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Chlorinated Hydrocarbons in British Wildlife

Polychlorobiphenyl compounds have been detected in British wildlife. In birds' livers and eggs they are often in greater quantities than organochlorine pesticide residues. Polychlorobiphenyls are known to be toxic, and their detection in wildlife raises the question of the adverse effect they may have.

by

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WHEN wild birds' eggs or the livers of British wildlife are examined by gas-liquid chromatography (GLC) using electron-capture detection, peaks corresponding to such organochlorine pesticides as BHC, dieldrin and DDT, and their metabolites and breakdown products, often appear on the resultant chromatograms. These peaks sometimes represent amounts which are significant from a toxicological point of view, but the sensitivity of modern instruments also enables completely insignificant amounts to be detected. Analysts concerned with the determination of organochlorine pesticides in wildlife, however, have long been aware that it is not unusual for an additional series of as many as ten peaks, corresponding to unidentified compounds, also to appear on these chromatograms[1-4]. Little is known of the structure and origin of these compounds, but Roburn[1] established that they are organochlorine in nature, with a substantial chlorine content, and consequently possess electron-capturing properties.

On silicone or 'Apiezon' GLC columns, the series of peaks for these compounds normally begins at the point at which *pp'*-TDE and *pp'*-DDT are emerging from the columns and extends, in terms of retention times, to four or five times that of *pp'*-DDT, which is the last of the commonly occurring pesticides to emerge.

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Before examination by GLC, samples have normally been subjected to a fairly rigorous clean-up procedure, such as that of de Faubert Maunder *et al.*[5], which includes a dimethylformamide–hexane partition and passage through a column of prepared alumina. Because these unknown compounds are similar in nature and properties to the organochlorine pesticides, they also pass through this and similar clean-up procedures; consequently, they often interfere seriously with determinations of *pp'*-TDE and *pp'*-DDT in wildlife because of overlapping of the chromatographic peaks. Suitable procedures have been devised to overcome this difficulty of interfering peaks. One method is to effect a better resolution of these particular peaks by choice of a suitable stationary phase for GLC[6]. Another method, which also demonstrates the presence of these compounds in the sample, is the use of a preliminary thin-layer chromatographic treatment[7]. The cleaned-up extracts are examined on silica gel chromatoplates using a 1 per cent solution of acetone in hexane as mobile phase[3]. Spots corresponding to the compounds can then be found at R_F values between 0·8 and 1·0, whereas the commonly occurring organochlorine pesticides and their metabolites produce spots of lower R_F values. A similar separation can also be shown with reverse phase paper chromatography[8] whereby the unknown compounds move only a very short distance from the base line compared with the pesticides and their metabolites.

Gas-liquid chromatography using dual channel detection systems with separate electron-capture and flame-ionization detectors has also been used to demonstrate the presence of large amounts of weakly electron-capturing material, in addition to known pesticides residue, in the eggs of oyster catchers (*Haematopus ostralegus*)[9].

Experience in this laboratory has shown that these compounds occur most frequently and in the largest proportions in the livers, fat and eggs of birds, particularly terrestrial predators such as sparrowhawks (*Accipiter nisus*) and kestrels (*Falco tinnunculus*) and marine feeders such as guillemots (*Uria aalge*) and kittiwakes (*Rissa tridactyla*); they also commonly occur in freshwater fish. We have detected small amounts in human and animal fat samples, but less frequently and in much smaller proportions than in avian samples.

A similar series of chromatographic peaks has been observed by Holden[10,11] when examining extracts of seals and fish taken in Scottish waters. He found that the peaks given by the extracts of fresh and sea-water fish represented fairly low concentrations, but those given by extracts of seal blubber corresponded to much larger proportions, of the order found by this laboratory in birds' eggs.

