. At the initial concentration of 0-1 mg/l, decrease of CFU count was not found and after one day, the number of bacteria increased ten times more than the initial population. The effects of DBT and MBT were also studied at stations F1 and F4 (Fig.IV-3). At an initial concentration of 10 mg/l DBT, log CFU of bacteria was decreased from 4.1 to 1.6 at station F1. However at station F4, the decrease of bacterial population was not observed at all. This time course of number of bacteria culture might be due to differences of bacterial populations at each location. After 5 days, the number of bacteria increased moderately. At an initial concentration of 1 mg/l DBT, CFU of bacteria did not decrease at stations F1 and F4. At the concentration of MBT ranging from 1 to 10 mg/l, the decrease of CFU was not found. It can be concluded that CFU is not decrease at each Bt concentration of 1mg/l or less.

The effect of phenyltin compounds on bacterial growth was also investigated in station F1 and station F4 (Fig.IV-4). Even at the concentration of 10 mg/l of TPT and DPT, decrease of CFU was not found. While, at 10 mg/l of MPT, the number of bacterial population of station F1 was remarkable decreased after one day by chance. It is clear that the sensitivity of bacteria to MPT is greatest among Pts.

Uchida (1993) reported that the susceptibility of estuarine bacteria to Ots was examined using the agar plate method by counting CFU for estuarine water samples gathered at Aburatubo and Kachidoki Bay. An inhibitory effects on colony forming ability of environmental bacteria occurred at 5-100 µg/l of TBT and TPT. In our experiment, it was clear that bacteria which could be multiplied at concentration of TBT more than 10 mg/l were existed in river water. Accordingly, the susceptibility of bacteria to Ots depend on the bacterial flora collected from area.

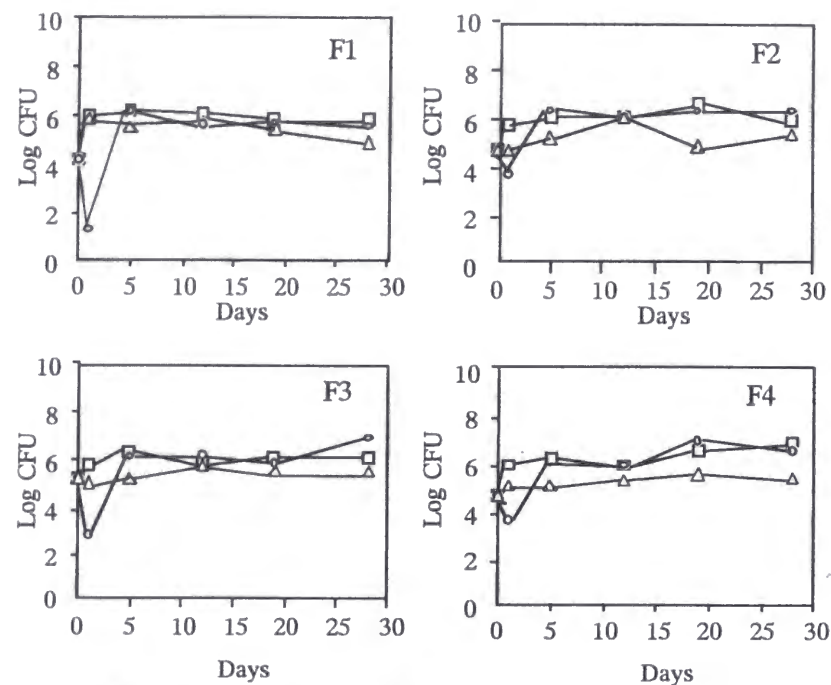


Fig.IV-2 Time course of number of bacteria culture in the river water containing TBT

The each value is the average of CFU on four plates.

○:10mg/l □:1mg/l △:none

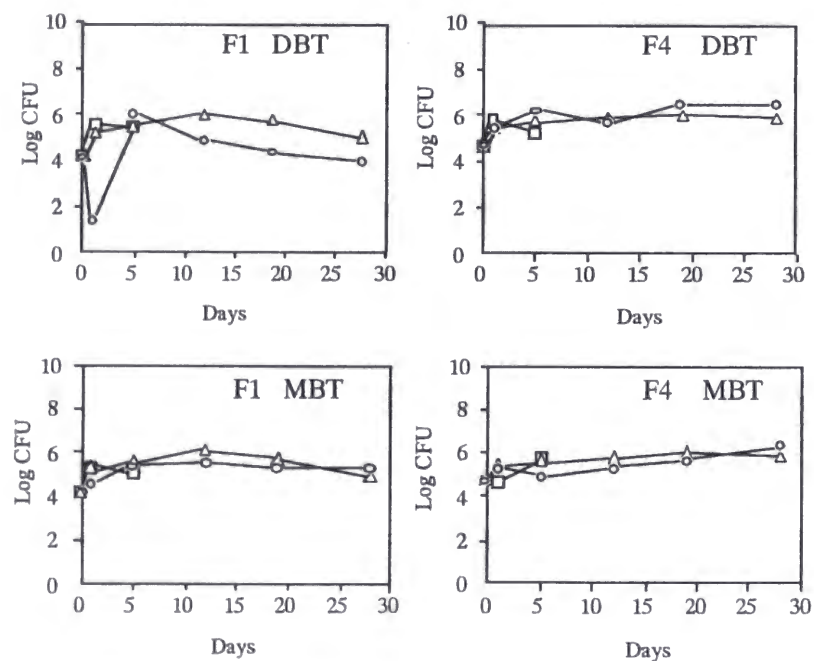


Fig.IV-3 Time course of number of bacteria culture in the river water containing DBT or MBT

The each values is the average of CFU on four plates

○:10mg/l □:1mg/l △:none

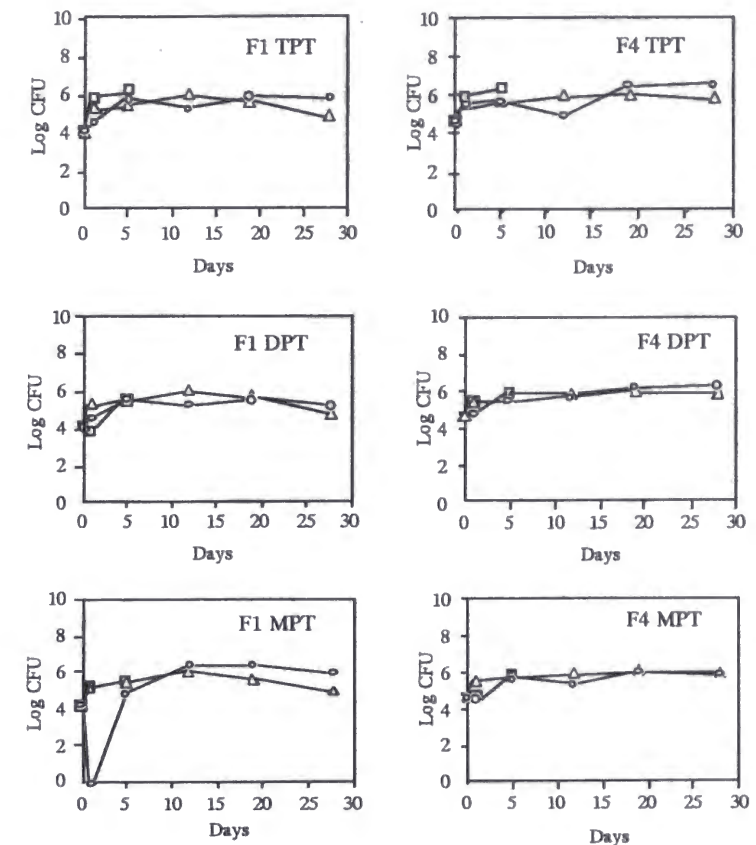


Fig.IV-4 Time course of number of bacteria culture in the river water containing Pts

The each values is the average of CFU on four plates

○:10mg/l □:1mg/l △:none

3-2 Comparison of tolerance for organotin compounds between bacteria in water and type strain

The tolerance of type strains for Ots was investigated. The strains were used *Escherichia coli*, *Bacillus subtilis* and *Alcaligenes faecalis*. Furthermore, *Pseudomonas diminuta* which is degraded tributyl phosphate (TBP), was also tested (Fig.IV-5). The growth of these type strains were inhibited at the initial concentration less than 10mg/l of TBT, DBT or TPT, and bacterial number was decreasing during the study period. Especially, all of *Pseudomonas dominuta* could not survive in the medium containing

every Ots within a day after incubation. The kind of Ots which inhibited the bacterial growth was different by type strain of bacteria. On the other hand, as described in toxicity test of bacteria in rivers, CFU of microorganisms in rivers decreased after one day at these Ots concentration, and then increased, showing that a number of bacteria which show tolerance to Ots popularly distribute in estuarine water.

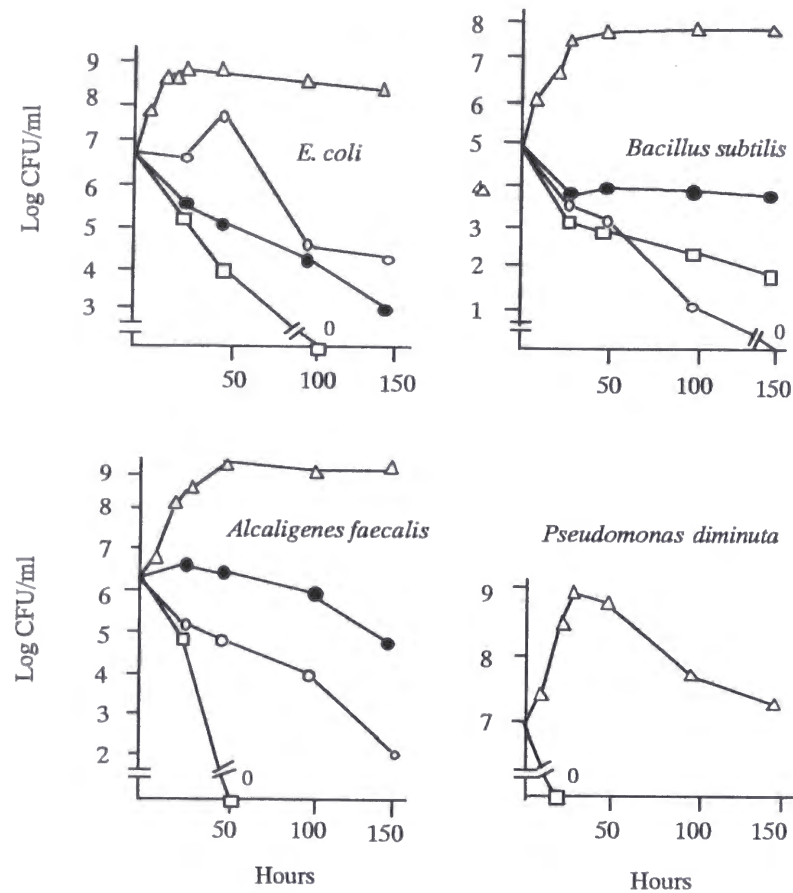


Fig.IV-5 Effect of Ots for type strains
 (●):TBT 10mg/l, (○):TPT 10mg/l, (□):DBT 10mg/l, (△): control

4. SUMMARY

At concentrations of DBT, TBT and MPT of 10 mg/l, decrease of the CFU count of rivers was observed after one day of incubation. However, the bacterial number increased

again after 5 days. Even at the concentration of 10mg/l MBT, DBT and TPT, decrease of CFU was not observed.

The susceptibilities of Ots for type strains (*E. Coli*, *Bacillus subtilis*, *Alcaligenes faecalis*, and *Pseudomonas diminuta*) were investigated. Type strains were inhibited at the initial concentration of 10mg/l of TBT, DBT or TPT. The growth of these results indicate that some of bacteria were inhibited by Ots but a number of bacteria which show tolerance to Ots popularly distribute in rivers.

SECTION II
BIODEGRADATION OF ORGANOTIN COMPOUNDS BY
MICROORGANISMS IN WATER

1. INTRODUCTION

The disappearance of Ots from the water environment is attributed to the biodegradation (Hattori *et al.*, 1988), photolysis (Maguire *et al.*, 1983, Soderquist and Crosby, 1980), biological accumulation (Yamada and Takayanagi, 1992), sedimentation and disappearance. It is reported that the aquatic microorganisms play an important role in the degradation of Ots (Lee *et al.*, 1989). Half-lives of TBT in water collected from a yacht harbour and a clean-water site were calculated to be 7 and 19 days, respectively (Seligman *et al.*, 1986a). Maguire and Tkacz (1985) reported that the half-life in water of Toronto Harbour was 35 ± 8 weeks at 20 °C in the dark at the initial TBT concentration of 2.44mg/l. Hattori *et al.* (1988) also investigated the degradation of TBT in polluted or unpolluted water samples by river die-away method (Barth, 1979, Saeger, 1979, 1980) measuring concentration of these organotin compounds which were added to water throughout the study period, and the half-lives of TBT in the polluted municipal river water and in the sea water from the highly polluted areas were 11 days. However, in sea water from the less polluted areas, this compound was little degraded. Thus, the half-lives of Ots by biodegradation by microorganisms varied with the quality of sample. There is not report concerning biodegradation of TPT in coastal waters. It is interesting to understand what extent the microorganisms in the water environment contribute to the disappearance of Ots.

In this section, in order to clarify the degradation mechanism of Ots in estuarine water, biodegradation process of TBT, DBT and TPT are investigated using river die-away method.

2. MATERIAL AND METHOD

2-1 Sampling description

The sampling area was shown in Fig. IV-1 of section I. As described in chapter III, section II, Station C1 is located in the mouth of Yodo River. Station C2 is a marina and has berths for 121 sailing boats. Station C3 is situated in the mouth of Shirinashi River, a zone of poor flushing. Station A1 is the outer portion of the Port of Osaka. Water samples collected at stations C1-C3 and A1 were used for the degradation study using river die-away method. The surface water at station C1-C3 and A1 were collected in June, 1992.

2-2 River die-away method

TBT, DBT, or TPT in DMSO was added to each estuarine water sample (50 ml). Each was prepared individually at the initial concentration of 10 or 100 µg/l, because Ots might be adsorbed on the glass wall. Autoclaved estuarine water samples were used as control. Bts and Pts in water samples were analyzed after shaking at 30°C in darkness for the study period. Bacterial cell number was also measured by counting CFU on agar plate after 0, 1, 5, 12, 19 and 28 days.

2-3 Analytical methods

Analysis of Ots was the same as that described in section I. Namely, fifty ml of water sample were extracted 0.1% tropolone-benzene solution after adding hydrochloric acid. After propylation with n-propylmagnesium bromide, the analytes were determined by GC-FPD. Detection limits of Bts in water were 0.2 µg/l.

