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PCB in mammals and birds in the Netherlands.

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In the Netherlands PCB's were observed for the first time in sandwich terns (Sterna sandvicensis) and eider ducks (Somateria mollissima) during a study of a case of bird mortality which was caused by industrial pollution with pesticides (mainly dieldrin and telodrin) (Koeman et al., 1967; 1969; 1972a; Koeman, 1971; Koeman and van Genderen, 1970; Swennen, 1972).

Since then it was shown in various studies that PCB's are present in many species both in the aquatic and terrestrial environment. In the present paper the main results will be summarized. Some data will be presented concerning an experimental study with small groups of cormorants (Phalacrocorax carbo) and herons (Ardea cinerea). The paragraph on seals, porpoises and dolphins includes data on dolphins which were obtained from New Zealand and Dutch Guiana.

For sample clean-up the techniques devised by Holden and Marsden (1969) and Armour and Burke (1970) were applied. The final detection was based on gaschromatography. For some species analysed, tissue extracts could be submitted to mass-spectrometric analysis by using a GC/MS combination (Koeman et al. 1969; ten Noever de Brauw and Koeman, 1973). A description of the methods currently in use for

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wildlife samples in the Netherlands was published by de Vos (1972). The quantitative estimation of PCB residues was carried out by peak-height comparisons using standard solutions containing a 60% technical PCB-mixture (Phénoclor DP 6).

1. PCB in birds of prey and owls.

In recent years various studies on birds of prey and owls in relation to pesticide contamination were carried out by the Research Institute for Nature management (Fuchs et al., 1971, 1972). The main purpose of these studies was to measure the change in residue levels following the official bans and restrictions on seed dressing applications on aldrin, dieldrin, heptachlor and methylmercury compounds which were enforced in 1968 and 1969. In the same studies the residue levels of PCB were measured. The average levels found are presented in table 1 (data from Fuchs et al. 1972). The levels found appeared to be particularly high in the long-eared owl (Asio otus) and the barn owl (Tyto alba). In one long-eared owl a liver level of 590 ppm was recorded. Among the birds of prey the levels were highest in the kestrel (Falco tinnunculus).

Table 1. Residue levels of PCB in the livers of birds of prey and owls. Mean residue levels are compared during three subsequent winters (data from Fuchs et al., 1972).

species	number of individuals in sample			PCB concentration (ppm wet weight)		
	68/69	69/70	70/71	68/69	69/70	70/71
buzzard	21	14	9	13.68	11.64	5.31
sparrow-hawk	5	11	16	9.20	5.12	6.81
kestrel	5	13	7	21.40	18.66	44.13
barn owl	5	5	3	43.82	28.80	3.02
long-eared owl	3	9	8	191.00	84.82	6.85

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3.2. PCB in marine birds.

Data on PCB levels were obtained for eider ducks and sandwich terns. The levels found in liver, brain and blood samples of eider ducks are presented in table 2. From the pesticide levels found in the tissues of the birds found dead or dying it could be concluded that poisoning by telodrin and dieldrin very probably was the main cause of death. Mortality only comprised female birds and occurred predominantly at the end of the incubation period. Female eiders consume little or no food when breeding. Apparently the chlorinated hydrocarbon compounds were mobilised from fat and other tissues in the course of incubation. In 1966 it could be demonstrated that the blood levels rose steeply (about twentyfold increase) (Koeman and van Genderen, 1970; Koeman, 1971). For this reason it must be realised that the PCB levels for dead and dying birds in table 2 also reflect the tissue distribution pattern at the end of the incubation period.

Table 2. Residue levels of PCB in tissues of eider ducks.

year	history	tissue	n	PCB concentration (ppm wet weight) mean and range
1967	found dead or dying	liver	10	63 (23-96)
		blood	10	24 (16-37)
	shot	liver	5	5.5 (2.1-12)
1968	found dead or dying	liver	9	89 (67-134)
		brain	4	55 (48-62)
	shot	liver	3	5.6 (3.9-6.5)

PCB residues in eggs of eider ducks and sandwich terns are given in table 3.

Table 3. Residue levels of PCB in eggs of eider ducks and sandwich terns.

species	year	n	PCB concentration (ppm wet weight) mean and range
eider duck	1968	10	16 (12-21)
	1970	5	5.8 (5.3-6.5)
sandwich tern	1967	10	4.5 (1.9-6.5)
	1969	15	11 (0.92-31)
	1970	10	7.1 (3.7-15)

PCB in cormorants and herons.

PCB levels found in cormorants and herons are summarized in table 4.

Table 4. Residue levels of PCB in tissues of cormorants and herons.

year	species and history	tissue	n	PCB concentration (ppm wet weight) mean and range or range
1970	cormorants found dead	liver	6	380 (93-470)
		total body	6	29-460
		brain[1)]	6	190
1970	cormorant nestlings	liver	6	0.38 (0.078-0.61)
		total body	6	2.4 (1.9-3.2)
		brain[1)]	6	0.69
1970/1971	heron	liver	18	7.7-712

[1)] residue level in mixed homogenates.

