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DISTRIBUTION AND TRANSFER OF ORGANOCHLORINES IN ADÉLIE PENGUINS (*PYGOSCELIS ADELIAE*) IN A BREEDING SEASON

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Abstract: PCBs and *p, p'*-DDE were analysed in the subcutaneous fat (SCF), abdominal fat (AF), muscle, liver, bone, brain and an egg sample of Adélie penguins collected from Antarctica during the breeding season of 1981. PCBs and DDE were largely retained in the fat-rich tissues of Adélie penguins. With advancement of starvation and resulting shrinkage of fat reserves, linear increase in the concentrations of both the compounds in the fat tissues and some sort of redistribution to other body parts were observed. Excretion via eggs, of organochlorines, was not found to be significant in Adélies. Elimination rates of all PCB isomers and congeners as well as DDE were found to be more or less the same. Loss and gain rates and biological half-life (BHL) of both the compounds were calculated on the basis of the feeding habits of Adélie penguins. BHL was found to be longer and loss rate lower in the case of DDE than PCB, indicating a faster clearance rate of the latter.

1. Introduction

Penguins can fairly well be utilized as the indicators of baseline pollution of basically pristine areas of the Southern Ocean, since their physiology prevents them from moving far north (STONEHOUSE, 1970). Being highest in Antarctic food chain and their feeding behaviour being rather simple, the transfer and bioaccumulation of organochlorines in the Antarctic ecosphere as well as in other wild birds can be easily evaluated by the analysis of these pollutant levels in penguins.

In a very coarse approximation, neglecting all biological facts, penguins can be regarded as "warm blooded fish" in terms of food-web and habitat (BALLSCHMITER *et al.*, 1981). They spend about six months in a year at the surface of the off-shore waters. In the breeding season every year they spend about a month completely starving and another month or so with only occassional feeding (DAVIS, 1982). During this breeding period, penguins use up most of their amassed fat reserves in their bodies and appear to maintain a delicate balance with the thermal demands of their environment (STONEHOUSE, 1970).

Organochlorines were found to be accumulated in the fat stores of various organisms like Weddell seals (HIDAKA *et al.*, 1983), Striped dolphins (TANABE *et al.*, 1981), bats (CLARKE and KRYNITSKY, 1983) and birds (BUSH *et al.*, 1974). So the analysis of the tissues and organs of penguins would reveal a clear pattern of accumulation, metabolism and redistribution, if any, of organochlorines, because of the specific behaviour

of penguins in amassing and utilizing large quantities of fat during feeding and starvation periods respectively.

Some variations were also observed in the ecology and behaviour of Adélies collected from various locations (CARRICK and INGHAM, 1970; SPURR, 1974; TAMIYA and AOYANAGI, 1982). MATSUDA (1964) observed some differences in the behaviour of Adélies in Ongulkalven (island) near Syowa Station from those of the penguins in other areas, because of the fact that the fast ice zone in his study area covers 100km or more and so those animals travelled longer distances to reach the water for feeding. In the present study also the open water was at a greater distance than normal from where the specimens were collected. This may have an effect on the concentrations of organochlorines in penguins' body by way of affecting the feeding and starvation periods.

Based on all these facts, the present study describes the effects of starvation on the concentration, body burden, redistribution etc., of PCBs and DDE in the bodies of Adélie penguins by analysing the concentrations of these compounds in various body parts and tissues of the Adélie specimens collected during a breeding season in the Antarctic region.

2. Materials and Methods

Adélie penguin specimens were collected at the Rumpa rookery, 18km SSW of Syowa Station (69°00'S, 39°35'E), Antarctica (Fig. 1) in different stages of their breeding