3. RESULTS AND DISCUSSION

3-1 Degradation of Organotin compounds by the microorganisms in water from the Port of Osaka

The concentrations of TBT in water at station C2 was 0.063 $\mu\text{g/l}$ in 1992 and Ots except TBT were not detected. It was reported that this station showed the highest level of TBT in Osaka harbour area (Harino *et al.*, 1990). The time-course of Bts degradation in water from station C2 during 60 days was shown in Fig. IV-5a when initial concentration of TBT was 9.3 $\mu\text{g/l}$. In this experiment, the initial TBT concentration was adjusted to the levels which CFU was not decreased throughout the study period. TBT decreased rapidly during the initial 20 days and was not detected after 40 days of incubation. DBT increased slightly during the initial 15 days, and then DBT also decreased slowly and was not detected after 30 days. On the other hand, MBT increased slowly and was kept constant level between 12 and 60 days. The compositions of Bts over 60 days are shown in Fig. IV-5b. The ratio of TBT to total Bts decreased during 60 days of incubation. On the contrary,

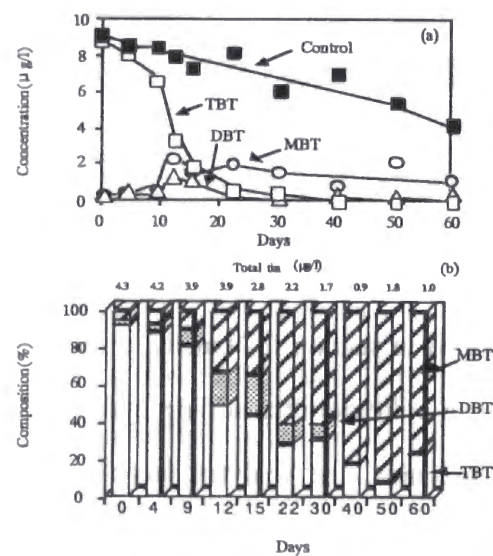


Fig.IV-5 Degradation of TBT by microorganisms in estuarine water from station C2
(a) Concentration of Bts during 60days
(b) Changes of the composition (%) of Bts during 60 days

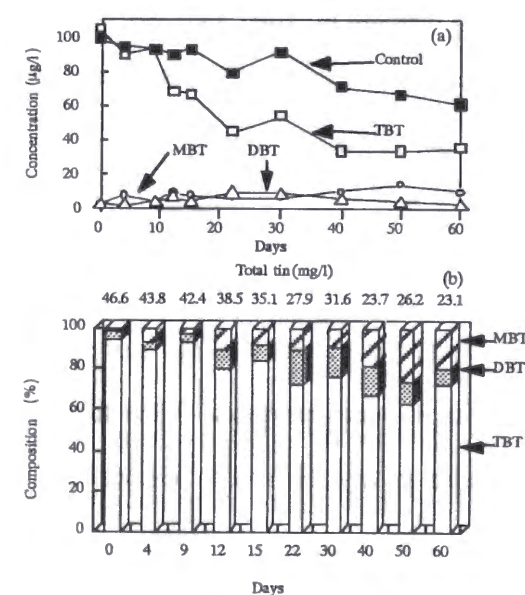


Fig.IV-6 Degradation of TBT by microorganisms in estuarine water from station C2
(a) Concentration of Bts during 60days
(b) Changes of the composition (%) of Bts during 60 days

the ratio of MBT to total Bts increased. The ratio of DBT increased between 0 and 15 days and thereafter decreased. This suggests that TBT is degraded to DBT, and DBT is subsequently debutylated to MBT. When the concentration of total Bts was calculated as inorganic tin, the concentration decreased from 4.3 to 1.0 $\mu\text{g/l}$ over 60 days. It is considered that MBT is further degraded to inorganic tin or it can be presumed that the other intermediates of Bts are produced. TBT concentration of the autoclaved control decreased gradually. From compositions of Bts, the ratio of MBT among Bts increased with decreasing of ratio of TBT. The ratio of TBT decreased from 93 to 61 % and those of MBT increased from 5 to 28 % throughout study period. Accordingly, it might be due to the chemical degradation. Fig.IV-6 shows the time course of Bts in water from station C2 at 104 $\mu\text{g/l}$ TBT concentration. TBT was degraded DBT following by MBT, similar to trend of TBT degradation at 9.3 $\mu\text{g/l}$. However drastic decrease of ratio of TBT was not observed and the ratio of TBT was 60 % after 60 days. Maguire and Tkacz (1985) reported that TBT of 2.44 mg/l was spiked to sea water. As a control, KCN was added to sea water in order to suppressed biodegradation. Bts concentrations in these samples were measured in 11 months at 20 °C. In accordance with the decrease of TBT, DBT and MBT increased throughout study period. This is agreement with our results of biodegradation test of TBT estuarine water by river die-away method.

The microbial degradation of DBT in water samples was also shown in Fig.IV-7a. The experiment was carried out in the same way as TBT at an initial concentration of 8.0 $\mu\text{g/l}$ DBT. DBT decreased rapidly during initial 11 days and disappeared after 22 days. The concentration of MBT increased for the first 11 days, and then decreased moderately. The compositions of Bts over 60 days are shown in Fig. IV-6b. The ratio of DBT to total Bts decreased from 100% to 19% between 0 and 15 days of incubation and was 0% after 22 days, and MBT accounted 100% of total Bts. The concentration of total Bts decreased from

4.4 to 0.2 $\mu\text{g/l}$. DBT concentration in control decreased slowly and the ratio of DBT decreased from 94 to 58% during the study period.

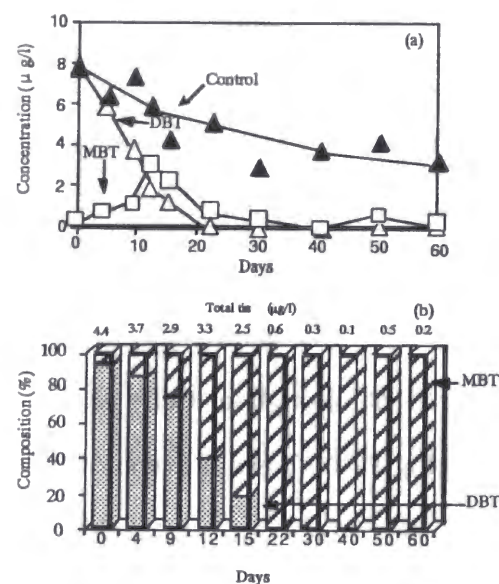


Fig.IV-7 Degradation of DBT by microorganisms in estuarine water from station C2
(a) Concentration of Bts during 60days
(b) Changes of the composition (%) of Bts during 60 days

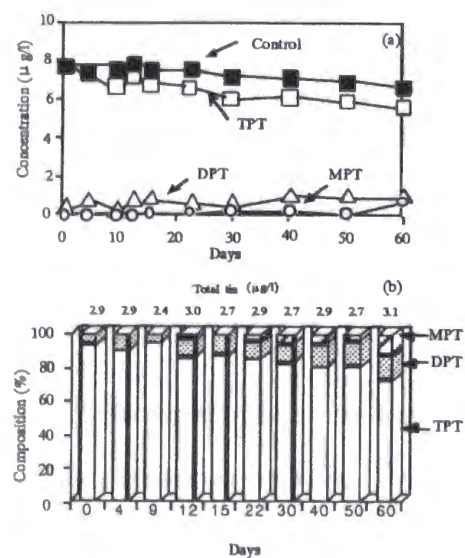


Fig.IV-8 Degradation of TPT by microorganisms in estuarine water from station C2
(a) Concentration of Pts during 60days
(b) Changes of the composition (%) of Pts during 60 days

The degradation of TPT in estuarine water from station C2 was also investigated by river die-away method. The initial concentrations of TPT were set at 8 $\mu\text{g/l}$ and 87 $\mu\text{g/l}$. The concentration of TPT scarcely changed after 60 days (Fig.IV-8 and 9). The compositions of Pts are shown Fig. IV-7b. The ratio of TPT decreased only 15% between 0 and 60 days and the ratio of DPT and MPT changed only slightly during 60 days. TPT concentrations in control did not decreased and the ratio of TPT ranged from 80 to 90%. It shows that the dephenylation of TPT does not occur or it occurs slow.

3-2 The half-lives of organotin compounds in water

The half-lives of organotin compounds in water samples were estimated from time course of organotin compounds which were corrected with the values in autoclaved control (Table IV-1). At the initial concentration of 9.3 $\mu\text{g/l}$, the observed half-life of TBT was 15 days. When TBT was adjusted to 104 $\mu\text{g/l}$, the half-life of TBT could not be calculated. The higher the TBT concentration, the slower the degradation rate. Kawai *et al.*(1988a) investigated degradation of TBP (tributylphosphate) at 1,5 and 10 mg/l TBP and reported

Table IV-1 Estimated half-lives of organotin compounds

Organotin compound	Initial concentration ($\mu\text{g/l}$)	Half-life (days)
TBT	9.3	15
TBT	104	> 60
DBT	8.0	10
TPT	8.0	> 60
TPT	87	> 60

Fig.IV-9 Degradation of TPT by microorganisms in estuarine water from station C2
(a) Concentration of Pts during 60days
(b) Changes of the composition (%) of Pts during 60 days

that Degradation rate of TBP become slow with increasing the initial TBP concentration. This were explained by relationship between enzyme and substrate. Namely, this relationship was obeyed equation as described below.

$$\text{Log } C_t = \text{Log } C_0 - Kt$$

C_t : concentration of substrate, C_0 : initial concentration of substrate,

t : hour,

K : constant

It is found that half-lives of chemical substances is increase with increasing of initial concentration from this equation,.

When the initial DBT concentration was 8 $\mu\text{g/l}$, the observed half-life was 10 days. It was obvious that debutylation rate of DBT occurred more rapid than that of TBT. In river water and seawater of the most polluted areas in Osaka Bay, DBT degraded more rapidly in comparison with TBT (Hattori *et al.*, 1988), which is in agreement with our results. The half-life of TPT could not be calculated, because this compound was scarcely degraded by the aquatic bacteria during 60 days of culture.

3-2 Comparison of the degradation of TBT in water at each station

Fig. IV-10 shows the concentrations of TBT in water samples from the Port of Osaka.

The concentration of TBT at stations

C1-C3 and A1 ranged from 0.004 to

0.081 $\mu\text{g/l}$. The highest

concentration of TBT was detected

at station C2 (marina) and the

median of TBT concentration was

0.062 mg/l. TBT at station C3 was

the second highest concentration in

this area. Stations C1 and A1 were

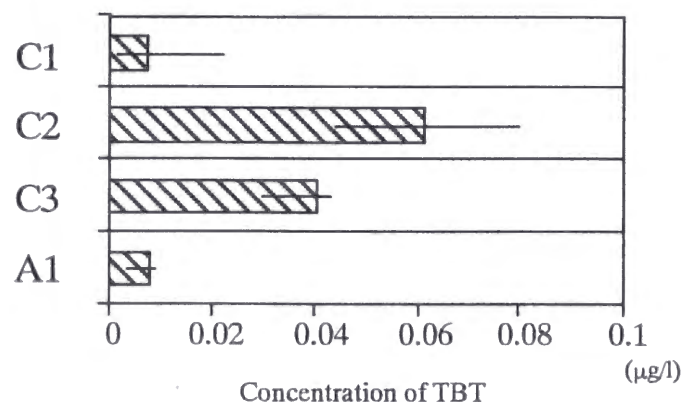


Fig. IV-10 TBT concentration in water at four sites from harbour area of Osaka City in 1992

not polluted by TBT and the level of TBT was in the range of 0.004 - 0.021 $\mu\text{g/l}$.

Therefore, it is regarded that in Osaka harbour area, stations C2-C3 are heavily polluted by TBT and stations C1 and A1 comprise non-polluted area.

TBT was added to estuarine water and initial concentration was adjusted to the range of 8.1 to 8.6 ng/l . Fig. IV-

11 shows the time-dependent

changes of the added TBT

concentration in water at four

stations. The TBT

concentration

decreased

rapidly at stations C1 and A1.

At stations C1, C3 and A1,

TBT was not detected after

18 days of culture. However,

TBT at station C2 could still

be determined at the level of

1 $\mu\text{g/l}$ after 42 days. Thus, the degradation rate of TBT by

microorganisms at station C2 was

slower than that at stations C1,

C3 and A1. Differences

in the degradation rate at stations

C1, C2, C3 and A1 were not

observed. The initial CFU at

stations C1, C2, C3 and A1 were

11000, 38000, 20900 and 2870,

respectively. Initial

CFU at station C2 was highest

among the four stations. At

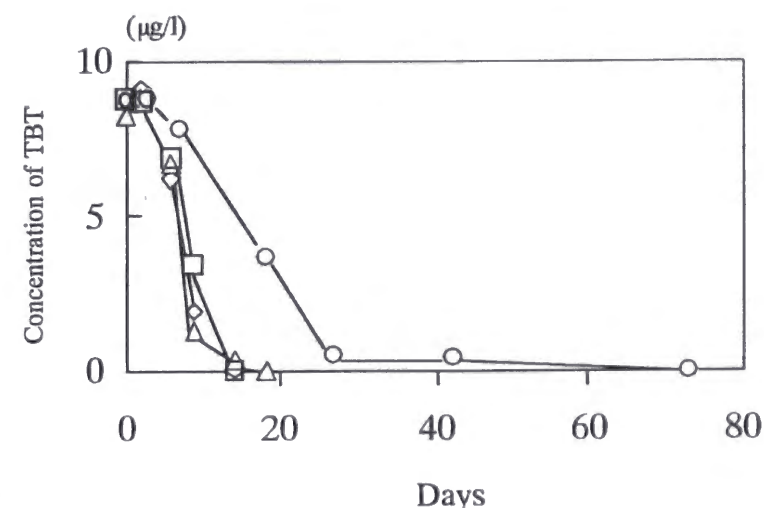


Fig. IV-11 Degradation of TBT by the microorganisms in water

□:station C1 ○:station C2 △:station C3 ◇:station A1

microorganisms at station C2 was slower than that at stations C1, C3 and A1. Differences in the degradation rate at stations C1, C2, C3 and A1 were not observed. The initial CFU at stations C1, C2, C3 and A1 were 11000, 38000, 20900 and 2870, respectively. Initial CFU at station C2 was highest among the four stations. At station C2, in spite of higher initial log CFU, degradation of TBT was slow. It shows that the degradation rate of TBT is not dependent on the initial CFU. On the other hand, as described in Fig. IV-10, the concentrations of TBT in water samples were high at stations C2-C3, and were low at stations C1 and A1. Kawai *et al.* (1986) report that the degradation rate of TBP by microorganisms in water sample of high TBP concentration was faster than those of low TBP concentration. The order of degradation rate of TBT at each station is not in accordance to the concentration of TBT in water sample. TBT concentration of water from

four station may be the level which did not give the effect for degradation activity of bacteria.

4. SUMMARY

Bacterial degradation of TBT, DBT and TPT was investigated in waters by river die-away method. TBT was degraded to DBT and MBT by a stepwise debutylation process. When the initial concentration of TBT was 9.3 $\mu\text{g/l}$, the observed half-lives of TBT was 15 days. However at the initial concentration of 104 $\mu\text{g/l}$, half-life of TBT was not observed after 60 days of incubation. The observed half-lives of DBT was 10 days at 8 $\mu\text{g/l}$ DBT. When the initial concentration of TPT was 8 $\mu\text{g/l}$, TPT was scarcely degraded during 60 days of incubation.

The differences of microbial degradation rate in four stations were investigated. Addition of 8 $\mu\text{g/l}$ TBT was degraded clearly at every stations during 0-64 days of culture. Except for station F6, TBT was not detected after 18 days at all sites. TBT at station F6 was still at least 0.39 $\mu\text{g/l}$ after 42 days. The order of degradation rate of TBT in water from each station is not in accordance to the TBT concentrations in water sample from each station. It is considered that TBT concentration in water from the Port of Osaka is not the level which give the effect for the bacterial flora.

SECTION III

BIODEGRADATION OF ORGANOTIN COMPOUNDS BY MICROORGANISMS DETACHED FROM SEDIMENT

1. INTRODUCTION

Bts was degraded by bacteria not only in water but also in sediment. Dowson *et al.* (1993b) investigated the degradation of TBT in contaminated freshwater and estuarine sediments. The decline of TBT concentration in aerobic sediment from Robertsons Boatyard (fresh water) and Tollesbury marina (estuarine) was observed as well as in water. According to them, TBT half lives in surface sediments were ranged from 360 to 775 days. The biological degradation of TBT has been reviewed by Stewart and Mora, (1990).

In section II, we showed that when the initial concentration of TBT was 9.3 and the observed half-life of TBT were 15 in water. The observed half life of DBT was 10 days at the initial concentration of 8.0 $\mu\text{g/l}$ DBT. On the other hand, TPT was scarcely degraded during 60 days of culture. In this section, the degradation process of Ots by microorganisms detached from sediment is clarified and the degradation rate of TBT of each station by microorganisms in water and from sediment were compared, in order to clarify the distribution and behavior of Bts in the harbour area of Osaka City.

2. MATERIAL AND METHOD

2-1 Sampling description

Detail sampling status were described in section II. Sediment samples collected from C1-C3 and A1 with Eckman birge grab sampler in November 1992. Degradation of TBT by using microorganisms isolated from sediment at stations F5-F8 was investigated and experiment was carried out within one day from collection.

