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CHROMATOGRAPHIC AND BIOLOGICAL ASPECTS OF POLYCHLORINATED BIPHENYLS

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I. INTRODUCTION

Despite the fact that polychlorinated biphenyls (PCB's) have been available commercially for forty years, it is only within the last five years that they have been recognized to be of environmental concern.

Polychlorinated biphenyls are produced by a comparatively small number of manufacturers, marketed under a number of commercial trade names, *e.g.*, Aroclor, Clophen and Phenoclor, and are represented as "a series of inert, chemically resistant, fire-retarding plasticizers compatible with a wide variety of resins, varnishes, waxes and paints; they vary from mobile, oily liquids to white crystals and hard transparent resins"[1]. The series of Aroclors (Monsanto) are marketed under various numbers and consist of mixtures of chlorinated biphenyls and terphenyls. The first two digits represent the molecular type: 12—chlorinated biphenyls; 25 and 44—blends of chlorinated biphenyls and chlorinated terphenyls (75% biphenyl and 60% biphenyl, respectively); 54—chlorinated terphenyls. The last two digits give the weight percent of chlorine, *e.g.*, Aroclor 1242 is a chlorinated biphenyl containing 42% chlorine.

The viscosity of the PCB's increases in direct proportion to the chlorine content from very fluid liquids to viscous products and solids. In the commercial process for PCB manufacture, biphenyls are chlorinated with anhydrous chlorine with either iron filings or ferric chloride as the catalyst, the byproduct is hydrogen chloride and

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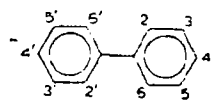
the product is a mixture of several PCB's. In the process of replacing hydrogen atoms with those of chlorine, a large number of substitution combinations arise, *viz.*,



For example, three monochlorobiphenyl isomers are possible, twelve dichlorobiphenyl isomers, twenty-one trichlorobiphenyl isomers, and so on. Theoretically, 210 compounds can be prepared by this substitution process. (A typical PCB example would be 2,4,6,2',4'-pentachlorobiphenyl.) Mass spectroscopic studies[2] of Aroclor 1260 revealed the presence of eleven isomers, *viz.*, five containing six chlorine atoms, five containing seven chlorine atoms and one containing eight, while Aroclor 1254 was found to contain eighteen compounds, *viz.*, one containing three chlorine atoms, four containing four chlorines, four containing five chlorines, five containing six chlorines, and four containing seven chlorines[3]. The annual production of PCB's in the Western world till most recently was estimated at 100 million pounds.

The chemical properties that make PCB's desirable industrial chemicals are their excellent thermal stability, their strong resistance to both acidic and basic hydroxides and action of corrosive chemicals and their *general* inertness. They are insoluble in water but possess a low finite vapor pressure. Their boiling points range from 278° for Aroclor 1221 to 415° for Aroclor 1268. All are stable to prolonged

TABLE 1

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SOME USES OF POLYCHLORINATED BIPHENYLS[4]

Material with which Aroclor is combined	*Aroclor used with (wt. %)*	*Use*
Polyvinyl chloride	Aroclor 1248, 1254 and 1260 (7–8%)	Secondary plasticizer to improve flame retardance and chemical resistance
Nitrocellulose lacquers	Aroclor 1262 (7%)	Co-plasticizer to enhance resistance
Polyvinyl acetate	Aroclor 1221, 1232 and 1242 (11%)	Improve quick-track and fiber-tear properties
Polyvinyl acetate	Aroclor 1254 (41%)	Pressure-sensitive adhesive
Epoxy resins	Aroclor 1221 and 1248 (20%)	Increase chemical and oxidation resistance and adhesive qualities
Polyester resins	Aroclor 1260 (10–15%) Aroclor 1260 (10–20%)	Effective and economical fire retardent Increases strength of fiber-glass reinforced polyester resins
Polystyrene	Aroclor 1221 (2%)	Plasticizer
Chlorinated rubber	Aroclor 1254 (5–10%)	Enhances resistance, flame retardance and improves electrical insulating properties
Styrene-butadiene copolymer	Aroclor 1254 (8%)	Improves chemical resistance
Paper coatings	Aroclor 1268 (10%) Aroclor 1268 (1.5%)	Fire retardant Injection moldings
Vinyl plastisols	Aroclor 1262 (5–50%)	Plasticizer in paint compositions
Varnish	Aroclor 1260 (25% solids)	Improves water and alkali resistance
Wax	Aroclor 1242 (5%)	Moisture and flame resistance

heating at 250° and the lower Aroclors can be distilled at atmospheric pressure without appreciable decomposition.

The largest single use of PCB's is related to their electrical properties, as coolant insulation fluids in transformers and capacitors. Other uses of PCB's include impregnation of cotton and asbestos for braided insulation of electrical wiring, plasticizers of vinyl chloride and polymer freons, as plasticizer in wire and cable coatings and in ballasts for fluorescent fixtures. Because of their thermal stability and fire resistance the PCB's also find application in high-pressure hydraulic fluids, heat transfer agents, machine tool cutting oils, specialized lubricants and gasket sealers. Miscellaneous uses include: formulations in epoxy paints, resins and chlorinated rubber, printing inks, waxes, synthetic adhesives, textile dyes, protective coatings for wood, metal and concretes, as sealers in water-proofing compounds and putty, and in carbonless reproducing paper.

PCB's have been incorporated into pesticide formulations, especially with such insecticides as DDVP, lindane[4,5], chlordane, aldrin, dieldrin and toxaphene to suppress their vaporization and hence extend their "kill-life" and have also been shown to increase the insecticidal properties of DDT[6].

Table 1 lists a number of uses of the Aroclor polychlorinated biphenyls and the material and percentages with which they are combined.

II. ECOLOGICAL ASPECTS

The first identification of polychlorinated biphenyls in regard to ecology was by JENSEN[7], who identified PCB's in the bodies of 200 pike taken from different parts of Sweden, in other fish and in eagle feathers collected in 1944. PCB's along with DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane] are now reported to be the most abundant of the chlorinated aromatic pollutants in the global ecosystem[8]. Extracts of sea eagles, pike and salmon[8] as well as in British[9] and Canadian[10–12] wildlife contained PCB's, and in the former instance it was found that in birds' liver and eggs the PCB residues were greater than the organochlorine pesticide residues. PCB's have also been found in fish, mussels and birds from the River Rhine and The Netherlands coastal areas[2] and in marine animals and wildlife in Sweden, England and the U.S.A.[2,8,11,13].

Polychlorinated biphenyls have also been found in human adipose tissue[14], samples of human milk[15] and in foods (margarine, vegetable oils and particularly fish[16])

Essentially, the same type of residue pattern is becoming apparent for the polychlorinated biphenyls that has been found for the persistent organochlorine insecticides. The PCB's are extremely stable, chemically fat soluble and hence persistent in the environment. The four possible pathways by which PCB's could be dispersed in the environment include: (a) because of their numerous manufacturing applications and general inertness, PCB's could be flushed as wastes into rivers, lakes, etc., to pollute fish and other wildlife; (b) via industrial smoke, in exhaust from aircraft engines, incineration and combustion of PCB-containing products, and leaching of plasticizer from plastic objects in waste disposal areas; (c) via insecticidal formulations to enhance kill-life (described above); and (d) via direct contamination of feeds and foodstuffs through leakage of heat transfer fluids.

Information relative to the biological decomposition of the PCB's is scant and

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TABLE 2

PATHOLOGIC CHANGES INDUCED BY PCB's[a]

Treatment	*Animal*	*Liver*	*Kidney*	*Pericardium and peritoneum*	*Other observable changes*	*Reference*
Single oral dose of 69 mg (42% Cl)	Guinea pig Rat Rabbit	Small fat droplets through lobules, slight to moderate central atrophy, focal necrosis noted in a few animals	Essentially normal	No noteworthy changes	Adrenals, spleen and pancreas showed no noteworthy changes	25
300 mg daily for 6 days (65% Cl)	Rat	Cells swollen, hyaline granules present, most died within few days				27
50 mg daily for up to 6 months (65% Cl)	Rat	Enlarged (33% weight increase), large number of hyaline globules in cytoplasm; several died during experiment				27
25, 50 and 100 p.p.m. in diet for 15 days (21-68% Cl Aroclors)	Rat	Increase in weight, effect increasing with increasing chlorine content. Aroclor 1232 10%, 1242 12%, 1254 14%, 1268 24% at 50 p.p.m.				32
100 p.p.m. in diet 200 p.p.m. in diet 400 p.p.m. in diet 800 p.p.m. in diet	Chicken	No effect No effect Enlarged and mottled Damaged	Damaged	Slight Hydropericardium Hydropericardium Hydropericardium, hydroperitoneum enlarged		29
200 and 400 p.p.m. in diet for 3 weeks (42%, Aroclor)	Chicken	No changes noted	Paleness at 200 p.p.m., extensive hemorrhage, and enlargement at 400 p.p.m.	Increased fluid in pericardial sac at the higher concentration	Paleness of pancreas, enlargement of adrenal and small spleen at low concentrations; at higher concentrations pale cream-colored pancreas, adrenals hemorrhagic	30
Various doses (54% Cl, Aroclor)	Bengalese finch	No weight changes	Weight was 32.4% of brain weight for controls and 53.5% for those dying from PCB poisoning	Slight weight increase, a few showed liquid in pericardial sac		33
400 p.p.m. in diet for 60 days (60% Cl[b])	Chicken	Centrolobular necrosis (cpds. 1 and 2); liver weight increased from 2.76 g/100 g to 4.31 g/100 g (cpd. 3); fatty degeneration	Tubular dilatation (cpds. 1 and 2); rare with cpd. 3	Hydropericardium common with cpds. 1 and 2; rare with cpd. 3.	Increased porphyria, spleen small with reduction of red pulp and atrophy of white pulp (cpds. 1 and 2); spleen decreased from 0.146 g/100 g to 0.130 g/100 g (cpd. 3)	34

[a] Phenoclor DP6 (cpd. 1), Clophen A60 (cpd. 2) and Aroclor 1260 (cpd. 3) were used. Differential effects noted under cpd. numbers. All chickens died on cpds. 1 and 2 within 60 days, only 15% mortality on cpd. 3.

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TABLE 3

TOXICITY OF AROCLORS[a]

	Aroclors									
	1221	1232	1242	1248	1260	1262	1268	4465	5442	5460	2565
Oral LD_{50}, mg/kg (rats)	3950[a]	4470[a]	8650[a]	11,000[a]	10,000[b]	11,300[b]	10,000[b]	16,000[b]	10,600[b]	19,200[c]	6,310[c]
Skin MLD, mg/kg (rabbits)	> 2000[a] < 3160[a]	> 1260[a] < 2000[a]	> 794[a] < 1260[a]	> 794[a] > 1260[a]	> 1260[b] < 2000[b]	> 1260[b] < 3160[b]	> 2500[c]	> 20000[b] < 31600[b]	> 12600[b] < 20000[b]	> 79,100[c]	> 20000[c] < 31600

[a] Undiluted.
[b] Administered as 50% solution in corn oil.
[c] Administered as 33⅓% solution in corn oil.

it was suggested[17] that they are likely more stable than DDT and its metabolites since the PCB's lack the ethane component between the aromatic rings which is the site of action of most of the transformations of DDT. This factor coupled with their physical and chemical characteristics for persistence indicates that these materials are capable of biological magnification up the food chain. (Although the concentration of the PCB's are generally in the p.p.b.* range in the environment, their high lipid solubility results in their accumulation in fatty tissues of lower animals and marine life.) Because of the very low aqueous solubility of the PCB's, when they are discharged into a river or lake, they will accumulate on the sediment in *relatively* high concentration and redissolve very slowly.

III. TOXICOLOGICAL ASPECTS

Compared to the chlorinated hydrocarbon pesticides, the toxicology of the polychlorinated biphenyls remains rather poorly known. The systemic effects in humans and deaths resulting from exposure to the PCB's and polychlorinated naphthalene was described by GREENBERG *et al.*[18] and the chloroacnegen effects reported by SCHWARTZ and co-workers[19-21], JONES AND ALDEN[22] and VON WEDEL *et al.*[23].

SAX[24] reported the toxicity of PCB's to man as moderate to high, especially when inhaled as vapors. Animal experiments with rats and guinea-pigs have shown that ingestion of PCB may give rise to liver injury[25,26]. Oral administration of chlorobiphenyls resulted in enlargement of the liver and vacuolar or fatty degeneration of liver cells.

BENNETT *et al.*[27] have previously shown that chlorinated biphenyl resulted in liver changes markedly different from those caused by other well known toxic agents such as carbon tetrachloride and chloroform poisoning and thought that this type of injury was persistent with slow recovery. The effects on mouse and monkey liver of long-term oral administration of chlorobiphenyls (1.5 mg/day or more) have been reported by NISHIZUMI[28].

One PCB has been shown to produce chick hydropericardial edema and growth depression proportional to dietary levels (200 and 400 p.p.m.) of the toxicant[29,30]. These changes were accompanied by such pathological changes as: enlarged hemorrhagic kidney, enlarged adrenal gland, small spleen, cream-colored pancreas, dermatitis, defeathering and transient changes in blood glucose, hemoglobin and hematocrit levels. Hydropericardium and abdominal edema have also been observed in Japanese quail[2] after PCB ingestion. Toxic and teratogenic effects of PCB's in chick embryos have also been reported by MCLAUGHLIN *et al.*[31].

Analogously with the chlorinated hydrocarbon pesticides, the most important effects are long-range sublethal effects. The pathologic changes in various organs are summarized in Table 2, illustrating some interesting differences between mammals and birds. For example, the most striking findings in mammals are alterations to the liver, whereas fluid in the pericardial sac, kidney damage and reduced spleen are found in birds.

The toxicity of eleven Aroclors in terms of oral LD_{50} (rats) and skin MLD (rabbits) is summarized in Table 3.

* Throughout this article the American (10^9) billion is meant.

Polychlorinated biphenyls and triphenyls have been found to be estrogenically active[36]. In a series of PCB's the compounds containing up to 48% chlorine were active. On a weight basis PCB compounds such as Aroclor 1221 were shown to have an estradiol-degrading potential (in pigeon liver homogenates) about five times that of *p,p'*-DDE or technical grade DDT[8,37]. Other examples of induction of hepatic hydroxylating enzymes by PCB's have been reported in the rat[32] and the American kestrel (*Falco sparverius*)[38].

VILLENEUVE *et al.*[35] studied the effects of PCB administration on microsomal enzyme activity in pregnant rabbits. The no-effect level of Aroclor 1254 for enzyme induction in the pregnant rabbit is between 1.0 and 10 mg/kg body weight when administered for twenty-eight days during gestation. Aroclor 1221 did not induce any enzyme activity in the does, foetus or placenta, so its no-effect level must be considered higher than that for Aroclor 1254. Placental transfer was shown to occur for both Aroclor 1254 and 1221 but does not cause any changes in the biochemical or physiological parameters measured, *e.g.*, total amount of Vitamin A stored per liver, protein levels, aniline hydroxylase enzyme activity, serum cholesterol, no effect in reproductive processes. The drug metabolizing enzymes aniline hydroxylase and aminopyrine *n*-demethylase were both induced by 10 mg/kg Aroclor 1254.

PCB's as inducers of hepatic enzymes (*e.g.*, increasing the rate of circulating estradiol in the liver) may be responsible for aberrations in calcium metabolism in certain species of birds[8] and are generally considered to be more of a potent threat than DDT to our declining bird populations, especially for predatory birds that accumulate fairly high levels of PCB's. ANDERSON *et al.*[11] have suggested evidence that PCB's affected eggshell thickness although to a lesser extent than DDE.

However, VERMEER AND REYNOLDS[40] found no significant correlation between shell thickness and PCB residues for the great blue heron (*Ardea herodias*) and PEAKALL[41] found no significant effect of PCB's in the eggshells of ring doves.

FRIEND AND TRAINER[12] reported on the interaction of PCB with duck hepatitis virus. Ten-day old mallard ducklings fed a polychlorinated biphenyl (Aroclor 1254) at concentrations of 25, 50 and 100 p.p.m. for ten days suffered no apparent clinical

TABLE 4

PERCENT INHIBITION OF HUMAN CELL[a] GROWTH OBTAINED WITH ENVIRONMENTAL CHEMICALS

Chemical	ID_{50} *(p.p.m.)*[b]	
	H	*F*
p,p'-DDT	5.5	50
o,p'-DDT	5.6	—
Parathion	4.4	6.4
PCB (Aroclor 1254)	6.3	110
Caffeine	480	650
Aspirin	600	1,000
Acetylsalicylic acid	370	—

[a] Human cells: H = HeLa established cell line; F = normal diploid skin fibroblasts.
[b] Concentration of chemical (in p.p.m.) that produced a 50% inhibition (ID_{50}) in cultures treated after 48 h of exposure.
[c] Commercial tablet.

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intoxication. When these birds were challenged five days later with duck hepatitis virus (DHV), they suffered significantly higher mortality than birds which were not exposed to the polychlorinated biphenyl (*e.g.*, 14% among virus controls *versus* 35 to 65% among groups receiving PCB plus DHV). This study illustrates one of the potential effects of sublethal concentrations of chemical pollutants and emphasizes the real differences that exist between "sublethal" and "no-effect" concentrations of pollutants.

The effects and interactions of environmental chemicals (aspirin, caffeine, Aroclor 1254, carbaryl, parathion, DDT and several insecticide metabolites) on human cells (HeLa and skin fibroblasts) in tissue culture were described by LITTERST AND LICHTENSTEIN[13]. The dosage causing a 50% inhibition in culture growth, and the interaction between these agents and their effect on protein and nucleic acid synthesis were all determined. Table 4 lists the percent inhibition of human cell growth obtained with selected chemicals and Table 5 shows the effect of aspirin, PCB and caffeine on macromolecule synthesis in human cell cultures after 4½ h of exposure to DDT and parathion. Both cell types responded nearly equally to the presence of an individual chemical. Aspirin and caffeine were eight to twenty times less toxic than the other agents studied while the PCB plasticizer Aroclor 1254 was as toxic as DDT.

IV. COLUMN AND THIN-LAYER CHROMATOGRAPHY OF PCB'S

Because of their similarity in structures and chemical properties, PCB's, if

TABLE 5

EFFECT OF ADDITIVES ON MACROMOLECULE SYNTHESIS IN HUMAN CELL CULTURES[a] AFTER 4½ h OF EXPOSURE TO INSECTICIDES

Additive	*Insecticide*					
	None		*DDT (75 p.p.m.)*		*Parathion (50 p.p.m.)*	
	H	*F*	*H*	*F*	*H*	*F*
Protein						
None	—	—	70[c]	51[c]	85	92
Aspirin (25 p.p.m.)	102	91	47[d]	45	78	105
PCB[b] (10 p.p.m.)	91	108	59[c]	59	82	66[d]
Caffeine (10 p.p.m.)	93	101	72	38[d]	66[d]	64[d]
RNA						
None	—	—	70[c]	89	81	67[c]
Aspirin (25 p.p.m.)	94	87	61	103	72	54
PCB (10 p.p.m.)	100	96	72	86	104[d]	55
Caffeine (10 p.p.m.)	100	94	88[d]	82	91	75
DNA						
None	—	—	77	91	64[c]	87
Aspirin (25 p.p.m.)	145[c]	86	64	86	43[d]	50[d]
PCB (10 p.p.m.)	93	107	64	86	59	67[d]
Caffeine (10 p.p.m.)	103	98	91	83	77	85

[a] Human cells: H = HeLa established cell line; F = normal diploid skin fibroblasts.
[b] Polychlorinated biphenyl plasticizer (Aroclor 1254).
[c] Differences between treated cultures (pesticide or additive) and control cultures (labeled treated) were significant at the 5% level.
[d] Differences between treated cultures (pesticide plus additive) and control cultures (pesticide treated only) were significant at the 5% level.

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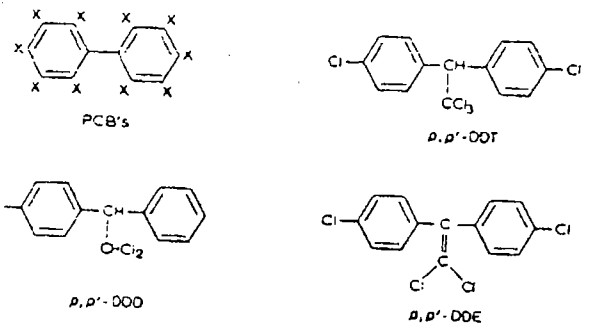
present in a sample, are carried through the usual pesticide extraction and screening procedures and are frequently mistaken for DDT and analogs in monitoring tests, *viz.*,



REYNOLDS[12,14] described an activated Florisil column technique which separated heptachlor, aldrin, DDE and PCB with the first elution (60 ml of *n*-hexane) from lindane; and heptachlor epoxide, DDD and DDT with the second elution (40 ml of 50% diethyl ether in hexane).

KOEMAN *et al.*[2] eluted a number of apolar compounds including DDE and PCB on activated Florisil columns using *n*-hexane, then dieldrin and endrin with 10% diethyl ether in hexane.

The separation of PCB's from DDT and its analogs and other common pesticides by column chromatography on silicic acid–Celite was reported by ARMOUR AND BURKE[45]. Aldrin and PCB were eluted with the first fraction (250 ml of petroleum ether) and lindane, heptachlor, heptachlor epoxide, dieldrin, endrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT and *p,p'*-DDD with the second fraction (200 ml of acetonitrile–

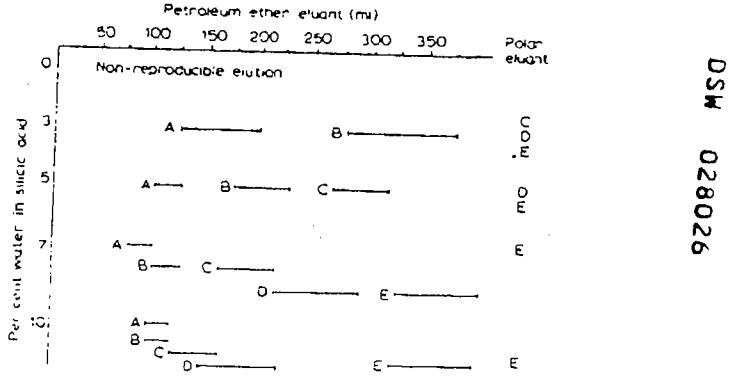


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Fig. 1. Effect of silicic acid–water content on the elution and separation of Aroclor 1254 and DDT analogs. Several fractions of petroleum ether eluate were analyzed by GLC; the respective compounds were found in the presence of various amounts of water by increasing the polarity of the eluant. Various polar eluants were used to elute the compounds: A = Aroclor 1260; B = *p,p'*-DDE; C = *o,p'*-DDT; D = *p,p'*-DDT; E = *p,p'*-DDD.

hexane–methylene chloride, 1:19:80). Determination of PCB's and pesticides could also be made on separate column eluates without cross-interference with recoveries of Aroclors 1254 and 1260 and of several chlorinated pesticides ranging from 76 to 90% and 80 to 100%, respectively. The effect of water content of silicic acid on elution and separation of Aroclor 1260 and DDT and analogs is shown in Fig. 1. The maximum margin of separation of PCB from pesticides with good reproducibility of elution pattern was obtained with silicic acid containing 3% water. A more polar eluent was required for the complete elution of *p,p'*-DDT and *p,p'*-TDE, when the water content was 5% less.

A semiquantitative determination of PCB's in tissue samples by TLC was described by MULHERN *et al.*[46]. Cleanup by hexane–acetonitrile partitioning and Florisil column chromatography was performed on samples before oxidative treatment[47] to convert DDE to DCBP (4,4-dichlorobenzophenone), then the PCB components were determined semiquantitatively (with a lower limit of sensitivity of 0.2 μg) by TLC with no prior separation from chlorinated pesticides required. TLC separation was accomplished on silver nitrate incorporated Aluminum Oxide G plates developed with 5% benzene in hexane. The spots were detected using a spray consisting of 5 ml of water and 10 ml of 2-phenoxy-ethanol diluted to 200 ml with acetone and containing one to five drops of 30% hydrogen peroxide, followed by exposure to germicidal UV light. The R_F values of Aroclor mixtures chromatographed by this TLC system

TABLE 6

R_F VALUES FOR AROCLOR MIXTURES AND PESTICIDES ON $AgNO_3$-INCORPORATED ALUMINUM OXIDE G PLATES DEVELOPED WITH 5% BENZENE IN HEXANE

Compound	*R_F value*
1242	0.91
1248	0.93
1254	0.93
1260	0.93
1262	0.94
DDE	0.93
DDT	0.88
DDD	0.74
Heptachlor epoxide	0.58
Dieldrin	0.48
DCBP	0.30

are listed in Table 6. Fig. 2 illustrates a chromatogram of a pelican egg sample containing oxidized samples as well as Aroclor 1254 and pesticide standards. The lower limit of detection of PCB by this TLC procedure is 0.2 μg.

Analytical data of pelican egg and eagle liver samples are shown in Table 7. PCB was determined by TLC and DDE data were obtained by the GLC procedure of REICHEL *et al.*[48]. The PCB/DDE ratio was approximately 1.0 except for the pelican eggs from California.

ARMOUR AND BURKE[49] used precoated aluminum oxide sheets with both *n*-heptane and acetone–*n*-heptane (2:98) as solvent systems for the separation of *p,p'*-DDT and *p,p'*-DDE from Aroclor 1254 and 1260. However, the above Aroclors and DDE were not resolved.

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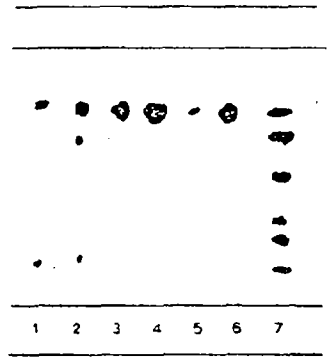

Fig. 2. Thin-layer chromatogram of a pelican egg sample. $AgNO_3$-incorporated Aluminum Oxide G, 5% benzene in hexane. 1 = 0.6 g of oxidized sample; 2 = 1.0 g of oxidized sample; 3 = 0.6 g of unoxidized sample; 4 = 1.0 g of unoxidized sample; 5 = 1 μg of Aroclor 1254; 6 = 5 μg of Aroclor 1254; and 7 = 5 μg of each of DCBP, dieldrin, heptachlor epoxide, DDD, DDT, and DDE (in ascending order).

