The polychlorobiphenyls, their occurrence and significance: a review

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Pollution of the global environment by man is rightly receiving considerable attention because of the increasing realisation of possible long term effects on both the physical environment and living organisms. Clearly it is necessary to distinguish between short term toxic effects and long term ones (*i.e.* reproductive and carcinogenic effects), these latter being more difficult to assess. The chlorinated hydrocarbons used as biocides are well documented in their short term toxic effects, and with knowledge of their facile accumulation in the environment due to their considerable chemical stability, more work is being done on the long term effects coupled with tighter control of their use. With the latter the main points of entry into the terrestrial environment are through crop spraying and seed dressings with consequent accumulation in the soil. From this point they can also reach the aerial environment secondarily by evaporation or 'co-distillation' and carriage in fine particulate matter. They are also systemic in plants to varying degrees. Aqueous run-off carries them through the drainage system together with industrial effluent sources to reach the marine environment above the continental shelves. It is thus possible for these compounds to reach the lower members of the many food chains. Man's domestic and industrial use of biocides for hygienic purposes leads to entry at various other points in the food chains. The very large concentration factor (10^3) possible in these food chains is well known and in the final members (*e.g.* marine mammals, predatory birds, man) the concentrations can reach alarming proportions. Lipid solubility means that a large percentage of these residues will be stored in the fat depots of the body.

The polychlorobiphenyls (PCBs), with a very widely dispersed use throughout industry, have very similar properties to the chlorinated hydrocarbon pesticides, but are even more thermally and chemically stable. An important difference, however, lies in the routes through which they contaminate the environment. Emphasis here is on industrial effluents reaching the marine medium, and possibly the burning of industrial waste contributes relatively more to the aerial distribution. Although recommended for use in conjunction with pesticides, there is no documented example of this known to the author, so that distribution through crop spraying should not be a contributory factor. This fits the known pattern of distribution of PCBs in the environment, the highest concentrations coming from the heads of fresh water food chains, marine organisms and sea birds. High levels are also known in terrestrial avian predators, but only recently has it been shown[1] that in one region they can be found at levels comparable to those for organochlorine pesticides. While relatively little toxicological work has been done on PCBs and some of the results are superficially conflicting, this can be explained and there can be no doubt that they have very similar effects on mammalian and avian metabolic processes to those of chlorinated pesticides, and indeed that synergistic effects can occur between the two classes.[2] If any control over the use of PCBs is ultimately attempted this will be much more difficult than with pesticides because of the very large number of uses to which they are put.

There exists considerable difficulty in the analytical field because these compounds cannot be separated easily from pesticide residues, and this has probably resulted in quite a few reports in the past giving falsely high results for some pesticide residues.[3] Unknown peaks in gas chromatography analyses were early recognised[4-6] but it was not until Jensen[7] applied mass spectrometry to the problem that some of them were identified as PCBs.

Properties and uses

The PCBs received early notice back in 1881[8] and went on widespread industrial use by 1930.[9] The biphenyls, terphenyls, naphthalenes, and mixtures of these are chlorinated in varying degrees according to the use to which they are put,[10] and are marketed under trade names such as 'Aroclor', 'Phenochlor', and 'Clophen'. The products are complex mixtures of the various compounds and isomers. They range from mobile liquids to crystalline materials or hard resins, and while the lower members are readily soluble in non-polar solvents they become progressively less so with increasing extent of chlorination. They are extremely stable compounds, not hydrolysed by water, acid or alkali, and with a thermal stability such that they are used in fire-proofing. They find use in elastomers, adhesives, paints, varnishes, printing inks, putty and as general fillers. The excellent dielectric properties lead to widespread use for electric insulators and as coolant/insulators in transformers. This stability and their low vapour pressures make them ideal as lubricants, hydraulic fluids, liquid seals, cutting oils and vacuum diffusion pump oils. Employment in packaging materials has led to the contamination of food.[11] There are a host of other uses, but finally it may be mentioned that both the Monsanto Company and the USDH have reported that they act as effective vapour suppressants in insecticide formulations and thus prolong the active life of an application. However, it has not been acknowledged that PCBs have actually been used for this purpose commercially. As an ancillary fact, a patent for the production of PCBs from lindane residues has been granted. This multiplicity of usage has repercussions in the analytical area because

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many solvents, some adsorbents and other analytical materials, and even the atmosphere of a laboratory can be contaminated by PCBs.

Occurrence in the environment

It is now well established that PCBs can be found in wildlife in many areas of the environment.[1,12–26] The highest levels are generally associated with the industrial areas and the adjacent freshwater and marine food chains. The distribution is inevitably not quite so simple as this because of the migratory habits of some of the species involved[19,24] and the fact of widespread aerial distribution.[28] Cohen and Pinkerton[27] reviewed the latter in respect of pesticides, mainly over the North American continent, and found higher levels in dust than rainfall. Risebrough *et al.*[25] studied the carriage of pesticides in the trade winds coming from Europe and Africa by collecting dust from the air over the Barbados Islands, and detected from 1-64 p.p.b. organochlorine residues. Dust collected over the Californian coast contained some thousand times greater concentration of organochlorines, but none could be found in dust collected from over the Central Pacific, although traces were inferred from the nature of the particulate matter. While no PCBs were detected in any of these samples, it was concluded that they were probably present in trace amounts. Tarrant and Tatton,[28] however, who found organochlorine pesticides in rainwater samples taken from all over the British Isles showed that levels were highest in the summer months (*cf.* Risebrough *et al.*[25]) and in populous areas, also found PCBs in all of them although they did not estimate the concentrations. The importance of aerial distribution of pesticides relative to the hydrospheric distribution has been stressed by Risebrough *et al.*, and it is inferred that PCBs have a similar pattern of distribution. However, it would seem that with PCBs more emphasis should be placed on the hydrospheric medium, because an important part in the transfer to the atmosphere, *via* dusts used as carriers in agricultural applications, can be considered as low in this case. If the vapour pressures of DDT and PCBs are taken as being of the same order, it would then be expected that atmospheric levels of PCBs would be low relative to DDT. The relative concentration of pesticides and PCBs in the hydrosphere and atmosphere have yet to be convincingly demonstrated.

