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BIOACCUMULATION AND METABOLISM OF PCBS AND DDE IN SHORT-TAILED SHEARWATER *PUFFINUS TENUIROSTRIS* DURING ITS TRANSEQUATORIAL MIGRATION AND IN THE WINTERING AND BREEDING GROUNDS*

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Abstract: PCBs and *p, p'*-DDE in Short-tailed Shearwater (*Puffinus tenuirostris*) from Tasmania, Japan and the northern North Pacific were measured. The mean concentrations of PCBs and *p, p'*-DDE found in the bind from Tasmania were 14 and 20 ng/g in chicks and 150 and 89 ng/g in adults respectively, on wet weight basis. The values of both the chemicals were found to be lower in chicks than in adults, and the degree of disparity in chemical levels between chicks and adults is greater in PCBs than in *p, p'*-DDE. Reflecting this, the DDE/PCB ratios were higher in chicks (1.3) than in adults (0.60). This is likely due to the greater accumulation of PCBs during the period of stay in the northern North Pacific feeding ground than in the southern South Pacific ones.

The shearwaters from Japan and the northern North Pacific seem to be post-fledgling or yearlings, since their DDE/PCB ratios (1.1 and 1.2) were found to be nearly the same as those in chicks (1.3) from Tasmania.

It is known that two types of metabolic enzymes (PB and MC type) are mainly associated with the PCB metabolism in higher animals. When compared the PCB compositions in the shearwaters from Japan and Tasmania, some members of PCBs metabolized mainly by the MC type enzyme systems were found to decrease or disappear in the bird from Japan, indicating that the activity of the MC type enzyme systems in the shearwater increased more than that of the PB type enzyme systems during the transequatorial migration of the bird.

1. Introduction

Some seabirds are known to be useful bioindicators reflecting the pollution levels of persistent chlorinated hydrocarbons in their habitats, since they feed mainly on the endemic species which accumulate the chemicals corresponding to the levels of pollutants in the habitats (TANAKA and OGI, 1984). Migratory birds reflect the contamination by chlorinated hydrocarbons of breeding grounds and/or wintering grounds.

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HENRY *et al.* (1982) suggested that the Peregrin Falcon (*Falco peregrinus*) breeding in northwestern North America was exposed to DDT in Latin America wintering grounds, since DDE levels in their blood plasma samples were found to be higher in spring migration than in fall migration. The Herring Gull (*Larus argentatus*) breeding in southwestern Finland was also suggested to be exposed to PCBs and DDTs in wintering grounds, viz, the southern Baltic Sea (LEMMETYINEN *et al.*, 1982). According to the monitoring survey of some Korean birds by MIN *et al.* (1984), the concentrations of chlorinated hydrocarbons in summer visitors were found to be higher than those in winter visitors and residents. They suspected that Southeast Asia, the wintering area for summer visitors is much more polluted than Siberia and Mongolia, the breeding area for winter visitors and Korea for permanent residents.

Short-tailed Shearwater (*Puffinus tenuirostris*) makes a long transequatorial migration between its southeast Australian breeding islands and North Pacific wintering grounds (SERVENTY, 1967; SERVENTY *et al.*, 1971; SHUNTOV, 1961). The eggs normally hatch by the end of January and chicks attain the fledgling stage by the beginning of May and they migrate to reach the northern North Pacific, wintering grounds, in the beginning of June. The adults arrive in the northern North Pacific, from the end of April to the beginning of May. Shearwaters feed mainly on small-sized organisms like the larvae and juveniles of fish and squid and zooplankton which are usually abundant in the surface layer of the pelagic environment (SERVENTY, 1967; SERVENTY *et al.*, 1971; OGI *et al.*, 1980).

Considering this, Short-tailed Shearwater is expected to be a useful bioindicator to know the pollution levels of chlorinated hydrocarbons in both hemispheres. Due to the long distance migration, the increased concentration of lipophilic xenobiotics resulting from the rapid consumption of body fat is also expected and this might induce some physiological changes in the body of the shearwater.

