POLYCHLORINATED BIPHENYLS IN WILD BIRDS IN BRITAIN AND THEIR AVIAN TOXICITY

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ABSTRACT

The amounts of polychlorinated biphenyl (PCB) compounds present in the livers and eggs of wild birds in Britain were determined by gas–liquid chromatography. Most of the 559 specimens came from predatory birds obtained between April 1966 and August 1968. PCB was found in terrestrial species from most regions of Britain, in all the individual and bulked samples of seabird eggs examined from one west coast and two east coast colonies and in most of the freshwater species collected from the midlands, east and south of England. The highest liver residues were found in freshwater fish-feeding birds (up to ca. 900 ppm) and bird-feeding raptors (up to 70 ppm). The levels present are similar to those of pp'-DDE. An indication of the avian toxicity of one PCB was obtained by feeding Arochlor 1254 to Bengalese finches. The estimated dose rate for 50% mortality at 56 days was 254 mg/kg day. At this dose rate the calculated mean liver content was 345 ppm. It has only 1/3 the toxicity of DDT, but could be more toxic at low doses because it appears to have a more gradual mortality curve than DDT. All birds dying from PCB had enlarged kidneys and before death some displayed apparent leg paralysis or body and wing trembling. It was concluded that PCB is unlikely to have caused widespread lethal toxity in wild predatory birds in Britain, but could be a component cause of the present breeding failure reported in several species.

INTRODUCTION

Studies of organochlorine insecticide residues in eggs of sea birds and in livers and eggs of predatory birds have been in progress at this experimental station since the early 1960s (Moore & Tatton, 1965; Prestt, 1966). The chemical analyses were made by gas–liquid chromatography, using electron capture detection. In addition

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to peaks corresponding to such organochlorine pesticides as BHC, dieldrin and DDT and its metabolites, it was not unusual for an additional series of peaks to appear on the chromatograms. There were as many as 10 of these peaks, corresponding to unidentified compounds. Roburn (1965) established that these were organochlorine in nature and for some time it was generally supposed that they were either further breakdown or condensation products of the organochlorine pesticides or possibly loose compounds of these with natural products such as protein matter. In 1966, however, Jenson succeeded in identifying a series of such peaks, in the tissues of 200 pike (*Esox lucius*) from different parts of Sweden and in the feathers of a Swedish white-tailed eagle (*Haliaeetus albicilla*), as corresponding to polychlorinated biphenyl (PCB) compounds. Following this Holmes *et al.* (1967) showed that the long retention time compounds occurring in British wild bird specimens were also PCBs and since 1966 these have been quantified in addition to the organochlorine insecticide residues.

This paper gives details of the amounts of PCB that have been found to date in wild birds and their eggs in Britain and also the results from a laboratory experiment in which a PCB was fed to the Bengalese finch (*Lonchura striata*).

ANALYTICAL PROCEDURE

The material obtained from the laboratory specimens was analysed at Monks Wood Experimental Station by M. C. French. First, all specimens were ground with sand and anhydrous sodium sulphate and extracted with hot n-hexane and acetone. The extract was cleaned up by the technique of de Faubert Maunder *et al.* (1964) and subjected to alumina column chromatography. The final hexane extracts were analysed using gas-liquid chromatography with electron capture detection. The columns used were packed with Silicone/Epikote on Diatomite CQ. The Arochlor 1254 fed to the laboratory birds gave a series of six main peaks on the chromatogram. As there were no changes in the relative peak heights compared with the standards (M. C. French, in preparation), quantification of the residue was simplified by comparing the height of peak 2 with that of the standard. Confirmation of the quantity was obtained by the second technique of comparing the total area under the peaks.

Wild bird specimens were analysed on our behalf by the Laboratory of the Government Chemist. The technique used was similar to the above but quantification of the PCB residue was made more difficult owing to the presence of organochlorine insecticides. These were separated from the PCB-type compounds by silica gel column chromatography prior to gas-liquid chromatography. Other methods of separation are discussed by Holmes *et al.* (1967) of the Laboratory of the Government Chemist. The lack of correspondence between relative peak heights on the chromatograms of the specimens and of commercial PCB preparations,

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and in some instances the complete absence of certain peaks from the former, prevents the determination of the amounts present by direct comparison. The assessment of the amounts of PCB was therefore based on peak heights produced by the electron capture detector related to standards of pp'-DDE and dieldrin (*see* similar technique used by Risebrough *et al.* (1968)). As the pattern of PCB peaks is frequently similar for different samples, and as the same method of computation is applied to all, the values reported reflect the relationship of the levels between samples rather than the absolute level in each. The figures shown for wildlife samples should therefore be interpreted in this light. Results up to 10 parts per million have been given to the nearest, between 10 and 30 ppm to the nearest 5 ppm, above 30 ppm to the nearest 10 ppm, and above 200 to the nearest 100 ppm.

STUDIES OF WILD BIRDS

Specimens examined

Analyses for PCB have been completed for a total of 196 livers from 33 species and of 363 eggs from 28 species (*see* Table 1). The specimens were obtained between April 1966 and August 1968. The seabird eggs were collected from Scolt Head, Norfolk; St Abbs Head, Berwickshire; the Mull of Galloway, Wigtownshire; and one of a great skua (*Stercorarius skua*) from Handa, Sutherland. They were part of the annual sample of seabird eggs collected for monitoring insecticides (Moore & Tatton, 1965; Moore, unpublished). Most of the heron (*Ardea cinerea*) and moorhen (*Gallinula chloropus*) specimens came from eastern England, being part of the material collected during a detailed study of the heron (Prestt, in press). The bird of prey specimens were sent throughout the year from various parts of Britain and represent part of the routine continuous collection being carried out to follow changes in the insecticide residues in British predatory birds (Prestt, 1967).

Most of the eggs were collected when fresh and after receipt at the laboratory the entire contents of each egg were blown into a glass phial and stored at −20 C until analysed. The livers were removed during autopsy and also stored in glass phials at −20°C until analysed.

Geographical distribution of PCB

Terrestrial.—Birds can only be used as indicators of local conditions if it is established that they are resident in a particular area. A total of 138 liver specimens from terrestrial species (*i.e.* birds occupying terrestrial habitats and feeding mainly on terrestrial organisms) has been analysed to date for PCB. All but eight were from birds of prey: 75 (54%) of the specimens were from largely sedentary species—golden eagle (*Aquila chrysaëtos*), sparrow-hawk (*Accipiter nisus*), peregrine (*Falco*

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TABLE I

PCB RESIDUES IN LIVERS AND EGGS OF WILD BIRDS IN BRITAIN (1966–8)