Despite considerable curiosity about the identity of these compounds, little progress has been made in identifying them, but there has long been a general supposition that

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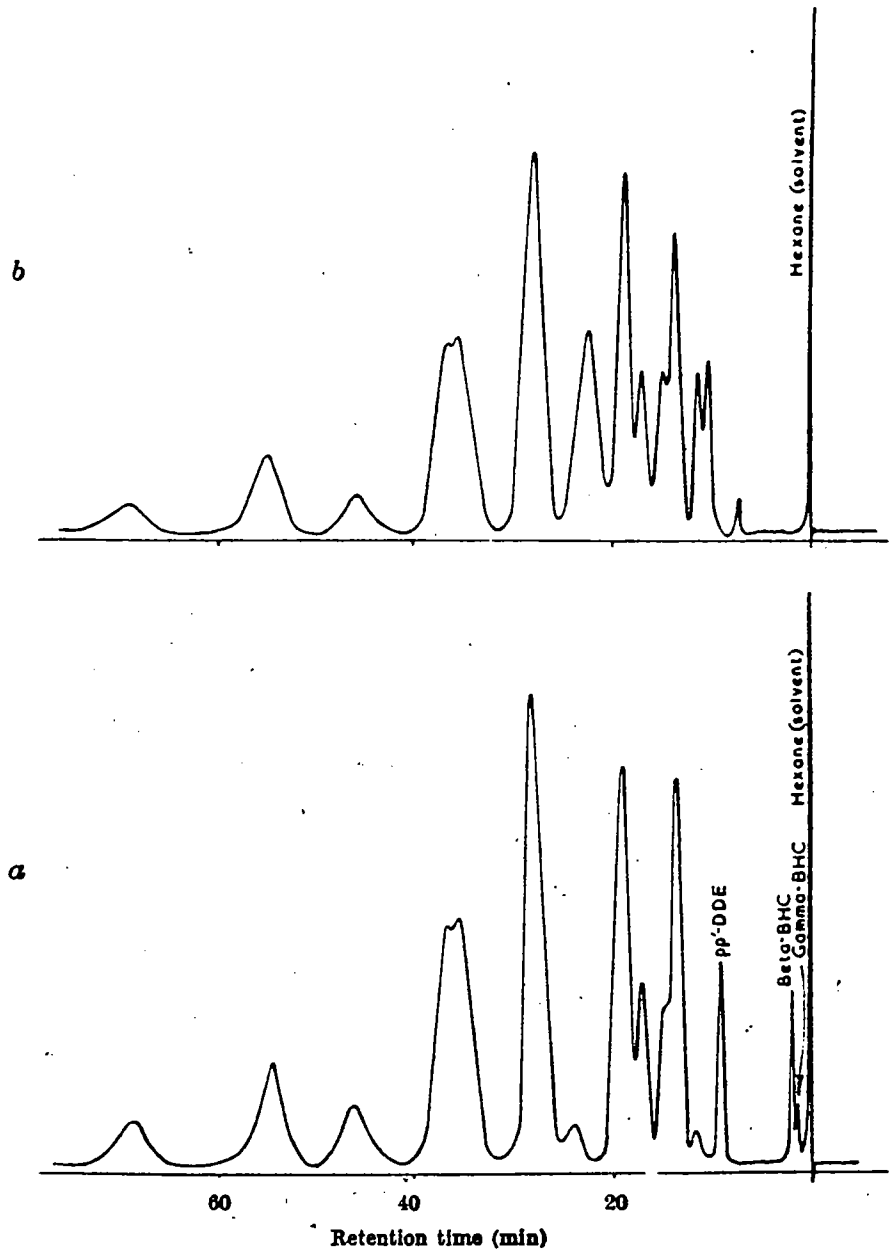

Fig. 1. Gas-liquid chromatogram of (*a*) extract of kestrel liver and (*b*) a commercial polychlorobiphenyl resin.

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.

Jensen, however, succeeded in identifying a series of such peaks as corresponding to polychlorobiphenyl compounds, in 200 pike taken in different parts of Sweden, and in an eagle[12]. We have now been able to show that

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Table 1. RELATIVE RETENTION TIMES ON THREE DIFFERENT GLC COLUMNS
(Excluding times for known pesticides)

Silicone *GE–SE* 52 column		(Dieldrin = 1·00) 'Apiezon *L*' column		Cyanosilicone *GE–XE* 60 column	
KLE	PCB	KLE	PCB	KLE	PCB
1·42	1·42	2·31	2·30	1·21	1·21
1·73	1·73	2·67	2·67	1·34	1·33
1·91	1·90	3·04	3·05	2·20	2·19
2·81	2·81	5·06	5·06	2·62	2·60
3·59	3·58	5·96	5·95	2·97	2·96
3·71	3·70	6·57	6·56	3·60	3·60
4·68	4·68			4·63	4·63
5·62	5·62			5·11	5·11
7·10	7·11				

KLE, Kestrel liver extract; PCB, polychlorobiphenyl resin.

the long retention time compounds occurring in British wildlife are also polychlorobiphenyl compounds.

