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Concentrations of Heavy Metals, Organochlorines, and Organotins in Horseshoe Crab, *Tachypleus tridentatus*, from Japanese Coastal Waters

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Abstract. Concentrations of heavy metals, organochlorine pesticides, polychlorinated biphenyls and organotins were determined in horseshoe crabs, *Tachypleus tridentatus*, collected from Japanese coastal waters. Heavy metal concentrations were high in the hepatopancreas, gill and egg. Residue levels of heavy metals were comparable to those recorded in most benthic organisms from Japanese coastal waters. Organochlorine concentrations were detected at a few ng/g and the residue pattern followed the order of polychlorinated biphenyls (PCBs) > chlordane compounds (CHLs) > hexachlorocyclohexanes (HCHs) > dichlorodiphenyltrichloroethane (DDTs) > hexachlorobenzene (HCB). Butyltin concentrations were high in the hepatopancreas, ranging from 350–2,270 ng/g in Hakata Bay and 570–5,000 ng/g (on a wet wt basis) in Habu Bay. Elevated concentrations of butyltins were also detected in the eggs of horseshoe crabs. High accumulations of butyltins in horseshoe crabs may pose a serious threat to their survival and therefore needs immediate attention to prevent their extinction.

Horseshoe crabs (Order: Xiphosura) belong to three genera (*Limulus, Tachypleus* and *Carcinoscorpius*) in the family Limulidae (regarded as "living fossils"). All inhabit shallow marine waters, generally on sandy bottoms where they move about or burrow just beneath the surface, preying on other animals. The three living genera of limulids have distinct geographical ranges. *Limulus* (*L. polyphemus*) is restricted to eastern North America, ranging from Nova Scotia to the Yucatan region of Mexico; *Tachypleus* (*T. tridentatus*) and *Carcinoscorpius* occur in southeast Asia (Mohan *et al.* 1984; Shuster 1985). The three living genera of limulids are disappearing along coastal areas where they thrived a decade ago, suggesting that they may not survive this century (Earle 1991). Recent observations have shown a rapid decline in the population of *T. tridentatus* in Japanese coastal seas (Itoh *et al.* 1991). Reproductive failure and habitat destruction have attributed to their population reduction. In some places people gather them for use as fertilizer and animal feed. Certain species of horseshoe crabs, *viz., Carcinoscorpius rotundicauda* and *Tachypleus gigas* inhabiting the Gulf of Thailand, are reported to be consumed by local human populations (Kungsuwan *et al.* 1987).

Despite their existence in polluted coastal waters, their sensitivity towards chemical pollution and other impacts are not clearly understood. Toxic effects of organochlorine compounds and organotins, such as physiological manifestations, reproductive failure, egg abnormalities and immune suppression have been described in aquatic biota, particularly for those species inhabiting coastal areas (Walker and Livingstone 1992). In the present study, horseshoe crabs (*T. tridentatus*) collected from western Japanese coastal waters were analyzed for the presence of heavy metals (Fe, Mn, Zn, Cu, Pb, Ni, Cd, Co and Hg), organochlorines [polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB) and chlordanes (CHLs)] and butyltin compounds including mono- (MBT), di-(DBT) and tributyltins (TBT) to determine present contamination levels, accumulation pattern and possible toxic effects.

Materials and Methods

Six crabs each from Hakata Bay and Habu Bay, Japan (Figure 1), were collected during 19-26, July 1992. Habu and Hakata are coastal towns, surrounded by industries and located in western Japan. Habu Bay is located within the Seto Inland Sea, whereas Hakata Bay is an open body of water with more circulation due to oceanic currents in the Japan Sea. The bottom sediment in Habu Bay is clay, whereas that of Hakata Bay is coarse sand. Hakata Bay is relatively shallow. Boat traffic is comparable in both bays.

After determining its sex, each crab was weighed and dissected. Total body weight ranged from 1,110 to 2,230 g for Habu Bay crabs and 900 to 2,100 g for those from Hakata Bay. Generally, females