The separation and identification of DDT analogs in the presence of polychlorinated biphenyls by two-dimensional TLC was described by FEHRINGER AND WESTFALL[30]. Separation resulted from the different migration patterns and migration distances relative to *p,p'*-DDE that mixtures of PCB's exhibit under two sets of conditions. Two-dimensional TLC was employed using MN-Kieselgel G-HR as the absorbent with *n*-heptane alone and *n*-heptane–acetone (98:2) as developing solvents and silver nitrate incorporated into the absorbent layer as the chromogenic agent. Fig. 3 illustrates a plate spotted for first-dimension development to be performed in a sandwich chamber with a mobile solvent of *n*-heptane. Fig. 4 shows a plate spotted for second-dimensional development to be performed in a partially saturated tank with *n*-heptane–acetone (98:2) as mobile solvent. Figs. 5 through 12 illustrate the two-dimensional TLC separation of DDT analogs from Aroclors 1254, 1260, 1248, 1242, 1232, 1228, 1262 and 4465, respectively. Fig. 13 depicts the two-dimensional separation of a crude fish sample containing PCB's, keltlane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE. Figs. 14 and 15 depict

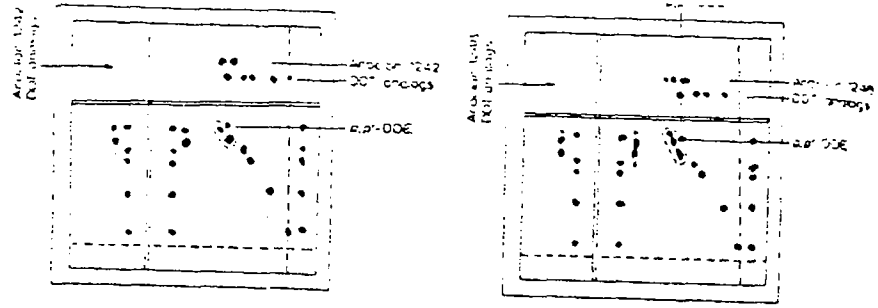

Fig. 9. Separation of Aroclor 1242 from the DDT analogs.

Fig. 10. Separation of Aroclor 1248 from the DDT analogs.

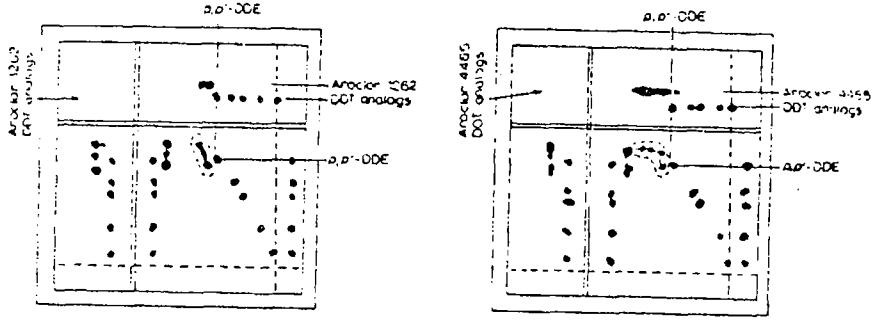

Fig. 11. Separation of Aroclor 1262 from the DDT analogs.

Fig. 12. Separation of Aroclor 4465 from the DDT analogs.

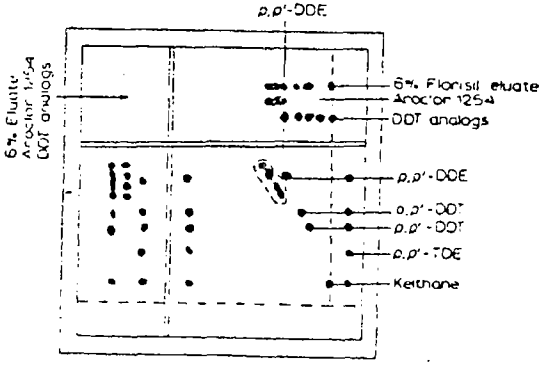

Fig. 13. Two-dimensional separation of a 6% Florisil eluate from a fish sample containing PCB's, kelthane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE.

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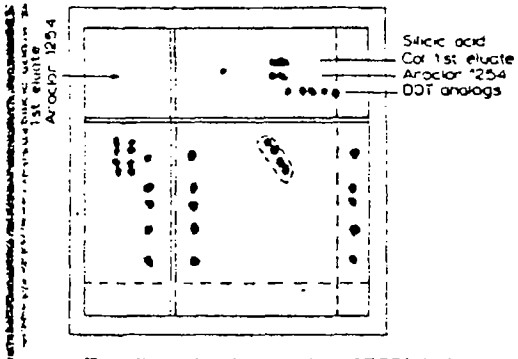

Fig. 14. Two-dimensional separation of PCB's isolated from a fish sample by the silicic acid column separation of ARMOUR AND BURKE[43].

respectively (both isolated from fish sample by the silicic acid column separation of ARMOUR AND BURKE[43]).

Aroclors 1254 and 1260 are the two of greatest interest in pesticide work because their GLC peaks elute in the same general region as the DDT analogs and chromatograph on many TLC systems at approximately the same R_F value as *p,p'*-DDE.

The synthesis and TLC of twenty-three chlorobiphenyls has been described by HUTZINGER *et al.*[51]. All chlorobiphenyls prepared were homogeneous on silica gel thin-

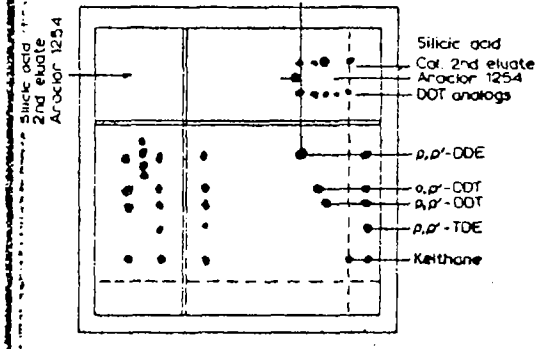

Fig. 15. Two-dimensional separation of kelthane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE isolated from PCB's in a fish sample by the silicic acid column separation of ARMOUR AND BURKE[43].

layer chromatograms developed in heptane and gave one peak when chromatographed on a 4% SE-30 column.

Table 8 lists the names, structural formulas, starting material sources, method of purification, melting points and R_F values of the individual chlorobiphenyls.

V. GAS–LIQUID CHROMATOGRAPHY

The discovery of PCB's in the environment was not accomplished until 1966

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TABLE 3

PHYSICAL AND TLC PROPERTIES OF CHLORODIPHENYLS

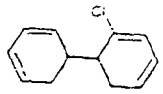
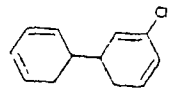
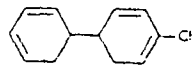
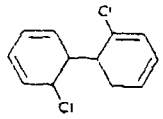
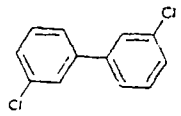
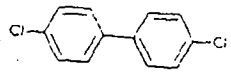
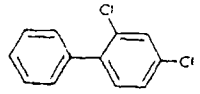
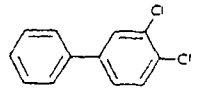
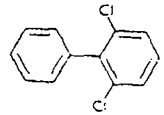
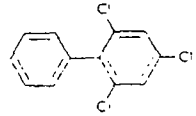
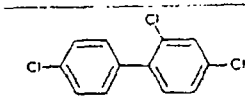
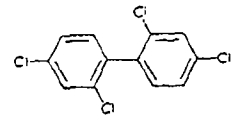
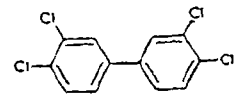
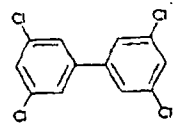
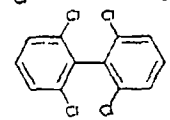
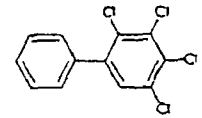
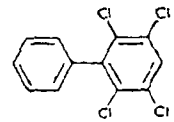
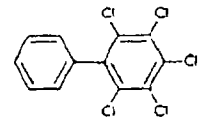
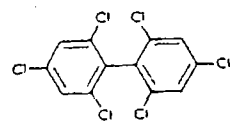
Compound[a]		Starting material (sources[b,c])	Method of purification[d]	Physical data	
Name	Formula			m.p. (°C)	R_F
Monochlorobiphenyls 2-		2-Aminobiphenyl (I)	TLC, MeOH aq.	31–31	55
3-		3-Chloroaniline (I)	Dist., MeOH aq.	16–17	49
4-			MeOH	77–78	54
Dichlorobiphenyls 2,2'-		2-Chloroiodobenzene (V)	TLC, MeOH	59–60	38
3,3'-		3,3'-Dinitrobiphenyl (II) ↓ 3,3'-Diaminobiphenyl	TLC, EtOH aq.	26–27	45
4,4'-		—	MeOH	148–149	31
2,3-		2,4-Dichloroaniline (I)	TLC, MeOH aq.	24–25	59
3,4-		3,4-Dichloroaniline (III)	TLC, MeOH	45–46	52
2,6-		2,6-Dichloroaniline (I)	Col., MeOH aq.	35–36	—
Trichlorobiphenyls 2,3,6-		2,3,6-Trichloroaniline (I)	Col., TLC, EtOH aq.	62–63	62

TABLE 3 (continued)

Compound[a]		Starting material (sources[b,c])	Method of purification[d]	Physical data	
Name	Formula			m.p. (°C)	R_F
2,4'-		4,4'-Dichloro-2-nitrobiphenyl[33] ↓ 2-Amino-4,4'-dichlorobiphenyl	MeOH aq.	57–58	53
Trichlorobiphenyls 2,6,4'-		2,2'-Dichlorobenzidine[28] or 2,4-Dichloroiodobenzene[29]	Dist., Col., EtOH aq.	41	58
3,3',4'-		3,4,3',4'-Tetraaminobiphenyl (VI) or 3,3'-Dichlorobenzidine (VIII)	TLC, EtOH aq.	173	41
3,3',5'-		3,5,3',5'-Tetrachlorobenzidine[28]	TLC, MeOH, hexane	164	65
2,6,2',6'-		2,6-Dichloroaniline (I) ↓ 2,6-Dichloroiodobenzene	Col., EtOH aq.	198	49
2,3,4,5-		2,3,4,5-Tetrachloronitrobenzene (IV) ↓ 2,3,4,5-Tetrachloroaniline	Col., MeOH	91	48
2,3,5,6-		2,3,5,6-Tetrachloronitrobenzene (II) ↓ 2,3,5,6-Tetrachloroaniline	Col., hexane	79	50
Pentachlorobiphenyl 2,3,4,5,6-		Pentachloronitrobenzene (I) ↓ Pentachloroaniline	Col., MeOH	123	51
Tetrachlorobiphenyls 3,4,6,2',6'-		3,4,6,2',6'-Tetrachlorobenzidine[28] or 2,3,6-Trichloroiodobenzene[31]	Col., MeOH/EtOH	113–113	60

(Continued on p. 362)

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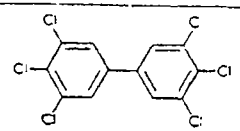
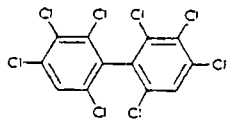
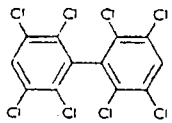
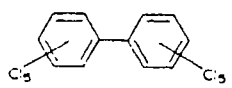
TABLE 3 (continued)

Compound[a]		Starting material (sources[b,c])	Method of purification[d]	Physical data	
Name	Formula			m.p. (°C)	
3,4,5,3',4',5'-		3,3',5,5'-Tetrachlorobenzidine[29]	TLC, EtOH aq.	201–202	
Octachlorobiphenyls 2,3,4,6,2',3',4',6'-		3,3'-Diamino-2,4,6,2',4',6'-tetrachlorobiphenyl[25]	TLC, EtOH	132	
2,3,5,6,2',3',5',6'-		2,3,5,6-Tetrachloronitrobenzene (I) ↓ 2,3,5,6-Tetrachloroaniline ↓ 2,3,5,6-Tetrachloro-4-iodobenzene	TLC, EtOH aq.	160–161	
Decachlorobiphenyl		Aroclor 1268 (VII)	Benzene	305–306	

[a] The following chlorobiphenyls are commercially available (source[b]): 2-chlorobiphenyl (IV, V, VIII), 3-chlorobiphenyl (IV, V, VIII), 4-chlorobiphenyl (I, IV, V, VIII); 2,2'-dichlorobiphenyl (IV, VIII), and 4,4'-dichlorobiphenyl (I, IV, VIII).

[b] Code for commercial suppliers: I = Aldrich Chem. Co.; II = Sapon Lab.; III = Eastman; IV = Chemical Procurement Labs.; V = K & K Lab.; VI = Burdick & Jackson Lab.; VII = Monsanto Chem. Co.; VIII = Pfalz & Bauer Chemicals.

[c] Prepared by method given in reference or commercially available.

[d] TLC = Preparative thin-layer chromatography (hexane); Col. = silica column (hexane). MeOH = methanol, EtOH = ethanol. Dist. = distillation at 0.5 mm.

[e] In heptane on commercially prepared thin-layer plates (Merck Silica Gel F_{254}).

for three basic reasons: (1) they were not deliberately distributed about the ecosystem, (2) their presence was not immediately evident due to their relatively low acute toxicities, and (3) the difficulty of analytical detection and separation. The polychlorinated biphenyls were first detected as interfering peaks in the GLC analysis of environmental samples being analyzed for chlorinated pesticide residues. This difficulty is shown in Fig. 16, which illustrates gas chromatograms of a standard pesticide mixture, Aroclor 1254, and a mixture of both Aroclor 1254 and chlorinated pesticides. REYNOLDS[12,41] reviewed the problem of pesticide residue analysis in the presence of PCB's. The reported GLC patterns indicating PCB interferences have shown marked resemblances with Aroclors 1254 and 1260[2,7,11,31,51]. This type of interference with pesticide residue analysis is shown with a standard mixture of organochlorine pesticides and a commercial PCB mixture (Aroclor 1254) in the chromatograms of Fig. 17. The results obtained that the presence of PCB's in the sample extracts

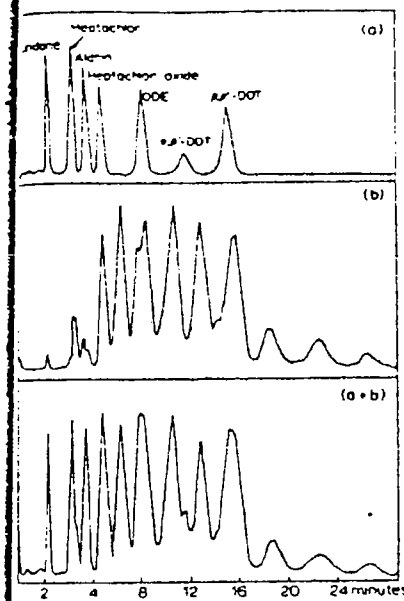


Fig. 16. Gas chromatograms of (a) a standard pesticide mixture, (b) Aroclor 1254, and (a + b) a 50:50 mixture of both. The concentration of Aroclor 1254 is approximately ten times those of the pesticides in (a). Gas chromatograph (Packard 7620) conditions: nitrogen gas flow, 80 ml/min; temperatures—oven 210°, inlet 230°, detector 218°; ^{63}Ni electron capture detector; 5 ft. × 1/4 in. glass column packed with 8% SE-30 on 80–90 mesh Chromosorb Q.

cause interference with pesticide residue analysis under these or similar operating conditions.

The GLC was carried out using a Varian Model 1200 gas chromatograph fitted with a tritium electron capture detector and a spiral glass column (6 ft. × 1/8 in. O.D.) packed with 6% QF-1 and 4% SE-30 on acid-washed Chromosorb W. (The number of theoretical plates for DDT = 2227.) The operating conditions were: column temperature 200°, injector temperature 250° and detector base temperature 250°; nitrogen flow rate 20–30 ml/min; Varian Aerograph Model 20 recorder of 1 mV full-scale deflection and a chart speed of 2/3 in./min.

It is of note that the peaks of the commonly found pesticides all have a corresponding PCB peak that would interfere if present in the same extract. The problem of the interferences of PCB's with the analysis of chlorinated pesticides has been largely overcome by prior column chromatography[2,12] or via an initial extraction with hexane as in regular pesticide analysis, partitioning with acetonitrile to remove fats, passage of cleaned-up extract through both Florisil and silicic acid columns, then finally analysis by GLC with preferably a chloride-specific detector. The pesticides are retained on the silicic acid column for later elution and identification.

The use of a column (30 cm × 2.5 cm O.D.) packed with 40 ml (*ca.* 10 g or 10 cm in height) Florisil (60–80 mesh, stored at 130° until use) and elution with 200 ml of hexane permitted the separation of the PCB's and organochlorine pesticides

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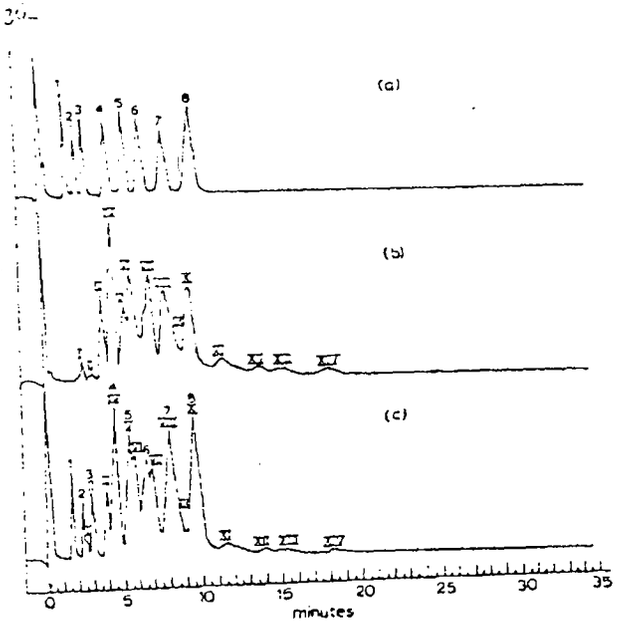

Fig. 13. PCB interference with organochlorine pesticide residue analysis in a borosilicate column (6 ft. × ¼ in. packed with 4% SE-30 and 6% QF-1 on 60–80 mesh Chromosorb W. (a) Standard mixture of organochlorine pesticides. 1 = 0.05 ng of lindane, 2 = 0.10 ng of heptachlor; 3 = 0.10 ng of aldrin; 4 = 0.14 ng of heptachlor epoxide; 5 = 0.20 ng of DDE, 6 = 0.20 ng of dieldrin; 7 = 0.30 ng of DDD; 8 = 0.50 ng of DDT. (b) 5 ng of Aroclor 1254 (the fourteen major peaks being numbered I to XIV). (c) Combination of the above organochlorine standard pesticide mixture and Aroclor 1254. Injector temp., 250°; column temp., 200°; detector base temp., 250°; nitrogen flow rate, 20–30 ml/min.

TABLE 9

PERCENT RECOVERIES OF PCB'S AND PESTICIDES FROM FLORISIL COLUMNS BY ELUTION WITH HEXANE[14]

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

PCB peak No. (GLC)	*Expt. I, 100 ml of hexane*	*Expt. II, 200 ml of hexane*	*Pesticide[a] peak*	*Expt. III, 100 ml of hexane*	*Expt. IV, 200 ml of hexane*
			Lindane	None	None
1	80.1	92.2	Heptachlor	None	92.7
2	86.7	103.1	Aldrin	62.8	94.1
3	65.6	100.0	Hept. epox.	None	None
4	98.2	101.0	DDE	29.5	97.5
5	42.1	100.0	Dieldrin	None	None
6	44.9	98.7	DDD	None	None
7	64.0	101.2	*p,p'*-DDT	None	None
8	90.8	105.2			
9	66.4	105.8			
10	72.6	103.8			
11	76.9	99.9			
12	57.2	100.0			
13	100.0	100.0			
14	71.4	100.0			

[a] Under these experimental conditions 250 ml of 20% ethyl ether in hexane is sufficient to elute the pesticides although 200 ml can quantitatively remove them.

TABLE 10

PERCENT RECOVERIES OF PCB'S AND PESTICIDES FROM A MIXTURE AFTER SEPARATION ON FLORISIL (EXPTS. V AND VI)[14]

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.

Eluted with 200 ml of hexane			*With 250 ml of 20% ether in hexane*		
PCB and/or pesticide peak	*% Recovery[a]*	*% Recovery[b]*	*Pesticide peak*	*% Recovery[a]*	*% Recovery[b]*
			Lindane	93.6	98.5
Heptachlor	92.7	98.1	Heptachlor	None	None
PCB1	104.0	101.7			
PCB2 + Aldr	102.1	96.6	Aldrin	1.3	4.0
PCB3	100.0	106.0			
PCB4	104.2	102.6	Heptachlor epoxide	96.4	102.2
PCB5 + DDE[c]	97.8	104.4	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	99.8	92.5
PCB11	100.0	105.3			
PCB12	100.0	100.0			
PCB13	100.0	100.0			
PCB14	96.2	100.0			

[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 of hexane, receiver was changed, and the column eluted with 250 ml of 20% ethyl ether in hexane.

[b] Same as in [a] except that the PCB's and pesticides were first mixed with an extract from a 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.

with the exception of DDE, aldrin and heptachlor)[12]. Table 9 shows percent recoveries of PCB's and pesticides from Florisil columns by elution with hexane and indicates almost quantitative removal of the PCB's was effected with 200 ml of hexane while under the same conditions only three of the eight pesticides tried showed evidence of elution. Further experiments indicated the feasibility of column separation of PCB's and pesticides when mixed and when they were present in the extract from an animal tissue (Table 10).

After the Florisil separation of PCB's and pesticides has been effected, the two eluates are chromatographed separately on an SE-30/QF-1 column and compared with appropriate standards for quantitation; this is then normally followed by confirmation of the identities of the pesticides.

Despite the separation of the PCB's and their elimination as sources of interference it is still necessary to confirm the identity of the pesticides by additional techniques because of the non-specific nature of the electron capture detector. The sequence recommended by REYNOLDS[12] involves: (a) the analysis of the pesticide fraction on a more polar liquid phase column, *e.g.*, polyester such as DEGS or DEGA

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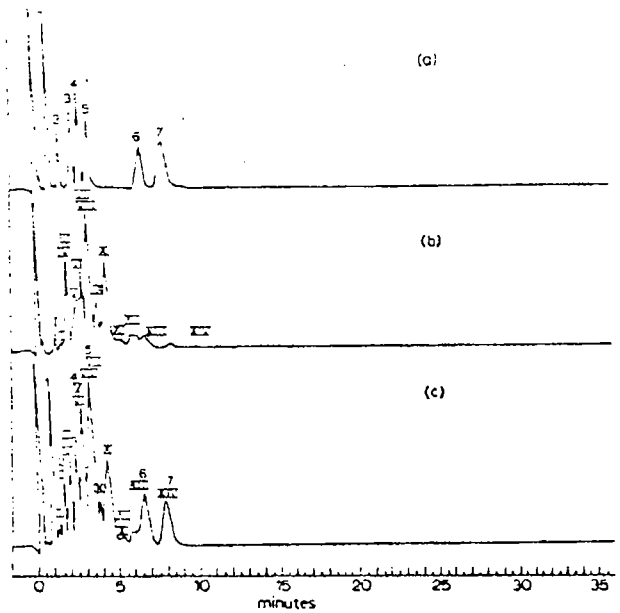

Fig. 18. Separation of PCB's and organochlorine pesticides on a polar-phase column (5% DEGS/2% H_3PO_4). (a) Standard mixture of organochlorine pesticides of the same amounts as in Fig. 17. 1 = heptachlor and aldrin; 2 = lindane; 3 = heptachlor epoxide; 4 = DDE; 5 = dieldrin; 6 = DDT; 7 = DDD. (b) 5 ng of Aroclor 1254. (The fourteen major peaks are numbered I to XIV as in chromatogram b of Fig. 17, assuming that the order of elution is unchanged.) (c) Combination of the organochlorine standard pesticide mixture and Aroclor 1254. Other GLC parameters as in Fig. 17.

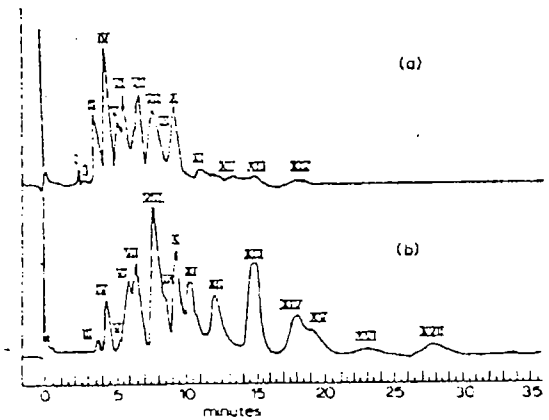

Fig. 19. Comparison of Aroclors 1254 and 1260. (a) 5 ng of Aroclor 1254; the fourteen major peaks are numbered I to XIV as in chromatogram (b) of Fig. 17. (b) 5 ng of Aroclor 1260; the seventeen major peaks are numbered I to XVII (the early peaks corresponding to those in Aroclor 1254). GLC parameters as in Fig. 17.

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and (b) derivatization and use of characteristic GLC retention times of the derivatives. For example, reaction with ethanolic potassium hydroxide yields products (via mainly dehydrochlorination) with shorter retention times. This technique is most effective for DDT, DDD and their isomers as well as α- and γ-BHC (the β-isomer is unaffected).

Typical GLC separation of PCB's, pesticides and a mixture of the two groups is shown in Fig. 18.

The two most commonly found PCB's in wildlife samples resemble Aroclor 1254 and 1260. Certain differences are exhibited in their GLC patterns that can be used to distinguish one from the other (Fig. 19). Aroclor 1260, with a higher chlorine content, shows about seventeen major peaks on an SE-30/QF-1 column compared to eleven with Aroclor 1254. Another major difference is the peak height ratio of peaks 10 and 13, *e.g.*, this ratio is approximately 9.3 and 1.0 for Aroclors 1254 and 1260, respectively.

Fig. 20 illustrates GLC chromatograms of a number of Aroclor mixtures determined on SE-30/QF-1 columns. The more highly chlorinated biphenyls (Aroclors 1254 and 1260) are easily detected while the compounds of the lower chlorinated mixtures, *e.g.*, Aroclors 1221, 1232 and 1242 and the higher-molecular-weight mix-

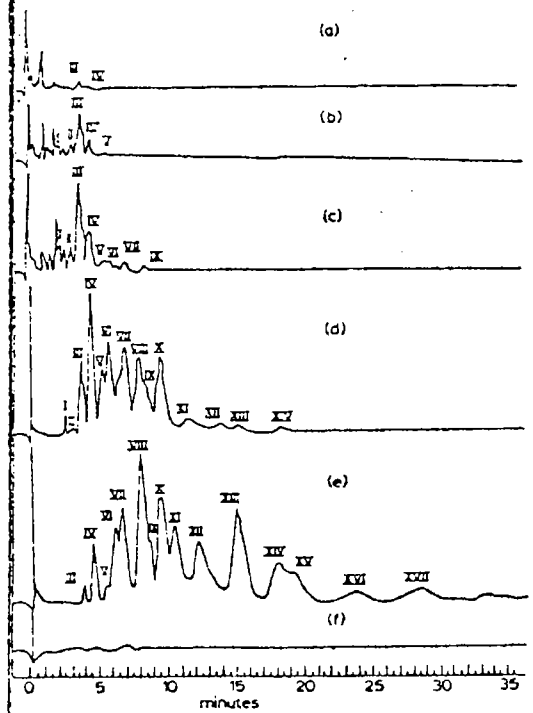

Fig. 20. GLC profiles of the more popular Aroclor mixtures under normal analytical conditions. All peak numbers correspond to those of Aroclor 1254. (a) 5 ng of Aroclor 1221. (b) 5 ng of Aroclor 1232; (c) 5 ng of Aroclor 1242. (d) 5 ng of Aroclor 1254; (e) 5 ng of Aroclor 1260; (f) 5 ng of Aroclor 1260. GLC parameters as in Fig. 17.