The present study describes the specific bioaccumulation and metabolism of PCBs and p,p'-DDE during the transequatorial migration of the Short-tailed Shearwater.

2. Materials and Methods

2.1. Samples

Ten chicks younger than three months old and fourteen adults of Short-tailed Shearwater (*Puffinus tenuirostris*) were collected from the breeding ground, Tasmania, Australia during November 1983 and April 1984. In May 1984, three shearwaters which fell down during migration from Tasmania to the northern North Pacific were collected from the beach of Kujûkuri, Pacific middle coast of Japan. In the northern North Pacific (51°34′N, 173°58′E), two shearwaters incidentally entangled in gill nets set for salmon were also sampled in June 1982. Sex, body weight and life stage of each sample are shown in Table 1. The samples were stored at -20°C or below until analysis.

2.2. Chemical analysis

The body of each shearwater was homogenized with the meat chopper and then the minced body samples were employed for PCBs and p,p'-DDE analyses, following the alkaline alcohol digestion method of WAKIMOTO *et al.* (1971). 10–30 g of the minced samples were refluxed in 100–300 m*l* 1 N KOH-ethanol solution for 1 h. PCBs and *p, p'*-DDE thus extracted into ethanol were transferred to 100 m*l* hexane. Subsequently, the hexane layer was concentrated and cleaned up by 1.5 g of silica gel (Wako gel S-1) packed in a glass column (10 mm i.d. $\times$ 200 mm length). PCBs and *p, p'*-DDE were eluted with 200 m*l* of hexane at an elution rate of 1 drop/s. The eluate was concentrated to 5 m*l* in a Kuderna-Danish concentrator and concentrated hexane was cleaned with 5% fuming sulfuric acid.

Aliquots of these solutions were injected into an electron capture gas chromatograph (GC-ECD), Shimadzu GC-7A, with a splitless system for the determination of PCBs and *p, p'*-DDE (*p, p'*-DDE present here includes *p,p'*-DDT, since *p, p'*-DDT is converted into *p, p'*-DDE during the alkaline alcohol digestion). The column used was WCOT glass capillary (0.23 mm i.d. $\times$ 30 m length) coated with OV-101 for both PCBs and *p, p'*-DDE analysis. The operating conditions for PCBs are as follows: Column temperature was initially kept at 180°C for 16 min and then raised to 230°C at a rate of 0.5°C/min and then held for 32 min. Injector and detector temperature were 250°C. Carrier flow of nitrogen was controlled at 0.7 m*l*/min. For *p, p'*-DDE column temperature was at 230°C isothermal and other GC conditions were the same as PCBs analysis.

A few representative samples were also injected into a Shimadzu 9020 DF gas chromatograph-mass spectrometer (GC-MS) equipped with an electron-impact ion source and a SCAP-1123 data-system for the measurement and identification of PCB isomers and congeners. The column was the same as in GC-ECD. The operating conditions of GC-MS are as follows: Column temperature was programed from 190° to 250°C at 0.5°C/min. Ion source temperature was kept at 280°C. PCB isomers and congeners were determined by selected ion monitoring at m/z 256, 292, 326, 360, 394 and 430 for tri-, tetra-, penta-, hexa-, hepta- and octachlorobiphenyls, respectively. Carrier gas is helium at a flow rate of 0.6 m*l*/min.

Overall procedural blanks of PCBs and *p, p'*-DDE were found to be negligible, less than 0.03 and 0.02 ng/g, respectively.