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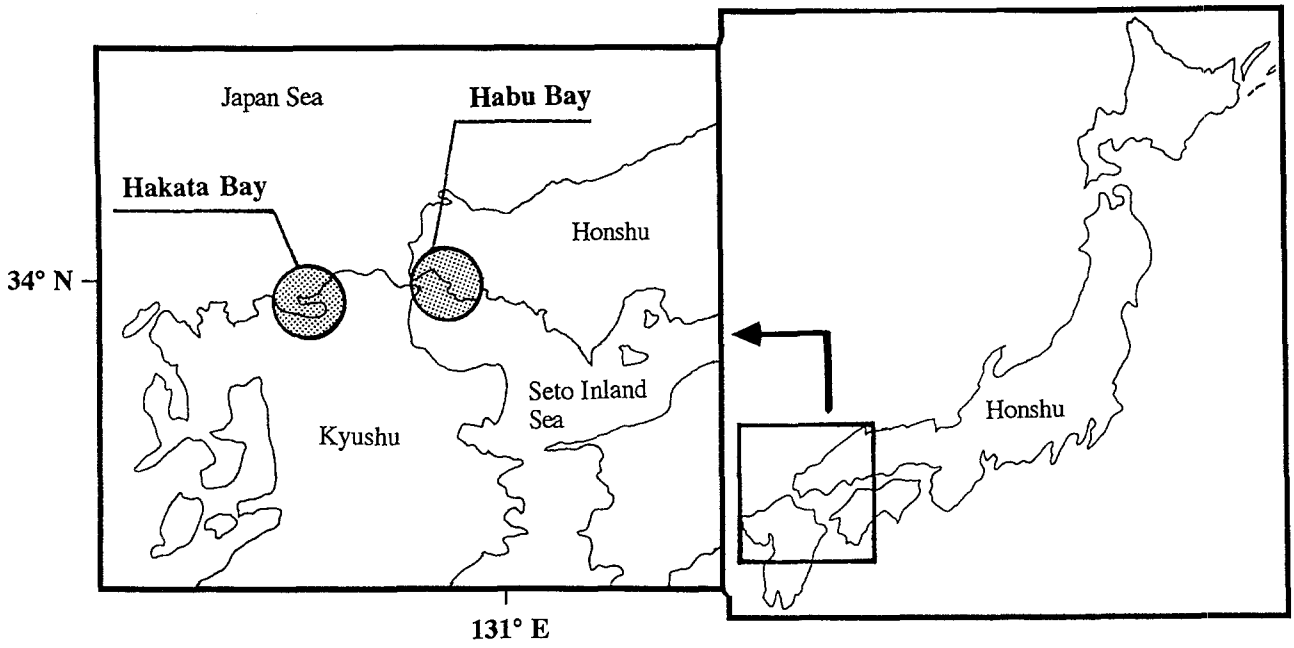

Fig. 1. Map of Japan showing the locations of sampling sites (Hakata Bay and Habu Bay)

were heavier (1,800–2,230 g) than males (900–1,240 g). Heavy metals were analyzed in many tissues/organs (dorsal carapace, ventral carapace, telson, leg and pedipalp, gill, soft bone, muscle, hepatopancreas, egg, heart, digestive tract, blood and oviduct) of two specimens (a male and a female) from Hakata Bay to understand the distribution pattern. Hepatopancreas, egg (in females) and muscle were analyzed in the remaining crabs, as these tissues were found to accumulate the highest concentrations of heavy metals. Hepatopancreas, a lipid-rich tissue, was examined for the presence of organochlorine residue levels, as they tend to accumulate preferentially in the fatty tissue. Similarly, organotins tend to accumulate in the hepatopancreas (Evans and Laughlin 1984). Hepatopancreas and eggs were analyzed for butyltin residues.

Heavy metals (Fe, Mn, Zn, Cu, Pb, Ni, Cd and Co) were analyzed by digesting the homogenized samples of tissues/organs in a mixture of nitric, perchloric and sulfuric acids (Honda *et al.* 1982). Concentrations of Fe, Mn and Zn were measured by atomic absorption spectrophotometry (F-AAS, Shimadzu Model AA-670, AA-680). Analyses of Cu, Pb, Ni, Cd and Co were performed after extraction with methyl isobutyl ketone (MIBK) and sodium diethyldithiocarbamate (DDTC) chelation. Mercury was analyzed by cold vapor UV spectrophotometry (Akagi and Nishimura 1991), after nitric, sulfuric and perchloric acid digestion. Analyses were made with a Sansou automatic mercury analyzer (Model HG-3000). Quality assurance was checked by using a standard reference material, NIES No. 1 (Okamoto *et al.* 1978), and the standard error in triplicate analyses was less than 5% for each element.

Organochlorine pesticides and polychlorinated biphenyls were analyzed by the method described by Tanabe *et al.* (1991). Samples were extracted with mixed solvents of diethyl ether and hexane in a Soxhlet apparatus. After Kuderna-Danish concentration, extracts were passed through Florisil®-packed dry columns to remove fat, and then through silica gel columns for clean up and separation. After concentration, the extracts were subjected to further clean up with 5% fuming sulfuric acid in concentrated sulfuric acid.