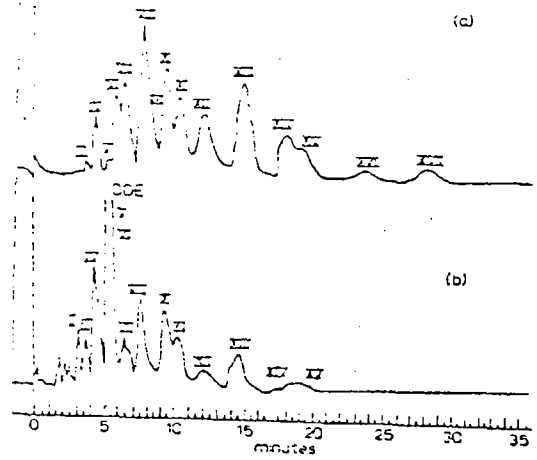

Fig. 21. Typical sample indicating the presence of PCB's of the Aroclor 1260 type in a duckling from the Toronto area. (a) 5 ng of Aroclor 1260 numbered I to XVII as in chromatogram b of Fig. 17. (b) The equivalent of 0.35 mg of duck sample (hexane portion of Florisil split). Note the DDE contribution to PCB peak V. GLC parameters as in Fig. 17.

tures (Aroclor 5460) are less responsive with the usual operating parameters and are thus more likely to go undetected.

Fig. 21 illustrates gas chromatograms of Aroclor 1260 found in a duckling and Fig. 22 indicates a resin powder extract from a fish hatchery trough indicating the presence of PCB's of the Aroclor 1254 type and illustrates an example of PCB's causing contamination as a direct result of industrial application.

The separation of PCB's from organophosphorus compounds by the Florisil technique has also been affected[12]. GLC utilizing a dual detector (electron capture plus a phosphate or thermionic) connected to a dual pen recorder permits the differentiation of the PCB's and organophosphates as shown in Fig. 23. The PCB's

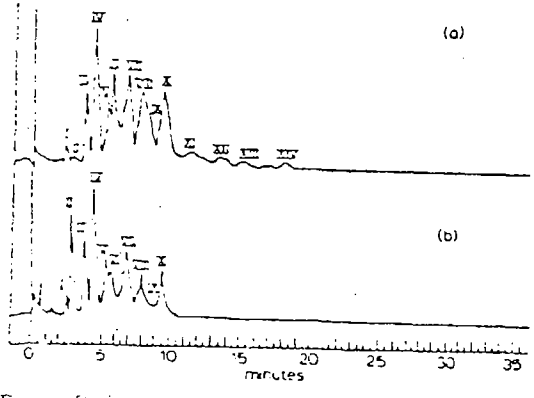

Fig. 22. Resin powder extract indicating the presence of PCB's of the Aroclor 1254 type. (a) 5 ng of Aroclor 1254 numbered I to XIV as in chromatogram b, of Fig. 17. (b) The equivalent of 0.2 mg of resin powder extract (hexane portion of Florisil split), numbered I to XIV as in Aroclor 1254. GLC parameters as in Fig. 17.

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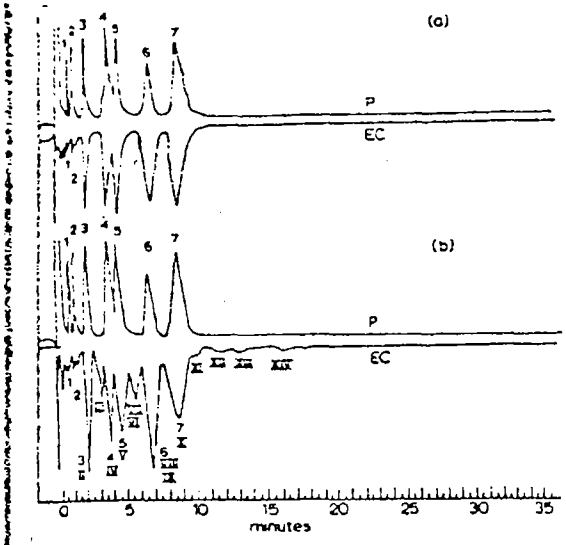

Fig. 23. Differentiation of Aroclor 1254 and organophosphorus pesticides using dual detectors and effluent split. (a) Organophosphorus standard pesticide mixture. 1 = 1.75 ng of phorate; 2 = 2.0 ng of diazinon; 3 = 3.0 ng Ronnel; 4 = 13.0 ng of malathion; 5 = 5.0 ng of parathion; 6 = 15.0 ng of methyl Trithion; and 7 = 10.0 ng of ethion. (b) Combination of organophosphorus mixture (chromatogram a) and 10 ng of Aroclor 1254 numbered I to XIV as in chromatogram b of Fig. 17 (a compromise of the EC response is necessary to obtain maximum response on the phosphorus detector). P = Phosphorus detector; EC = electron capture detector. Column 4% OV-100/6% OV-210 on 60–80 mesh acid-washed Chromosorb W. Other GLC parameters as in Fig. 17.

similarly to the organochlorine pesticides are not detected on the phosphate detector.

The estimation of PCB's following GLC analysis has been generally achieved via the following techniques: (1) the PCB estimation based on the peak of Phenoclor DP6 having R_T (relative retention time with dieldrin = 1) equal to 1.45 (ref. 2), (2) the PCB's are estimated on the assumption that they have similar electron capture responses to *p,p'*-DDE and a factor is applied to fit the assumed 54% chlorine content of the PCB's[54], (3) estimates of the PCB's are reported as the *sum* of all the PCB components[13], and (4) estimation of PCB's based on an average of two peaks[2].

A GLC study[45] of Aroclors 1221, 1232, 1254, 1260, 1262, 4465, 5442 and 5460 on columns of 10% DC-200 and 15% QF-1/10% DC-200 with electron capture detection showed the Aroclors to be multicomponent mixtures with retention times throughout and beyond the retention time range of common chlorinated pesticides. With a detector sensitivity producing $\frac{1}{2}$ full scale recorder response to 1 ng of heptachlor epoxide, significant responses were obtained with about 10–20 ng of the various Aroclors.

In the above study of ARMOUR AND BURKE[45] the efficacy of silicic acid columns for the separation of Aroclors and chlorinated pesticides added to trout and salmon extracts was also elaborated. Figs. 24 and 25 illustrate gas chromatograms of the mixtures of Aroclors and DDT analogs in trout and salmon, respectively, before and after column separation. Determinations by electron capture GLC showed the salmon

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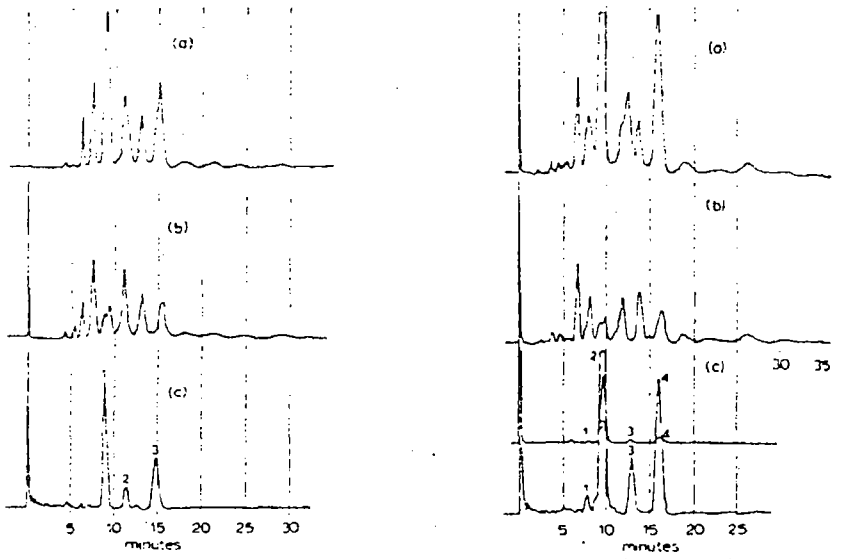

Fig. 24. GLC curves of brown trout extract fortified with 2.0 p.p.m. Aroclor 1254, 0.3 p.p.m. *o,p'*-DDT, 0.15 p.p.m. *p,p'*-TDE, and 0.13 p.p.m. *p,p'*-DDE (plus 0.19 p.p.m. residue) before and after separation on silicic acid column. (a) Before separation; (b) petroleum ether eluate from silicic acid column containing PCB; (c) polar eluate from silicic acid column containing (1) *p,p'*-DDE, (2) *p,p'*-TDE, and (3) *p,p'*-DDT. 10-mg sample injected for each curve.

Fig. 25. GLC curves of Lake Michigan coho salmon extract containing residues of PCB and DDT and analogs before and after separation on silicic acid column. (a) Before separation; (b) petroleum ether eluate from silicic acid column containing 14.6 p.p.m. PCB (as Aroclor 1254); (c) polar eluate from silicic acid column containing (1) 0.1 p.p.m. *o,p'*-DDE, (2) 10.4 p.p.m. *p,p'*-DDE, (3) 1.3 p.p.m. *o,p'*-DDT, and (4) 3.2 p.p.m. *p,p'*-DDT. 2.7 mg of sample injected for curves a, b, and c; 0.16 mg of sample injected for curve c, used for *p,p'*-DDE quantitation.

to contain 14.6 p.p.m. PCB, 3.2 p.p.m. *p,p'*-DDT, 1.3 p.p.m. *o,p'*-DDT, 10.4 p.p.m. *p,p'*-DDE and 0.4 p.p.m. *o,p'*-DDE. The PCB residue was measured in terms of Aroclor 1254, using total area of the GC response.

HOLMES *et al.*[9] described the detection of PCB's in British wildlife. In birds' livers and eggs they are often in greater quantities than organochlorine pesticide residues. GLC of kestrel liver was achieved utilizing three columns, *viz.*, SE-52, Apiezon-L and XE-60. The actual retention times recorded for the peaks on these columns are shown in Table II. Fig. 26 shows a chromatogram of an extract from a kestrel liver indicating the peaks corresponding to *p,p'*-DDE (0.43 ng), *β*-BHC (0.33 ng) and *γ*-BHC (0.03 ng). The other peaks form a series from PCB's. Fig. 26 also gives the analogous chromatogram of a commercial PCB resin and illustrates the similarity of peaks of high retention times. The peaks on the chromatogam for the kestrel liver shown in Fig. 26 were estimated to be equivalent to about 12 ng of polychlorobiphenyl compounds. This is contrasted against the organochlorine pesticide residues, which total about 0.8 ng, of which 0.33 ng is *β*-BHC, an isomer of BHC which is generally considered as non-toxic to wildlife. Further confirmation of the components of

TABLE II

RELATIVE RETENTION TIMES ON THREE DIFFERENT GLC COLUMNS (EXCLUDING TIMES FOR KNOWN PESTICIDES)[a]

KLE = Kestrel liver extract; PCB = polychlorobiphenyl resin.

Silicone GE/SE-52 column		*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
3.50	3.58	5.96	5.93	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				

[a] Dieldrin = 1.00.

using 1% acetone in hexane as developing solvent. Spots corresponding to the PCB's are 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 reversed-phase paper chromatography[55], whereby the unknown compounds (PCB's) have very much lower R_F values compared with the chlorinated pesticides and their metabolites.

HOLDEN AND MARSDEN[10] reported that seals and porpoises in Scotland and

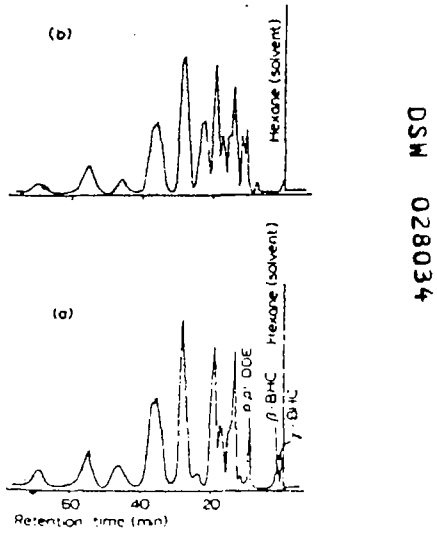

Fig. 26. Gas-liquid chromatogram of fat an extract of kestrel liver and the a commercial polychlorobi

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Canada, far from the sites of application of pesticide, can accumulate high concentrations of residues in their blubber. It was stressed that these chemicals are spreading through the long food chain which ends with seals and porpoises and of massive amounts can be confined to their place of discharge.

The cleaned-up hexane extracts[36] were analyzed by GLC on a Varian Aerograph 205-2B instrument employing two glass columns 5 ft. × 1/8 in. O.D. One column was packed with 10% DC-200 silicone oil on acid-washed, DMCS-treated 80–100 mesh Chromosorb W and the other with the same support coated with 5% DC-200 + 7.5% QF-1. The oven temperature was 200°, the nitrogen gas flow 50 ml/min and the column resolution for dieldrin equivalent to 1600 theoretical plates. While complete separation of the common pesticide residues was possible on one or the other of these columns, interference by PCB residues[5,9] with *p,p'*-TDE and *p,p'*-DDT necessitated a further analytical stage (alcoholic potassium hydroxide hydrolysis) which quantitatively converts *p,p'*-DDT to *p,p'*-DDE and *p,p'*-TDE to *p,p'*-MDE. Hydrolysis also destroys α- and β-BHC where present but PCB's are unaffected. A cyanosilicone (XE-60) column[37] has also been used to separate *p,p'*-DDT and *p,p'*-TDE from PCB interference, confirming the latter.

Unidentified peaks on the chromatograms appeared to be similar to those produced by PCB's and to enable comparison to be made, the ratios (R_x) of the retention times of all regularly occurring peaks (relative to dieldrin = 100) were calculated for the operating column temperature of 200° (these values being temperature dependent). The R_x values of PCB compounds from commercial PCB formulations were also de-

TABLE 12

RELATIVE RETENTION VALUES (R_x) OF RESIDUES[a]

DC-200 column			*DC 200/QF-1 column*		
Residue		*Sample*	*Residue*		*Sample*
PCB	= 68.5	68	PCB	= 68.5	70
p,p'-DDE	= 106	100	*p,p'*-DDE	= 84	83.5
Dieldrin			Dieldrin	= 100	100
PCB	= 121	125	*p,p'*-TDE	= 121	121
p,p'-TDE	= 129	128	PCB	= 123	122
PCB	= 148	149	PCB	= 146.5	—
p,p'-DDT	= 170	171	*p,p'*-DDT	= 147	147
PCB	= 174	175	PCB	= 234	231
PCB	= 205	205	PCB	= 282	278
PCB	= 282	287			
PCB	= 330	338			

[a] Dieldrin = 100.

termined for both types of column (Table 12). At least seven PCB-type peaks were found, including those which interfere with *p,p'*-TDE and *p,p'*-DDT.

The GLC analysis of organochlorine and heavy metal residues in bald eagle eggs was described by KREITZ *et al.*[35]. Bald eagle eggs collected in 1965 from nests in Wisconsin, Maine and Florida all contained residues of DDE, DDD, dieldr[illegible]

TABLE 13

DDT[a] AND PCB RESIDUES IN MARINE BIRDS[b] AND IN THE PEREGRINE FALCON[c] (ref. 59)

Species, locality, date	*Total DDT[d] (p.p.m.)*	*% DDE*	*PCB (p.p.m.)*	*DDT/PCB*
Cassin's auklet[e]	5.8	98	0.16	36
Ancient murrelet[f]	0.75	90	0.15	5
Fulmar[g]	0.41	76	0.08	5
Fulmar[g]	3.4	89	0.34	10
Red phalarope[h]	0.78	79	0.10	8
Rhinoceros auklet[i]	2.7	97	0.36	8
Slender-billed shearwater[j]	32.0	92	2.1	15
Sooty shearwater[k]	12.3	94	1.2	10
Sooty shearwater[k]	10.3	86	0.0	12
Peregrine falcon				
Breast muscle, second-year female, migrant from Arctic	104	99	22	4.5
Breast muscle, immature, California	13	99	10.5	1.2
Breast muscle, adult female, California	112	98	109	1.0

[a] From RISEBROUGH *et al.*[48] and RISEBROUGH *et al.*[9].
[b] Entire bird analyzed, except peregrine falcons.
[c] From RISEBROUGH *et al.*[9].
[d] Includes *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDMU, *p,p'*-DDU; p.p.m., wet weight.
[e] *Ptychoramphus aleuticus*, adult female, Farallon Islands, April, 1966.
[f] *Synthliboramphus antiquus*, Monterey Bay, Nov. 1, 1966.
[g] *Fulmarus glacialis*, Monterey Bay, Nov. 1, 1966.
[h] *Phalaropus fulicarius*, Monterey Bay, Nov. 1, 1966.
[i] *Cerorhinca monocerata*, Monterey Bay, Nov. 1, 1966.
[j] *Puffinus tenuirostris*, Monterey Bay, Dec. 12, 1966.
[k] *Puffinus griseus*, Monterey Bay, Nov. 1, 1966.

of chlorine per molecule). Many also contained traces of DDT. Fractions were analyzed on OV-17/3% XE-(8) according to REICHEL *et al.*[38]. Confirmatory residue analyses were performed on a 12% DEGS on Anakrom SO (100–110 mesh) column with a temperature of 100° and a nitrogen flow rate of 85 ml/min (the retention time of dieldrin was 9.5 min).

The widespread distribution of PCB's and chlorinated hydrocarbons in marine ecosystems of the Pacific Ocean has been reported by RISEBROUGH and co-workers[8,9]. Birds contain higher concentrations of these chemicals than fish, e.g., tissues of the peregrine falcon have contained the highest amounts which have so far been recorded. The PCB's have thus far not been detected in samples of airborne particulates, but their observed distribution in the sea indicates that they are dispersed by wind currents and that their fallout pattern is similar to that of the DDT compounds. Tables 13 and 14 depict the DDT and PCB residues in marine birds and in peregrin falcon and in the eggs of several bird species, respectively. Table 15 shows the DDT and PCB residues in marine fish. Of the commercial PCB preparations, those having the following retention times relative to *p,p'*-DDE on DC-200 and QF-1 columns have been detected in marine fish and birds:

DC-200: 1.35, 1.48, 1.75, 2.05, 2.11, 2.50, 2.90, 3.11, 3.88, 5.51

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Dieldrin: 1.05; *p,p'*-DDD: 1.27; *p,p'*-DDT: 1.98

Others: 1.19, 1.33, 1.50, 1.65, 1.72, 2.12, 2.53, 3.23, 3.88, 4.84

Dieldrin: 1.20; *p,p'*-DDD: 1.75; *p,p'*-DDT: 1.91

The retention times of three of the principal peaks are underlined. Similar values for the DC-200 column have been reported from extracts of seals from the North Sea.

TABLE 14

DDT AND PCB CONTENT IN EGGS OF SEVERAL BIRD SPECIES[59]

Species, locality	*No.*	*Total DDT*[a] *(μg)*	*p,p-DDE (%)*	*PCB*[a] *(μg)*	*DDT/PCB*
Brandt's cormorant[b]					
(Ekala resseay penicillatus)					
Farallon Islands	17	326	91	113	2.9
Pelagic cormorant					
(Phalacrocorax pelagicus)					
San Mateo Co., Calif.	2	128 (125–130)	99	62	2.1
Murre					
Uria aalge					
Farallon Islands	6	1945 (932–3621)	96	558 (364–1019)	3.5
Pigeon Guillemot					
Cepphus grylle					
Farallon Islands	1	110	93	20	5.5
San Mateo Co.	1	103	91	62	1.7
Cassin's auklet					
Ptychoramphus aleuticus					
Farallon Islands	2	147 (127–167)	97	15	10
Western Gull					
Larus occidentalis					
Farallon Islands	1	423	95	118	3.6
San Mateo Co.	1	235	94	112	2.1
San Francisco Bay	1	458	87	480	0.95
Black-crowned night heron					
Nycticorax nycticorax					
San Francisco Bay	1	541	89	330	1.6
	1	869	99	24	36
Caspian tern					
Hydroprogne caspia					
San Francisco Bay	2	1260 (1210–1320)	89	805 (960–950)	1.7 (1.3–2.0)
San Diego Bay	5	1440	88	1010 (550–1600)	1.4
Forster's tern					
Sterna forsteri					
San Diego Bay	2	1165 (510–732)	89	114 (91–137)	5.8
Least petrel					
Halocyptena microsoma					
Baja California	2	30 (23–37)	82	3.1 (1.2–5.0)	10
Peregrine falcon					
Falco peregrinus					
Baja California	1	2830	98	471	10

[a] Fat basis, wet wt.

[b] Sample 1 pooled for PCB analysis; *p,p'*-DDE content ranged from 81–112 p.p.m.

[c] From Risebrough *et al.*[59]

lantic[19]. Concentrations of the chlorinated hydrocarbons, both DDT and PCB, tend to be an order of magnitude higher in marine birds than in fish (Tables 13 and 15). As shown in Tables 13 and 14, PCB is widely distributed among marine birds which are the terminal carnivorals of a complex mesh of food chains in the sea.

The chlorine content of extracts of other birds and fish was determined with

TABLE 15

DDT[a] AND PCB RESIDUES IN MARINE FISH[59]

Species, locality, date	*No.*	*Mean wt. (g)*	*Total DDT (p.p.m.)*	*p,p'-DDE (%)*	*PCB (p.p.m.)*	*DDT/PCB*
Northern anchovy						
Terminal Island	44	11.7	14.0	83	1.0	14
June 25, 1965		(6.5–20)	±1.9			
Shiner perch						
San Francisco Bay	14	5.5	1.0	28	1.2	0.8
October 20, 1965		(4–8)	±0.1			
San Francisco Bay	10	26.7	1.4	35	0.4	3.5
October 20, 1965		(10–48)	±0.3			
San Francisco Bay	15	15.3	1.1	33	1.2	0.9
November 4, 1965		(8–19)	±0.1			
English sole						
San Francisco Bay	18	14.3	0.55	25	0.11	5
July 29, 1965		(5–35)	±0.07			
San Francisco Bay	33	17.3	0.55	24	0.11	5
November 4, 1965		(7.5–53)	±0.12			
San Francisco lightship	15	253	0.19	63	0.05	4
December 1, 1965		(175–306)	±0.04			
Monterey	15	195	0.76	70	0.04	19
February 15, 1966		(89–262)	±0.16			
Jack mackerel						
Channel Islands	31	81.8	0.56	57	0.02	28
November 22, 1965		(45–141)	±0.10			
Hake						
Puget Sound	22	281	0.18	23	0.16	1.1
January 29, 1966		(185–350)	±0.05			
Channel Islands	6	384	1.8	68	0.12	15
February 24, 1966		(61–872)	±1.1			
Bluefin tuna						
Body muscle	7	—	0.56	45	0.04	14
			±0.24			
Liver	9	—	0.22	45	0.04	6
			±0.13			
Yellowfin tuna						
Liver	13	—	0.07	13	nd	> 7
			±0.02			
Liver	13	—	0.62	30	0.04	15
			±0.19			
Skipjack tuna						
Liver	3	—	0.057	23	0.1	0.6
Body muscle	13	—	0.051	18	nd	>30
			±0.014			
Liver	25	—	0.050	11	nd	>30
			±0.021			
Liver	12	—	0.020	41	nd	>20
			±0.008			

[a] From Risebrough *et al.*[59]; concentrations in wet weight, p.p.m., means, standard errors, 95% confidence limits.

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a 3 H tritium source electron capture detector and a method of quantification of the PCB compounds was devised based on peak heights produced in the electron capture detector related to the standard p,p'-DDE[61]. The DDT compounds were destroyed by nitration[61] and p,p'-DDT, DDD and toxaphene dehydrochlorinated by saponification with alcoholic potassium hydroxide (PCB was not degraded by either procedure).

Methods for the determination of PCB's were surveyed by RISEBROUGH *et al.*[62]. Several of the major GC peaks obtained for PCB on 10% DC-200/Chromosorb W and 5% QF-1 Chromosorb W columns at 195° were found to interfere with the determinations of DDT and DDD. The three major peaks generally have approximately the same height on chromatograms obtained with an electron capture detector. Only negligible amounts of p,p'-DDD and p,p'-DDT are present, if the PCB peak with a retention time of 1.48 on a DC-200 column is approximately as high as the DDD and DDD peaks (retention times of 1.27 and 1.68, respectively). However, when the DDT and or the DDT peaks were higher than the PCB peak with a retention time of 1.48, the amounts of p,p'-DDD and p,p'-DDT could be estimated from the changes in peak height after a 5-min saponification with 5% potassium hydroxide in ethanol.

PARSLOW *et al.*[63] described aspects of polychlorinated biphenyls in wild birds in Great Britain and their avian toxicity. The amounts of PCB's in the livers and eggs of wild birds were determined by GLC using electron capture detection and columns of Silicone/Epikote on Diatomite CQ following prior silica gel column separation of organochlorine insecticides. PCB's were found in terrestrial species from most regions of Great 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 fresh water species collected from the Midlands, East and South of England. The highest liver residues were found in fresh water fish-feeding birds (up to *ca.* 900 p.p.m.) and bird-feeding raptors (up to 70 p.p.m.) with the levels present similar to those of p,p'-DDE.

The avian toxicity of Aroclor 1254 fed to Bengalese finches (estimated dose rate for 50% mortality at fifty-six days) was 252 mg/kg/day. At this dose rate the calculated mean liver content was 345 p.p.m. Aroclor 1254 has only 1/13 the toxicity of DDT, but could be considered more toxic at low doses since 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 although PCB is unlikely to have caused widespread lethal toxicity in wild predatory birds in Great Britain, it could be a component cause of the present breeding failure reported in several species.

The source of PCB contamination in the marine environment (Glasgow and Clyde areas) was studied by HOLDEN[62]. Although the extraction of organochlorine compounds from sewage solids using a mixture of *n*-hexane and isopropanol is believed to be less efficient than extraction from water, it was found by HOLDEN[62] that many sludge samples gave gas-liquid chromatograms similar to those of PCB mixtures.

