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Distribution Patterns of Hexachlorocyclohexanes and Other Organochlorine Compounds in Muscles of Fish from a Japanese Remote Lake During 2002–2003

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Lake Mashu (43°35' N, 144°32' E), is an oligotrophic crater lake with a circumference of 19.8 km and a mean water depth of 138 m, is located in eastern Hokkaido, Japan (Figure 1). Water input into the lake comes from precipitation and permeation of ground water through cracks. The lake water possesses the highest transparence in Japan. In addition to this, Lake Mashu is a remote lake situated far from emission sources of organic pollutants. However, it has been reported that remote ecosystems in the Arctic and Europe receive large amounts of organochlorine compounds (OCs) through long-range atmospheric transport and deposition (Barrie et al. 1992; Carrera et al. 2002); therefore, the same observation may apply to Lake Mashu ecosystem. For example, there have been comprehensive investigations of Lake Baikal (Iwata et al. 1995), but the study of Lake Mashu, which is one of the Asian background lakes, has been superficial. Surprisingly few studies have so far been made at the contaminant concentrations of trophic level species in Lake Mashu. Monitoring OCs of biota inhabiting this lake provides us with a major pathway for the introduction of these pollutants to the ecosystem.

Three fish species (rainbow trout, *Oncorhynchus mykiss*; kokanee, *Oncorhynchus nerka*; Japanese dace, *Tribolodon ezoe*) were caught as analytical targets. In particular, rainbow trout are the top predators in this freshwater system; the diversity in feeding habit permits them to represent the overall OC contamination through a food chain. The objectives of this study were to: measure the OC concentrations in three fish species; and compare this data with that for the major OC contaminants found in other regions.

MATERIALS AND METHODS

We employed dioxin-analytical grade solvents (Wako Pure Chemical Industries Ltd., Osaka, Japan). Authentic standards were purchased from AccuStandard Inc. (CT, USA). All isotope labeled standards 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.

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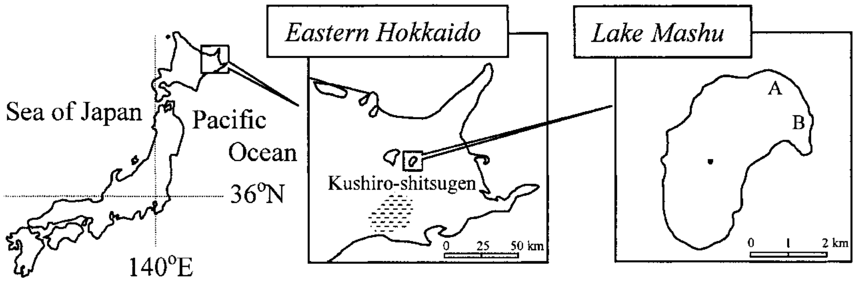

Figure 1. Map of Japan showing Lake Mashu. A and B: sampling locations.

In August 2002 and September 2003, we carried out a sampling survey of Lake Mashu in Hokkaido. Net casts from a fishing boat captured fishes. A total of 22 rainbow trout, 15 kokanee, and nine Japanece dace were collected for analysis. Length and weight of each fish were immediately measured on the boat. Muscle tissue of the fishes was separated by dissection at sampling sites; further, sex was judged from the gonad. The fishes were stored at –20°C in sealed glass containers prior to analysis. Weight and length of these fishes were: trout (116–826 g, 37–44 cm), kokanee (48–82 g, 17–20 cm), and dace (80–472 g, 16–20 cm). All samples were analyzed on the basis of the isotope dilution method; therefore, ^{13}C-labeled surrogate OC standards (10 ng) were added to all the samples. Five grams of homogenized samples containing Hydromatrix® (Varian Inc., CA, USA) for moisture absorption were transferred to a steel column for pressurized fluid extraction (ASE-200; Dionex Corp., CA, USA). Extraction was carried out twice with acetone/hexane (1:1; v/v) at 100°C for 10 min. The volume was reduced in a rotary evaporator. A column (25 mm i.d., 500 mm length) for gel permeation chromatography was packed with 50 g of pre-washing Bio-Beads® S-X3, 200–400 mesh (Bio-Rad Laboratories, Inc., CA, USA). OCs were eluted with 150 ml of dichloromethane/cyclohexane (1:1; v/v) corresponding with the fraction between 125 ml and 275 ml. The eluate was concentrated to 5 ml. Hexane was added to replace dichloromethane/cyclohexane. The concentrate was purified with a 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, 18 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, endrin and dieldrin). Fraction 1 was then evaporated to 5 ml for a silica gel column. 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, 18 h), 2 g of anhydrous sodium sulfate, and a quartz wool plug. The concentrate was loaded; OCs were then eluted first with 30 ml of hexane (Fraction 3, HCB, aldrin, and mirex). Fraction 4 containing the other OCs was collected with 30 ml of diethyl ether/hexane (25:75; v/v). Fractions 2, 3, and 4 were transferred to centrifuge tubes and reduced with a gentle stream of dry nitrogen. We added 500 pg of $^{13}C_{12}$-labeled PCB 153 as an internal standard to the final concentrate.