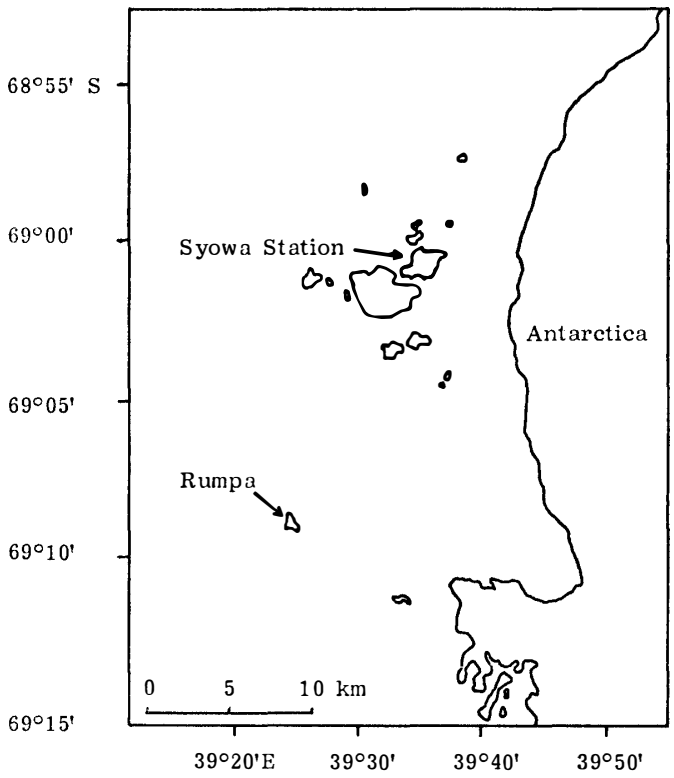

Fig. 1. Map showing Rumpa (island), Antarctica–sampling site of Adélie penguins (Pygoscelis adeliae).

period (mating, egg-laying, incubation and chick-hatching) between November and December 1981, during the 22nd Japanese Antarctic Research Expedition (JARE-22).

Chemical analysis of PCBs and DDT compounds (as *p,p'*-DDE) in Adélie penguins was made by the alkaline alcohol digestion method of WAKIMOTO *et al.* (1971). Required amounts of the samples were refluxed in 1N KOH-C_2H_5OH solution for one hour. PCBs and *p,p'*-DDE (*p,p'*-DDE values reported here include *p,p'*-DDT also since *p,p'*-DDT is converted to *p,p'*-DDE during the alkaline alcohol digestion) thus extracted were transferred to 100 m*l* hexane by shaking in a separatory funnel. Subsequently, the concentrated hexane layer was cleaned up by passing through 1.5 g of silica gel (Wako gel S-1) packed in a glass column (10 mm i.d. × 20 cm). PCBs and 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 KD (Kuderna-Danish) concentrator and further microconcentrated to 200 μl in a stream of nitrogen whenever necessary.

Aliquots of these solutions were used for injection by a splitless technique glass capillary gas chromatography with ^{63}Ni electron capture detection using Shimadzu 7-A capillary gas chromatograph. The column consisted of 30 m × 0.23 mm glass capillary —WCOT OV 101 for both PCB and DDE analysis. Operating conditions for PCBs and DDE are as follows: For PCBs the column temperature program was 180 to 230°C at a rate of 0.5°C/min with an initial 8 min and final 32 min hold. The temperature of both injector and detector was 250°C. For DDE the column temperature was 230°C isothermal. Injector and detector temperature was the same as in PCB analysis. Nitrogen was used both as a carrier and make up gas.

Some samples were injected into Shimadzu 9020 DF gas chromatograph-mass spectrometer equipped with an electron-impact ion source and the SCAP-1123 data system for the measurement and identification of PCB isomers and congeners in detail. PCBs were determined by selected ion monitoring at *m/z* 222, 256, 292, 326, 360, 394 and 430 for di-, tri-, tetra-, penta-, hexa-, hepta- and octachlorobiphenyls respectively. Carrier flow of helium was controlled at 0.6 m*l*/min. The same type of column used in GC-7A glass capillary gas chromatograph was used in GC-MS also. Operating conditions for GC-MS are as follows: Column oven temperature was programmed as 190 to 230°C at a rate of 0.5°C/min. Injector and ion source temperatures were kept at 250 and 280°C respectively.

3. Results and Discussion

3.1. Variations of PCBs and DDE in the subcutaneous fat (SCF) and abdominal fat (AF) samples with starvation

The concentration values of PCBs and DDE in the SCF and AF samples of the male and female specimens are shown in Tables 1 and 2 respectively. It is clear from this data that in the specimens collected at the beginning stage of starvation (1M, 2M, 3M and 2F, 3F—mating stage in Fig. 2) concentrations were almost the same to each other and the values did not vary between the SCF and AF samples. With advancement of starvation, in the specimens collected after 15 days (5M and 5F—egg-laying stage in Fig. 2) concentrations increased with a concordant decrease in fat reserves (Tables 1 and 2).