Table 16 shows the proportions in terms of relative peak heights of the major peaks in typical chromatograms, as identified by their R_x values (retention time of 200 relative to dieldrin = 100). The column used was packed with 10% DC-200 silicone on Chromosorb W. PCB compounds were initially separated from all major organochlorine pesticide residues, except p,p'-DDE on silica columns[64].

TABLE 16

RELATIVE PEAK HEIGHTS IN GAS-LIQUID CHROMATOGRAMS

Sample	R_x										
	69	*81*	*100*[a]	*121*	*145*[b]	*171*	*209*	*234*	*283*	*333*	*379*
Raw sludge A	38	63	51	104	100	92	32	19	21	10	5
Raw sludge B	39	24	17	51	100	66	70	53	84	29	25
Aroclor 1254	30	72	59	110	100	92	26	12	19	8	4
Aroclor 1260	3	14	4	30	100	69	70	55	87	30	23

[a] This peak includes p,p'-DDE in sludge.
[b] Peak height = 100 for reference.

The peak pattern for sludge A closely resembles a PCB formulation containing about 50% by weight of chlorine (Aroclor 1254) but the pattern for sludge B suggests Aroclor 1260. On the basis of wet weight of raw sludge the estimated concentrations of PCB's using Aroclor 1254 as reference in fifteen samples were in the range <0.1 to 14 p.p.m. In terms of dry matter the values ranged from 1 to 185 p.p.m. It was estimated that an average concentration of PCB of the order of 1 p.p.m. would be equivalent to a discharge in the Clyde estuary of the order of one ton of PCB's per year. PCB compounds found in wildlife, using GLC at about 200°, have been shown to contain mostly six to seven chlorine atoms[2], although compounds with more chlorine atoms may not be sufficiently volatile to be detectable at this temperature.

The chronic toxicity, uptake and retention of Aroclor 1254 in two estuarine fishes, pinfish (*Lagodon rhomboides*) and spot (*Leiostomus xanthurus*) was described by HANSEN *et al.*[61]. The results suggested that chronic exposure to Aroclor 1254 increased susceptibility of the test fish to disease and also appeared to be toxic to these fish. This PCB is rapidly stored by pinfish and spot and persists in tissues for approximately three months. Chemical analyses for residues of Aroclor 1254 in pooled samples of fish tissue were performed by GLC following: (a) elution and concentration from a Florisil column[65] with 6% ethyl ether in petroleum ether, and (b) extraction of samples of less than 1 g by a modification of the ENOS[66] micromethod. The column eluates were analyzed by electron capture gas chromatographs equipped with DC-200 columns. The multiple-peaked Aroclor 1254 was quantitated by averaging the heights of five major peaks having retention times relative to aldrin of 1.31, 1.55, 2.32, 2.74, and 3.27. Interference from DDT and its metabolites was negligible due to relatively high concentrations of Aroclor 1254 found in the samples.

Spot exposed to 1 p.p.b. Aroclor 1254 for fifty-six days rapidly stored this PCB, with maximum levels being attained in fourteen to twenty-eight days. Thereafter the relative amount (p.p.m.) was generally constant, whereas the absolute amount (μg) continued to increase as the fish grew. The liver concentrated the greatest relative amount of this PCB followed in decreasing order by the gills, whole fish, heart, brain, and muscle. Maximum concentrations in whole spot were 3.7×10^4 times that in the test water. In earlier work with DDT[67] it was shown that when pinfish and Atlantic croaker (*Micropogon undulatus*) were exposed to 0.1 and 1.0 p.p.b. p,p'-DDT in water, the DDT content increased for two weeks, then remained constant at 10^4 to 3.8×10^4 times the exposure concentration. Table 17 lists the test conditions,

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TABLE 17

TEST CONDITIONS, MORTALITY OF FISHES, AND UPTAKE OF AROCLOR 1254 IN FLOWING WATER BIOASSAYS

Fish	*SL*[a] *(mm)*	*Number*[b] *exposed*	*Concentration (p.p.b.)*	*Mortality*[c] *25% day*	*50% day*	*Terminal percent*	*Days exposed*	*Aroclor 1254 (p.p.m.)*[d] *in fish after exposure*	*Water temperature (°C)*	*Salinity (‰)*
Spot	25	150	1	-	-	17	33	17	11–16	16–32
Spot	40	150	1	-	-	1	59	27	23–32	10–33
Spot	24	60	5	12	18	51	20	46	11–18	16–32
Spot	24	300	5	23	30	53	26	120	8–16	20–32
Spot	24	50	5	28	38	62	45	152	28–33	23–31
Pinfish	30	50	5	9	12	66	14	44	16–22	20–32
Pinfish	27	54	5	17	—	41	35	109	22–32	14–34

[a] SL = Sample length.
[b] An equal number of fish were held in control aquaria; control mortality never exceeded 7%.
[c] Mortality of spot exposed to 5 p.p.b. Aroclor was significantly greater in all tests ($P < 0.001$) than that of control fish (χ^2 values were all greater than 10.83).
[d] Milligrams per kilogram.

TABLE 18

LEVELS OF AROCLOR 1254 IN p.p.m., WET WEIGHT, IN SPOT EXPOSED TO 1 p.p.b. OF THIS CHEMICAL (EACH SAMPLE CONSISTED OF TISSUES FROM TEN FISHES)

Total micrograms of Aroclor in parentheses.

Tissue	*Number of days exposed*							*Number of days of flushing*					
	0	*3*	*7*	*14*	*28*	*42*	*56*[a]	*14*	*28*	*42*	*56*	*70*	*84*[a]
Brain	0.4	3.6	6.0	8.6	8.4	12.0	8.3	7.6	5.8	5.0	3.5	3.4	2.9
	(0.1)	(1.1)	(2.1)	(3.9)	(3.7)	(5.9)	(5.2)	(5.6)	(4.7)	(4.1)	(3.5)	(3.4)	(3.1)
Gills	0.2	9.0	11.0	29.0	40.0	39.0	46.0	30.0	30.0	24.0	12.0	14.0	12.0
	(0.4)	(8.3)	(13.0)	(41.0)	(64.0)	(82.0)	(74.0)	(75.0)	(72.0)	(65.0)	(35.0)	(46.0)	(37.0)
Heart	ND[b] (< 0.1)	5.5	6.8	10.0	12.0	17.0	13.0	10.0	7.8	6.1	5.3	4.7	2.5
		(0.3)	(0.5)	(1.0)	(0.8)	(1.6)	(1.5)	(1.7)	(1.0)	(0.8)	(1.0)	(0.8)	(0.6)
Liver	1.2	34.0	75.0	96.0	210.0	107.0	83.0	65.0	52.0	28.0	17.0	29.0	22.0
	(0.1)	(12.0)	(42.0)	(92.0)	(126.0)	(86.0)	(72.0)	(83.0)	(60.0)	(38.0)	(30.0)	(58.0)	(35.0)
Muscle	0.1	1.0	1.8	3.6	7.4	7.6	6.5	6.9	4.8	3.4	3.3	2.5	3.0
	(0.1)[c]	(1.8)	(4.5)	(17.0)	(24.0)	(36.0)	(45.0)	(65.0)	(45.0)	(34.0)	(41.0)	(40.0)	(32.0)
Whole fish	0.1	3.5	7.2	17.0	37.0	30.0	27.0	25.0	19.0	12.0	9.3	9.3	7.3[d]
	(1.5)	(72.0)	(201.0)	(661.0)	(1417.0)	(1553.0)	(1983.0)	(2452.0)	(2041.0)	(1373.0)	(1369.0)	(1486.0)	(782.0)

[a] The same tissues analyzed from control spot contained no detectable Aroclor 1254 (<0.1 to <1.0 p.p.m. depending on sample weight).
[b] ND = not detected.
[c] Total micrograms in sample; sample approximately 26% of total amount of muscle.
[d] Other tissues analyzed on day 84 were: gall bladder 1.9 (0.9), gonad 0.9 (0.3), gut 2.5 (7.8), rest of muscle 4.5 (133.0), and skin 12.

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toxicity of fish, and uptake of Aroclor 1254 in flowing water bioassay and Table 18 depicts the tissue levels (brain, gills, heart, liver, muscle) and whole fish in specimens exposed to 1 p.p.b. of Aroclor 1254.

Kitta *et al.*[84] studied the effects of uptake of Aroclor 1242 on growth, nucleic acids and chlorophyll of the marine diatom *Cylindrotheca closterium*.

Following exposure of the cells to 0.1 and 0.01 p.p.m. levels of the PCB, the PCB was extracted from a dried lyophilized pellet with acetone and analyzed by GLC using two column systems: (a) a 6 ft. × 1/4 in. O.D. glass column packed with 4%, SE-30/2%, QF-1 on Chromosorb XXX and (b) a 6 ft. × 1/2 in. O.D. glass column packed with 1.5%, OV-17/1.95% QF-1 on Chromosorb XXX. The operating parameters were: inlet temperature 235°, column temperature 200°, detector temperature 250°, and carrier gas flow 60 ml/min. Identification of Aroclor 1242 was accomplished by measuring relative retention times of the five major peaks along with relative peak heights. Quantification was accomplished by comparison of the area under the curve of the two late eluters with the standard Aroclor. Verification of the peak identities by TLC positively identified Aroclor 1242 as the major PCB component of the sample. Some PCB materials not common to the known Aroclor 1242 mixture were isolated. These materials, in all cases early GLC eluters, were believed to be possible metabolic products of Aroclor 1242.

Cylindrotheca closterium were found to absorb and concentrate Aroclor 1242 600 to 1000 times above the level in sea water. At 0.1 p.p.m. concentration in sea water PCB's inhibited growth and diminished levels of RNA and chlorophyll of this marine diatom.

The occurrence of Aroclor 1254 in the water, sediment and biota of Escambia Bay, Fla., was described by Duke *et al.*[69]. Tissue of fish, crabs, oysters and shrimp were extracted for 4 h with petroleum ether in a Soxhlet apparatus following initial mixing with anhydrous sodium sulfate. Extracts were concentrated, partitioned with acetonitrile, the acetonitrile then evaporated just to dryness, the residue transferred to a Florisil column[85] with petroleum ether, and the Aroclor 1254 eluted from the column with 6% ethyl ether in petroleum ether.

Sediment samples were dried at room temperature and extracted for 4 h with 10% acetone in petroleum ether in a Soxhlet apparatus, extracts evaporated to dryness and the residues eluted from a Florisil column as described above. The extracts of all substrates were identified and measured by electron capture GC using three columns of different polarity, *e.g.* DC-200, QF-1 and mixed DC-200 and QF-1 to confirm identification. In a few samples, TLC was employed for additional confirmation and to assess the amount of DDT present. Interference from DDT was negligible due to the relatively high residues of Aroclor 1254 found in the samples. Quantitation of this multiple peak compound was approximated by averaging the peak height of five major peaks indicating recovery rates above 80%.

Additional acute studies indicated that juvenile shrimp were the most sensitive to Aroclor 1254 and were killed when exposed to 5.0 p.p.b. in flowing sea water. The Aroclor content in water from Es Amble Bay, even near the mouth of the river, contained less than 1 p.p.b. and shrimp collected from the bay contained a maximum of 2.5 p.p.m. The above study illustrated the need for both conducting continued surveillance of estuaries in order to preserve these nursery grounds for valuable fisheries resources as well as conducting long-term tests on the effect of sublethal concentrations

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TABLE 19

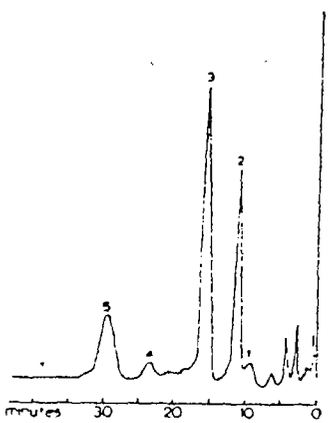
ORGANOCHLORINE RESIDUES (ng/g FRESH WEIGHT) IN BREAST MUSCLES OF ROBINS

	µg of PCB ingested	*p,p'*-DDE	*p,p*-DDT	PCB
Birds fed with PCB (Clophen A60)	60	95	7	375
	55	96	25	467
	65	75	25	341
	55	64	21	164
	60	91	22	365
	55	56	2	287
Control birds	—	72	23	120
	—	69	27	70
	—	70	30	90
	—	66	26	51

tions of PCB's on estuarine organisms in sensitive stages of their life history.

The effect of PCB on the nocturnal activity in caged robins (*Erithacus rubecula* L.) was described by Ulfstrand *et al.*[70].

The breast muscles of six birds fed a diet containing Clophen A50 were analyzed by GLC utilizing a Varian Aerograph 204 instrument equipped with electron capture detectors and three columns containing SF-96 (4%), QF-1 (8%) and SF-96/QF-1 (3:1) as the stationary phases on 100–120 mesh Gas-Chrom P. Table 19 depicts the organochlorine residues (*p,p'*-DDE, *p,p'*-DDT and PCB) found in breast muscles. The PCB level in the experimentally contaminated birds was four times that found in



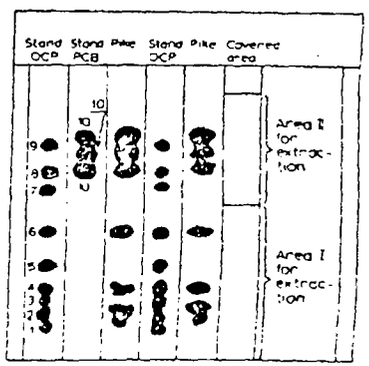


Fig. 27. Gas chromatogram of an extract of human milk (heptachlor epoxide added as internal standard). 1 PCB peak 2; 2 heptachlor epoxide; 3 *p,p'*-DDE, disturbed by PCB peaks 4 and 5; 4 PCB peak 8; 5 *p,p'*-DDT, disturbed by PCB peak 10. *Cf.* Fig. 30.

Fig. 28. Thin-layer chromatograms of standards and extracts of pike. 1 Dieldrin; 2 heptachlor epoxide; 3 *p,p'*-DDD; 4 lindane; 5 α-BHC; 6 *p,p'*-DDE; 7 *o,p'*-DDD; 8 *o,p'*-DDE; 9 *p,p'*-DDT; heptachlor; 11 aldrin; 10 PCB.

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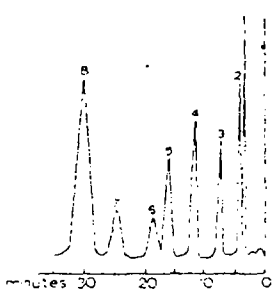
the controls, but it was in unexpectedly low concentrations, indicating that much of it had been excreted or stored elsewhere than in the breast muscles.

WESTÖÖ AND NORÉN[71] reported the determinations by GLC and TLC of organochlorine pesticides in animal foods in the presence of polychlorinated biphenyls. The extraction of organochlorine pesticides and PCB's from animal foods, routine clean-up of the extract as well as main GLC and TLC procedures were performed according to NORÉN AND WESTÖÖ[72].

Figs. 27 and 28 illustrate a gas chromatogram of an extract of human milk and a thin-layer chromatogram of standards and extracts of pike, respectively, and show peaks and spots often observed for various PCB's and organochlorine pesticides.

Figs. 29 and 30 show gas chromatograms of some chlorinated pesticides and a PCB standard, respectively, and illustrate the interference seen in food analysis,



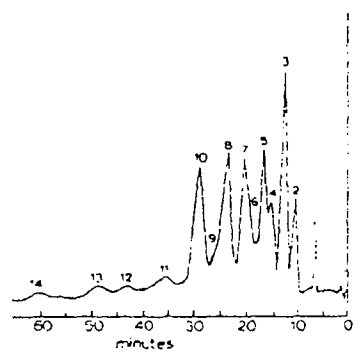


Fig. 29. Gas chromatogram of some chlorinated pesticides. 1 = α-BHC; 2 = lindane; 3 = aldrin; 4 = heptachlor epoxide; 5 = p,p'-DDE; 6 = dieldrin; 7 = p,p'-DDD; 8 = p,p'-DDT.

Fig. 30. Gas chromatogram of a PCB standard.

especially in fish where the PCB level is occasionally high. Fig. 31 depicts gas chromatograms for the analysis of PCB and chlorinated pesticides in a sample of herring before and after oxidation with chromic acid at room temperature (p,p'-DDE is decomposed by oxidation without affecting the PCB).

Both Varian Aerograph Hy-Fi Models 600 and III-1200 gas chromatographs were used equipped with electron capture detectors. The following columns were used: a 5 ft. × 1/8 in. Pyrex glass column packed with 5% DC-11 on 60-80 mesh Chromosorb W (nitrogen flow rate, 75 ml/min; column, injector and detector temperatures, 105°, 210° and 105°, respectively) and a 5 ft. × 1/8 in. Pyrex glass column packed with a mixture of equal parts of 5% DC-11 on 60-80 mesh Chromosorb W and 15% QF-1 on 60-80 mesh Chromosorb W (nitrogen flow rate, 30 ml/min; column, injector and detector temperatures, 171°, 200° and 200°, respectively).

For TLC[72] Aluminum Oxide G plates were developed with n-hexane hydrous diethyl ether (2010 8). Detection was accomplished using a silver nitrate spray consisting of 0.10 g of silver nitrate in 1 ml of water and 10 ml of 2 phenoxyethanol diluted to 200 ml with acetone and treated with one drop of 30% hydrogen peroxide[73].

GRANT et al.[74] described the oral metabolism of Aroclor 1254 in male animal

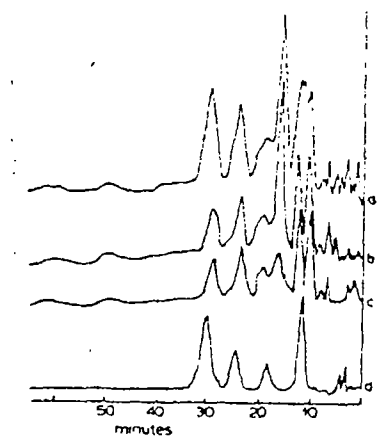


Fig. 31. Gas chromatograms performed for the analysis of PCB and chlorinated pesticides in a sample of Baltic herring (heptachlor epoxide added as internal standard). For standards, cf. Figs. 29 and 30. (a) original extract; (b) extract of Area II, Fig. 28, before oxidation; (c) extract of area II, Fig. 28, after oxidation; (d) extract of area I, Fig. 28.

and carbon tetrachloride-treated rats. Hexane extracts of tissues were analyzed by GLC–electron capture analysis on a Varian Aerograph Model 600D gas chromatograph fitted with a coiled 4 ft. × 1/4 in. O.D. glass column containing 4% SE-30 and 6% QF-1 on 80-100 mesh Chromosorb W. The nitrogen flow rate was 120 ml/min, with column and injection temperatures of 193° and 225°, respectively.

Residues were found in all tissues analyzed (blood, heart, kidney, brain, liver and fat), with the greatest concentration in the fat. The GLC–EC pattern of the residues was different from the standard mixture administered, indicating that all components were not metabolized at the same rate. Higher residues were found in the carbon tetrachloride-treated rats. Aroclor 1254 residues in the brain, liver, spleen, blood, testes, heart, kidney and fat were reduced by 90, 84, 80, 79, 78, 76, 64 and 33%,

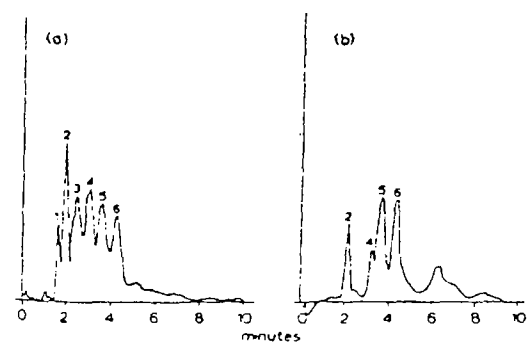


Fig. 32. GLC–EC tracings from (a) 24 ng of Aroclor 1254 and (b) the residue found in the liver of a Group 2 (cf. Table 20).

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TABLE 20

THE AMOUNT (%) THAT EACH OF THE SIX MAJOR PEAKS OF THE AROCLOR 1254 STANDARD CONTRIBUTES TO THE TOTAL RESIDUES IN THE TISSUES[a]

	Peak No.						Peak No.					
	1	2	3	4	5	6	1	2	3	4	5	6
	Group 1						Group 2					
Spleen	6	21	9	22	21	21	3	17	2	11	34	33
Testes	3	15	6	21	27	28	1	19	4	11	35	32
Liver	2	11	3	30	28	27	0	25	2	18	26	29
Kidney	8	21	11	21	19	19	0	18	2	14	34	32
Fat	7	25	11	25	17	16	1	19	2	20	28	29
Brain	1	13	5	22	30	29	2	19	4	12	34	29
Blood	4	13	6	24	26	26	1	20	3	12	33	31
Heart	4	17	6	21	26	26	1	17	2	8	31	39
	Group 3						Group 4					
Spleen	7	25	14	19	18	17	—	—	—	—	—	
Testes	6	23	14	19	19	19	1	20	4	12	31	32
Liver	4	21	11	21	22	21	0	19	4	29	26	31
Kidney	8	22	15	18	18	17	1	21	4	8	32	34
Fat	10	29	16	21	13	12	1	20	5	20	26	28
Brain	6	25	13	20	18	18	1	22	4	11	30	31
Blood	5	22	14	22	18	18	2	22	3	11	29	33
Heart	5	27	15	18	18	17	1	20	3	10	32	34

[a] The six major compounds are present in the following percentages: (1) 12%; (2) 25%; (3) 18%; (4) 17%; (5) 15%, and (6) 13%.

respectively, in twenty days. Aroclor 1254 significantly increased the size of the liver and also the percent lipid in the liver as well as to potentiate the toxicity of carbon tetrachloride in the rat.

Fig. 32 shows the chromatograms from 24 ng of Aroclor 1254 and from the residue found in the liver of a rat. The amount (%) that each of the six major peaks of the Aroclor contributes to the total residues in the tissues is presented in Table 20.

Fig. 32 and Table 20 show that the components of the Aroclor 1254 mixture with the shorter retention times, peaks 1, 2 and 3, and presumably with the lowest chlorine contents[2] were metabolized to a greater degree than those with the longer retention times. This observation agrees with that reported in studies with Phenoclor DP6 fed to Japanese quail[3]. Expression of the Aroclor 1254 residues in the tissues relative to those in blood (Table 21) shows that the ratios depend on the length of time following the oral dose (kidney, brain, liver and fat, Group 1 vs. 2) and the metabolic activity of the liver (liver and fat, Group 1 vs. 3). Table 22 depicts residues in tissues of rats forty-five days after being orally administered a single dose of Aroclor 1254 (500 mg/kg).

A comparative toxicologic study with PCB's in chickens with special reference to porphyrin, edema formation, liver necrosis and tissue residues was described by DE VOS AND KOLMAN[4]. Three of the chlorinated PCB's were used in a comparative subacute oral toxicity test (400 p.p.m.) in chickens, e.g., Phenoclor DP6 (I), Clophen A60 (II) and Aroclor 1260 (III). Using mortality, mean survival time, mean weight gain, pathological observations (hydropericardium, abdominal and subcutaneous edema

TABLE 21

CONCENTRATIONS OF THE AROCLOR 1254 RESIDUES IN TISSUES RELATIVE TO THOSE IN BLOOD

	Group			
	1	2	3	4
Blood	1.00	1.00	1.00	1.00
Testes	9.81	10.24	8.62	22.48
Heart	12.33	13.88	16.21	24.68
Spleen	14.88	13.86	9.51	—
Kidney	15.89	26.67	14.90	44.32
Brain	20.40	9.35	10.89	23.84
Liver	59.17	44.88	206.88	75.16
Fat	508.24	1601.57	233.89	4576.20

and centrolobular liver necrosis) as parameters, a significant difference in toxicity was found, e.g., I and II showed the highest and III the lowest. Microscopically centrolobular liver necrosis was found in chicks fed compounds I and II. Atrophy of the spleen was found in all test groups and chemical porphyrin was found as a general PCB effect: increased fecal excretion of coproporphyrin and protoporphyrin and fluorescence of tissues occurred in all test groups. This porphyrogenic effect was also observed in Japanese quail and rats treated with 2000 p.p.m. of compound I. GLC analysis of liver and brain of dead chicks gave PCB levels that varied from 120 to 2900 p.p.m. Tissues were extracted with petroleum ether in a Soxhlet extraction apparatus after drying with anhydrous sodium sulfate. Clean-up of the samples was performed by liquid–liquid partition with dimethylformamide and column chromatography using activated Florisil. The PCB preparations in the final extract were measured by GLC using a Varian Aerograph Model 204-1B with electron capture detection and a Pyrex glass column (5 ft. × 1/8 in.) filled with 10% DC-200 on 80–100 mesh Gas-Chrom Q operated at 200° with nitrogen as carrier gas at a flow rate of 50 ml/min. For quantitation one peak, considered to be representative, was selected (the peak with R_x = 1.45 relative to dieldrin). The approximate amount of total PCB

TABLE 22

RESIDUES IN TISSUES OF RATS 45 DAYS AFTER BEING ORALLY ADMINISTERED A SINGLE DOSE OF AROCLOR 1254 (500 mg/kg)

Group 2 of Experiment II killed on day 47.

	Residue				
	Wet tissue (p.p.m.)			Relative to blood	
Blood	0.458[a]	±	0.05[b]	1.00	
Heart	2.71	±	0.61	15.09	
Kidney	3.39	±	0.29	18.83	
Brain	4.19	±	0.44	23.28	
Liver	16.11	±	3.79	89.11	
Fat	197.40	±	41.86	1207.22	

[a] Mean of five values.
[b] Standard error of the mean.

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residue was calculated from the height of this peak with compound I (Phenoclor DP6) as the standard.

In a number of tissue extracts the amounts of PCB were measured also by a Dohrmann C 250A microcoulometric detection system equipped with a T-[illegible]-S-halogen cell coupled to a Microtek MT-220 gas chromatograph and employing a 6 ft. × 1/2 in. Pyrex column filled with a mixed-bed column packing QF-[illegible] DC-200[illegible]. With this system 0.6 μg of Phenoclor DP6 gave about half-scale response on a 1-mV recorder (microcoulometer range: 200 ohms). By this method, the content of chlorine in the extract is determined by addition of the chlorine contents of the different PCB peaks. Since the average chlorine contents of the compounds used were 60%, the total residue could be calculated.