3. Results and Discussion

3.1. Concentrations of PCBs and p, p'-DDE in Short-tailed Shearwater

The results of the analysis are shown in Table 1. The concentrations of PCBs and *p, p'*-DDE in Short-tailed Shearwater from Tasmania were found to be in the ranges of 3.6 to 27 ng/g (mean: 14 ng/g) and 3.2 to 50 ng/g (mean: 20 ng/g) in chicks and of 88 to 290 ng/g (mean: 150 ng/g) and from 31 to 180 ng/g (mean: 89 ng/g) in adults on wet weight basis, respectively. The levels of both the chemicals were higher in adults than in chicks, which indicates the accumulative characteristics of these chemicals with increased age of Short-tailed Shearwater. Concentrations of PCBs and *p, p'*-DDE in adults were 11 and 4.5 times higher, respectively. DDE/PCB ratios were lower in adults (range: 0.35–0.91; mean: 0.60) than in chicks (range: 0.79–1.8; mean: 1.3). This is most likely due to the exceedingly greater accumulation of PCBs in adults during the period of stay in the northern hemisphere, indicating higher contaminated feeding

Table 1. Concentrations of PCBs and p, p′-DDE in the minced body samples of Short-tailed Shearwater from Tasmania, Japan and the northern North Pacific.*

Specimen No.	Sampling date	Sex	Body weight (g)	Stage**	Fat (%)	Concentration (ng/g) wet weight basis PCBs	wet weight basis p,p'-DDE	fat weight basis PCBs	fat weight basis p,p'-DDE	DDE/PCB
Tasmania										
M5075	840227	F	445	C	20.6	21	28	100	140	1.4
M5081	840227	M	515	C	24.2	3.6	3.9	15	16	1.1
M5082	840227	F	447	C	32.9	12	10	37	31	0.85
M5083	840227	M	315	C	20.2	4.1	3.2	20	16	0.79
M5034	840322	F	735	C	36.6	11	19	30	50	1.7
M5037	840322	M	629	C	43.0	15	20	34	47	1.4
M5041	840322	M	661	C	46.9	8.7	16	19	34	1.8
M5060	840420	M	585	C	52.4	27	50	52	95	1.8
M5062	840420	M	655	C	55.8	20	26	37	47	1.3
M5063	840420	M	543	C	53.5	21	23	38	43	1.1
M5043	831123	F	626	A	15.4	140	69	900	450	0.50
M5045	831123	M	695	A	23.5	110	47	460	200	0.43
M5049	831123	M	715	A	24.1	130	120	550	500	0.91
M5005	831223	F	514	A	10.5	88	31	840	290	0.35
M5007	831223	F	533	A	12.6	110	51	870	410	0.47
M5009	831223	M	555	A	15.0	220	180	1500	1200	0.81
M5020	840126	F	591	A	31.9	120	79	370	250	0.66
M5021	840126	F	565	A	16.0	290	150	1800	960	0.53
M5022	840126	M	596	A	18.7	97	57	520	310	0.59
M5024	840227	F	526	A	18.7	160	130	850	680	0.81
M5028	840227	F	562	A	20.7	120	81	560	390	0.70
M5031	840227	M	560	A	17.8	200	98	1100	540	0.50
M5064	840321	M	565	A	15.4	170	91	1100	580	0.52
M5067	840321	F	557	A	19.6	97	56	490	280	0.58
Japan										
J-1	840530	F	237	***	0.54	22	27	4100	5000	1.2
J-2	840530	F	249	***	0.68	38	38	5500	5600	1.0
J-3	840530	M	230	***	0.66	22	27	3400	4100	1.2
Northern North Pacific										
NNP-1	820630	M	583	***	13.1	39	44	300	330	1.1
NNP-2	820630	F	522	***	12.5	44	59	350	480	1.3

* Tasmania: excluding feather, wings, head, leg, major breast muscle, liver, kidneys and uropygial gland. Japan: excluding feather. Northern North Pacific: excluding uropygial gland.
** C: chick, A: adult.
*** Probably post-fledgling or yearling.

grounds in the northern North Pacific than in the southern South Pacific. BENNINGTON *et al.* (1975) found that the concentrations of PCBs in Diving Petrel (*Pelecanoides urinatrix*) collected from the sub-Antarctic region were 30–40 times lower than those in auklets from the northern North Pacific, whereas DDE levels were comparable in both species. Similar observations were also reported by TANABE *et al.* (1983) on marine mammals and surface waters.