2-2 Colony counting

Total viable counts were determined using agar plates of purchased from Nissui Co. Ltd. containing meat extract 5 g, peptone 10 g, sodium chloride 5 g and agar 15 g in 1 liter of distilled water. The plates were incubated at 30 °C for 3 days before counting colonies.

2-3 River die-away method

Water sample containing microorganisms from sediment were prepared by treating 50 g of sediment in 500 ml of autoclaved estuarine water with sonification for 5 min. In addition, after adding 700 ml of autoclaved estuarine water, the mixture was left to stand for 60 min. The volume was determined as the volume which separated both of water and sediment after standing for 30 min. Log CFU in supernatant at C1, C2, C3 and A1 was 3.16, 3.21, 2.79 and 1.92, respectively. The supernatant was carefully sampled and then divided into 2 groups. One group was analyzed after adding 0.5 ml of TBT standard solution to 50 ml of the supernatant and then shaken at 30 °C in the dark

TBT standard solution was adjusted to 1000 µg/l in dimethylsulfoxide(DMSO) was prepared and 0.5 ml of this solution was added to 50 ml of each water sample. This process resulted in the initial concentration being adjusted to 10 µg/l. Autoclaved estuarine water samples were used as a control. In order to prevent the adsorption of organotin compounds on the glass, each sample was prepared individually. Water samples were incubated while shaking at 30 °C in the dark for 73 days.

The second group was used as a control solution after sterilization by autoclave.

2-4 Analytical method

Analytical procedures were the same as those described in section II. Namely, fifty

ml of water sample were extracted 0.1% tropolone-benzene solution after adding hydrochloric acid. After propylation with n-propylmagnesium bromide, the analytes were determined by GC-FPD. The detection limit of each Bts for a signal - to - noise ratio of three was 0.2 µg/l.

3. RESULTS AND DISCUSSION

3-1 Degradation of TBT by microorganisms from sediment

The degradation process of TBT by the microorganisms from sediment of the Port of Osaka was similar to that in water. Namely, TBT degraded to DBT and MBT (Fig.IV-12). The change of Bts composition is shown in Fig.IV-13. The ratio of TBT at station C1 was decreased from 90 % to 20 % during 0-67 days of culture. In contrast to TBT, MBT increased from 7 % to 67 % and DBT remained in the range of 10-16 %. The ratio of TBT in control sample decreased from 90 % to 53 % during 0-67 days. It is due to the chemical degradation. The compositions of Bts at station A1 also

showed a similar tendency to that at station C1. That is to say, MBT increased with the decrease of TBT. Of particular note is that TBT was not detected after 67 days at station A1. The half lives of TBT in sediment samples were estimated from the time course of TBT concentrations which were corrected with values in autoclaved control. The

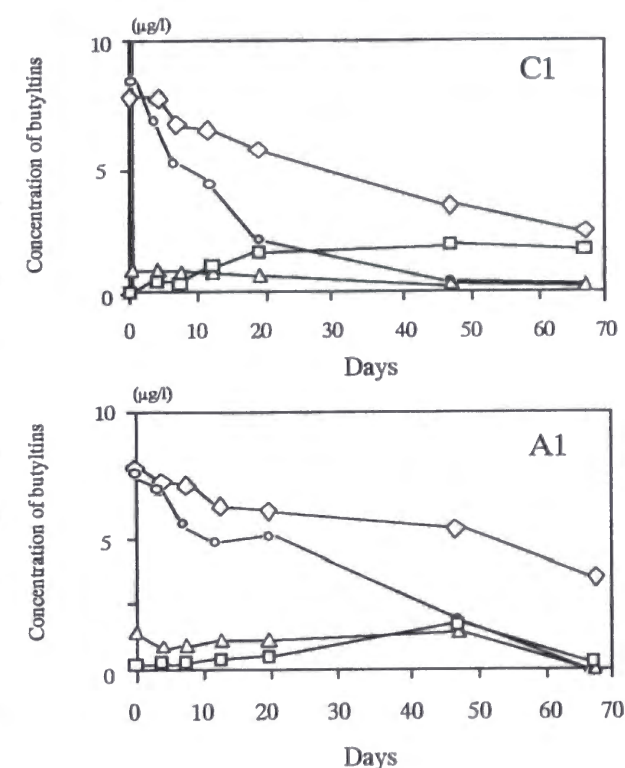


Fig.IV-12 Degradation of TBT by the microorganisms from sediment
○:TBT △:DBT □:MBT ◇:control

estimated half - life of TBT was 8 days at station C1 and that at station A1 was 11 days. The sum of MBT, DBT and TBT was calculated, and it was decreased from 9.7 to 2.7 $\mu\text{g/l}$ at station C1 and from 9.8 to 0.4 $\mu\text{g/l}$ at station A1 during 0 - 67 days. As to the

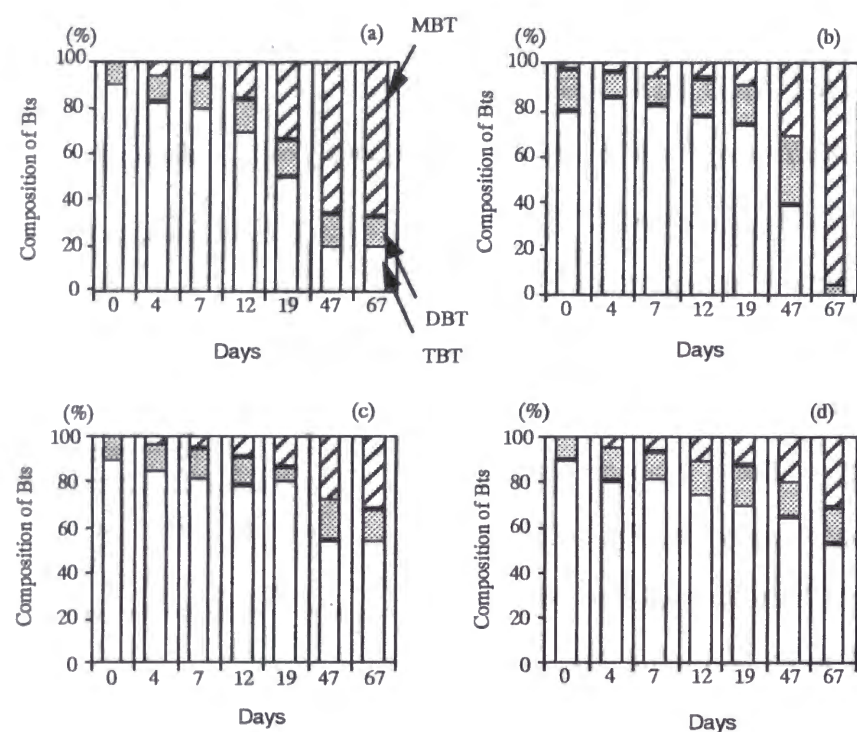


Fig.IV-13 Composition of Bts with the degradation of TBT by the microorganisms from sediment

(a):station C1, (b):station A1, (c):station C1 control, (d):station A1 control

formation of DBT from TBT by living organism, it has been reported that the formation of β -(hydroxybutyl) tributyltin occurred, which in turn was degraded to butyltin with the release of butene (Kimmel *et al.* 1977, Lee *et al.*, 1989). In the formation of MBT, β -(hydroxybutyl) dibutyltin might be formed, and followed by degradation to inorganotin. Though butyltin bonding hydroxyl group or inorganotin was not measured in our experiment, it is presumed that these compounds mentioned above were produced in the

process of microorganisms degradation.

3-2 half lives of TBT by microorganisms from sediment

The degradations of TBT by the microorganisms from sediment at stations C1-C3 and A1 were shown in Fig.IV-14. The initial log CFU from sediment samples of stations C1-C3 and A1 were in the range of 1.9 - 3.2. When TBT was adjusted to 7.5 $\mu\text{g/l}$, estimated half-lives by microorganisms from sediment from stations C1, C2, C3 and A1 were 7, 9, 9 and 9 day, respectively. it is considered that though CFU at station C2 was about a hundred times higher than that at station A1, the difference of half-life at each station is not observed.

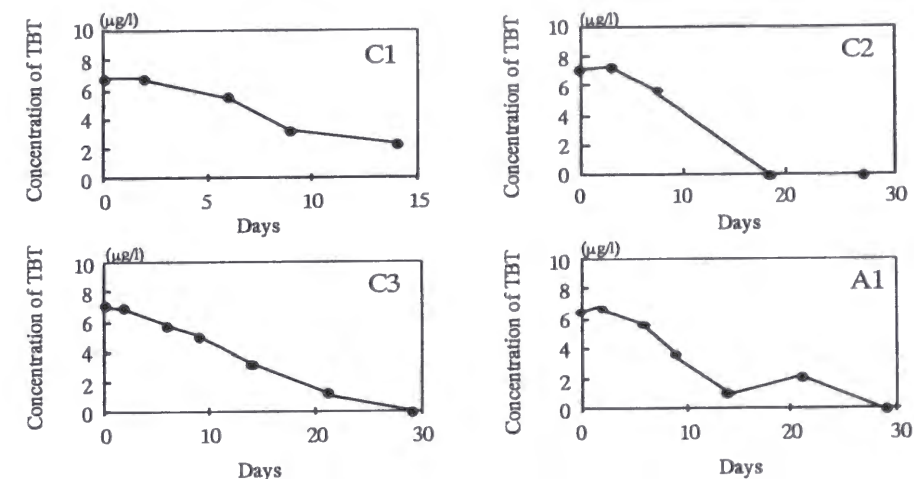


Fig.IV-14 Comparison of TBT degradation by the microorganisms and from sediment

4. SUMMARY

TBT were determined in water and sediment samples collected from four stations in harbour area of Osaka City. TBT in surface waters ranged from 0.008 to 0.116 $\mu\text{g/l}$. TBT concentrations in sediment samples were in the range of 0.005-2.36 mg/kg dry. The highest concentration of TBT in surface water and sediment were found at a marina and

the zone of poor flushing.

Degradations of TBT by the microorganisms from sediment in the four stations were also investigated. The degradation process of TBT by the microorganisms from sediment was similar to that in water, namely, TBT was degraded to DBT and MBT and the marked differences of degradation rate of TBT were not observed at four station.

SECTION IV

CONCLUSION

In this chapter, susceptibility of bacterial populations to Bts and Pts has been firstly described. At concentration of DBT, TBT and MPT of 10mg/l, decrease of the CFU count was observed after a day of incubation. However, the bacterial populations increased again after 5 days. It was found that a number of bacteria which have tolerance to Ots exist in estuarine water. Secondly, degradations of TBT, DBT and TPT in water were investigated by river die-away method. TBT was degraded to DBT and MBT by a stepwise debutylation process. When the initial concentration of TBT were 9.3 the observed half-life of TBT were 15 days. The observed half life of DBT was 10 days at the initial concentration of 8 μ g/l DBT. The order of degradation rate of TBT at each station is not in accordance to the TBT concentration in water sample. TPT was scarcely degraded during 60days of incubation at the initial concentration of 8 μ g/l TPT. The degradation process of TBT by the microorganisms from sediment was similar to that in water and the half lives of TBT did not attribute to the TBT concentration in sediment.

CHAPTER V
BIOCONCENTRATION OF ORGANOTIN COMPOUNDS IN THE
MARINE ORGANISMS

SECTION I
THE LEVELS OF ORGANOTIN COMPOUNDS IN FISH

1. INTRODUCTION

There are many reports concerning Ots concentration in aquatic organisms. Elevated concentrations of TBT were noticed in mollusks (Morcillo *et al.*, 1997). Becker *et al.* (1992) found TBT up to 4 mg/kg wet and TPT up to 1mg/kg wet in zebra mussels. Kannan *et al.* (1995a,b) reported that concentrations of Bts in horseshoe crab (*Hepato pancreas*) from Hakata Bay ranged from 0.35 to 2.27 mg/kg wet and those from Hakata Bay ranged from 0.57 to 5 mg/kg wet and concentrations of Pts in the crab were 2-fold higher than that of Bts. It was also reported that the concentrations of TBT in fish from the lake in Netherland were ranged on ND-2.5 mg/kg dry and TPT concentrations ranged from 0.038 to 2.6 mg/kg dry, respectively (Stab *et al.*, 1996). Fent and Hunn (1991) report a TBT concentration of 0.17 mg/kg in muscle from Switzerland. Stab *et al.* (1996) also report the concentrations of TBT and TPT in Roach, Silver Br, Rufe, Smelt, Tench Bream, Perch, Pike and Pike perch from Grote Poel Lake in Netherland were in the range of ND-2.5 mg/kg dry and 0.038-2.6 mg/kg dry, respectively. TBT levels in the range from 0.114 mg/kg wet to 0.196 mg/kg wet were found in muscle of blue catfish, gafftopsail catfish and hardhead catfish from the Gulf of Mexico (Kannan *et al.*, 1997). The continued presence of TBT in fish from coastal area is of concerning matters and it is important that the difference of TBT concentration in the kind of fish is clear

Environmental agency of Japan is investigated TBT and TPT concentration in mussel and fish in 1994 (Environmental agency, Japan, 1996). TBT concentrations in

mussel (*Mytilus edulis*) from 4 sites in coastal area in Japan were in the range of ND - 0.10 mg/kg wet and TPT concentrations were ND - 0.04 mg/kg wet, respectively. TBT and TPT levels in sea bass (*Lateolabrax japonicus*) from 7 sites in these areas were in the range of ND - 0.17 mg/kg wet and ND - 0.28 mg/kg wet, respectively. Thus Ots levels were relatively higher in marine organisms. While, considering the human effect for Ots, it is important to understand the current status of these in fish and shellfish.

In this section, the concentration of Bts and Pts in muscle of several fish species from the Port of Osaka are measured and the difference of Ots concentration in fish species are discussed. Further, the relationship between Ots concentration and the total length or lipid contents of fish are studied.

2. MATERIAL AND METHOD

2-1 Sampling Description

Fish were collected at station F4 and station A1 in the Port of Osaka in 1996 (Fig.V-1). Station A1 and station F4 are located in the outer part of the Port of Osaka and in downstream of Yodo River. Both stations are in a zone of well flushing. Salinity at station A1 is 18.06 and that at station F4 is 0.36.

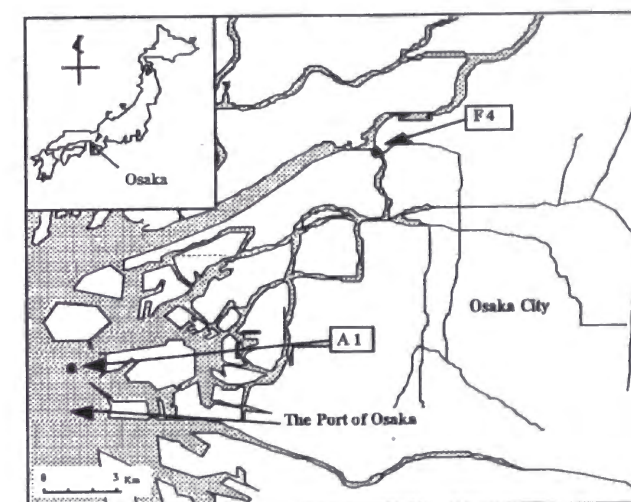


Fig.V-1 Sampling site

Japanese sea perch (*Lateolabrax japonicus*), white croaker (*Pennehia argentatus*), yellow tail (*Seriola quinqueradiata*), bandfish (*Trichiurus japonicus*), silver whiting (*Sillago japonica*), butter fish (*Psenopsis anomala*), file fish (*Stephanolepis cirrhifer*) and tripped puffer (*Takifugu xanthopterus*) were captured with drift net at station A1 and gray mullet (*Mugil cephalus*), Japanese barbel

(*Hemibarbus barbus*) and bluegill (*Lepomis macrochirus*) with casting net at station F4. Fish samples analyzed in the presents study were stored below -20 °C until chemical analysis was carried out. Total length and body weight of fish species analyzed were shown in Table V-1. Ots concentration in muscle of fish were measured in this section. In order to study the relationship between Ots concentrations and lipid contents or total length, Japanese sea perch of 11 female and 8 male were collected from station A1 in 1996. Total length of Japanese sea perch ranged from 31 cm to 39 cm and the body weight of these fish ranged from 0.29 kg -0.58 kg.

