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FDA Defends Handling Of Chemical, Opposes Call for Outright Ban

Substances Called PCBs, Made Only by Monsanto, Have Caused Concern Over Possible Hazards

By a WALL STREET JOURNAL Staff Reporter

WASHINGTON — Food and Drug Administration officials defended their handling of the problem of PCBs, a widespread environmental contaminant, and opposed an outright ban on the chemical.

Dr. Charles Edwards, FDA commissioner, argued that unnecessary consumer confusion and alarm have arisen about the potential hazards of the chemical, which has been found in eggs, poultry and several packaged-food products.

"We are taking specific, adequate and positive steps to deal with whatever problem, or potential problem, that PCBs are likely to pose to human health," Dr. Edwards told a news conference. "We reject the need and, in fact, the feasibility, as some have proposed, for an outright ban on the substances. Although the use of PCBs requires control, an outright ban isn't feasible and wouldn't be in the best interest of the consumer," he added.

PCBs or polychloride biphenyls, are a class of industrial compounds manufactured in the U.S. only by Monsanto Co. They are widely used as a heat-exchange medium and for other industrial purposes because of their favorable properties, which include lack of flammability.

But since 1966, the substances have been detected as an environmental contaminant. PCBs, much like the pesticide DDT to which they are closely related, are persistent and relatively impervious to heat, chemicals and other forms of biological breakdown. They have been shown in laboratory tests with animals to cause liver damage and other adverse effects at high-dose levels. But the dangers of long-term chronic exposure to humans, at low levels, isn't clearly known.

Dr. Edwards and other FDA officials insisted that PCBs don't pose any immediate health hazard. They argued that the agency is taking the required steps to minimize the presence of the compounds in the food chain and reduce the exposure of consumers.

"Based on the levels of PCBs we have found in our scientific evaluation of the health hazard, except for accident-type situations, we don't have an imminent health hazard problem; but PCBs are a potential hazard," James Grant, deputy FDA commissioner, stated.

Mr. Grant said Monsanto was no longer selling PCBs for use in the food industry and was taking steps to replace them with other substances. The company will continue to market PCBs for industrial applications, particularly in transformers and other electrical equipment that are totally closed and can be made leak-proof, the FDA said. One federal expert said these applications constitute about 85% of PCBs' use.

FDA officials argued that substitutes for PCBs, particularly in electrical equipment, could pose undue hazards, particularly from the risk of fire. "They have important flame-resistant properties and, properly used, directly assist government and industry in protecting the consumer against fire," Dr. Edwards declared.

The FDA disclosed it had detected PCBs in 5% of a total of 18,000 raw agricultural commodities sampled. Most of these were fish and levels ranged from 0.01 parts per million to three parts per million. The agency has set a safety margin of less than five parts per million for PCBs in fish and poultry, less than 0.5 parts per million in eggs; and less than 0.2 parts per million in milk. These levels are more stringent for foods consumed by infants and children.

A second FDA survey of foods purchased by the consumer in the marketplace turned up PCBs at levels ranging from trace amounts to 0.56 parts per million in 60 of 720 samples. Most of the problem was in meat, poultry and fish.

More recently, the agency found the chemical as a contaminant in 36 of 60 samples of packaged foods at levels ranging from trace amounts to three parts per million. Foods included spaghetti and noodle dinners, and rice, grits and various cereals.

The agency said it wouldn't specify individual products, because the spot checking was random and the health hazard unproven.

However, the agency is continuing a more exhaustive survey of PCBs in packaged goods that it hopes to conclude by November. Then, if some products show consistently high levels, warnings might be issued, an FDA official said.

But the FDA argued that much more must be known about the extent of the PCB problem in packaged food before regulatory action is taken. The chemical has been traced to the packaging material, itself, a form of reprocessed paper. One major source of contamination in reprocessed paper, carbonless carbon paper that contains PCBs to hold the ink, is being eliminated by the paper manufacturers. But the FDA experts note the PCBs are so widely distributed in the environment that other sources may contribute to the contamination of packaging materials.

Technically, PCBs are banned from packaging materials as a toxic substance under FDA regulations. But agency experts said this restriction, which could require the removal of such packaging materials from the market, isn't being enforced.

The current survey of the extent of contamination of packaged products will help the agency decide whether to enforce this requirement or, perhaps, set guidelines for the amounts of PCBs that can be contained in packaging materials.

A key issue is the extent to which the chemical migrates from the paper into the food. One possible solution would be to require that companies insert a PCB-free barrier on wrapping paper between the packaging material and the food product.

But it's clear the FDA has decided to avoid precipitate action. Dr. Albert C. Kolbye Jr., deputy director of the agency's Bureau of Foods, insisted that consumer exposure to foods containing as much as five parts per million of PCBs is infrequent and sporadic. "To our knowledge, we aren't aware of any foods contaminated at significant levels on a regular and consistent basis," he said.

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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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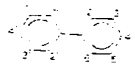
[illegible] several PCB's. [illegible] of replacing hydrogen atoms [illegible] a large number of substitution combinations arise [illegible]



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

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

TABLE 1

[illegible] USES OF POLYCHLORINATED BIPHENYLS[1]

[illegible]	Aroclor used with (wt %)	Use
[illegible] chloride	Aroclor 1248, 1254 and [illegible]	Secondary plasticizer to improve flame retardance and chemical resistance
[illegible]	[illegible]	Co-plasticizer to enhance resistance
[illegible]	Aroclor [illegible] and [illegible]	Improve mock track and fiber tear properties
[illegible] vinyl acetate [illegible] resins	Aroclor [illegible]	Pressure sensitive adhesive
	Aroclor 1221 and 1248 [illegible]	Increase chemical and oxidation resistance and adhesive qualities
[illegible] resins	Aroclor [illegible]	[illegible] and economical fire retardant
	Aroclor [illegible]	Increases strength of fiberglass [illegible] polyester resins
[illegible]	Aroclor 1221 [illegible]	[illegible]
[illegible]	[illegible]	[illegible] resistance, flame retardance and improves electrical insulation properties
[illegible]	Aroclor [illegible]	Improves chemical resistance
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

[illegible]ating at 150° 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 poly*chlorinated 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*
[illegible] gle oral dose of fervor (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	[illegible]
[illegible] mg daily for 6 days (64% Cl)	Rat	Cells swollen, hyaline granules present, most died within few days				27
[illegible] 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 18%, 1254 13%, 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 [illegible]	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 0.136 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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it was suggested[17] that they are likely to be 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 Arochlors in terms of oral LD_{50} (rats) and skin MLD (rabbits) is summarized in Table 3.

* Throughout this article the American (10⁹) billion is meant.

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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 LITTLEFIELD AND LICHTENSTEIN[19]. 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

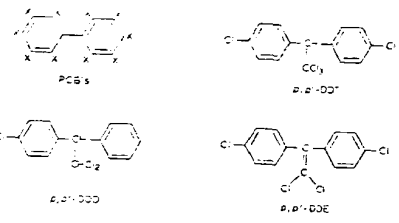
Additive	*Insecticide*					
	None		*DDT (75 p.p.m.)*		*Parathion (50 p.p.m.)*	
	H	*F*	*H*	*F*	*H*	*F*
Protein						
None	—	—	70[c]	51[c]	85	92
Aspirin (25 p.p.m.)	102	93	47[d]	45	78	105
PCB[b] (10 p.p.m.)	91	108	50[c]	59	82	86[d]
Caffeine (10 p.p.m.)	93	101	72	58[d]	69[d]	64[d]
RNA						
None	—	—	70[c]	89	81	67[c]
Aspirin (25 p.p.m.)	94	87	61	104	72	84
PCB (10 p.p.m.)	100	90	72	86	104[d]	95
Caffeine (10 p.p.m.)	100	94	88[d]	82	91	75
DNA						
None	—	—	77	91	94[c]	87
Aspirin (25 p.p.m.)	115[c]	86	91	86	43[d]	59[d]
PCB (10 p.p.m.)	93	105	94	86	50	62[d]
Caffeine (10 p.p.m.)	105	98	91	84	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 + additive) and control cultures (pesticide treated only) were significant at the 5% level.

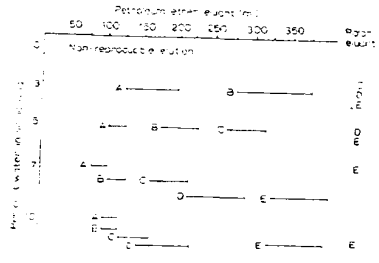
[d] Differences between treated cultures (pesticide plus additive) and control cultures (pesticide treated only) were significant at the 5% level.



KEANS described an activated Florisil column technique which separates aroclors, dieldrin, DDE and PCB with the first elution (60 ml of *n*-hexane), endrin, and heptachlor epoxide, DDD and DDT with the second elution (40 ml of 50% diethyl ether in hexane).

KOBLINTZ *et al.*[8] 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.

For separation of PCBs from DDT and its analogs and other common pesticides by column chromatographic on silicic acid–Celite was reported by ARGAUER AND BOWERS[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 *o,p'*-DDD with the second fraction (200 ml of acetonitrile–



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[illegible]

[illegible]

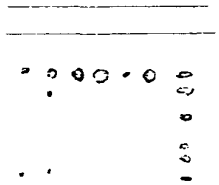


Fig. 2. Thin-layer chromatogram of [illegible] sample. AgNO3-impregnated Aluminagram Sil G. 1. [illegible] of solvent. 2. [illegible] of sample. 3. [illegible] of sample. 4. [illegible] of sample. 5. [illegible] of Aroclor 1254. 6. [illegible] Aroclor 1248. 7. [illegible] of each of DCBP, dieldrin, heptachlor epoxide, DDD, DDT and DDE (in ascending order).

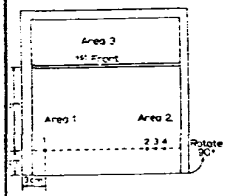
The separation and identification of DDT analogs in the presence of polychlorinated biphenyls by two-dimensional TLC was described by FEHRINGER AND WUSTRAU[20]. Separation resulted from the different migration patterns and migration distances relative to *p,p'*-DDE that mixtures of PCB's exhibit under two sets of conditions. Two-dimensional TLC was employed using MN-Kieselgel G-HR as the ab-

TABLE 7

EXAMPLES OF PCB QUANTITATION BY TLC

Sample	Location	PCB (p.p.m.)	DDE (p.p.m.)	PCB/DDE
Fish oil	Ga.	420	133	3.16
Fish oil	Calif.	300	70	4.3
Fish oil	N.J.	1250	175	7.14
Fish oil	N.C.	500	200	2.5
Fish oil	Ga.	500	200	2.5
Fish oil	Fla.	500	60	8.33
Fish oil	Fla.	350	200	1.75
Duck liver	Ark.	350	55	6.36

sorbent with a [illegible] mixture of *n*-heptane–acetone (98:2) as developing solvent and silver nitrate impregnation of the dissolution layer as the chromogenic agent. Fig. 1 illustrates a plate spotted for first-dimension development to be performed in a sandwich chamber. Fig. 2 shows a plate spotted for second-dimension development to be performed in a partially saturated chamber. Fig. 3 shows a plate spotted for second-dimension development to be performed in a partially saturated chamber. Mobile solvent was *n*-heptane–acetone (98:2) as mixed solvent. Figs. 5 through 12 illustrate separations of Aroclors 1254, 1260, 1248, 1242, 1232, 1221 and 1016 from DDT analogs. Fig. 13 depicts the two-dimensional separation of a mixture of eight compounds containing PCB's, dieldrin, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT. Figs. 14 and 15 show the two-dimensional separation of [illegible] DDT analogs from PCB



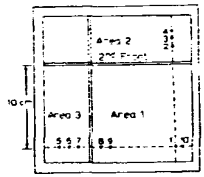
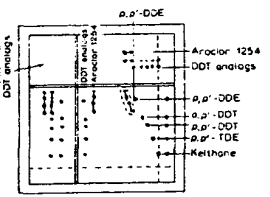


Fig. 3. Plate spotted for first-dimension development to be performed in a sandwich chamber. Mobile solvent *n*-heptane.