		Liver			Eggs		
		No. specimens	*Range (ppm) min-max*	*Arithmetic mean (ppm)*	*No. specimens*	*Range (ppm) min-max*	*Arithmetic mean (ppm)*
Podicipidae	Great Crested Grebe *Podiceps cristatus*	4	30–40	36	1	40	
	Slavonian Grebe *P. auritus*	2	1–1				
	Little Grebe *P. ruficollis*	2	4–15				
Phalacrocoracidae	Shag *Phalacrocorax aristotelis*				11	4*	
Ardeidae	Heron *Ardea cinerea*	16	0–ca900	98	101	0–80	5
	Heron nestlings	14	1–30	5			
	Bittern *Botaurus stellaris*	2	1–10				
Anatidae	Whooper Swan *Cygnus cygnus*	2	0				
Falconidae	Golden Eagle *Aquila chrysaëtos*	5	0–15	4	20	0	
	Buzzard *Buteo buteo*	10	0–5	0·5	5	0–1	
	Rough-legged Buzzard *B. lagopus*	3	0				
	Sparrow-Hawk *Accipiter nisus*	16	1–40	9	48	0–10	3·1
	Kite *Milvus milvus*	3	0–2		3	0	
	Hen-Harrier *Circus cyaneus*	1	3		3	0–2	
	Osprey *Pandion haliaetus*	2	5–20		2	0–2	
	Hobby *Falco subbuteo*	1	0		1	1	
	Peregrine *F. peregrinus*				28	0–6	1·3
	Merlin *F. columbarius*	2	0–70		3	2–4	
	Kestrel *F. tinnunculus*	47	0–25	3	3	0–2	
Balearicidae	Water Rail *Rallus aquaticus*	1	1				
	Moorhen *Gallinula chloropus*				13	0–15	2·4
Haematopodidae	Oystercatcher *Haematopus ostralegus*				9	0–1	0·3

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Charadriidae	Lapwing *Vanellus vanellus*				1	0	
	Ringed Plover *Charadrius hiaticula*				5	1·1	
Scolopacidae	Woodcock *Scolopax rusticola*				1	0	
Stercorariidae	Great Skua *Stercorarius skua*				1	25	
Laridae	Herring-Gull *Larus argentatus*				1	1	
	Black-headed Gull *L. ridibundus*	1	0				
	Kittiwake *Rissa tridactyla*				13	5*	
	Common Tern *Sterna hirundo*	1	0		14	1·2	1·6
	Sandwich Tern *S. sandvicensis*				40	1·10	2·8
Alcidae	Razorbill *Alca torda*				11	7*	
	Little Auk *Plautus alle*	1	1				
	Guillemot *Uria aalgae*	1	8		16	5*	
Strigidae	Barn-Owl *Tyto alba*	22	0·50	6	4	0·5	
	Little Owl *Athene noctua*	9	0				
	Tawny Owl *Strix aluco*	3	0·7				
	Long-eared Owl *Asio otus*	1	0		4	0·4	
	Short-eared Owl *A. flammeus*	3	0·2				
Alcedinidae	Kingfisher *Alcedo atthis*	14	0·40	11			
Hirundinidae	Swallow *Hirundo rustica*	1	1				
Corvidae	Raven *Corvus corax*	2	0				
	Carrion Crow *C. corone*	1	0		1	0	
	Rook *C. frugilegus*	1	3				
Laniidae	Great Grey Shrike *Lanius excubitor*	1	6				
Sturnidae	Starling *Sturnus vulgaris*	1	0				
	Total	196			363		

* Denotes specimens bulked

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peregrinus), barn-owl (*Tyto alba*), little-owl (*Athene noctua*), tawny-owl (*Strix aluco*), long-eared owl (*Asio otus*), raven (*Corvus corax*), carrion crow (*C. corone*), and rook (*C. frugilegus*); 49 (36%) came from species that are partial migrants—merlin (*Falco columbarius*) and kestrel (*F. tinnunculus*); and only 14 (10%) were from migratory species rough-legged buzzard (*Buteo lagopus*), hobby (*Falco subbuteo*), hen-harrier (*Circus cyaneus*), short-eared owl (*Asio flammeus*), woodcock (*Scolopax rusticola*), swallow (*Hirundo rustica*), great grey shrike (*Lanius excubitor*), and starling (*Sturnus vulgaris*). Because such a high proportion of the specimens were from largely sedentary birds and because they had been obtained from many different parts of Britain, it was considered that they provided an adequate sample from which an indication could be obtained of the geographical dispersion of PCB in the terrestrial environment of Britain.

Figure 1 shows the geographical location from which the specimens of terrestrial species were obtained, together with an indication of the amount of PCB present. It can be seen that individuals with PCB levels of between 10–100 ppm in the liver were obtained from widely separated parts of Britain, *e.g.* the Hebrides in West Scotland, the Scottish Highlands, and the north-east, north-west, south-east and south-west of England. Specimens with smaller amounts have been obtained from north and south Wales, the midlands and south of England. It is also evident that specimens with no, or very little (< 1 ppm), PCB came from the same widely scattered localities. These include individuals from species in which PCB has been found, as well as species in which PCB was not found. Of the total of 116 eggs from terrestrial species that have been analysed, 108 (93%) were from the sedentary species, 6 (5%) were from partial migrants, and only 2 from migratory species. Although the eggs were collected from fewer localities, they support the general conclusions drawn from the livers. If therefore it is correct that these sedentary species of birds are indicative of the region from which they were obtained, it would appear that PCB is available in all regions of Britain and its distribution appears to be random rather than by area.

Freshwater.—Most of the specimens from freshwater fish-feeding species examined came from the midlands, east and south-east England, so consideration of the geographical distribution of PCB in freshwater aquatic systems must be confined to this part of the country. Of the specimens examined all the species with the exception of the heron are sedentary. British herons are sedentary but since migrants, particularly from the Scandinavian countries, come to Britain, some of the results may refer to migrant individuals. However, almost half the heron liver residues were from nestlings so these, like the heron eggs, must reflect local conditions. It can only therefore be a small proportion of the analyses that could refer to other than British herons and the results must to a large extent be indicative of the situation in the more southerly and eastern parts of England.

The geographical distribution of the freshwater specimens and an indication of the amount of PCB present in them is given in Fig. 2. This shows that specimens

