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Levels of Organochlorine Compounds in Bivalves from the Japanese Coastal Waters: Results from the Environmental Specimen Bank in Japan

Y. Takazawa, A. Tanaka, Y. Shibata, M. Morita

National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki
305-8506, Japan

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Environmental pollution caused by organochlorine compounds (OCs) representing persistent organic pollutants (POPs) is a global problem. Although use of products containing OCs has been banned or restricted in almost all countries, OC persistence in biota and in abiotic environments has been confirmed. Because of their bioaccumulative capacity as a sink of OCs, bivalves are reasonable bioindicators for clarifying the presence of OCs in the aquatic environment. OC bioconcentration factors (BCFs) of bivalves to the surrounding water reach 10,000 (Verschueren 1996). For that reason, OCs have easy access to bivalves' tissues. Advantages of bivalve monitoring are implied by some of their characteristics: They have high tolerance to various pollutants. They are comparatively long-lived and show a response to variation of OC concentrations in the surrounding environment. They are also easily collected. Bivalves tend to reflect the mean OC concentration for the selected coastal area.

Recently, there has been renewed interest in "OC global monitoring" since the Stockholm Convention on POPs of May 2004. That convention also argued for bivalves as biological monitors of POPs; in fact, the species choice is now widely accepted for assessing aquatic environmental quality (Monirith et al. 2003). Bioaccumulation of OCs through the food chain provides wildlife and human beings with toxicological effects (e.g. influence on endocrine homeostasis) (de Felip et al. 2004). Therefore, dietary exposure should be noteworthy. Monitoring toxic OCs has important implications for protection of human health: bivalves are a commercially available seafood.

We have conducted sampling surveys of bivalves at several locations in Japan for the environmental specimen bank, which is a part of the "time capsule project" at our institute. Concentrations of toxaphene, mirex, and other OCs (heptachlor, heptachlor epoxide, hexachlorocyclohexanes (HCHs), DDT-related compounds (DDTs), chlordane-related compounds (CHLs), hexachlorobenzene (HCB), aldrin, endrin, and dieldrin) were determined using gas chromatography-mass spectrometry (GC-MS) based on negative ion chemical ionization. This study is intended to present the latest persistent organic contamination in Japan using bivalves as bioindicators in coastal waters. The emphasis falls on the determination

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Correspondence to: Y. Takazawa

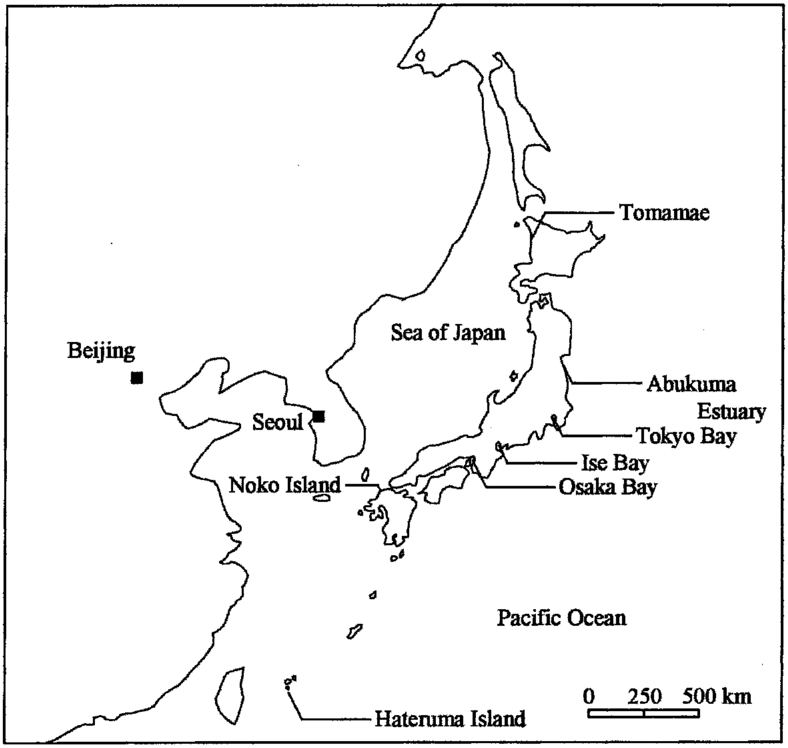

Figure 1. Map of seven sampling sites in Japan.

