Polychlorobiphenyls (PCB's) and their Interference with Pesticide Residue Analysis

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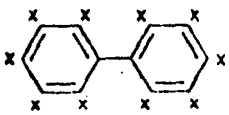
Introduction

The PCB's  (X indicating the possible chlorine positions) were studied as early as 1881 (1) and by 1930 (2) were in wide use. They are known to be quite toxic, especially to liver cells. As early as 1935, Jones and Aldom (3) reported that men employed in the production of PCB's developed acne-type skin eruptions. Three years later, Greenburg and coworkers (4) reported that PCB's and polychlorinated naphthalenes were responsible for the deaths of three workers.

Residue chemists, especially in Europe, have recently become interested in these PCB's as well as the polychlorinated triphenyls, naphthalenes, terpenes, and other related compounds, since

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Jensen (5) in Sweden reported their presence in wildlife tissues about two years ago.

The PCB's and related compounds (although in the rest of this paper reference will be made to the PCB's only, the other related compounds are also quite important) have very numerous and important industrial uses, but are not used as pesticides. Because of their similarities in structure and properties to the DDT pesticide group, the PCB's, if present, are carried through the usual pesticide extraction and screening procedures, and since they possess electron absorbing properties, will interfere with gas liquid chromatographic electron capture (GLC-EC) analysis of the organochlorine compounds.

Before any discussion of the type of interference encountered it would be appropriate to mention briefly some of the properties and uses of the PCB's.

They are produced and marketed under a number of commercial trade names e.g. 'Aroclor', 'Clophen A50', etc. The PCB's are available as liquids, resins, or solids; insoluble in water; thermoplastic; non-drying; stable on long heating at 150°C.; electrically non-conducting; not affected by boiling with NaOH solution; do not support combustion when alone above 260°C.; are easily soluble in most common organic solvents and drying oils.

They are used in protective coatings, as plasticizers and extenders, as sealers in water-proofing compounds and putty, in asphaltic materials, printing inks, waxes, and synthetic

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adhesives.

Liquid PCB's are used as dielectrics, as hydraulic fluids, in thermostats, in cutting oils, as extreme pressure lubricants, as grinding fluids, and as heat transfer media.

Solid PCB's are used to impregnate carbon resistors, as sealers or impregnating agents for electrical apparatus.

Obviously, the stability of these compounds makes them extremely useful and versatile for a great number of applications. Considering their stability - not affected by boiling with H_2SO_4 or nitric acid, not metabolized in living organisms, and nonflammable if containing more than four chlorine groups, it is as Jensen (6) pointed out, difficult to explain how these compounds find their way into living organisms.

However, with the numerous applications, it is not inconceivable that fish and other wildlife could be polluted as a result of the flushing of wastes into rivers, lakes, etc. It is also possible that contamination could proceed via the atmosphere when wastes containing these compounds are burnt.

However, a third and more likely source is the possibility that some companies might be using PCB's in pesticide formulation to increase the kill-life of insecticides. The Monsanto Company, which manufactures the Aroclors, stated back in 1965 (7) that the Aroclors can "trap" and hold more volatile ingredients making volatile insecticides and repellents last longer in residual activity. The most pronounced effect for increasing the

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kill-life of insecticides was obtained with lindane, chlordane, and benzene hexachloride (BHC). A ten-fold effectiveness for lindane was reported by the U.S.D.A. by including 5-25% PCB's in the formulation. Attempts to determine whether this idea had been put into practice by some companies have so far been unsuccessful. But there is no doubt that, if the PCB's are being used in pesticide formulation, then this would certainly explain their presence in wildlife tissues and other samples.

Jensen (6) has used a nitration procedure in order to differentiate the PCB's from the pesticide residues. He treated the cleaned-up extract with a mixture of concentrated HNO_3 and concentrated H_2SO_4 (1:1) for 5 min. at 0°C. After the addition of crushed ice, he extracted the reaction mixture with hexane and reinjected the extract. He states that the method should leave PCB's, lindane, and BHC unaffected. Our attempts to repeat this reaction have not been fully successful. There appears to be some loss of the more volatile (early emerging) PCB's, heptachlor epoxide is not affected, and peaks with longer retention times appear.