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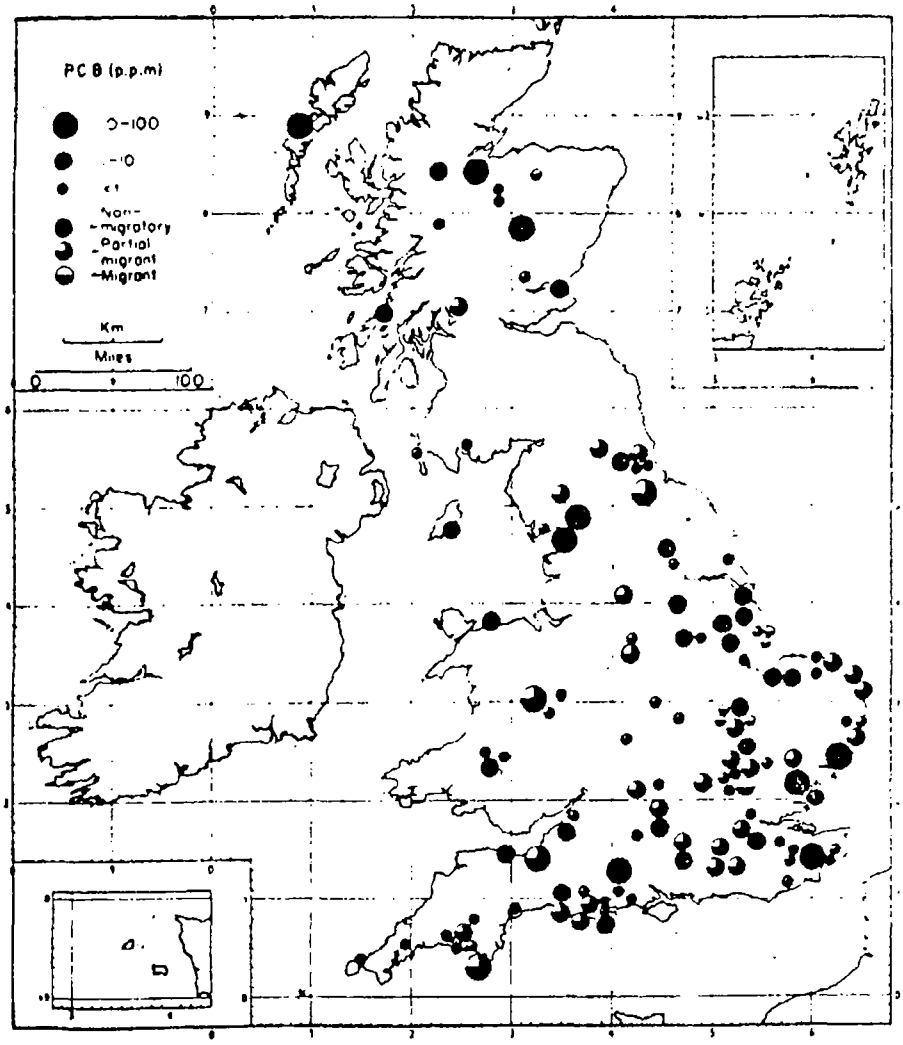

Fig. 1. Showing the geographical localities and PCB residues found in the livers of terrestrial predatory wild birds. The specimens were collected during 1966–68 and were found dead or dying. A different symbol has been used to distinguish non-migratory birds, partial migrants and migrants.

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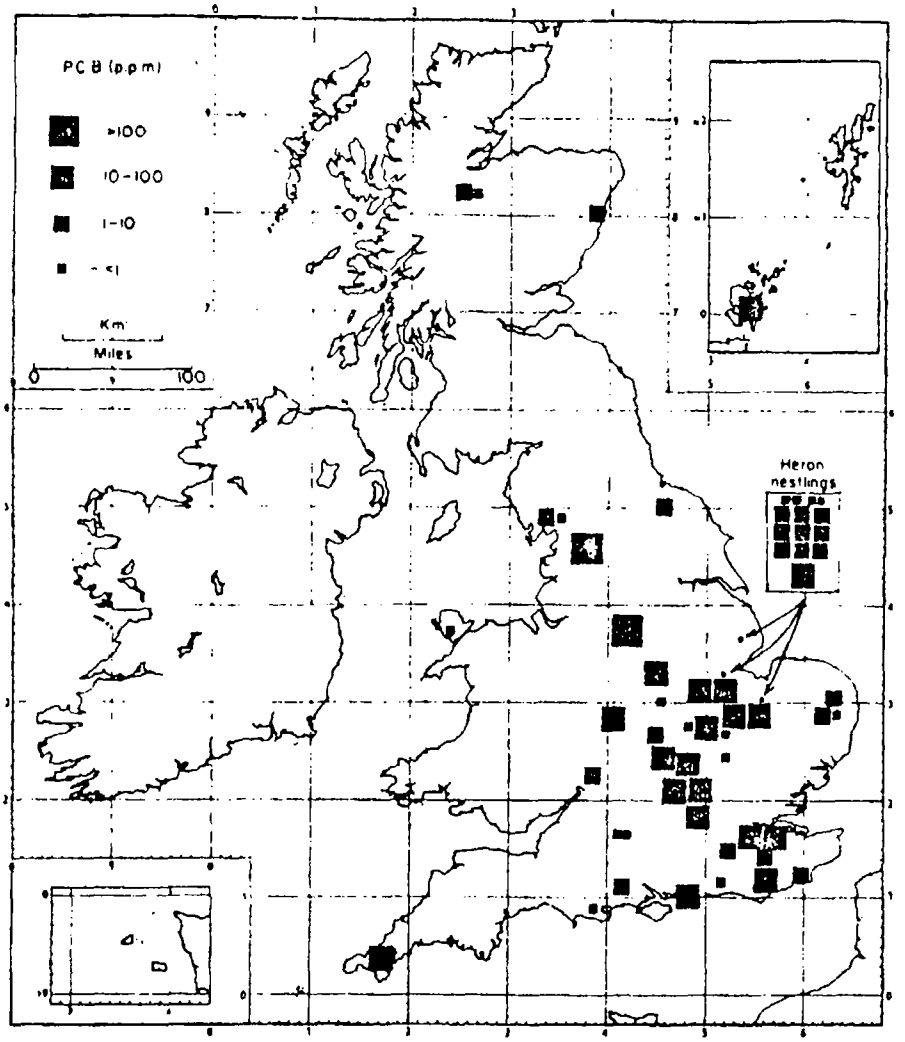

Fig. 2. Showing the geographical localities and PCB residues found in the livers of freshwater fish-feeding birds. The specimens were collected during 1966–68 and were found dead or dying. Most of the species included are non-migratory, but they could include some Herons which had migrated to Britain.

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with more than 10 ppm PCB present in the liver have been obtained from all parts of the area represented by the sample and only 12 (27%) of the 45 specimens had less than 1 ppm. Again the egg analyses give a similar result to those from the livers, with < 1 ppm only being found in 38 (37%) out of 102 eggs analysed. These findings show that PCB has been obtained by freshwater fish-feeding species in all parts of the midlands, east and south-east England, and suggest that about two-thirds of the individuals in this area may contain PCB in considerable amounts.

Marine.—The Scolt Head egg sample contained 10 sandwich tern (*Sterna sandvicensis*) eggs analysed individually, and 10 common tern (*S. hirundo*) eggs bulked: the Mull of Galloway sample contained 7 bulked guillemot (*Uria aalgae*) eggs, 6 bulked shag (*Phalacrocorax aristotelis*) eggs, 6 bulked razorbill (*Alca torda*) eggs and 6 bulked kittiwake (*Rissa tridactyla*) eggs; and the St. Abbs Head sample 5 bulked shag eggs, 8 bulked guillemot eggs, 1 single guillemot egg, 1 herring-gull (*Larus argentatus*) egg, 5 bulked razorbill eggs and 7 bulked kittiwake eggs. PCB was present in all the bulked samples of all species, it was also present in all the 10 individual sandwich tern eggs and the single guillemot, herring-gull and skua egg. These results show PCB to have been present in all of several species sampled at three widely separated seabird colonies round the British coast. The organochlorine residues in four species examined in 1967 on both the east and west coasts were:

		St Abbs Head (east coast)				*Mull of Galloway (west coast)*		
		PCB (ppm)	*pp'-DDE (ppm)*	*dieldrin (ppm)*		*PCB (ppm)*	*pp'-DDE (ppm)*	*dieldrin (ppm)*
Guillemot	8 eggs	3	0·7	0·1	7 eggs	8	2·0	0·3
Razorbill	5 eggs	6	1·0	0·5	6 eggs	7	1·1	0·5
Shag	5 eggs	3	1·6	1·0	6 eggs	5	2·2	1·6
Kittiwake	7 eggs	3	0·2	0·1	6 eggs	8	0·7	0·2

PCB related to diet

The terrestrial species examined have been divided into five categories on the basis of their main food preferences: bird-feeders; mammal-feeders; carrion-, bird- and mammal-feeders; and insect-feeders. The amount of PCB present appears to vary between the categories. This is suggested by the differences in the ranges of residues present (Table 2), the highest levels (70 ppm and 50 ppm) being in the bird- and mammal-feeders compared with 15 ppm and 1 ppm in the other two categories. The same differences are found in the egg residue figures, residues as high as 10 ppm being found in the 84 eggs of bird-feeding species (merlin 3, long-eared owl 4, peregrine 29, sparrow-hawk 48), up to only 5 ppm in mammal-feeders (barn owl 4, kestrel 3), and none being recorded in the 23 eggs from species in the carrion-, mammal- and bird-feeding category (kite (*Milvus milvus*) 3, golden eagle 15, buzzard 5). The 15 golden eagle eggs that contained no PCB were from inland

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TABLE 2

LIVER RESIDUES OF PCB RELATED TO DIET IN TERRESTRIAL SPECIES OBTAINED THROUGHOUT BRITAIN

PCB	*Principal food*			
(ppm)	Birds	Mammals	Mammals, Birds, Carrion	Insects
Range (min-max)	0-70	0-50	0-15	0-1
No. specimens	18	75	21	11
Species (with number of each shown in brackets)	Merlin (2) Sparrow-hawk (16)	Short-eared Owl (3) Tawny Owl (3) Kestrel (47) Barn Owl (22)	Buzzard (10) Kite (3) Rough-legged Buzzard (3) Golden Eagle (5)	Hobby (1) Swallow (1) Little Owl (9)

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eyries; in contrast an eaglet from North Uist, where the golden eagle's prey includes seabirds, proved to contain 17 ppm PCB.

The freshwater fish-feeding birds examined were mostly obtained from the midlands, east and south-east of England, so in Table 3 the liver residues from these have been compared to those in specimens of resident bird-feeding and mammal-feeding species obtained from the same area. The numbers examined of bird- and mammal-feeders, particularly of the former, are small but the results are of a similar order to those obtained from specimens from these same categories

TABLE 3

LIVER RESIDUES OF PCB RELATED TO DIET, IN SPECIMENS FROM THE MIDLANDS, EAST AND SOUTH OF ENGLAND

PCB	*Principal food*		
(ppm)	*Birds*	*Mammals*	*Fish (Mainly freshwater)*
Range (min max)	1–40	0–50	0–*ca* 900
No. specimens	6	18	46
Species (with number of each shown in parentheses)	Sparrow-hawk (6)	Tawny-owl (3) Barn-owl (15)	Bittern (2) Kingfisher (12) Great Crested Grebe (4) Little Grebe (1) Heron (27)

collected from all parts of Britain (*see* Table 2). The wide range and very high levels found in some of the fish-feeders, *e.g.* ca. 900 ppm in a heron from Hathersage in Derbyshire and ca. 300 ppm in a heron from Settle in Yorkshire, indicate that PCB has apparently been very much more readily obtained and or retained by predatory birds in the freshwater habitats in this area of England, than by terrestrial predatory birds in this or any other part of Britain.

A comparison between the amounts of PCB and organochlorine insecticides

A sufficiently large sample of heron eggs and kestrel livers has now been examined to provide an indication of the variation in amounts of the organochlorine compounds present within one species. Figure 3A shows the residues of dieldrin, pp'-DDE and PCB, the only compounds present in significant amounts, found in 101 heron eggs obtained in 1968 from 6 colonies in east England. Of the three compounds the dieldrin residues are the most limited in range and do not exceed 6·5 ppm, whereas those for both PCB and pp'-DDE extend over a greater range to 80 ppm and 26·5 ppm respectively. There is, however, a larger number of eggs with < 1 ppm PCB (60) than with less than the same amount of dieldrin (42) and pp'-DDE (15), suggesting that of the three PCB is generally present in smaller amounts in these herons.

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A similar comparison of the distribution of residues of the same three compounds in 47 kestrel livers is given in Fig. 3B. Again the dieldrin residues have a more limited range compared with those of PCB and pp'-DDE, particularly as two of the specimens with high dieldrin residues (15 and 18 ppm) probably died from dieldrin

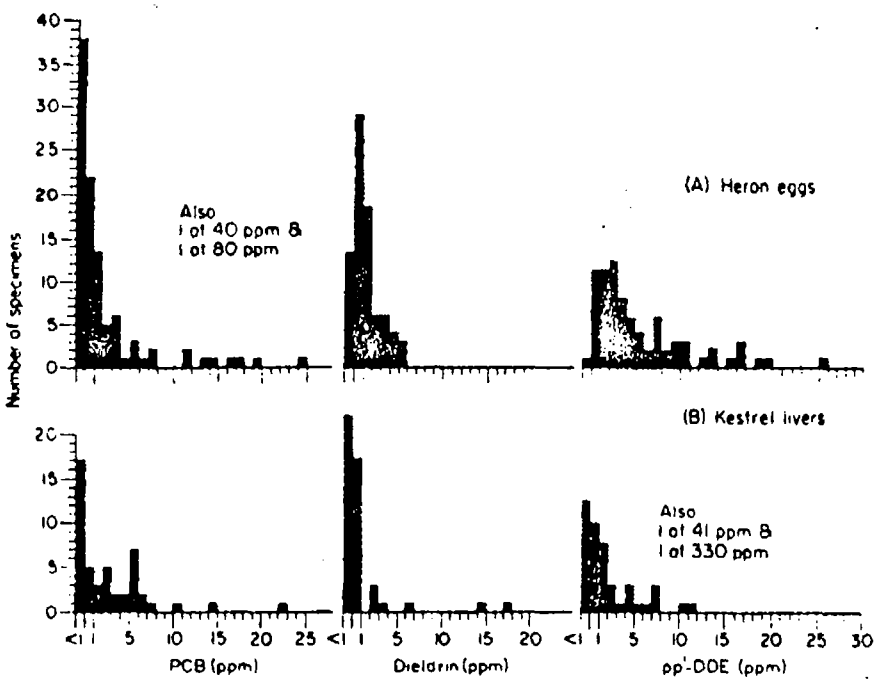

Fig. 3. Showing the residues of PCB, dieldrin and pp'-DDE in parts per million in (A) heron eggs (entire contents, wet weight) and (B) kestrel livers (wet weight), obtained in Britain during 1966–68.

poisoning (Prestt *et al.*, 1968). Unlike the heron eggs, however, there is a larger number of liver specimens with < 2 ppm dieldrin (40) than with less than this amount of PCB (22) or pp'-DDE (23), suggesting that there is a greater ratio of PCB to dieldrin and pp'-DDE in the kestrel livers than in the heron eggs.