Ingestion of rice bran oil contaminated with chlorobiphenyls (Kanechlor [illegible])

TABLE 23

METHOD OF EXTRACTION AND CLEAN-UP OF CHLOROBIPHENYLS FROM RICE OIL

3 g of rice oil
Dissolve in 12 ml of *n*-hexane
Extract with 30 ml of acetonitrile saturated with hexane (four times)

Acetonitrile layer
Extract with 100 ml of hexane in 700 ml of 2% saline solution

Hexane layer
Discard

Saline solution layer
Extract with 100 ml of hexane

Hexane layer

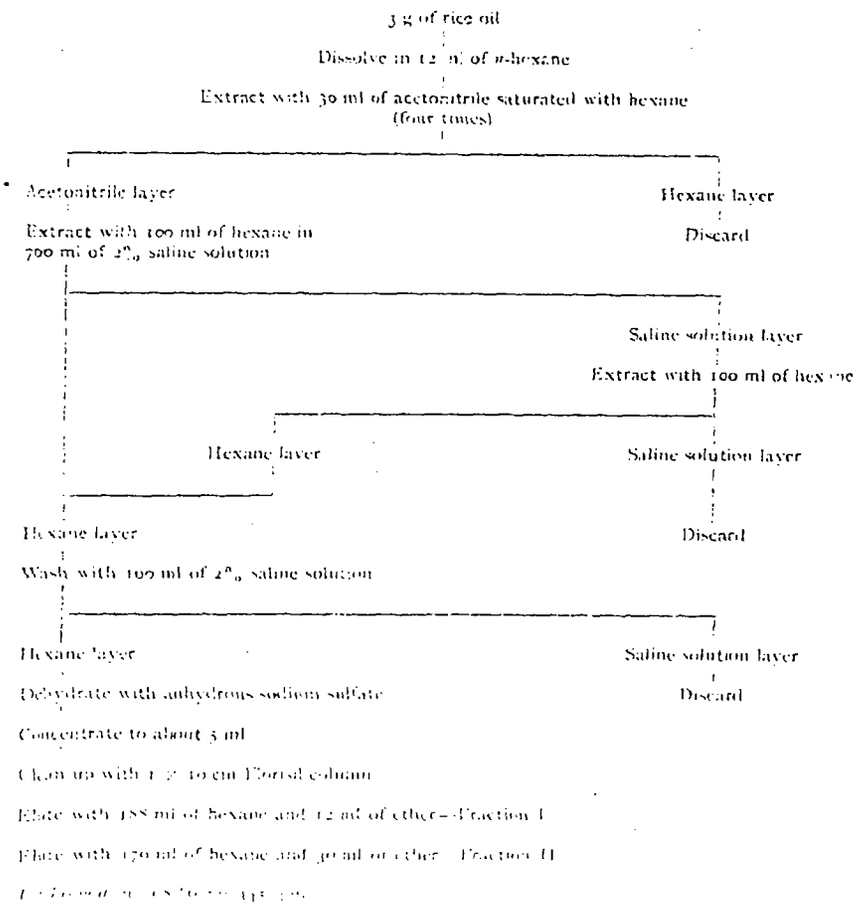
Saline solution layer
Discard

Hexane layer
Wash with 100 ml of 2% saline solution

Hexane layer
Dehydrate with anhydrous sodium sulfate
Concentrate to about 5 ml
Clean up with 1 × 10 cm Florisil column
Elute with 188 ml of hexane and 12 ml of ether—Fraction I
Elute with 170 ml of hexane and 30 ml of ether—Fraction II

Saline solution layer
Discard

[illegible]



CHROMATOGRAPHIC AND BIOLOGICAL ASPECTS OF PCB'S 387

TABLE 24

EXTRACTION METHOD FROM BIOLOGICAL MATERIALS

From 0.5 to 1 g of material
Chop up into small pieces
Add 5 ml of ethanol and filter

Residue
Extract with 5 ml of ethanol on a water-bath for 15 min and filter after cooling (twice)

Residue
Extract with ether samely as with ethanol and filter (once)

Residue
Extract with *n*-hexane samely as with ethanol and filter (twice)

Residue
Discard

Filtrate

Filtrate

Filtrate
Evaporate to dryness

Evaporate to dryness
Dissolve in few ml of *n*-hexane and filter

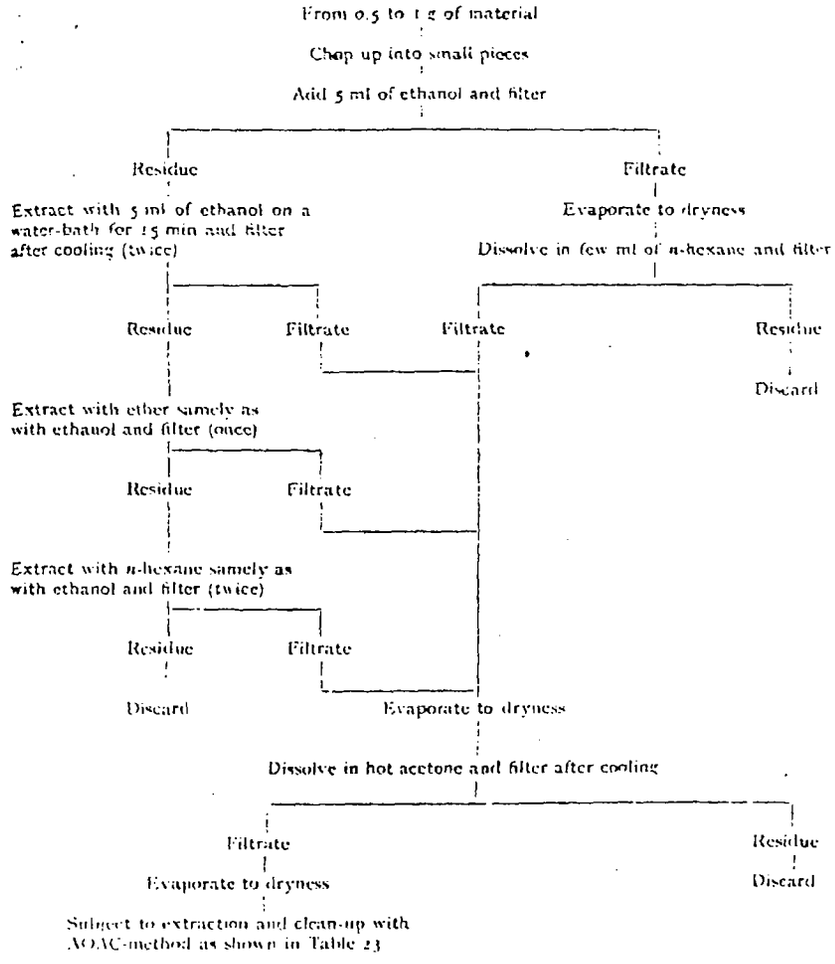
Residue
Discard

Filtrate

Evaporate to dryness
Dissolve in hot acetone and filter after cooling

Filtrate
Evaporate to dryness
Subject to extraction and clean-up with AOAC-method as shown in Table 23

Residue
Discard



produced poisoning among the general population in Western Japan from summer to fall of 1968[76], with a total of 600 patients to date. It was found that contamination of the oil occurred by both the leakage of chlorobiphenyls into the rice bran oil through pin holes in a pipe used for heat exchange and deionizing in the manufacturing process. It was also demonstrated that some newborn and still-born infants with darkish skin were born from the poisoned mothers.

KOJIMA *et al.*[77] described the utility of GLC for the analysis of chlorobiphenyls from the skin of a still-born infant who was born from a patient suffering from chlo-

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...rbiphenyl poisoning and also showed that chlorobiphenyls passed through the pla... cenia barrier and accumulated in the fetus. Kaneclore 400 is a mixture of di-, tri-, tetra- and pentachlorobiphenyls (chlorine content approximately 48%). Approximately 2300 p.p.m. was concluded to be the concentration of Kaneclore 400 in the patient administered with rice oil. Histologically, many melanine pigments were demonstrated in the darkish skin of the still-born infant and this pigmentation could possibly be caused by chlorobiphenyls since some components of Kaneclore 400 were detected in the darkish skin of the infant by GLC. Parallel experiments with mice confirmed the fact that chlorobiphenyls passed through the placenta barrier and accumulated in the fetus. GLC analyses were carried out using a Shimadzu GC-1C instrument equipped with an electron capture detector of pulse type. The glass column (2 × 262.5 mm) was packed with 1.5% SE-30 on 60–80 mesh Chromosorb W. The operating temperature for the column and detector was 200°; the carrier gas was nitrogen with a flow rate of 65.5 ml/min. Tables 23 and 24 illustrate the method of extraction and clean-up of chlorobiphenyls from rice oil and biological materials, respectively. Fig. 33 depicts gas chromatograms of the patient administered with rice

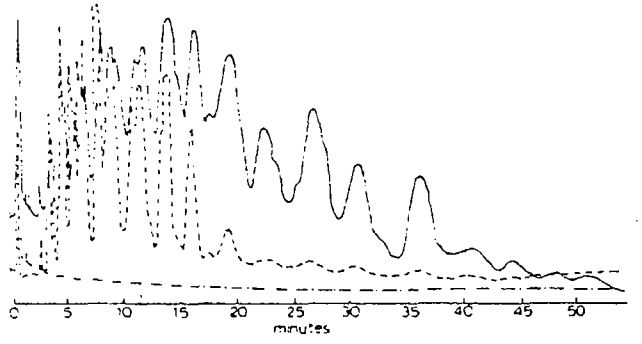

Fig. 33. Gas chromatograms of rice oil. ———, Rice oil (material); — · — · —, rice oil (control); — — —, Kaneclore 400.

oil, of the control rice oil and Kaneclore 400; Figs. 34 and 35 show chromatograms of the skin of a still-born infant and control and the skin of infantile mice (material and control compared with chlorobiphenyls in rice oils, respectively, and Fig. 36 illustrates a chromatogram of the skin of maternal mouse (material) and control compared with chlorobiphenyls in rice oil.

The biological interaction between a series of polychlorinated biphenyl compounds and dieldrin and DDT was studied by LICHTENSTEIN *et al.*[6]. Many PCBs were found toxic to *Drosophila melanogaster* Meigen and housefles *Musca domestica* L., but to a lesser extent than dieldrin or DDT. Their toxicity increased with *decrease* in their chlorine content and moreover, sublethal dosages of several of the PCB plasticizers increased the toxicity of dieldrin and DDT. Since the PCBs are in the environment, their potential effects on biological systems, especially in combination with other synthetic chemicals, should thus be considered. The chromatographic behavior of eleven PCBs was examined by both TLC and GLC. The latter method

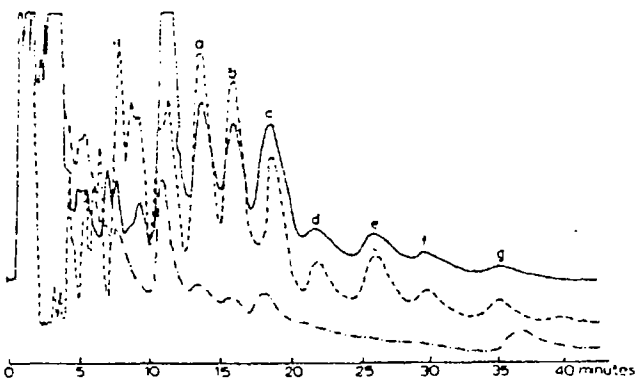

Fig. 34. Chromatograms of the skin of a still-born infant and control compared with chlorobiphenyls in rice oil. ———, Skin of still-born infant; — · — · —, skin of infant (control); — — —, chlorobiphenyls in rice oil.

two systems. One instrument was a Packard Model 7834 gas chromatograph equipped with a 150 mCi tritium affinity ionization detector operated at 50 V and a 1.83 m × 4.0 mm I.D. glass column packed with 5% SE-30 on 60–80 mesh acid-washed, DMCS-treated Chromosorb W (conditioned for seven days at 250° before use) with a column pressure of 25 p.s.i. of nitrogen (flow rate, 125 ml/min). The injection, oven and detector cell temperatures were 240°, 190°, and 215°, respectively. The second instrument was a Jarrell-Ash Model 28-700 gas chromatograph equipped with a 100 mCi tritium electron affinity ionization detector and operated at 20 V. A 1.22 m × 4.0 mm I.D. glass column contained a 1:1 mixture of 5% QF-1 and 5% DC-200 coated on 80–90 mesh Anakrom AS and conditioned for seven days at 250° before use (column pressure 16 p.s.i.; nitrogen flow rate 100 ml/min). The injection port, oven and detector temperatures were 250°, 190° and 210°, respectively. For TLC,

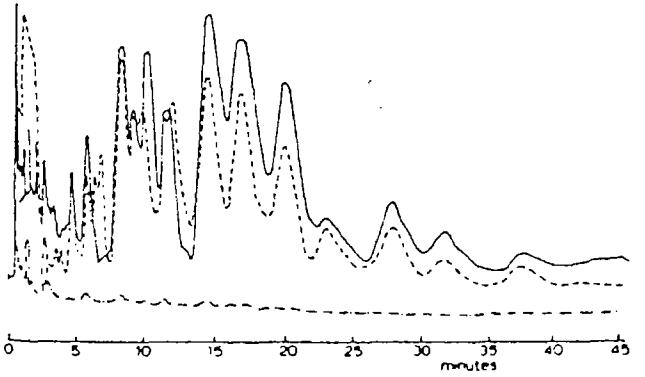

Fig. 35. Chromatograms of the skin of infantile mice and control compared with chlorobiphenyls in rice oil. ———, Skin of infantile mice (material); — · — · —, skin of infantile mice (control); — — —, chlorobiphenyls in rice oil.

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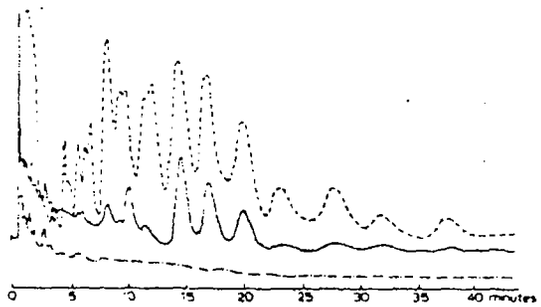

Fig. 36. Chromatograms of the skin of maternal mouse and control compared with chlorobiphenyls in rice oil. ———, Skin of maternal mouse (material); — · — · —, skin of maternal mouse (control); — — —, chlorobiphenyls in rice oil.

Aluminum Oxide G plates were developed with *n*-heptane, sprayed with the MITCHELL reagent[38] and exposed to UV light for 30 min.

Table 25 summarizes the GLC behavior of eleven Aroclor PCB's and a number of insecticides analyzed with two different columns in separate gas chromatographs. The observed retention times were compared with those obtained after the injection of lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, DDT, DDE and TDE. The GLC of nearly all of the plasticizers resulted in chromatograms containing a multitude of peaks, in some cases as many as fifteen.

Figs. 37 and 38 illustrate the TLC of plasticizers on Aluminum Oxide G. It

TABLE 25

COMPARISON OF PLASTICIZERS AND INSECTICIDES BY GLC

Chromatographic conditions: (I) Packard gas chromatograph; column 5%, SE-30 on 60–80 mesh Chromosorb W; oven temp., 190° (II) Farrell-Ash gas chromatograph, column 5%, QF-1/5%, DC-200 (1:1) on 80–90 mesh Anakrom AS; oven temp., 190°

Abbreviations: LI = lindane; HC = heptachlor; HO = heptachlor epoxide; AL = aldrin; DL = dieldrin; DT = *p,p'*-DDT; DE = DDE; TD = TDE.

Plasticizer Aroclor		*% weight chlorine*	*GLC I*		*GLC II*	
			No. of peaks	*Identical with*	*No. of peaks*	*Identical with*
1221-a	A	21	1	None	5	LI
1232-a	B	32	15	LI,HC,AL,DE,DL,TD,DT	15	LI,HC,HO,DE
1242-a	C	42	14	LI,DE,AL,DE,DL,TD,	14	LI,HC,HO,DE
1248-a	D	48	15	LI,DE,AL,DE,DE,TD,DT	15	LI,HC,HO,DT,
1254-a	E	54	11	AL,DE,DL,TD,DT	10	HO,DE, TD
1260-a	F	60	13	TD,DT	13	DE,TD
1262-a	G	62	12	TD	13	DE,TD
1268-a	H	68	7	None	8	None
1254-A	I	68	11	TD	11	DL
5442-A	J	42	3	None	7	LI,HC,HO
5460-A	K	60	1	None	0	None

* A = Chlorinated biphenyls; a = Chlorinated triphenyls.

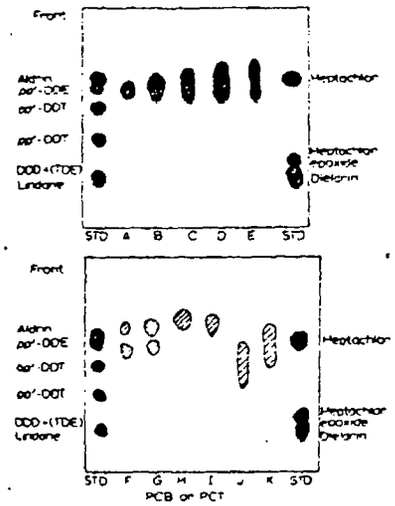

Fig. 37. Thin-layer chromatogram of plasticizers A, B, C, D, and E on Aluminum Oxide G. Solvent: 1% acetone in *n*-heptane. STD = Insecticide standards for comparison.

Fig. 38. Thin-layer chromatogram of plasticizers F, G, H, I, J, and K on Aluminum Oxide G. Solvent: 1% acetone in *n*-heptane. STD = Insecticide standards for comparison.

is likely that these PCB's were not fully separated or resolved in the above system since only one or two spots were found per plasticizer.

Polychlorinated biphenyls have been detected in all samples of rainwater obtained over a 12-month study in Great Britain from seven stations[39]. Hence, aerial fallout is one source of PCB in the sea. Aliphatic chlorinated hydrocarbons identified in surface waters of the North Atlantic are derived in part from barge dumping of waste products of the vinyl chloride industry[39].

PCB contamination of Escambia Bay, Fla., has been traced to an industrial plant on the Escambia river by DUKE *et al.*[40]. PCB's have been found in sewage sludge in Sweden[41], and it has been estimated by HOLDEN[42] that approximately a ton of PCB a year is entering the Clyde estuary in Scotland as a component of raw sewage sludge and that an equivalent amount enters the estuary of the Thames in sewage sludge.

The input of PCB's into California coastal waters from urban sewage outfalls has been described by SCHMIDT *et al.*[43].

Aliquots of concentrated extracts were analyzed by GLC using a Microtek 220 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 3% QF-1 column on 80–100 mesh, acid-washed, DMCS-treated Chromosorb. The column, injection port and detector temperatures were 190°, 230° and 250°, respectively. Nitrogen was used as a carrier and purge gas; flow through the column was 95 ml/min

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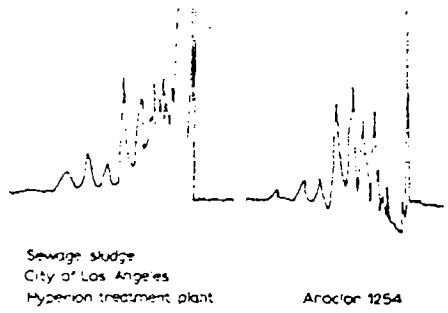

Fig. 30. GLC analysis of sewage sludge.

TABLE 26

PCB AND DDT COMPOUNDS IN URBAN SEWAGE OUTFALLS IN CALIFORNIA

Sampling station (flow in m.g.d.)[a]	*Sample No.*	*PCB type*	*Parts per billion*		*Estimated kg/day*[b]	
			PCB	*DDT*[c]	*PCB*	*DDT*
Richmond	1	ND	—	0.040	—	0.005
21.5	2	ND	—	0.071		
	3	ND	—	0.068		
	4	ND	—	0.063		
EBMUD	1	1260	3.8	0.041	2.0	0.020
155	2	1260	3.2	0.036		
	3	1260	3.6	0.041		
	4	1260	3.1	0.024		
San Francisco	1	1260	3.8	0.020	0.6	0.003
33.5	2	1260	5.8	0.033		
Oxnard	1	ND	—	0.068	—	0.006
10.0	2	ND	—	0.15		
Hyperion waste	1	1254	0.16	0.023	0.4	0.05
water, 340	2	1254	0.37	0.057		
Hyperion sludge	1	1254	94.3	ND	1.6	—
5.0	2	1254	78.5	ND		
White Point	1	Mixture,	70[d]	68.1	100	97
350	2	1242 and		96.7		
	3	1254		57.0		
	4			58.6		
Terminal Island	1	1242	12.8	0.098	0.35	0.002
0.3	2	1242	5.8	0.020		
Orange County	1	1242	0.21	0.071	0.18	0.030
130	2	1242	0.64	0.058		
	3[e]	1242	0.23	0.093		
San Diego	1	ND	—	0.087	—	0.022
80	2	ND	—	0.055		

[a] Millions of gallons per day, on day of collection, not averaging supplied by plant personnel.
[b] Based on average concentrations.
[c] Includes *p,p'*-DDD, *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDD and *o,p'*-DDE.
[d] Concentrate from pooled samples.
[e]

and flow through the purge was 45 ml/min. PCB was identified by the presence of profile on chromatograms identical to those of one of the commercial PCB preparations (Aroclor 1254) (Fig. 30). Other chromatograms were identical except for minor peaks to chromatograms of Aroclor 1242, mixtures of Aroclor 1242 and 1254 or of Aroclor 1260. PCB was quantified by direct comparison with standard preparations of these commercial mixtures. The profile of PCB peaks was unchanged by either saponification or passage through a sulfuric acid-Celite column[83] used for additional clean-up of extracts.

Table 26 lists the PCB and DDT compounds in urban sewage outfalls in California. It is noted that daily outputs of PCB in the order of 2 kg are equivalent to a ton a year and because of the low solubility of these compounds in water and their high affinity for lipids, a large fraction of the PCB can be expected to pass into marine food chains. The highest output was recorded from Los Angeles County, one of the most industrialized areas of the state.

The utility of reversed liquid–liquid partition and GLC in the determination of PCB's and chlorinated pesticides in water and sewage sludge was described by AHLING AND JENSEN[81]. In this method the water is passed through a filter (3 g) containing a mixture of *n*-undecane and Carbowax 4000 monostearate on Chromosorb W, and the absorbed pesticides eluted with petroleum ether (10 ml). When detected by means of GLC with electron capture detection, the sensitivity is 10 ng/m^3 of lindane with a sample size of 200 l. The recovery of added pesticides was 50–100% (DDT, 80%) and for PCB (Clophen 40 and A50, Bayer), 93–100%. The filter column was 30 cm × 1 cm I.D. with a glass filter disk G 1 (100–120 μ). GLC was carried out using a Varian Aerograph Model 204 chromatograph equipped with an electron capture detector and a 160 cm × 0.20 cm borosilicate glass column containing 4% methylsilicone oil or 8% fluorosilicone oil (QF-1) on HMDS-treated 80–100 mesh Chromosorb W. The detector and injector temperatures were 205° and 220°, respectively; the carrier gas was nitrogen (purified with a 6-in. molecular sieve) at a flow rate of 30 ml/min. The column temperature was chosen to give DDT a retention of 20 min (about 190°).

Table 27 lists the results from recovery experiments using a variety of filters for the separation of a number of chlorinated pesticides and PCB and shows the superiority of 60–80 mesh Chromosorb W, covered with a mixture of Carbowax monostearate and *n*-undecane. Figs. 40a and b show gas chromatograms of extracts from distilled water and natural water, respectively, analyzed on SF-96 columns. Fig. 41

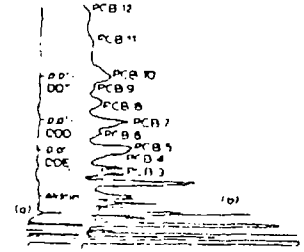

Fig. 40. (a) Gas chromatogram from an SF-96 column of an extract from distilled water. (b) Gas chromatogram from an SF-96 column of an extract from natural water.

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TABLE 27

RESULTS FROM RECOVERY EXPERIMENTS

Filter	Filter (g)	Water (l)	Flow speed, (ml/min)	Standard pesticides[a] (µl/l)	Petroleum ether (ml)	Recoveries							
						BHC	Lindane	Aldrin	Dieldrin	DDE	DDD	DDT	PCB
Chromosorb W + ca 80% undecane	1.5	6	65	5[b]	10	trace	20	19	80	56	69	50	
10% Carbowax	1.5	6	65	5	10	trace	trace	28	[b]	37	17	63	
Chromosorb W + ca 80% undecane	1.5	6	65	5	10	11	51	11	75	75	77	70	
10% Carbowax + 10% undecane	2	6	65	5	5	45	10	31	[b]	31	86	58	
10% Carbowax + 30% undecane	2	6	130	5	5	45	30	31	[b]	31	70	58	
10% Carbowax + 30% undecane	3	6	65	5	5	68	46	50	97	65	101	80	
10% Carbowax + 30% undecane	4	6	65	5	8	88	67	56	69	100	100	90	
10% Carbowax + 30% undecane	2.5	18	65	1.33	5	64	42	43	62	100	100	68	
10% Carbowax + 30% undecane	5	11	65	0.18 PCB[a]	5	156	111	100	100	90	86	93	
10% Carbowax + 30% undecane	1	5	65	10	5								53–80
10% Carbowax + 30% undecane	3	5	65	10	5								93–100

[a] The standard had the following composition and concentration in ng/µl: BHC, 1.0; lindane, 1.0; aldrin, 2.0; DDE, 1.0; dieldrin, 3.0; DDD, 8.0; DDT, 12.0; and PCB (Clophen A30), 10.0.

[b] The extract was treated with sulfuric acid, which destroyed the dieldrin.

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Fig. 41. Gas chromatogram from an SF-96 column of an extract from sewage sludge.

illustrates a gas chromatogram from an SF-96 column of an extract from sewage sludge.

ZITKO[84] described the solubilization of PCB in water by non-ionic surfactants as well as the determination of concentration of the solubilized PCB by UV spectrophotometry and fluorescence and GLC and the toxicity of PCB to Atlantic salmon (*Salmo salar* parr.).

GC was carried out on a Varian 600D instrument equipped with a 250-mCi tritium electron capture detector, using a 5 ft. × 1/8 in. glass column containing 1% SE-30 on 100–120 mesh Chromosorb W operated at 180°. UV and fluorescence spectra were recorded on a Beckman DK-2A and on a Perkin-Elmer MPF-2A instrument, respectively. Retention times and electron capture responses have been reported for mono- and dichlorobiphenyls[85,86]. EMERY AND GASSER[87] have described the GLC separation on polyphenyl thioethers of mono- and some di- and trichlorobiphenyls. As a general rule, chlorine substitution in position 2 shortens and vicinal disubstitution lengthens the retention time. The reported relative electron capture detector responses are 1, 0.2, 2.3, 7.6, 5.8, and 3.5 for 4-, 3-, and 2-mono-, and 4,4'-, 3,3'- and 2,2'-dichlorobiphenyl, respectively[86]. The retention times and electron capture detector responses of a number of PCB's have been reported by ZITKO *et al.*[88].