Table 1. Concentrations and burdens of PCB and p, p'-DDE in the tissues and organs of male Adélie penguins.

Sample No. stage and date of collection	Body wt. (g) Body length (mm)	Fraction analysed	Fat content (%)	Total wt. of fraction (g)	ng/g wet wt.		ng/g fat wt.		Burden (μg)		Ratio (DDE /PCB)
					PCB	DDE	PCB	DDE	PCB	DDE	
1M-Mating 811106	5020 501	SCF	89.9	1078	29.0	166	32.3	185	31.3	179	5.7
		AF	97.3	69.8	30.7	172	31.6	177	2.15	12.0	5.6
2M-Mating 811107	5500 509	SCF	78.5	1077	27.1	157	34.5	200	29.2	169	5.8
		AF	96.3	51.3	30.7	172	31.9	179	1.58	8.84	5.6
3M-Mating 811107	5860 528	SCF	87.9	1243	28.7	144	32.7	164	35.7	179	5.0
		AF	94.6	70.4	27.3	157	28.9	166	1.92	11.1	5.8
		Bone	14.6	541	2.88	17.8	19.7	122	1.56	9.64	6.2
		Muscle	2.38	2600	0.18	1.43	7.56	60.1	0.47	3.72	7.9
		Liver	3.78	110	0.28	0.52	7.41	13.8	0.031	0.058	1.9
		Brain	4.84	22	0.36	1.37	7.44	28.3	0.008	0.030	3.8
5M-Egg-laying 811122	4110 530	SCF	68.3	667	49.3	261	72.2	382	32.9	174	5.3
		AF	92.3	21.1	59.2	384	64.1	416	1.25	8.11	6.5
		Bone	9.1	509	4.58	28.6	50.3	314	2.33	14.5	6.2
		Muscle	1.89	1874	0.47	4.23	24.9	224	0.85	7.73	9.1
		Brain	5.86	18.4	0.53	3.65	9.04	62.3	0.01	0.01	6.7
7M-Incubation change-811211	3740 498	SCF	46.6	333	41.5	375	89.1	805	13.8	125	9.1
		AF	80.8	14.1	89.7	497	111	615	1.27	7.01	5.5
		Bone	12.7	424	5.29	38.3	41.6	301	2.24	16.2	7.2
		Muscle	2.16	1945	0.74	9.06	34.3	419	1.44	17.6	12.2
		Liver	3.36	79.5	0.83	5.56	24.7	165	0.066	0.441	6.7
		Brain	6.00	16.5	0.80	6.52	13.3	108	0.013	0.107	8.2

SCF: subcutaneous fat, AF: abdominal fat.

Table 2. Concentrations and burdens of PCB and p, p'-DDE in the tissues and organs of female Adélie penguins.

Sample No. stage and date of collection	Body wt. (g) Body length (mm)	Fraction analysed	Fat content (%)	Total wt. of fraction (g)	ng/g wet wt.		ng/g fat wt.		Burden (μg)		Ratio (DDE /PCB)
					PCB	DDE	PCB	DDE	PCB	DDE	
2F-Mating 811107	4710 483	SCF	87.2	832	36.4	223	41.8	256	30.3	185	6.1
		AF	92.8	61.8	41.0	259	44.2	279	2.54	16.0	6.3
3F-Mating 811107	5420 518	SCF	87.4	1213	32.4	179	37.1	205	39.3	217	5.5
		AF	98.6	77.8	37.9	204	38.4	207	2.95	15.9	5.4
5F-Egg-laying 811122	3850 474	SCF	74.6	371	79.7	398	107	534	29.6	148	5.0
		AF	80.8	26.6	99.9	588	124	728	2.67	15.7	5.9
		Egg-yolk	26.3	33.4	19.6	98.2	74.5	373	0.65	3.28	5.0
7F-Incubation change-811211	4540 512	SCF	82.3	931	29.3	143	35.6	174	27.3	133	4.9
		AF	94.9	40.8	40.1	143	42.3	151	1.64	5.84	3.6
8F-chick hatching-811222	3790 499	SCF	68.3	654	42.4	210	62.1	308	27.8	138	5.0
		AF	94.3	33.4	54.1	261	57.4	277	1.81	8.73	4.8

SCF: subcutaneous fat, AF: abdominal fat.