The concentration ratios of DDE/PCB in the individuals of shearwater were 1.2, 1.0 and 1.2 from Japan and 1.1 and 1.3 from the northern North Pacific (Table 1).

These values were found to be nearly the same range as the DDE/PCB ratios in chicks from Tasmania (range: 0.79–1.8; mean: 1.3). So the shearwaters from Japan and the northern North Pacific shown here seem to be post-fledgling or yearlings.

BOGAN and NEWTON (1977) reported that the brain lipid contents were relatively constant with decreasing body lipids in full range of nutritional state of Sparrowhawk (*Accipiter nisus*) collected from Great Britain, while the percentage of DDE located in brain increased slowly in birds with progressively less fat but was markedly higher in birds which contained less than 1.5% fat in their bodies. They suggested that a starving bird is more at risk not only from the increased circulating chlorinated hydrocarbons released from adipose tissue but also from the brain accumulating more than its 'share' of this relocated chemicals. As shown in Table 1, the body lipid contents in the shearwaters from Japan were found to be 0.54–0.68% that were extremely lower than those in chicks from Tasmania (20.2–55.8%). This fact might give an insight of the increased risk of chlorinated hydrocarbons as speculated in Sparrowhawks.

3.2. *Specific metabolism of PCBs in Short-tailed Shearwater during its transequatorial migration*

Technical formulation of PCBs is a complex mixture including approximately 100 isomers and congeners of different numbers and positions of chlorine atoms in a biphenyl ring. A wide range of their physicochemical and biochemical properties have led to heterogenous PCB compositions in environmental media and biota. In general, biodegradability of PCBs increases with decrease in number of chlorine atoms. Thus, the analyses of PCB compositions give a useful information to find out the specific biological and physiological processes in wild animals.

Figure 1 shows the PCB isomer and congener compositions in Short-tailed Shearwater from Japan, the northern North Pacific and Tasmania. About 50 isomers and congeners, mainly penta- and hexachlorobiphenyls, were detected. Some isomers and congeners were found to decrease or disappear in the shearwaters from Japan and Tasmania (adult) when compared with those from Tasmania (chicks) and the northern North Pacific. This suggests the accelerated metabolism of some PCB members in the body of shearwaters during migration.

It is known that two types of metabolic enzymes are related to the metabolism of xenobiotics in higher animals, one is the PB type enzyme systems induced by phenobarbital and the other is the MC type enzyme systems induced by 3-methylcholanthrene. In the case of PCBs, the members having chlorine-unsubstituted area at both *meta* and *para* positions in the biphenyl ring (P area) are metabolized by PB type enzyme systems, and those having chlorine-unsubstituted area at both *meta* and *ortho* positions (M area) are metabolized by MC type enzyme systems (SAEKI *et al.*, 1983).

Table 2 shows the relative loss rates of some penta- and hexachlorobiphenyls, the major residual members of PCBs, in Short-tailed Shearwater during transequatorial migration. The higher loss rates were found not only in PCB members with P area (peak Nos. 62, 54 and 65) but also in isomers with M area (peak Nos. 59, 55 and 514), which indicates the active metabolism of PCBs by both PB and MC type enzyme systems. It is known in wild birds that the PB type enzyme systems are generally predominant as compared with MC type enzyme system (WATANABE *et al.*, 1985). Con-