The outstanding feature of the distribution is the occurrence in marine organisms on the continental shelves round North West Europe and North America, and also the extraordinarily high levels found in some freshwater avian predators, all associated with effluent from industrial activity.[13,14,16,17,20,21,26] Along the west coast of the USA PCBs have been detected in estuarine and coastal waters in San Francisco Bay, San Diego Bay and Puget Sound (Washington). In areas remote from industrial activity round the Bay of California and parts of the Panama coast for example, birds' eggs contained 20-30μg PCB each, but near the above mentioned industrial locations the eggs contained up to several hundred and sometimes over a thousand ppt PCB egg. The east coast of the USA has yielded similar evidence for contamination by PCBs. Holden and Marsden[13] detected PCBs in seals from the Gulf of St Lawrence in shallow waters (Magdalen Islands) and in the deeper waters of the Cabot Straits. They have also been qualitatively detected in fish and shellfish from Texas and Georgia, and in the south, in the Gulf of Mexico, in estuarine waters at Charleston, S. Carolina, levels of less than 0·1 p.p.m. were found in fish and blue crabs.[20] Duke *et al.* also monitored the results of an industrial leakage into the Escambia River which was carried through several inner bays through to the Gulf of Mexico off Florida. Maximum levels at source ranged from 486 p.p.m. in sediment to 20 p.p.m. in fish, crustacea and sediment in the outer bays. The best indicators of contaminant levels were static organisms like oysters (up to 3 p.p.m.).

Round the coasts of North West Europe the situation is similarly documented. Jensen *et al.*[17] investigated levels in marine ecosystems in Swedish waters. The closed nature of the Baltic area perhaps leads to somewhat higher concentrations than those generally found on Atlantic and American coasts. Levels tended to be lower in waters off West Sweden and to the north of the Baltic proper, in the Gulf of Bothnia, and higher going south from the Stockholm Archipelago to the South Baltic. The levels ranged from 0·002 to 1·0 p.p.m. in mussels and fish up to 150-240 p.p.m. in white tailed eagles. The concentrations were equivalent to the total DDT levels found. Koeman *et al.*[16] qualitatively identified PCBs along the Netherlands coast from the Wadden Sea southwards to, and up, the Rhine, in mussels, fish and birds and their eggs. The uniformity of GC patterns suggested that they all originated from pollution of the Rhine. Holmes, Simmons and Tatton[12] reported the presence of PCBs in marine birds and their eggs collected from round the British Isles, and Holden and Marsden[13] showed them to be present in seals and porpoises round the coasts of Scotland, being highest in those from the east coast (see below). Bonner,[21] investigating the deaths of seal pups on the Cornish coast, found PCBs here (160 p.p.m. in blubber) higher than in comparable northern samples (34-48 p.p.m.), but less than in East Anglian specimens (350-520 p.p.m.). Presst, Jefferies and Moore, surveying PCB levels in British wildlife, reported an average of 4 (1-25) p.p.m. in the eggs of marine birds, and comparing the east and west coasts of Scotland in respect of two sets of twenty five eggs somewhat higher levels (7 as against 3·6 p.p.m.) in the western samples. No PCBs were detected in whales analysed by Wolman and Wilson,[29] and this is compatible with a distribution pattern confined to the continental shelves. However, this may be a rather naive use of the evidence because the water solubility of PCBs, especially the higher chlorinated ones, is extremely low and the bulk of the PCB is more likely to be associated with sediment on the bottom and entering into the food chains mainly *via* bottom living organisms. Most whales feed on plankton, and thus might in any case be expected to show low levels. A further point of perspective is also given by the fact that loss of lower PCBs from aqueous suspensions when aerated has been observed.[49]

Holden[26] has traced aqueous pollution back to one type of source. The work on seals in Scotland having shown that levels were highest in the Firth of Forth region, water samples from the surface and at depth from the estuary and river up to Glasgow showed no detectable amounts

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and only trace quantities in trunk sewer effluents. The zooplankton contained less than 0·03 p.p.m., and fish up to 2·6 p.p.m. However, industrial effluents are usually passed to the sewage works together with domestic sewage, and so the sludge, which is dumped offshore, was examined. The fifteen samples analysed ranged from 1·185 p.p.m. PCB on dry weight, with two sewage plants giving consistently high levels (50-160 p.p.m.). Sludge from Manchester exhibited somewhat lower levels, and that from London less again, but taking into account the varying outputs, each area was estimated to contribute roughly 1 ton/year PCB contamination to the marine environment from sewage sludge. In the case of inland areas sludge can be disposed of by spreading over the land surface, and so offer a route to terrestrial food chains. The question of why it is that relatively low levels of PCB are found in man and his domesticated animals, posed in 1967[30] perhaps now has some answer.