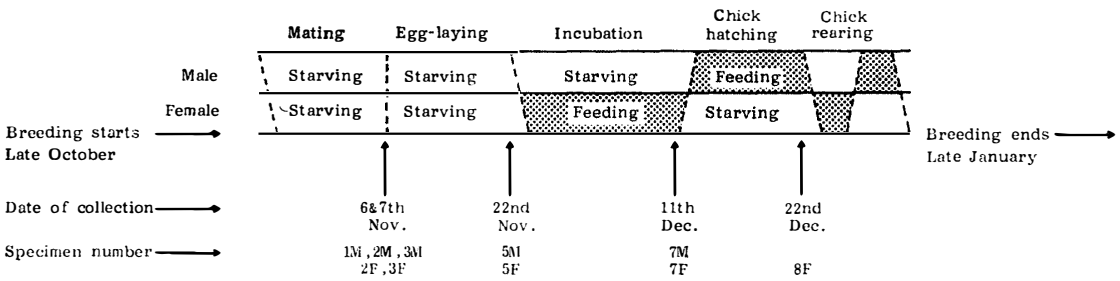

Fig. 2. Schematic representation of the breeding ecology and sampling dates of Adélie penguins (Pygoscelis adeliae).

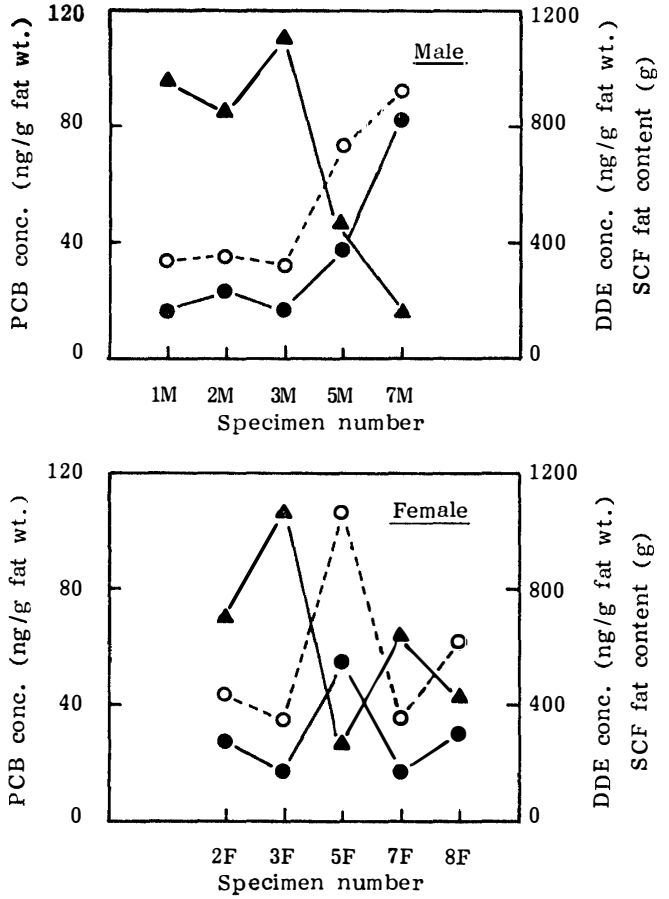

Fig. 3. Variations in the concentrations of PCB and DDE with SCF in male and female Adélie penguins.

After these days (*i.e.* after egg-laying) female birds normally go to sea for feeding, while the males continue their starvation during the first incubation spell. Female birds return about 16.6 days later on an average (Davis, 1982) and so in the specimens collected after 34 days (Fig. 2), the male (7M) contained the highest concentrations of PCBs and DDE in the drastically reduced fat reserves; but at the same time in the female (7F) concentrations of both the chemicals decreased in the replenished fat reserves (Tables 1 and 2; Fig. 3).

CLARKE and KRYNITSKY (1983) found such an increase of DDE in the starved specimens of hibernating bats. BOGAN and NEWTON (1977) also reported the same pattern of increase in the percentage of DDE in the brains of migrating sparrowhawks. The observed increase of organochlorine levels in Adélie penguins during starvation is an indication of the probable hazardous effects of these chemicals in the migrating and hibernating animals having very high concentrations of these compounds, in case such a phenomenon occurs in them also.