Table V-1 Biological data of fish in the Port of Osaka

Specimen	Sampling date	Sampling locality	Number of sample	Total length (cm)	Body weight (kg)
Japanes sea perch	18 Oct. 96'	Station A1	2	62 - 72	1.6 -3.1
female	18 Oct. 96'	Station A1	11	30 - 40	0.3 -0.6
male	18 Oct. 96'	Station A1	8	30 - 40	0.3 -0.4
White croaker	18 Oct. 96'	Station A1	2	36 - 46	0.7 -1.0
Yellowtail	18 Oct. 96'	Station A1	2	38 - 39	0.7
Bandfish	18 Oct. 96'	Station A1	2	68 - 70	0.2
Silver whiting	18 Oct. 96'	Station A1	3	24 - 25	0.1
Butter fish	18 Oct. 96'	Station A1	3	12 - 15	0.1
File fish	18 Oct. 96'	Station A1	1	23	0.2
Triped puffer	18 Oct. 96'	Station A1	1	28	0.5

Gray mullet	11 Oct. 96'	Station F4	2	20 - 22	0.1 -0.2
Japanese barbel	11 Oct. 96'	Station F4	3	18 - 24	0.1 - 0.2
Bluegill	11 Oct. 96'	Station F4	3	12 - 16	0.1 - 0.2

2-2 Lipid content

Five grams of muscle of fish were extracted twice with 40 ml of Folch solution (chloroform:methanol = 1:1) after centrifuged, the supernatants were mixed and 60 ml of distilled water added to the mixtures. The chloroform layer was separated and dried with anhydrous Na₂SO₄. The solvent was removed using a rotary evaporator and dried in a desiccator. Lipid content was measured by gravimetric technique.

2-3 Analytical method

Reagent was used as described in chapter II. The detection limit for aquatic organisms for each Ots was 0.001 mg/kg wet.

3. RESULTS AND DISCUSSION

3-1 Concentration of butyltin and phenyltin compounds in various fish species

Levels of TBT and TPT in the muscle of fish are shown in Fig.V-2. TBT in fish from sea areas were detected in the range of 0.052-0.190 mg/kg wet and TBT in fish from estuary and rivers ranged from 0.011 to 0.022 mg/kg. TBT concentrations in fish were high in order of silver whiting>triped puffer>band fish>white croaker>yellowtail>butter fish>Japanese sea perch>file fish. The ratio of TBT to total Bts was also highest in this order (Fig.V-3). TPT in fish from sea areas were detected in the range of 0.030-0.124 mg/kg wet and TPT in fish was not detected. TPT levels in each fish showed the same order as TBT levels roughly, but highest concentration of TPT was observed in white croaker in fish from seawaters and rivers.

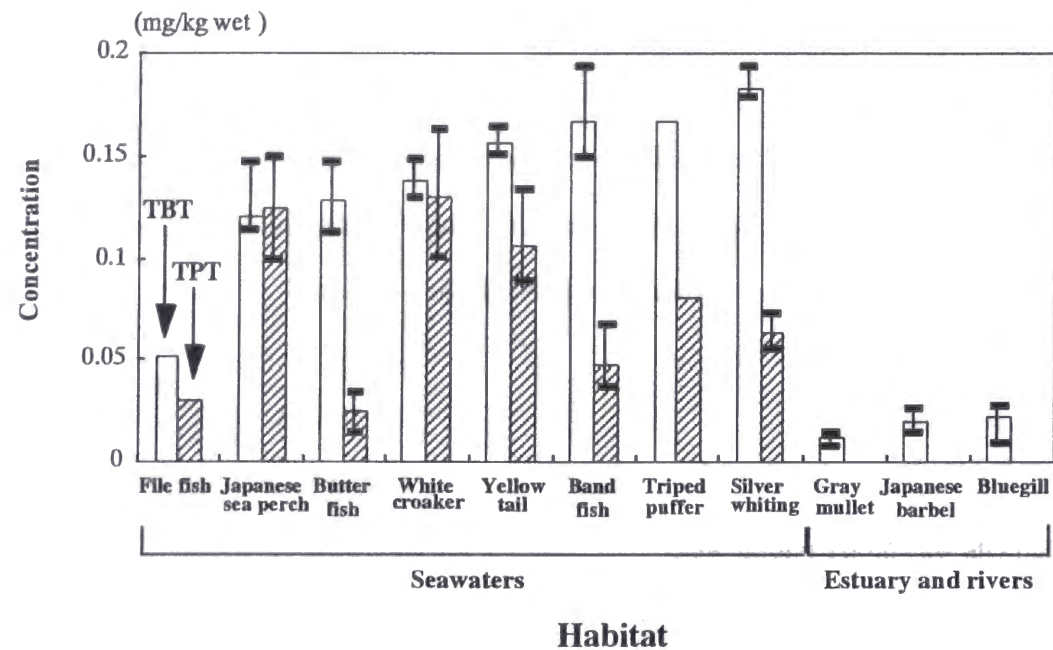


Fig.V-2 Concentration of TBT and TPT in several fish species
Bars indicate ranges.

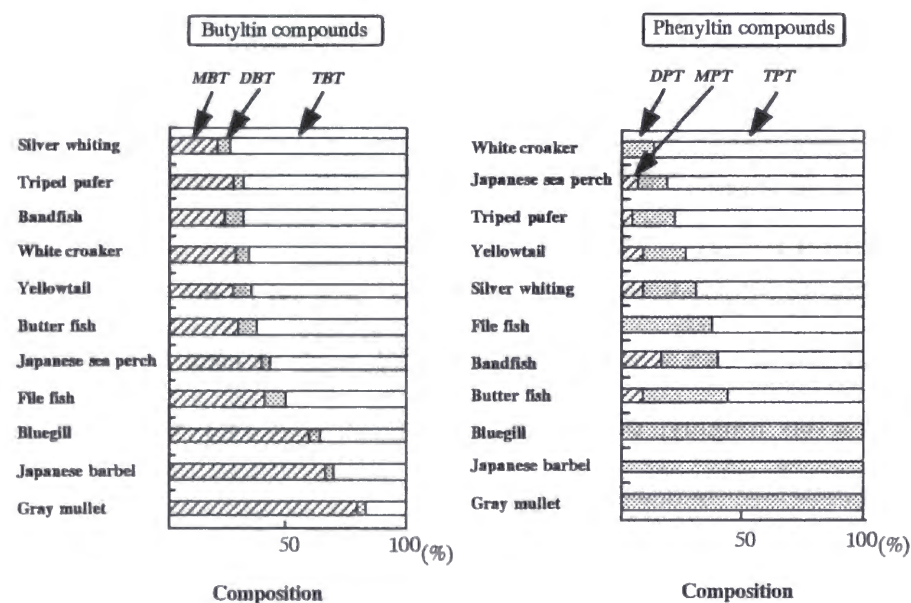


Fig.V-3 Composition of Bts and Pts in several fish species
Compositions of Ots were calculated from means of concentration of that in fish.

TBT and TPT levels were compared between fish from sea areas and rivers (Fig.V-2). TBT and TPT concentrations in sea waters were higher than those in rivers. the ratio of TBT to total Bts was also higher in fish from sea areas (Fig.V-3). TBT concentrations in water and sediment from sea areas were 0.013 $\mu\text{g/l}$ and 0.07 mg/kg dry, respectively. TPT was not detected in water and was 0.007 mg/kg dry in sediment. Meanwhile, TBT concentrations in water and sediment from rivers were 0.008 $\mu\text{g/l}$ and 0.007 mg/kg dry, respectively and TPT was not detected in water or sediment from rivers. It is therefore concluded that the difference in Ots concentration in fish is related to Ots contaminants in their habitat. While, TPT was detected in fish in spite of not detection of TPT in water. Stab *et al.* (1996) reported that TBT is more easily eliminated or metabolized as the di- and mono-Ots levels than TPT. Judging from these result, TPT is more persisted than TBT in fish.

Acceptable daily intake (ADI) for TPT (0.5 mg/kg/day) in fish was proposed by World Health Organization. If a person whose weight is 60 kg, eats 110g of fish muscle each day, the acceptable concentration of TPT in fish is 0.24 mg/kg wet. While, ADI for TBT has not been proposed. If ADI for TBTO (1.6 TBTO mg/kg/day) by the Ministry of Welfares is applied to TBT, the acceptable concentration of TBT is 0.42 mg/kg wet. The concentrations of TBT and TPT in muscle of fish from the Port of Osaka are lower than the estimated acceptable concentration from ADI.

3-2 Comparison of TBT and TPT concentration between male and female of Japanese sea perch

The sex difference of Ots concentrations in muscle of Japanese sea perch (the total length, 30-40 cm) from the Port of Osaka was studied (TableV-2). TBT concentrations ranged from 0.068 to 0.835 mg/kg in males and from 0.175 to 0.840 mg/kg in females. On the other hand, TPT concentrations were 0.050-0.426 mg/kg and 0.050-0.247 mg/kg,

respectively. In Japanese sea perch, no significant differences in TBT and TPT levels were not noted ($p < 0.05$).

Table V-2 TBT and TPT concentration in female and male of Japanese sea perch

TBT		TPT	
female	male	female	male
0.835	0.563	0.144	0.247
0.107	0.666	0.050	0.114
0.254	0.644	0.121	0.127
0.377	0.633	0.124	0.089
0.497	0.175	0.027	0.065
0.255	0.297	0.084	0.113
0.187	0.209	0.129	0.050
0.484	0.840	0.125	0.225
0.736		0.426	
0.352		0.205	
0.068		0.070	

(mg/kg wet)

3-3 Correlation between concentration of organotin compounds and lipid contents in Japanese sea perch

Relationship between Ots concentration and lipids content in muscle of Japanese sea perch was also studied. No correlation of TBT or TPT concentration to lipid content was observed (Fig. V-4). Yamada *et al.* (1992) also report no correlation between lipid content and accumulated TBT or TPT content. This shows that TBT and TPT are not accumulated in fat. It is well known that organic compounds such as PCBs do accumulate in fat of fish. Kawai *et al.* (1988b) report that distribution of chemical substances is accounted for by lipid composition. Therefore, the difference in distribution mechanism between TBT and

PCBs may be due to the formation of complexes between trialkyl compounds and protein as well as methylmercury (Yamada and Takayanagi, 1992). For example, levels of PCBs corrected positively with triglyceride content and total cholesterol content. Judging from these results, it is seen that the distribution mechanism of Ots in fish is different from that of hydrophobic substances such as PCBs. Trialkyltin compounds are report bind to amino acids, peptides and proteins (Davies and Smith, 1980).

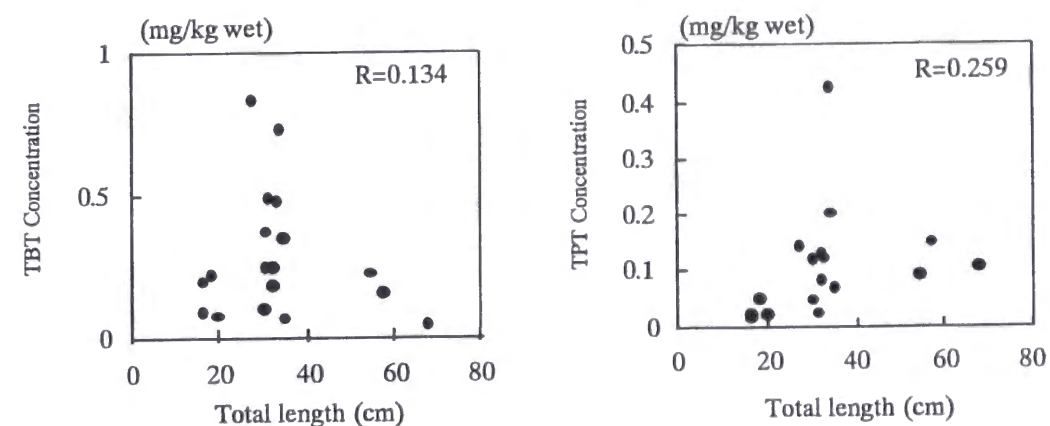


Fig.V-4 Correlation between lipid contents and concentration of TBT or TPT in muscle of Japanese sea perch

3-3 Correlation between concentration of organotin compounds and total length in Japanese sea perch

The relationship between Ots concentration and total length in Japanese sea perch (*Lateolabrax japonicus*) described previous section was analyzed (Fig.V-5). No correlation between the total length and TBT or TPT concentration was observed. Though concentration of TBT and TPT were decreased with decrease of total length of fish, those were not increased with increase of total length. In the case of PCBs, concentration of di-, tri- and tetra-PCBs decreased with increase mean body size, while the reversed trend was observed in penta-, hexa- and hepta-PCBs (Subramanian *et al.*, 1983). The result for Ots

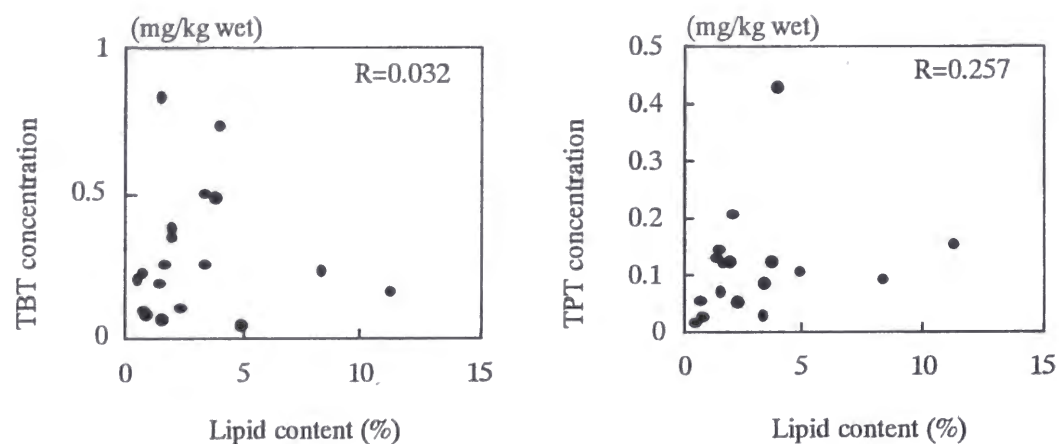


Fig.V-5 Correlation between total length and concentration of TBT or TPT muscle of in Japanese sea perch

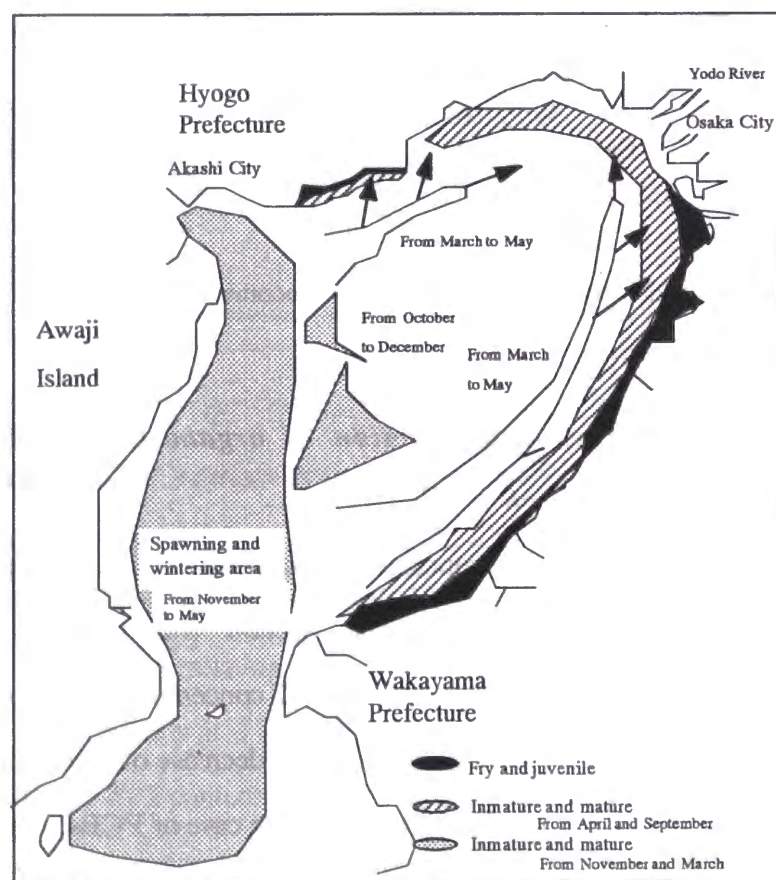


Fig. V-6 Activity range of Japanese sea perch (Osaka prefectural fisheries experimental station)

did not coincident with those for PCBs. Activity of Japanese sea perch in life stage is shown in Fig.V-6. Japanese sea perch inhabit the fringe of Osaka bay and active range is wide. It is presumed that TBT and TPT levels were differ in each coastal area in Osaka Bay. The levels of TBT or TPT may be due to activity range.