Fig. 4. Plate spotted for second-dimension development to be performed in a partially saturated tank. Mobile solvent *n*-heptane–acetone (98:2). Locations of spots after first-dimension development are not indicated since spots are not visible at this point.



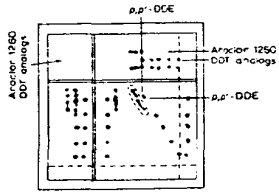
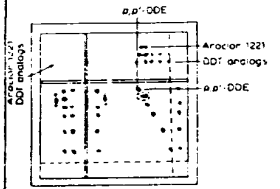


Fig. 5. Separation of Aroclor 1254 from the DDT analogs.

Fig. 6. Separation of Aroclor 1260 from the DDT analogs.



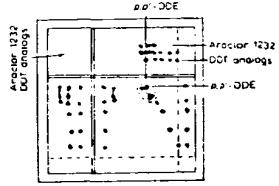


Fig. 7. Separation of Aroclor 1221 from the DDT analogs.

Fig. 8. Separation of Aroclor 1232 from the DDT analogs.

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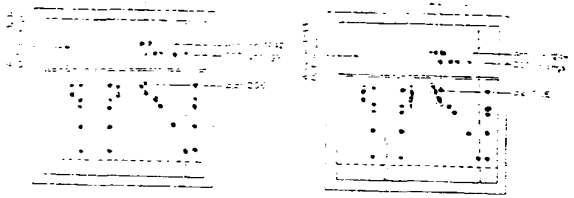

Fig. 9. Separation of Aroclor 1242 from the DDT analogs.

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

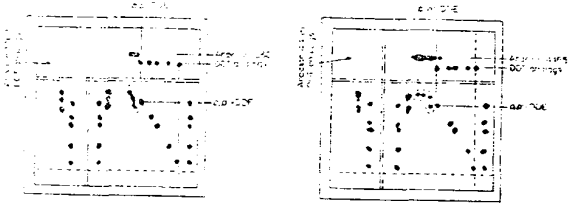

Fig. 11. Separation of Aroclor 1260 from the DDT analogs.

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

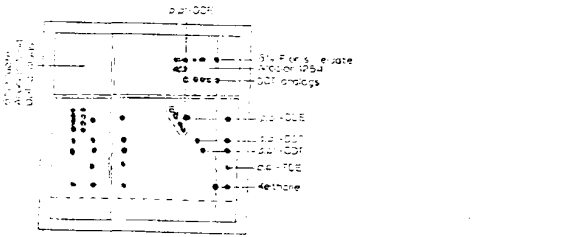

Fig. 13. Two-dimensional separation of PCB's isolated from a fish sample containing [illegible] Aroclor 1254 and *p,p'*-DDE.

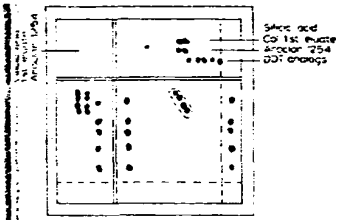

Fig. 14. Two-dimensional separation of PCB's isolated from a fish sample by the silicic acid column separation of ARMOUR AND BURKE[43].

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-

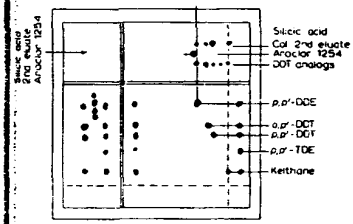

Fig. 15. Two-dimensional separation of kelthane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE isolated from PCB's in a fish sample by the silicic acid column separation of ARMOUR AND BURKE[43].

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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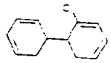
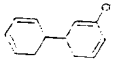
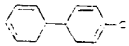
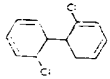
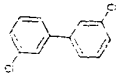
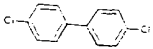
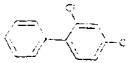
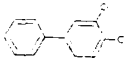
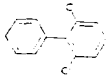
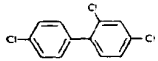
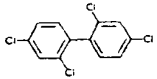
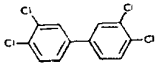
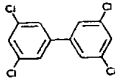
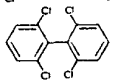
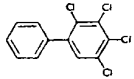
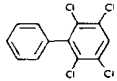
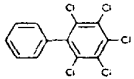
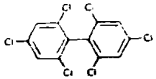
[illegible] (continued)

[illegible]	Formula	Starting material (source[illegible])	Method of purification[illegible]	Physical data m.p. (°C)	[illegible]
[illegible]		2-Aminobiphenyl (I)	TLC, MeOH aq.	53–[illegible]	[illegible]
[illegible]		3-Chloroaniline (II)	Dist., MeOH aq.	19–17	[illegible]
[illegible]			MeOH	77–78	[illegible]
[illegible]		2-Chloroiodobenzene (V)	TLC, MeOH	59–60	[illegible]
[illegible]		3,3'-Dinitrobiphenyl (II) ↓ 3,3'-Diaminobiphenyl	TLC, EtOH aq.	26–27	15
[illegible]		—	MeOH	148–149	[illegible]
[illegible]		2,4-Dichloroaniline (I)	TLC, MeOH aq.	24–25	[illegible]
[illegible]		2,4-Dichloroaniline (III)	TLC, MeOH	15–[illegible]	[illegible]
[illegible]		[illegible]	Col., MeOH aq.	35–[illegible]	[illegible]
[illegible]		[illegible]	Col., TLC, EtOH aq.	62–64 62–5[illegible]	[illegible]

[illegible]	Formula	Starting material (source[illegible])	Method of purification[illegible]	Physical data m.p. (°C)	Rf
[illegible]4'-		4,4'-Dichloro-2-nitrobiphenyl[18] ↓ 2-Amino-4,4'-dichlorobiphenyl	MeOH aq.	57–58	53
[illegible]chloro-[illegible]phenyls [illegible]6',4'-		2,2'-Dichlorobenzidine[20] or 2,4-Dichloroiodobenzene[20]	Dist., Col., EtOH aq.	41	58
[illegible]3',4'-		3,4,3',4'-Tetraaminobiphenyl (VI) or 3,3'-Dichlorobenzidine (VIII)	TLC, EtOH aq.	173	44
[illegible]3',5'-		3,5,3',5'-Tetrachlorobenzidine[20]	TLC, MeOH, hexane	164	65
[illegible]6,2',6'-		2,6-Dichloroaniline (I) ↓ 2,6-Dichloroiodobenzene	Col., EtOH aq.	19[illegible]	40
[illegible]3,4,5-		2,3,4,5-Tetrachloronitrobenzene (IV) ↓ 2,3,4,5-Tetrachloroaniline	Col., MeOH	91	4[illegible]
[illegible]3,5,6-		2,3,5,6-Tetrachloronitrobenzene (I) ↓ 2,3,5,6-Tetrachloroaniline	Col., hexane	79	50
[illegible]tachloro-[illegible]phenyl [illegible]3,4,5,6-		Pentachloronitrobenzene (I) ↓ Pentachloroaniline	Col., MeOH	123	51
[illegible]tachloro-[illegible]phenyls [illegible]4,5,2',4',6'-		2,6,2',6'-Tetrachlorobenzidine[20] or 2,4,6-Trichloroiodobenzene[21]	Col., MeOH, EtOH	112–113	[illegible]

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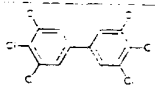
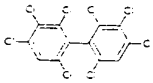
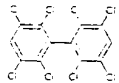
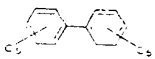
(Continued on p. [illegible])

[illegible]	[illegible]	[illegible]	[illegible]	Physical [illegible] m.p. (°C)	[illegible]
[illegible]		3,[illegible]-Tetrachlorobenzidine[illegible]	TLC, EtOH aq.	201–202	[illegible]
[illegible]		[illegible] 2,2,6,2',4'-[illegible]chlorobiphenyl[illegible]	TLC, EtOH	112	[illegible]
[illegible]		2,3,5,6-Tetrachlorobenzene (I); 2,3,5,6-Tetrachloroaniline			
[illegible]		2,3,5,6-Tetrachloroiodobenzene	TLC, EtOH aq.	160–161	[illegible]
[illegible]		Aroclor 1208 (VII)	Benzene	305–306	[illegible]

[illegible] chlorobiphenyl [illegible] (IV, V, VIII) [illegible] 2-chlorobiphenyl (IV, VIII), and [illegible] (IV, VIII).

[illegible] Aldrich Chem. Co., II = Sapon Lab., III = Eastman, IV [illegible] K & K Lab., VI = Baldick & Jackson Lab., VII = Monsanto [illegible]

[illegible] by method [illegible] commercially available.

[illegible] Preparation [illegible] chromatography (hexane) [illegible] silica column (hexane) [illegible] at 0.5 mm.