3.2. Total body burden and redistribution of PCBs and DDE

Total body burdens of three male specimens at different stages of starvation are shown in Table 3. As observed in Striped dolphins (TANABE *et al.*, 1981) and in Weddell seals (HIDAKA *et al.*, 1983), more than 80% of PCBs and DDE were found to remain in the SCF and AF samples of Adélie penguins. So it is reasonable to assume that, in Adélies, the residual amounts of chlorinated hydrocarbons in the fat reserves can be considered as total body burdens.

An increase in the percentage of organochlorine burden was observed in all the body parts with a slight decrease in the residues in fat during advancement of starvation (Table 3), indicating a clear redistribution of organochlorines from SCF to other body parts and tissues, the bone and muscle being the major recipients. This finding agrees fairly well with the suggestions of MATTHEWS and DEDRICK (1984), that a change in the PCB concentration or tissue volume of any one tissue will result in a corresponding change of concentrations in all tissues; *i.e.* the concentrations of these

Table 3. Percentage to the total burden of PCBs and DDE in the tissues and organs of male Adélie penguins.

Specimen and stage	Days of starvation	Tissue or organ	Percent to total	
			PCB	DDE
3M Mating	—	SCF	90	87.9
		AF	4.84	5.45
		Bone	3.93	4.74
		Muscle	1.18	1.83
		Liver	0.08	0.03
		Brain	0.008	0.02
5M Egg-laying	15	SCF	88.1	85.1
		AF	3.35	3.97
		Bone	6.24	7.09
		Muscle	2.28	3.78
		Liver	ND	ND
		Brain	0.024	0.03
7M Incubation	34	SCF	73.3	75.1
		AF	6.75	4.21
		Bone	11.9	9.74
		Muscle	7.65	10.60
		Liver	0.35	0.27
		Brain	0.07	0.06

ND: not determined.

componuds increased simultaneously in fat reserves as well as in other tissues in Adélie penguins when the body weight decreases. As a result, when the fat reserves declined beyond a critical level, percentage of the burdens in other tissues and organs increased, clearly because of redistribution.

On redistribution, even if the brain and liver receive more than their 'shares' it cannot be expected to have any hazardous effects as observed in big brown bats by CLARKE and PROUTY (1977) and in sparrowhawks by BOGAN and NEWTON (1977), because the concentrations of DDE observed in the brain of Adélie penguin is far lower than the values reported by these authors.

3.3. Excretion via egg of PCBs and DDE

As shown in Table 2, the amounts of PCB and DDE residues excreted via egg from mother were found to be very low. So the excretion via eggs in Adélie is negligible (1.3 μg PCB and 6.56 μg DDE/2 eggs—4% individually of both PCBs and DDE to that of the burden in mother), whereas LEMMETYINEN *et al.* (1982) found a considerably higher

