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Toxaphene and Other Organochlorine Compounds in Pintails (*Anas acuta*) from Saitama Kamoba in Japan during Oct 2000–Feb 2002

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Widespread agricultural use and industrial use of organochlorine compounds (OCs) had led to heavy contamination of OCs in water, air, and soil. OCs are generally characterized by strong persistency, bioconcentration through food webs, and long-range global transport; in addition, the compounds are known to affect the immune systems of wildlife (Bustnes et al., 2004). Over recent decades, the effects of dichlorodiphenyldichloroethylene (DDE) on eggshell quality and embryo hatchability have been the subjects of controversy; many researches on OC toxicity have been reported (McCarty and Borgert, 2006). Therefore, it is necessary to estimate OCs that accumulates to wildlife to consider countermeasures related to protection of wildlife.

Saitama Kamoba, located at the Imperial Wild Duck Preserve, Japan, in the Saitama prefecture (Fig. 1), has a pond covering an area of 13,000 m^2. In the winter, although over 10,000 wild ducks migrate their, little attention has been given to chemical contamination of the ducks inhabiting the pond. The Arctic, including the breeding grounds of migratory birds, has become a sink for OCs from lower latitudes; in particular, this is a serious problem for avian species having long life spans. We carried out extensive sampling surveys at Saitama Kamoba to collect over 40 pintails from Siberia during Oct 2000–Feb 2002. The purpose of this paper is to clarify OC residue level of pintails and compare our data with those obtained from other regions.

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Materials and Methods

Acetone, hexane, dichloromethane, cyclohexane, and diethyl ether were of the highest grade available (Wako Pure Chemical Industries, Osaka, Japan) and were used without further purification. Hexachlorobenzene (HCB), aldrin, endrin, dieldrin, cis-/trans-chlordane, cis-/trans-nonachlor, oxychlordane, heptachlor, o,p'-/p,p'- dichlorodiphenyldichloroethane (DDD), o,p'-/p,p'-DDE, o,p'-/p,p'-dichlorodiphenyltrichloroethane (DDT), and heptachlor epoxide were purchased from AccuStandard (CT, USA). Five toxaphene congeners (B8-1413, B7-515, B9-1679, B9-1025, and B10-1110) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). $^{13}C_6$-HCB (99%), $^{13}C_{12}$-aldrin (99%), $^{13}C_{12}$-endrin (99%), $^{13}C_{12}$-dieldrin (99%), $^{13}C_{10}$-cis-/trans-nonachlor (99%), $^{13}C_{10}$-trans-chlordane (99%), $^{13}C_{10}$-heptachlor (99%), $^{13}C_{10}$-heptachlor epoxide (99%), $^{13}C_{10}$-oxychlordane (99%), d_8-p,p'-DDD (98%), $^{13}C_{12}$-o,p'-/p,p'-DDE (99%), $^{13}C_{12}$-o,p'-/p,p'-DDT (99%), and $^{13}C_{12}$- polychlorinated biphenyl (PCB) 153 (99%) were purchased from Cambridge Isotope Laboratories (MA, USA).

Pintails were captured at Saitama Kamoba in Oct 2000, Feb 2001, Oct 2001, and Feb 2002, using a thrown net. After determination of their biological parameters, the pintails were dissected and stored at –20°C in sealed glass containers before analyses. One gram of fat tissue 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 for 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

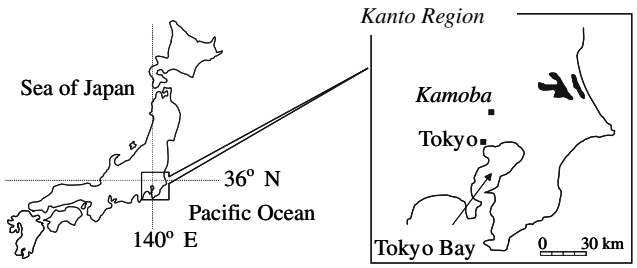

Fig. 1 Map of Japan showing Saitama Kamoba

Bio-Beads® S-X3, 200–400 mesh (Bio-Rad, CA, USA) using a mixed solution of dichloromethane/cyclohexane (1:1 v/v). A fraction of 125–275 ml was transferred to a rotary evaporator, and 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 at 130°C for 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, Darmstadt, Germany: activated at 130°C for 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. The results were obtained by duplicate analysis.