Distribution in terrestrial and freshwater ecosystems has largely been assayed in terms of the avifauna because of the effects of organochlorine pesticides and almost certainly PCBs on some bird populations. The DDT/PCB ratio has been used by Risebrough *et al.* as an environmental index,[14,24,25] but when data is compared consideration must be made of the various biological factors, such as tissue distribution, species metabolism, migration, diet, health and age, which all affect residue concentrations. These authors have shown that the DDT/PCB ratio is low, below 2, near industrial areas, and increases with the distance away to become greater than 10 in remote regions. The geographical distribution in terrestrial and freshwater wildlife will be reviewed within the framework of these factors. Risebrough *et al.*[14] examined peregrine falcons and their prey, centring round the Bay of California. Falcons freshly migrated from the Arctic had low levels which increased with residence time in California; the levels in the Californian birds increased with age; and the indigenous adults showed the highest levels. The maximum values in Table I can be used to show the differences in tissue distribution.

Table I
Tissue distribution of DDT and PCB in peregrines and their prey

		Total DDT p.p.m.	BCP p.p.m.	DDT/PCB ratio
Peregrines:	unhatched egg	102	10·2	10·0
	muscle	100	98	1·0
	liver	77	57	1·3
	body fat	50	3·2	15·6
Prey:	egg	151	45	3·3
	whole body	59	9·8	5·5
	muscle	26·4	0·098	270

The same authors[24] determined chlorinated hydrocarbon residues in breeding pelicans and cormorants in lakes to the north-west of the Great Lakes (Table II). As the birds used the same food sources the clear differences in results demonstrated a species difference probably due to variation in residue levels in the migration areas, but possibly metabolic differences in terms of rates of excretion of organochlorines into the eggs may be involved. Birds have also been studied in Britain[12,19] and the extensive survey (599 samples) by Presst, Jefferies and Moore[19] illustrates some of the factors involved; firstly habitat and migration (Table III). Secondly; the eggs from marine birds from two colonies widely separated from one another demonstrates very convincingly (Table IV) specific differences related to migration habits. The guillemot spends the longest time in coastal waters, the Kittiwake the shortest time there, for breeding purposes only, and the Shag is essentially sedentary. The effect of health on relative and absolute levels is seen in the report by Bonner[21] on seal pup deaths. (Table V).

Table II
Chlorinated hydrocarbon residues in pelicans and cormorants

Eggs	pp'DDE p.p.m.	PCB p.p.m.	DDE/PCB ratio
Cormorant	10·4	8	1·3
Pelican	1·7	0·6	2·9
Fish (whole)	0·08	0·5	0·16

Table IV
Specific differences related to migration habits

	DDT/PCB ratio St Abbs Head	Galloway
Guillemot	0·233	0·250
Razorbill	0·500	0·455
Shag	0·625	0·727
Kittiwake	0·666	0·875

Table V
Effect of chlorinated hydrocarbon levels on seal pup health

		Total DDT p.p.m.	PCB p.p.m.	DDT/PCB ratio
Liver:	Cornish	2·4	46	0·05
	Northern (well fed)	0·6	4	0·15
	Northern (starving)	2·0	6	0·33
Blubber:	Cornish	11·1	160	0·07
	Northern (well fed)	9·8	34	0·29
	Northern (starving)	18·1	48	0·38

Table III
Habit and migration factors affecting chlorinated hydrocarbon residues

Bird class	Liver No samples	Liver Mean p.p.m.	Egg No samples	Egg Mean p.p.m.
(a) Terrestrial predators:				
static	75	4·5	115	1·7
partial migrants	49	4·3	6	2·0
migrants	9	1·0	5	1·0
(b) Freshwater (mostly static and mostly herons)	57	345	124	4·7
(c) Marine	4	2·5	96	4·0

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One of the most striking features of freshwater species is the extraordinarily high levels in herons at the head of a food chain where up to 900 p.p.m. have been found[19] in British material. As the British heron is sedentary, occurs nearly all over the country, and there are few migrants from the continent, this species might be expected to be a good geographical index of PCB distribution. Unfortunately nearly all the samples obtained in the survey of Presst *et al.*[19] came from the Midlands and South East so that no clear pattern emerged. However, the age factor is well illustrated by this species as the average liver levels for nestlings was found to be 5 p.p.m. while that for adults was 98 p.p.m. Diet is of course an important consideration, and means that different species from the same area can exhibit very different PCB concentrations. Thus, while birds feeding on insects have shown 0·1 p.p.m. (liver), raptors feeding on mammals yielded 0·50 p.p.m. It is necessary then, when comparing or interpreting data, to take into consideration all these factors. Terrestrial restriction of PCBs to near industrial areas would seem to be reinforced by the failure of Holden[26] to find them in freshwater fish in Scotland, and by Davis[31] who failed to find them in beetles, earthworms, slugs and grasshoppers of one agricultural area in England.

To what extent are PCBs found in human beings and foodstuffs? Jensen in 1966 first reported[7] the presence of PCBs in human hair, and later reports[12,23] indicated that levels in man and his foodstuffs were lower than those found in birds, and below 1 p.p.m. One exceptional case has been recorded,[22] however. Recently a very extensive survey of foodstuffs in Sweden sampled between 1967 and 1969, and analysed for organochlorine pesticides and PCB's, has been published.[1]

In a wide range of foods and food materials, PCB levels were found to be below 0·1 p.p.m. (90 per cent of 1400 samples) and comparable to DDT levels. The higher concentrations came from freshwater and marine fish, especially those with a high fat content. Another case where relatively high levels were found was with milk. Cows excrete little DDT in their milk and here all eleven samples were below 0·03 p.p.m. DDT and 0·1 p.p.m. PCB on whole milk. In human milk relatively more DDT is excreted and the average over 22 samples was 0·107 p.p.m. DDT and 0·16 p.p.m. PCB. Other relatively high samples are eggs and butter. (Table VI)