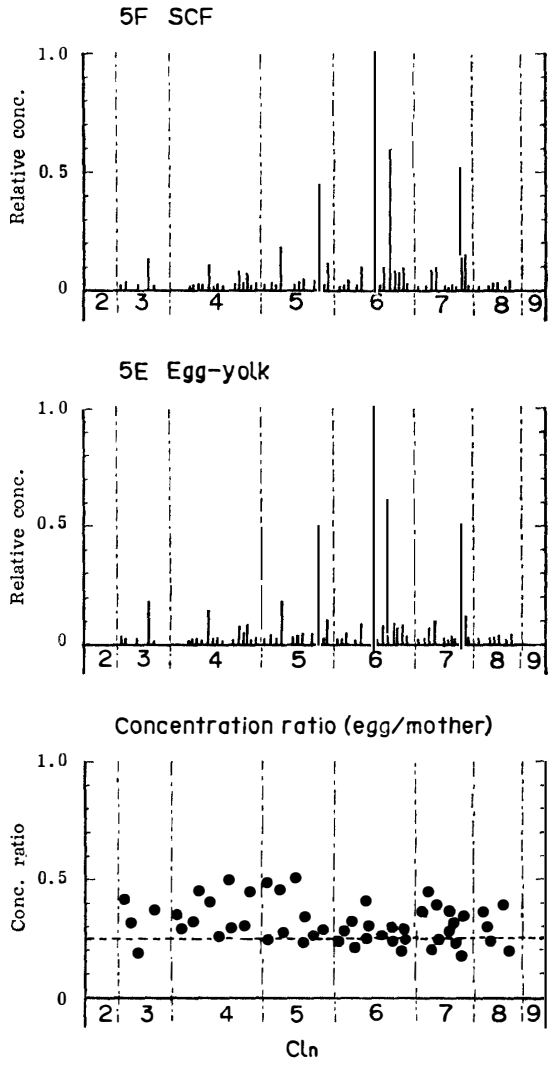

Fig. 4. PCB isomer and congener compositions in 5F SCF (mother) and 5E (egg-yolk) and their concentration ratios (CR). Relative concentration shows the ratio of individual chlorobiphenyl concentrations to the concentration of maximum peak. The PCB value of maximum peak was treated as 1.0. Horizontal broken line in the lower figure shows the CR of DDE (egg/mother).

percentage of excretion of these compounds via eggs in Arctic terns (21.9% DDT and 44.9% PCB) and Herring gulls (30.5% DDT and 23.9% PCB). BOGAN and NEWTON (1977) also presumed a substantial loss of DDE (52% of the total content in the mother at egg-laying) via eggs in sparrowhawks. The present fact of very low amounts of excretion of PCBs and DDE from mother to egg in Adélie is because of the smaller percentage of the eggs weight (clutch of two eggs) to mother's weight at the time of laying was only about 7%, whereas LEMMETYINEN *et al.* (1982) reported 32.4% of the same value for a clutch of two eggs in Arctic tern and 29.2% for a clutch of three eggs in Herring gulls.

Moreover, it is interesting to note that the composition of PCB isomers and congeners is strikingly the same in the SCF samples of the mother (5F) as well as in the egg (Fig. 4). Concentration ratios of the individual members are almost the same for all biphenyls, indicating no preferential elimination of any particular PCB isomer. This is in contrast to the observation by TANABE *et al.* (1982) in Striped dolphin, where the concentration ratios were found to decrease from di- to octachlorobiphenyls. Concentration ratio of DDE (egg/mother) was also found to be almost in the same range as PCBs (Fig. 4). The preferential transfer found in mammals has been explained as a function of placental barrier. So, considering this non-preferential transfer of PCB isomers and congeners, *p*, *p'*-DDE in Adélie penguin is certainly due to the absence of placenta.

3.4. Accumulation via food and loss and gain rates of PCBs and DDE

Feeding habits of Adélie penguins (EMISON, 1968) and their feeding and starvation periods (STONEHOUSE, 1970; DAVIS, 1982) are well known. So, assuming certain other facts, the amounts of PCBs and DDT compounds accumulated every year from food by Adélies can fairly well be calculated.

To attain this value, three samples of euphasiids (main food item of penguins) collected during the BIOMASS research program in January–February 1984 were analysed for PCB and DDE concentrations and the values were averaged (0.021 ng/g PCB and 1.17 ng/g DDE on wet weight basis). Average values of the data presented by SUBRAMANIAN *et al.* (1983) on some species of fish from Antarctica were taken as the values representing the concentrations in fishes on which the Adélies feed (ave. 0.29 ng/g PCB and 0.75 ng/g DDE on wet weight basis). Based on these and on the basis of Adélie's food consumption for one feeding trip (EMISON, 1968), an Adélie penguin receives 89 μg of DDT compounds and 29 μg of PCBs every year via food.

On the basis of the growth rates of Adélie chicks (STONEHOUSE, 1970), the food consumption of one chick in the first year of its life was assumed to be half of an adult. Also, on the basis of the breeding habits of Adélies and by the fact that the concentrations of PCBs and DDE in their fat reserves are more or less the same, the specimens 1M, 2M and 3M are considered as of the same age, about 5 years old.

In such a case and at the rate of accumulation calculated already, a five year old Adélie penguin should have a burden of 400 μg of DDE and 130 μg of PCB, in its body, if there is no excretion. But the actual burdens are far less (Table 3). From these values the loss and gain rates and biological half-life (BHL) of both the compounds were calculated using the formulae,