A Packard A7901 gas chromatograph with a 6 ft. × 4 mm glass column containing 1% SE-30 on 100–120 mesh acid-washed Chromosorb operated at 200° was used. The injector and detector temperatures were 210° and the carrier gas was nitrogen at a flow rate of 60 ml/min. D.C. voltage in the electron capture detector was 95 V and meter sensitivity was 1 × 10^{-8} A. Solutions of chlorinated biphenyls in pesti-

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cide-grade hexane were used in concentrations of 1–260 μg/ml for injections on column and volumes from 2 to 5 μl were used to obtain peak heights of 30–70% of the full-scale pen deflection. Relative retention times and electron capture detector responses of chlorinated biphenyls and Aroclor 1254 and 1260 are shown in Table 2. Chlorine substitution in positions 2 and 6 shortens and vicinal substitution lengthens the retention time also in tri- and tetrachlorobiphenyls. In some instances these effects overshadow the effect of the number of chlorines in the molecule, which increases the retention time. The detector response increases strongly with increasing number of chlorine atoms in the molecule (*e.g.*, the response of decachlorobiphenyl is 500 times stronger than that of 4-chlorobiphenyl). PCB's found in wildlife are generally of the Aroclor 1254 or 1260 type[15,34,74]. In the study of ZITKO *et al.* above[58] these Aroclors gave fourteen and sixteen peaks with relative retention times from 0.48 to 3.28 and from 0.72 to 6.80, respectively. The presented data indicate that the electron capture response of all chlorinated biphenyls with four to nine atoms of chlorine per molecule is not likely to exceed the value of 1.6 relative to *p,p'*-DDE under the described conditions.

DE VOS AND PEET[69] described the reversed-phase partition TLC and GLC of the polychlorinated biphenyls.

Kieselguhr plates impregnated with 8% liquid paraffin (b.p. 40–60°) were developed with acetonitrile–acetone–methanol–water (40:18:40:2), detected with

TABLE 28

RETENTION TIME AND ELECTRON CAPTURE DETECTOR RESPONSE OF CHLOROBIPHENYLS

Compound	*Relative retention time (p,p'-DDE = 1.00)*	*Relative response per ng ± standard deviation (p,p'-DDE = 1.00)*
4-Chlorobiphenyl	0.17	0.0033 ± 0.00005
2-Chlorobiphenyl	0.11	0.0030 ± 0.00004
3-Chlorobiphenyl	0.14	0.0006 ± 0.00001
4,4'-Dichlorobiphenyl	0.30	0.0152 ± 0.0002
3,4'-Dichlorobiphenyl	0.25	0.0155 ± 0.0001
2,2'-Dichlorobiphenyl	0.15	0.0130 ± 0.0001
3,4-Dichlorobiphenyl	0.28	0.0388 ± 0.0002
2,4-Dichlorobiphenyl	0.19	0.0150 ± 0.0004
2,6-Dichlorobiphenyl	0.16	0.0815 ± 0.0049
2,4,4'-Trichlorobiphenyl	0.39	0.298 ± 0.010
2,4,6-Trichlorobiphenyl	0.24	0.270 ± 0.006
2,2',4,4'-Tetrachlorobiphenyl	0.51	0.200 ± 0.003
3,3',4,4'-Tetrachlorobiphenyl	0.66	0.770 ± 0.027
2,2',6,6'-Tetrachlorobiphenyl	0.33	0.0403 ± 0.0016
2,3',5,5'-Tetrachlorobiphenyl	0.70	0.625 ± 0.045
2,3,4,5-Tetrachlorobiphenyl	0.60	0.715 ± 0.010
2,3,5,6-Tetrachlorobiphenyl	0.51	0.505 ± 0.010
2,3,4,5,6-Pentachlorobiphenyl	1.00	1.30 ± 0.017
2,2',4,4',6,6'-Hexachlorobiphenyl	0.78	0.545 ± 0.020
3,3',4,4',5,5'-Hexachlorobiphenyl	2.98	1.15 ± 0.061
2,2',3,4,4',6,6'-Octachlorobiphenyl	2.02	1.58 ± 0.020
2,2',3,4',5,5',6,6'-Octachlorobiphenyl	2.43	1.53 ± 0.070
Decachlorobiphenyl	8.20	1.61 ± 0.086
Dieldrin (1.35)		0.910 ± 0.005
Aroclor 1254		1.15 ± 0.010

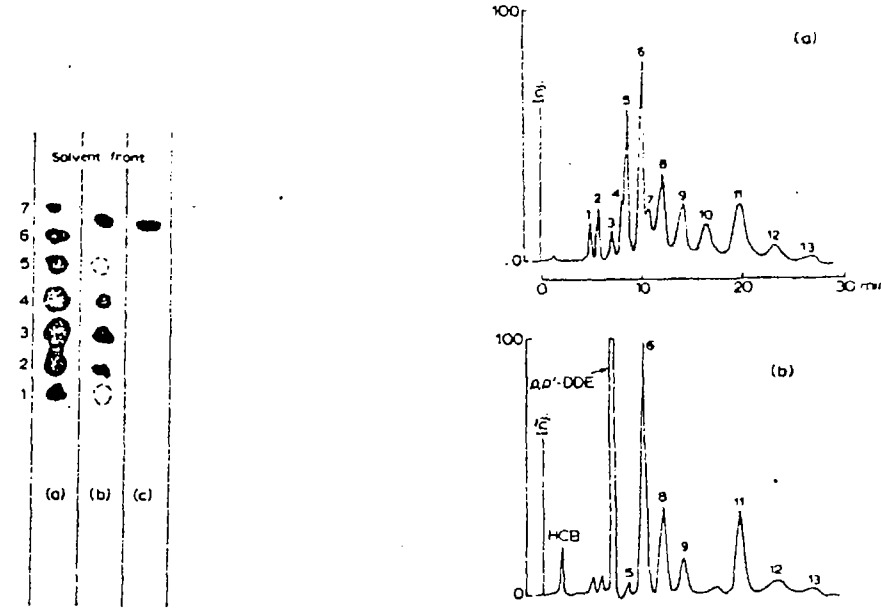

Fig. 42. Thin-layer chromatograms of (a) technical PCB mixture, (b) extract of owl liver, and (c) *p,p'*-DDE. Kieselguhr impregnated with liquid paraffin, developed three times with acetonitrile–acetone–methanol–water (40:18:40:2), and visualized with silver nitrate and UV light.

Fig. 43. Gas chromatograms of (a) Phenochlor DP6 (0.6 μg) and (b) a hexane extract of owl liver. Instrumental conditions are given in Table 29.

TABLE 29

RELATIVE RETENTIONS (DIELDRIN = 1.00) OF PCB PEAKS 1–13 IN FIG. 43a

Instrument: Microtek MT 220. Column: glass, 6 ft. × 1/4 in. O.D., packed with 3% OV-1 on 80–100 mesh Gas-Chrom Q. Operating temperatures: column, 200°; injector, 220°; transfer line (Teflon-coated aluminum), 220°. Detection system: Dohrmann C 250 A microcoulometer, with oxidative pyrolysis furnace and T 300 S titration cell. Sensitivity: 200 ohms. Gain: low (200). Recorder sensitivity: 1 mV. Chart speed: 1 cm/min.

Peak No.	*Relative retention*
1	0.71
2	0.84
3	1.03
4	1.18
5	1.23
6	1.47
7	1.50
8	1.73
9	2.01
10	2.33
11	2.80
12	3.33
13	3.76

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silver nitrate (1.7 g in 200 ml 96% ethanol) and irradiated with UV (Philips TUV 15 W) at a distance of about 15 cm. (Black spots on a white background usually appeared within 20 min.) Fig. 42 shows thin-layer chromatograms of a technical PCB mixture (Phenochlor DP6 containing 60% chlorine, an extract of owl liver, and a sample of *p,p'*-DDE).

Fig. 43 illustrates gas chromatograms of both Phenochlor DP6 and a hexane extract of owl liver. The instrumental conditions employed and the relative retention of the peaks in Fig. 43a are summarized in Table 29.

Peak numbers 5, 6, 8, 9, 11, 12 and 13 in Fig. 43b have the same retention times as the corresponding peaks in Fig. 43a. Previous studies[2] utilizing a combination gas chromatograph–mass spectrometer have shown that in the chromatogram of the technical PCB mixture containing 60% chlorine, peaks 1 and 2 are produced by pentachlorobiphenyls, peaks 3, 4, 5, 6 and 8 by hexachlorobiphenyls, peaks 7, 9, 10, 11 and 12 by heptachlorobiphenyls, and peak 13 by octachlorobiphenyl. Table 30

TABLE 30

RELATIONSHIP BETWEEN SPOTS OBTAINED BY THIN-LAYER CHROMATOGRAPHY (FIG. 42a) AND PEAKS OBTAINED BY GAS CHROMATOGRAPHY (FIG. 43a) OF PHENOCHLOR DP6.

Spot No.	*Corresponding peak numbers*
1	13
2	11
3	6, 9, 12
4	7, 8, 10
5	2, 4, 5
6	3, 6
7	1, 3

shows the relationship between spots obtained by TLC (Fig. 42a) and peaks obtained by GLC (Fig. 43a) of Phenochlor DP6. Of the chlorinated pesticides that are frequently detected in wildlife samples only the fungicide hexachlorobenzene (HCB) interferes with one of the PCB spots (No. 3). The lowest detectable amounts obtained with the above TLC system are of the order of 100 ng of single compounds such as DDE and HCB, and a few micrograms for PCB mixtures such as Phenochlor DP6.

The application of carbon-skeleton chromatography to the qualitative differentiation between PCB's and DDT was demonstrated by Asai *et al.*[19]. PCB's and biphenyl yield identical carbon-skeleton chromatograms that are strikingly different from that of DDT. Comparisons of the relative retention times of products yielded by the PCB's with known compounds, plus partial identification by their UV spectra suggested that the products formed at 300° catalyst temperature were cyclohexylbenzene and biphenyl, and at 260° were cyclohexylbenzene and a small amount of bicyclohexyl. The apparatus consisted of a NHL-Beroza carbon-skeleton determination (National Instrument Laboratories, Inc., Rockville, Md.) attached to the injection port of an Aerograph Model A-600-B gas chromatograph equipped with a flame ionization detector. For the electron capture gas chromatograms, the same chromatograph was equipped with a tritium detector. A sodium chloride neutral [illegible] packed with DMCS-treated 80–100 mesh [illegible]

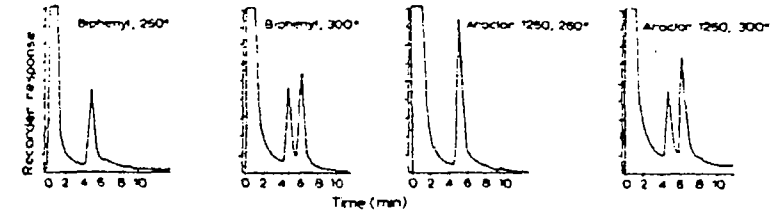

Fig. 44. Carbon-skeleton chromatograms of biphenyl and Aroclor 1260 at two catalyst temperatures.

Chrom Q)[91] was used to change the carbon-skeleton determinator. The temperature of the catalyst bed was maintained at either 260° or 300°. The carbon-skeleton chromatograms were obtained using a 3 ft. × 1/8 in. stainless-steel column packed with 5% DC-200 on DMCS-treated 80–100 mesh Gas-Chrom Q. The column temperature was held at 105° and the hydrogen flow rate was 20 ml/min. The carbon-skeleton chromatograms of Aroclor 1260, biphenyl, bicyclohexyl- and cyclohexylbenzene were obtained on a 3 ft. × 1/8 in. stainless-steel column packed with 80–100 mesh

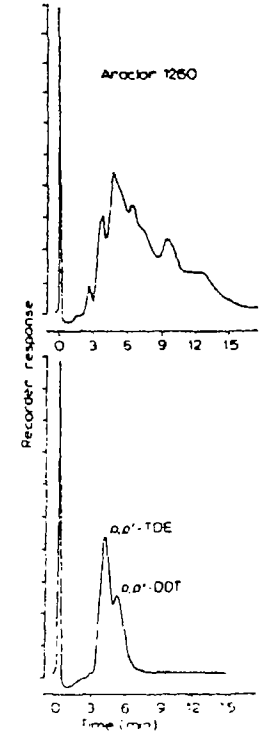

Fig. 45. Gas chromatograms of Aroclor 1260 and a mixture of *p,p'*-TDE and *p,p'*-DDT using the electron capture detector.

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Carbowax 400 on Porasil S. The column temperature was 178° and the hydrogen flow rate was 20 ml/min. For the electron capture determinations, a 2 ft. × 1/8 in. stainless-steel column packed with 10% DC-200 on DMCS-treated 80–100 mesh Gas-Chrom Q was used with a column temperature of 180° and a nitrogen flow rate of 85 ml/min. Fig. 44 illustrates carbon-skeleton chromatograms of biphenyl and Aroclor 1260 at two catalyst temperatures, viz., 260 and 300°. The two peaks (cyclohexylbenzene and biphenyl) observed at the higher catalyst temperature were due to incomplete hydrogenation of the biphenyl (hydrogenation is favored at lower temperatures)[72].

Gas chromatograms for Aroclor 1260 and a mixture of *p,p'*-TDE and *p,p'*-DDT obtained with the electron capture detector are shown in Fig. 45 and illustrate how the PCB's can interfere with the analysis of the DDT pesticide group. Carbon-skeleton chromatograms obtained at 260° and 300° are depicted in Fig. 46 for the

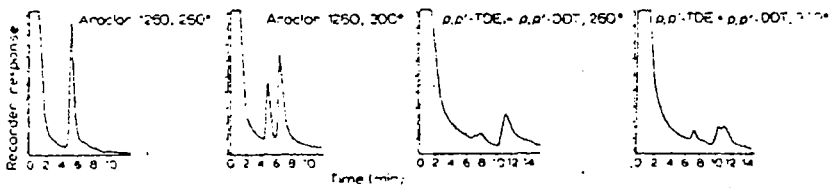

Fig. 46. Carbon-skeleton chromatograms at two catalyst temperatures of Aroclor 1260, and a mixture of *p,p'*-TDE and *p,p'*-DDT.

samples at much higher concentrations. These chromatograms suggest that this technique can be an extremely useful means for ascertaining the presence or absence of PCB's in samples, which from gas chromatographic responses show the presence of the DDT pesticide group.

The qualitative identification of PCB's in metabolism studies and some surveillance situations by carbon-skeleton chromatography should require samples of a microgram or less. The response of this technique for a specific Aroclor depends upon its chlorine content; the greater the chlorine content, the larger will be the amount required for detection. For 1 μg of Aroclor 1260, with the electrometer attenuator at 8×, a response of 13% of full scale deflection was obtained. For 1 μg quantities of Aroclors with less chlorine contents than Aroclor 1260, responses should increase as the chlorine content decreases.

VI. GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROSCOPY

The GC–mass spectrometric behavior of PCB's in human adipose tissue was described by Bikos *et al.*[73]. The GC column was a stainless-steel capillary, 100 ft. × 0.020 in. I.D. coated with OV-1 silicone oil. Programmed temperature analyses were made both for the six Aroclor 1200 series standards and the tissue extracts. Figs. 47 and 48 illustrate total ion current monitor chromatograms obtained for Aroclors 1254 and 1260 and detail the programming conditions used. The molecular separator and gas inlet temperatures were maintained at 210° and 215°,

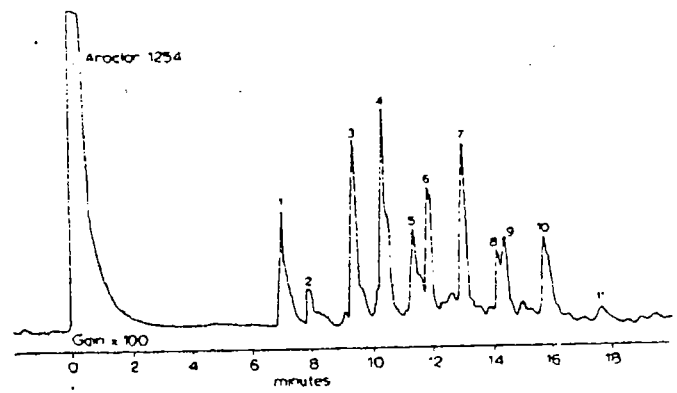

Fig. 47. TICM chromatogram of a standard Aroclor 1254 mixture of polychlorinated biphenyls. Programmed temperature analysis: 2 min at 185°, to 210° at 5°/min, isothermal at 210°. (See text for remaining instrumental parameters and partial peak identification.)

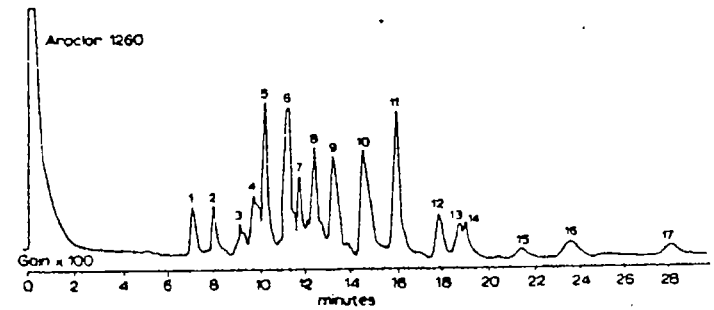

Fig. 48. TICM chromatogram of a standard Aroclor 1260 mixture of polychlorinated biphenyls. Programmed temperature analysis: 2 min at 200°, to 230° at 5°/min, isothermal at 230°. (See text for remaining instrumental parameters and partial peak identification.)

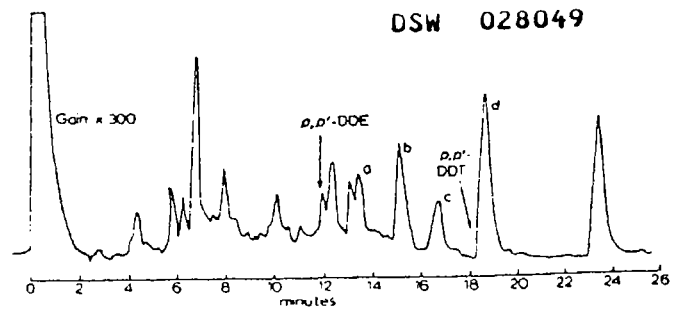

Fig. 49. TICM chromatogram of human adipose tissue extract A. Programmed temperature analysis: 5 min at 180°, to 210° at 5°/min, isothermal at 210°.

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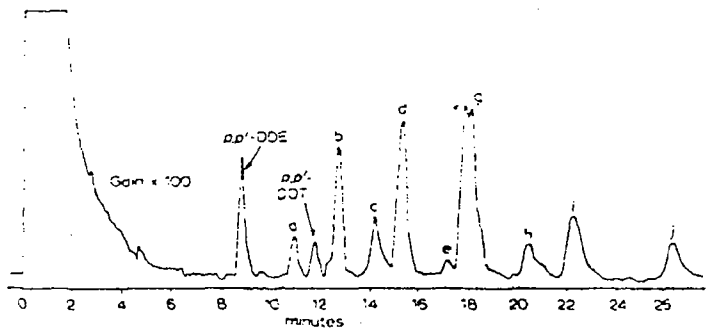

Fig. 50. TICM chromatogram of human adipose tissue extract B. Programmed temperature analysis: 2 min at 190°, to 230° at 5°/min, isothermal at 230°.

spectively. The mass spectra were recorded at 80 eV electron energy with 230-eV accelerating voltage and the filament emission current was 100 μA. Helium carrier gas flow rate was 4 ml/min and the injector temperature was 175°. The mass spectra were scanned magnetically over the range *m/e* 5 to *m/e* 500 in 6 sec. Figs. 49 and 50 illustrate the total ion current monitor chromatograms of human adipose tissue. Previous analysis by microcoulometric and electron capture GLC had indicated the presence of PCB residues and a modified MILLS' procedure[23] was used for the preparation of the samples. Samples designated A and B (Figs. 49 and 50, respectively) were estimated to contain 200 p.p.m. and 600 p.p.m. total PCB as determined by electron capture GC.

This study indicated the enhancement of separation efficiency for the individual PCB compounds when analyzed by capillary column GC–mass spectrometry with no evidence of thermal degradation of any of the PCB compounds. All components of the Aroclors gave molecular ion groups of high intensity as would be expected from highly chlorinated biphenyl structures. The characteristic isotopic distribution pattern[24] corresponding to the number of chlorine atoms in the parent ion and chlorine-containing fragment ions was observed.

Two noteworthy features of the spectra were the relatively intense fragment ions produced by consecutive loss of chlorine atoms from the parent ion and the occur-

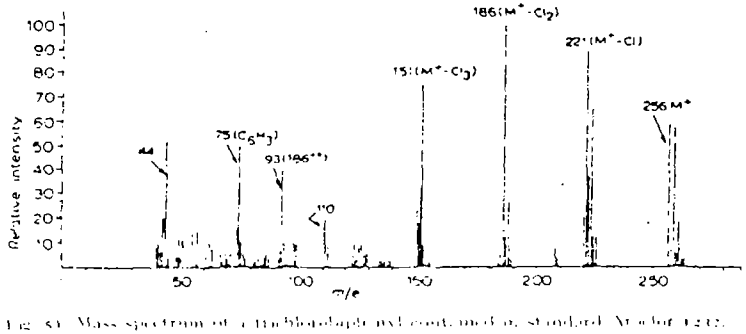

Fig. 51. Mass spectrum of a trichlorobiphenyl contained in standard Aroclor 1232.

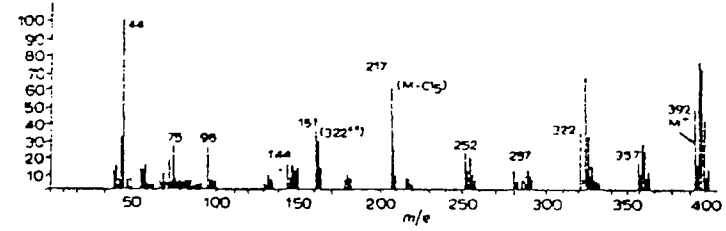

Fig. 52. Mass spectrum of a heptachlorobiphenyl found in standard Aroclor 1260.

ence of intense doubly charged fragments within the mass spectra of most of the PCB compounds.

Figs. 51 and 52 show mass spectra obtained for a trichlorobiphenyl isomer in Aroclor 1232 and a heptachlorobiphenyl isomer in Aroclor 1260, respectively. Thus, in the chromatograms of Figs. 47 and 48 peaks 1 and 2 were shown to be tetrachlorobiphenyls; peaks 3–6, hexachlorobiphenyls; peaks 7 and 9–12, heptachlorobiphenyls; peaks 13 and 14, octachlorobiphenyls; peaks 15 and 16, nonachlorobiphenyls; and peak 17, decachlorobiphenyl.

The mass spectra of the components of adipose tissue of sample extract A (Fig. 49) revealed that peaks a, b, c, and d were polychlorinated biphenyls identical with those obtained respectively for peaks 6, 7, 9 and 10 of Aroclor 1254 (Fig. 47).

The mass spectrum of peak d (an isomer of hexachlorobiphenyl, mol. wt. 358) is shown in Fig. 53.

The mass spectra of components of adipose tissue B (Fig. 50) showed that peaks a through j were identical to those obtained for peaks 7 and 9–17 in the Aro-

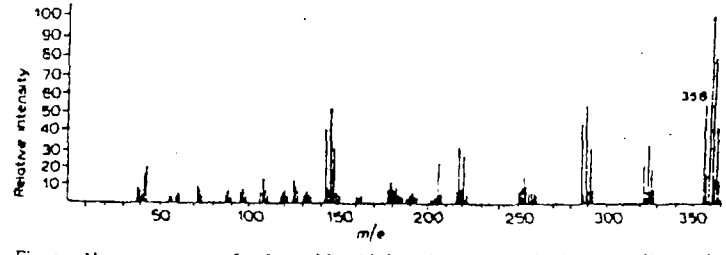

Fig. 53. Mass spectrum of a hexachlorobiphenyl contained in human adipose tissue sample A (peak d, Fig. 49).

clor 1260 standard (Fig. 48). In addition, *p,p'*-DDE and *p,p'*-DDT, which were present at higher levels than those encountered in sample A, were also confirmed in this adipose tissue sample.

A system for the identification of multiple pesticide residues in foods by combined GC–mass spectroscopy was described by BELLMAN AND BARRY[25] and this technique was used to demonstrate the absence of PCB's as an intermediate in the quantitation of *p,p'*-DDT and *p,p'*-DDE in fresh and smoked chubs. A Barber-Colman Model 5000 gas chromatograph was used equipped with a flame ionization de-

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tector and a 5-mV potentiometric recorder. The column was a glass U-tube, 6 ft × 2 mm I.D., packed with either 10% DC-200 on 80–100 mesh HP Chromosorb W or a mixed phase consisting of 2% OV-17/2% QF-1 on 80–100 mesh HP Chromosorb W, with helium as the carrier gas. Separations were performed isothermally with column temperatures of 180–215°, depending on the pesticides being separated with the total column effluent split between the mass spectrometer and the flame ionization detector.

The mass spectrometer was an A.E.I. MS-12 with an electron bombardment ion source. The electron trap was set at 100 μA and the source temperature maintained at *ca.* 200° by the heat of the electron-emitting filament. The electron bombardment energy ion 24 eV has the advantage over the more customarily used 70 eV of increasing the relative intensity of the molecular ion chlorine isotope cluster. The ion-accelerating voltage was 8 kV with the electron multiplier detector set at a gain of 7×10^4 with mass spectra scanned magnetically from *ca. m/e* 500 to *m/e* 12 in about 12 sec. A total ion current monitor detector was situated between the ion source and the mass analyzing magnet. (This detector intercepts a portion of the ion beam and displays it as a voltage signal on a 10-mV potentiometric strip chart recorder.) The interface between the GLC and the mass spectrometer was essentially as reported by MARKEY[98].

Cheese and cocoa beans were prepared for analysis by the fatty food procedure described in the *Pesticide Analytical Manual*[97]. The pesticides in the isolated fish sample (fresh and smoked chubs) were extracted with petroleum ether and partitioned into acetonitrile and cleaned up successively over Florisil and silicic acid columns. The latter utilized the procedure of ARMOUR AND BURKE[45] to remove any PCB that might have been present. Fig. 54 illustrates the flame ionization detection (FID) and total ion current monitor (TICM) chromatograms obtained from a smoked chub sample after silicic acid chromatography and shows several peaks, two of which have the same retention times as those of *p,p'*-DDE and *p,p'*-DDT. It was determined

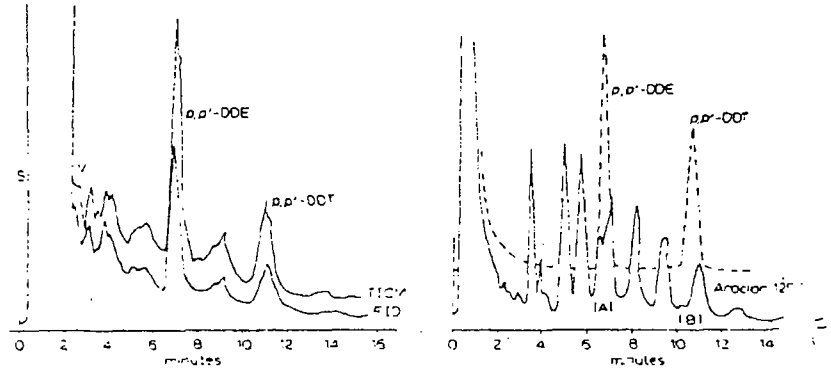

Fig. 54. FID and TICM chromatogram obtained from a smoked chub sample after silicic acid column chromatography. S = Solvent front; V = point at which glass valve is opened. Electron impact (24 eV) mass spectra of peaks marked MS were recorded on a 30% SE-30 on HP Chromosorb W; 210°.