of toxaphene and mirex. Little attention has been given to the point in Japan because neither compound has ever been registered as a pesticide. In addition, we evaluated detected OC residue levels on the basis of the acceptable daily intake (ADI) as established by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO). Those data that were obtained will elucidate OC residues' daily impact on human health.

MATERIALS AND METHODS

We employed dioxin-analytical grade solvents (Wako Pure Chemical Industries Ltd., Osaka, Japan). Toxaphene standards of twenty-two congeners and other OC standards (purity, 97–99%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and AccuStandard Inc. (CT, USA), respectively. All isotope labeled standards (purity, 99%) were purchased from Cambridge Isotope Laboratories Inc. (MA, USA). All other chemicals (Wako Pure Chemical Industries Ltd., Osaka, Japan) were of the highest grade available and were used without further purification.

Two bivalve species, blue mussels (*Mytilus galloprovincialis*) and oysters (*Saccostrea mordax*) were collected during 2002–2004. The sampling sites were seven coastal areas: Hateruma Island, Noko Island, Osaka Bay, Ise Bay, Tokyo Bay, Abukuma Estuary, and Tomamae (Figure 1). Blue mussels were collected from six sites, excepting Hateruma Island; oysters were collected there. The bivalve bodies were removed from their shells at the sampling sites and were transported to the laboratory after being packed in dry ice. Homogenized whole body tissues were stored in liquid nitrogen until analysis. The sample (5 g) mixing with anhydrous sodium sulfate was transferred to a quartz thimble for the Soxhlet extraction and was extracted with diethyl ether/hexane (3:1; v/v, 150 ml) for 12 h. After the extraction was finished, surrogate standards (10 ng) were added to the extract. Diethyl ether/hexane was substituted with hexane, while the volume of extract was reduced in a rotary evaporator. The column (25 mm i.d., 500 mm length) for gel permeation chromatography was packed with 50 g of Bio-Beads® S-X3, 200–400 mesh (Bio-Rad Laboratories, Inc., CA, USA) using a mixture solution of dichloromethane/cyclohexane (1:1; v/v). The eluate between 125 ml and 275 ml was transferred to a flask and was concentrated to 3 ml. Hexane was added to replace dichloromethane/cyclohexane. The concentrate was purified with the pre-rinsed glass column (15 mm i.d., 300 mm length) containing, from top to bottom: 2 g of anhydrous sodium sulfate, 10 g of Florisil® (Wako Pure Chemical Industries Ltd., Osaka, Japan: activated 130°C, 12 h), 2 g of anhydrous sodium sulfate, and a quartz wool plug. After the sample was loaded, OCs were eluted with 100 ml of diethyl ether/hexane (5:95; v/v, Fraction 1) and 100 ml of diethyl ether/hexane (20:80; v/v, Fraction 2 containing endrin and dieldrin). The Fraction 1 was then evaporated to 3 ml for a silica gel column chromatography. The pre-rinsed silica gel column (12 mm i.d., 300 mm length) contained, from top to bottom: 2 g of anhydrous sodium sulfate, 5 g of Silica gel 60 (Merck Inc., Darmstadt, Germany: activated 130°C, 12 h), 2 g of anhydrous sodium sulfate, and a quartz wool plug. The concentrate of Fraction 1 was loaded; OCs were then eluted first with 30 ml of hexane (Fraction 3 containing HCB, aldrin, and mirex). Fraction 4 containing toxaphene and other OCs was recovered with 30 ml of diethyl ether/hexane (25:75; v/v). The Fractions 2, 3, and 4 were evaporated to 2 ml and were transferred to centrifuge tubes. Five hundred picograms of $^{13}C_{12}$-labeled 2,2',4,4',5,5'-hexachlorobiphenyl as an internal standard was added into the tubes; final concentrates (100 μl) were obtained with a gentle stream of dry nitrogen.