Samples were analyzed with a Hewlett-Packard 5890 series II gas chromatograph equipped with a ^{63}Ni electron capture detector and moving needle-type injection system (splitless, solvent cut mode). Fused silica capillary columns consisting of DB-1 (J & W Scientific, CA, USA) having a film thickness of 0.25 μm (30m × 0.25 mm i.d.) were used for the determination of polychlorinated biphenyls. Capillary columns, DB-1701 (J & W Scientific, CA, USA) having the same dimensions and film thickness were employed for the analysis of organochlorine pesticides. The column oven temperature was programmed from 160 to 240°C at a rate of 2°C/min with a final hold of 20 min. Injector and detector temperatures were maintained at 280°C. Helium was the carrier gas (flow rate; 1.3 ml/min) and nitrogen was the make-up gas (flow rate; 60 ml/min). An equivalent mixture of Kanechlor preparations (KC-300, KC-400, KC-500, and KC-600) with known PCB composition and content was used as a standard. Concentrations of individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentrations. Organochlorine pesticides were quantified by comparing individually resolved peak heights to corresponding standard peak heights.

Methods for butyltin analysis were described in detail by Iwata *et al.* (1994). Butyltin was extracted from the hepatopancreas with 40 ml of 0.1% tropolone-acetone and 10 ml of 1N HCl, and transferred to 0.1% tropolone-benzene. The extract was passed through a column packed with 35 g of anhydrous Na_2SO_4 to remove moisture and then concentrated to 5 ml using a rotary evaporator (40°C). The extract was propylated by adding *n*-propyl magnesium bromide (2 mol/L in THF solution, Tokyo Kasei Kogyo Co. Ltd., Japan) as a Grignard reagent and the mixture was shaken at 40°C for 30 min. The excess Grignard reagent was destroyed with 20 ml of 1N H_2SO_4 and the derivatized (propylated) extract was transferred to 10% benzene-hexane and rotary evaporated to near dryness and made up to 5 ml with hexane. The extract was passed through a 20 g Florisil®-packed dry column to remove lipids, rotary evaporated and passed through a 6 g Florisil®-packed wet column for final cleanup.

A gas chromatograph-flame photometric detector (Hewlett-Packard 5890 Series II) with a moving needle type injection system and a tin mode filter (610 nm) was used for quantification. A fused silica capillary column coated with 0.25 μm of DB-1 was used. The column oven temperature was programmed from 80°C (1 min hold) to 160°C at a rate of 15°C/min and then at a rate of 5°C/min to a final temperature of 260°C and held for 5 min. Helium was used as the carrier gas at a flow rate of 1.3 ml/min. Hydrogen, air and nitrogen were passed at 160, 120 and 10 ml/min for the flame photometric detector. Injector and detector temperatures were maintained at 200 and 270°C, respectively. Butyltin trichloride, dibutyltin dichloride and tributyltin chloride of known amounts (0.1 μg) were spiked into fish liver (cod from the Pacific Ocean containing undetectable levels of butyltin residues), passed

Table 1. Tissue distribution of heavy metal concentrations (μg/g wet wt) in male and female horseshoe crabs from Hakata Bay, Japan

Tissue	Weight (g)	Fe	Mn	Zn	Cu	Pb	Ni	Cd	Co	Hg
Female										
Dorsal carapace	373	14.1	0.198	4.00	1.67	0.162	0.286	<0.001	<0.005	0.012
Ventral carapace	65.0	21.0	0.202	3.45	1.17	<0.01	0.345	0.031	<0.005	0.027
Telson	32.4	23.7	0.281	3.53	5.92	<0.01	<0.005	<0.001	<0.005	0.015
Leg and Pedipalp	242	16.6	0.189	32.8	15.4	<0.01	<0.005	<0.001	<0.005	0.027
Gill	156	403	7.34	13.9	16.7	7.54	0.262	0.050	0.057	0.067
Soft bone	14.0	2.97	0.345	25.9	4.70	<0.01	<0.005	0.039	<0.005	0.043
Muscle	120	1.37	0.114	163	2.51	<0.01	0.070	0.009	<0.005	0.081
Hepatopancreas	205	26.2	4.28	57	7.07	0.287	0.458	1.56	0.169	0.152
Egg	354	7.98	1.72	32.7	23.1	<0.01	<0.005	<0.001	<0.005	0.055
Heart	5.94	9.70	0.581	27.7	5.00	<0.01	0.119	0.096	0.112	NA[a]
Digestive tract	28.6	13.2	0.545	32.5	21.2	0.797	0.099	0.094	0.086	0.048
Blood	295	<0.05	0.061	1.83	24	<0.01	<0.005	<0.001	<0.005	0.010
Oviduct	4.58	4.02	0.434	55.1	4.01	<0.01	<0.005	0.037	<0.005	NA
Male										
Dorsal carapace	297	34.9	0.381	7.83	2.99	0.152	0.360	0.015	<0.005	0.034
Ventral carapace	32.0	41.9	0.788	32.6	6.91	0.265	0.362	0.114	0.120	0.035
Telson	21.6	21.1	0.401	2.85	8.87	<0.01	<0.005	<0.001	<0.005	0.057
Leg and pedipalp	168	27.1	0.477	62.0	20.6	<0.01	0.046	0.013	0.067	0.035
Gill	86.7	1510	6.35	15.2	22.8	13.2	0.704	0.117	0.090	0.045
Soft bone	5.60	8.13	0.639	52.9	5.40	<0.01	<0.005	0.048	<0.005	0.020
Muscle	77.7	2.66	0.307	97.2	8.02	<0.01	0.108	0.027	<0.005	0.064
Hepatopancreas	124	45.4	3.54	84	35.6	0.151	0.545	1.55	0.220	0.174
Heart	4.89	16.0	0.864	37.0	8.97	<0.01	0.208	0.169	0.166	NA
Digestive tract	19.6	16.8	0.508	30.5	17.1	0.235	0.114	0.084	0.146	0.037
Blood	161	0.91	0.539	2.85	26.0	<0.01	<0.005	<0.001	<0.005	0.042