Concentrations were presented as mean values based on a wet weight basis (w.w.). Mean recoveries of labeled surrogate standards through the analytical procedure were determined as: 72% for α-hexachlorocyclohexane (α-HCH), 93% for β-HCH, 70% for γ-HCH, 52% for δ-HCH, 71% for hexachlorobenzene (HCB), 59% for aldrin, 105% for endrin, 91% for dieldrin, 89% for *p,p'*-DDE, 85% for *o,p'*-DDE, 105% for *p,p'*-DDE, 86% for *o,p'*-DDT, 96% for *p,p'*-DDT, 63% for heptachlor, 97% for heptachlor epoxide, 86% for *trans*-chlordane, 94% for *cis*-nonachlor, 85% for *trans*-nonachlor, 87% for oxychlordane, and 109% for mirex. Method detection limits of OCs ranged from 0.0002 to 0.04 ng/g. Operation blanks 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. Mean concentration of all major OCs were not significantly different for sex and sampling date with each fish species (*t*-test at $p < 0.05$).

Determination of OCs was carried out using 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 fused silica capillary column. Helium was employed as a carrier gas at a flow rate of 1 ml/min. Temperatures of the 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, to 280°C at a rate of 2.5°C/min, and maintained at 280°C for 1 min. Methane served as the reagent gas. Temperatures of the ion source and a quadrupole were maintained at 150°C and 106°C, respectively.

RESULTS AND DISCUSSION

Table 1 presents the result of OC residue levels. Total concentrations of measured OCs ranged from 1.70 to 16.8 ng/g w.w. (12 rainbow trout, 2002), from 0.49 to 6.74 ng/g w.w. (15 kokanee, 2002), from 2.88 to 7.85 ng/g w.w. (10 rainbow trout, 2003), and from 2.71 to 5.53 ng/g w.w. (nine Japanese dace, 2003). Thirty-six samples (i.e., eight rainbow trout, 2002; 11 kokanee, 2002; eight rainbow trout, 2003; nine Japanese dace, 2003) among all the samples (46 samples) showed the following residue pattern: HCHs > DDTs > CHLs > HCB > DRNs = heptachlor epoxide = heptachlor = mirex. On the other hand, remaining 10 samples provided no identical tendency among HCHs, DDTs, and CHLs, whereas the other OCs were accumulated with the order of HCB > DRNs = heptachlor epoxide = heptachlor = mirex. However, α-HCH and *p,p'*-DDE were obviously dominant in all the fishes from Lake Mashu. The proportion based on the sum of mean OC concentrations was: 23.5–46.2% (α-HCH) and 15.9–41.5% (*p,p'*-DDE). The mean concentration ranges of HCHs (1.37–3.23 ng/g w.w.) and DDTs (0.56–3.50 ng/g w.w.) were similar to those of Canadian lake trout muscle (DDTs: 2–10 μg/kg

Table 1. Mean concentrations (ng/g w.w.) of OCs in muscle tissue of fishes from Lake Mashu.