[illegible] prepared from layer plates (Merck Silica Gel [illegible])

[illegible] distributed about the [illegible] due to their relatively low acute [illegible] and [illegible] detection and separation. The [illegible] in the GLC analysis [illegible] pesticide residues. This [illegible] of a standard [illegible] Aroclor 1254 and chlorinated [illegible] pesticide residue analysis in the [illegible] PCB's [illegible] interferences have [illegible] This type of interference [illegible] mixture of organochlorine [illegible] 1254 in the [illegible]

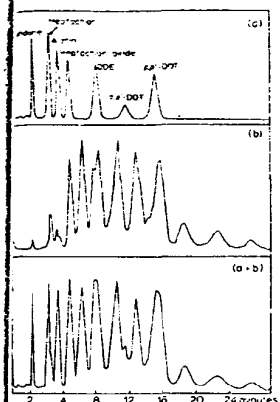


Fig. 16. Gas chromatograms of (a) a standard pesticide mixture, (b) Aroclor 1254, and (a + b) a 50/50 mixture of both. The concentration of Aroclor 1254 is approximately ten times those of the pesticides in (a). Gas chromatograph (Packard 7620) conditions: nitrogen gas flow, 80 ml/min; temperatures—oven 210°, inlet 230°, detector 218°; ^{63}Ni electron capture detector; 5 ft × 1/8 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[illegible] 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. 19 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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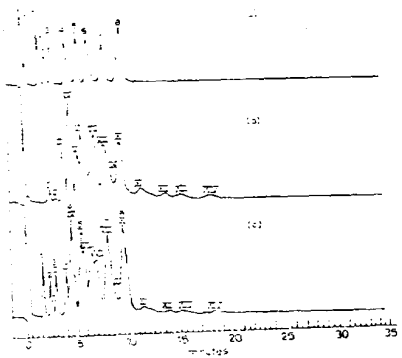

FIG. 12. (a) Electropherogram of a mixture of chlorinated pesticides resolved by analysis in a borosilicate column (1.5 m × 2 mm I.D.) packed with 4% SE-30 and 6% OV-210 on 100–120 mesh Chromosorb W (by standard gas chromatography). Peaks: 1 = 0.05 ng of lindane; 2 = 0.10 ng of heptachlor; 3 = mixture of equimolar lindane, pentachlor (I.S.) and aldrin; 4 = 0.20 ng of heptachlor epoxide; 5 = 0.20 ng of DDE; 6 = 0.20 ng of dieldrin; 7 = 0.40 ng of endrin; 8 = 0.40 ng of DDD; 9 = 0.80 ng of DDT. (b) 5 ng of Aroclor 1254 (the fourteen major peaks) + 0.05 ng of lindane + 0.10 ng of heptachlor + 0.20 ng of heptachlor epoxide + 0.20 ng of DDE + 0.20 ng of dieldrin + 0.40 ng of endrin + 0.40 ng of DDD + 0.80 ng of DDT. Conditions of the above organochlorine standard pesticide mixture and Aroclor 1254 injector temp., 250°; column temp., 200°; detector base temp., 250°; 50% argon + 5% methane flow rate, 200–400 ml/min.

TABLE 9

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

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

PCB peak No. (IUPAC)	Expt. I: 100 ml (1 fraction)	Expt. II: 2 × 100 ml (2 fractions)	Pesticides & etc.	Expt. III: 150 ml of hexane	Expt. IV: 200 ml of hexane
1	98.5	98.5	Lindane	None	None
2	80.5	99.5	Heptachlor	None	92.7
3	95.0	100.0	Aldrin	62.8	94.1
4	95.5	100.5	Heptachlor epoxide	None	None
5	92.5	100.0	DDE	26.3	97.5
6	84.0	99.5	Dieldrin	None	None
7	84.0	99.5	DDD	None	None
8	91.5	100.0	DDT	None	None
9	97.5	100.0			
10	97.5	100.0			
11	97.5	100.0			
12	97.5	100.0			
13	97.5	100.0			
14	97.5	100.0			

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[illegible]

TABLE 10

ELUTION PATTERNS OF PCB'S AND PESTICIDES FROM A MIXTURE AFTER SEPARATION OF FLORISIL SALTS V AND VI[a]

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 3% ether			Eluted 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
CB1	101.0	101.7			
CB2 + Aldrin[c]	102.1	96.6	Aldrin	4.3	4.0
CB3	100.0	100.0			
CB4	104.2	102.6	Heptachlor epoxide	96.4	101.2
CB5 + DDE[c]	97.8	102.5	DDE	None	None
CB6	101.3	100.6			
			Dieldrin	100.0	100.0
CB7	97.8	105.1			
CB8	100.0	100.0	DDD	102.3	98.0
CB9	91.6	97.0			
CB10	104.7	104.3	DDT	90.8	92.5
CB11	100.0	103.5			
CB12	100.0	100.0			
CB13	100.0	100.0			
CB14	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.

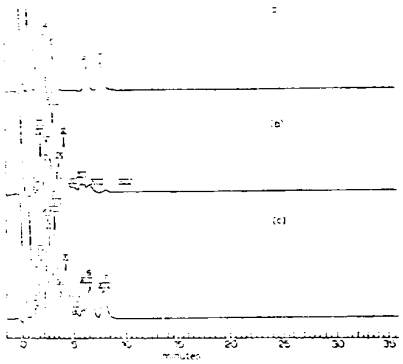
[b] Same as in [a] except that the PCB's and pesticides were first mixed with an extract from a animal tissue which was known to be essentially free of pesticides.

[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.

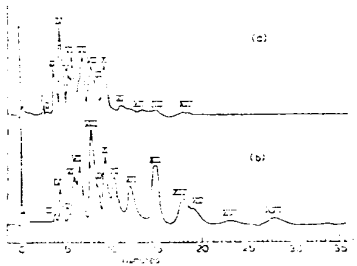
with the exception of DDE, aldrin and heptachlor)[12]. Table 9 shows percent recoveries of PCB's and pesticides from Florisil columns by elution with hexane and indicates almost quantitative removal of the PCB's was effected with 200 ml of hexane while under the same conditions only three of the eight 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 eluates are chromatographed separately on an SE-30 or 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 fate on a more polar liquid phase column, e.g., polyester such as DEGS or DEGA;



[illegible] chromatograms of chlorinated pesticides on a non-polar phase column (5% DC-[illegible]). [illegible] mixture of organochlorine pesticides and PCBs (5 ng of each component). (b) Same as (a) but with 5 ng of Aroclor 1254. (c) Same as (a) but with 5 ng of Aroclor 1260. Peaks: 1 = [illegible]; 2 = [illegible]; 3 = heptachlor epoxide; 4 = DDE; 5 = dieldrin; 6 = DDD; 7 = DDT; 8 and 9 = Aroclor 1254. The fourteen major peaks are numbered I to XIV (according to retention time) in Fig. 19, assuming that the order of elution is unchanged [i.e., compared to that of the organochlorine standard pesticide mixture and Aroclor 1254. Other GLC parameters as in Fig. 17.



[illegible] chromatograms of Aroclor 1254 and 1260. (a) 5 ng of Aroclor 1254, the fourteen major peaks [illegible] (b) 5 ng of Aroclor 1260, the seven major peaks corresponding to those in Aroclor 1254. [illegible] GLC parameters as in Fig. 17.

(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 0.3 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-

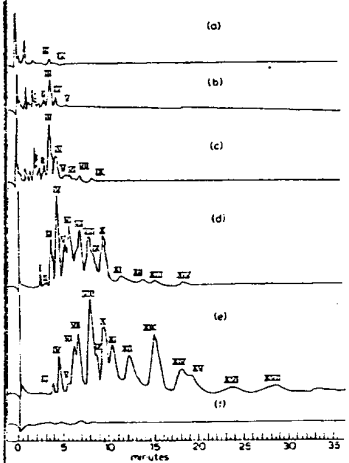


Fig. 20. GLC profiles of the more popular Aroclor mixtures under normal analytical conditions. All peak numbers correspond to those of Aroclor 1254. (a) 5 ng of Aroclor 1221. (b) 5 ng of Aroclor 1232. (c) 5 ng of Aroclor 1242. (d) 5 ng of Aroclor 1254. (e) 5 ng of Aroclor 1260. (f) 5 ng of Aroclor 5442. GLC parameters as in Fig. 17.

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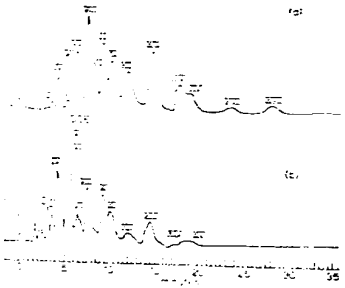

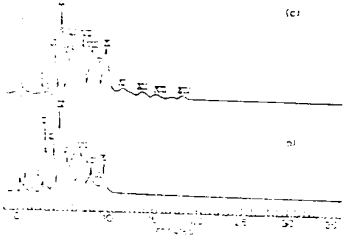

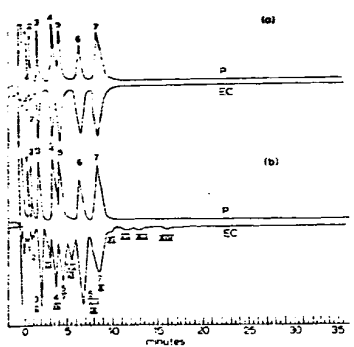

Fig. 23. Differentiation of Aroclor 1254 and organophosphoras pesticides using dual detectors and effluent split. (a) Organophosphoras standard pesticide mixture: 1 = 1.75 ng of phorate; 2 = 3.0 ng of diazinon; 3 = 3.0 ng Ronnel; 4 = 13.0 ng of malathion; 5 = 5.0 ng of parathion; 6 = 15.0 ng of methyl 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-101/6%, 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 Phenocler DP6 having R_E (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[24], (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[15] 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 ½ 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[15] 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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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*		*Cyanoethyl-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	3.01	3.05	2.20	2.19
2.81	2.81	5.06	5.06	2.62	2.60
3.59	3.58	5.90	5.65	2.97	2.90
3.71	3.70	6.57	6.50	3.60	3.90
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

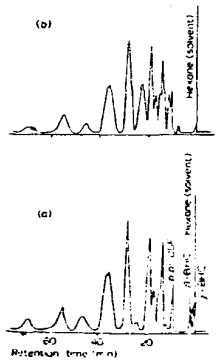

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TABLE 13

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

Species, locality, date	*Total DDT[a] (p.p.m.)*	*p,p'-DDE*	*PCB (p.p.m.)*	*DDT/PCB*
Cassin's auklet[d]	5.8	98	0.10	36
Ancient murrelet[e]	0.75	90	0.15	5
Fulmar[c]	0.41	79	0.08	5
Fulmar[g]	3.4	89	0.34	10
Red phalarope[h]	0.78	79	0.10	8
Rhinoceros auklet[i]	4.7	97	0.56	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	80	0.8	12
Peregrine falcon				
Breast muscle, second-year female, migrant from Arctic	104	90	22	4.5
Breast muscle, immature, California	13	99	10.5	1.2
Breast muscle, adult female, California	112	98	100	1.0

[a] From RISEBROUGH *et al.*[58] and RISEBROUGH *et al.*[8].
[b] Entire bird analyzed, except peregrine falcons.
[c] From RISEBROUGH *et al.*[8].
[d] Includes *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDMU, *p,p'*-DDE, 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%, NE-60 according to RICHEL *et al.*[38]. Confirmatory residue analyses were performed on a 12% DEGS on Anakrom SO (100–110 mesh) column with a temperature of 190° and a nitrogen flow rate of 85 ml/min (the retention time of dieldrin was 0.5 min).