[a]NA = Not analyzed

through the whole analytical procedure and used as an external standard. Concentrations were estimated by comparing peak heights of butyltins in samples with those in external standards.

Results and Discussion

Heavy Metal Concentrations and Distribution in Various Tissues

Concentrations of heavy metals in tissues of male and female horseshoe crabs collected from Hakata Bay are shown in Table 1. Fe, Mn and Pb concentrations were highest in gill and Zn in muscle; concentrations of Ni, Cd, Co and Hg were greatest in the hepatopancreas of both sexes. The highest concentrations of Cu were found in the egg and blood of females and the hepatopancreas of males. In general, no significant differences were found between sexes concerning the tissue distribution of most heavy metals. Based on tissue weight, the body burden of heavy metals was estimated and the results are shown in Figure 2. Generally, heavy metal burdens were considerably higher in the gill, hepatopancreas and egg than other tissues. Gills had the greatest burdens of Fe and Mn. The hepatopancreas retained considerable Mn, Pb, Ni, Co, Cd and Hg loads. Zinc and Hg were present in notable quantities in the muscle. Eggs had considerable burdens of Mn, Zn, Cu, and Hg.

Mean concentrations of heavy metals in the hepatopancreas, egg and muscle of horseshoe crabs from Habu Bay and Hakata Bay are shown in Table 2. Concentrations of Fe, Mn, Zn, Cu, Pb, and Ni were relatively higher in the hepatopancreas of Habu Bay samples, whereas Cd and Hg were greatest in Hakata Bay samples. Egg and muscle from both locations contained almost comparable concentrations of heavy metals except for Hg, where Hakata Bay samples showed comparatively higher values.

To our knowledge, no information is available on heavy metal levels and toxicity in horseshoe crabs from other regions for comparison with the present values. However, it may be presumed that the observed concentrations of toxic heavy metals are not high enough to cause concern from the ecotoxicological view point. The concentrations were rather comparable with those reported for several coastal benthic organisms, such as fish (including crustaceans) and bivalves (Furness and Rainbow 1990).

Organochlorine Pesticide and Polychlorinated Biphenyl Concentrations

Organochlorine residue patterns in both locations followed the order of PCBs > CHLs > HCHs > DDTs > HCB (Table 3). The concentrations of PCBs were 22–70 ng/g wet wt (mean: 40) in Hakata Bay and 33–97 ng/g net wt (mean: 63) in Habu Bay samples. The respective mean concentrations of CHLs, HCHs, DDTs and HCB were, 13, 3.6, 2.0, and 0.02 for Hakata Bay and 7.3, 3.2, 1.9, and 0.12 ng/g wet wt for Habu Bay samples.

Between sex and sampling location, variations in organochlorine concentrations were compared (Table 4). Comparisons were made for lipids because of the lipophilic nature of organochlorines. Generally, male samples contained higher concentrations than females. The transfer of organochlorine residues