Table VI

	No	*Total DDT*	*PCB*	*DDT/PCB (p.p.m. whole material)*
Human milk	22	0·107	0·16	0·668
Hen. yolk	20	0·21	0·027	7·78
Butter	12	0·1	0·03	3·33

Toxicology

Soon after the beginnings of industrial use of the chlorinated naphthalenes and biphenyls early in this century, human exposure was seen to lead to skin eruptions (chloracne) and in animal inhalation and feeding experiments (1919), it was shown that liver damage occurred with the chlorinated naphthalenes. As a result of three human deaths, investigations were continued in the 1930s, and it was further demonstrated in work on rabbits that in the naphthalene series the higher chlorinated mixtures were the most toxic. The work was expanded by Bennett *et al.*[32,33] who also used mixtures including PCBs, and PCBs alone. A PCB containing 65 per cent chlorine was used, and later one containing 55 per cent. The substances were applied to rats by inhalation, subcutaneous injection, and oral means, and it was confirmed that the high chlorinated naphthalenes were more toxic than the lower ones, but that the PCB was more toxic still. Here, feeding at a lower rate (0·05g/rat every other day) produced extensive mortality, and as with the naphthalenes resulted in yellowing and enlargement of the livers. Liver damage caused by these compounds had not disappeared two months after dosing had ceased. This damage caused the animals to be extremely sensitive to damage by other compounds, *e.g.* carbon tetrachloride (used as a solvent for these mixtures), and much smaller doses than normal were rapidly fatal. Miller in 1944[34] experimented with a PCB containing 42 per cent chlorine and worked on guinea pigs, rats and rabbits, using feeding, subcutaneous, skin and corneal applications. He used single doses ranging from 69 to 690mg and courses totalling up to 1380mg (over up to 54d) and found that guinea pigs were the most sensitive, rabbits less so, and rats least sensitive in respect of liver damage. Damage was largely confined to the liver but in rats there was also hypertrophy of the spleen. The occurrence of hyaline bodies in the liver was confined to the rat. Von Oettingen in 1955[35] found that even 0·05g/d fed to rats caused liver damage. Skin lesions were similar to those found in man.

Man's exposure to these chlorinated hydrocarbons leads initially to dermatitis, then liver damage with resultant jaundice, and Greenburg,[36] reviewing the situation in 1939, concluded that there had probably been at least six human deaths from this cause. Bennett *et al.*[32] had measured the air concentration at thirty industrial plants and suggested a maximum allowable concentration (MAC) of 0·5mg/m^3 for tetrachlorinated and higher chlorinated naphthalenes, with the observation that these levels had certainly been grossly exceeded over the previous twenty years. This work resulted in increased awareness of the hazards from vapours of these compounds and elementary improvements in industrial conditions has largely eliminated this source of danger (but see Biros[22]). The American Conference of Government and Industrial Hygienists in 1953[37] laid down an MAC for the lower chlorinated PCBs of 1·0mg/m^3, and for the higher chlorinated ones ('Arochlors' 1254–) 0·5mg m^3. This appears to presume parallelism with the naphthalenes, and these figures are perpetuated in handbooks of industrial toxicology,[38] but recent papers[2,45] point to the reverse situation (see below). These MACs are to be compared with those for 'Aldrin' and 'Dieldrin' (0·25mg/m^3, tentative) and parathion (0·1mg/m^3). The oxidised chlorinated biphenyls are more toxic than the parent compounds.[38]

McLaughlin in 1963 published the results of toxicological studies on a number of chemicals which might be found in food[39] using a sophisticated method based on injection into fertile hens' eggs. 25,000 eggs were used over three years and strict controls set up. Among the compounds tested was the

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'Arochlor 1242' injected at 0·025 and 0·1ml giving a 0 and 5 per cent hatch as compared with over 95 per cent in the control group. Teratogenic effects were also noted in some of the chicks which did hatch. These results may be compared with those for 'Heptachlor', where 0·05 and 0·10ml of a 1 per cent solution in propylene glycol (non-toxic) gave a 75 and 65 per cent hatch respectively.

Flick *et al.*[40] endeavouring to identify the factor causing the chick oedema disease, following reports that the factor was highly chlorinated and that a plasticiser (PCB) used as a cage wire coating fed at 0·01 per cent or higher caused oedema in chicks, investigated the effect of 'Arochlor 1242'. Groups of twelve chicks were fed on diets containing 200 or 400 p.p.m. over a three week period, changes in weight being noted. Weight gain was lower with the 400 p.p.m. diet and indeed loss took place at the end of the period. Three chicks on this diet died. Here the hydropericardium volume was ten times larger than with the 200 p.p.m. level or the controls, the pancreas, kidneys and adrenals affected, and haemoglobin levels were lower. The PCB produced similar effects to the oedema factor, but was not so potent, and gas chromatography demonstrated that it was not the same.

Two groups of workers, Risebrough, Peakall *et al.* in the US[19,24,41–3] and Jefferies, Moore, Presst *et al.* in Great Britain,[19,44] have actively pursued the effects of organochlorine pesticides and PCBs on birds. The decline in certain bird populations, especially raptors, has been attributed to the presence of organchlorine pesticides, but it is also possible that PCBs have played a part in this, not only because of their intrinsic toxicity but because it has been shown (in insects,[2] and below) that there are synergistic effects between some PCBs and DDT and 'Dieldrin'. Lethal toxicity to the adult bird is not the only factor because it has been shown[14,41] that organochlorine pesticides at the order of one p.p.m. are effective in inducing steroid hydroxylases in avian liver, thus leading to lowered oestrogen levels and reduced reproductive capacity. Added to this DDE has an entirely different effect on the reproductive system, that of causing eggshell thinning, which causes added failures in reproduction. Teratogenic effects may also occur because of the excretion/concentration factor in the eggs. It is thus necessary to review the effects of the organochlorine pesticides on birds in conjunction with those of the PCBs.