The widespread distribution of PCB's and chlorinated hydrocarbons in marine ecosystems of the Pacific Ocean has been reported by RISEBROUGH and coworkers[8,39]. 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 peregrine falcons and in the eggs of several land species, respectively. Table 15 shows the DDT and PCB residues in marine fish. Of the commercial PCB preparations, these having the following retention times relative to *p,p'*-DDE on SE-30 and QF-1 columns have been detected in marine fish and birds:

SE-30: 1.25, 1.48, 1.75, 2.05, 2.11, 2.50, 2.60, 3.11, 3.88, 4.81

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[illegible] uncertainties are standard deviations unless otherwise indicated. Similar values [illegible] from analyses of seals from the North [illegible]

[illegible] RESIDUES IN SEALS OF NORTHERN PACIFIC[illegible]

[illegible]	No.	Total DDT[a] (µg)	p,p'-DDE (%)	PCBs (µg)	DDT/PCB
[illegible]	17	320	91	113	2.9
[illegible]	2	278 (125–430)	90	62	2.1
[illegible]	9	1925 (953–3911)	99	553 (364–1030)	3.5
[illegible]	1	110	93	20	5.5
[illegible]	1	103	91	62	1.7
[illegible]	2	147 (147–147)	97	15	10
[illegible]	1	143	93	118	1.0
[illegible]	1	235	91	112	2.1
[illegible]	1	138	87	480	0.93
[illegible]	1	533	88	330	1.0
[illegible]	1	2[illegible]	99	24	1.0
[illegible]	2	[illegible]	89	805 (700–910)	1.7 (1.3–2.0)
[illegible]	5	[illegible]	88	[illegible] (355–[illegible])	1.1
[illegible]	2	[illegible] (1[illegible]–[illegible])	80	114 (91–137)	3.8
[illegible]	2	97 ([illegible]–[illegible])	80	13 (12–13)	10
[illegible]	1	170	92	171	1.0

[illegible] 1.15 [illegible] p,p'-DDE + traces of [illegible] Ref. (12) 0.02

lantic[13]. 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[a]

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	22	11.7	14.0	83	1.0	14
June 25, 1965		(5.5–20)	±1.9			
Shiner perch						
San Francisco Bay	13	5.5	1.0	78	1.1	0.8
October 20, 1965		(4–8)	±0.1			
San Francisco Bay	10	26.7	1.3	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		(8–49)	±0.1			
English sole						
San Francisco Bay	18	14.3	0.55	85	0.11	5
July 29, 1965		(5–35)	±0.07			
San Francisco Bay	33	17.3	0.55	21	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	0.70	70	0.04	19
February 15, 1966		(80–262)	±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	43	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						
Belly muscle	7	—	0.50	45	0.04	14
			±0.24			
Liver	9	—	0.22	45	0.04	6
			±0.13			
Yellowfin tuna						
Liver	13	—	0.02	15	nd	> 7
			±0.02			
Liver	13	—	0.62	30	0.04	15
			±0.19			
Skipjack tuna						
Liver	1	—	0.057	21	0.1	0.6
Body muscle	14	—	0.054	18	nd	< 30
			±0.014			
Liver	25	—	0.930	11	nd	> 30
			±0.024			
Liver	12	—	0.020	21	nd	< 20
			±0.005			

[a] From Risebrough *et al.*[66] concentrations in wet weight, p.p.m. [illegible] 95% confidence limits.

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TABLE

RELATIVE PEAK HEIGHTS IN GAS-LIQUID CHROMATOGRAMS

Sample	R_E										
	69	81	100[a]	121	145[b]	171	200	234	283	333	379
Raw sludge A	35	63	51	104	100	92	32	10	21	10	3
Raw sludge B	30	23	17	37	100	66	20	53	84	20	25
Aroclor 1254	30	74	59	110	100	92	29	12	19	8	4
Aroclor 1260	3	14	4	30	100	69	70	55	87	30	21

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

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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.*[44]. 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[45] with 6% ethyl ether in petroleum ether, and (b) extraction of samples of less than 1 g by a modification of the ENOS[46] 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. The latter the relative amount (p.p.m.) was generally constant, whereas the absolute amount (μg) contained 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[47] 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,

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: 25% day	Mortality: 50% day	Mortality: 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	14–16	16–[illegible]
Spot	30	150	1			1	56	27	23–32	10–[illegible]
Spot	24	50	5	12	18	51	20	46	11–[illegible]	16–[illegible]
Spot	24	300	5	24	36	53	20	120	8–[illegible]	20–[illegible]
Spot	24	50	5	28	38	62	45	152	28–33	23–[illegible]
Pinfish	30	50	5	9	12	66	14	14	16–[illegible]	20–[illegible]
Pinfish	27	54	5	17	[illegible]	41	35	109	22–32	14–[illegible]

[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 = [illegible]) than that of control fish (χ^2 values were all greater than [illegible]).
[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: 0	3	7	14	28	42	56[a]	Number of days of flushing: 14	28	42	56	70	84[a]
Brain	0.1	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	30.0	46.0	30.0	30.0	24.0	12.0	14.0	12.0
	(0.1)	(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)	(1.0)	(1.5)	(1.7)	(1.0)	(0.8)	(1.0)	(0.8)	(0.0)
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	2.0
	(0.1)[c]	(1.8)	(4.5)	(17.0)	(24.0)	(36.0)	(45.0)	(65.0)	(45.0)	(34.0)	(44.0)	(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	9.1	7.2[d]
	(1.5)	(72.0)	(201.0)	(661.0)	(1417.0)	(1553.0)	(1083.0)	(2452.0)	(2041.0)	(1373.0)	(1309.0)	(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 (13.0), and skin 12.

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TABLE IV

ORGANOCHLORINE RESIDUES (ppm) PRESENT IN BREAST MUSCLES OF ROBINS

	No. of PCB injected	p,p'-DDE	p,p'-DDT	PCB
Birds fed with PCB (Clophen A50)	60	93	7	475
	55	99	25	197
	65	75	25	441
	65	64	41	164
	60	91	22	305
	55	50	2	257
Control birds	—	75	23	120
	—	69	47	70
	—	70	30	99
	—	60	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 IV 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

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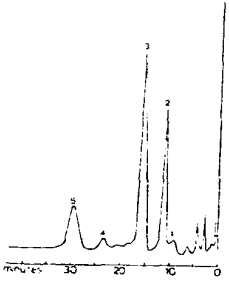

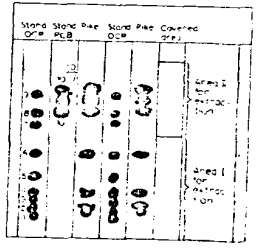

Fig. 2. Gas chromatographic separation of human milk extract. Other chlorinated hydrocarbons present in small amounts. 1 = PCB peak; 2 = heptachlor epoxide; 3 = *p,p'*-DDE. Analysis by PCB peak 1 = 4.0 ppm; 2 = PCB peak 3 = 2.5 ppm; *p,p'*-DDE distributed by PCB peak as 0.6 ppm.

Fig. 3. Thin layer chromatographic analysis of extracts of grain. 1 = PCB; 2 = heptachlor epoxide; 3 = *p,p'*-DDE; 4 = dieldrin; 5 = aldrin; 6 = *p,p'*-DDT; 7 = *o,p'*-DDT; 8 = *p,p'*-DDD. Area I = *p,p'*-DDE; Area II = PCB; Area III = *p,p'*-DDT; Area IV = *o,p'*-DDT; Area V = *p,p'*-DDD; Area VI = unidentified.

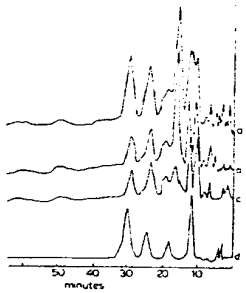

Fig. 4. Gas chromatograms performed for the analysis of PCB and chlorinated pesticides in a sample of Baltic herring, heptachlor epoxide added as internal standard. For standards, *cf.* Figs. 2a and 3a. (a) original extract; (b) extract of Area II, Fig. 2a, before oxidation; (c) extract of area II, Fig. 2a, after oxidation; (d) extract of area I, Fig. 2a.

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 Supelcoport (100–120 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 99, 84, 80, 70, 78, 70, 64 and 33%, respectively.

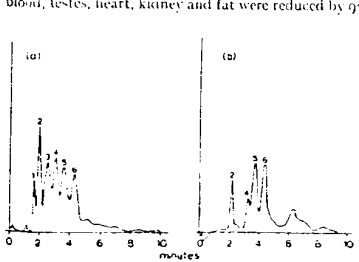

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TABLE 21

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	0.81	10.23	8.62	22.48
Heart	12.13	14.88	19.21	21.08
Spleen	14.88	14.80	9.57	—
Kidney	15.80	20.07	14.00	14.32
Brain	20.40	9.35	10.80	33.84
Liver	59.17	34.88	206.88	75.16
Fat	508.24	1001.57	433.89	1540.20

and centrolobular liver necrosis) as parameters, a significant difference in toxicity was found, *e.g.*, I and H showed the highest and III the lowest. Microscopically centrolobular liver necrosis was found in chicks fed compounds I and H. 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 2000 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 202-1B 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_t = 1.45 relative to dieldrin). The approximate amount of total PCB

TABLE 22

RESIDUES IN TISSUES OF RATS 15 DAYS AFTER BEING ORALLY ADMINISTERED A SINGLE DOSE OF AROCLOR 1254 (500 mg/kg)

Group 2 of Experiment II killed on day 17

	Residue	
	Basis of wet tissue (p.p.m.)	Relative to blood
Blood	47.85	1.00
Heart	2.51	13.00
Kidney	4.01	18.54
Brain	4.19	21.28
Liver	37.1	204.44
Fat	695.50	3205.22

[a] Mean of two values.
[b] Sacrificed because of the toxicity.

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[illegible] compound I Phenoclor [illegible]

[illegible] of tissue extracts the amounts of PCB were measured also [illegible] system equipped with a T-[illegible] Microtek MT-[illegible] gas chromatograph and [illegible] column packing [illegible] Wide-system [illegible] Phenoclor DP6 have about half-scale [illegible] By this method, the [illegible] the extract is determined by addition of the chlorine contents [illegible] Since the average chlorine contents of the compounds used [illegible] could be calculated.