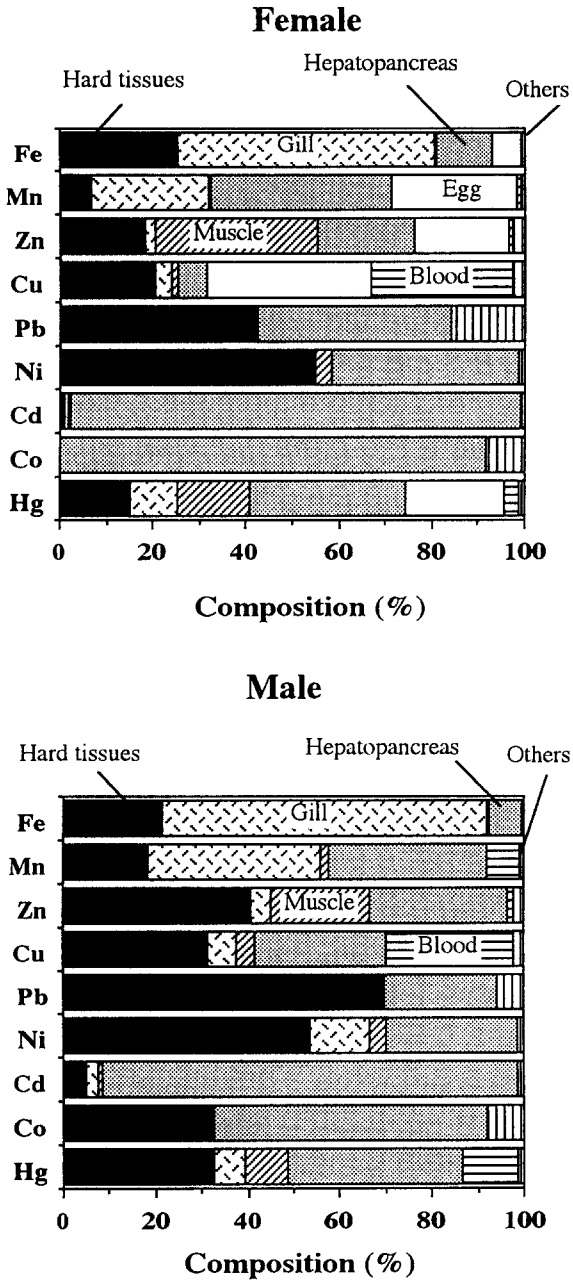

Fig. 2. Tissue distribution of heavy metal burden in male and female horseshoe crabs (hard tissues include carapace, telson, legs and pedipalp)

through eggs may be a plausible explanation for the lower levels in females. A comparison of organochlorine residue levels between Hakata Bay and Habu Bay samples revealed the presence of 2–4 times higher levels of PCBs in the latter. Concentrations of other organochlorine pesticides were comparable in both the locations.

The ratios of isomers/metabolites of CHLs, HCHs and DDTs are shown in Figure 3. Oxychlordane, a CHLs metabolite, constituted 70% of total CHLs in both the locations followed by *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and *trans*-chlordane. β-HCH occupied an average of 62% of Σ HCH followed by α- (35%) and γ- (3%) isomers. Likewise, *p,p'*-DDE comprised 72% of Σ DDT, followed by *p,p'*-DDD (18%) and *p,p'*-DDT (10%). These results imply that there is no fresh large scale input of these compounds. DDT and HCH were banned in Japan in the early 1970s and chlordanes were banned in 1986 (Loganathan *et al.* 1989). In lower trophic level species such as fish, *trans*-nonachlor constituted a higher proportion whereas in humans and birds, the oxychlordane proportion was the highest (Kawano *et al.* 1988). The presence of higher levels of oxychlordane probably reflects a modified drug metabolizing enzyme system (probably higher metabolic potential) than fishes or it may suggest discontinued use of chlordane in these locations.

The organochlorine residue levels detected in horseshoe crabs in the present study were comparble to those reported for fish from the River Nagaragawa (Loganathan *et al.* 1989) and other market fish and shellfish from Osaka, Japan, during the mid-1980s (Kuwabara *et al.* 1989). In general, organochlorine residue levels detected in horseshoe crabs were low except for PCBs, and organochlorine contamination is probably not the major cause of the decline in populations. More toxicological data (laboratory and field tests) are needed to understand the sensitivity of horseshoe crabs towards organochlorine compounds.

Accumulation of Butyltin Residues

In contrast to organochlorines, butyltin residues were considerably higher (Table 5). Mean concentrations of total butyltins in horseshoe crab hepatopancreas from Hakata Bay and Habu Bay were 1,150 ng/g (range: 350–2,270) and 2,100 (range: 570–5,000) ng/g wet wt, respectively, suggesting greater contamination in Habu Bay samples. In Hakata Bay, males showed two fold higher concentrations of butyltins than females, whereas in Habu Bay the sexes were similar. However, females had higher burdens of butyltins in the hepatopancreas (Table 5) due to larger hepatopancreases in females than in males. Higher body burdens in females may cause greater transfer to the eggs, thus affecting hatchability and survival as well as future generations. Butyltin residues in eggs and the hepatopancreas of a female crab from Hakata Bay as well as Habu Bay are shown in Table 6. Concentrations in eggs were proportional to respective concentrations in the hepatopancreas. Moreover, the concentration pattern of mono-, di-, and tributyltin in eggs was similar to that found in the hepatopancreas, *i.e.*, organisms with high concentrations of TBT in the hepatopancreas had high levels in the egg. Recorded concentrations in eggs were comparable to those reported for marine fish eggs (26 to 470 ng/g) from Japan (Suzuki *et al.* 1992). The mean mass of eggs present in ovaries of crabs from Habu and Hakata Bays was 460 and 103 g, respectively. Accordingly, the elimination of butyltins via egg laying was calculated to be 120 and 3.5 μg for Habu and Hakata Bay organisms, respectively. These values are 3 and 12% of the hepatopancreas burden of butyltins. It is not clear whether the observed concentrations of butyltins in eggs have adverse effects on hatchability and survival of early life stages of crabs. Only a few data are available on the effects of TBT on early life stages of fish. Fent (1992) demonstrated embryotoxic effects of TBT at concentrations of 0.69–0.82 μg/L in freshwater minnow, *Phoxinus phoxinus*.