LABORATORY EXPERIMENTS ON THE TOXICITY OF A PCB

Experimental procedure

The avian toxicity of one of the polychlorinated biphenyls was tested on the Bengalese finch, a domesticated form of the sharp-tailed finch (*Lonchura striata*) (*Estrildinae*). This bird was chosen as it is easily maintained in the laboratory and

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has been used at Monks Wood Experimental Station for long-term testing of the sublethal and lethal toxicities of pp'-DDT (Jefferies, 1967).

A proprietary cage-bird rearing food (composed of biscuit meal, dried milk, soya bean meal and maw seed) was used as the vehicle for the PCB. The PCB used was one of the more highly chlorinated types—Arochlor 1254 (biphenyl with 54% w/w of chlorine). This PCB was chosen as the pattern of peaks produced on a gas–liquid chromatogram was similar to that found in wildlife specimens examined at Monks Wood Experimental Station. A 20,000 ppm 'master-mix' of this material in the rearing food was made as follows: 4 g of the PCB was weighed into a foil boat and this was then placed in a beaker containing 70 ml of acetone and the solution stirred with a glass rod. The contents of the beaker were then tipped into a 2 lb Kilner jar containing 196 g of the rearing food ground to a powder (as PCB is a resinous material the difficulty of weighing out a definite amount was overcome by altering the weight of rearing food to the amount of PCB weighed out). The beaker and glass rod were then washed with two separate 20 ml amounts of acetone which were also added to the contents of the Kilner jar. The acetone was evaporated from the rearing food for 10 hours at room temperature, when no smell of acetone could be detected. Finally, the 'master-mix' was blended thoroughly by turning the jar on a tumbler mixer for 2 hours. A control diet was treated in the same way.

Eight other dietary levels from 10,000 to 75 ppm (*see* Table 4) were made up in Kilner jars by diluting the 'master-mix' with finely-ground rearing food. Each mixture was then tumbled for 2 hours. A fresh 'master-mix' was made at approximately weekly intervals and all mixtures were stored in the sealed jars at 4°C. A test analysis of 0·9367 g of a 5,000 ppm mixture showed a concentration of 5,028 ppm PCB.

Thirty-eight adult (*i.e.* over 4 months) Bengalese finches, mean weight 14·17 ± 0·17 g, were placed separately in cages 61 × 41 × 38 cm and maintained at 16°C. Each cage was provided with cuttlefish, grit, water and the standard diet of canary and millet seed. The numbers of birds on each of the 10 dietary levels are indicated in Table 4. The PCB-rearing food mixtures were given to the test birds as only part of their diet. This technique was used to overcome the possible problem of the PCB rendering the food unacceptable to some individuals, which may prefer to starve rather than eat the contaminated food and starvation could obscure any real effects of the PCB. Thus the standard seed diet was removed from each cage at 14.00 hours and one hour's starvation given before the PCB/rearing food mixture was presented for two hours. A mixture of 1·5 g of PCB/rearing food with 1·5 g of water was given each time, mixed to a dry paste. At 17.00 hours the standard seed diet was replaced. It was found necessary to measure the intake of the test diet, as the consumption varied considerably within each concentration fed. An evaporation control, set up each day, allowed compensation for this loss and the calculation of the PCB consumption for each bird in μg/day (*see* means in Table 4).

With this form of feeding, the actual parts per million of PCB in the total diet are much lower than that in the PCB/rearing food mixtures given. An earlier series

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of tests using exactly the same maintenance at various temperatures showed that the relationship between food consumption (*i.e.* weight of seed minus husks plus dry weight of rearing food), body weight and temperature was given by the equation $y = 395{\cdot}50 - 5{\cdot}25x$ (where y = mg consumption of dry food/gram of body weight/day and x = mean daily temperature in °C). Using this equation the concentrations of PCB in the total diet (4·414 g of food/bird/day) were calculated for purposes of comparison with other work (*see* Table 4).

TABLE 4

THE RANGE OF TEST DIETS CONTAINING PCB FED TO 38 LABORATORY-MAINTAINED BENGALESE FINCHES FOR 56 DAYS AS PART OF THEIR NORMAL DIET. THE MEAN WEIGHED INTAKE OF PCB AND THE CALCULATED TOTAL DIETARY LEVEL ARE ALSO GIVEN

Partial dietary level (ppm)	*Number of birds used* ♂	♀	*Mean PCB consumption in µg/day*	*Calculated total dietary level (ppm)*
Control	6	–	0	0
75	2	–	26	6
250	2	1	73	17
500	2	1	125	28
1,000	4	1	217	49
1,500	4	–	367	83
2,000	2	1	502	114
5,000	2	1	1,011	229
10,000	4	1	1,331	302
20,000	4	–	1,940	440

The 38 birds were maintained on the above test diets for 8 weeks. Those still alive at the end of this period were killed with ether except for 5 test birds (75–2,000 ppm diets) and 3 controls which were starved for 21 hours prior to being killed. This was done in order to evaluate the amount of PCB released from the depot fat into more sensitive organs when a bird is under stress, as in bad weather or after long flights or illness. All birds were examined and the brain, liver, kidneys and heart weighed. The livers of all test birds and the brains of most were analysed for PCB concentration.

Avian toxicity of PCB

Seven birds died during the 56 days on the test diets of PCB. Deaths occurred at 17, 18, 21, 26, 32, 40 and 42 days with no apparent correlation of time with dose rate. No mortality occurred in the control.

One of the earlier symptoms of PCB poisoning was an apparent lack of balance at perching. Later three birds which died showed a form of paralysis in the legs, with one leg carrying all the weight whilst the other was splayed out to one side with foot closed. Before death some birds showed body trembling with shaking wings.

As the consumption of PCB varied considerably at each dietary level, the birds were regrouped before calculation of the LD50 according to their individual

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consumption in mg PCB kg body weight day. Five dosed groups were used besides the control. These were 0–12, 12–36, 36–72, 72–144 and >144 mg kg day. The percentage survival in each group, together with mean dose rate and number in the group are shown in Fig. 4. None of these dose rates produced 100% mortality. The highest mortality, one out of two birds, was shown at the highest dose rate