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

[illegible]

[illegible] OF EXTRACTION AND CLEANUP OF CHLOROBIPHENYLS FROM RICE OIL

[illegible] of rice oil

Dissolve in [illegible] ml of n-hexane

Extract with [illegible] ml of acetonitrile saturated with hexane (four times)

Hexane layer

Discard

Saline solution layer

Extract with 100 ml of hexane

Hexane layer

Saline solution layer

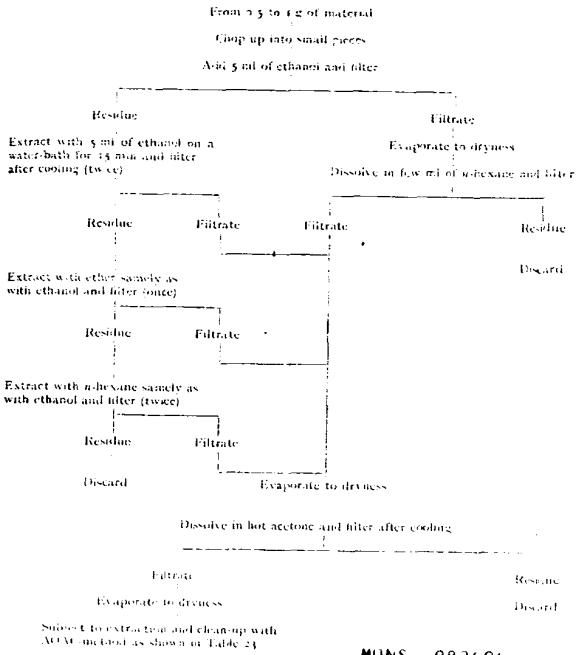
Discard

Saline solution layer

Discard

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

Filtrate

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

Evaporate to dryness

Dissolve in few ml of n-hexane and filter

Residue

Filtrate

Filtrate

Residue

Discard

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

Residue

Filtrate

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

Residue

Filtrate

Discard

Evaporate to dryness

Dissolve in hot acetone and filter after cooling

Filtrate

Residue

Evaporate to dryness

Discard

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

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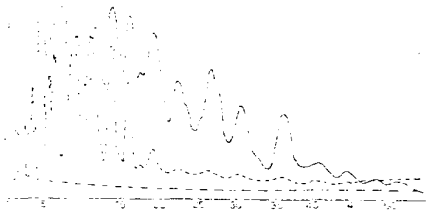
produced poisoning among the general population in Western Japan from summer to fall of 1968[illegible], with a total of 600 patients to date. It was found that contamination of the oil occurred by both the leakage of chlorobiphenyls into the rice bran oil through pin holes in a pipe used for heat exchange 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.*[illegible] 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-



[illegible]

[illegible] 34 and 35 show chromatograms of [illegible] respectively, and Fig. 36 illustrates [illegible] and control compared [illegible]

[illegible] polychlorinated biphenyl [illegible] *et al.*[illegible] Many [illegible] *Mascoma* [illegible] PCB's [illegible]

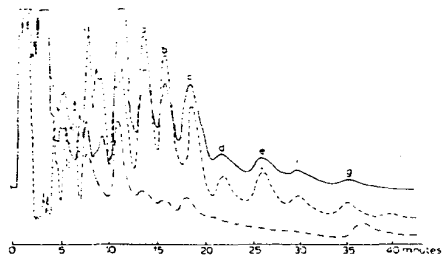


Fig. 34. Chromatograms of the skin of a still-born infant and control compared with chlorobiphenyls in rice oil. ——, Skin of still-born infant. —·—·—, skin of infant (control). - - - -, chlorobiphenyls in rice oil.

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 10 p.s.i.; nitrogen flow rate 100 ml/min). The injection port, oven and detector temperatures were 250°, 190° and 210°, respectively. For TLC,

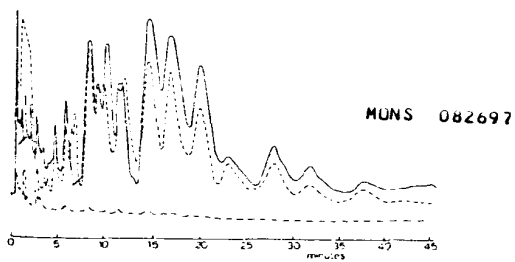


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Fig. 35. Chromatograms of the skin of infantile mice and control compared with chlorobiphenyls in rice oil. ——, Skin of infantile mice (mat. fat). —·—·—, skin of infantile mice (control). - - - -, chlorobiphenyls in rice oil.

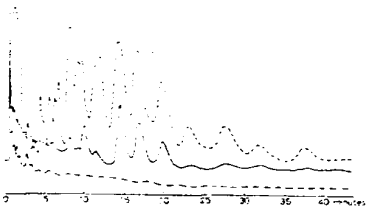

Fig. 36. Gas-liquid chromatogram of the neutral fraction of a sample of Atlantic surface water collected off the coast of [illegible] and concentrated with chloroform. [illegible] Column: [illegible] Temperature: [illegible] Sample: [illegible] of material from [illegible] litres of water.

Aluminum Oxide G plates were developed with n-heptane, sprayed with the Mitchell reagent[28] and exposed to UV light for 5 min.

Table 25 summarizes the Gas Chromatography of seven Aroclor PCB's and a number of chlorinated pesticides carried with two different columns in separate gas chromatographs. The observed retention times were compared with those obtained after the injection of [illegible] together with [illegible] where possible, aldrin, dieldrin, DDT, DDE and TDE (see G.L.C. Manual on the pesticides provided in Chromatograms containing mixtures of peaks in some cases as many as seven).

Figs. 37 and 38 illustrate the T.L.C. of plasticizers on Aluminum Oxide G. It

2. METHODS

[illegible] Chromatography [illegible] Plasticizers [illegible] Gas-liquid chromatography [illegible] Thin-layer chromatography [illegible] Infrared spectroscopy [illegible] Ultraviolet spectroscopy [illegible] Mass spectrometry [illegible] Nuclear magnetic resonance spectroscopy [illegible]

Gas-liquid chromatography [illegible] Plasticizers [illegible] Gas-liquid chromatography [illegible] Thin-layer chromatography [illegible] Infrared spectroscopy [illegible] Ultraviolet spectroscopy [illegible] Mass spectrometry [illegible] Nuclear magnetic resonance spectroscopy [illegible]

[illegible]

and flow through the purge was 45 ml/min. PCB was identified by the presence of peaks on chromatograms identical to those of one of the commercial PCB preparations (Aroclor 1254, Fig. 3c). 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[93] 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[94]. 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/m³ 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

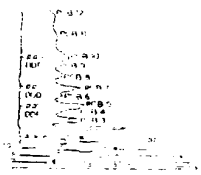

TABLE 2

RETENTION DATA ON MIXED PCB COLUMNS

Packing	Packing (%)	Weight (g)	Phase loading (% of adsorbent)	Standard* (μg/ml)	Polychlor. biphenyls (μg/ml)	Retention times							
						BHC	Lindane	Aldrin	Dieldrin	DDE	DDD	2,4'-DDT	PCB
Chromosorb W, AW, DMCS, 80–100 mesh	1.5	6	0.09	5**	10	Front	20	40	80	50	69	90	
1% SE-30 + 1% QF-1 on Chromosorb W, AW, DMCS, 80–100 mesh	1.5	6	0.03	5	10	front	20	35	80	45	67	85	
1% SE-30 on Chromosorb W, AW, DMCS, 80–100 mesh	1.5	6	0.03	5	10		18	81	11	75	75	72	2
1% SE-30 on Chromosorb W, AW, DMCS, 100–120 mesh		6	0.03	5	5		15	69	31	80	31	106	1
1% SE-30 on Chromosorb W, AW, DMCS, 100–120 mesh	2	6	0.06	5	5		15	30	31	80	31	70	4
1% SE-30 on Chromosorb W, AW, DMCS, 100–120 mesh	3	6	0.03	5	5		68	30	90	97	65	105	20
1% SE-30 on Chromosorb W, AW, DMCS, 100–120 mesh	1	6	0.03	5	8		86	67	90	69	100	100	10
1% SE-30 on Chromosorb W, AW, DMCS, 100–120 mesh	2.5	18	0.03	1.33	5		64	42	41	62	140	130	68
1% SE-30 on Chromosorb W, AW, DMCS, 100–120 mesh	5	41	0.05	0.38 (5)***	5		130	111	100	100	90	86	93
10% Carbowax 20M on Chromosorb W, AW, DMCS, 80–100 mesh	1	5	0.03	10	5								44, 85
10% Carbowax 20M on Chromosorb W, AW, DMCS, 80–100 mesh	3	5	0.03	10	5								93, 99

* The standard had the following composition and concentration in mg/l: BHC, 1.0; lindane, 1.0; aldrin, 2.0; DDE, 4.0; dieldrin, 3.0; DDD, 8.0; DDT, 12.0; and PCB (Aroclor 1260, 10.0).

** The extract was diluted with solvent, and, which dilution of the skeleton

Fig. 4. Gas chromatogram from an SE-96 column of an extract from sewage sludge

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

ZITKO[81] 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 4% 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 di-substitution 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.*[84]. A Packard A7901 gas chromatograph with a 6 ft × 4 mm glass column containing 4% 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 iso-

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[illegible] 2 [illegible] 70% of the [illegible] detector [illegible] 1254 [illegible] 1260 [illegible] in Table 2 [illegible] In some instances these [illegible] the molecule, which increas[illegible] The detector response increases strongly with increasing numb[illegible] 500 times [illegible] PCB's found in wildlife are generally of [illegible] 1254 or 1260 [illegible] In the study of ZITKO *et al.* above[illegible] these Aroclors [illegible] with relative retention times from 0.48 to 3.28 and [illegible] 72 to [illegible] respectively. The presented data indicate that the electron capture [illegible] of all chlorinated biphenyls with four to nine atoms of chlorine per molecule [illegible] to 1.6 relative to *p,p'*-DDE under the described [illegible]

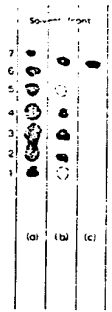
[illegible] Vos and Piet[illegible] described the reversed-phase partition TLC and GLC of [illegible] chlorinated biphenyls.

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 (p,p'-DDE = 1.00)	Relative response per mg standard [illegible] (p,p'-DDE = 1.00)
[illegible]	0.17	0.0033 ± 0.00005
[illegible]	0.13	0.0030 ± 0.00004
[illegible]	0.13	0.0006 ± 0.00001
[illegible]	0.39	0.0152 ± 0.0002
[illegible]	0.25	0.0155 ± 0.0001
[illegible]	0.15	0.0131 ± 0.0002
[illegible]	0.15	0.0155 ± 0.0002
[illegible]	[illegible]	0.0[illegible] ± 0.0003
[illegible]	[illegible]	0.0[illegible] ± 0.0010
[illegible]	[illegible]	0.2[illegible] ± 0.0010
[illegible]	0.51	0.2[illegible] ± 0.0004
[illegible]	0.06	[illegible] ± 0.0027
[illegible]	[illegible]	0.0[illegible] ± 0.0016
[illegible]	[illegible]	0.0[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.0[illegible] ± 0.0[illegible]
[illegible]	[illegible]	1.0[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]
[illegible]	[illegible]	0.1[illegible] ± 0.0[illegible]



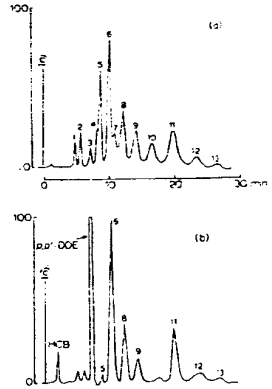


Fig. 42. Thin-layer chromatograms of (a) technical PCB mixture, (b) extract of owl liver, and (c) *p,p'*-DDE. Kieselguhr impregnated with liquid paraffin, developed three times with acetonitrile–acetone–methanol–water (40:18:40:2), and visualized with silver nitrate and UV light.

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	1.24
6	1.47
7	1.59
8	1.73
9	2.01
10	2.33
11	2.86
12	3.33
13	3.7[illegible]

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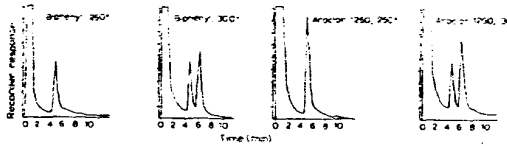

Fig. 44. Carbon-skeleton chromatograms of biphenyl and Aroclor 1260 at two catalyst temperatures.