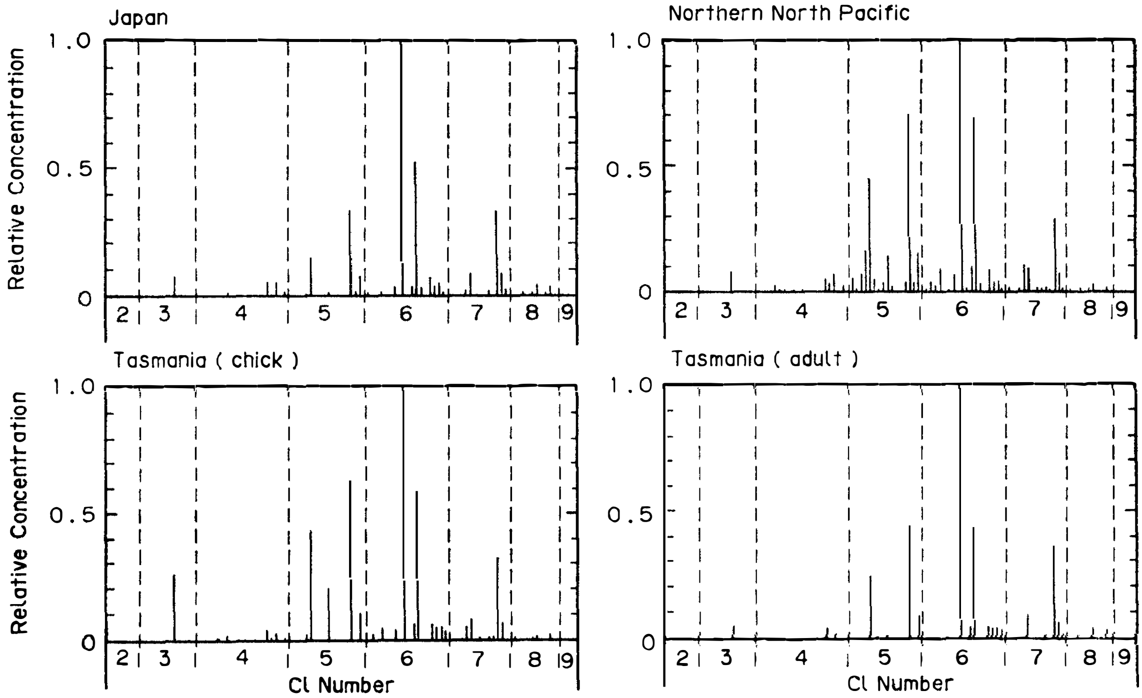

Fig. 1. PCB isomer and congener compositions in Short-tailed Shearwaters from Japan, the northern North Pacific and Tasmania. Each bar indicates the relative concentration of individual PCB isomers and congeners measured by mass fragmentography. PCB value of maximum peak (peak No. 610) was treated as 1.0.

Table 2. Chemical structures of some penta- and hexachlorobiphenyls found in the body of Short-tailed Shearwater and their relative loss rate during migration.

Peak No.	IUPAC No.	Structure	Relative concentration (peak No. 610=1)		Relative loss rate* (%)
			Tasmania (chick) (A)	Japan (B)	
59	89	2, 2′, 3, 4, 4′	0.21	0.013	94
62	151	2, 2′, 3, 5, 5′, 6	0.025	0.0021	91
54	101	2, 2′, 4, 5, 5′	0.029	0.0067	77
65	149	2, 2′, 3, 4′, 5′, 6	0.042	0.0098	77
55	99	2, 2′, 4, 4′, 5	0.44	0.15	65
514	108	2, 3, 3′, 4, 5′	0.64	0.35	45
612	137	2, 2′, 3, 4, 4′, 5	0.068	0.040	41
618	159	2, 3, 3′, 4, 5, 5′	0.056	0.038	31
68	133	2, 2′, 3, 3′, 4, 6′	0.040	0.031	23
619	156	2, 3, 3′, 4, 4′, 5	0.058	0.046	20
614	138	2, 2′, 3, 4, 4′, 5′	0.59	0.54	9
610	153	2, 2′, 4, 4′, 5, 5′	1.0	1.0	0