Following work by Peakall,[41] which demonstrated that pigeons fed with DDT at 10 p.p.m. and/or 'Dieldrin' at 2 p.p.m. for one week had higher levels of testosterone or progesterone metabolites than controls, Risebrough, Peakall *et al.*[19] compared the effect of DDE, DDT and PCB ('Arochlor 1262') in increasing the levels of polar metabolites in oestradiol metabolism, again using pigeon liver isolates in vitro. Their results are given in Table VII.

Table VII

	Polar metabolites formed (mμ moles)
Control	29
DDE (40mg/kg)	76
DDT (40mg/kg)	93
PCB (20mg/kg)	160

This PCB has about five times the capacity to reduce oestradiol levels than DDT or DDE. The effect of a single dose of DDT may last for over two months. This work was extended to compare the effects of two different PCBs in kestrels, again using liver microsome isolates.[19] The kestrels were fed at 0·5 and 5·0 p.p.m. levels with 'Arochlor 1254' and 'Arochlor 1262', and the results were as in Table VIII.

Table VIII

	Polar metabolites (nmoles/g microsomal fraction)	
	'1254'	'1262'
Control	0	0
0·5 p.p.m.	13·65	19·05
5·0 p.p.m.	60·5	47·95

While these figures do not clearly indicate the relative activity of these PCBs, it has been shown that 'Dieldrin' and PCB are much more active in reducing oestrogen levels than either DDT or DDE.[42] That pesticides reduce oestrogen levels in mammals has also been demonstrated.

Presst, Jefferies and Moore[44] investigated the lethal effects of 'Arochlor 1254' on Bengalese finches. Food containing higher levels of PCB (440 p.p.m.) was refused by the birds (100 per cent mortality not occurring) but it was still possible to calculate the LD_{50} as 253·6mg/kg/d. Seven out of the 38 birds died. Enlarged kidneys was the main feature, with in two cases increased volume of pericardial fluid. While there was a correlation between dose rates and liver concentrations, there was also considerable variation in levels (3-697 p.p.m.). Whether a particular concentration will be fatal will depend, as with organochlorine pesticides, on the condition of an animal. Considerable storage in fat is possible and until this reservoir is saturated, concentrations will probably not rise to lethal concentrations in susceptible organs. So danger can arise in times of stress (*e.g.* starvation or with the breeding cycle) when the fat reserves are mobilised releasing large quantities of PCB or whatever. The liver by itself in these birds may thus not be a good index because the liver fat reserves may vary considerably and will themselves be mobilised ultimately. The authors therefore suggest the brain/liver percentage as a useful index, being high in those birds that died (83·7 per cent and low in the survivors (26·4 per cent). The LD_{50} for DDT is some thirteen times lower than for this PCB, but consideration has to be given to the fact that while the DDT mortality curve is steep (no deaths at 1000 and all dead at 1200mg/kg/d), the PCB curve is shallow, so that at low levels PCB may be more toxic than DDT. The authors conclude on the basis of this experimental work, and reasonably assuming that no drastic change in useage of PCBs is likely to have taken place over the past two decades, that few bird deaths have been caused by PCBs. However they state, without citing evidence, that the PCBs do not vary markedly in toxicity and this is at variance with recent work[2,45,46] (see below), the PCB used here being of relatively low toxicity. Additionally the authors do not consider the possibility of synergistic effects.[2] It was recognised that the position with sublethal effects may be different.

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Very little work[20,47–9] has been done on the effect of PCBs (and terphenyls) on aquatic organisms, despite the fact that it is known that some marine crustacea and fish are very sensitive to organochlorine pesticide residues.[44] For example *Crangon vulgaris* (brown shrimp) has an LC_{50}/48h/15 c for DDT of 0.003 – 0·1 p.p.m., and levels as low as 0·0001 p.p.m. may be detrimental to some species of mollusc. One difficulty in the study of toxicity to aquatic organisms is the extremely low solubility of these compounds in water and the difficulty of obtaining sufficiently stable emulsions.[47–9] The use of solubilising agents, of low toxicity, overcomes this. Zitko,[49] using 'Corexit 7664' to solubilise 'Arochlor 1221' and 'Arochlor 1254', carried out 192 hour tests with these compounds using Atlantic salmon parr. He found that concentrations above 2mg/l were lethal. Wildish[48] using 'Arochlor 1254' in emulsions, or solubilising with 'Corexit 7664', carried out 30d tests with *Gammarus oceanicus* and found lethal threshold values of 0·001 to 0·01mg/l ('Corexit') and 0·01 to 0·1mg/l (emulsions). Guthrie *et al.*[47] tested mixtures containing chlorinated terphenyls and their oxidation products and found lethal threshold values of about 5mg/l and pertinently that low oxygen levels in the water enhanced the toxicity. Duke *et al.*[20] who monitored PCB levels in biota and sediment in the Escambia Bay area of Florida as a result of an industrial leakage of PCB into the Escambia River, conducted both acute toxicity tests and 20d bioassays. 48h tests on pinfish and juvenile shrimps at the 100 p.p.b. level was not toxic to the fish but caused 100 per cent mortality in the shrimps. Oysters received a 96h test and the effect was measured in terms of arrest of shell growth. Here 1 p.p.b. resulted in 19 per cent decrease in shell growth and 100 p.p.b. 100 per cent. Resultant whole body concentrations for the three organisms were 17, 3·9 and 33 p.p.m. respectively. It should be noted that the oysters when replaced in PCB free water for three weeks recovered their normal growth rates. In the 20d assays using a concentration of 5 p.p.b., a 72 per cent mortality occurred in juvenile shrimps and 5 per cent in juvenile crabs. It would appear then from all this work that some crustacea and molluscs are as sensitive to PCBs as they are to organochlorine pesticides.