Analyses were performed with a portable mass spectrometer 5973N Mass Selective Detector (Agilent Technologies Inc., DE, USA) equipped with a 6890 series gas chromatograph (Agilent Technologies Inc., DE, USA). HT8 (50-m length, 0.22-mm i.d., 0.25 μm film thickness; SGE Japan Inc., Kanagawa, Japan) was selected for a capillary column. Helium flowed as a carrier gas at 1 ml min^{-1}. Methane served as a reagent gas. Temperatures of an ion source and a quadrupole were maintained at 150°C and 106°C, respectively. For OCs except toxaphene, one microliter of a final concentrate was injected with an autosampler 7673 (Agilent Technologies Inc., DE, USA) under a splitless mode. Temperatures of an injector port and a transfer line in the gas chromatograph were maintained at 260°C and 280°C, respectively. The column temperature was maintained at 50°C for 0.3 min, ramped to 200°C at a rate of 20°C min^{-1}, to 280°C at a rate of 2.5°C min^{-1}, and maintained at 280°C for 1 min. In the analysis of toxaphene, a pulsed splitless mode was selected. Temperatures of an injector port and a transfer line were maintained at 220°C and 280°C, respectively. The column temperature was maintained at 60°C for 1 min, ramped to 170°C at a rate of 23°C min^{-1}, 7.5 min isothermal, to 275°C at a rate of 3°C min^{-1}, and maintained at 275°C for 12 min.

Concentrations were presented as medians based on a lipid weight basis (l.w.). Mean recoveries of labeled surrogate standards through the analytical procedure were determined as: 101% for α-HCH, 96% for β-HCH, 96% for γ-HCH, 64% for δ-HCH, 80% for HCB, 107% for aldrin, 117% for endrin, 120% for dieldrin, 78% for *p,p'*-DDD, 80% for *o,p'*-DDE, 86% for *p,p'*-DDE, 83% for *o,p'*-DDT, 97% for *p,p'*-DDT, 115% for heptachlor, 97% for heptachlor epoxide, 80% for *trans*-chlordane, 74% for *cis*-nonachlor, 77% for *trans*-nonachlor, 96% for oxychlordane, 109% for mirex, and 90% for toxaphene. Method detection limits of OCs, except *p,p'*-DDT (2.3 ng g^{-1}), ranged from 0.01 to 0.8 ng g^{-1}. Blanks for the procedure were measured to check contamination originating in the laboratory. SPSS version 10.0J (SPSS Inc., Chicago, IL, USA) and FreeJSTAT 8.2 (Free software provided by M. Sato, Japan) were used for statistical analysis. Non-parametric multiple comparison using the Kruskal-Wallis test was employed to evaluate OC concentrations of the sampling locations.