	Rainbow trout, 2003				Japanese dace, 2003				Rainbow trout, 2002				Kokanee, 2002			
	male, $n = 4$		female, $n = 6$		male, $n = 3$		female, $n = 6$		male, $n = 3$		female, $n = 9$		male, $n = 12$		female, $n = 3$	
Compounds	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
α-HCH	1.81	1.08	1.66	0.86	1.13	0.14	1.63	0.70	2.07	1.53	1.42	0.59	0.90	0.41	1.35	0.59
β-HCH	0.50	0.25	0.47	0.23	0.19	0.03	0.27	0.07	0.48	0.41	0.34	0.11	0.20	0.12	0.31	0.10
γ-HCH	0.52	0.30	0.47	0.22	0.44	0.01	0.55	0.11	0.64	0.48	0.42	0.16	0.27	0.11	0.41	0.15
δ-HCH	0.16	0.14	0.11	0.05	0.00	0.00	0.00	0.00	0.05	0.09	0.00	0.00	0.00	0.01	0.00	0.00
HCB	0.26	0.16	0.29	0.18	0.18	0.01	0.24	0.17	0.33	0.25	0.25	0.10	0.20	0.08	0.29	0.07
aldrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
endrin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.11	0.00	0.00
dieldrin	0.02	0.05	0.04	0.06	0.00	0.00	0.00	0.00	0.03	0.06	0.00	0.00	0.02	0.08	0.00	0.00
p,p'-DDD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p,p'-DDE	1.49	0.44	1.45	0.93	0.66	0.11	0.56	0.19	3.50	4.24	1.41	0.64	1.05	0.47	1.67	0.32
p,p'-DDT	0.00	0.00	0.27	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
heptachlor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.07	0.00	0.00
heptachlor epoxide	0.05	0.03	0.07	0.04	0.00	0.00	0.01	0.03	0.14	0.12	0.11	0.05	0.08	0.02	0.10	0.04
cis-chlordane	0.04	0.05	0.07	0.06	0.07	0.03	0.05	0.01	0.20	0.25	0.12	0.05	0.22	0.19	0.26	0.07
trans-chlordane	0.03	0.04	0.06	0.05	0.12	0.10	0.08	0.03	0.16	0.18	0.14	0.07	0.25	0.27	0.18	0.07
cis-nonachlor	0.05	0.03	0.08	0.05	0.03	0.04	0.03	0.01	0.19	0.21	0.08	0.03	0.10	0.04	0.19	0.01
trans-nonachlor	0.14	0.10	0.22	0.11	0.12	0.01	0.10	0.04	0.51	0.52	0.27	0.12	0.30	0.15	0.48	0.01
oxychlordane	0.04	0.04	0.08	0.06	0.00	0.03	0.01	0.02	0.14	0.12	0.14	0.05	0.07	0.03	0.12	0.02
mirex	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total HCHs	2.98	1.77	2.71	1.36	1.75	0.17	2.45	0.86	3.23	2.48	2.19	0.81	1.37	0.63	2.40	0.91
Total DRNs	0.02	0.05	0.04	0.06	0.00	0.00	0.00	0.00	0.03	0.06	0.00	0.00	0.07	0.16	0.00	0.00
Total DDTs	1.49	0.44	1.72	1.04	0.66	0.11	0.56	0.19	3.50	4.24	1.27	0.75	1.05	0.47	1.67	0.32
Total CHLs	0.31	0.26	0.50	0.29	0.33	0.13	0.28	0.10	1.20	1.24	0.72	0.33	0.94	0.65	1.23	0.13

Total HCHs = α-HCH + β-HCH + γ-HCH + δ-HCH; Total DRNs = aldrin + endrin + dieldrin; Total DDTs = *p,p'*-DDD + *p,p'*-DDE + *p,p'*-DDT; Total CHLs = *cis*-chlordane + *trans*-chlordane + *cis*-nonachlor + *trans*-nonachlor + oxychlordane; S.D.: standard deviation.

