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Comparison of Organochlorine Compounds Among Fat, Muscles, and Livers of Pintails (*Anas acuta*) from Lake Hyoko, Japan

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An international convention aiming to restrict persistent organic pollutants (POPs) was formally adopted in May 2001, and global actions to reduce and eliminate releases of the pollutants have been recommended. These POPs consist of twelve organochlorine compound (OC) groups containing unintentional by-products. The OCs known as the "dirty dozen" have already had a strong impact on wildlife and human beings (Choi et al. 2001). Avian species tend to be particularly vulnerable to the effects of these compounds. Chlorinated pesticides have been shown to give rise to estrogen-dependent reproductive effects in several avian species (Fry 1995); further, dichlorodiphenyltrichloroethane (DDT) and its metabolites have also been shown to cause obstruction of Ca^{2+} metabolism in birds (Lundholm 1994). However, for the clarification of the toxic effects of OCs, basic information such as the distribution of OCs among organs (or tissues) is still insufficient. The distribution ratios obtained through this study will enable us to develop an easy and effective means of analyzing wildlife for contamination and provide insight into the global migration of these toxic chemicals.

Pintail (*Anas acuta*) is a migratory bird that commonly winters in Japan, arriving here from Siberia. Since the so-called breeding grounds possess a relatively low population density, the region around Siberia is independent of specific contamination, at least that based on human activities. Until now, little knowledge of OCs has been reported in avian species from the Far East. Damage by chemical substances in migrants has been traced to pesticide use on wintering grounds (White et al. 1981). This fact implies that the Japanese ecosystem might have a disproportionate impact on the wildlife inhabiting Siberia. To evaluate southern Japan as a pollution source, we have to perform a comprehensive investigation of residues in biota from the Far East. One of the effective uses of such investigation would be to understand the realities of OC movements by migrants like pintails.

In January 2003, we carried out a sampling survey of pintails at Lake Hyoko, Niigata, Japan (Figure 1). Concentrations of nineteen OCs containing their metabolites were determined by gas chromatography-mass spectrometry (GC-MS) based on negative ion chemical ionization. Distribution patterns of these OCs among fat, breast muscles, and livers are discussed herein.

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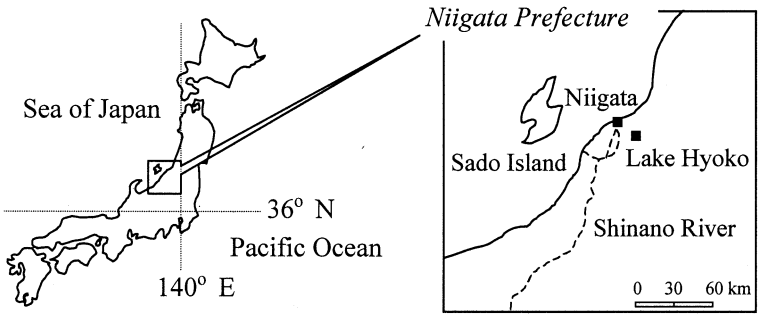

Figure 1. Map of Japan showing Lake Hyoko, Niigata.

MATERIALS AND METHODS

Solvents for the dioxin-analysis grade (Wako Pure Chemical Industries, Osaka, Japan) were employed. Authentic standards were purchased from AccuStandard (CT, USA). All isotope labeled standards were purchased from Cambridge Isotope Laboratories (MA, USA). All the other chemicals (Wako Pure Chemical Industries, Osaka, Japan) were of highest grade available and were used without further purification.

After determination of biological parameters, the pintails were dissected, and were stored at –20 °C in sealed glass containers before analyses. The sample (fat, 1 g; a breast muscle, 5 g; a liver, 5 g) containing a dehydrating agent was transferred to a steel column for the pressurized liquid extraction system (ASE-200, Dionex, CA, USA). Extraction was carried out with acetone/hexane (1:1; v/v) at 100 °C within 10 min. All the samples were extracted twice, and surrogate standards (10 ng) were added. 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 Bio-Beads® S-X3, 200–400 mesh (Bio-Rad, CA, USA) using mixture solution of dichloromethane/cyclohexane (1:1; v/v). A fraction between 125 ml and 275 ml was transferred to a rotary evaporator, and the fraction 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, 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). The 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) containing from top to bottom: 2 g of anhydrous sodium sulfate, 5 g of Silica gel 60 (Merck, Darmstadt, Germany: activated 130 °C, 18 h), 2 g of anhydrous sodium sulfate, and a quartz wool plug. The concentrate was loaded, and 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. Five hundred picograms of $^{13}C_{12}$-labeled PCB 153 as an internal standard was added to the final concentrate.