Although Jensen did not elaborate as to the fate of the pesticides, we have demonstrated that apparently, nitration does occur. This was shown for DDT when a large peak (probably due to the tetranitro derivative) appeared on the chromatogram about 7 hours after injection of the nitrated extract.

Of course, this reaction is a modification of the old

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Schechter-Haller (8) DDT method in which more drastic conditions (fuming HNO_3 and concentrated H_2SO_4 with heating on steam bath) were used to ensure oxidation and removal of interfering biological materials. The nitrated pesticides were extracted with ether and a colorimetric method was used in the final determinative step.

Erro et al. used this technique to determine toxaphene in the presence of DDT, on the basis that the chromatographic pattern of toxaphene is not affected by nitration while the nitrated DDT does not chromatograph under the specified conditions.

Obviously, nitration does not appear to be the answer for complex mixtures of pesticides and PCB's since some pesticides (lindane, BHC, toxaphene, 'Strobane', etc.) apparently will not nitrate while some of the PCB's might nitrate. Although we have not used Jensen's column packing (the liquid phase SF-96 is a methyl silicone), it is impossible to avoid complication and interference from the nitro derivatives formed, especially when the pesticides are present in large amounts.

There are three main reasons why we prefer an approach different from Jensen's:

1. It is preferable to separate the two groups rather than destroying one, especially when it is the pesticides that are being destroyed.

2. The nitration approach tends to complicate the interpretation of the chromatograms, since the nitro derivatives

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possess greater electron absorbing power and with their longer retention times, should emerge and interfere with subsequent injections.

3. We have been unable to repeat Jensen's clear-cut differentiation, apparently partly because of the nitration of some of the PCB's.

Interference of PCB's

We have attempted a more ideal approach to differentiate the two groups by separation followed by the separate analysis of each group.

The GLC work was carried out under the following conditions:

Gas Chromatograph: Varian Model 1200, fitted with tritium-electron capture detector; column: glass, spiral, 6' x 1/[illegible], packed with 6% QF-1 and 4% SE-30 on Chromosorb W (AW). No. of theoretical plates for DDT = 2227.

Operating Conditions: Column temperature 190°C.; injector temperature 245°C.; detector (base) temperature 240°C.; N_2 flow rate, approximately 40 ml./min.; volume injected, 5µl. Recorder: Varian Aerograph Model 20, 1 mV, full scale deflection. Chart speed: 2/3" per min.

Fig. 1 indicates the degree of separation of 8 pesticides in a standard mixture. The excellent separation obtained for DDE and dieldrin in this column which was first used by McCully and McKinley (10) should be noted.

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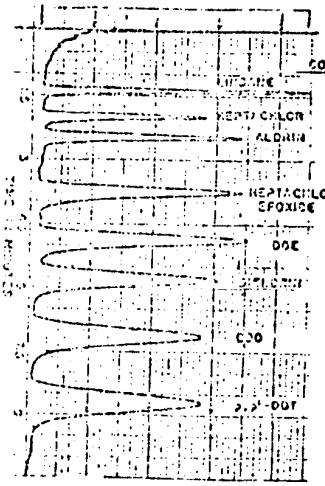

FIG. 1 CHROMATOGRAM OF STANDARD MIXTURE OF ORGANOCHLORINE PESTICIDES.