Fig. 55. FID chromatogram of Aroclor 1254 standard and *p,p'*-DDE and *p,p'*-DDT mixture. The letters A and B mark the width of *p,p'*-DDE peak, B = width of *p,p'*-DDT peak. Column: 6% SE-30 on [illegible] Chromosorb W; 210°.

(using the procedure of ARMOUR AND BURKE[45]) that the original PCB interference in the chubs was caused by Aroclor 1254. An FID chromatogram of Aroclor 1254 (Fig. 55) shows several peaks that can interfere with the determination of *p,p'*-DDE and *p,p'*-DDT. The regions A and B represent the width of the *p,p'*-DDE and *p,p'*-DDT peaks, respectively. The centers of the regions represent the peak maximum for these pesticides. Mass spectra were obtained for the GLC components of Aroclor 1254 in the center of regions A and B, *i.e.*, Aroclor 1254 components with the same retention

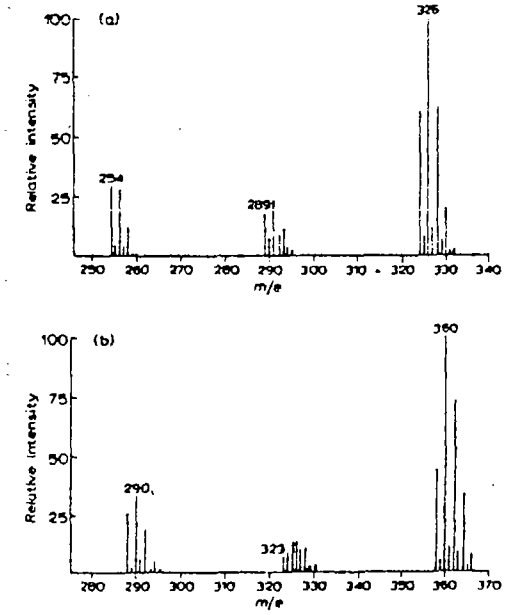

Fig. 56. Mass spectra of (a) Aroclor 1254 components with the same retention time as that of *p,p'*-DDE and (b) Aroclor 1254 components with the same retention times as that of *p,p'*-DDT.

time as that of *p,p'*-DDE (Fig. 56a) and Aroclor 1254 components with the same retention time as that of *p,p'*-DDT (Fig. 56b). Fig. 57 shows that mass spectra of (a) *p,p'*-DDE standard at 24 eV and (b) *p,p'*-DDE in a sample showing PCB carry-over after a silicic acid column separation procedure. The *m/e* 326 peak shows the presence of PCB. This carry-over of PCB into the pesticide-containing fraction occurred when extracts representing more than 0.4 g of fat were chromatographed over silicic acid.

Another objective of the study of BELLMAN AND BARRY[95] was to determine if eluates obtained from the *Pesticide Analytical Manual*[97] procedure could be used to confirm the identification of pesticide residues by combined GLC–MS. The FID and TICM chromatograms (Fig. 58) were obtained from a 6% eluate of a frog leg sample and show that the mass spectra of the four GLC peaks are identical. (The mass spectra

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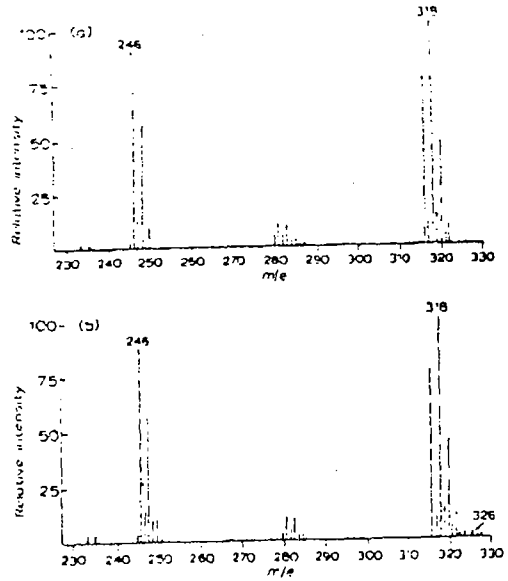

Fig. 57. Mass spectra of (a) a *p,p'*-DDE standard recorded at 24 eV and (b) *p,p'*-DDE in a sample showing PCB carry-over after a silicic acid column separation procedure. The *m/e* 326 peak shows the presence of PCB.

of standard α-, β-, γ- and δ-isomers of HCH* also appear to be identical and match the mass spectra of the four GLC peaks.) Hence identification of which isomers of HCH were present in the sample required the combination of GLC retention time and the mass spectra produced.

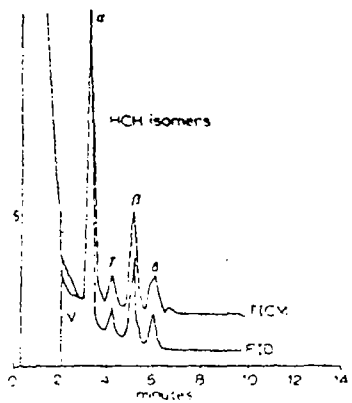

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Fig. 58. ECD and FICD chromatograms obtained from the 6% eluate of frog leg sample. [illegible] cent front. [illegible] = amount at which the glass cube is expected. Blips seen on FICD peaks mark MS [illegible]. Column: 2% OV-17/2% QF-1 on HP Chromosorb W, 180°.

* HCH is the common name for the mixture of isomers of 1,2,3,4,5,6-hexachlorocyclohexane

The occurrence and distribution of PCB's in the River Rhine and the coastal areas of The Netherlands has been reported by KOEMAN *et al.*[3]. The identification was carried out by GC and mass spectrometry. For this purpose a Varian MAT Model CH 4 mass spectrometer was coupled to a Varian Aerograph Type A550-B oven utilizing an interface under conditions described by TENNOEVER DE BRAUW AND BRUNNEE[98]. The relative retention times (R_r) were estimated with a Varian Aerograph Model 204-1B gas chromatograph with electron capture detection. Pyrex glass column (5 ft. × 1/8 in.) filled with 10% DC-200 on 80–100 mesh Gas-Chrom Q were used in both gas chromatographs, and the columns were operated at 200° with helium or nitrogen as carrier gas. The tissues of the indicator organisms used were extracted with petroleum ether in a Soxhlet extraction apparatus after drying with anhydrous sodium sulfate. The samples were cleaned up by dimethylformamide partition[99] and column chromatography over activated Florisil. The apolar compounds including the PCB's were eluted with hexane and then a 10% diethyl ether in hexane solution was used to elute dieldrin and aldrin. Tables 31 and 32 list the mass numbers and numbers of chlorine atoms per molecule of peaks with relative retention times (relative to dieldrin = 1.00) longer than 0.20 and shorter than 1.00 and of peaks longer than 1.00, respectively. Mass spectra were taken from all peaks present in the total ion current chromatogram obtained with the double ion source of the CH 4 mass spectrometer. In Figs. 59a and b gas chromatograms are shown of an eider extract and a technical PCB mixture (Phenoclor DP6), illustrating a strong resemblance between the electron capture chromatograms and the ion current chromatograms. Both from the mass numbers and the numbers of chlorine atoms per molecule, it was concluded that most of the compounds present in the extracts could be identified as PCB's

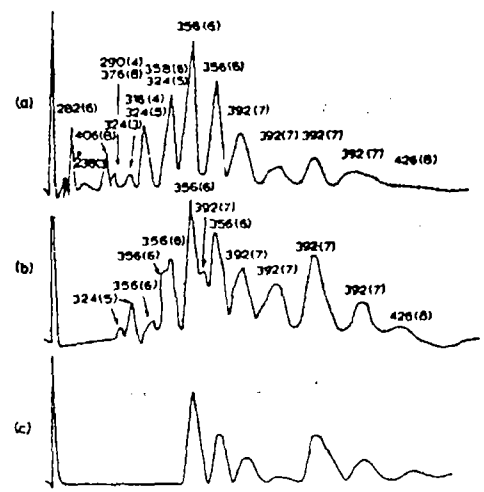

Fig. 59. Gas chromatograms of (a) an eider extract, (b) a technical PCB mixture (Phenoclor DP6) and (c) a grey seal liver and brain extract.

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TABLE 31

MASS NUMBERS AND NUMBERS OF CHLORINE ATOMS PER MOLECULE (IN PARENTHESES) OF PEAKS WITH RELATIVE RETENTION TIMES (RELATIVE TO DIELDRIN = 1.00) LONGER THAN 0.20 AND SHORTER THAN 1.00

With the exception of HCB, telodrin and compound 376 (8) all compounds identified are conformable to PCB's. A 10% DC-200 on Supelcoport Gas Chrom Q column was used, temperature, 200°; nitrogen flow rate, 45 ml/min. Mass number calculations are based on Cl = 35. HCB = Hexachlorobenzene, telodrin = 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzofuran, + = at the given retention time a peak is present in the chromatogram.

Sampling area	*Type of sample*	R_x 0.25	0.32	0.33	0.34	0.40	0.48	0.57	0.61	0.65	0.71	0.75	0.84	0.86
1	Roach (*Leuciscus rutilus*) total body extracts 1967, 1968	HCB 282 (6)	256 (3)	256 (3)		256 (3) + 290 (4)	290 (4)	290 (4)	290 (4)		290 (4)		324 (5)	324 (5)
1	Groundling (*Gobio gobio*) total body extracts 1968	+	+	+		+	+		+		+		+	+
2	Mussel (*Mytilus edulis*) 1968	+			+	+				+			+	
3	Sand eel (*Ammodytes lancelatus*) total body extracts 1966	+		+		+	+	+	+		+			+
3	Sandwich tern (*Sterna sandvicensis*) tissue and egg extracts 1965, 1966	HCB 282 (6)				256 (3)	290 (4)	290 (4)	Telodrin 406 (8)		290 (4) + 376 (8)		324 (5)	
3	Elder (*Somateria mollissima*) tissue and egg extracts 1966, 1967	HCB 282 (6)				256 (3)			Telodrin 406 (8)		290 (4) + 376 (8)		324 (5)	
	Phenochlor DP6											324 (5)	324 (5)	
	Arochlor 1260											324 (5)	324 (5)	
	Clophen A60											324 (5)	324 (5)	

TABLE 32

MASS NUMBERS AND NUMBERS OF CHLORINE ATOMS PER MOLECULE (IN PARENTHESES) OF PEAKS WITH RELATIVE RETENTION TIMES (RELATIVE TO DIELDRIN = 1.00) OF PEAKS LONGER THAN 1.00

With the exception of DDE all compounds identified are conformable to PCB's. + = At the given retention time a peak is present in the chromatogram. For further information, see Table 31.

Sampling area	*Type of sample*	R_x 1.01	1.17	1.23	1.45	1.57	1.69	1.96	2.27	2.67	3.20	3.58
1	Roach (*Leuciscus rutilus*) total body extracts 1967, 1968	DDE 316 (4) + 324 (5)	358 (6)	358 (6)	358 (6)	392 (7)	358 (6) 392 (7)	392 (7)	392 (7)	392 (7)	392 (7)	426 (8)
1	Groundling (*Gobio gobio*) total body extracts 1968	+	+	+	+	+	+	+	+	+	+	+
2	Mussel (*Mytilus edulis*) 1968	+	+	+	+	+	+	+	+	+	+	+
3	Sand eel (*Ammodytes lancelatus*) 1966	+	+	+	+	+	+	+	+	+	+	+
3	Sandwich tern (*Sterna sandvicensis*) tissue and egg extracts 1965, 1966	DDE 316 (4) + 324 (5)		358 (6) 324 (5)	358 (6)		358 (6)	392 (7)	392 (7)	392 (7)	392 (7)	426 (8)
3	Eider (*Somateria mollissima*) tissue and egg extracts 1966, 1967	DDE 316 (4) + 324 (5)		358 (6) + 324 (5)	358 (6)		358 (6)	392 (7)	392 (7)	392 (7)	392 (7)	426 (8)
	Phenoclor DP6	358 (6)	358 (6)	358 (6)	358 (6)	392 (7)	358 (6)	392 (7)	392 (7)	392 (7)	392 (7)	426 (8)
	Arochlor 1260	358 (6)	358 (6)	358 (6)	358 (6)	392 (7)	358 (6)	392 (7)	392 (7)	392 (7)	392 (7)	426 (8)
	Clophen A60	358 (6)	358 (6)	358 (6)	358 (6)	392 (7)	358 (6)	392 (7)	392 (7)	392 (7)	392 (7)	426 (8)

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(with a strong probability of Phenoclor DP6, Clophen A60 and Aroclor 1260 being present). Other chlorinated compounds found were hexachlorobenzene (HCB), *p,p'*-DDE and telodrin.

Tables 31 and 32 indicate that the lower chlorinated PCB's occur more frequently in the tissues of the roach than in the seabirds. This suggests the possibility that the lower chlorinated PCB's are less persistent, and this was confirmed by an experiment with Japanese quail which were fed a diet containing 2,000 p.p.m. of Phenoclor DP6 (chromatogram in Fig. 50b). A chromatogram representing the residue in liver and brain of these birds is shown in Fig. 50c and shows that a number of the compounds present in the original mixture were metabolized, particularly the lower chlorinated ones. Residues in the brains and livers of the quail were measured on a semiquantitative scale by peak height comparison of peak $R_T = 1.45$ using the Phenoclor DP6 mixture as a standard. The residue levels calculated in this manner were about twenty times higher in the quail tissues than in the livers and brains of the eiders. The quail developed hydropericardia with a dose of 2,000 p.p.m. Peakall *et al.*[29] also have reported hydropericardia in White Leghorn cockerels dosed with 400 p.p.m. in their diet.

Bagley *et al.*[3] described the identification of polychlorinated biphenyls in two bald eagles by combined GLC–mass spectrometry. The separation and identification of the unknown components were obtained with an LKB Model 9000 GLC–MS equipped with the stainless-steel molecule separator system of Ryhage[199]. The 9 ft. × 0.25 in. spiral glass column was packed with 1% SE-30 on 100–120 mesh Gas-Chrom Q. The operating temperatures were: flask heater 220°, GLC oven 180°, separator 240°, and ion source 270°. The carrier gas was helium at a rate of 35 ml/min, the ionization potential was 70 eV, trap current 60 μA, accelerating voltage 3.5 kV and the scan time was 12 sec from *m/e* 2–400. TLC on silica gel as described by Mulhern[196] was

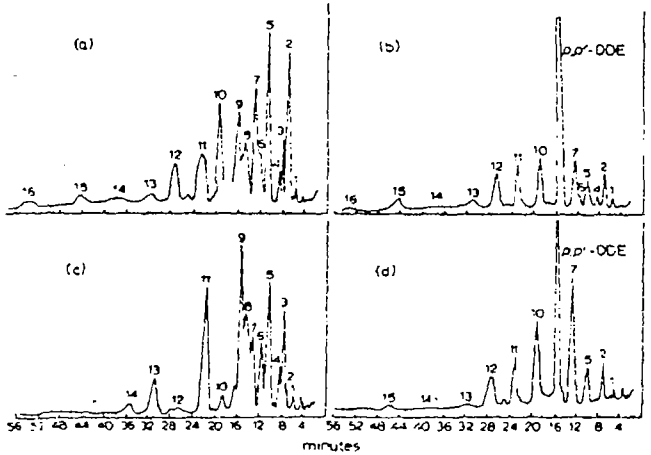

Fig. 60. Total ion current chromatogram of: (a) standard PCB mixture (Arochlor 1254, 0.5 μg); (b) eagle carcass 68-056, TLC zone IV; (c) eagle carcass 68-056, TLC zone III; and (d) eagle carcass 68-050, TLC zone IV. GLC conditions: 9 ft. × 0.25 in. 1% SE-30 column; oven temperature, 180°; helium flow, 35 ml/min.

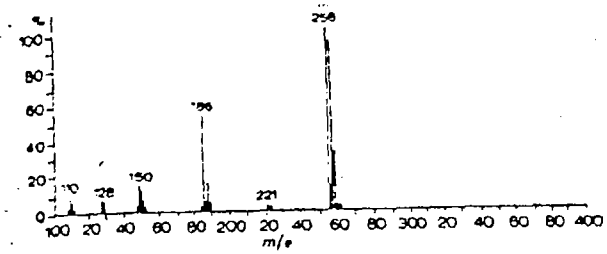

Fig. 61. Mass spectrum of peak 1 (Figs. 60a, b, and d), empirical formula $C_{12}H_7Cl_3$.

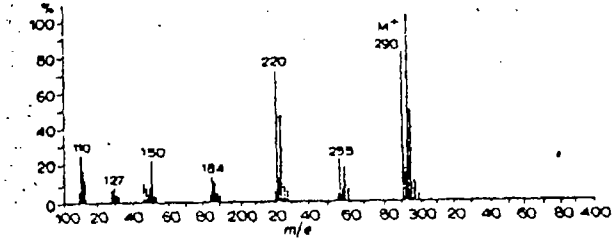

Fig. 62. Mass spectrum of peak 2 (Figs. 60a, b, and d), empirical formula $C_{12}H_6Cl_4$.

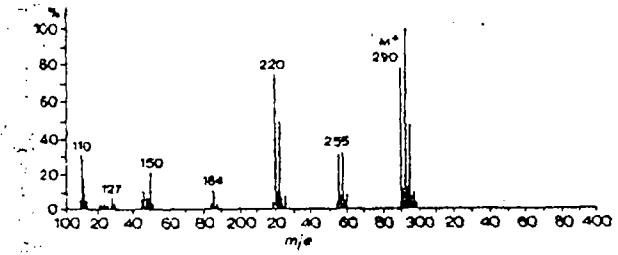

Fig. 63. Mass spectrum of peak 3 (Figs. 60a–c), empirical formula $C_{12}H_6Cl_4$.

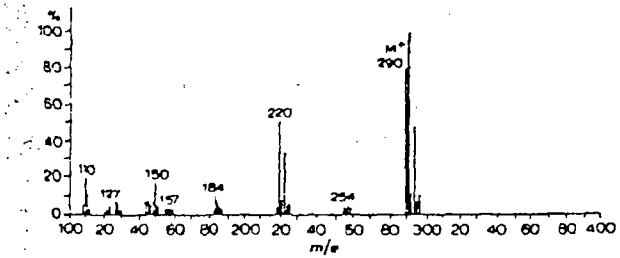

Fig. 64. Mass spectrum of peak 4 (Figs. 60a and c), empirical formula $C_{12}H_6Cl_4$.

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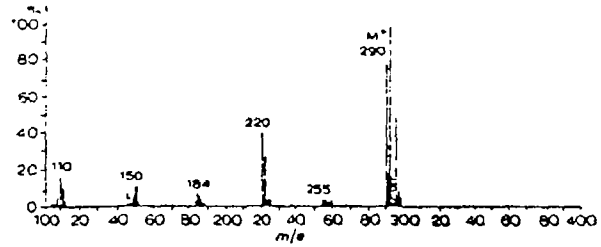

Fig. 65. Mass spectrum of peak 5 (Figs. 60a, b, and d), empirical formula $C_{12}H_6Cl_4$.

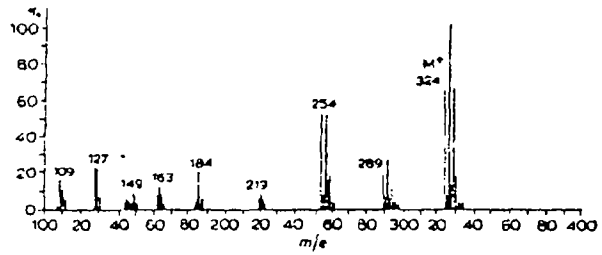

Fig. 66. Mass spectrum of peak 5 (Fig. 60c; 1254-III (total ion current chromatogram not shown for Aroclor 1254, TLC zone III)), empirical formula $C_{12}H_5Cl_5$.

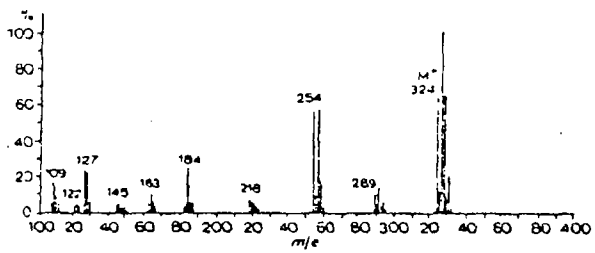

Fig. 67. Mass spectrum of peak 7 (Figs. 60a, b, and d), empirical formula $C_{12}H_5Cl_5$.

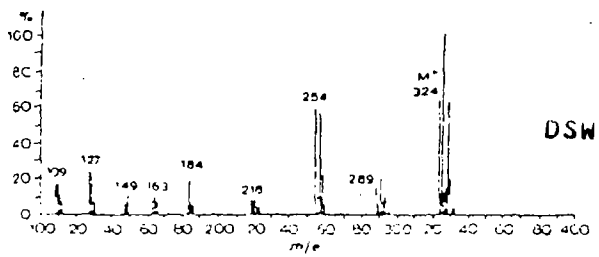

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Fig. 68. Mass spectrum of peak 8 (Figs. 60a and c), empirical formula $C_{12}H_5Cl_5$.

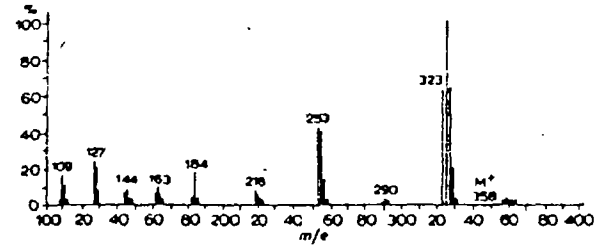

Fig. 69. Mass spectrum of peak 9 (Figs. 60a and c), empirical formula $C_{12}H_4Cl_6$.

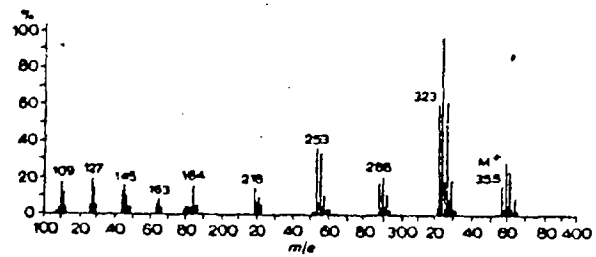

Fig. 70. Mass spectrum of peak 10 (Figs. 60a, b, and d), empirical formula $C_{12}H_4Cl_6$.

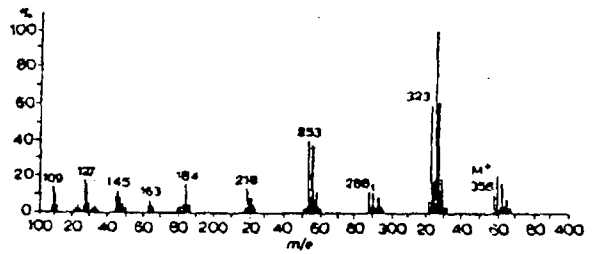

Fig. 71. Mass spectrum of peak 11 (Fig. 60c; 1254-III (total ion current chromatogram not shown for Aroclor 1254, TLC zone III)), empirical formula $C_{12}H_4Cl_6$.

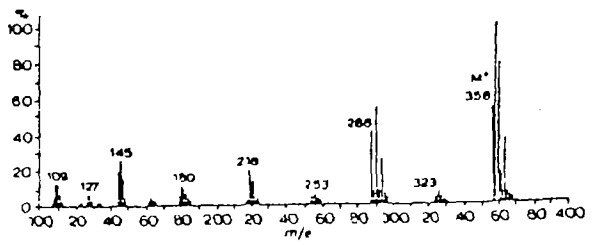

Fig. 72. Mass spectrum of peak 12 (Figs. 60a, b, and d), empirical formula $C_{12}H_3Cl_7$.

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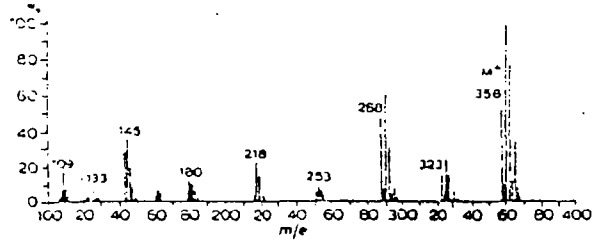

Fig. 73. Mass spectrum of peak 12 (Figs. 6oa, b, and d), empirical formula $C_{12}H_4Cl_6$.

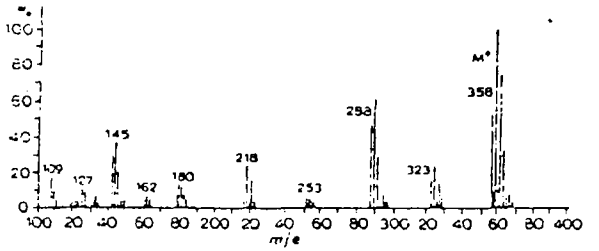

Fig. 74. Mass spectrum of peak 13 (Figs. 6ob, and d), empirical formula $C_{12}H_4Cl_6$.

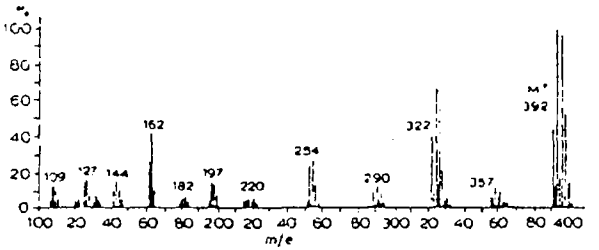

Fig. 75. Mass spectrum of peak 15 (Figs. 6oa, b, and d), empirical formula $C_{12}H_2Cl_8$.

used to separate interfering organochlorine pesticides. The major peaks as shown in the total ion current chromatogram for Aroclor 1254 (Fig. 6oa) are numerically labeled in order of their retention time. The peaks in total ion current chromatograms for eagle samples (Fig. 6od) are labeled as they relate to the Aroclor standard and show that peaks 1–16 have approximately the same retention times as identically numbered peaks in Aroclor 1254. Figs. 61–75 show mass spectra for peaks in the Aroclor standard and corresponding peaks in eagle tissue samples.