Determination was carried out by a portable mass spectrometer 5973N Mass Selective Detector (Agilent Technologies, DE, USA) equipped with a 6890 series gas chromatograph (Agilent Technologies, DE, USA). HT8 (SGE Japan, Kanagawa, Japan: 50 m length, 0.22 mm i.d., 0.25 μm film thickness) was selected for a fused silica capillary column. Helium was employed as a carrier gas at a flow rate of 1 ml min^{-1}. Temperatures of an injector port and a transfer line in the gas chromatograph were maintained at 260 °C and at 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. Methane was employed as a reagent gas. Temperatures of an ion source and a quadrupole were maintained at 150 °C and 106 °C, respectively.

RESULTS AND DISCUSSION

SPSS version 10.0J (SPSS Inc., Chicago, IL, USA) and FreeJSTAT 8.2 (Free software provided by M. Sato, Japan) were used for the statistical analysis. Non-parametric multiple comparison using the Kruskal-Wallis test was employed to compare OC concentrations in tissues. The concentrations were presented as medians on a lipid weight (l.w.) basis. Mean lipid contents (mean ± standard deviation) were as follows: 81.9±15.2% (fat), 1.5±0.8% (muscles), and 5.5± 2.1% (livers). Mean recoveries of labeled surrogate standards ranged from 60.9% to 119% with relative standard deviations of 10% or less.

Table 1 summarizes all of the data. Residue patterns in fat and muscles were as follows: ∑DDTs > ∑CHLs > ∑HCHs = HCB > heptachlor epoxide > ∑DRNs >> mirex (DDT-related compounds, DDTs; chlordane-related compounds, CHLs; hexachlorocyclohexane-related compounds, HCHs; hexachlorobenzene, HCB; drin-related compounds, DRNs). In contrast with these patterns, livers presented a slightly different tendency: ∑CHLs > ∑DDTs = heptachlor epoxide > ∑DRNs > ∑HCHs = HCB >> mirex. ∑DDTs were detected in the range of 72.1–1594 ng g^{-1} l.w. (fat), 89.0–2716 ng g^{-1} l.w. (muscles), and 44.1–510 ng g^{-1} l.w. (livers). DDTs tended to be accumulated in fat; probably, the decrease in lipid content of muscles by migration played a role in concentrating the DDT residues of muscles. In particular, the concentration in muscles was significantly higher than that in livers ($p < 0.05$) and was similar to concentrations (e.g., 30–4600 ng g^{-1} l.w.) in muscles of migratory birds from Lake Baikal, Russia (Kunisue et al. 2002). Relative compositions of major OCs are illustrated in Figure 2. The frequency of DDTs in the present study approximated the following order: *p,p'*-DDE >> *p,p'*-DDD > *p,p'*-DDT. Furthermore, it was impossible to detect *p,p'*-DDT in livers. The fact that chemical and biological processes transform DDT into DDD or DDE in natural environments (Baxter 1990) is now widely accepted; the concentrations of

Table 1. Concentrations (ng g^{-1} l.w.) of OCs in fat, breast muscles, and livers.

	Fat, $n = 11$			Breast muscles, $n = 11$			Livers, $n = 11$		
Compounds	median	25% tile	75% tile	median	25% tile	75% tile	median	25% tile	75% tile
α-HCH	0.5	0.3	1.1	3.4	1.5	7.5	1.0	0.7	1.9
β-HCH	29.9	20.6	41.1	52.8	25.1	93.2	16.7	12.0	28.8
γ-HCH	0.1	0.1	0.2	1.0	0.4	1.6	0.0	0.0	0.0
δ-HCH	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HCB	29.0	20.0	48.0	83.4	35.1	141	23.4	12.9	54.6
aldrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
endrin	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	1.5
dieldrin	9.8	2.7	17.8	17.5	13.7	27.7	39.0	14.3	115
p,p'-DDD	5.8	2.4	17.3	0.0	0.0	18.6	0.0	0.0	16.9
p,p'-DDE	167	133	491	528	190	1221	96.8	59.5	276
p,p'-DDT	37.7	10.1	83.3	0.0	0.0	0.0	0.0	0.0	0.0
heptachlor	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0
heptachlor epoxide	14.6	7.1	36.0	35.2	7.7	46.1	112	53.6	229
cis-chlordane	1.6	0.8	2.4	4.8	2.4	6.8	1.3	0.6	2.7
trans-chlordane	12.6	7.4	47.6	31.1	10.8	45.0	13.9	2.9	29.5
cis-nonachlor	4.3	0.5	13.9	4.4	2.1	13.1	1.4	0.3	8.2
trans-nonachlor	18.2	4.0	47.4	21.6	12.1	42.1	22.6	6.0	56.3
oxychlordane	40.1	17.3	76.5	52.4	13.9	103	102	43.6	223
mirex	0.0	0.0	0.0	0.0	0.0	8.1	0.0	0.0	0.0
Total HCHs	32.1	21.0	41.6	57.0	28.7	108	17.1	12.6	31.4
Total DRNs	9.8	2.7	18.3	17.5	13.7	27.7	41.2	14.3	115
Total DDTs	252	181	657	528	190	1246	120	59.5	276
Total CHLs	61.7	46.0	151	147	50.5	172	149	70.7	301

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.