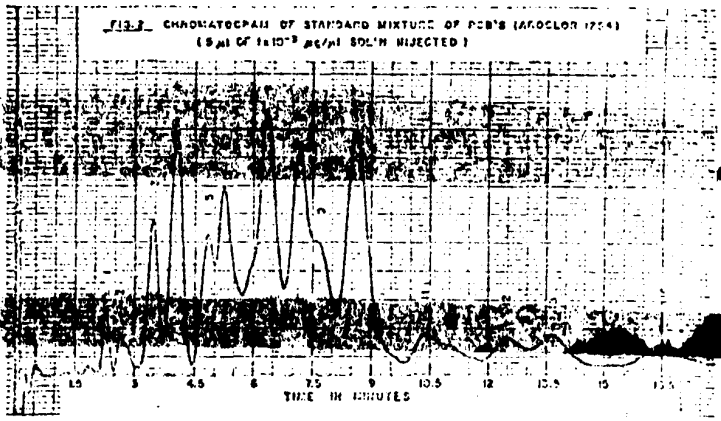

FIG. 8 CHROMATOGRAM OF STANDARD MIXTURE OF PCB'S (AROCLOR 1254)
(5 µl OF 1×10^{-3} µg/ml SOLN INJECTED)

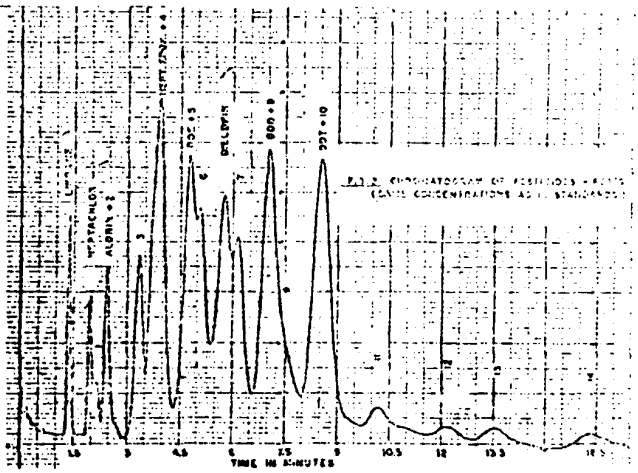

FIG. 9 CHROMATOGRAM OF PESTICIDES + PCB'S
(EQUAL CONCENTRATIONS AS IN STANDARDS)

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Figure 2 shows the number of peaks and the separation obtained for a sample of PCB's ('Aroclor' 1254) while Figure 3 demonstrates the degree of interference encountered when the pesticides are mixed with the PCB's.

It is interesting to note that the peaks of the commonly found pesticides all have a corresponding PCB peak that would interfere if present in the same extract. This is in agreement with Jensen's work.

Separation of PCB's from Pesticides by the Use of Florisil

With thin layer chromatography (TLC) it was observed that the PCB's ('Aroclor' 1254) tended to run towards the solvent front on the TL plates. Bearing this in mind and the fact that our cleanup procedures for pesticide residues in animal tissues usually involve a final Florisil step, we experimented to see if the PCB's could be eluted from the Florisil column with n-hexane knowing that most of the pesticides are not eluted under these specific conditions.

Four preliminary experiments were carried out to test the feasibility of this separation on Florisil. In Expt. I, 5 ml. of standard PCB preparation was added to the glass column (30 cm. x 2.5 cm. O.D.) packed with 40 ml. (ca. 19 gm. or 10 cm. in height) Florisil (60-100 mesh, Floridin Co., stored at 130°C. until ready for use) and topped with an 1/2" layer of anhydrous Na_2SO_4. Elution was carried out with 100 ml. n-hexane, and the percentage recoveries were determined. This experiment was

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repeated but the elution was effected with 200 ml. hexane (Expt. II). The same experiments were carried out with the standard pesticide mixture eluting with 100 (Expt. III) and 200 ml. (Expt. IV) hexane respectively.