In contrast to organochlorines, butyltin concentrations in horseshoe crabs were not proportional to fat content of the tissue (hepatopancreas or egg). Higher concentrations in the

Table 2. Heavy metal concentrations (μg/g wet wt; mean ± SD) in hepatopancreas, egg and muscle of horseshoe crabs from Hakata and Habu Bays[a]

Tissue	Location	Fe	Mn	Zn	Cu	Pb	Ni	Cd	Co	Hg
Hepatopancreas	Hakata	36 ± 10	3.6 ± 0.47	83 ± 30	59 ± 61	0.18 ± 0.06	0.63 ± 0.24	1.3 ± 0.34	0.27 ± 0.14	0.17 ± 0.03
	Habu	48 ± 19	22 ± 11	117 ± 142	75 ± 42	0.38 ± 0.11	0.76 ± 0.20	0.79 ± 0.48	0.28 ± 0.15	0.08 ± 0.03
Egg	Hakata	7.2 ± 1.4	0.95 ± 0.68	32 ± 3.4	20 ± 3.4	ND[c]	0.07 ± 0.01	0.01 ± 0.006	0.08[b]	0.05 ± 0.01
	Habu	4.9 ± 1.3	1.0 ± 0.28	32 ± 3.2	20 ± 2.7	ND[c]	0.1 ± 0.01	0.01[b]	ND[c]	0.02 ± 0.01
Muscle	Hakata	1.8 ± 0.5	0.18 ± 0.07	124 ± 23	9.9 ± 7.0	ND[c]	0.08 ± 0.02	0.02 ± 0.01	0.20 ± 0.25	0.08 ± 0.01
	Habu	3.4 ± 1.0	0.56 ± 0.31	122 ± 11	12 ± 3.9	ND[c]	0.09 ± 0.04	0.01 ± 0.003	ND[c]	0.04 ± 0.01

[a] Six samples of hepatopancreas and muscle and three samples of egg were analyzed from each location
[b] Only one sample contained detectable residues
[c] ND = Not detected

Table 3. Organochlorine concentrations (ng/g wet wt) in horseshoe crab hepatopancreas from Habu Bay and Hakata Bay, Japan

Location	Sex	n	Fat (%)	PCBs	DDTs	HCHs	CHLs	HCB
Hakata Bay	F	3	24	37	2.8	3.2	14	0.03
			(18–32)	(36–38)	(2.3–3.3)	(2.2–3.7)	(7.7–23)	(0.01–0.05)
	M	3	11	42	1.2	3.9	12	0.01
			(6.9–19)	(22–70)	(0.43–2.0)	(1.6–5.7)	(8.1–17)	(<0.01–0.02)
Habu Bay	F	3	13	62	1.4	1.6	7.0	0.18
			(5.7–18)	(36–97)	(0.64–2.5)	(0.61–2.3)	(2.7–9.8)	(<0.01–0.54)
	M	3	12	65	2.4	4.8	7.5	0.05
			(6.1–19)	(33–95)	(1.5–4.1)	(3.2–7.3)	(3.8–11)	(0.02–0.10)

HCHs = α + β + γ isomers of hexachlorocyclohexanes
DDTs = *p,p'*-DDE + *p,p'*-DDD + *p,p'*-DDT
CHLs = oxychlordane + *trans*-chlordane + *cis*-chlordane + *trans*-nonachlor + *cis*-nonachlor + heptachlor
Figures in parentheses indicate the range

Table 4. Mean (range) organochlorine concentrations (ng/g fat wt) in horseshoe crab hepatopancreas from Hakata Bay and Habu Bay, Japan

Location	Sex	n	Fat (%)	PCBs	DDTs	HCHs	CHLs	HCB
Hakata Bay	F	3	24	163	12	14	56	0.14
			(18–32)	(120–200)	(10–13)	(10–20)	(43–72)	(0.04–0.22)
	M	3	11	377	11	36	110	0.16
			(6.9–19)	(320–420)	(6.2–16)	(23–56)	(89–120)	(0.05–0.20)
Habu Bay	F	3	13	540	12	12	55	1.1
			(5.7–18)	(290–690)	(6.1–18)	(10–16)	(47–70)	(<0.07–3.0)
	M	3	12	790	30	57	85	0.45
			(6.1–19)	(170–1,600)	(7.9–67)	(17–120)	(35–170)	(0.11–0.91)

HCHs = α + β + γ isomers of hexachlorocyclohexanes
DDTs = *p,p'*-DDE + *p,p'*-DDD + *p,p'*-DDT
CHLs = oxychlordane + *trans*-chlordane + *cis*-chlordane + *trans*-nonachlor + *cis*-nonachlor + heptachlor
Figures in parentheses indicate the range

hepatopancreas suggest preferential accumulation of these compounds in this excretory organ.