4. SUMMARY

Ots levels in muscle of fish from rivers and sea areas were described. TBT and TPT in fish were detected in the range of 0.001-0.190 mg/kg wet and ND to 0.159 mg/kg wet, respectively. TBT concentrations in fish from sea areas were higher than rivers and TBT was the dominant compounds among Bts. The concentrations of TBT in muscle of fish were reflected on those in waters and sediment. In spite of TPT not being detected in waters from sea areas, relatively high concentration of TPT was detected in fish. No correlation of TBT or TPT concentration to the total length or lipid content was observed, suggesting that the distribution mechanism of Ots in fish is difference from those of hydrophobic substances such as PCBs.

SECTION II

COMPARISON OF PARTITION BETWEEN WATER AND AQUATIC ORGANISMS

1. INTRODUCTION

In previous section, it was found that the higher levels of TBT and TPT were observed in muscle of fish in comparison with water levels and TBT in fish was level to cause health problem. Therefore, it is important to know the extent of partition of Ots to aquatic organisms. Here, BCF were defined as concentration in aquatic organisms / concentration of water. BCF in fish is reported to be in the range of 10^2 - 10^4 (Tuda *et al.*, 1987b, 1988, Martin *et al.*, 1989, Yamada *et al.*, 1992). From these papers, it is concluded that Ots accumulates in fish easily. Contaminants of Ots for marine products which are important human foods are therefore matters of concern.

2. MATERIAL AND METHOD

BCF of fish was calculated from TBT concentration in water and aquatic organisms which described in section I, and in water. BCF of scallop in Otsuchi Bay was calculated from TBT concentration which described in chapter III section III. BCF of fish and shellfish were compared to that in other bay area in section II.

3. RESULTS AND DISCUSSION

3-1 Partition of tributyltin compounds in muscle of fish

BCF of TBT calculated by dividing TBT concentration in the aquatic organisms (mg/kg wet) by concentration in water ($\mu\text{g/l}$). Log BCF of TBT in fish from each area is shown in Fig.V-7. Log BCF of fish from sea areas and rivers ranged from 3.6 to 4.2 and from 2.9 to 3.5, respectively. Log BCF in sea areas was thus significantly higher than that in rivers ($P < 0.05$). Yamada *et al.* (1992) report that the BCF of TBTO for marine fish

is greater than that of freshwater fish, and our findings were agree with Yamada's observation. BCF calculated from field samples was compared with that reared in laboratory. Test of BCF for various chemicals have shown Log BCF in range of 3.5-4.0 in marine fish (*Sillaga japonica*, *Rudarius ercodes* and *Pagrus majer*) (Yamada and Takayanagi, 1995) and 2.7 in guppies (Tsuda *et al.*, 1990). Log BCF calculated from values in field was the same ranges as that in laboratory.

BCF of MBT, DBT and TBT in fish was compared (Fig. V-7). Medians of BCF in fish from sea water were 3.11 for MBT, 4.01 for DBT and 4.04 for TBT. Namely, BCF in fish from sea areas was high in the order of TBT, DBT and MBT. Medians of BCF in fish from rivers were 2.88 for MBT, 2.89 for DBT and 3.30 for TBT. Though BCF of TBT and DBT in fish from rivers was lower than that from sea water, the orders of BCF of Bts were the same as those in fish from sea waters. As showed in chapter III, section II, K_{pw} and K_{mw} were high in the order of TBT, DBT and MBT. The hydrophobic property also increased in the order of TBT>DBT>MBT. Accordingly, BCF of fish is attributed to hydrophobic property.

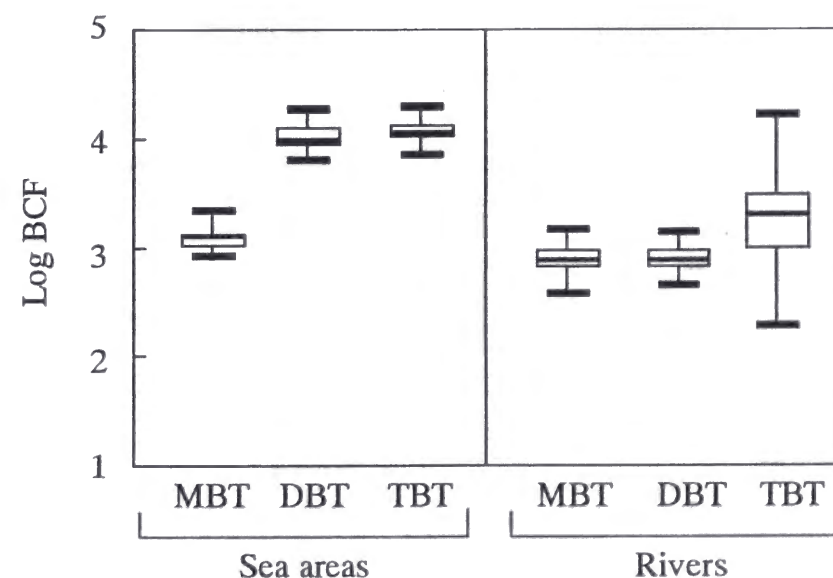


Fig.V-7 Box-and whisker displays of BCF of TBT in fish

Medians with quartiles in boxes, 90% confidence limits as bars.

3-2 Partition of tributyltin compounds in soft tissues of shellfish

BCF of TBT in mussels and scallops was calculated from the values in chapter III. Log BCF of TBT in mussels was the same as in scallops and at 4.1. Concerning BCF in mussels, there have been many reports. BCF of zebra mussels in Barcelona harbour in Spain has been reported 4.5-4.7 (Tolosa *et al.*, 1992). The values in mussels (*Dreissena polymorpha*) calculated from field samples in Westeinder of Netherland are reported at 6.0 (Becker and Tarradellas, 1994). From these papers, it is estimated that Log BCF of TBT in mussels is ranged from 4.0 to 6.0. This levels is the same as BCF in the Port of Osaka. BCF of mussel was high in the order of TBT, DBT and MBT as well as fish and medians of BCF were 3.30 for MBT, 4.40 for DBT and 4.13 for TBT. BCF of scallops were 3.84 for MBT, 4.03 for DBT and 4.10 for TBT. The order of BCF of Bts in mussel and scallop may attribute to hydrophobic property.

BCF in fish from sea areas was compared to that in shellfish. The median values of BCF in fish and shellfish were the same and BCF in fish from rivers was lower than that in fish from sea areas.

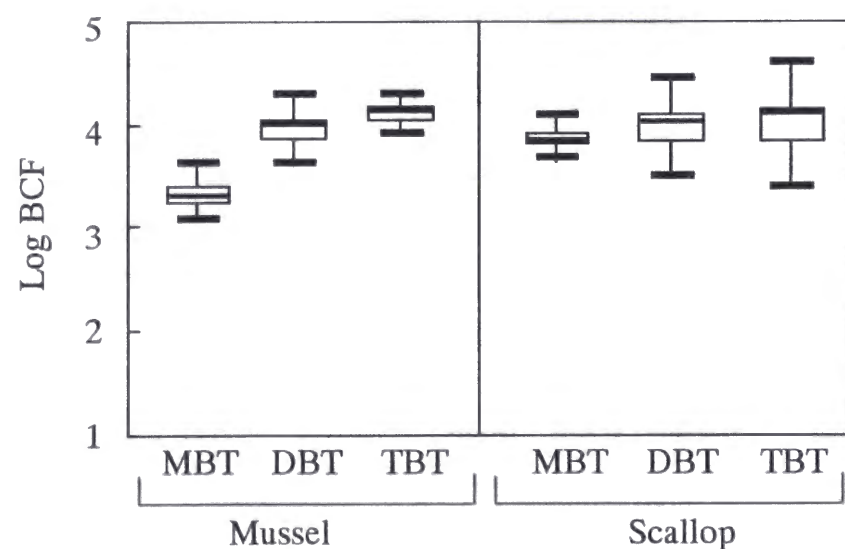


Fig.V-8 Box-and whisker displays of BCF of TBT in shellfish
Medians with quartiles in boxes, 90% confidence limits as bars.

4. SUMMARY

BCF of TBT was calculated from TBT concentration in water and aquatic organisms. Log BCF of fish from sea areas and rivers ranged from 3.6 to 4.2 and from 2.9 to 3.5, respectively. BCF of TBT in fish from sea areas was higher than those from rivers. BCFs of MBT, DBT and TBT in fish were compared. BCF in fish from sea areas and rivers were high in the order of TBT, DBT and MBT.

Log BCF of TBT in mussels was the same as in scallops and at 4.1. The orders of BCF of Bts in mussel and scallop were high TBT, DBT and MBT.

BCF in fish from sea areas was compared to that in shellfish. The median values of BCF in fish and shellfish were the same and BCF in fish from rivers was lower than that in fish from sea areas.

SECTION III

THE LEVELS OF ORGANOTIN COMPOUNDS IN DIFFERENT ORGANS AND TISSUES OF FISH AND SHELLFISH

1. INTRODUCTION

It is well known that the concentrations of chemical substances accumulated in fish are different from the organs and tissues. The highest concentrations of mercury among the organs and tissues of gray mullet was found in liver (Einaga *et al.*, 1978). Yoshida *et al.* (1973) investigated the distribution of PCBs in tissues of carp by liquid scintillation measurement using [¹⁴C] PCBs. PCBs localized mainly in the gall bladder, adipose tissue and heptapancreas but only slight radioactivity was detected in muscle. Thus, tissues and organs accumulated different chemical substances. Section I showed that TBT and TPT in muscle of fish from sea areas were detected in the range of 0.052-0.190 mg/kg wet and 0.030-0.159 mg/kg wet, respectively.

In this section, the levels and distribution of Ots in organs and tissues of fish and scallop are presented.

2. MATERIAL AND METHOD

2-1 Sampling Description

In October 1996, three species of fish including Japanese sea perch (*Lateolabrax japonicus*), white croaker (*Pennahia argentatus*) and yellow tail (*Seriola quinqueradiata*) were collected from station F4 of the Port of Osaka (Fig.V-1), and scallops (*Patinopecten yessoensis*) were taken from scallop culture of sea waters in Iwate prefecture (Fig.III-18). All species were stored below -20 °C until chemical analysis was carried out.

Heart, gill muscle, scale, spleen, liver, stomach, kidney, brain, skin, gonad and vertebra in these fish and muscle, mantle, gill and liver in scallop were analyzed.

2-2 Analytical method

Analytical method was obeyed as described in section I.

Detection limit of MBT, DBT, TBT, MPT, DPT and TPT for S/N=3 were 0.001 mg/kg wet, respectively.

3. RESULTS AND DISCUSSION

3-1 Concentration and composition of butyltin and phenyltin compounds in different organ and tissues of fish

The concentration of Bts and Pts of each organ and tissue of fish were shown in Fig.V-9. The concentration of TBT in the organs and tissues in Japanese sea perch, White croaker and yellowtail were ranged of 0.074 - 0.321 mg/kg wet, 0.026-0.276 mg/kg wet, 0.068-0.262 mg/kg wet, respectively. TBT concentration in liver was the highest among organs

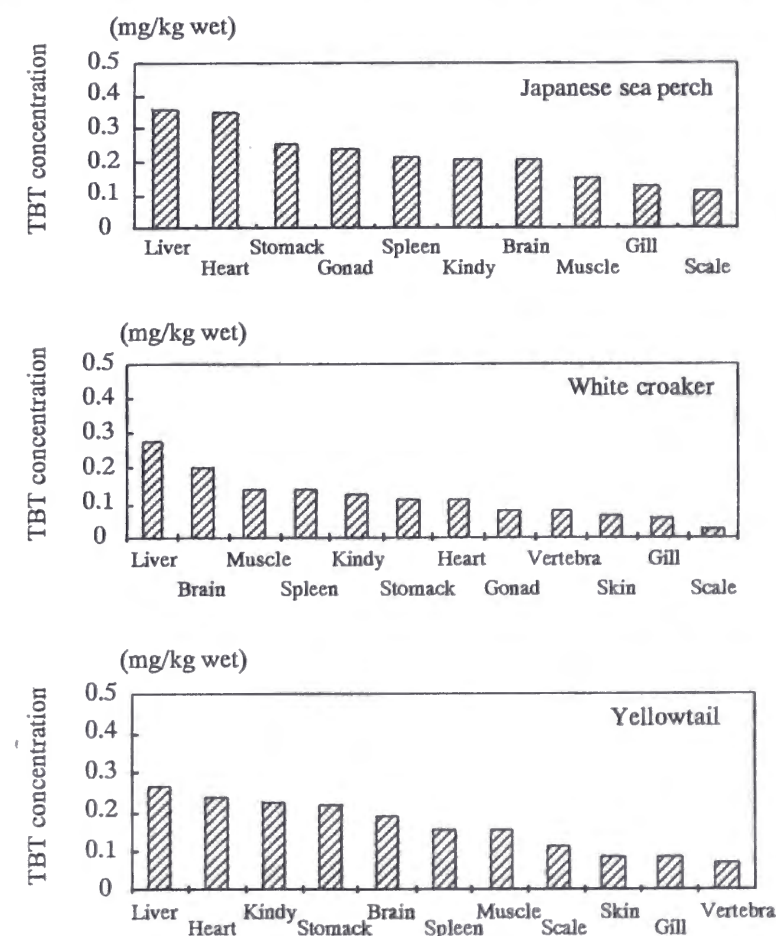


Fig.V-9 Concentration of TBT in each organ and tissue of fish

and tissues of in three fish. It was reported that Bts concentration in liver were up to one order of magnitude higher than in muscle of 17 fish species from Australia, Papua New Guinea and Solomon Islands, suggesting preferential accumulation of these compounds in excretory organs (Kannan *et al.*, 1995a). Suzuki *et al.* (1992) reported that the higher concentration of Bts in liver than in muscle was observed in yellowtail. These reports agreed with our results. Higher concentrations of TBT were observed in heart and brain. Oshima *et al.* (1997) was reported that the relative higher concentration of TBT was observed in blood of fish. It is considered that these results were obtained, because most of analyzed heart was blood. Generally, TBT concentration was low in scale, gill, vertebra and skin.