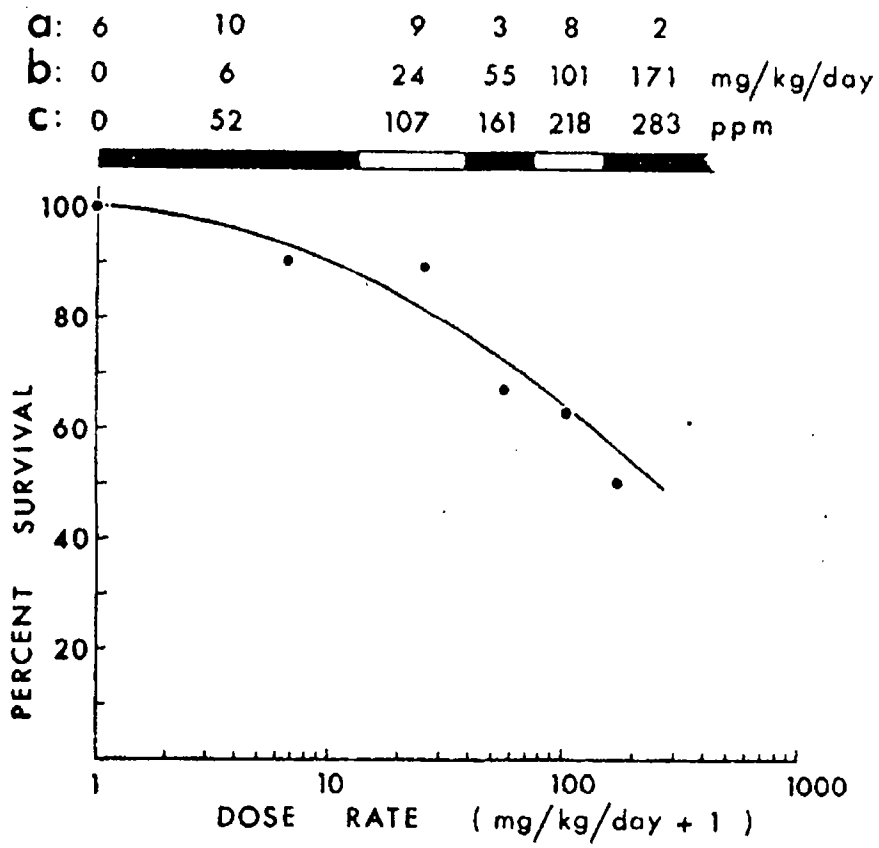

Fig. 4. The percentage survival within each of the five dose rate groups and control (a) numbers in each group; (b) mean dose rate in each group and the calculated mortality curve. The calculated liver concentrations in ppm wet weight are also shown (c).

with a mean of 170·7 mg kg day. The factors limiting the production of higher mortality figures were the low toxicity of the material and the apparent unacceptability of very high concentrations in the food. There was a significant correlation between the concentration of PCB in the food and the amount eaten ($y = 0{\cdot}5327 - 0{\cdot}0975x$, where x = log ppm PCB in test diet and y = grams dry rearing food

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eaten per day: $r = -0{\cdot}6581$: DF = 30: $P < 0{\cdot}1\%$). Thus as the concentration in the test diet increased, the daily consumption decreased. Increasing the top dietary level from 20,000 to 40,000 ppm would not have greatly increased the daily intake of PCB.

The mortality curve and regression equation were calculated by probit analysis (*see* Fig. 4). The equation was found to be $y = 2{\cdot}8826 + 0{\cdot}8807x$ (where x = log dose rate in mg/kg/day and y = percentage mortality in probits) and the estimated LD50 56 days was $2{\cdot}4042 \pm 0{\cdot}5763$ log units (= 253·6 mg/kg day or a total of 14,200 mg/kg for the 56 days). Estimated dose rates for 75% and 99% mortality using this equation are 1,462 and 112,100 mg/kg/day respectively. These quantities would be difficult to achieve using the present feeding technique

Of the five starved PCB birds, one died after 20 hours and a second after 21 hours starvation. At this time the remaining three were on their cage floors showing signs of stress and two were trembling; all starved birds were then killed with ether. The control birds were perching and one was flying normally after the same period of starvation.

There is very little published work on the avian toxicity of the PCBs with which to compare these findings. Flick *et al.* (1965) fed Arochlor 1242 at levels of 200 and 400 ppm in the diet to one-day-old white leghorn cockerels in order to compare the response with the symptoms of chicks suffering from a new pathological disorder known as 'Chick Edema Disease' (Schmittle *et al.*, 1958). Unfortunately the Arochlor was fed for three weeks only, by which time three out of 24 birds had died on the 400 ppm diet. This result is somewhat similar to that of the present experiment, where three out of the seven birds which died on diets up to 440 ppm had done so by three weeks. Koeman *et al.* (1969) fed a diet containing 2000 ppm of Phenochlor DP6 to 20 Japanese quail (*Coturnix coturnix*), all of which died in 55 days. This total dietary level was not achieved in the present experiment but, using the data in Table 4, an approximation would suggest that this might have resulted in an intake of 10,000 μg/day. Figure 4 shows that this would have produced a similar, almost complete mortality in a similar number of Bengalese finches.

Body weights, organ weights and post-mortem findings

During the 56-day period of the experiment the controls increased in body weight by a mean of 3%. The 20 dosed birds killed at the end of the experiment also had a net mean gain (6%). Birds dying of PCB poisoning had a net mean loss of 13%, which was similar to the mean loss of 14% shown by the birds which were starved for 21 hours.

The weights of the liver, heart and kidneys of each bird were related to its brain weight (mean $0{\cdot}453 \pm 0{\cdot}005$ g; this forms a better indicator of body size than the more variable body weight) by calculating the percentages organ weight/brain weight × 100/1. The liver percentage was similar in controls (139·5%), PCB birds killed (136·6%) and birds dying of PCB poisoning (143·0%). However,

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in PCB birds starved for 21 hours, the liver percentage was considerably decreased to 88·7%, presumably due to the loss of organ fat. The heart percentage was slightly increased from 38·3% in the controls to 40·9% in the PCB birds killed and to 44·0% in those dying of PCB poisoning. Two of the males which died had heart percentages of 51 and 52% which are outside the range of normal heart percentages for males of this species (Jefferies, 1969). The kidney percentage was similar in the controls (32·4%) and the PCB birds killed (30·8%) but was very much larger in the birds which died (53·5%). There was probably a slight reduction in the kidney weight of the starved birds (28·0%).

The most obvious gross pathological change seen on post-mortem examination of all the birds dying from PCB poisoning was the enlargement of the kidneys. Also, two of these birds showed considerable amounts of fluid in the pericardial sac. Hydropericards were mentioned by both Flick *et al.* (1965) and Koeman *et al.* (1969) in chickens and quail poisoned with PCBs. Flick *et al.* also found considerable kidney enlargement. Very little depot fat was found in the birds dying from PCB poisoning and none in those starved for 21 hours.

Concentration of PCB in the organs of living and dead birds

The brain and liver of one of the control birds were analysed at the end of the experiment and no PCB was detected.

The concentrations of PCB in the livers of the 20 birds killed immediately after the end of the experiment ranged from 3 to 634 ppm, that in the seven birds dying from PCB poisoning from 70 to 697 ppm. These concentrations are shown plotted against dose rate in Fig. 5. The concentrations found in birds killed and birds dying overlap considerably. There was a significant correlation between the dose rate and the concentration of PCB in the liver ($y = 1{\cdot}336 + 0{\cdot}500x$, where y = log ppm PCB in liver and x = log dose rate in mg/kg/day; $r = 0{\cdot}5414$; $DF = 25$; $P < 1\%$). Using this equation the liver concentrations for each of the dose rate groups were calculated and are shown in Fig. 4. The variation in the liver PCB concentration at any one dose rate is extremely large (the 95% confidence limits are 0·9490 units of y above and below the regression line—*e.g.* for the LD50 of 253·6 mg/kg/day the liver PCB concentration would be 345 ppm with limits from 39 to 3,070 ppm). This may be due partly to variation in fat content since the liver concentration in the starved birds, as well as being much higher (69 to 1,214 ppm) was not so variable (*see* top of Fig. 5). However, the variation is too great for this to be the sole cause.