Table 33 lists the relative retention time of PCB's and some commonly occurring organochlorine pesticides obtained using a 1% SE-30 column. The similarity of relative retention times (compared to *p,p'*-DDE) of a number of PCB peaks and the organochlorine pesticides prohibits the utility of GLC identification alone

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TABLE 33

RELATIVE RETENTION TIME OF PCB'S AND SOME COMMONLY OCCURRING ORGANOCHLORINE PESTICIDES (*p,p'*-DDE = 1.00)

PCB		*Relative retention time*	*Compound*	*Relative retention time*
Peak No.	*Mol. wt.*			
1	256 (3)[a]	0.39	Heptachlor	0.43
2	290 (4)	0.49		
3	290 (4)	0.53	Aldrin	0.53
4	290 (4)	0.58		
5	290 (4), 324 (5)	0.70	Heptachlor epoxide	0.66
6	324 (5)	0.79		
7	324 (5)	0.83	Dieldrin	0.96
8	324 (5)	0.97	*o,p'*-DDD	1.02
9	358 (6)	1.04	Endrin	1.08
10	358 (6)	1.23	*p,p'*-DDD	1.27
11	358 (6)	1.46	*o,p'*-DDT	1.35
12	358 (6)	1.72	*p,p'*-DDT	1.69
13	358 (6), 392 (7)	2.01		
14	392 (7)	2.32		
15	392 (7)	2.80		
16	392 (7)	3.38		

[a] The number in parentheses following the molecular weight is the number of chlorine atoms in the molecule.

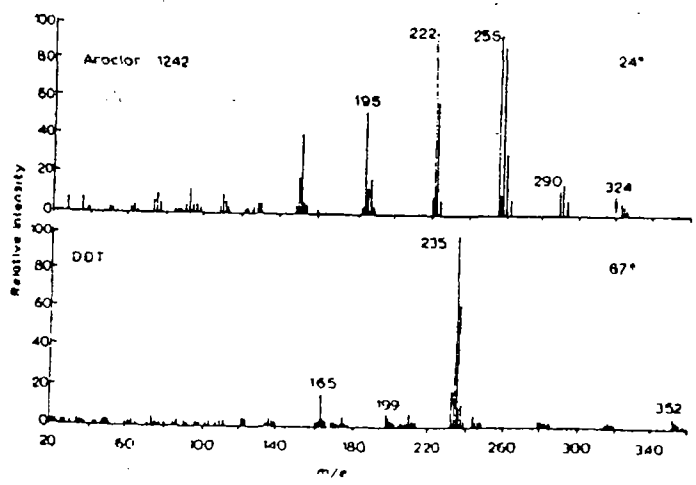

Fig. 76. 70 eV mass spectra (CEC 21-110B instrument) of a GC mixture of Aroclor 1242 and DDT at different sample temperatures. Isotope clusters at *m/e* 222, 256, 290, and 324 result from individual components of "Aroclor", the group of peaks at *m/e* 150 are mainly fragment ions from the...

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TABLE 34

MASS SPECTRA OF POLYCHLORINATED BIPHENYLS

The first line in the heading is sample, the second peak number, and the third molecular w- ht with the number of chlorine atoms in the molecule in parentheses.

m/e	1254 III 6 324 (5)	68–050 III 9 324 (5)	66–050 III 10 358 (6)	1254 14 392 (7)	68–056 IV 14 392 (7)	1254 14 392 (7)	68–056 IV 16 392 (7)	1254 13 392 (7)
98	10.2	5.4	6.3	8.2	10.9	10.9	7.6	10.8
99	7.0	3.7	4.8	4.1	5.4	5.0	4.1	4.1
107	3.9	1.0	2.1	4.1			3.4	4.0
108	4.3	2.2	5.3	11.8	15.9	13.6	10.7	11.9
109	20.2	12.5	16.0	15.3	20.0	16.4	10.0	22.8
110	17.0	8.3	11.3	13.8	14.1	14.5	8.3	16.1
111	9.5	3.3	4.0	5.3	6.4	6.8	4.1	5.8
119			1.2		4.1	4.5	3.1	2.8
120	3.2	1.3	2.5	5.9	6.4	6.4	5.2	5.5
121	3.9	1.5	2.8	5.3	5.4	7.3	4.8	5.3
122	6.4	3.4	5.7	7.1	8.2	6.8	5.9	7.1
123	6.6	3.0	3.1		5.9		4.1	3.3
125	3.6							
126		1.0	9.1	17.1	18.9	19.1	15.2	19.2
127	29.5	22.9	11.2	22.3	20.7	25.4	16.2	21.9
128	27.3	21.2	6.0	14.1	9.5	14.5	6.7	9.7
129	9.3	5.6	1.8			5.4		
131			1.7		5.9		3.4	1.2
132		1.6	3.8	8.8	10.0	10.0	7.2	8.3
133	4.8	2.4	3.7	8.2	9.1	10.0	5.9	7.2
134		1.4	2.1	4.4	5.9		3.4	3.9
135	3.9	1.6	1.8	2.9	4.5		3.1	3.2
136								
137	2.7							
138	5.2							
143				10.3	17.3	11.8	10.3	8.9
144	6.6	4.6	27.4	18.2	29.5	20.9	11.7	37.5
145	9.8	5.8	34.0	16.2	26.4	17.3	11.0	38.9
146	6.8	4.2	18.9	14.4	16.8	13.6	9.3	23.9
147	7.3	3.7	8.5	6.5	8.6	10.0	5.2	11.1
148	5.0	2.0	3.2	3.2			2.1	3.3
149	13.4	5.0	2.8	2.9			3.1	2.8
150	5.7			3.5				2.5
150			1.5	4.4			4.5	1.2
157			2.3	2.9			2.8	1.9
158			1.7	4.3				2.8
161			2.5	25.3	29.1	27.3	26.7	17.5
162	9.3		3.8	42.9	47.7	43.6	48.3	29.1
163	13.2		3.7	30.6	31.4	30.0	31.0	21.0
164	9.3		1.9	14.3	11.4	10.9	9.8	7.8
165	3.2							
167	3.2							
168								
169								
170								
171								
179				9.4	5.9	9.1	8.2	6.0
180				16.9	7.3	13.2	7.7	6.9
181				15.1	8.2	10.9	9.1	6.5
182	2.0	1.5	11.3	8.8	11.7	10.0	9.5	11.2
183	6.1	4.6	8.5	5.6	9.4	7.3		4.8
191	25.2	17.5	5.7	9.1	5.9	10.0	4.3	7.5
195	6.8	3.7	3.8	3.2		5.1		4.9

TABLE 34 *(continued)*

m/e	1254 III 6 324 (5)	68–050 III 9 324 (5)	68–050 III 10 358 (6)	1254 14 392 (7)	68–056 IV 14 392 (7)	1254 16 392 (7)	68–056 IV 16 392 (7)	1254 13 392 (7)
196	9.1	5.8	1.1	3.2				1.9
196				8.8	9.1	8.2	7.6	5.0
197				17.6	19.5	12.7	13.8	11.4
198				15.6	17.3	13.6	13.4	11.7
199				9.1	8.6	6.4	9.0	5.1
216			1.2	6.5	7.7	5.4	5.5	5.0
217	7.9		3.7	8.2	8.6	7.3	6.5	7.5
218	9.5	6.7	23.6	11.5	16.4	10.9	6.0	25.8
219	16.6	6.2	6.6	7.1	7.7	8.2	5.5	8.3
220	7.3	5.8	16.0	12.3	11.8	15.4	4.6	19.2
221	8.6	4.2	2.9	3.8		4.5		4.4
222	2.7	1.4	2.8	6.8	4.5	7.3		5.5
251								2.1
252			5.7	25.9	28.2	25.4	25.5	20.8
253			6.6	8.2	9.8	9.1	6.9	9.3
254	59.1	54.2	11.3	40.0	32.7	40.0	25.5	28.6
255	10.2	7.5	7.5	8.8	7.7	9.1	5.7	10.4
256	61.4	53.7	8.5	21.8	14.1	25.4	10.7	15.5
257	9.3	7.3	3.4	5.3	4.5	6.4	2.2	5.3
258	20.4	15.8	2.7	7.1	3.6	9.1	2.4	4.7
259	4.1	3.3	1.4					
260	4.2	2.7						
261	1.8	1.1						
262	1.9							
286				3.5	4.5	4.5	4.6	5.3
287				5.9	7.3	7.3	5.9	52.8
288			46.2	12.3	24.5	13.6	9.0	13.6
289	29.5	14.2	7.5	13.3	13.1	11.8	9.3	69.4
290	20.4	3.7	59.4	23.5	34.1	26.4	10.7	14.2
291	43.2	17.9	10.4	10.0	10.0	10.9	5.5	43.0
292	23.9	3.7	3.0	18.8	20.9	23.6	7.1	8.9
293	22.7	0.9	5.7	5.9	5.7	6.8	3.1	12.2
294	11.4	1.5	8.0	9.4	7.0	10.0	2.8	3.6
295	5.7	2.1	2.6	2.3				3.0
296	2.9		1.7	4.9				
297	0.7							
322				38.8	40.9	40.9	40.3	44.5
323			12.3	9.7	9.1	10.4	8.6	18.6
324	63.6	62.5	13.4	82.3	68.2	82.3	69.0	52.8
325	10.4	9.2	20.7	17.6	13.6	17.3	13.0	33.3
326	100.0	100.0	19.8	66.5	50.4	74.5	48.3	44.4
327	13.9	12.9	15.1	14.7	10.0	16.4	9.0	10.7
328	63.6	62.5	12.3	32.9	20.9	36.4	17.9	21.4
329	8.9	7.9	5.7	7.1	5.0	9.1	4.0	8.6
330	21.6	18.3	3.8	10.6	6.4	12.3	5.2	6.4
331	2.7	2.6	1.2	3.5				
332	3.4	2.7		4.9				
333	0.6							
337				14.1	38.6	13.6	9.0	55.5
358			51.8	11.2	35.2	11.8	5.9	21.1
359			9.4	27.6	72.7	24.5	17.9	100.0
360			100.0	20.6	14.7	19.1	10.2	23.9
361			16.0	22.9	54.1	20.0	16.2	81.9

(Continued on p. 418)

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TABLE 34 (continued)

m/e	1254 III 6 351 (5)	68-050 III 9 354 (5)	68-050 III 10 358 (6)	1254 14 392 (7)	68-056 II 14 392 (7)	1254 16 392 (7)	68-056 II 16 392 (7)	1254 13 392 (7)
362			80.2	15.0	17.9	17.3	7.6	15.0
363			12.3	10.6	23.6	10.0	7.2	36.1
364			34.0	7.1	6.8	7.3	3.4	6.1
365			4.7	3.2	6.4	3.6	1.7	8.6
366			8.5	2.1				
367			1.1					
368			1.2					
392				47.1	43.2	47.3	51.7	29.7
393				10.6	10.4	13.6	10.7	6.1
394				100.0	100.0	100.0	100.0	61.3
395				17.6	17.3	20.0	17.6	10.8
396				97.1	95.4	90.0	96.5	58.3
397				14.7	13.9	16.4	15.2	8.0
398				52.9	46.8	51.2	53.4	28.6
399				7.6	7.7	10.0	7.9	4.1
400				15.9	16.6	17.3	16.5	10.3
401				2.6	2.5	2.7	2.4	1.4
402				3.5	3.2	3.6	2.9	1.9

peaks have similar retention times although in some cases mass spectral data show the compounds to be isomers rather than the same compound.

The identification of PCB's and DDT in mixtures by mass spectrometry was reported by HUTZINGER *et al.*[102]. Fig. 76 illustrates 70 eV mass spectra of a 1:1 mixture of Aroclor 1242 and DDT at different sample temperatures. The peaks arising from the individual major components of the Aroclor mixture can easily be distinguished from major peaks found in the most common chlorinated insecticides. A useful spectrum of Aroclor 1242 could be obtained at low temperatures by direct introduction with the aid of a temperature-controlled probe[101], and fractional sublimation from many chlorinated pesticides directly in the ion source seems possible (see Fig. 76).

VII. ANALYSIS OF CHLORINATED NAPHTHALENES AND DIBENZOFURANS

Chlorinated naphthalenes possess a variety of suggested uses related to their electrical, flame-retardant and fungus-resistant properties, stability, and compatibility with other materials[103]. Many of their physical properties and suggested uses are similar to those of polychlorinated biphenyls[3]. The behavior of chlorinated naphthalenes in analytical methods for organochlorine pesticides and PCB's was studied by ARMOUR AND BURKE[104]. The chlorinated naphthalenes studied were representative Halowax (Koppers Co., Inc.) samples, *e.g.*, Halowax 1014, a mixture of tetra-, penta- and hexachloronaphthalenes; Halowax 1051, octachloronaphthalene; and Halowax 1099, a mixture of trichloro- and tetrachloronaphthalenes. GLC of chlorinated naphthalenes showed that their behavior is similar to that of the organochlorine pesticides. Retention time and response data for the three Halowax [illegible] with electron capture detection are given in Table [illegible]

TABLE 35

GLC DATA FOR CHLORINATED NAPHTHALENES

GLC columns: glass, 6 ft. × 4 mm I.D. 10% DC-200 on 80–100 mesh HP Chromosorb W, and 10% DC-200/15% QF-1 (1:1) on 80–100 mesh HP Chromosorb W. Operating conditions: nitrogen flow rate, 120 ml/min; column and detector temperature, 200°; injector temperature, 225°. Concentric design electron capture detector operated at d.c. voltage to produce 1/4 full scale deflection (f.s.d.) for 1 ng of heptachlor epoxide when full scale deflection is 1×10^{-8} A.

Compound	Response (ng) (Approx. amount for 1/4 f.s.d.)	Retention times relative to aldrin	
		10% DC-200 column	10% DC-200/15% QF-1 (1:1) column
Halowax 1099	25	0.45, 0.57, 0.65, 0.71, 0.79, 0.86, 0.91, 1.04, 1.14, 1.23, 1.44, 1.57, 1.88	0.49, 0.65, 0.73, 0.82, 0.96, 1.14, 1.26, 1.46, 1.62, 1.96
Halowax 1014	25	0.45, 0.79, 1.04, 1.43, 1.57, 1.82, 2.85, 3.12, 3.53, 3.73, 6.0	0.49, 0.83, 1.15, 1.45, 1.61, 1.93, 2.80, 3.16, 3.68, 3.84, 5.9
Halowax 1051	10	11.3	10.8

GC conditions are those used for organochlorine pesticide residue analysis[106]. The components of Halowax 1014 chromatograph throughout the retention time region of the organochlorine pesticides (retention times relative to aldrin, 0.5 to 6). Halowax 1099 exhibits peaks in the region of early eluting pesticides (retention times relative to aldrin, 0.5 to 2), and Halowax 1051 chromatographs beyond the retention times of common pesticides (retention time relative to aldrin, 11). Study of the Florisil column chromatographic clean-up[106] showed that Halowax 1014 and 1099 were completely eluted by 200 ml of 6% diethyl ether–petroleum ether. Halowax 1051 was found in both eluates from successive 200 ml elution with 6% and 15% diethyl ether–petroleum ether. A number of the organochlorine pesticides, including DDT and its analogs are also eluted by 6% diethyl ether–petroleum ether. The chromatographic procedure on silicic acid columns developed for separating PCB from organochlorine pesticides[42] was evaluated for the separation of the chlorinated naphthalenes from pesticides. Each of the Halowax samples (*e.g.*, 1014, 1051 and 1099) was completely eluted by 250 ml of petroleum ether eluent. (PCB's are also recovered in this eluate.) No Halowax was found in the acetonitrile–hexane–methylene chloride eluate, where most of the common organochlorine pesticides are recovered.

Fig. 77 shows gas chromatograms of brown trout extract fortified with 2.5 p.p.m. Halowax 1014, 0.3 p.p.m. *p,p'*-DDT and 0.2 p.p.m. *p,p'*-TDE and containing 0.19 p.p.m. *p,p'*-DDE residues before and after separation on silicic acid columns.

The identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial polychlorinated biphenyls was described by VOS *et al.*[105]. In a previous study[107] a significant difference in toxicity was found between three commercial PCB preparations, high mortality, liver necrosis and chick edema-like lesions being associated with two of the compounds tested. In the study of VOS *et al.*[105], column chromatography and GLC demonstrated the presence of polar compounds in the 25% diethyl ether fraction of each of two PCB

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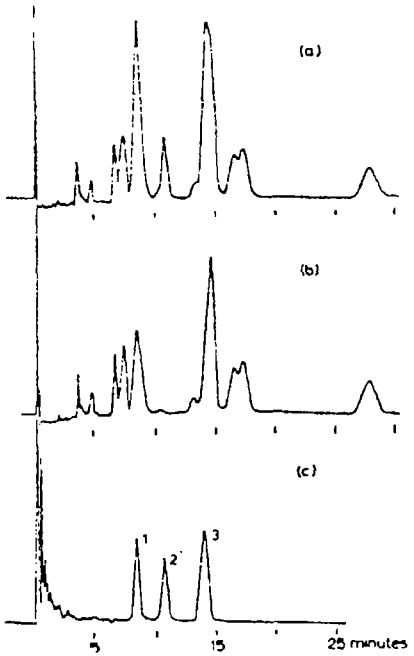

Fig. 77. GLC curves of brown trout extract fortified with 2.5 p.p.m. Halowax 1014, 0.3 p.p.m. *p,p'*-DDT, and 0.2 p.p.m. *p,p'*-TDE and containing 0.19 p.p.m. *p,p'*-DDE residue, before and after separation on a silicic acid column. (a) Before separation. (b) petroleum ether eluate from silicic acid column containing chlorinated naphthalene. (c) Polar eluate from silicic acid column containing. (1) *p,p'*-DDE, (2) *p,p'*-TDE, and (3) *p,p'*-DDT. 10-mg sample injected for each curve. GLC conditions: same as those given in Table 35.

preparations which contained an average of 60% chlorine (Phenoclor DP6 and Clophen A60), and a chick embryo assay[109] confirmed the high toxicity of this fraction. The polar compounds were not found in the corresponding fraction of a third PCB (Aroclor 1260). The identity of the polar compounds which included tetra- and pentachlorodibenzofuran was confirmed by mass spectrometric and microcoulometric analyses.

Aliquots obtained from the column chromatography (Florisil) of the PCB's

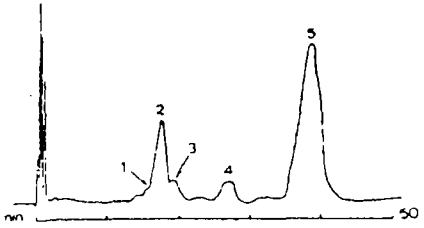

Fig. 78. Gas chromatogram of the third fraction of Clophen A60 (showing a pattern identical to that of the third fraction of Phenoclor DP6). Data for peaks 1–5 are given in Table 36.

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TABLE 36

RETENTION DATA AND COLLECTED MASS SPECTROMETRIC DATA OF PEAKS SHOWN IN FIG. 78

Peak No.	*Relative retention*[a]	*Mass Nos.*[b] *and No. of chlorine atoms/molecule*	*Identity of compounds*
1	1.40	304 (4 Cl)	Tetrachlorodibenzofuran
2	1.58	332 (6 Cl)	Hexachloronaphthalene
3	1.74	358 (6 Cl)	Hexachlorobiphenyl
		392 (7 Cl)	Heptachlorobiphenyl
4	2.42	338 (5 Cl)	Pentachlorodibenzofuran
		392 (7 Cl)	Heptachlorobiphenyl
5	3.48	366 (7 Cl)	Heptachloronaphthalene

[a] Retention related to dieldrin = 1.00.
[b] Mass number calculations based on Cl = 35.

were analyzed by GLC employing a 204-1B Varian Aerograph gas chromatograph with electron capture detection. The 5 ft. × 1/8 in. Pyrex glass column was filled with 10% DC-200 on 80–100 mesh Gas-Chrom Q and operated at 200° with nitrogen as carrier gas at 50 ml/min. Mass spectrometry was used to identify compounds present in the polar fraction (25% diethyl ether-hexane) from the column fractionation of PCB's. For this purpose a CH 4 mass spectrometer (Varian MAT) was coupled to a Varian Aerograph type A550B oven, with a 5 ft. × 1/8 in. Pyrex glass column filled with 10% DC-200 on 80–100 mesh Gas-Chrom Q. The design of the interface between the above gas chromatograph and the mass spectrometer has been described by TENNOEVER DE BRAUW AND BRUNNEE[88].

High-resolution mass spectra were taken on a double-focus spectrometer type SM (Varian MAT). Exact mass measurements were made by the peak-matching method and measurements were also made from spectra which were recorded on photographic plates processed by a Varian MAT on a Leitz comparator SAM1.

Quantitative GLC information was obtained via microcoulometric analysis of the third polar Florisil column fractions using a Dohrmann microcoulometer coupled to a Microtek Model MT220 gas chromatograph with a 6 ft. × 1/4 in. glass column containing the same 10% DC-200 columns packing described above. The

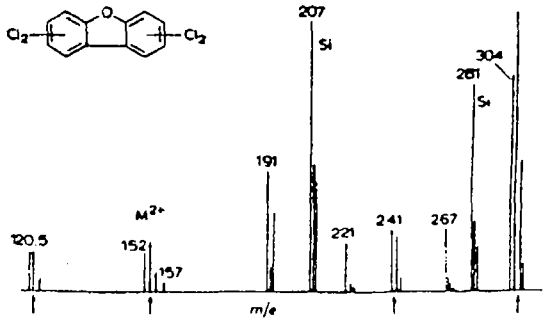

Fig. 79. Mass spectrum of tetrachlorodibenzofuran (mass No. 304) obtained from the third fractions of Clophen A60 and Phenoclor DP6 (peak 1 in Fig. 78).

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column effluents were oxidized in a Dohrmann combustion furnace and the hydrochloric acid produced by chlorinated compounds was detected and measured quantitatively in a T-300-S titration cell.

The microcoulometric gas chromatogram of the third fraction (25% diethyl ether–hexane eluates) obtained with a 10-mm Florisil column is shown in Fig. 8 and reveals the presence of polar compounds in Phenoclor DP6 and Clophen A60. (Data for peaks 1–5 are given in Table 36.)

Fig. 79 shows the mass spectrum of tetrachlorodibenzofuran (mass No. 304) obtained from the third fractions of Clophen A60 and Phenoclor DP6 (peak 5 in Fig. 78). Fig. 80 depicts a mass spectrum of pentachlorodibenzofuran (mass No. 338) obtained from the third fractions of Clophen A60 and Phenoclor DP6 (peaks 4 in Fig. 78). The spectrum of heptachlorobiphenyl (mol. wt. 392) is also present.

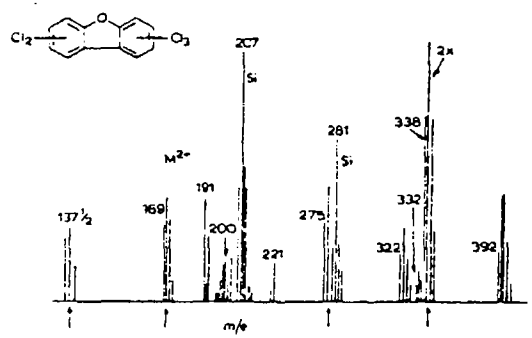

Fig. 80. Mass spectrum of pentachlorodibenzofuran (mass No. 338) obtained from the third fractions of Clophen A60 and Phenoclor DP6 (peak 4 in Fig. 78). The spectrum of heptachlorobiphenyl (mol. wt. 392) is also present.

The peak of the tetrachlorodibenzofuran (mass No. 304) was too masked by the proximity of the hexachloronaphthalene peak (peak No. 2, Fig. 78) to allow quantitative calculations. Similarly, peak No. 4 in the chromatogram (Fig. 78), found to be a mixture of heptachlorobiphenyl and pentachlorodibenzofuran, could not be measured directly. However, by assuming that the whole peak was produced by the dibenzofuran compound alone, a certain maximum level could be indicated. This level was found to be 5 p.p.m. of pentachlorodibenzofuran in Clophen A60 and 20 p.p.m. in Phenoclor DP6. The minimum level could be indicated with the estimated lower limit of detection (1 p.p.m.) of the mass spectrometric equipment.

The halodibenzofurans are extremely toxic. Tri- and tetrachlorodibenzofuran in a single oral dose of 0.5–1.0 mg/kg caused severe and often lethal necrosis in rabbits[110,111] and chloracne[110]. Table 37 shows the results of the chick embryo assay of the chlorinated biphenyls Aroclor 1260, Phenoclor DP6 and Clophen A60 and the 25% diethyl ether fraction of Clophen A60. The similarity between the mortality levels in groups 3 and 5 (Table 37) suggests a strong indication that the polar residue in the latter determines the toxicity of the commercial PCB's. Chlorinated naphthalenes are far less toxic than chlorinated dibenzofurans[111] and the latter may thus be assumed to determine the toxicity of the third fraction. The occasional occurrence of hydropericardia in chicks fed Aroclor[29] may indicate the presence of a very small quantity of a toxic factor in this preparation.

TABLE 37

CHICK EMBRYO ASSAY OF THE CHLORINATED BIPHENYLS AROCLOR 1260, PHENOCLOR DP6 AND CLOPHEN A60 AND THE 25% DIETHYL ETHER FRACTION (THE THIRD FRACTION) OF CLOPHEN A60

Test compound	Group No.	Dose[a] (mg/egg)	No. of eggs treated	No. of infertile eggs	Percentage hatch (% of fertile eggs)	Remarks
Aroclor 1260	1	3.5	15	0	80	
Phenoclor DP6	2	3.5	15	0	0	Most embryos died during first half of incubation period.
Clophen A60	3	3.5	20	1	5	
Clophen A60 (third fraction)	4	3.5	15	0	0	All embryos died during first four days of incubation period.
	5	3.5	15	0	7	Most embryos died during first half of incubation period.
	6	0.35	15	2	92	
	7	0.035	15	2	100	
–	8	0	20	2	94	Ethanol control
–	9	—	20	0	90	Untreated control

[a] The doses, each dissolved in 0.05 ml of ethanol, were injected into the air cell of fresh eggs before incubation.

The origin of the toxic factors in Clophen A60 and Phenoclor DP6 (e.g., tetra- and pentachlorodibenzofuran) can possibly be explained by a consideration of their manufacture and particularly of the procedure for the distillation of crude PCB in which sodium hydroxide can be used[112].

For example, PCB can react with sodium hydroxide at elevated temperatures to yield phenolic compounds which may be saponified to polychlorohydroxybiphenyls by sodium hydroxide in a polyhydric alcohol medium[113]. Dibenzofuran derivatives could then be formed via a further loss of hydrochloric acid.

VIII. SUMMARY

The chromatographic (column, thin-layer and gas–liquid chromatography and combined gas–liquid chromatography and mass spectroscopy) and biological aspects of the polychlorinated biphenyls have been reviewed with primary focus as to their utility, ecological distribution and toxicity as well as diverse techniques for their separation from chlorinated pesticides and subsequent analysis.

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