TABLE 4

Percent recovery of PCB's and Pesticides from Florisil columns by elution with hexane (a)

PCB peak no. (GLC)	Expt. I 100 ml hex	Expt. II 200 ml hex	Pesticide peak	Expt. III 100 ml hex	Expt. IV 200 ml hex
1	80.1	92.2	Lindane	None	None
2	86.7	103.1	Heptachlor	None	92.7
3	65.6	100.0	Aldrin	62.8	94.1
4	98.2	101.0	Hept. epox.	None	None
5	42.1	100.0	DDE	20.5	97.5
6	44.9	98.7	Dieldrin	None	None
7	64.0	101.2	DDD	None	None
8	96.3	105.2	p,p'-DDT	None	None
9	60.4	105.8			
10	72.6	103.3			
11	76.7	90.9			
12	57.2	100.0			
13	100.0	100.0			
14	71.4	100.0			

a. Recoveries are based on peak height comparisons and each value represents the average of duplicate determinations.

b. Under the experimental conditions, 200 ml. of 20% ethyl ether in hexane is used normally to elute the pesticides although 200 ml. can quantitatively remove them.

The experimental results which are shown in Table I indicate that separation on a Florisil column is feasible. Almost quantitative removal of the PCB's is effected with 200 ml. hexane, while under the same conditions only three of the 8 pesticides tried showed evidence of elution (heptachlor 92.7%, aldrin 94.1%, and DDE 97.5%). It is interesting to note that these three pesti-

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-cides showing some elution from Florisil with hexane, are, like the PCB's, quite mobile under our TLC conditions.

Two further experiments were carried out to see if the separation was still effective when PCB's and pesticides were mixed (Expt. V) and when they were present in the extract from an animal tissue (Expt. VI). The first elution was made with 200 ml. hexane, the receiver was changed, and the second elution was carried out with 250 ml. of 20% ethyl ether in hexane to remove the pesticides.

The results of the two experiments are shown in Table II, and confirm our earlier finding that with the exception of DDE, aldrin, and heptachlor, a clear-cut separation of the PCB's and pesticides can be made by the use of a Florisil column.

The fact that DDE is eluted with the PCB's by pure hexane can be used to advantage in the confirmation and quantification of DDT by dehydrochlorination. The estimation of small amounts of DDT in the presence of interference (for example, a PCB) is enhanced if DDE is previously removed. The DDE produced by dehydrochlorination can then be used to estimate the amount of DDT originally present. In the presence of comparatively large amounts of DDE, this approach is not very dependable.

Discussion

The results of the above experiments coupled with the work of Jensen indicate that there are serious problems confronting residue analysts. However, as far as the writer is aware, there

TABLE II

Percent Recoveries of PCB's and Pesticides from a Mixture after Separation on Florisil (Expts. V & VI)*

Eluted with 200 ml. hexane			With 250 ml. 20% ether in hexane		
PCB and/or pest. peak	% Recov.(a)	% Recov.(b)	Pesticide peak	Recov.(a)	Recov.(b)
			Lindane	93.6	98.5
Heptachlor	92.7	98.1	Heptachlor	None	None
PCB1	104.0	101.7			
PCB2 + Ald.[c]	102.1	96.6	Aldrin	1.3	4.0
PCB3	100.0	100.0			
PCB4	104.2	102.6	Hept. epox.	96.4	102.7
PCB5 + DDE[c]	97.8	102.4	DDE	None	None
PCB6	101.3	100.0			
			Dieldrin	100.0	100.0
PCB7	97.8	105.1			
PCB8	100.0	100.0	DDD	102.3	98.9
PCB9	91.6	97.0			
PCB10	104.7	104.3	DDT	99.0	92.5
PCB11	100.0	105.5			
PCB12	100.0	100.0			
PCB13	100.0	100.0			
PCB14	96.2	100.0			

* The peaks are arranged in order of their emergence (increasing retention time) from the GLC column, and where a PCB and a pesticide peak appear in the same line (horizontally) they have similar retention times.

a. A standard mixture of PCB's and pesticides in pure hexane was placed on the Florisil column; first elution was made with 200 ml. hexane, receiver was changed, and the column eluted with 250 ml. 20% ethyl ether in hexane.

b. Same as in (a) except that the PCB's and pesticides were first mixed with an extract from an animal tissue which was known to be essentially free of pesticides.

c. Since a single peak was obtained, the recovery was calculated by a comparison of the peak height against that in the combined standard mixture of PCB and pesticide. In all other cases the peak height was compared to that in the standard injected separately.