Chrom Q[34] 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

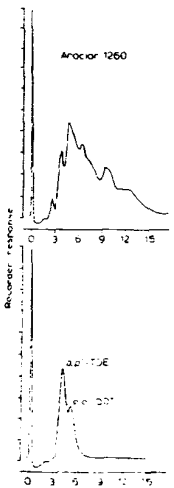

Fig. 45. Gas chromatograms of Aroclor 1260 and a mixture of *p,p'*-TDE and *p,p'*-DDT using the electron capture detector.

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[illegible] and the hydr[illegible] [illegible] 2 ft [illegible] [illegible] nitrogen flow [illegible] Fig. 44 [illegible] grams of biphenyl [illegible] [illegible] peaks [illegible] and [illegible]. The two peaks [illegible] observed at the higher catalyst temperature were due [illegible] [illegible] hydrogenation is favored at lower temp[illegible]

The chromatograms for Aroclor 1260 and a mixture of p,p'-TDE and [illegible] DDT obtained with the electron capture detector are shown in Fig. 45 and illustrate [illegible] PCB [illegible] interfere with the analysis of the DDT pesticide group. Carbon [illegible] chromatograms obtained at 200° and 230° are depicted in Fig. 46 for the

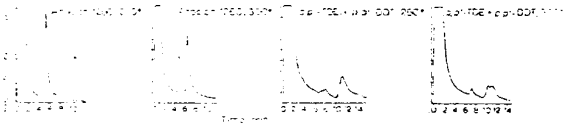

Fig. 46. Carbon-skeleton chromatograms at two catalyst temperatures of Aroclor 1260, and a mixture of p,p'-DDE and p,p'-DDT.

samples at much higher concentrations. These chromatograms suggest that this technique can be a 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 [illegible] or less. The response of this technique for a specific Aroclor depends [illegible] the greater the chlorine content, the larger will be the [illegible] required for detection. For 1 mg of Aroclor 1260, with the electronic [illegible] of 8[illegible], a response of [illegible] was obtained. For [illegible] of Aroclors with less chlorine contents than Aroclor 1260, responses should increase as the chlorine content decreases.

[illegible]

The [illegible] of PCB's in human adipose [illegible] [illegible] stainless steel [illegible] Programmed temperature [illegible] [illegible] and 215° [illegible]

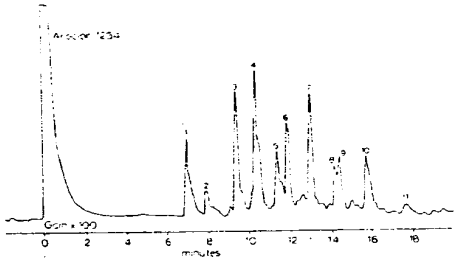

Fig. 47. TICM chromatogram of a standard Aroclor 1254 mixture of polychlorinated biphenyls. Programmed temperature analysis: 2 min at 185° to 210° at 5 °/min, isothermal at 210°. (See text for remaining instrumental parameters and partial peak identification.)

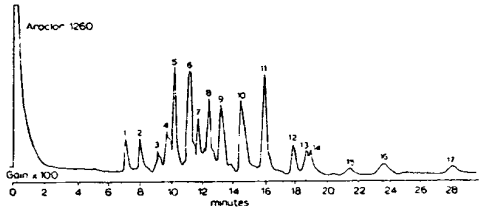

Fig. 48. TICM chromatogram of a standard Aroclor 1260 mixture of polychlorinated biphenyls. Programmed temperature analysis: 2 min at 200° to 230° at 5 °/min, isothermal at 230°. (See text for remaining instrumental parameters and partial peak identification.)

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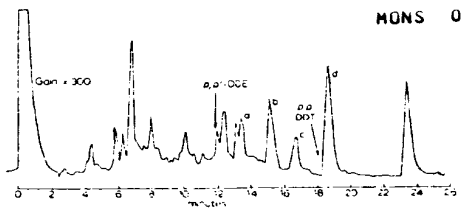
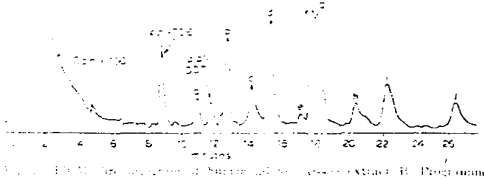


Fig. 49. TICM chromatogram of human adipose tissue extract A. Programmed temperature analysis: 5 min at 180° to 230° at 5 °/min, isothermal at 230°.

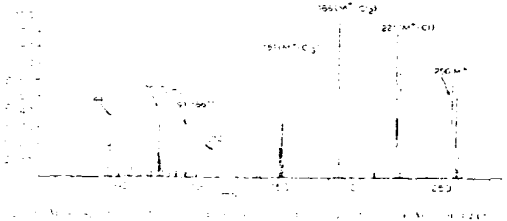


[illegible] Programmed temperature [illegible] 175°. Peaks 1–4 [illegible]

[illegible] at 70 eV electron energy with 2.5 kV [illegible] and the GLC [illegible] was 200 μA. Helium [illegible] was [illegible] into the [illegible] temperature was 175°. The mass spectra [illegible] continually over the range [illegible] 500 to 0 sec. Figs. 49 and 50 [illegible] the peak [illegible] monitor chromatograms of human adipose tissue [illegible] by microcoulometric and electron capture GLC had indicated the [illegible] of PCB residues and a modified Mestres procedure[8] was used for the prepa- [illegible] of the samples. Samples designated A and B (Figs. 49 and 50, respectively) [illegible] estimated to contain 200 p.p.m. and 600 p.p.m. total PCB as determined by [illegible] capture GC.

This study is limited by the [illegible] of separation efficiency for the individual PCB components analyzed by capillary column GC–mass spectrometry with no [illegible] of their fragmentation pattern of any of the PCB compounds. All components of [illegible] were found to give mass spectra of high intensity as would be expected from [illegible] GC–mass spectrometry studies. The characteristic isotope distribution [illegible] corresponding to the number of chlorine atoms in the parent ion and chlorine- [illegible] fragment ions was observed in all cases.

Two noteworthy features of the spectra were the relatively intense fragment [illegible] and the [illegible] of [illegible] ions from the parent ion and the [illegible]



[illegible]

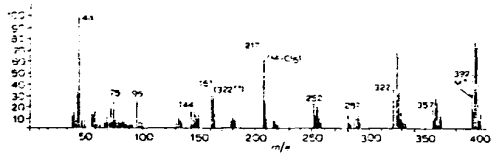


Fig. 52. Mass spectrum of a heptachlorobiphenyl found in standard Aroclor 1260.

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; peak 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-

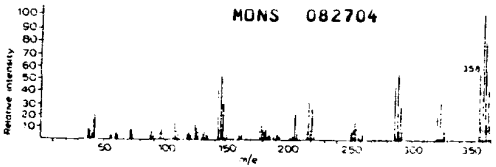


Fig. 53. Mass spectrum of a hexachlorobiphenyl component in human adipose tissue sample A (peak d, Fig. 49).

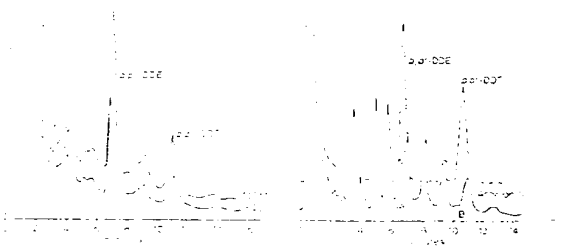
clor 1260 standard (Fig. 48). In addition, *p,p'*-DDE and *p,p'*-DDT, which were present at higher levels than those encountered in sample A, were also confirmed in this adipose tissue sample.

A system for the identification of multiple pesticide residues in foods by combined GC–mass spectroscopy was described by BELLEAU AND BARAY[9] and this technique was used to demonstrate the absence of PCBs as an interference in the quantitation of *p,p'*-DDT and *p,p'*-DDE in fresh and smoked shads. A Barber Coleman Model 5000 Gas Chromatograph was used equipped with a flame ionization de-

[illegible] a glass [illegible] [illegible] HP Chromosorb [illegible] [illegible] HP Chromosorb [illegible] [illegible] formally with [illegible] [illegible] separated with [illegible] [illegible] flame ionization [illegible]

The mass spectrometer was an A.E.I. MS-12 with an electron bombardment [illegible] The electron trap was set at [illegible] μA and the source temperature maintained [illegible] [illegible] of the electron emitting filament. The electron bombardment [illegible] 24 eV has the advantage over the more customarily used 70 eV of [illegible] [illegible] of the molecular ion cluster. The ion-[illegible] voltage was 8 kV with the electron multiplier detector set at a gain of [illegible] Mass spectra scanned magnetically from *ca. m/e* 500 to *m/e* 12 in about [illegible] A total ion current monitor detector was situated between the ion source and [illegible] analyzing magnet. This detector intercepts a portion of the ion beam and [illegible] a voltage signal on a 10 mV potentiometric strip chart recorder. The [illegible] between the GLC and the mass spectrometer was essentially as reported [illegible]

Cheese and corona beans were prepared for analysis by the fatty food procedure described in the *Pesticide Analytical Manual*[illegible]. The pesticides in the isolated fish sample (fresh and smoked chubs) were extracted with petroleum ether and [illegible] into acetonitrile and cleaned up successively over Florisil and silicic acid columns. The latter utilized the procedure of ARMOUR AND BURKE[illegible] 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



[illegible]

(using the procedure of ARMOUR AND BURKE[illegible]) 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

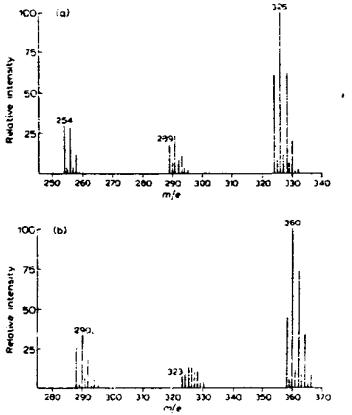


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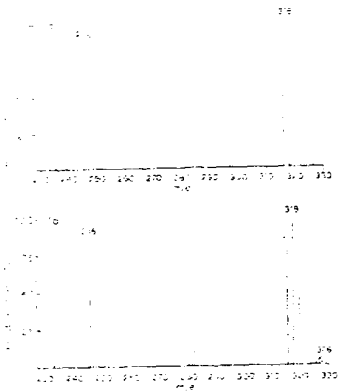
Fig. 56. Mass spectra of (a) Aroclor 1254 components with the same retention time as that of *p,p'*-DDE and (b) Aroclor 1254 components with the same retention times as that of *p,p'*-DDT.