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It could be calculated that some of the cormorants found dead contained as much as approximately 1 g of PCB in their bodies. In order to assess the possible toxicological implications of PCB on the cormorant an experimental study was carried out with 5 cormorants which were taken from their nest in the spring of 1971. In the autumn of 1971 all 5 birds were treated with PCB (Clophen A 60). The dose rate at the beginning of the experiment was 100 ppm calculated on an amount of about 250 g fish which was administered to each cormorant every day. In the course of the experiment the dose rate was increased stepwise to 500 ppm after 14 weeks. The main results are given in table 5.

Table 5. Residue levels in tissues of cormorants poisoned with PCB (Clophen A 60) under experimental conditions.

no	sex	total dose (g)	survival time (days)	residue level (ppm wet weight) brain	liver	fat	total body 1)	total body content (g)	% of dose
1	♀	2.3	55	115	210	n.a.[2]	830	1.12	49
2	♂	3.5	75	160	230	n.a.	1820	2.73	79
3	♀	6.7	106	76	245	10,300	2750	4.85	72
4	♀	7.1	110	180	290	20,500	2150	3.34	47
5	♂	9.1	124	120	285	n.a.	2580	4.15	46

1) total body minus bill, feathers, legs and feet.

2) n.a. = not analysed

It appears from the table that the rate of retention of PCB in the body was high and varied from 46 to 79 percent. The actual amount must have been slightly more because several parts of the birds were not included in the total homogenate. Average levels of 130 and 252 ppm PCB were detected in the brains and livers respectively. No correlation was found between the concentrations in these tissues and the time of survival, which suggests that these represent threshold concentrations for the cormorant. Comparing these levels with those

in the birds found dead (table 4) it seems highly probable that the wild birds had died as a consequence of PCB poisoning. A more detailed discussion on this experiment was given elsewhere (Koeman et al. 1973).

Liver necrosis and hepatic porphyria are a general finding after PCB poisoning in various species of laboratory animals (Vos and Koeman, 1970; Vos and Beems, 1971; Vos et al., 1971). These symptoms were not seen in the cormorant. Hydropericardium and subcutaneous edema, symptoms observed in chickens which were fed the same lot of technical PCB as was given to the cormorants, were not observed in the latter species. These symptoms were due to the presence of chlorinated dibenzofurans in the mixture used (Vos et al., 1970). Apparently the cormorants did no respond to these compounds.

In cormorant eggs a significant correlation was found between shell thickness and the concentrations of PCB, DDE and hexachlorobenzene (HCB). The correlation was not significant for dieldrin (table 6).

Table 6. Residue levels of PCB and some other chlorinated hydrocarbon compounds in 61 cormorant eggs which were divided into 8 groups according to egg shell thickness[1]

egg shell thickness ($mm.10^{-2}$, mean and range)	number of eggs	residue level in ppm (wet weight) PCB	DDE	HCB	dieldrin
47.5(46.4-49.2)	9	40	3.0	0.43	0.53
45.4(44.9-46.1)	9	42	3.2	0.50	0.35
42.2(41.2-44.0)	9	46	4.0	0.31	0.28
40.4(39.9-41.0)	9	85	5.8	0.53	0.48
38.5(37.4-39.4)	9	50	4.4	0.63	0.44
36.9(36.2-37.4)	9	97	6.4	0.84	0.48
34.8(32.6-36.0)	8	130	12	1.5	5.2
27.6	1	205	14	1.8	6.8

1) correlation significant for HCB ($P < 0.005$), DDE ($P < 0.005$) and PCB ($P < 0.005$), not significant for dieldrin ($P > 0.05$). Kendall Rank correlation test according to Wilcoxon and Wilcox (1964).

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many data from the literature, which will not be reviewed here, strongly suggest that DDE could be one of the main causal factors involved in eggshell thinning in wild birds.

The presence of PCB in cormorant tissues was confirmed by mass-spectrometry. Apart from PCB a new environmental contaminant was detected in the tissues of the cormorant. In a previous study (Koeman et al., 1969) we reported the presence of a compound with massnumber 376 and 8 chlorine atoms per molecule in the tissues of eider ducks. By using a computer GC/MS system and comparison with reference compounds the unknown substance could be identified as octachlorostyrene. Two heptachlorostyrenes were also found (ten Noever de Brauw and Koeman, 1973). Quantitative estimations are in progress. Polychlorinated dibenzofurans were not detected so far.

As can be seen in table 4 comparably high concentrations were also found in herons. An experimental study with three herons which were poisoned with PCB under experimental conditions revealed that this species apparently is less susceptible than the cormorant. A much higher dose rate had to be applied in order to induce symptoms. At death the following concentrations were recorded in brain and liver. Brain: 420, 430 and 445 ppm respectively; liver: 1140, 1275 and 1430 ppm respectively. The adipose tissue of the latter bird contained 56,400 ppm. As in the case of the cormorant the characteristic symptoms observed in various laboratory animals after PCB poisoning were not found in the heron.