* Relative loss rate=(1−B/A)×100.

sidering this, the increased activity of the metabolic enzyme systems, particularly MC type enzymes, found in the shearwater may indicate the specific physiological changes in the period of migration. The metabolic enzyme systems are known to be induced by the lipophilic xenobiotics (SAFE, 1984), hence the results obtained here suggest that some residual xenobiotics in the shearwater induced the prominent MC type enzyme systems due to the increase in their concentrations resulting from the consumption of body fat during the long distance migration. In fact, the concentrations of PCBs and p,p'-DDE in the shearwater from Japan were found to be about one hundred times higher than those in chicks from Tasmania on fat weight basis (Table 1), implying the probable increased concentrations of other xenobiotics which can induce the metabolic enzyme systems. CARLSON (1980) reported in the rats experiment that starvation enhanced the enzymatic activity induced by PCBs, and also noted that this might be caused by the increased level of PCBs in blood and liver due to their mobilization from storage sites in adipose tissue. He suggested that starvation leading to enhanced enzymatic activity may be a common factor in the case of other lipophilic xenobiotics. The induction of the metabolic enzyme systems, particularly MC type enzymes, is also expected not only in shearwaters but also in some other wild animals that have a starvation period in their life cycles.

4. Conclusions

Figure 2 shows the schematic description of the relationship between the specific bioaccumulation and metabolism of PCBs in Short-tailed Shearwater and its transequatorial migration. The shearwater accumulates more PCBs during feeding in the northern North Pacific than in Tasmania, which reflects the status of global marine pollution by PCBs. PCBs are metabolized by the PB type enzyme systems all over the life cycle of the shearwater. During transequatorial migration, the MC type enzyme systems are induced by some chlorinated hydrocarbons and other lipophilic xenobiotics due to the increase in their concentrations resulting from the consumption of body fat and metabolize PCBs which are not normally metabolized by PB type enzyme systems. Results obtained here indicate the following:

1) Short-tailed Shearwater is a suitable bioindicator to know the global pollution of chlorinated hydrocarbons.

2) Persistent chlorinated hydrocarbons can be used as chemical indicators to make clear the biological processes, like aging and drug-metabolism in Short-tailed Shearwater.

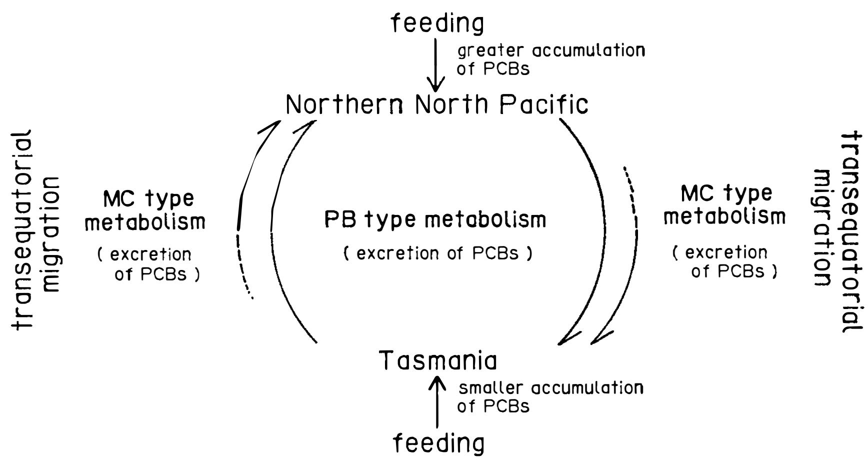

Fig. 2. Probable metabolism of PCBs during transequatorial migration of Short-tailed Shearwater.

3) Toxic effects of chemicals are manifested under specific biological processes like migration, breeding, etc., by means of higher lipid consumption, reallocation of chemicals to various organs and by the induction of enzyme systems. Hence in evaluating the toxicity of chemicals, these factors should be considered.

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