Some toxicological work has been effected using insects.[2,46] Moriarty[2] dosed fourth instar nymphs of *Chorthippus brunneus* (grasshopper) topically with 'Arochlor 1254' at 12·5, 50 or 200μg levels, but found that definite mortality was only associated with the 200μg group plus a possible sub-lethal effect associated with moulting (fat mobilisation). The maximum amounts of PCB in individuals of the latter group were 14μg (male) and 8μg (female). The work of Lichtenstein, however, is much more revealing as to the relative toxicity of the various PCBs and the synergistic effects with DDT and 'Dieldrin'. Groups of 50 *Drosophila melanogaster* were placed in contact with vessels coated with a layer of the substance or mixture of substances, and groups of 15 *Musca domestica* were dosed topically. Tests of the individual substances showed that 'Dieldrin' was some 25-30 times more toxic than DDT, and 1-8000 times more toxic than the PCBs. The higher chlorinated biphenyls, and the terphenyls, gave rise to no mortality in 0–24 or 48h tests, but toxicity increased with decrease in chlorine content in the lower half of the series. These results raise doubts as to the relevance of the many papers which have used 'Arochlor 1254', which here has little or no effect under these conditions. The synergistic effects with 'Dieldrin' were positive with some of lower 'Arochlors' and uniformly positive with *pp'*DDT up to 'Arochlor 1254'. However, with the higher chlorinated PCBs and PCTs a reduction of toxicity was observed. While it is improper to transpose results from one phylum to another and short term toxicity may have little bearing on long term effects, nevertheless this paper is a strong indication that considerable care should be taken in planning these toxicological studies.

Work using mammals is equally sparse. A preliminary study by Sabotka,[50] using 'Arochlor 1254' and 'Arochlor 1260' or DDT in single doses of 1-10mg/kg ('Arochlor's) or 0·25mg/kg (DDT) carried out behaviour tests on mice over periods of 4 to 24h after administration. There is a clear difference between the two groups of substances, DDT probably attenuating and PCBs probably enhancing central inhibitory systems. The paper by Bitman and Cecil[45] surveying the oestrogenic activity of DDT analogues and PCBs in terms of the 18h glycogen response of immature rat uterus assumes considerable importance in relation to a lot of the work cited above. The stereochemical requirements are quite strict, and in general for diphenyl-methane and -ethane, and triphenylmethane compounds activity is restricted to those where either the *pp'* positions are vacant or filled by hydroxy or methoxy groups, being inactive where halogen or alkyl groups are present. So the *pp'* compounds perthane, kelthane, DDE, DDD and methoxychlor are inactive. However, *pp'*DDT is active and the *op'*isomer some sixteen times more so, while of the others mentioned only *op'*DDE gains a response. 'Arochlor's 1221-48' are about half as active at *pp'*DDT, but the higher members are inactive. (*cf.* Lichtenstein). In perspective though these compounds only have something like 10^{-3} times the activity of natural oestrogens, but this fits well with the observed pattern of steroid hydroxylase induction in birds, where DDT and PCB are very active but DDE is less so. Perhaps another significant point is that the *oo'* and *pp'*biphenols have an equivalent oestrogenic activity. The lower chlorinated PCBs could be degraded in the atmosphere as has been shown by laboratory experiments where the greatest diminution in one gas chromatograph peak was found where PCBs on an aqueous surface were subject to UV irradiation.[51] At the same time it has been observed[12,17,51] that the lower chlorinated PCBs tend to be absent in wildlife samples, particularly in birds, and it may be speculated to what degree there may be differential excretion or *in vivo* breakdown, and what effect this has. A recent study by Vos *et al.*[52] reveals another important aspect. They discovered that two out of three 60 per cent chlorinated PCBs contained highly toxic oxygenated impurities. These two commercial preparations ('Phenochlor DP6', 'Clophen A60') when injected into hens' eggs at the 3·5mg level caused almost 100 per cent mortality whereas the third sample ('Arochlor 1260') gave a relatively low mortality. A fraction containing no PCBs was isolated from one of them by 'Florisil' column chromatography and shown to be toxic.

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Gas chromatography and mass spectra showed this fraction to contain tetrachloro and pentachloro-dibenzofurans which were, however, masked by hexachloronaphthalene and heptachlorobiphenyl respectively, thus limiting the accuracy of quantification. The pentachlorodibenzofuran was estimated to be at about the 5 and 20 p.p.m. level in the two samples. That PCB exerted a similar effect to that of the chick oedema factor (CEF) had been noted previously,[40] and that toxicological studies using PCBs had produced variable results. 1,2,3,7,8,9,-hexachloro dibenzodioxin has now been identified as one of the oedema factors[52] and it may be recalled that the toxic factor in the weedkiller 2,3,5-T was shown to be a tetrachloro dioxin.[53] In fact these chlorinated dioxins and dibenzofurans are very highly toxic groups of compounds. 0·2μg pentachlorodibenzofuran is needed to cause 100 per cent mortality in hen eggs as compared to 0·05μg of hexachlorodibenzo-*p*-dioxin. The occurrence of the dibenzofurans in PCBs is associated with the use of sodium hydroxide in the distillation of erude PCB. Pentachlorophenol (PCP) and 2,3,4,6- tetrachlorophenol are possible precursors of CEF and have been found to be present in toxic oleic acids.[54]

It is evident then that other compounds besides the PCBs themselves should be sought for in wildlife and that individual compounds as well as commercial preparations should be used in toxicological studies. Unfortunately the individual PCB compounds are not readily available.