The relationship of the concentration of PCB in the brain to that in the liver was examined for each group of experimental birds by calculating the percentage, brain ppm/liver ppm × 100/1. In the PCB birds killed at the end of the experiment it was low, a mean of 26·4 ± 6·1%, whereas in those dying from PCB poisoning it was over three times higher, 83·7 ± 7·4%. In the starved PCB birds the percentage was between the two at 47·6 ± 7·3%.

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One bird (15·04 g), fed 1,500 ppm PCB and killed at the end of the experiment, was submitted to total body analysis to find the percentage storage of the PCB consumed. The brain (0·42 g), liver (0·63 g) and depot fat (0·23 g) were analysed

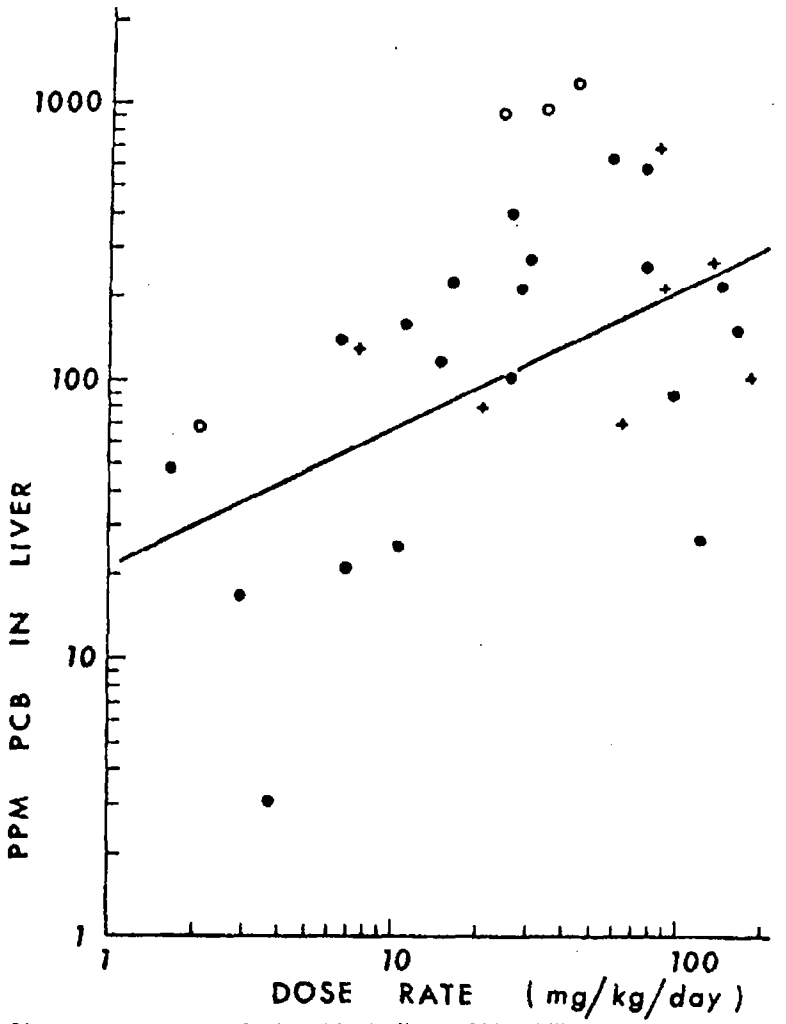

Fig. 5. The concentrations of PCB found in the livers of birds killed (●), birds dying (·), and birds starved (○) correlated with their dose rates.

separately. The rest of the body was weighed (12·20 g) and analysed together. The total body weight was made up by gut contents (0·84 g) and feathers (0·72 g). PCB content of the brain (6μg; 14 ppm), liver (16 μg; 25 ppm), fat (132 μg; 574

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ppm) and rest of body (750 μg; 61 ppm) was 904 μg. This bird consumed 10,248 μg of PCB and so stored 9% of the PCB it had eaten. It is stored in the depot and organ fat of the body.

Comparison of the toxicity of PCB with that of organochlorine insecticides

Long-term experiments on the sublethal and lethal toxicity of DDT and its metabolites have been carried out using this species with the same experimental technique. Although not completed, the LD 50, 56 days of pp'-DDT has been found to be approximately 1100 mg kg (19 mg kg day). Thus the PCB Arochlor 1254 has only 1/13 of the toxicity of DDT (DDT in turn has only 1·6 to 1/14 of the toxicity of dieldrin, De Witt *et al.*, 1960). However, whereas DDT has a steep mortality curve (*i.e.* no deaths at approx. 1000 mg kg, all dead at 1200 mg kg using 132 birds; 18 and 21 mg/kg day respectively), this curve is much more gradual with PCB. This means that at low dose rates, below 18 mg kg day, PCB may have a comparatively greater toxicity than similar amounts of DDT (*i.e.* to the 5-10% of the population which is sensitive to these low amounts).

Detecting mortality from PCB poisoning

On intake of PCB this material accumulates in the organs of the body, particularly the depot fat, in concentrations commensurate with the dose rate. It would appear that death follows enlargement of the kidneys and hydropericardium. The liver remains normal in size at death as the bird dies before the organ and depot fat have been completely used. Some body weight loss occurs, possibly due to lack of feeding prior to death. The PCB from the depot fat which is used at this time appears to go to the brain and increases the quantity there considerably. When a bird containing PCB starves (as would occur in the field during bad weather or illness) the same thing happens and the brain concentration increases. However, the concentration in the liver also rises due to the reduction in liver weight with loss of organ fat (the highest content of PCB in the liver of a bird which died was 740 μg, 697 ppm – the highest content in a starved bird was similar at 670 μg although the concentration had risen to 1214 ppm).

With materials such as DDT where the correlation between dose rate and liver content is very good (Jefferies & Walker, 1966) and the mortality curve has a steep slope, an indication of whether a bird has died after poisoning from that compound may be gauged from the concentration in the liver. With PCB, however, the opposite is the case. There is a hundred-fold variation in the liver concentration at each dose rate and the mortality curve has a gradual slope so that over a considerable range of dose rates the lethal range of liver concentrations is paralleled by a similar range of non-lethal concentrations. For example, the highest lethal concentration in the present experiment was 697 ppm but one bird on a similar dose rate lived with 634 ppm in the liver. Thus there is no certainty, given the liver residue alone, whether a

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particularly large concentration has caused death or not. If of a similar sensitivity to *L. striata*, one in thirty birds would die with liver residues from 5 to 360 ppm (mean 40 ppm), one in three with 22 to 1780 ppm (mean 200 ppm) and one in two with 39 to 3070 ppm (mean 345 ppm). It is unlikely that death would occur at amounts much lower than 5 ppm.