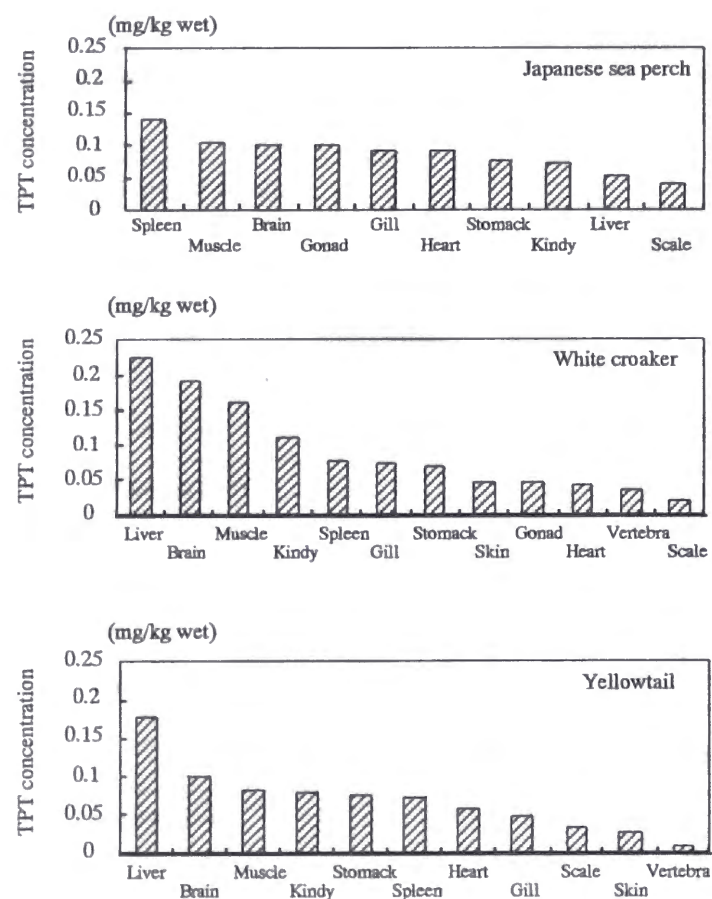


Fig.V-10 Concentration of TPT in each organ and tissue of fish

The concentration of TPT in the organs and tissues of Japanese sea perch, white croaker and yellowtail were in the range of 0.039-0.138 mg/kg wet, 0.005-0.226 mg/kg wet, 0.009-0.176 mg/kg wet, respectively (Fig.V-10). The levels of TPT in organs and tissues were lower than those of TBT. TPT was high in liver of white croaker and yellowtail as well as TBT. It is interesting trend that TPT in brain was also high. In general, the concentration of chemical substances such as heavy metals in brain was significantly lower than those in other organs, because of "blood-brain barrier". However, these results were reversed, suggesting that TBT and TPT is able to pass through the blood-brain barrier. Methyl mercury were detected in brain of carp (Yamanaka *et al.*, 1974). DDE, DDT and PCB were found in brain of harp seal (*Pagophilus groenlandicus*) and porpoise (*Phocaena phocaena*) (Holden and Morsder, 1967, Jones *et al.*, 1976). Morcillo *et al.* (1997) reported that the concentration of TPT decreased in the order liver>gill>digestive tube and no Ots residue was detected in muscle of red mullet (*Mullus barbatus*). Yamada *et al.* (1994) examined the TPT concentration in organs and tissues of red sea bream which were given the feed containing TPTCl. With dietary uptake, TPT concentration is the highest in liver and it decreases in the order of digestive tract, gill, head, skin and muscle. TPT concentration by direct uptake from water in the highest in liver and it decreases in the order of gills, digestive tract, head skin and muscle. These papers indicate that TPT concentration in liver is the highest among organs and tissues.

Fig.V-11 shows the composition of Bts and Pts in livers of the three fish. In Japanese sea perch, white croaker and yellowtail, ratio of TBT were about 25% and the combined ratio of DBT and MBT were higher than that of TBT. Kannan *et al.* (1995c) reported that ratio of MBT was higher than that of DBT and TBT in liver of 11 fish (rubberlip morwong, shovelnose ray, blue groper, sea mullet, striped seaperch, Atlantic salmon, sea mullet, mud flathead, silver tremally and sea bass) from Australia. This report was agreed with our results. DBT and MBT were detected in water samples in addition to TBT (Chapter III section II). It considered that DBT and MBT were also accumulated in liver of fish.

TPTs in liver of Japanese sea perch, white croaker and yellowtail were dominant species among Pts. It is concluded that TPT is not metabolized.

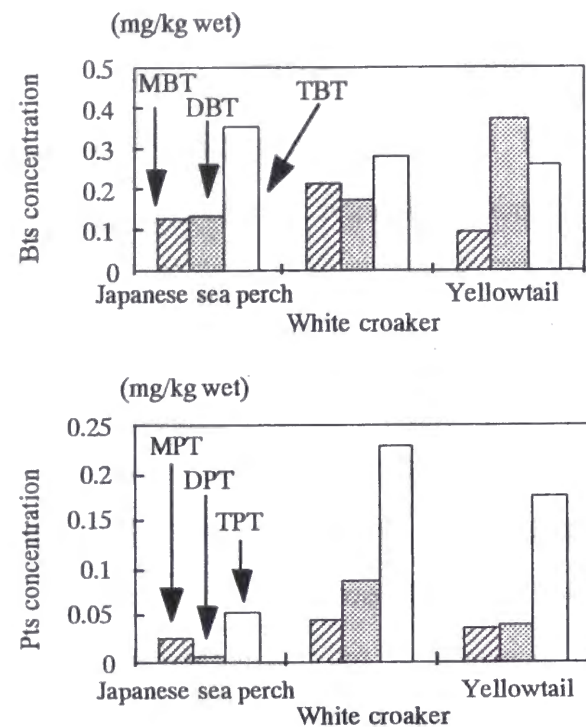


Fig.V-11 Composition of Bts and Pts in liver of fish

3-2 Butyltin and phenyltin compounds in different organs and tissues of scallop

Scallop was divided into four portions : muscle, gill, mantle and liver. The concentrations of TBT and TPT in four portions of scallop were ranged from 0.006 to 0.13 mg/kg wet and from 0.002 to 0.014 mg/kg wet (Fig. V-12). The levels of TBT decreased in the order of muscle > liver > mantle > gill. To our knowledge, there is no papers concerning the concentration of Bts in scallop. Accordingly, our data could not be compared to other papers. Guolan and Yong (1995) investigated the accumulation of TBT in mussel. TBT concentrations in gill in mussel were higher than those in muscle and mantle. This report was different from our results. This difference may attribute to the difference of kind of shellfish. In general, the ratios of TBT in tissues except for gill were

higher than the other Bts. The ratio of DBT in organs and tissues of scallop was lower than that of MBT and TBT. The order of TPT in scallop was difference from that of TBT. TPT concentration was high in the order of liver, mantle, gill and muscle. This attribute the differences of metabolism and accumulation of Ots in organs and tissues of scallop. In composition of Pts in scallops, the ratio of TPT was the highest among Pts in gill, mantle and liver of scallops.

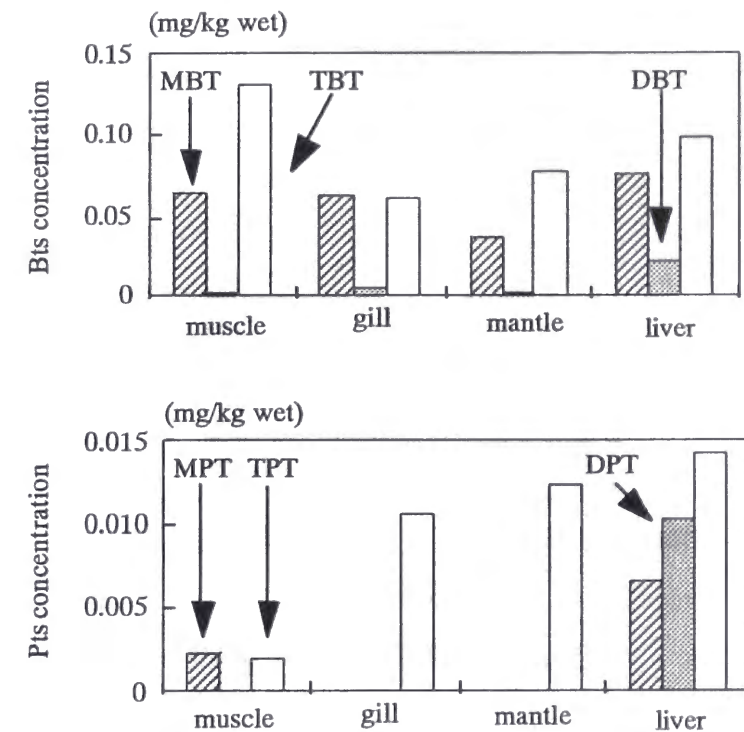


Fig.V-12 Composition of Bts and Pts in organs and tissues of scallops

3-3 Comparison of bioconcentration factor of butyltin in liver of fish and scallop

BCF of each Bt in liver of fish and shellfish was calculated (Fig. V-13). BCF in liver of fish ranged from 4.1 to 4.4 for TBT, from 4.0 to 4.3 for DBT and from 3.3 to 3.7 for MBT. BCFs of Bts in liver of Japanese sea perch, white croaker and yellowtail were high

in the order of TBT, DBT and MBT. This orders of Bts in liver of fish were the same as those in muscle of fish. It is considered that hydrophobic interaction make relatively more contributions to BCF in liver of fish. Tsuda *et al.* (1988) reported that BCFs of TBT and DBT in liver of carp (*Cyprinus carpio Linne*) were 2.1 and 1.0, respectively. The orders of BCF of TBT and DBT were roughly agreed with our results. BCFs of TBT in liver of scallops were the highest among Bts and BCFs of MBT were the same as that of DBT. The orders of BCFs of Bts in liver of shellfish were different from those of fish a little. Though BCF of DBT in scallop was lower than that in fish. BCFs of MBT and TBT in shellfish were the same levels as those in fish. These results show that TBT was accumulated in liver of fish and shellfish more easily.

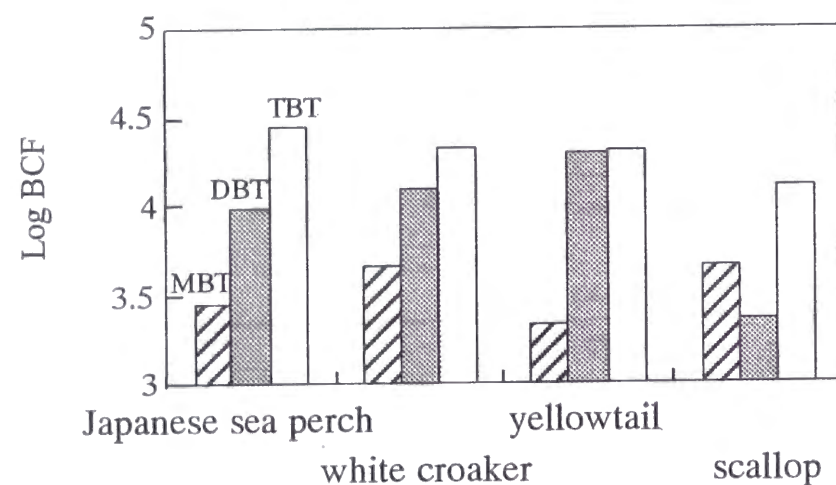


Fig.V-13 BCF of Bts in liver of fish and shellfish

4. SUMMARY

Considerable concentration of TBT and TPT were detected in every organs and tissues of fish. The concentrations of TBT and TPT in liver of fish were highest among organs and tissues. DBT and MBT were also accumulated in liver of fish. Further, distribution of TBT and TPT in organs and tissues is characterized that relative high concentrations of TBT and TPT are observed in heart and brain.

The levels of TBT in scallop decreased in the order of muscle > liver > mantle > gill. The ratios of TBT in organs and tissues except for gill were high than the other Bts. The levels of TBT in scallop decreased in the order of muscle > liver > mantle > gill and the ratio of TPT was the highest among Pts in four portions of scallops. BCFs of Bts in liver of fish were high in the order of TBT, DBT and MBT and those in scallop were high in the order of TBT, MBT and DBT.

SECTION IV CONCLUSION

Ots levels in muscle of fish from rivers and sea areas were described. The concentrations of TBT in fish from sea areas were higher than those from rivers and the ratio of TBT was the highest among Bts. In spite of no detection of TPT in waters from sea areas, relatively higher concentration of TPT was detected in muscle of fish. These results mean that Ots accumulate in fish easily and in particular, TPT is persisted for a long time. On the other hand, no correlation between TBT or TPT concentration and the total length or lipid contents were observed, suggesting that the distribution mechanism of Ots in fish is difference from those of hydrophobic substances.

BCF of TBT in fish from sea areas was also higher than those from rivers. BCF of TBT in fish from sea areas was the same levels as that in mussel and that in mussel was the same as scallops.

Ots were detected in every organs and tissues of fish. Ots levels in liver of fish were highest among organs and tissues and relative high concentration of TBT and TPT were observed in heart and brain. The levels of TBT in scallop decreased in the order of muscle > liver > mantle > gill and the ratio of TPT was the highest among Pts in four portions of scallops. BCF of TBT in liver of fish and shellfish was the highest among Bts. These findings indicate that Ots levels must be investigated not only in muscle but also in liver of fish, in order to conserve aquatic organisms.

CHAPTER VI CHARACTERIZATION OF DISTRIBUTION OF ORGANOTIN COMPOUNDS IN AQUATIC ENVIRONMENT - COMPARISON BETWEEN ORGANOTIN COMPOUNDS AND ORGANOCHLORINE COMPOUNDS

SECTION I COMPARISON OF PARTITION TO SEDIMENT AND AQUATIC ORGANISMS IN FIELD

1. INTRODUCTION

Ots released to water from antifouling paint are deposited to sediment and concentrated to aquatic organisms. Accordingly, it is essential to estimate the characterization of partition to sediment and aquatic organisms. There are many reports concerning partition of organic compounds such as PCB to each compartment. It well known that 94% of the total PCBs present was in sediments, and a further 4% was found in seawater and fish (Tanabe *et.al.*,1989). These results show that most of PCBs accumulate in sediment. In this section, the partition of Ots in aquatic environment was characterized by comparison with that of PCBs.

2.MATERIAL AND METHOD

2-1 Sampling description

Fig.VI-1 shows sampling sites. water samples were collected using bucket from 4 sites in the Port of Osaka in February 1995. Sediment and plankton samples were taken with Eckman birge grab sampler and plankton net (NXX17 horizontal haul) in February and August 1995. Ignition loss of sediment ranged 11-12 %. In this study, kind of net

plankton was not identified. Mussel samples were collected from station C2 in the Port of Osaka. Shell length of mussel ranged from 2.1 cm to 6.7 cm. TBT and PCBs in these samples were measured. Water samples were maintained at 5 °C and other samples were stored at -20 °C until chemical analysis were conducted.

2-2 Analytical methods

Organotin compounds

Analysis of Ots was conducted as described in chapter II.

PCBs

PCBs were analyzed by the method described by Wakimoto *et al.* (1971) with some modifications. Ten liter of water samples were extracted twice by shaking with hexane for 10 min. After drying with anhydrous Na₂SO₄, the extracts were concentrated up to 5ml using a Kuderna-Danish (KD) and then were passed the silica gel chromatography. The analytes were

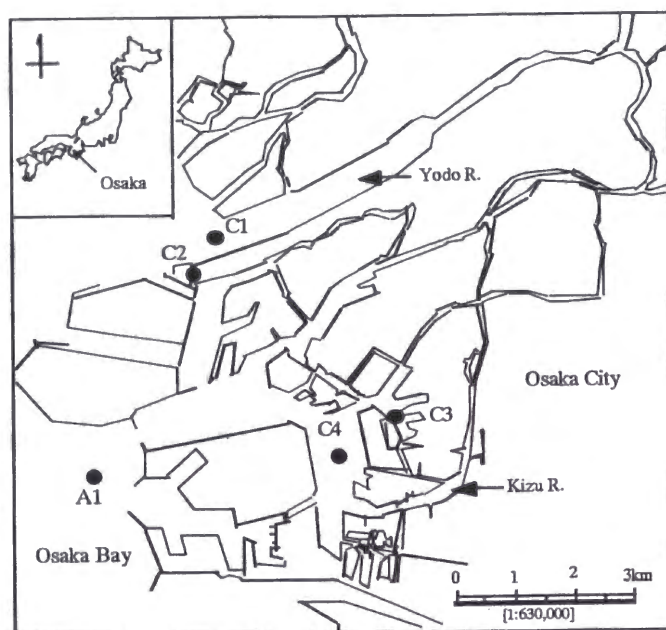


Fig. VI-1 Sampling area

eluted with 150 ml of hexane and elutes were concentrated up to 4 ml. Five ml of 1N NaOH/ethanol were added to the eluate and stand for over night. After separation, 5ml of 7% sulfuric acid were added to the organic layer. The organic layer was washed with 5ml of 7% fuming sulfuric acid and neutralized with 0.1N KOH solution. After washing with distilled water, the organic layer concentrated with a stream of nitrogen. The analytes were conducted on a gas chromatography equipped with ECD.