4. PCB in seals, porpoises and dolphins.

During 1970 and 1971 a number of common seals (Phoca vitulina) and harbour porpoises (Phocoena phocoena) found dead along the Dutch coast were analysed for PCB and other compounds (in particular metals).

In addition two bottle-nosed dolphins (Tursiops truncatus) were analysed which died in a dolphinarium in the Netherlands. By mediation of the Zoological Museum and Zoological Laboratory of the University of Amsterdam samples were obtained from common dolphins (Delphinus delphis) and dusky dolphins (Lagenorhynchus obscurus) caught in fishing nets along the coast of New Zealand and a Surinam dolphin (Sotalia guianensis) which also died in a fishing net.

The results of the blubber (subcutaneous fat) analyses of 7 seals are presented in fig. 1. It can be seen that the PCB levels do far exceed those of DDT. The difference between DDT and PCB levels is far less for the porpoises (fig. 2.). In absolute terms the concentration of the DDT group compounds (DDT + DDE + DDD) was higher in porpoises than in seals. In 1968 Risebrough reported that the DDT/PCB ratio appeared to be higher in marine birds from remote areas than in those caught in less remote areas (Risebrough et al., 1968).

Considering that seals generally live much closer to the shore than porpoises, the higher DDT/PCB ratio for the latter group is well in agreement with Risebroughs finding. The levels in the blubber of the bottle-nosed dolphins were of the same order as those in porpoises. It is interesting to note that PCB's could not be detected in the blubber of the dolphins from New Zealand and Dutch Guiana (fig. 3.). However, the DDT group levels in these Pacific dolphins are of the same order of magnitude as the levels found in porpoises from the North Sea.

The toxicological evaluation of the PCB in the tissues of seals and whales cannot be made at present. In the adult seals the brain levels varied from 13 to 89 ppm on a wet weight basis.

Recently Ringer et al.(1972) reported levels of 11 ppm in mink (Lutreola lutreola) which died after being fed either dietary PCB containing coho salmon or ocean fis with supplemental PCB (basal

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diet supplemented with 10 ppm each of Aroclors 1242, 1248 and 1254). The authors also found an effect on reproduction (serious drop in number of producing females and kit mortality)(see also Aulerich et al., 1971). As compared to these data the levels in the marine carnivorous mammals reported above merit serious attention. The data on metals were reported elsewhere (Koeman et al., 1972b).

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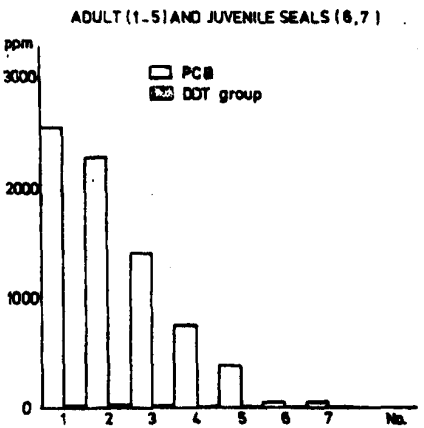

Fig. 1. PCB and DDT group concentrations in blubber (subcutaneous fat) of 7 seals found dead in 1970 and 1971 (the hexane extractable lipid content varied from 19-68 %)

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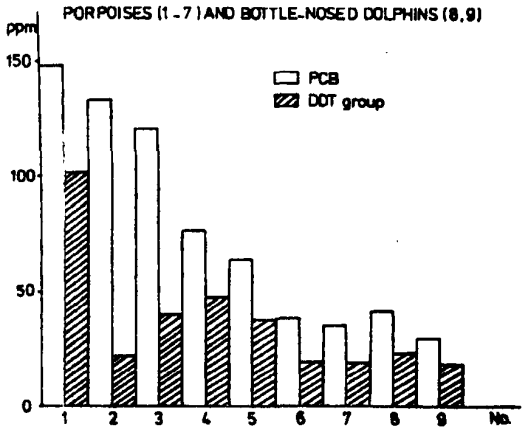

Fig. 2. PCB on DDT group concentrations in blubber of stranded porpoises and bottle-nosed dolphins from a delphinarium (the hexane extractable lipid content varied from 64-96 %)

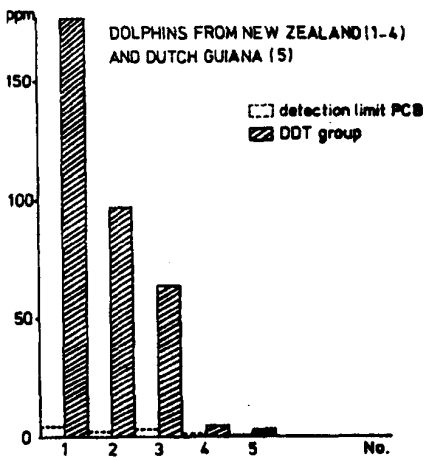

fig. 3. DDT group concentrations in blubber of dolphins caught in fishing nets in New Zealand and Dutch Guiana (the hexane extractable lipid content varied from 40-80 %).