RESULTS AND DISCUSSION

Table 1 shows concentrations of OC residues obtained in whole tissues of bivalves. The highest concentrations were found in DDTs (the sum of *o,p'-/p,p'*-DDD, *o,p'-/p,p'*-DDE, and *o,p'-/p,p'*-DDT) and in CHLs (the sum of oxychlordane, *cis-/trans*-chlordane, and *cis-/trans*-nonachlor). Furthermore, the two OC groups possessed significant dominance against toxaphene, HCB, heptachlor, heptachlor epoxide, and mirex ($p < 0.01$). Residue patterns of OCs, except for those of DDTs and CHLs, implied this approximate order: HCHs (the sum of α-/β-/γ-/δ-HCH) > DRNs (the sum of aldrin, endrin, and dieldrin) > toxaphene (the sum of Parlar 26, Parlar 40, Parlar 41, and Parlar 50) = HCB = heptachlor = heptachlor epoxide = mirex. On the other hand, total OC concentrations were observed as: 118 ng g^{-1} (Hateruma Island), 448 ng g^{-1} (Noko Island), 803 ng g^{-1} (Osaka Bay), 274 ng g^{-1} (Ise Bay), 221 ng g^{-1} (Tokyo Bay), 1283 ng g^{-1} (Abukuma Estuary), and 252 ng g^{-1} (Tomamae). For example, the contribution ratios of DDTs and of CHLs to the total OC concentrations were 14–74% (min: at the Abukuma Estuary, max: at Hateruma Island) and 17–62% (min: at Tomamae, max: at the Abukuma Estuary), respectively. Consequently, the sum of DDTs and CHLs occupied 76–98% of the total OC concentrations in each site. These results engendered our conclusion that the overall tendency to OC accumulation in bivalves inhabiting Japanese coastal waters is attributable particularly to DDTs and CHLs. Sampling sites are classifiable as three types: remote areas with extremely low population density (Hateruma Island), rural areas (Tomamae), and industrial areas (Noko Island, Osaka Bay, Ise Bay, Tokyo Bay, and Abukuma Estuary). Hateruma Island, which

Table 1. Concentrations (ng g^{-1} l.w.) of OCs in bivalves from the coastal waters.

Compounds	Hateruma I. 05/2004 $n = 10$	Noko I. 04/2003 $n = 2$	Osaka B. 11/2003 $n = 3$	Ise B. 11/2003 $n = 3$	Tokyo B. 04/2003 $n = 3$	Abukuma E. 06/2004 $n = 3$	Toma. 09/2002 $n = 1$
α-HCH	nd	3.2	4.8	3.2	5.8	18	4.1
β-HCH	1.1	10	24	20	13	24	8.1
γ-HCH	0.1	4.3	2.3	2.0	1.8	10	1.9
δ-HCH	nd	nd	nd	nd	0.0	4.8	0.6
HCB	0.4	4.2	4.3	3.0	3.7	88	1.4
Aldrin	nd	nd	nd	nd	0.0	2.0	nd
Endrin	nd	0.4	nd	1.8	0.0	22	0.3
Dieldrin	nd	4.4	20	6.1	3.8	111	1.6
o,p'-DDD	5.3	51	35	4.9	19	21	23
p,p'-DDD	10	63	37	6.0	17	34	79
o,p'-DDE	1.6	5.7	6.1	1.0	7.3	8.2	3.7
p,p'-DDE	35	15	125	41	47	39	18
o,p'-DDT	12	33	14	3.9	3.5	30	21
p,p'-DDT	24	39	36	12	5.4	47	39
Heptachlor	nd	0.4	8.5	nd	3.3	nd	nd
Heptachlor epo.	nd	0.7	4.4	3.0	2.0	16	0.8
cis-Chlordane	5.9	64	138	51	25	428	13
trans-Chlordane	7.7	60	146	52	27	200	11
cis-Nonachlor	3.8	29	60	19	9.5	30	5.3
trans-Nonachlor	10	52	126	40	21	118	13
Oxychlordane	0.3	1.6	7.5	3.1	1.4	23	0.8
Mirex	nd	5.9	1.5	1.1	0.9	5.7	2.0
Parlar 26	nd	0.3	nd	nd	1.2	1.4	1.7
Parlar 40	0.2	0.3	0.1	0.1	0.6	0.5	0.6
Parlar 41	nd	nd	nd	nd	1.3	nd	1.4
Parlar 50	0.6	0.7	0.4	0.3	1.7	1.1	2.1

nd: not detected; Heptachlor epo.: Heptachlor epoxide; Toma.: Tomamae.