A more certain conclusion about the probability of death from PCB poisoning may be reached if the brain is analysed as well as the liver (to obtain the relationship of the liver/brain residues) and the body is given an autopsy paying particular attention to enlargement of the hydropericardium and kidneys.

CONCLUSIONS AND DISCUSSION

The majority of our samples tested for PCB have come from predatory birds. Studies of organochlorine pesticides have shown that larger residues of these compounds are present in predatory species than in omnivorous or herbivorous birds (Moore & Walker, 1964; Prestt, 1967). The organochlorine pesticides and the polychlorinated biphenyls are similar in several ways. The presence of PCB in such a large number of specimens from diverse habitats throughout the country, in relatively large amounts, suggests that they, like the organochlorines, are persistent. Also, both accumulate in the body fat of animals. Thus one might expect that predatory animals at the top of food chains would also be the best group to indicate the presence and the geographical distribution of the PCBs and to suggest whether or not they represent a hazard to British wild birds.

Other studies (Risebrough *et al.*, 1968; Koeman *et al.*, 1969; Jensen *et al.*, 1969) have suggested that residues of PCBs, like residues of certain organochlorine pesticides, may now be widespread. Our study confirms that they are widely distributed in Britain. In the terrestrial environment they were present in birds and their eggs from all parts of the country. In seabirds they were found in all species sampled at three coastal colonies, one off the Irish sea and two off the North sea. The largest amounts discovered were in lake and river birds from the midlands, east and south of England. The highest of these, ca. 300 and ca. 900 ppm, are the largest liver residues of PCB yet reported in wild animals from any part of the world. Like pp'-DDE and dieldrin, PCB is present in larger amounts in the bird-feeding and fish-feeding species. The highest levels found in Swedish waters by Jensen *et al.* were in the fish-feeding white-tailed eagle and heron.

The level of PCB found was also similar to that of pp'-DDE. The average residues of dieldrin, pp'-DDE and PCB for four predatory species obtained in Britain from 1963 to 1968 are given below and show this similarity (data for pp'-DDE and dieldrin, from Prestt in press and for PCB from Table 1 of present paper).

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	No. of specimens	*pp'-DDE (ppm)*	*dieldrin (ppm)*		*PCB (ppm)*
Heron	42	30·18	9·33	Freshwater fish-feeders*	40
Sparrow-hawk	57	14·5	4·4	Bird-feeders*	10
Kestrel	159	5·0	3·1	Mammal-feeders*	4
Barn-owl	102	4·2	2·6		

* For categories included in each group see Tables 2 and 3.

In view of the similarities of the distribution and levels of PCB to those of pp'-DDE and dieldrin, the immediate question is whether PCB could have caused the effects on predatory birds in Britain previously attributed to the organochlorine pesticides. As far as lethal poisoning is concerned, there are several reasons for considering this unlikely. First, our toxicological studies show one PCB (and comparisons with the other published toxicological studies suggest that the toxicities of the different PCB compounds may not vary very greatly) to have only about 1·13 the toxicity of pp'-DDT. DDT has only 1·6 to 1·14 the lethal chronic toxity of dieldrin (De Witt *et al.*, 1960) and our studies have shown that pp'-DDE has an even lower lethal toxicity than DDT to the Bengalese finch. Very few of the specimens examined are likely, therefore, to have died from either PCB (or DDE) poisoning. As there is no reason to suppose a greater use of PCB in Britain prior to the period during which the present samples were obtained, it is unlikely it has ever had any widespread acute effects in Britain. Secondly, while the population crashes of the peregrine and sparrow-hawk were coincident in space and time with the introduction of aldrin, dieldrin and heptachlor, *i.e.* in the mid 1950s (Ratcliffe, 1963; Prestt, 1965), the introduction and use of PCB is not. PCB was first used in 1930 and, while it probably became more available to wildlife after the 1939–45 war, there is no reason to suppose it might suddenly have produced an exceptional effect in the late 1950s. In the same way, while the recovery of the sparrow-hawk and peregrine started in the mid 1960s following certain bans on aldrin, dieldrin and heptachlor, there is no reason to suppose there may have been a reduction in the amount of PCB available at this time (it should be remembered that the dieldrin figures shown above were obtained after the bans). The geographical distribution of PCB appears to be general in the terrestrial environment in Britain, so in this way also there is no connection with the population declines which were more severe in the south and east of Britain. Nothing in the evidence available about PCB, therefore, contradicts the previously advanced hypothesis that lethal poisoning by aldrin, dieldrin and heptachlor is the most likely explanation of the sudden declines in certain birds of prey in Britain in the late 1950s (Jefferies & Prestt, 1966).

As regards the sublethal effects on bird populations the situation may be different. There has been a widespread breeding failure among predatory birds in Britain

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and the USA in post war years (Ratcliffe, 1963, 1967a; Lockie & Ratcliffe, 1964, Hickey, 1969). Also many species (again particularly the predatory birds) have been shown to be laying thin-shelled eggs since approximately 1947 (Ratcliffe, 1967b, Hickey & Anderson, 1968). Laboratory experiments here show that the reproductive system of birds can be adversely affected by organochlorines such as DDT (Jefferies, 1967, 1969, De Witt, 1955) and the same compound has been shown experimentally to cause the production of thin-shelled eggs in the American kestrel *Falco sparverius* (Porter & Wiemeyer, 1969). Recent work by Peakall (1967) has shown that one of the mechanisms by which this may be brought about is the induction of steroid hydroxylating enzymes in the liver (see discussion in Anderson *et al.*, 1969). As PCB is now known to produce a similar reaction (Risebrough *et al.*, 1968) it seems possible that PCB may be one of the component causes of the present breeding failure. This breeding failure is probably an important factor adversely affecting the recovery of the bird of prey populations. Anderson *et al.* (1969), in their study on the effects of organochlorine compounds on breeding pelicans (*Pelecanus erythrorhynchos*) and cormorants (*Phalacrocorax auritus*), reached a similar conclusion that there was no evidence of acute toxicity from PCB or pp'-DDE, the two main compounds present, but both correlated with change in eggshell thickness.

PCBs are produced and marketed under a number of commercial trade names e.g. Arochlor, Clophen, and are available as liquids, resins or solids. They are insoluble in water, but have a low finite vapour pressure. They already have many uses: protective coatings, plasticisers, sealers in waterproofing, printing inks, synthetic adhesives. In liquid form they are used as hydraulic fluids, in thermostats, cutting oils, grinding fluids and they are incorporated into electrical apparatus. There are thus many ways in which they can become widely dispersed in the environment – into the atmosphere with industrial smoke, or in exhaust from aircraft engines and into rivers as factory effluent. It has even been suggested they may have been incorporated in pesticide formulations to increase the persistence of insecticides (Reynolds, 1969). In view of the large number of different uses of PCB and its apparent persistence, it is not surprising that it is now present in a large number of wild birds. Moreover, as it is an industrial pollutant, its control will present an entirely different problem to that of the control of the organochlorine pesticides.

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