Holden and Marsden[55] have already noted the presence of unidentified polar compounds in wildlife extracts. It is to be hoped that the current FDA programme[56] on the toxicology of PCBs takes into account all these factors.

Analysis

PCBs are extracted together with chlorinated hydrocarbon pesticides in routine residue analyses,[57] and being complex mixtures of compounds with GC retention times on non-polar columns almost identical to a number of pesticides, notably of the DDT group, present an analytical problem which is not completely soluble by gas chromatographic means alone. Chemical conversion of the pesticides, leaving the PCBs almost unchanged, provides a partial but not completely satisfactory solution, but group separation is fundamentally sounder, this of course providing additional evidence of identity. Both thin layer chromatography and column chromatography have afforded quite good partial separation of PCBs from pesticides and the best scheme[58] claims to leave only 'Aldrin' with the PCBs, with which there is very little interference by the PCBs. A number of workers[18,22,59,60] have confirmed the presence of PCBs in living tissues by means of mass spectrometry, but apart from this means the quantification of PCBs presents severe problems because of the large number of compounds involved and that the individual compounds are not readily available, so that most authors have only been able to calculate approximate figures.

Gas chromatography retention data for PCBs is now adequately documented[12,16,18,26,50,61] (and also[1,2,13,14,20,24,25,30,39]) and on non-polar columns the main region of interference of PCBs and organochlorine pesticide residues with one another may vary from the point of emergence of *pp'*DDE that of *pp'*DDT. PCBs may continue to emerge with retention times up to four or five times that of DDT. It has been noted in this laboratory that response to PCBs relative to organochlorine pesticides can be small on some columns, and from the viewpoint of determining pesticide residues, can ameliorate the situation. However, using more polar columns, *e.g.* 'QF-1' or 'XE-60',[12,23,59,62] PCBs emerge largely prior to the DDT group, which allows simple quantification of the latter, but may still leave an unsatisfactorily complex chromatogram. Improved resolution of PCB compounds has been claimed[63] using novel stationary phases.

One alternative has been to alter the retention time of pesticides by derivatisation, leaving the PCBs intact, and of course aiding the identification of the pesticides. Jensen[59] used a mild nitration technique adapted from Erro *et al.* and originating from the Schechter-Haller method, claimed that the PCBs remained unaltered while members of the DDT group were effectively removed, although other organochlorines (*e.g.* 'Toxaphene', heptachlor epoxide, lindane and BHC) are not removed. This scheme (1:1 HNO_3/H_2SO_4 for 5min at 0°c) was followed by Risebrough *et al.*,[14,50] but Reynolds[61] found that DDT derivative peaks ultimately emerged on the chromatogram making for an unprofitably long analysis time. Moreover, some lower chlorinated PCBs appeared to have been affected by the procedure. Saponification with alcoholic potash[13,14,50] leads to the dehydrochlorination of *pp'*DDT, *pp'*TDE, and 'Toxaphene', with the derivative peaks appearing earlier in the chromatogram, and the PCBs reportedly unaffected. BHC is destroyed by this procedure. PCBs have also been differentiated from DDT using carbon-skeleton chromatography.[64]

Separation prior to gas chromatography is to be preferred not only because simpler chromatograms can be obtained but because this will give nearly as much evidence as to identity as derivation. Thin layer chromatography, reverse phase paper chromatography[12] and column chromatography afford means of obtaining partial separations of organochlorine pesticides from PCBs and fractions can easily be obtained where only 'Aldrin', 'Heptachlor', and *pp'*DDE remain with the PCBs. The former two are not very important in respect of the gas chromatography separation because there is usually little interference between them and PCBs, but *pp'*DDE can emerge with the major PCB peaks using non-polar columns. Thin layer chromatography has given this sort of separation using silica gel[15] and alumina,[1,2] and in column chromatography Holden and Marsden[26,45] claimed good separation of PCBs using either alumina, silica gel, or both in series with long narrow columns. Reynolds[61] documented a separation with 'Florisil', while Bevenue and Ogata[62] commented on their failure to reproduce this work in terms of pesticide separations only, and noted the differences in activity between 'regular' and 'PR Florisil'. This reviewer has found no difficulty in obtaining this separation using 'Florisil'. Armour and Burke[58] in a carefully documented paper showed that it was possible to carry out a separation leaving only 'Aldrin' with the PCBs. For this they used silicic acid partly deactivated with 3 per cent water mixed with celite and eluted the

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PCBs ('Arochlor's 1254 and 1260') with petroleum ether. 'Arochlor 1254' needed the larger volume of eluant and the separation was more critical. The cautionary note is added that any trace of polar solvent in the sample will make the separation impossible and *pp'*DDE will emerge with the PCBs. Also, the separation is only possible provided that the column (20+5g) is not over loaded (*i.e.* not exceeding 20μg PCB). This then solves the main separation problem leaving on non-polar gas chromatography columns only a small PCB peak which may interfere with the estimation of 'Aldrin'. These authors have also documented the separation of chlorinated naphthalenes.[65]