For sediment analysis, 10 grams were refluxed with 1N KOH ethanol solution for 6

hours. The analytes were extracted twice with 100 ml of hexane after adding 500 ml of distilled water. The combined organo layer concentrated up to 5ml with KD after drying with anhydrous Na₂SO₄. Further treatment was the same as described for water samples.

Ten of wet aquatic organisms were placed together with 30 ml of acetone and the mixture was extracted for 10 min in a mechanical shaker. After removal of the supernatant with centrifuge at 3,000 r.p.m. from 10 min, the residue was re-extracted for 20 min with 50 ml of acetone and then the mixture was centrifuged. The organic layer was separated after shaking the combined supernatants with 500 ml of 20% NaCl solution and the analytes were extracted with 100 ml of dichloromethane twice for 10 min. The combined organic layer were concentrated up to 5ml using KD after addition of 100 ml of hexane. Further procedure was the same as described for water sample. PCBs concentrations were displayed as total PCBs.

3. RESULTS AND DISCUSSION

3-1. Comparison of horizontal distribution of PCBs and tributyltin in the Port of Osaka

PCBs concentration in water from 4 sites of the Port of Osaka ranged from 0.008 µg/l to 0.32 µg/l (Fig. VI-2). Maritime safety agency, Japan (1997) in Japan was investigated PCB concentrations at 13 sites in Japan. According to them, PCBs levels in Japan were less than 0.0001µg/l. PCBs levels in the Port of Osaka is highest among Japanese areas. PCBs concentration in water at station C3 was the highest among the stations in the Port of Osaka. PCBs was detected in the range of 0.0005-0.10 mg/kg dry from 13 sites in Japan except for the Port of Osaka (Maritime safety agency, 1997). PCBs levels in sediment in the range of 0.1-2.4 mg/kg dry (Fig. VI-2). PCBs levels in sediment in the Port of Osaka are also higher than other areas in Japan. PCBs concentrations in sediment at C3 was highest among those at the stations in the Port of Osaka. PCBs levels in

plankton from the Port of Osaka ranged of 0.85-4.3 mg/l (Fig. VI-2). PCBs concentration at station C3 was higher than other sites as well as water and sediment.

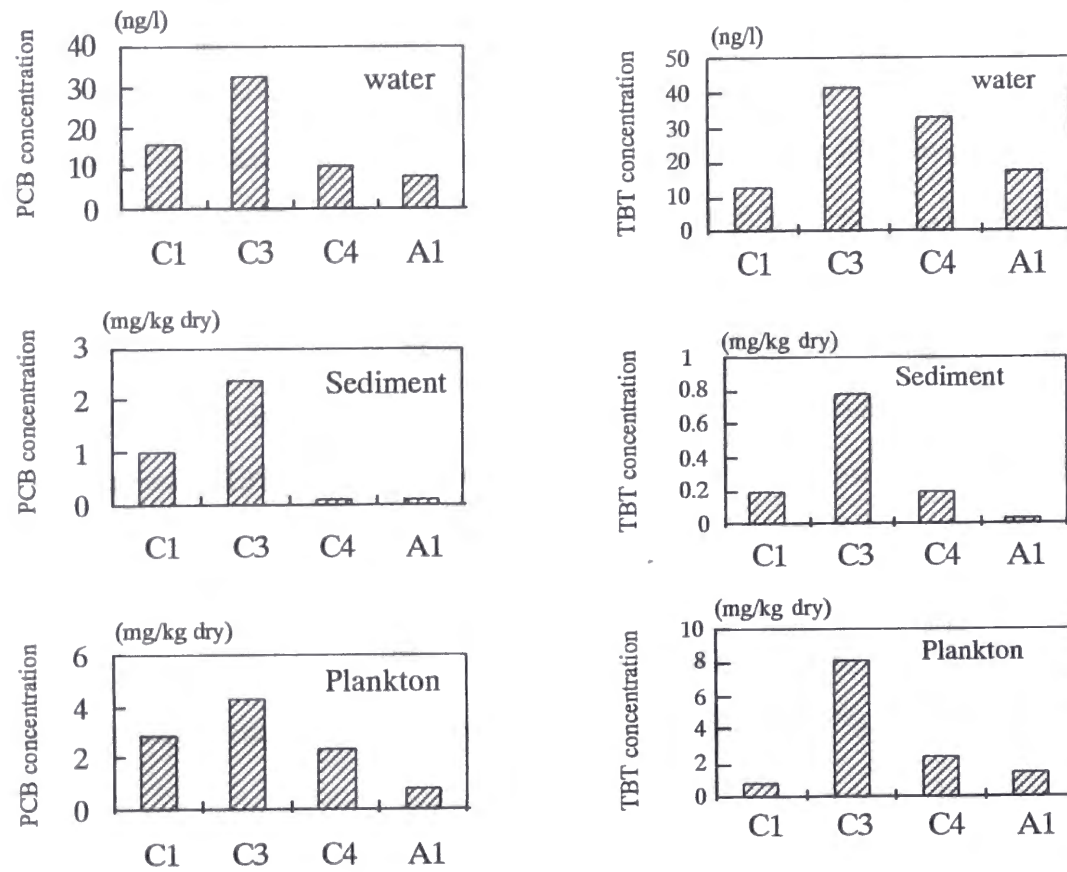


Fig.VI-2 Horizontal distribution of PCBs in the Port of Osaka in February 1995.

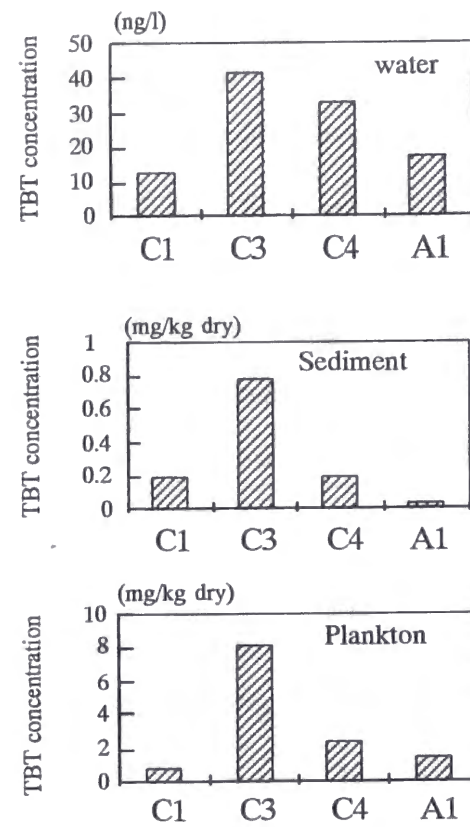


Fig.VI-3 Horizontal distribution of TBT in the Port of Osaka in February 1995

TBT levels in the Port of Osaka are shown in FigVI-3. TBT concentrations in water , sediment and plankton were detected in the range of 0.013-0.042 µg/l , 0.04-0.78 mg/kg dry and 0.78-8.2 mg/kg dry, respectively. TBT levels at station C3 is highest among stations as well as PCBs levels. Thus horizontal distribution of TBT in the Port of Osaka was roughly agreed with that of PCBs.

Mussel samples were collected seasonally at C2. PCBs and TBT concentration in mussel from the Port of Osaka were detected in the range of 0.1 - 0.25 mg/kg wet and

0.09 - 0.19 mg/kg wet, respectively (Table VI-1).

Table VI-1 The concentrations of PCBs and TBT in mussel samples in 1995

	PCBs	TBT
February	0.16	0.19
June	0.18	0.09
August	0.10	0.14
November	0.25	0.14

3-2. Comparison of partition to sediment in field

K_{sw} of TBT and PCBs were compared from field data in 1993 (Fig.VI-4). Log K_{sw} of TBT ranged from 3.6 to 4.3 and median was 4.00. Partition to sediment of PCBs was calculated and median of log K_{sw} was

4.4 and ranges were from 4.1 to 4.9. It is concluded that K_{sw} of TBT were lower than

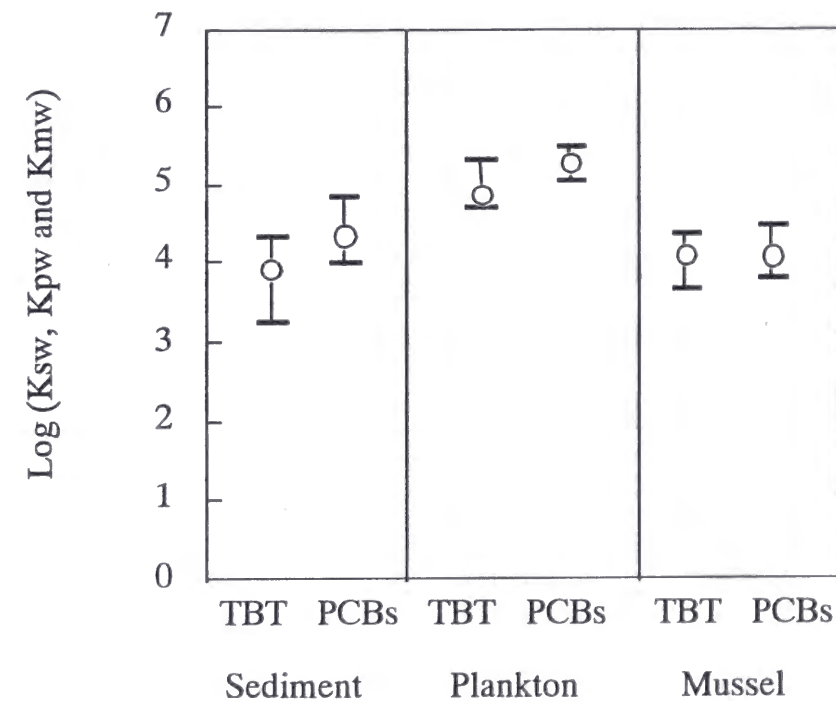


Fig.VI-4 Partition to sediment and aquatic organisms Comparison between TBT and PCBs

PCBs in the Port of Osaka. In general, organic substances desorbed to sediment more easily with increase of ignition loss. Partition of TBT in sediment is due to desorption to surface particles. organic substances are rich because ignition loss ranged from 11 to 12 %. on the other hand, log P_{ow} of TBT and PCBs were in the range of 2.8 - 3.8 (Tsuda *et al.*, 1990, Laughlin *et al.*, 1986a) and 6.1 - 7.4 (Hawker and Connell., 1988), respectively. These P_{ow} values show that PCBs have more hydrophobic substances than TBT. Therefore, it is considered that K_{sw} of PCBs was higher than that of TBT.

3-3 Comparison of concentration to aquatic organisms in field

Based on field data in the Port of Osaka, partition coefficients of TBT and aquatic organisms were compared to those of PCBs (Fig.VI-4). K_{pw} and K_{mw} of TBT were calculated by dividing their TBT concentration in aquatic organisms by those in waters in field. Median and ranges of log K_{pw} of TBT were 4.9 and ranges were from 4.8 to 5.3. Median of log K_{mw} of PCB were 5.2 and ranges from 5.0 to 5.3. Median of log K_{mw} of PCBs was 4.0 and ranges were from 3.8 to 4.2. Median of log K_{mw} of TBT was 4.1 and ranges were from 3.9 to 4.2. These results show that partitions of TBT to plankton were lower than those of PCBs. In the case of plankton, partition is due to adsorption to surface of plankton and intake in plankton. However, the adsorption make more contribution to partition, because surface area of plankton is large. These K_{pw} of PCBs were higher than that of TBT as well as K_{sw} . K_{mw} of TBT was the same as PCBs. This reason is not clear.

4. SUMMARY

Preliminary, K_{sw} of TBT were compared to those of PCBs. Log K_{sw} of TBT ranged from 3.6 to 4.3 and median was 4.0. While, log K_{sw} of PCBs was in the range of 4.1 - 4.9 and median was 4.4. It is concluded that K_{sw} of TBT was lower than those of PCBs.

Secondly, K_{pw} and K_{mw} of TBT were compared to those of PCBs. Log K_{pw} of TBT and PCBs were 4.9 and 5.2, respectively. While, log K_{mw} of TBT and PCBs were 4.1 and 4.0, respectively. K_{pw} of TBT was lower than that PCBs and K_{mw} of TBT was the most same as that of PCBs.

SECTION II
ESTIMATION OF DISTRIBUTION FROM PHYSICO CHEMICAL
PARAMETER

1. INTRODUCTION

The environmental fate of contaminants is determined by the joint action of several factors ascribable either to the nature of the substance or to the environment. As representative parameter express the physico-chemical properties, water solubility and octanol-water partition coefficient are used frequently. Water solubility was widely used to estimate sorption by sediment (Karickhoff, 1978). In general, partition coefficient to sediment was increasing with decreasing of water solubility. Water solubilities and octanol-water partition coefficients (P_{ow}) have been also used to estimate bioconcentration of organic compounds from water (Veith, 1979). BCF was increasing with decreasing of water solubility or increasing of octanol - water partition. The aim of this section is to examine whether the behavior of Ots can be estimated from physico-chemical properties as well as organic compounds such as PCBs.

2. ESTIMATION OF PARTITION TO SEDIMENT FROM WATER SOLUBILITY

Negative correlation between water solubility and K_{sw} is reported. For example, Chiou *et al.*(1979) proposed equation (1).

$$\text{Log } K_{sw} = - 0.557 \text{ X Log WS(mg/l) + 4.040} \quad \text{---- (1)}$$

Water solubility (WS) of PCBs ranged from 0.037 to 0.42 mg/l (Tanabe and Tatsukawa, 1981). K_{sw} was estimated by substituting water solubility of PCBs into equation (1). Estimated Log K_{sw} ranged from 4.3 to 4.8 and median was 4.5 (Table VI-2). As described in section I, range of log K_{sw} of PCBs was from 4.08 to 4.9 and median was 4.4 in field data. This values were agreed with estimated K_{sw} .

Water solubility of TBT ranged from 5 - 50 mg/l (Watanabe, 1994). K_{sw} was calculated by substituting water solubility of TBT into equation (1). Estimated K_{sw} from water solubility of TBT ranged 3.1-3.7 and median was 3.3 (Table VI-2). Log K_{sw} of TBT was 4.50 in the Port of Osaka. Although estimated Log K_{sw} of PCBs was the same as observed one, in the case of TBT, estimated log K_{sw} was lower than observed one.

Table VI-2 Estimation of partition of sediment from water solubility

	PCBs	TBT
Observed Log Ksw	4.1 - 4.9 (4.4) ^a	3.4 - 4.3 (4.0)
Estimated Log Ksw	4.3 - 4.8 (4.5)	3.1 - 3.7 (3.3)

a : median

Table VI-3 Variation of water solubility of TBTO with pH
(Maguire *et al.*, 1983)

PH	Solubility, mg/L	
2.0	glycine hydrochloride	60±3
2.4	glycine hydrochloride	37±1
2.6	glycine hydrochloride	30±2
2.8	glycine hydrochloride	22±2
3.0	formate	12±2
4.0	acetate	10±1
5.0	acetate	7±1
5.6	acetate	5±2
6.0	phosphate	0.75±0.5
6.6	phosphate	0.75±0.5
7.0	phosphate	4±1
7.6	phosphate	1±0.5
7.8	phosphate	1.5±0.5
8.1	Tris-HCl	31±1
9.2	Tris-HCl	29±2
10.0	glycinate	18±6
10.8	carbonate	14±1

Table VI-3 shows that the water solubility of Ots is at minimum of 0.75 mg/l at pH 6.0-6.6 and increases with both decreasing and increasing pH (Maguire *et al.*, 1983). Thus, it is considered that water solubility of TBT change as TBTO, because TBT is ionic chemical substances. This means that chemical forms of TBT are changed by pH. Therefore, K_{sw} could not be estimated from water solubility.