In Japan, the use of tributyltin as an antifouling agent in marine paints has been restricted by the Department of Fisheries since 1991. However, this restriction is yet to be imposed for use on ocean liners and other vessels. The observed pattern of butyltin residues (an increased proportion of TBT rather than MBT and DBT) in horseshoe crabs suggests that boats are major sources other than urban or industrial wastes. Analysis of several fish and shellfish species from different areas of Japan showed the presence of up to 1,340 ng/g in the liver of sea bass from the Seto Inland Sea (Environment Agency of Japan 1988; Higashiyama *et al.* 1991; Yonezawa *et al.* 1993). A comparison of butyltin concentrations recorded in the hepatopancreas of horseshoe crabs with those in the liver of marine fish collected in and around Japan (Suzuki *et al.* 1992) revealed a 2 to 10-fold higher concentration in crabs (Figure 4). Furthermore, the concentrations in horseshoe crabs were in the higher ranges of those reported for lower trophic aquatic biota from other areas in the world (International Programme on Chemical Safety 1990).

Ratios of mono-, di-, and tributyltin concentrations in the hepatopancreas of male horseshoe crabs from Habu and Hakata Bays were 76%, 10%, 14%, and 50%, 21%, 29%, respectively. Females from Habu Bay exhibited a butyltin composition of 50%, 14% and 36% for mono-, di-, and tributyltin, respectively, whereas those from Hakata Bay exhibited 40%, 15%, and 45%, respectively. Males contained a higher proportion of monobutyltin and a lower proposition of tributyltin than the females. The presence of MBT and DBT in the horseshoe

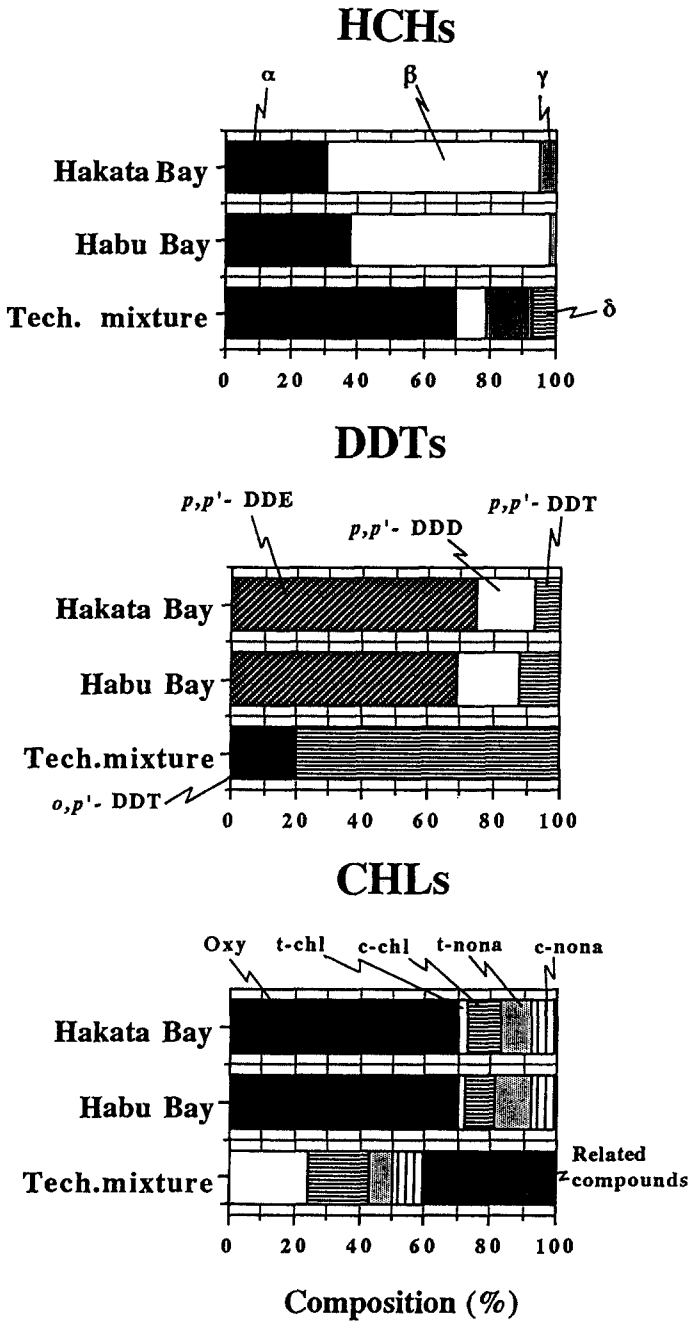

Fig. 3. Mean composition percent of isomer/metabolites of hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethanes (DDTs) and chlordanes (CHLs) in the hepatopancreas of horseshoe crabs