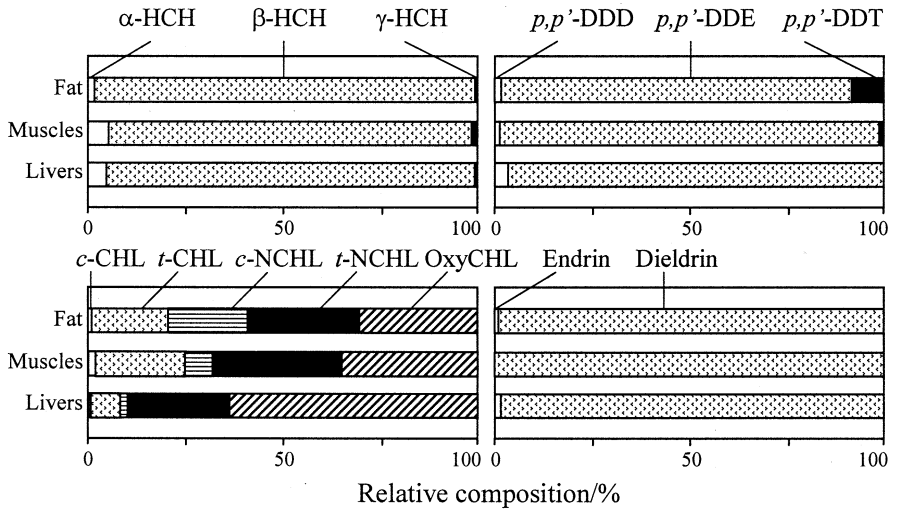

Figure 2. Relative compositions of major organochlorines found in pintail from Lake Hyoko. CHL: chlordane; NCHL: nonachlor; OxyCHL: oxychlordane.

p,p'-DDE in pintails occupied 82–98% of the ∑DDTs. Keith and Gruchy (1972) have supposed that *p,p'*-DDE remains in wildlife at higher concentrations than other organic pollutants. Our data were in accord with their report; this dominant DDT transformation product was thought to provide the characteristic contamination of birds through bioaccumulation. Although use of technical DDT products in Japan has been prohibited since the early 1970s, *p,p'*-DDT was significantly more dominant in fat (15% in ∑DDTs) than in the other tissues ($p < 0.01$). The presence of *p,p'*-DDT indicates that this species was recently exposed to *p,p'*-DDT in its breeding grounds, stopover sites, or wintering grounds. In fact, use of DDT products as a malaria vector control has been prevalent in Southeast Asia for a long time; high concentrations of ∑DDTs (max. 54000 ng g^{-1} l.w. in Chinese mussels) are still being detected in species from that region (Monirith et al. 2003). Therefore, we concluded that *p,p'*-DDT originated in Southeast Asia or China (atmospheric transportation). Comparison of OC residues in black-tailed gulls from Japanese remote areas also indicated the existence of a "DDT hot spot" around Japan (Choi et al. 2001). According to bioaccumulation through the food web (i.e., differences in dietary exposure), it is assumed that herbivorous pintails possess lower OC concentrations than piscivorous and insectivorous migrant species. Table 1 indicates that the concentrations of ∑DDTs (fat, 252 ng g^{-1} l.w.; muscles, 528 ng g^{-1} l.w.; livers, 120 ng g^{-1} l.w.) were less than that of ∑DDTs (1907 ng g^{-1} l.w.) in piscivorous black-tailed gulls.

CHL products have been widely used as insecticides against termites in East Asian countries. Although the use of CHL products has been restricted in Japan since 1986, CHLs were present in all the samples. Figure 2 implies that oxychlordane (43–68% in ∑CHLs) and *trans*-nonachlor (21–24% in ∑CHLs) were the major contributors to the overall CHL contamination; in particular, total