is inferred to be a background area for this OC study, showed lower total concentrations of OCs than other areas, but measurable DDT residues (830–54,000 ng g^{-1} l.w.) are still detected in mussels from China (Monirith et al. 2003). Nakata et al. (2002) reported similar results (34,000 ng g^{-1} l.w. in Chinese mussels). Therefore, even though China is located near Hateruma Island, that country's pesticide use appears to have little influence on that island's surrounding marine environment. This fact requires further explanation. For example, two unregistered pesticides (toxaphene and mirex) in Japan were present at low concentrations (total toxaphene: 0.4–5.8 ng g^{-1}, mirex: 0.9–5.9 ng g^{-1}). That observation suggests transboundary movement of both OCs. Probably, the Japanese environment appear to be no less exposed to pesticides derived from the East Asian countries on the basis of ocean current and atmospheric flow. If we ignore the accumulation process (i.e., metabolism, excretion, and intake) of OCs in

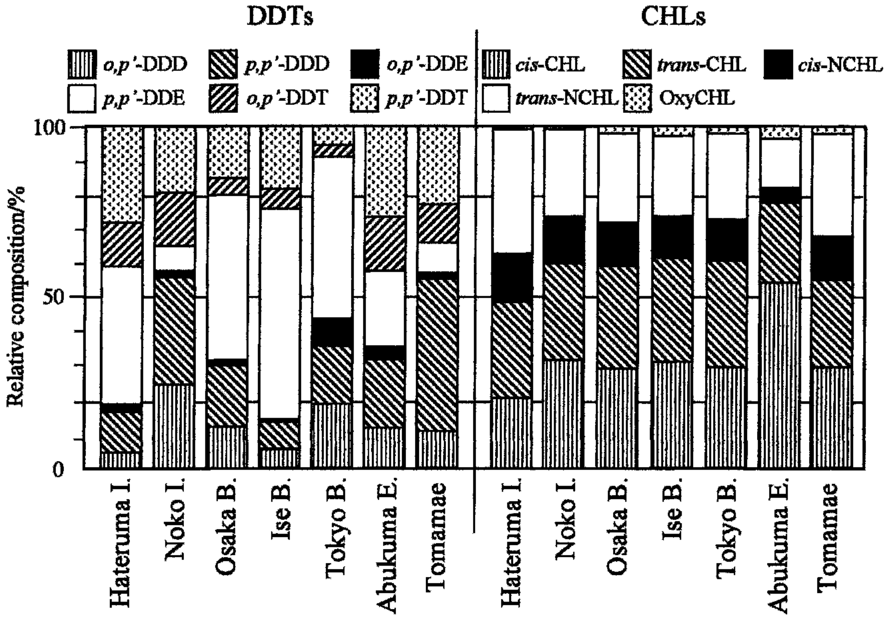

Figure 2. Relative composition of DDTs and of chlordane-related compounds.

bivalve species, one explanation for these data gaps between our results and the DDT concentrations that are shown for China may be that the residue levels of OCs depend on volatility, water solubility, and decomposition. China is both a heavy consumer and major producer of technical HCH (producing 4.46 million metric tons) in East Asia (Li et al. 1998). Nevertheless, HCHs are minor contributors found in the range of 11–110 ng g^{-1} l.w. in Chinese mussels (Monirith et al. 2003). Judging from the observations above and the low volatility of DDTs, it seems likely that oysters inhabiting Hateruma Island are influenced only slightly by DDTs from neighboring countries such as China. No significant differences were observed in the tendencies of OC accumulation for blue mussels and oysters (Ueno et al. 1999). We concluded that OC concentrations in bivalves are related strongly to more localized pollution sources compared to OC concentrations detected in the other marine species (e.g. pelagic fishes).

Figure 2 indicates relative compositions of analyzed isomers concerning DDTs and CHLs. The DDT compositions were characterized by *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT, whereas *o,p'*-DDE showed a significant correlation to the three compounds ($p < 0.05$). Comparisons of OCs among the sampling sites clarified the different DDT patterns of Noko Island and of Tomamae; the ratios of *p,p'*-DDT at the two sites were greater than those of *p,p'*-DDE. This fact indicates the recent use of technical DDT from a geographical point of view (i.e., both sites face the Sea of Japan): Russia, China, and South Korea might possess DDT hot spots. This fact demands further consideration. On the other hand, CHLs showed a similar residue pattern at all sites: the sum of *cis*-chlordane and *trans*-chlordane ranged from 49 to 79% among the total CHLs, whose concentrations were in the range of 28–799 ng g^{-1}. Total CHL concentrations in this study were higher than those from other East Asian countries such as Singapore, Indonesia, and South Korea (Monirith et al. 2003). The quotient of the *trans*-nonachlor to *cis*-chlordane concentrations was close to 1.0 for the five sampling sites aside from the Abukuma Estuary and Hateruma Island. We presume that aerial deposits caused CHL contamination at the five sites.