Confirmation of identity has been achieved by combined gas chromatography/mass spectrometry,[16,22,59,60] but generally quantification is not simple because of the large number of compounds involved and because these are not readily available individually for reference purposes. If it can be assumed that a fraction contains only PCBs (*i.e.* in a column chromatography or gas chromatography effluent) and that the composition on a gas chromatography trace conforms to that of a commercial mixture, then microcoulometric estimation will give an answer. Jensen *et al.*,[17] using a combination of mass spectrometry, ECGC and microcoulometry obtained results which were only accurate to about a factor of two. With the same assumptions electron capture detection can be used, referring to commercial PCB mixtures[26] either by estimating the total peak area[19,59,63] or basing the calculation on one[1,16,19] or more[20] suitable peaks. *pp'*DDE or 'Dieldrin' may be used as an internal standard.[16,50] One empirical approach calculated one PCB peak as *pp'*DDT and multiplied by a factor of ten to obtain a result;[24] another[66] took the total area:weight ratio to be equivalent to that for DDE; and another [19,50] assumed the response of each peak to be equivalent to DDE and applied a correction factor from the use of microcoulometric detection (the authors did not have permanent access to a microcoulometric detector). Gregory[67] studying the electron capture coefficients of mono- and dichloro- and methylbiphenyls showed that there is over a twentyfold variation between the chloro compounds. Zitko[49] determined the concentrations of PCB in fresh and salt water by means of ultraviolet spectrophotometry and fluorescence. While the lower chlorinated PCBs can be prepared easily[68] and only simple mixtures formed when using the Ullmann reaction, the higher members are more difficult to make singly.

Discussion

The decline of certain bird populations was associated with the use of 'Dieldrin' seed dressings and has been attributed to lethal toxic effects. With increasing awareness of these dangers, better monitoring and usage control, at least in developed countries, lethal toxic effects are progressively less likely to occur. Rather, the significance of PCBs (and pesticides) to man and other organisms must now be seen in the context of sub-lethal effects having long term influence on populations through the media of reproductive processes (reduction in fertility, delays in breeding), teratogenic and carcinogenic effects on both the parent and later generations. LD_{50} tests, even over a long period, are irrelevant from this viewpoint. Certainly it is true that severe mammalian liver damage and death can result from high PCB levels but these are only reached in a few foodchain heads, and the normal levels found in man are not of this order. To be set against this, however, is the finding (in LD_{50} terms) that while with DDT there appears to be a definite 'no effect' level this is apparently not true for PCB. Precisely what compounds are responsible for the toxicity of PCBs is not yet clear, but it seems certain that it concerns the lower chlorinated compounds and probably the oxidation products *in vivo* or *in vitro*. It also emerges that impurities in some commercial mixtures are certainly highly toxic, and if the fact of synergistic effects, though demonstrated as yet in one area only, is taken into account then the picture becomes a very complicated one. Clearly it is extremely difficult to carry out strictly controlled toxicological studies at a feeding level of the order of 1 p.p.m. over a long period, and in terms of multigeneration tests. The need for multigeneration tests may be related to DDT, where, despite many years study, it has only recently been indicated that, with only the original parents dosed, carcinogenic effects may occur in later generations (6th generation mice). DDT may again be used to illustrate the sort of effect which may be looked for. In California, Oregon and Washington, genetic changes in the fruit fly population have been shown to result from heavy spraying programmes in these areas, and in the latter two states were tied to two specific forest spraying programmes. The pattern of these genetic changes can also be correlated with spread by winds over long distances. With PCBs the major dispersal medium is probably water, but otherwise there are no essential differences in the situation. The PCBs are spread all over the continental shelves which are fed by industrial effluents.

The toxicology of the weedkiller 2,4,5-T[53] and of mercury residues[69] has recently come to the fore and they present some parallels with the PCBs. While in contrast to PCBs there is a definite, but small, contribution to agricultural pollution, by mercury from seed dressings, the industrial contribution must lead to a similar marine distribution to PCBs, although as yet very little is known about this area. Mercury, in contrast to PCB, is only cumulative in living tissue to a limited extent, and there is a definite natural contribution from the sea floor. The massive aerial use of the weedkiller 2,4,5-T as a defoliant by the Americans resulted in teratological as well as direct toxic effects due to traces of a chlorinated dibenzodioxin. It is as well that PCBs are not used in such fashion because it has now been shown that some commercial preparations contain traces of chlorinated dibenzofurans which are also highly toxic.

Water pollution is receiving considerable attention within the traditional framework of oxygen deficiency, reduced and oxidised nitrogen, temperature and so on, but the type of pollution problem presented by PCBs, DDT and so on is largely ignored. In a recent official report on toxic chemicals, which recommends further work to appraise the situation in fresh, estuarine and coastal waters, there appears to be a large conceptual gap. Commenting on the presence of DDT in raw sewage, this is dismissed as a significant source of riverine pollution because DDT is solid attached and largely remains in the sewage sludge, but what happens to the sludge is blithely ignored. The ironic situation is of

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course that the sludge is commonly dumped in estuaries or even spread out over the land so that pollutants such as the PCBs are recirculated through the food chains.

These are but a few aspects of environmental pollution which can ultimately lead to man's own detriment. In many cases the users of pollutant materials, industrial or domestic, have no conception of their own insidious contribution to pollution, and in the case of the very useful PCBs it is very difficult to see how their disposal can be controlled to minimise pollution. From the point of view of restricting types of use to those least likely to result in pollution, one producer, Monsanto, has made a very responsible move[70] in this direction, but as PCB manufacture is no longer covered by patents this may not be effective. It is obvious that with their ubiquitous presence the effects of PCBs and associated compounds must be investigated with great thoroughness. It is not only to preserve bird populations or other wildlife but to prevent long term changes in man himself that such searches are directed.

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