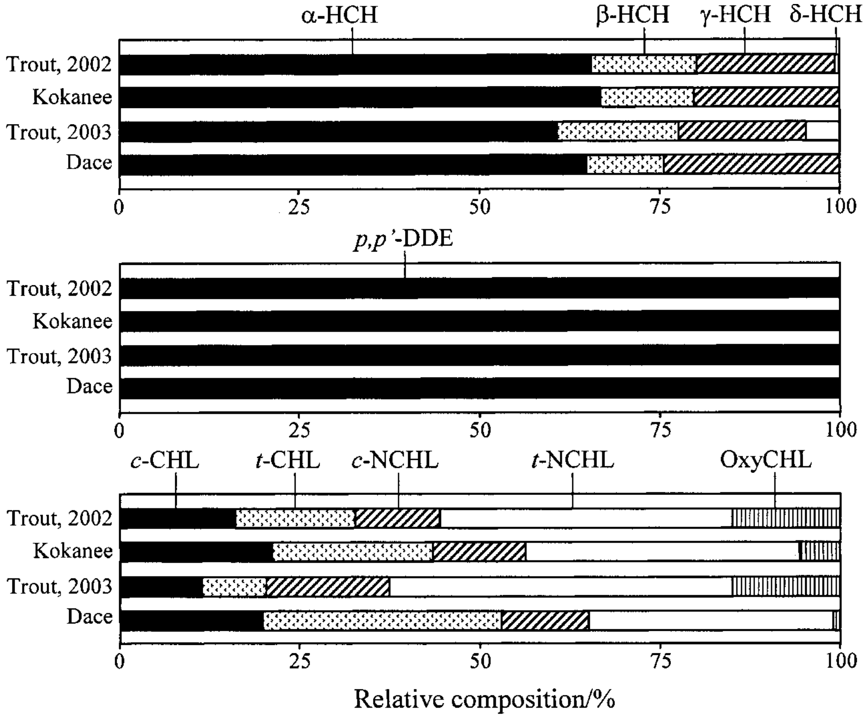

Figure 2. Relative composition of HCHs, DDTs and CHLs found in fishes from Lake Mashu. CHL: chlordane; NCHL: nonachlor; OxyCHL: oxychlordane.

w.w., HCHs: 0.2–0.5 μg/kg w.w.), of Arctic char muscle (HCHs: 0.2–2.4 ng/g w.w.) in Greenland, and of Canadian whitefish muscle (HCHs: 0.3–1.1 ng/g w.w.) (Wilson et al. 1995; Cleemann et al. 2000; Lockhart et al. 1992), whereas the mean concentrations of γ-HCH (lindane) and DDTs in the present study were lower than those (γ-HCH: 0.024 μg/g w.w., DDTs: 0.014 μg/g w.w.) of rainbow trout collected from Spanish fish farms (Sahagún et al. 1998).

Figure 2 indicates the relative compositions of HCHs, DDTs, and CHLs. In biota, β-HCH possesses the highest degree of bioaccumulation among HCHs, whereas the obtained HCH distribution delivered a markedly different pattern. The World Health Organization refers to the fact that technical HCHs are mainly composed of α-HCH (65–70%), β-HCH (7–10%), γ-HCH (14–15%), and δ-HCH (approx. 7%); with excretion as well as metabolism of α-HCH in fishes being considerably faster than that of β-HCH (WHO 1992). Although Lake Mashu is located in a remote area having no use of pesticides in the past, a close look at Figure 2 (top) will reveal that the HCH residue pattern was similar to the relative composition of technical HCH. The α- to γ-HCH ratios ranged from 2.6 to 3.5 in this study. The influence of HCH contributions accumulated in northern latitudes has also been observed in other regions involving remote lakes; for example, HCHs in the

European mountain lake waters which received only atmospheric inputs were found at considerably high levels (990–2900 pg/L) compared with those of other OCs (HCB, DDTs, and PCBs) (Vilanova et al. 2001). Further, Bidleman et al. (1995) have reported the higher content of α-HCH in the Arctic Lakes. Technical HCH is one of the most widely distributed OCs in the world. It is known that heavy usage of technical HCH has been carried out in China when compared with Japan. In fact, the strong impact of HCHs (in particular, α-HCH) on the Japanese atmospheric environment has been observed (Murayama et al. 2003). In addition to this, the previously investigated atmospheric HCH concentrations tended to show rather higher values of α-HCH in the Japanese peripheral sea compared with inland areas (Iwata et al. 1993), Judging from these data, it seems reasonable to suppose that evaporating HCHs from seawater occurred in Japan and that HCHs were distributed to lake water of Lake Mashu which is located at a higher latitude.