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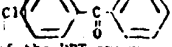
has been no positive confirmation of the presence of PCB's in wildlife tissues by techniques other than chromatography. This leaves doubts that the presence of the unidentified peaks (UIP's) is actually due to PCB's. There is the possibility that some or all of the peaks are due to condensation products of the metabolites of pesticides like DDT. For example, 4,4'-dichlorobenzophenone (DCB)  is known to be a metabolite of the DDT group.

The presence of the keto group makes it quite feasible for condensation to take place.

There are at least two points that lend support to this possibility.

1. The UIP's (being called PCB's) are usually observed only when large amounts of the DDT group are present.

2. Jensen checked eagle feathers collected since 1939 and first detected PCB (not confirmed) in an eagle from 1944. It might be a coincidence, but this is approximately the time that DDT use came into prominence. It should be noted also that the PCB's were in wide use as early as 1930 (2). Thus until positive confirmation (e.g. with mass spectra) is obtained, there will remain some doubt that these UIP's are due to PCB's - especially if PCB's are not used in pesticide formulations.

It is certainly true, however, that whether or not the UIP's are PCB's their presence leads to difficulties. The results obtained for some samples of fat recently analyzed in our labora-

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-tory are typical of the problem. The sample containing the highest levels of residues contained the following pesticides in p.p.m.: DDE - 1.42, dieldrin - 2.13, DDD - 5.61, and p,p'-DDT - 2.60.

Even prior to subjection to TLC confirmation, the DDD value appeared unusually high when it is considered that its presence in tissues is usually accounted for by three main routes:

a) It is used as a pesticide, but not extensively.

b) It is one of the metabolites of DDT - however, the DDT->DDE pathway is much more prevalent than DDT->DDD, with the latter usually occurring in the liver, hence the fat tissue is an unlikely location for large amounts of DDD.

c) It is a frequent contaminant of technical DDT used in spray programs.

When confirmation of the pesticides was attempted, the TLC plates showed no DDD, although the apparent amount present should have given a distinct spot on the plate. However, a spot was observed running near the solvent front, a considerable distance from DDD. When this spot was scraped off the TLC plate, eluted, and reinjected into the gas chromatograph, a peak having retention time identical to DDD was observed. Although this interfering material has not been identified - it could be a PCB since its retention time coincides with one of the PCB's - it is obvious how easily one could report false results, especially if use is made of the GLC-EC results without further confirmation.

TLC continues to be our main confirmatory method, but there are times, especially with smaller (but significant) amounts of pesticides, when it is impossible to make a positive confirmation with this technique alone.

The determination of GLC retention times on two or more stationary phases is quite useful in some cases, but as Robinson (11) has pointed out, it cannot be regarded as an independent parameter of identity since it may be shown that various organochlorine pesticides on different stationary phases are significantly correlated.

Bearing in mind these problems and the difficulty of applying infrared, mass spectra, and other spectroscopic methods for confirmation of small amounts of pesticide residues, more emphasis and reliance should be given to chemical modification of the pesticides and reinjection into the gas chromatograph, using the retention times of the products as means of confirmation.

With our SMI technique (S = Separation of PCB's on Florisil, M = Modification of the pesticide by chemical means, I = Injection of the extract containing the product into the GLC apparatus) we have observed some cases where a single GLC peak indicating the pesticide was in fact a mixture consisting of the pesticide plus some other PCB-type unknown having the same retention time. With TLC as the sole confirmatory method, one could quantify the whole as being due to the pesticide and be out by many factors depending on the ratios of the two compounds giving rise to the

single GLC peak.

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