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 the flow rate of 1 ml/min. One microliter of a final concentrate was injected with an autosampler 7673 (Agilent Technologies, DE, USA) under a splitless mode (under a pulsed splitless mode in the case of toxaphene). The temperatures of the injector port and transfer line in the gas chromatograph were maintained at 260°C (220°C in the case of toxaphene) and 280°C, respectively. The column temperature for toxaphene analysis was maintained at 60°C for 1 min, ramped to 170°C at a rate of 23°C/min, 7.5 min isothermal, to 275°C at a rate of 3°C/min, and maintained at 275°C for 12 min. The column temperature for the other persistent organic pollutants (POPs) analysis 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 was employed as a reagent gas for negative ion chemical ionization under pressure of 2.4×10^5 kPa. The temperatures of an ion source and a quadrupole were held at 150°C and 106°C, respectively. The mass spectrometer was operated on the basis of selected ion monitoring (SIM).

SPSS version 10.0J (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis, and significant differences were evaluated at $p < 0.05$. Preliminary statistical analyses provided no significant differences in mean concentrations between males and females (or adults and juveniles). Therefore, comparisons were limited to differences between levels of various OCs and sampling periods (October and February); the comparisons were carried out using Student's *t*-test assuming different variances. The mean lipid weight fraction of pintail fat tissues ($n = 41$) was 89.6 ± 9.6%. The obtained data were divided into two parts; October was just after the birds had flown in, while February was the time for the birds to fly off again. The OC concentrations in each season are represented as means ± standard deviations (SD). The method detection limits ranged from 0.0002 to 0.04 ng/g. Mean recoveries of labeled surrogate standards ranged from 61 to 111%.

Results and Discussion

Table 1 summarizes all of the data. The concentrations are shown on a lipid weight basis. The dominance of detected OCs showed the approximate order: CHLs (the sum of cis-/trans-chlordane, cis-/trans-nonachlor, oxychlordane, heptachlor, and heptachlor epoxide) = DDTs (the sum of o,p'-/p,p'-DDD, o,p'-/p,p'-DDE, and o,p'-/p,p'-DDT) > HCB > DRNs (the sum of aldrin, endrin, and dieldrin) > > TOX (the sum of B8-1413, B7-515, B9-1679, B9-1025, and B10-1110). DDTs were detected in the range 5.1–667 ng g^{-1} during Oct 2000–Feb 2002, which was narrower than that (11–9785 ng g^{-1} on a dry weight basis) of pintails captured in southern California and Mexico during Dec 1981–Mar 1982 (Mora et al., 1987). The detection frequency of DDTs approximately followed the order: p,p'-DDE > p,p'-DDD > p,p'-DDT > o,p'-DDD = o,p'-DDE = o,p'-DDT. p,p'-DDE accounted for more than 78% of the total concentrations of DDTs in this study, whereas the mean concentrations of DDTs in our herbivorous pintails were 20 times

Table 1 Mean concentrations (ng/g l.w.) of OCs in pintail fat

	Oct 2000		Feb 2001		Oct 2001		Feb 2002	
	N = 12		*N* = 10		*N* = 10		*n* = 9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HCB	23	16	31	16	21	13	25	15
DRNs	3.2	6.1	14	14	3.2	1.7	10	10
DDTs	53	63	126	193	92	105	111	126
CHLs	71	118	195	284	31	17	96	85
TOX	0.12	0.14	0.18	0.08	0.52	0.52	0.30	0.55

SD: standard deviation.

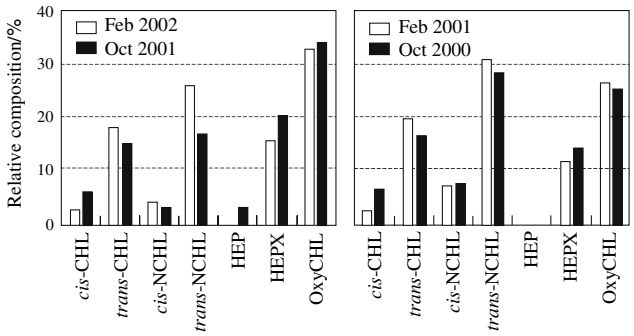

Fig. 2 Relative compositions of CHLs found in pintail fat tissues. CHL: chlordane; NCHL: nonachlor; HEP: heptachlor; HEPX: heptachlor epoxide

smaller than those in piscivorous black-tailed gulls(1377–2459 ng g^{-1}) (Choi et al., 2001).

Figure 2 shows the relative compositions of CHL-related compounds. The compositions were basically the same over the period Oct 2000–Feb 2002, and relatively high concentrations were detected. Oxychlordane (26–34%) and trans-nonachlor (17–31%) were the major contributors to the overall CHL contamination, in agreement with results for albatrosses from the North Pacific Ocean (Muir et al., 2002). Although heptachlor epoxide (1.6–227 ng g^{-1}) was found in all the samples, heptachlor was detected in the range 0.68–2.5 ng g^{-1} with a low frequency (17%). This result led to the conclusion that pintails captured in Saitama Kamoba showed advanced metabolism for heptachlor.