Polychlorinated biphenyl has been in common commercial use for some time as a plasticizer in paints, resins and plastics. It also has electrical insulating properties and can be used for a number of other protective purposes. Commercial preparations of this material are usually graded according to the degree of chlorination, but all of them are substantially complex mixtures of polychlorobiphenyl compounds that could be expected to resemble the organochlorine pesticides in their chemical and physical properties. In the prescribed extraction and GLC conditions, these mixtures produce a series of chromatographic peaks of the type in question.

The compounds occurring in British wildlife correspond to the more highly chlorinated biphenyl compounds rather than the more lightly chlorinated types. These highly chlorinated biphenyls also occur to some extent in the analogous polychloroterphenyl preparations which have commercial uses similar to those of polychlorobiphenyl. It is possible that some of the compounds with very long retention times occurring in British wildlife are lightly chlorinated polychloroterphenyl compounds.

Fig. 1*a* shows the gas-liquid chromatogram of an extract from a kestrel liver. The peaks corresponding to *pp'*-DDE (0·43 ng), beta-BHC (0·33 ng) and gamma-BHC (0·03 ng) are labelled. The other peaks form the series referred to above and until now would have been regarded simply as "unknown organochlorine compounds not corresponding to any known pesticide or its metabolites". Fig. 1*b* shows the analogous chromatogram of a commercial polychlorobiphenyl resin. So far as retention times are concerned and apart from the pesticide peaks, there is exact matching for all but a few peaks in the two chromatograms.

The chromatograms shown were obtained by use of a silicone gum (*GE–SE* 52) column, but this precise correspondence between the retention times of the peaks for the unknown compounds and those from the polychlorobiphenyl resin has also been demonstrated on 'Apiezon *L*'

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and cyanosilicone columns. The actual retention times recorded for the peaks on these three types of column are given in Table 1.

Further confirmation as to the identity of these compounds has been obtained by a study of their behaviour on thin-layer chromatoplates, alumina and silica gel absorption columns and reverse phase paper chromatograms. In addition, these compounds have been shown to resemble the polychlorobiphenyl compounds in their chemical inertness. Thus they are not easily modified by simple chemical reactions to produce readily identifiable shifts in their retention times on GLC as can be done, for instance, to *pp'*-DDT by hydrolysing it to *pp'*-DDE or to aldrin by oxidizing it to dieldrin.

A number of livers and eggs taken from birds in the British Isles or their coastal waters have been examined in this laboratory with results similar to those obtained for the kestrel liver referred to earlier. While the identification of these compounds in British wildlife may solve the mystery of these peaks on GLC chromatograms, it raises, however, many other questions. Why, for example, do these compounds seem to be accumulated, most frequently and in the largest proportions, in certain types of wildlife such as birds, seals and fish, and only at the most in very small proportions in man or his domestic animals such as cows or sheep? In this respect they seem to differ markedly from the organochlorine pesticides which seem to have invaded nearly all aspects of our environment and to which they bear a strong resemblance chemically and physically.

Some of the samples of birds examined in this laboratory have seemed to contain higher proportions of polychlorobiphenyl compounds than organochlorine pesticides. For example, the peaks on the chromatogram for the kestrel liver shown in Fig. 1 were estimated to be equivalent to about 12 ng of polychlorobiphenyl compounds. This must be contrasted against the organochlorine pesticide residues listed above. These total only about 0·8 ng of which 0·33 ng is beta-BHC, an isomer of BHC which is generally regarded as non-toxic to wildlife.

Great concern has often been expressed by conservationists about the effects of pesticides on wildlife, particularly birds. The identification of these other organochlorine compounds in wildlife prompts enquiries as to the possible toxic effects they may be having. That these compounds are toxic is generally agreed[13,14]; the polychlorobiphenyls are in fact regarded as an industrial hazard and threshold limits for them in air have been advised[15].

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