3. ESTIMATION OF BIOCONCENTRATION FACTOR FROM WATER SOLUBILITY

BCF can be also estimated from water solubility. Fukushima *et al.* (1996) proposed the equation (2) in order to estimate the BCF from water solubility of PCBs.

$$\text{Log BCF} = 0.55 \times \text{Log WS (mg/l)} + 3.80 \text{ ---- (2)}$$

Estimated log BCF of PCBs ranged from 4.0 to 4.6 and median was 4.2 (Table VI-4). Observed log BCF calculated from field data, ranged from 5.0 to 5.3 (median : 5.2). Estimated log BCF were a little lower than observed log BCF. Log BCF of TBT were estimated from the water solubility. Ranges of calculated log BCF was from 2.9 to 3.4 and median was 3.1 (Table VI-4). Observed log BCF in plankton ranged from 4.8 to 5.3 and median was 4.9. In the case of TBT, estimated log BCF was remarkably different

Table VI-4 Estimation of BCF of plankton from water solubility

	PCBs	TBT
Observed Log BCF	5.0 - 5.3 (5.2) ^a	4.8 - 5.3 (4.9)
Estimated Log BCF		
WS → BCF	4.0 - 4.6 (4.2)	2.9 - 3.4 (3.1)
Pow → BCF	4.5 - 5.6 (5.0)	2.2 - 2.9 (2.6)

a : median

from the observed log BCF.

Log BCF in mussel were estimated by equation (3) (Geyer *et al.*, 1982).

$$\text{Log BCF} = -0.682 \times \text{Log WS}(\mu\text{g/l}) + 4.94 \text{ ---- (3)}$$

In the case of PCBs, estimated log BCF ranged from 4.5 to 5.6 and median was 5.0 (Table VI-5). Observed log BCF calculated from field data ranged from 3.8 to 4.2. Estimated log BCF were value near observed log BCF. On the other hand, estimated log BCF of TBT ranged from 1.7 to 2.0 and median was 2.0 (Table VI-5). Observed log BCF ranged from 3.9 to 4.2 and median was 4.1. Estimated BCF was considerably lower than observed BCF.

4. ESTIMATION OF BIOCONCENTRATION FROM OCTANOL WATER PARTITION

There are many reports concerning estimation of BCF from Log P_{ow} . Log P_{ow} indicate the extend of TBT which is concentrated in plankton and mussels. For example, relationships between BCF and Log P_{ow} such as equation (4) are reported for plankton by Southworth *et al.* (1978).

$$\text{Log BCF} = 0.700 \times \text{Log Pow} + 0.260 \text{ ---- (4)}$$

Log P_{ow} of PCBs is reported to be ranged of 6.1 -7.4 (Hawker and Connell, 1988). From these values, estimated log BCF of plankton was in the range of 4.5-5.6 and median was 5.0 (Table VI-4). From field data, range of log BCF of plankton was 5.0-5.3 and median was 5.1. Concerning PCBs, estimated values roughly coincided with observed ones. On the other hand, log P_{ow} of TBT reports to range from 2.8 to 3.8 (Tsuda *et al.*, 1990, Laughlin *et al.*, 1986a). Estimated log BCF of TBT in plankton ranged from 2.2 to 2.9 and median of log BCF was 2.6 (Table VI-4). Log BCF of TBT in plankton from the Port of Osaka ranged from 4.8 to 5.9 and median was 2.6. The observed log BCF was remarkably higher than the estimated one from log P_{ow} .

Table VI-5 Estimation of BCF of mussel from log P_{ow}

	PCBs	TBT
Observed Log BCF	3.8 - 4.2 (4.0) ^a	3.9 - 4.2 (4.1)
Estimated Log BCF		
WS → BCF	3.2 - 3.9 (3.4)	1.7 - 2.0 (2.0)
Pow → BCF	4.5 - 5.6 (5.0)	1.6 - 2.4 (2.0)

a : median

Geyer *et al.*, (1982) examined the relevance of P_{ow} for predicting the tendency of variously structured organic chemicals to bioconcentration in the mussel (*Mytilus edulis*). As a result, linear regression between P_{ow} and BCF was obtained.

$$\text{Log BCF} = 0.858 \text{ X } \log P_{ow} - 0.808 \quad \text{----- (5)}$$

Log BCF of PCB in mussel was estimated by equation (5). Estimated log BCF ranged from 4.5 to 5.6 and median was 5.0 (Table VI-5). In the Port of Osaka, range and median of log BCF was 3.8-4.2 and 4.0 respectively. Namely, the observed log BCF of PCB was a little lower than the estimated one from log P_{ow} .

Log BCF of TBT estimated from equation (4) ranged from 1.7 to 2.4 and median was 2.0 (Table VI-5). The observed log BCF of TBT was remarkably higher than the estimated one from log P_{ow} as well as plankton. Namely, the difference that between observed value and estimated values in TBT is lower than those in PCBs.

Positive correlation between BCF and Log P_{ow} of hydrocarbon such as PCBs was observed. However BCF of TBT in plankton and mussel did not reflect log P_{ow} .

Laughlin *et al.*(1986a) report that octanol-water partition coefficients for TBT vary as a function of salinity and the lowest value, 5500, was measured in 25‰ and increased in higher of lower salinities to a maximum of 7000 in deionized water. Further P_{ow} was also depended on pH and salinity (Arnold *et al.*, 1997). On the other hand, PCBs were independent of the condition of water. These findings show that accumulation mechanism of TBT in aquatic organisms can not estimate from P_{ow} .

4. SUMMARY

Observed BCF of PCB agreed with the estimated one from P_{ow} , however, BCF of TBT in plankton and mussel did not reflect P_{ow} . It may depend on ionic and hydrophobic groups of Ots. In general, K_{sw} can estimate from water solubility. For example, estimated K_{sw} of PCBs coincided with observed log K_{sw} . While, estimated K_{sw} of TBT did not agree with observed one. Furthermore, BCF can be also calculated from P_{ow} . Log BCF of PCBs was estimated from P_{ow} , however, in the case of TBT, estimated BCF was lower than observed one.

SECTION III

DIFFERENCE OF DISTRIBUTION BETWEEN FIELD AND LABORATORY EXPERIMENT

1. INTRODUCTION

Under field condition, environmental variables (e.g., temperature, pH, light wavelength and irradiation intensity, water and air turbulence, living organisms) are quite complex. Therefore, it is difficult to be clear significant changes in environmental behavior of contaminants. So, in order to explain the behavior of chemical substances in environment, the results in laboratory experiment are used frequently.

In this section, it is discussed whether data of Ots in laboratory experiment can be reflected values in field by comparison with the case of PCBs.

2. PARTITION TO SEDIMENT

K_{sw} of TBT in field were shown in section I. K_{sw} in field is defined as TBT concentration in sediment / TBT concentration in water. K_{sw} of TBT in the Port of Osaka ranged from 2.3×10^3 to 5.8×10^3 and median of K_{sw} was 10^4 . Fent and Hunn (1991) report to be in the range from 2.9×10^2 to 1.7×10^3 at three sites in Lake Lucerne of Switzerland. Unger *et al.* (1988) report the K_{sw} of TBT from estuarine and freshwater sediment of Hampton Roads were 2.5×10^3 and 5.5×10^3 , respectively. From these results, it is estimated that K_{sw} in field is in general ranged from 10^2 to 10^4 . On the other hand, in laboratory experiment, Unger *et al.* (1988) also reported that sorption coefficient of TBT was estimated to be between 1.1×10^2 and 8.2×10^3 from isotherm for TBT on Chesapeake Bay sediment. Kram *et al.* (1989) report that K_{sw} of TBT ranged from 10^3 to 5.0×10^3 in sediment from Pear Harbour after shaking for 24 hr in a lighten room. Maguire *et al.*, (1985) report that to extent of binding of TBT to sediment, 100 ng of wet sediment were shaken with 10ml of a solution of $0.1 \mu\text{g}$ of Sn / L of TBT for periods of

1-16 days. K_{sw} was 1.8×10^3 - 2.5×10^3 at 20°C after 4 days of shaking. Judging from these results, TBT partition coefficient was estimated in the range of 10^3 - 10^4 . As regards TBT, K_{sw} estimated from field data was roughly agreed with that in laboratory. Ranges of K_{sw} from field data were more wide than those from laboratory data. Maguire *et al.* (1985) report that adsorption of TBT to sediment report to depend on salinity and pH in water or grain size distribution, clay mineralogy and percentage of organocarbon of sediment. Therefore, in the case of TBT, wide ranges of K_{sw} due to the condition in aquatic environment.

On the other hand, K_{sw} of PCBs was ranged from 1.1×10^4 to 7.5×10^4 (median, 3.7×10^4) and in the Port of Osaka. Hiraizumi *et al.* (1979) report that K_{pw} of PCB for Freundlich type isotherm equation was ranged from 2.0×10^4 to 4.0×10^4 in active sludge. K_{sw} of PCBs from laboratory experiment was agreed with those of K_{sw} of PCBs in field.

3. BIOCONCENTRATION FACTOR

K_{sw} of TBT to plankton ranged from 6.2×10^4 to 7.8×10^4 and median was 7.6×10^4 in field. While, there is not any report about partition of TBT to plankton in laboratory experiments. Therefore these values could not be compared. Log BCF of TBT in mussel was ranged from 7.2×10^3 to 1.5×10^4 and median was 9.2×10^3 in the Port of Osaka. Zuolian and Jensen (1989) reported that K_{mw} ranged of 0.5×10^4 to 6×10^4 after 51 days in seawater from Rungsted marina in Denmark and that it depended on the concentration of TBT in the water. While, BCF of TBT have been estimated in laboratory experiments using radioactive tracer over 47 days accumulation. As a result that BCFs of TBT were only up to 6×10^3 (Laughlin *et al.*, 1986b). Namely, BCF in laboratory experiments agreed with those calculated from field data. It is of particular interesting that at TBT concentration of 45 ng/l or less, the tissue burden becomes stable after several days in laboratory experiments, however, the constant after several days, was not observed in

field experiments.

About PCB, range and median of $\log K_{pw}$ was from 1.0×10^5 to 2.2×10^5 and 1.5×10^5 in this study area. Kawai *et al.* (1984) report that partition of PCBs ranged from 9.7×10^4 to 1.2×10^5 in the Port of Osaka. In laboratory experiment, Hiraizumi *et al.* (1979) reports that $\log K_{pw}$ of PCBs ranged from 10^4 to 5×10^4 in zoo plankton. Log BCF from the field data was roughly agreed with that from laboratory data. BCF in mussel ranged from 0.6×10^4 to 1.6×10^4 and median was 1.1×10^4 in the Port of Osaka. To our knowledge, it is not reported that concerning bioconcentrations of PCBs to mussels in laboratory experiments. BCF in field can not be therefore compared to those in laboratory experiments. Accordingly, it is not possible to compared BCF obtained in the field with those obtained in laboratory experiment.

4. SUMMARY

K_{sw} of TBT estimated from field data roughly agreed with that in laboratory as well as PCBs. While, BCF of PCBs and TBT in field data is also the same as that in laboratory experiments. These results shows the importance of laboratory experiment, in order to explain the distribution of Ots.

SECTION IV

CONCLUSION

Preliminary, partition coefficients of TBT to sediment were compared to those of PCBs. Partition coefficient of TBT to sediment ranged from 3.4 to 4.3 and median of that was 4.0. While, partition coefficient to sediment of PCBs was in the range of 4.1-4.9 and median of K_{sw} was 4.4. It is concluded that the partitions of TBT to sediment are lower than those of PCBs. While, partition coefficient of TBT and PCBs to plankton ranged from 4.9 and 5.2, respectively. From these results, Partition of TBT to plankton was lower than that of PCBs, Furthermore, partition of TBT to mussel was the same as PCBs.

The observed \log BCF of PCB agreed with the estimated one from $\log P_{ow}$, however, BCF of TBT in plankton and mussel did not reflect $\log P_{ow}$.

As regards TBT, K_{sw} estimated from field data roughly agreed with that in laboratory. Moreover, BCF of TBT in field were also the same as in laboratory experiments as well as PCBs. From reports concerning BCF of mussel, it is found that the burden of TBT in soft tissues becomes stable after several days in laboratory experiments however, this stabilization after several days, was not observed in field experiments.

CHAPTER VII

GENERAL CONCLUSION

The object of this study is to make clear the distribution of Ots in aquatic environment.

Preliminary, we developed the analytical method of organotin compounds and the current status of Ots in Osaka Bay was investigated. The results of this investigation showed that higher concentration of TBT in coastal area was observed. The levels and temporal trend of Ots in water, sediment, and plankton and mussels as a biological indicator from the Port of Osaka were therefore monitored. The Port of Osaka was contaminated heavily by Ots, and in spite of regulation for Ots, TBT levels were not decreased during 1992-1996 and those in water were level which cause the imposex. Furthermore, the levels of Ots in the Port of Osaka was compare to those in Otsuchi Bay. TBT levels in the Port of Osaka were higher than those in Otsuchi Bay and higher concentration of TPT in Otsuchi Bay was detected. these data suggest that the difference of utilization of the port reflect the Ots levels.

Secondly, degradation of Ots by microorganisms in investigated in order to elucidate the temporal changes of Ots. TBT is degraded faster by microorganisms in water than those from sediment with those expected.

It is well known that Ots accumulate in aquatic livings easily, Thirdly, Ots in fish and shellfish were therefore investigated. The levels of Ots in fish from sea areas were higher than from rivers and the higher level of Ots in liver of fish was observed among organs and tissues of fish.

Finally, the distribution of Ots was compared to that of PCBs and the characterization of Ots in aquatic environment was found. The behavior of Ots in aquatic environment found to be effected by quality of water such as pH, salinity and etc..

In the near future, organometallic compounds will be used increasingly in the various field and be released to environment. Analysis of organometallic compounds is in general very difficult because of unstable substances. Moreover distribution of them are not

estimated easily because change of solubility by condition of environment. This study will therefore become the basic data of behavior of organometallic compounds.

CHAPTER VIII
LIST OF RESULTS

The results in this study are described below.

- 1) Analytical method of Ots was developed. The analytical procedure was extraction with 0.1% tropolone-benzene followed by propylation, clean up with florisil column and the determination by GC/FPD. The detection limit of Ots in water, sediment and biological samples was 0.003mg/l, 0.005 mg/kg dry and 0.005 mg/kg wet, respectively.
- 2) The relative high concentration of TBT was observed in water and sediment from Osaka Bay.
- 3) In the Port of Osaka, the highest concentration of TBT was observed at stations which located at marina and mooring of small- and medium-hull vessels.
- 4) TBT concentrations in water and mussels have decreased, ones in sediment and plankton were uniformed during 1989-1996.
- 5) The current status of Ots in Otsuchi Bay were investigated. TBT concentration in sediment was about thousand times more than that of water and TBT levels in mussels were the same as those in scallops and TBT in fish were the lowest among all aquatic organisms.
- 6) TBT levels in water from the Port of Osaka were lower than those from Otsuchi Bay, however TBT in sediment, plankton, mussels from the Port of Osaka were higher than those from Otsuchi Bay.
- 7) TPT levels in Otsuchi Bay were higher than those in the Port of Osaka.
- 8) At the concentration of DBT, TBT and MPT of 10mg/l, decrease of the CFU count was observed after a day of incubation.
- 9) TBT was degraded MBT and DBT by microorganisms in water and from sediment.
- 10) TBT concentrations in fish from sea areas were higher than those from rivers.
- 11) In spite of no detection of TPT in water, TPT was detected in fish.
- 12) No correlation between TBT or TPT concentration and the total length or lipid

contents were observed.

- 13) BCF of fish in sea areas was higher than that in rivers significantly.
- 14) BCF of TBT in mussels was the same values as that in scallops.
- 15) Ratio of mono- and di- Ots were high in liver of fish.
- 16) K_{sw} and K_{pw} of TBT were lower than those of PCBs, however K_{tw} of TBT was agreed with those of PCBs.
- 18) Although K_{sw} , K_{pw} and K_{tw} of PCBs could be roughly estimated from water solubility and P_{ow} . TBT could not be estimated them, because solubility of TBT change by the condition.
- 19) TBT in field experiment coincided with ones in laboratory experiment as well as PCBs.

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