concentration of both the compounds in livers occupied on average 89% of the ∑CHLs. These compounds also dominated among the CHLs found in the fat of albatrosses from the North Pacific Ocean (Muir et al. 2002). Oxychlordane was shown to be a more stable metabolite than heptachlor epoxide, transformed from heptachlor, in birds as well as mammals (Nomeir and Hajjar 1987). Figure 2 emphasizes the accumulation of oxychlordane in livers; however, no significant differences in oxychlordane content were found among the tissues ($p > 0.05$). Heptachlor was present at a higher detection frequency (45%) in fat, and the maximum concentration delivered 2.5 ng g^{-1} l.w. The detection frequencies of heptachlor in muscles and livers were 18% and 9%, respectively. Heptachlor epoxide was found in all the samples in the range of 2.6–85.2 ng g^{-1} l.w. (fat), 4.0–228 ng g^{-1} l.w. (muscles), and 16.4–773 ng g^{-1} l.w. (livers). These residue levels tended to be slightly higher than those in comprehensive investigations of migratory birds including pintails from California and Mexico (Mora et al. 1987). The concentration of heptachlor epoxide in livers differed significantly from that in fat ($p < 0.01$) and muscles ($p < 0.05$), and the action of metabolic enzymes concerning heptachlor provided the characteristic concentration for livers. For migratory birds, it is impossible to estimate accurately the CHL burden in only the wintering area. However, the fact that *trans*-chlordane appears in slightly high ratios in Figure 2 signifies at the least, recent exposure to technical CHLs in the Far East. On the basis of the Asia-Pacific mussel watch program (Monirith et al. 2003), Japanese aquatic environment possess relatively high CHL concentrations compared with the other OCs. Therefore, the aquatic ecosystem around Lake Hyoko might cause CHLs to accumulate in pintails.

In Japan, HCB was produced before 1979 as a synthetic raw material. This compound was also unintentionally formed by industrial activities such as the synthesis of chlorinated solvents and waste incineration. It can be assumed that most of the countries contribute emission sources of HCB at present. In this study, HCB was detected in the range of 16.4–119 ng g^{-1} l.w. (fat), 22.3–489 ng g^{-1} l.w. (muscles), and 10.3–79.6 ng g^{-1} l.w. (livers). Judging from a previous study (Hansen et al. 1978), the concentrations were too low to have an overt adverse effect on birds. A significant difference was present between muscles and livers ($p < 0.05$); this result suggested that the lipid in the breast muscle as well as subcutaneous fat became a final reservoir of HCB.

With its various uses (insecticides and vector control, and others), technical HCH is one of the most widely distributed organochlorines in the world. It is known that heavy usage of technical HCH (an estimated 4.5 million t) has been carried out in China (Li et al. 1998); that country alone is a sufficient emission source of HCHs for the entire Far East. The distribution tendency of HCHs in tissues was similar to that of HCB; that is, breast muscles showed the highest concentrations (13.0–132 ng g^{-1} l.w.). Although technical HCH is mainly composed of α-HCH (65–70%), β-HCH (7–10%), γ-HCH (14–15%), and δ-HCH (approx. 7%), the total concentration (∑HCHs) was controlled completely by β-HCH. The strong persistency of β-HCH can be attributed to its rate of degradation, as the compound

has been shown to possess the slowest rate among HCHs in broilers (δ-HCH > γ-HCH > α-HCH > β-HCH) (WHO 1992). The concentrations in muscles and livers showed a significant difference ($p < 0.05$).

Endrin was found with low frequencies in fat (45%) and livers (36%); further, the contents in both tissues also were lacking in dominance (fat, nd–0.6 ng g^{-1} l.w.; livers, nd–5.7 ng g^{-1} l.w.). Aldrin was absent in these tissues. As to why aldrin was not detected, the compound may have been transformed into dieldrin in wetlands. It has been reported that amino acid and humic acid in aquatic environments possess the ability to epoxidate aldrin under natural light (Ross and Crosby 1985). After wildlife was exposed to aldrin (dietary exposure), the epoxidation (i.e., converting aldrin into the corresponding epoxide, dieldrin) would tend to develop in the liver. The residue levels of dieldrin were in stark contrast with those of aldrin. Dieldrin was detected in all the samples (fat, 1.5–126 ng g^{-1} l.w.; muscles, 2.6–913 ng g^{-1} l.w.; livers, 4.7–243 ng g^{-1} l.w.) and was significantly more prevalent in livers than in fat ($p < 0.05$). Dieldrin was the most dominant compound among the compounds mentioned above; however, the contribution ratio of "dieldrin transformed from its precursor" to detected dieldrin was unclear.

Comparison of organochlorines in fat, breast muscles, and livers of pintails was useful for the estimation of characteristic distribution of several OCs. Reflection on some of these will make clear that the decrease of lipid contents cause the concentration of most OCs. As an overall tendency, in particular, lipid in breast muscles was shown to be capable of being a final sink for migratory birds; we may, therefore, reasonably conclude that the distribution of OCs stems from the motion of breast muscles during migration. In addition, it was noted that the compounds (heptachlor epoxide, oxychlordane, dieldrin, and endrin) containing oxygen in their structures tended to be present in higher concentrations in livers than in breast muscles.

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