β-HCH contributed to the total HCHs considerably; the isomer accounted for 41–91% of the total HCHs. Although technical HCH mainly comprises of α-HCH (65–70%), β-HCH (7–10%), γ-HCH (14–15%), and δ-HCH (approx. 7%), the ratios of α-HCH were less than 32% through all analyses. We infer that there was no recent use of technical HCHs at these sites.

HCB is an unintentional by-product that is formed by industrial activities such as waste incineration and synthesis of chlorinated solvents. It is no exaggeration to say that most countries possess emission sources of this compound. In fact, our data (0.4–4.3 ng g^{-1}), except for data from the Abukuma Estuary (88 ng g^{-1}), closely resembled those for 11 East Asian countries (Monirith et al. 2003). HCB and HCHs are representative OCs of long-range transport via air, whereas no correlation between the concentrations in our bivalves and latitudes of the sampling sites was established. A more noteworthy case is toxaphene. Very few studies have addressed toxaphene residues in East Asia.

We observed that the residues were dominated by three congeners: Parlar 26, Parlar 40, and Parlar 50. In particular, Parlar 40 and Parlar 50 were detected from all analyzed samples. Parlar 41 was present in blue mussels from Tokyo Bay and Tomamae. However, other toxaphene congeners were not identifiable. These four congeners were included among six notable toxaphene congeners in fish oils that were reported by Kimmel et al. (1998). Toxaphene was a minor contributor to overall OC contamination, as we have seen: total toxaphene ranged from 0.4 to 5.8 ng g^{-1}. Nevertheless, we are now in a position to say that the surrounding environment in Japan has been exposed to toxaphene as well as the North Atlantic countries.

Dieldrin was the most dominant compound among DRNs. It was detected at the six sites; concentrations ranged from 1.6 to 111 ng g^{-1}. Endrin and aldrin did not contribute to OC burdens; moreover, aldrin was detected at only one site. Probably, epoxidation of aldrin in aquatic environments helps account for that result (Ross and Crosby 1985). Heptachlor and heptachlor epoxide were also detected in the range of 0.4–8.5 ng g^{-1} and 0.7–16 ng g^{-1}, respectively. Comparison of OCs (in particular, α-HCH, γ-HCH, HCB, CHLs, and DRNs) among the sampling sites led to the suggestion that OC hot spot may be present around the Abukuma Estuary.

On the basis of the ADI proposed by FAO/WHO, the mean daily intake of bivalves was calculated for dominant DDTs and CHLs in Japan: 0.01 mg/kg/day for DDTs and 0.0005 mg/kg/day for CHLs. We assumed 10 g of soft tissues (fat content: 1%) as the mean daily intake for a human with 50 kg body weight. If a human eats 62.6 kg (for DDTs) and 10 kg (for CHLs) of bivalves on a wet-weight basis every day, the daily intakes of both toxic OC groups would exceed the ADI; therefore, the anticipated intakes were considerably lower than the ADI. Consequently, consumption of Japanese bivalves would not affect human health.

Several points were clarified from these remarks. First, DDTs and CHLs are major contributors of OC contamination in Japanese coastal waters. Next, pesticides that are unregistered in Japan were also detected at low concentrations. Furthermore, the monitoring of OCs using bivalves tended to represent localized contamination. In other words, concentrations may vary at respective sampling sites, even if sampling surveys were carried out in an identical shore. Finally, the daily intake values of DDTs and CHLs from Japanese bivalves were much lower than those of ADI.

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