HCB was present in all the samples within the range 4.4–67 ng g^{-1}; the mean concentrations during Oct 2000–Feb 2002 were 23 ± 16 ng g^{-1} (October 2000), 31 ± 16 ng g^{-1} (February 2001), 21 ± 13 ng g^{-1} (October 2001), and 25 ± 15 ng g^{-1} (February 2002). In the short period from October to February in the investigated years, an obvious seasonal variation ($p > 0.05$) of concentrations was absent. The World Health Organization (WHO, 1997) has suggested that HCB is formed unintentionally by industrial activities such as the synthesis of chlorinated solvents and waste incineration. It can therefore be assumed that most countries continue to emit HCB at present. In addition, this compound, having a high vapor pressure and a wide range of half-lives, is easily transported in the atmosphere. These facts probably explain the constant concentrations of HCB in this study.

Endrin was found in small amounts (0.44 ng g^{-1} and 0.77 ng g^{-1}) in only two samples. In contrast, dieldrin was present in almost all of the samples. The mean concentration of DRNs in each sampling period was 3.2–14 ng g^{-1}; the mean concentration in February 2001 (14 ± 14 ng g^{-1}) was significantly higher than that in October 2000 (3.2 ± 6.1 ng g^{-1}).

Toxaphene, like the DDT products, was distributed worldwide, and yet was never registered as a pesticide in Japan. In particular, large quantity of commercial pesticides including toxaphene was spread in the former Soviet Union and the United States in the past (Voldner and Li, 1993). We determined concentrations of toxaphene through a commercially available standard mixture containing five toxaphene congeners. As seen in Table 1, the mean residue levels (0.12–0.52 ng g^{-1}) of toxaphene were extremely low compared to the other OCs. The minor contribution of toxaphene contrasted with a stronger toxaphene contribution in the data (for albatross fat tissues from the North Pacific Ocean) reported by Muir et al. (2002). As we know, however, avian species and sampling locations have been deemed the causes of the major differences in toxaphene contribution between the two sets of data. Only two prominent congeners (B8-1413 and B9-1679) were found among five analyzed congeners in pintails. B9-1679 could be detected as several research groups have reported the selective bioaccumulation of the congener in nonachlorobornanes (Herzke et al., 2002; Buser and Müller, 1994). B9-1679 and B8-1413 levels ranged from 0.03–0.64 ng g^{-1} (detection frequency 61%) and 0.02–1.0 ng g^{-1} (detection frequency 85%). These concentrations were considerably lower than those in Antarctic penguin fat (B8-1413, 34 ng g^{-1}; B9-1679, 23 ng g^{-1}) (Vetter et al., 2001). In this study, the ratio of [B8-1413]/[B9-1679] ranged from 1.1–2.9, contrasting with those (approximately 1.0) detected in albatrosses from the northern hemisphere (Muir et al., 2002). Although the reason for the dominance of B8-1413 was unclear, we expected that these ratio originated from feeding habits or differences among species. Our data indicate that toxaphene contamination has spread to the Far East, including Japan.

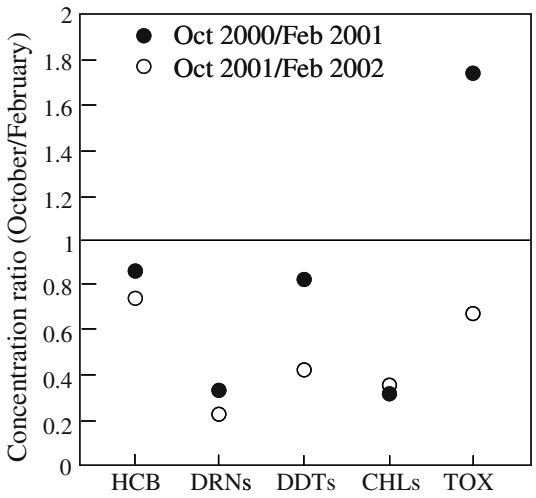

Fig. 3 Seasonal variations of detected OCs

Figure 3 shows seasonal OC concentration ratios ([OCs detected in October]/[OCs detected in February]) in our pintail fat tissues. There are two accumulative pathways to explain the ratios. One is the increase of observed OC concentrations due to the loss of internal lipid weight (exercise by migrant), and the other is OC exposure in wintering grounds. Here, as seen in Fig. 3, concentrations of OCs (except TOX) in the period Oct 2000–Feb 2001 were higher in February than in October. Seasonal variations were more apparent for DRNs and CHLs. CHL products were used for termite control in Japan until 1986. On the other hand, Tanabe et al. (1998) have pointed out the possibility that CHLs in migratory birds are excreted relatively promptly when the bird begins to fly off for the season. It will be clear from these examples that migrant pintails are unintentionally exposed to high levels of CHL contamination in Japan rather than in their breeding grounds.

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