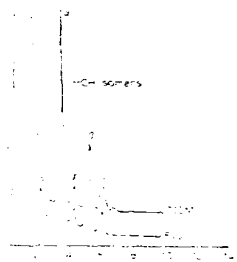
time as that of *p,p'*-DDE (Fig. 56a) and Aroclor 1254 components with the same retention time as that of *p,p'*-DDT (Fig. 56b). Fig. 57 shows that mass spectra of (a) *p,p'*-DDE standard at 24 eV and (b) *p,p'*-DDE in a sample showing PCB carry-over after a silicic acid column separation procedure. The *m/e* 320 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 BELLEMAN AND BARRY[illegible] was to determine if eluates obtained from the *Pesticide Analytical Manual*[illegible] 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



[illegible] Mass spectra (obtained with a Varian MAT CH4 instrument) recorded at 24 eV and (in A) at 70 eV of (A) a sample [illegible] (B) [illegible] extract of a cricket and (C) [illegible] purification procedure. The m/e 320 peak [illegible] (p,p'-DDE).

[illegible] and isomers of HCH also appear to be identical and in each [illegible] spectra of the four GLC peaks. Hence identification of which isomers of [illegible] were present in the sample required the combination of GLC retention times and [illegible] mass spectra produced.



[illegible] Gas chromatograms of [illegible] (A) a sample extract, (B) a [illegible] of PCB Aroclors 1260 and 1254, and (C) a [illegible] and Dieldrin.

The occurrence and distribution of PCBs in the River Rhine and the coast of areas of The Netherlands has been reported by Koeman *et al.*[3]. The identification was carried out by GC and mass spectroscopy. For this purpose a Varian MAT Model CH 4 mass spectrometer was coupled to a Varian Aerograph Type A550 Argon ionization detector under conditions described by Tindler and Biros[24]. The relative retention times (R_R) were estimated with a Varian Aerograph Model 204-1B gas chromatograph with electron capture detection. Pyrex glass columns (5 ft × 0.8 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[26] 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 3f and 3g 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. 5ya 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

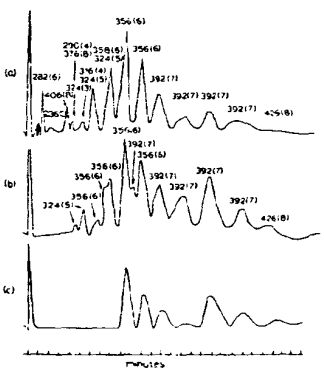


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Fig. 5y. Gas chromatograms of (a) an eider extract, (b) a solution of PCB Aroclors 1260 and 1254, and (c) a solution of Dieldrin and Aldrin.

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 1.00 OF SPECIFIC HCB PEAKS

With the exception of HCB, chlordane and compound A (MS), all compounds identified are conformable to PCBs. + = At the given retention time a peak is present in the chromatogram. Gas Chromatographic column: 1.5% OV-1 + 1.95% OV-210 on Gas-Chrom Q (80–100 mesh), 200 cm × 2 mm I.D. glass column; temperature programmed from 180° to 270° at 4°/min; nitrogen flow rate 30 ml/min. For further information, see Table 30.

Sampling area	Type of sample	R_x											
		0.51	0.62	0.63	0.64	0.75	0.78	0.81	0.85	0.91	0.95	0.97	1.00
1	Roach (*Leuciscus rutilus*) total body extracts 1967, 1968	HCB 252 (6)	256 (8)	256 (8)		256 (8) 290 (5) 290 (7)	290 (5)	290 (7)	290 (5)		290 (8)		324 (5) 358 (6) 358 (6)
1	Groundling (*Gobio gobio*) total body extracts 1968		+	+			+				+		+ +
2	Mussel (*Mytilus edulis*) 1968	+			+	+				+			+
3	Sand eel (*Ammodytes lanceolatus*) total body extracts 1967, 1968	+		+		+	+			+			+
3	Sandwich tern (*Sterna sandvicensis*) tissue and egg extracts 1965, 1966	HCB 252 (6)				256 (8)	290 (5)	290 (7)	Toxichlor 290 (5)		290 (8) 324 (5)		324 (5)
3	Eider (*Somateria mollissima*) tissue and egg extracts 1966, 1967	HCB 252 (6)				256 (8)			Toxichlor 290 (5)		290 (8) 324 (5)		324 (5)
	Phenochlor DP6											DDE 316 (4) + 324 (5)	DDE 316 (4) + 324 (5)
	Aroclor 1260											PCB (5) 358 (6)	PCB (5) 358 (6)
	Clophen A60											PCB (5) 358 (6)	PCB (5) 358 (6)

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 PCBs. + = 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	1.21	1.45	1.57	1.80	1.95	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)	304 (7)	158 (6) 304 (7)	304 (7)	304 (7)	304 (7)	304 (7)	120 (5)
1	Groundling (*Gobio gobio*) total body extracts 1968	+	+	+	+	+	+	+	+	+	+	+
2	Mussel (*Mytilus edulis*) 1968	+	+	+	+	+	+	+	+	+	+	+
3	Sand eel (*Ammodytes lanceolatus*) 1967	+	+	+	+	+	+	+	+	+	+	+
3	Sandwich tern (*Sterna sandvicensis*) tissue and egg extracts 1965, 1966	DDE 316 (4) + 324 (5)		358 (6) 324 (5)	358 (6)		358 (6)	304 (7)	304 (7)	304 (7)	304 (7)	120 (5)
3	Eider (*Somateria mollissima*) tissue and egg extracts 1966, 1967	DDE 316 (4) + 324 (5)		358 (6) 324 (5)	358 (6)		158 (6)	304 (7)	304 (7)	304 (7)	304 (7)	120 (5)
	Phenochlor DP6	358 (6)	358 (6)	358 (6)	358 (6)	304 (7)	158 (6)	304 (7)	304 (7)	304 (7)	304 (7)	120 (5)
	Aroclor 1260	358 (6)	358 (6)	358 (6)	358 (6)	304 (7)	158 (6)	304 (7)	304 (7)	304 (7)	304 (7)	120 (5)
	Clophen A60	358 (6)	358 (6)	358 (6)	358 (6)	304 (7)	158 (6)	304 (7)	304 (7)	304 (7)	304 (7)	120 (5)

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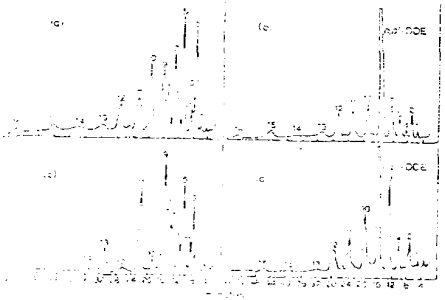

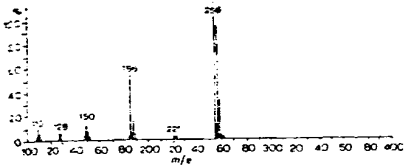

Fig. 61. Mass spectrum of peak 1 (Figs. 6a, b, and d), empirical formula $C_{14}H_8Cl_2$.

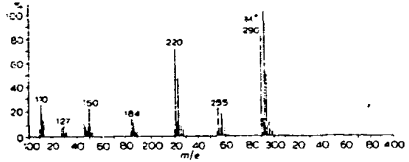

Fig. 62. Mass spectrum of peak 2 (Figs. 6a, b, and d), empirical formula $C_{14}H_6Cl_4$.

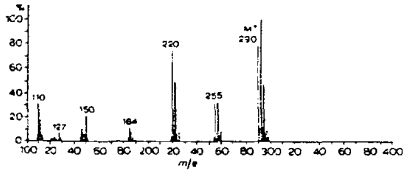

Fig. 63. Mass spectrum of peak 3 (Figs. 6a–c), empirical formula $C_{14}H_6Cl_4$.

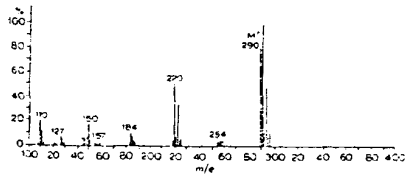

Fig. 64. Mass spectrum of peak 4 (Figs. 6a and c), empirical formula $C_{14}H_6Cl_4$.

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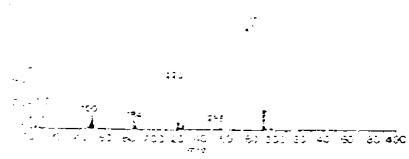
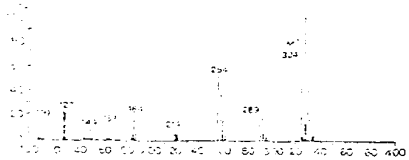
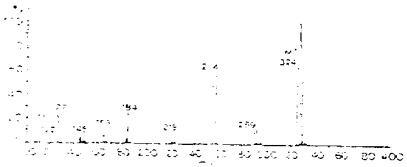
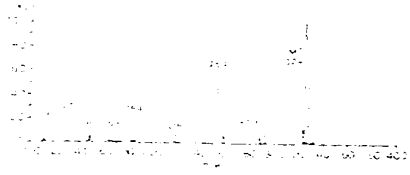








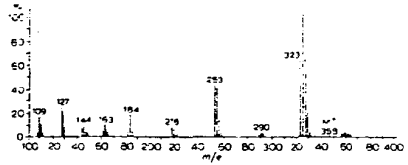


Fig. 69. Mass spectrum of peak 9 (Figs. 6ba and c), empirical formula $C_{12}H_3Cl_7$.

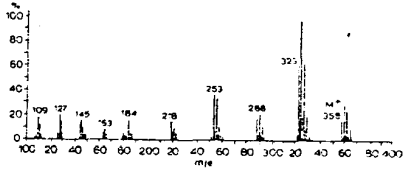


Fig. 70. Mass spectrum of peak 10 (Figs. 6ba, b, and d), empirical formula $C_{12}H_3Cl_7$.

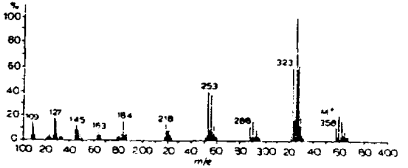


Fig. 71. Mass spectrum of peak 11 (Fig. 6bc; 1254-III (total ion current chromatogram not shown for Aroclor 1254, HLC zone III)), empirical formula $C_{12}H_3Cl_7$.

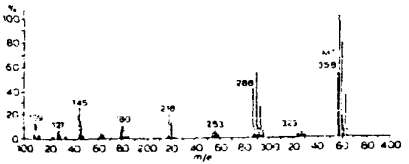
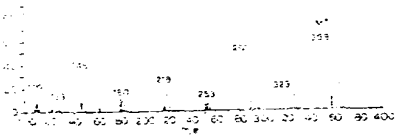
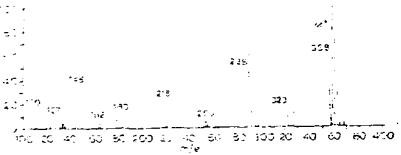


Fig. 72. Mass spectrum of peak 12 (Figs. 6ba, b, and d), empirical formula $C_{12}H_3Cl_7$.