Table 5. Butyltin concentrations (ng/g wet wt) and burden (μg) in horseshoe crab hepatopancreas from Hakata Bay and Habu Bay, Japan

Location	Sex	n	Fat (%)	ΣBT conc.	ΣBT burden
Hakata Bay	F	3	24 (18–32)	860 (350–1,560)	250 (110–480)
	M	3	11 (6.9–19)	1,400 (860–2,270)	160 (92–260)
Habu Bay	F	3	13 (5.7–18)	2,100 (570–5,000)	540 (230–990)
	M	3	12 (6.1–19)	2,000 (970–3,880)	370 (240–630)

ΣBT = mono-, di-, and tributyltin
Figures in parentheses indicate the range

Table 6. Butyltin concentrations (ng/g wet wt) in the female horseshoe crab egg and hepatopancreas[a]

Location	Tissue	Fat (%)	MBT	DBT	TBT	ΣBT
Hakata Bay	Egg	12	20	5.9	8.5	34
	Hepatopancreas	23	140	92	115	350
Habu Bay	Egg	9.4	45	31	180	260
	Hepatopancreas	14	2,100	730	2,200	5,000

[a]One egg and corresponding hepatopancreas sample from each location.

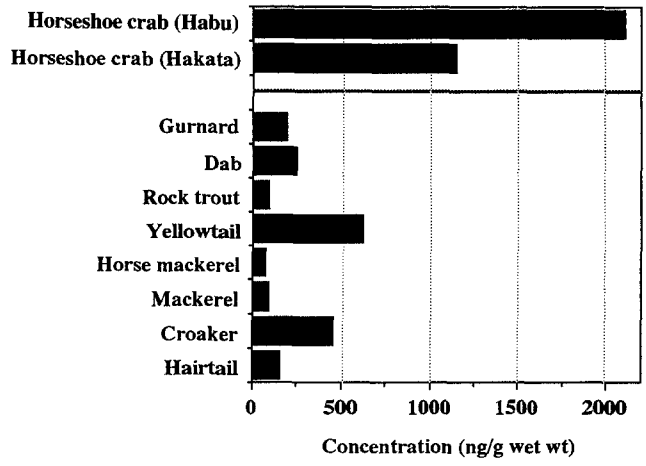

Fig. 4. Butyltin concentrations in horseshoe crab hepatopancreas compared to marine fish livers from Japan (fish data from Suzuki *et al.* 1992)

crabs may be due to the metabolic breakdown of TBT by microsomal enzymes. According to Lee (1991), butyltins were metabolized in fish and crustaceans, leading to the formation of DBT, MBT and inorganic tin.

Tributyltin has been associated with mortality, failure of settling of bivalve larvae, reduced growth, shell thickening and other malformations in oysters and imposex in mud snails and dogwhelks (Alzieu *et al.* 1989). According to Evans and Laughlin (1984), the bioconcentration factor for TBT in the hepatopancreas of mud crab, *Rhithropanopeus harrisii*, was 4,400. Water concentration ranges of tributyltin in Dokai Bay (near Habu Bay) and Hakata Bay were 7–14 ng/L and 12–28 ng/L, respectively (Environment Agency of Japan, 1993). Considering an average value of 10 ng/L for Habu Bay and 20 ng/L for Hakata Bay, the bioconcentration factors for tributyltin in horseshoe crabs were estimated to be 26,000–40,000, suggesting a higher bioaccumulation potential for these compounds (tributyltin concentrations in horseshoe crab hepatopancreases were 530 ng/g for Habu Bay and 400 ng/g for Hakata Bay). Higher bioconcentration factors estimated in this study may also suggest considerable intake via food chain and exposure to contaminated sediments.

The no observed effect level (NOEL) of tributyltin in water is 20 ng/L for freshwater and 2 ng/L for saline water (Alzieu *et al.* 1989). Even at a low concentration of 50 ng/L, TBT induced shell malformation in oysters (Alzieu *et al.* 1989). Chronic effects of TBT on the immune system of fish have been reported at a few hundred ng/L (Wester and Canton 1987). Acute toxic effects on fish occur at a few μg/L with early life stages of fish being more susceptible (Bushong *et al.* 1988; Martin *et al.* 1989; Fent 1992).

Therefore, it is probable that the higher concentrations of butyltins observed in horseshoe crabs may imply certain physiological manifestations. It should be noted that the number of samples analyzed in this study is too small (because of limitations in capturing threatened organisms) to establish conclusive evidence. Further studies are needed to determine the susceptibility of horseshoe crabs to organotin pollution as their coastal habitat is prone to intense boating activity and to the disposal of polluted wastes from the terrestrial environment.

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