Use of technical DDT products in Japan has been prohibited since the early 1970s; *p,p'*-DDT and *p,p'*-DDD were lower than their detection limits in this study. In contrast to both those compounds, *p,p'*-DDE was found in all analyzed samples. Its residual levels was similar to that of Canadian lake trout muscle (mean 2.8 ng/g w.w.) (Wilson et al. 1995), whereas the previous data of lake trout inhabiting Lake Tahoe (154 ppb w.w.) were 45-fold greater than our data (Datta et al. 1999). It is noteworthy that DDT products are widely used as a malaria vector control in the Southeast Asian countries; for example, investigation of several biota samples in India illustrates a typical pattern (i.e., a high *p,p'*-DDT ratio) of DDT use (Pandit et al. 2002). Chemical and biological processes transform DDT into DDD or DDE in natural environments; in particular, DDE is dominant in high trophic biota. On the other hand, DDT decomposition by microbial activity is not expected to be vigorous in the case of a crater lake such as Lake Mashu; further, the mean water temperature throughout the year is constantly low (ca. 5°C). Catalan et al. (2004) estimated that the average residence time for *p,p'*-DDT and *p,p'*-DDE in brown trout are 2–3 years and 1 year, respectively; the time is longer than that (a few weeks) of HCHs. Therefore, the absence of DDT in Figure 2 (middle) indicates that this lake is independent of recent serious DDT input sources, including atmospheric deposition. DDTs are all characterized by 5–10 fold lower vapor pressures than α-HCH (Suntio et al. 1988). We conclude that the DDT levels in Lake Mashu's fishes reflect the lower volatility of this compound.

CHL products have been used widely in the East Asian countries as insecticides for termite control. The use of CHL products has been restricted since 1986 in Japan, but *trans*-chlordane was present in all samples except two. Figure 2 (bottom) indicates that *trans*-nonachlor (36–44% of CHLs) was a major contributor to the overall CHL contamination. This compound is a representative of technical CHL detected in higher trophic levels (Weber and Goerke 2003). As seen in Table 1, the total CHL concentrations ranged from 0.28 to 1.23 ng/g w.w.; the residue level was slightly lower than the level (0.001–0.024 μg/g w.w.) detected in Japanese marine fishes (MOE 2003). Further, the result also agreed with those of Canadian lake trout muscle (0.2–2.0 ng/g w.w.) and of US lake trout muscle (1 ng/g w.w.) (Wilson et al. 1995; Allen-Gil et al. 1997). Oxychlordane and heptachlor epoxide (i.e., a metabolite of CHL and of heptachlor) in Japanese dace had low detection frequencies (11% for the two OCs) compared with those of rainbow trout (86% for oxychlordane; 82% for heptachlor epoxide) and of kokanee (93% for oxychlordane; 86% for heptachlor epoxide). It is considered that the difference in concentrations of CHL metabolites is dependent on the metabolic rate of each species.

Correlation between the HCH isomer concentrations (ng/g w.w.) and lipid content (g) in extract of rainbow trout showed good linearity ([α-HCH] = 23.2 [lipid content], $r^2 = 0.91$; [β-HCH] = 6.35 [lipid content], $r^2 = 0.88$; [γ-HCH] = 6.52 [lipid content], $r^2 = 0.89$; [δ-HCH] = 1.77 [lipid content], $r^2 = 0.68$). Although lipophilic OCs tend to be accumulated in lipid, no significant correlation was found in plots of the other OCs for concentration versus lipid content of muscle. One explanation for this result may be that the accumulation ratio of each OC is organ or tissue dependent; however, the reason is unclear.

In toxic DDTs and γ-HCH (lindane), the mean daily intakes were estimated on the basis of the ADI proposed by FAO/WHO (1985) (20 μg/kg/day for DDTs and 10 μg/kg/day for γ-HCH). We have assumed 300 g of trout muscle as the mean daily intake for a human with 50 kg body weight. As a result, the anticipated intakes (0.0034–0.021 μg/kg/day for DDTs and 0.0016–0.0038 μg/kg/day for γ-HCH) were very far from the ADIs for DDTs and γ-HCH. It is concluded that the residue levels in this study would not affect human health.

Fish species inhabiting Lake Mashu have considerably accumulated HCHs and *p,p'*-DDE; the levels are similar to those of Arctic fishes. The human body burden of DDTs and of γ-HCH is not a serious situation for human consumption. Strong persistence of α-HCH having great volatility and short residence time in fishes indicates that technical HCH affects the closed Lake Mashu ecosystem. We conclude that atmospheric deposition causes OC contamination in Lake Mashu.

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