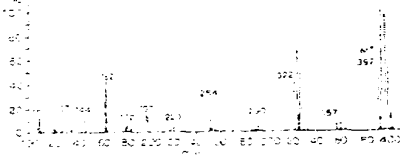


TABLE 3

RELATIVE RETENTION TIMES OF PCB'S AND SOME COMMONLY OCCURRING ORGANOCHLORINE PEST[illegible]

PCB Peak No.	Mol. wt.[a]	Relative retention time	Compound	Relative retention time
1	256 (3)*	0.39	Heptachlor	0.43
2	290 (4)	0.49		
3	290 (4)	0.53	Aldrin	0.51
4	290 (4)	0.58		
5	290 (4), 324 (5)	0.70	Heptachlor epoxide	0.66
6	324 (5)	0.79		
7	324 (5)	0.85	Dieldrin	0.90
8	324 (5)	0.97	o,p'-DDD	1.02
9	358 (6)	1.04	Endrin	1.05
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)	2.86		
16	392 (7)	3.38		

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

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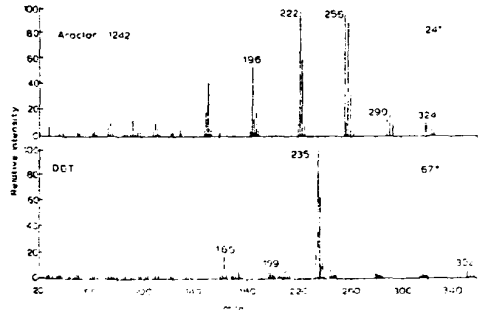

TABLE 24 (continued)

m/e	6 324 (5)	7 324 (5)	10 334 (6)	12 392 (7)	15 392 (7)	16 392 (7)	18 392 (7)	13 392 (7)
186	9.1	5.8	11	3.4				1.9
160				8.8	9.1	8.2	7.6	5.0
197				17.9	19.5	12.7	13.8	11.4
198				15.9	17.3	13.0	13.4	11.7
199				9.1	8.6	6.4	6.9	5.4
216			1.2	6.3	7.7	5.4	5.3	5.0
217	7.9		3.7	8.2	8.6	7.3	6.5	7.3
218	9.5	6.7	23.6	10.4	10.4	10.0	6.9	25.8
219	16.9	6.2	6.6	7.1	7.7	8.2	5.5	8.1
220	7.3	5.8	10.9	12.1	11.8	13.4	4.6	19.2
221	8.6	4.2	2.9	3.8		4.6		2.4
222	2.7	1.4	2.5	6.8	4.5	7.3		5.5
251								2.2
252			5.7	25.9	28.2	23.4	25.5	20.7
253			6.9	8.2	9.8	9.1	9.9	9.3
254	59.1	51.2	11.3	40.9	34.7	40.0	45.5	28.6
255	10.2	7.5	7.5	8.8	7.7	9.1	5.7	10.4
256	61.4	51.7	8.5	41.8	44.1	43.4	10.7	15.5
257	9.3	7.3	3.1	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	4.7						
261	1.8	1.1						
262	1.9							
286				3.5	4.5	4.5	4.6	5.2
287				5.9	7.3	7.3	5.9	5.8
288			46.2	12.3	24.5	13.6	9.0	13.9
289	26.5	14.2	7.5	12.3	13.2	11.8	9.3	69.1
290	20.4	3.7	59.4	23.5	34.1	20.4	10.7	14.2
291	5.2	17.9	10.4	10.0	10.0	10.9	5.5	13.0
292	23.9	3.7	3.0	18.8	25.0	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
299	2.6		1.7	2.9				
297	0.7							
322				38.8	40.0	40.0	40.3	22.5
323			12.3	9.7	9.1	10.4	8.6	15.0
324	93.0	62.5	13.2	82.4	65.2	82.5	60.0	52.5
325	10.1	9.2	20.7	17.6	13.0	17.3	13.0	33.3
326	100.0	100.0	10.8	60.5	50.1	74.5	45.3	11.1
327	13.0	12.9	15.1	11.7	10.0	10.1	9.0	10.7
328	61.6	62.5	12.1	32.9	25.0	39.4	17.6	23.4
329	8.0	7.0	5.7	7.1	5.0	9.1	4.0	8.6
330	21.6	18.1	3.8	10.0	6.1	12.1	5.2	6.1
331	4.7	2.6	1.2	3.5				
332	4.1	2.7		2.0				
333	0.6							
357				24.1	18.6	13.0	9.0	35.5
358			52.5	10.2	15.2	11.5	5.0	23.1
359			9.1	27.5	22.5	22.7	12.0	100.0
360			100.0	20.0	12.7	10.1	10.2	23.7
361			10.0	22.0	54.1	20.0	10.2	84.0

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TABLE 35

GLC OF HALOWAX CHLORINATED NAPHTHALENES

GLC conditions: glass coil, 1.5 mm I.D. × 1.8 m, DC-200 (50 cSt) on 100–120 mesh HP Chromosorb W, 1.5% (w/w); DC-200 (15 cSt) + QF-1 (1:1) on Supelcoport (80–100 mesh) HP Chromosorb W. Operating conditions: nitrogen flow rate, 120 ml/min; oxygen and hydrogen for detector; 200 °C injector temperature; 225 °C. Compounds eluting were detected by electron capture detector operated at 63Ni voltage to produce $I_{1/2}$ of 1 nA for electron flow for 1 ng of heptachlor epoxide when full scale deflection = 10^{-9} A.

Component	Response (wt) (Halowax, mineral fuel $I_{1/2}$ f.s.d.)	Relative time relative to aldrin	
		15% DC-200 column	15% DC-200 + 15% QF-1 (1:1) column
Halowax 1099	25	0.45, 0.57, 0.63, 0.71, 0.79, 0.86, 0.93, 1.04, 1.11, 1.23, 1.42, 1.57, 1.88	0.49, 0.65, 0.73, 0.84, 0.96, 1.14, 1.26, 1.46, 1.62, 1.96
Halowax 1014	25	0.45, 0.70, 1.03, 1.11, 1.57, 1.82, 2.85, 3.12, 3.33, 3.73, 6.0	0.50, 0.83, 1.15, 1.45, 1.80, 1.93, 2.80, 3.16, 3.98, 3.84, 5.9
Halowax 1051	10	1.13	1.08

GC conditions are those used for organochlorine pesticide residue analysis[98]. 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[196] 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[33] 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. PCBs 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. 37 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.*[197]. In a previous study[198] 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.*[197], 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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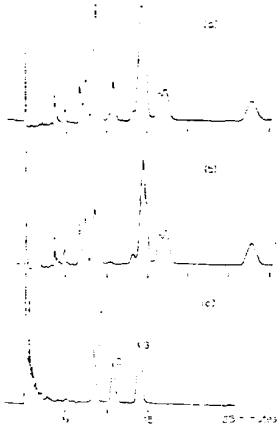
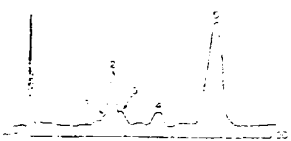


[illegible] Halowax 1014, 0.3 p.p.m. [illegible] p,p'-DDE residue before and [illegible] (b) petroleum ether eluate from [illegible] Polar eluate from silicic acid column [illegible] p,p'-DDE [illegible] p,p'-DDT 10-mg sample injected for each curve [illegible] Table 15

[illegible] which contained an average of 60% chlorine (Phenoclor DP6 and [illegible] Aroclor 1260) [illegible] the high toxicity of this [illegible] The polar compounds were not found in the corresponding fraction of a third [illegible] Aroclor 1260. The identity of the polar compounds which included tetra- and [illegible] dibenzofuran was confirmed by mass spectrometric and microcoulometric [illegible]

[illegible] obtained from the column chromatography (Florisil) of the P[illegible]



[illegible] Aroclor 1260 [illegible] Table [illegible]

TABLE 15

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

Peak No.	Relative retention[a]	Mass No.[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[98].

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

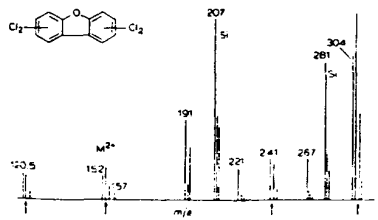


Fig. 79. Mass spectrum of tetrachlorodibenzofuran (mass No. 304) obtained from the third fractions of Clophen A60 and Phenoclor DP6 (peak 1 in Fig. 78).

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TABLE 37

CHICK EMBRYO ASSAY OF THE CHLORINATED BIPHENYLS Aroclor 1254, Phenoclor DP6, and Clophen A60 AND THE 25% DIELDRIN, ETHER FRACTION (THIRD FRACTION) OF CLOPHEN A60

Test compound	Group No.	Dose* (mg/egg)	No. of eggs treated	No. of infertile eggs	Percentage hatch (% of fertile eggs)	Remarks
Aroclor 1254	1	3.5	15	0	80	Most chicks showed curling first hour or second hour period.
Phenoclor DP6	2	3.5	15	0	0	
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 hour or second hour of incubation period.
	6	0.35	15	2	92	
	7	0.035	15	2	100	
—	8	0	20	2	91	Ethanol control
—	9	—	20	0	90	Untreated control

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

lences are far less toxic than chlorinated dibenzofurans[33] and the latter may thus be assumed to determine the toxicity of the third fraction. The occasional occurrence of hydropericardia in chicks fed Aroclor® may indicate the presence of a very small quantity of a toxic factor in this preparation.

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[32].

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[33]. 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–mass spectroscopic 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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MONS 082716

PCB Toxicity

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Volume 192, No. 4240

PCB's: How Toxic?

Polychlorinated biphenyls (PCB's) are recognized as ubiquitous environmental contaminants. In 1973, this worldwide problem resulted in a decision by the Organization for Economic Cooperation and Development to control the use and disposal of PCB's (*1*). At present, both the U.S. and Canadian governments are preparing legislation for the control of toxic substances.

The concern over PCB's is based on two factors, namely their environmental persistence and their toxicity. Recent results, however, cast doubts on the latter. Bowes *et al.* (*2*) observed concentrations of highly toxic polychlorinated dibenzofurans (PCDF's) ranging from 0.1 to 0.5 microgram per gram in all but one of the North American PCB's (Aroclor). Earlier studies by Vos *et al.* (*3*) had indicated that only PCB's manufactured in Japan (Kanechlor) and Europe (Clophen, Phenochlor) contained such impurities. In addition, PCDF's and other by-products were recently found in "pure" PCB isomers (*4*).

The toxicity of PCDF's exceeds that of PCB's by approximately four to six orders of magnitude. Their presence in PCB's has, therefore, significant bearing on toxicity studies on PCB's, commercial mixtures, and isomer preparations alike. Yet, in only a small proportion of the scientific reports on this subject is the problem of PCDF impurities in PCB's discussed. Obviously, the degree of this contamination is variable with the origin and probably also with other details of the manufacturing processes.

I strongly recommend, therefore, that in all future toxicity studies and for as many past studies as can be documented, precise information on the PCB's used (source, date of manufacture, lot number, and so forth) be recorded. I further recommend that past experiments for which such information is available be reevaluated in view of the strong possibility of the presence of PCDF's and their overriding toxic effects.

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