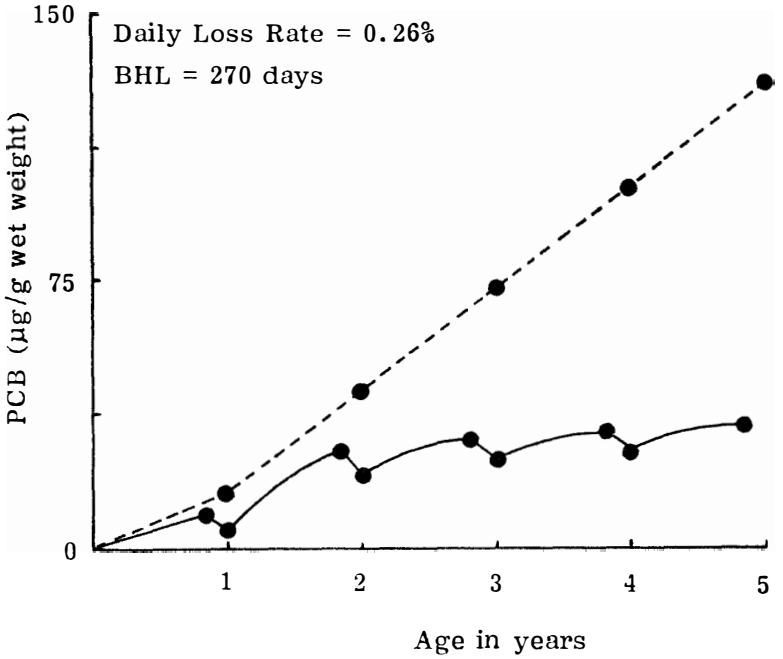

Fig. 5. Gain and loss rates of PCB with age in Adélies. Broken line shows expected concentrations every year if there is no excretion. Straight line shows loss and gain rates during feeding and starvation.

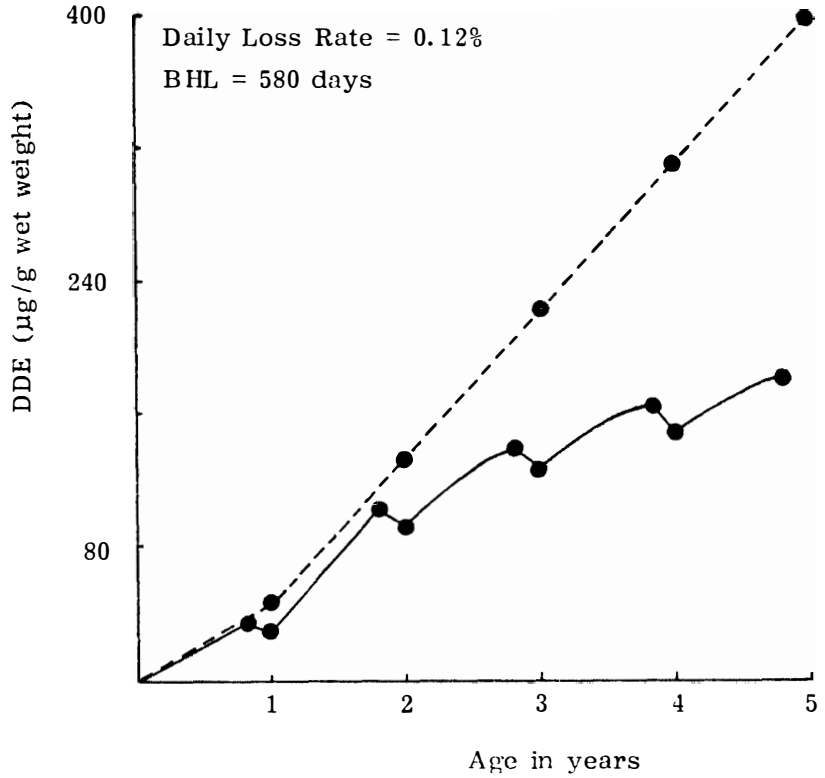

Fig. 6. Gain and loss rates of DDE with age in Adélies. Broken line shows expected concentrations every year if there is no excretion. Straight line shows loss and gain rates during feeding and starvation.

$$B=(B_n-DI)e^{-\lambda},$$
$$BHL=\ln\tfrac{1}{2}/-\lambda,$$

where B: burden, n: number of days, DI: daily intake, λ: daily loss rate.

The results are shown in Figs. 5 and 6. Lower loss rates and longer biological half-life for DDE than PCBs have resulted probably from the metabolism of lower chlorinated members of PCBs. The increase in the DDE/PCB ratio in all the body parts of specimen 7M, which had gone through the longest period of starvation among all the birds analysed here (Table 1, Fig. 2), also indicates that during starvation PCBs are cleared at a faster rate than DDE.

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