Distribution and Metabolism of 2,4,5,2′, 5′-Pentachlorobiphenyl

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Single doses of 2,4,5,2′,5′-pentachlorobiphenyl uniformly labeled with ^{14}C have been administered intravenously and orally to mice. Whole-body autoradiograms and scintillation counting of tissue samples have shown that most radioactivity leaves the circulation for the tissues within one hour. Peak concentrations varied, being highest in brown fat, which after 24 hours comprised the major reservoir of the unchanged compound in the body. Radioactivity disappeared rather rapidly from most other tissues, although the longest retention occurred in bronchial epithelium and some parts of the renal tubules.

The excretion of radioactivity was mainly through the bile, into feces, with a half-time of six days. There was little unchanged compound in the feces, the major metabolite was a hydroxylated derivative, both free and conjugated.

Earlier investigations into the retention, metabolism, and excretion of the various chlorinated biphenyls (PCBs) were based on analysis of tissues and excreta from animals dosed with one of the commercial PCB mixtures, or from animals or man that had absorbed those component PCBs that are persistent in the environment. Such investigations have shown that PCBs containing four chlorine atoms or less, which form the major part of PCBs entering the environment, largely disappear in the lower stages of food chains, and that the retention of higher PCBs by mammals is influenced by their extent of chlorination.[1-4] These conclusions have been confirmed by the study of the fate of single components of the commercial PCB mixtures[5] and of a commercial mixture labeled with tritium.[6]

The most reliable information on the fate of PCBs in an ecosystem is obtained from the use of single component PCBs labeled with a radioactive isotope. Such investigations have been reported with dichlorobiphenyl,[7] with tetrachlorobiphenyl,[8] and in the early stages of the present investigation with a pentachlorobiphenyl.[9] 2,4,5,2′,5′-Pentachlorobiphenyl is a suitable compound for an investigation into the relation between structure and storage, metabolism, excretion, and toxicity, as it is a major component of the commercial mixtures such as Arochlor 1254,[10] and it has been stated to be present in samples of human fat.[11]

METHODS

2,4,5,2′,5′-Pentachlorobiphenyl

A sample uniformly labeled with ^{14}C (30 millicuries/gm) in the 2′,5′-dichlorophenyl ring was supplied in benzene solution (0.67 millicurie/ml) and gave a single PCB peak by gas liquid chromatography (glc) at a retention time of 6.2 minutes. The thin layer chromatogram (tlc) R_f value was 0.6 (see below).

Preparation of the Dose

The PCB was administered to CBA mice as a solution in dimethylsulfoxide (DMSO) or in an aqueous emulsion. For the DMSO solution, a measured volume of the PCB solution was evaporated to dryness and the residue dissolved in sufficient DMSO to give 10 microcuries in 0.05 ml. For the emulsion, a measured volume of the benzene solution was evaporated in a polypropylene tube and the residue dissolved in the lipid-phosphatide phase of a pharmaceutical emulsion (Intralipid) 20% (Vitrum AB [Stockholm]). This solution was emulsified in 4 volumes of 2.5% aqueous glycerol by pumping it several times through a needle attached to a syringe, and this primary emulsion was then homogenized by placing the tube in an ultrasonic bath (Varian) maintained at 60 C. The droplet size of the emulsion so produced was about the same as that of Intralipid, most droplets being less than 1μm diameter.

Whole Body Autoradiography

For the whole body autoradiograms, 10μ curies of the DMSO solution or of the emulsion was injected into the tail vein, or 10 microcuries of the emulsion was administered orally by stomach tube. This dose of PCB corresponds to about 15 mg/kg body weight. Animals were killed 20 minutes, one, four, and 24 hours after an injection, and 1, 8, 16, and 32 days after an oral dose, and autoradiograms were prepared from whole body sections by the method of Ullberg.[12] Exposure of the sections to the photographic emulsion was performed at −15 C, as earlier experiments had shown that a considerable diffusion of radioactivity from fat occurred at room temperature.

The isotope "staircase" scale in Fig 1 and 2 was prepared by the method of Berlin and Ullberg,[13] using ^{14}C-labeled NA_2CO_3 solutions varying by a factor 2.

Determination of Radioactivity in Tissues and Excreta

Mice were dosed orally with 5 microcuries of PCB emulsion (about 7 mg/kg body weight), groups of three were killed 1, 4, 8, 16, and 32 days after dosing, and the organs were removed for radioactivity measurements. Organs were also included from the mice dosed with 10 microcuries for the autoradiographic experiments. Another group of three mice, each receiving 5 microcuries was maintained in a metabolism cage and the urine and feces collected separately.

A sample of each tissue was weighed on filter paper, dried, and ignited in an oxygen flask containing 1 M sodium hydroxide solution. The daily output of feces was dried and weighed, then ground in a mortar and a weighed amount similarly ignited in the oxygen flask. Diluted urine and the sodium hydroxide solutions were added to Instagel and the radioactivity measured with a scintillation counter (Packard Series 3000).

Samples of fat were taken from seven of the mice, with survival times ranging between one hour and eight days; they were bulked together and analyzed for PCB by the method of Jensen et al.[14]

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Metabolism

Four mice were dosed orally each with 6 mg of PCB containing 0.4 microcurie ^{14}C-PCB. Feces were collected from the mice for seven days and dispersed in water. The suspension was fractionated by the scheme given below. Thin layer chromatograms were on silica gel F254 (Merck) plates, eluted with toluene containing 1% volume per volume (v/v) ethanol. Column chromatography was on 25 × 1 cm columns of silica gel 60-200 mesh (Sigma Chemical Co, Type 1) or florisil 60-100 mesh activated at 110 C for five hours. Gas-liquid chromatograms were made (Varian 1400 instrument with an EC detector). A 6 foot × ¼ inch column was used, packed with 4% SF96 on Chromosorb W HP 80/100 (Varian). Column, injector, and detector temperatures were 175, 200 and 192, C respectively. The carrier gas was N, at 30 ml/min.

Mass Spectroscopy

A mass spectrograph (LKB 9000) was used with direct inlet of the sample and the following conditions; acceleration voltage, 3,500 v; ion source temperature, 270 C; electron energy 70 electron volts; and injection temperature, 50 to 60 C.

RESULTS

Whole Body Autoradiography

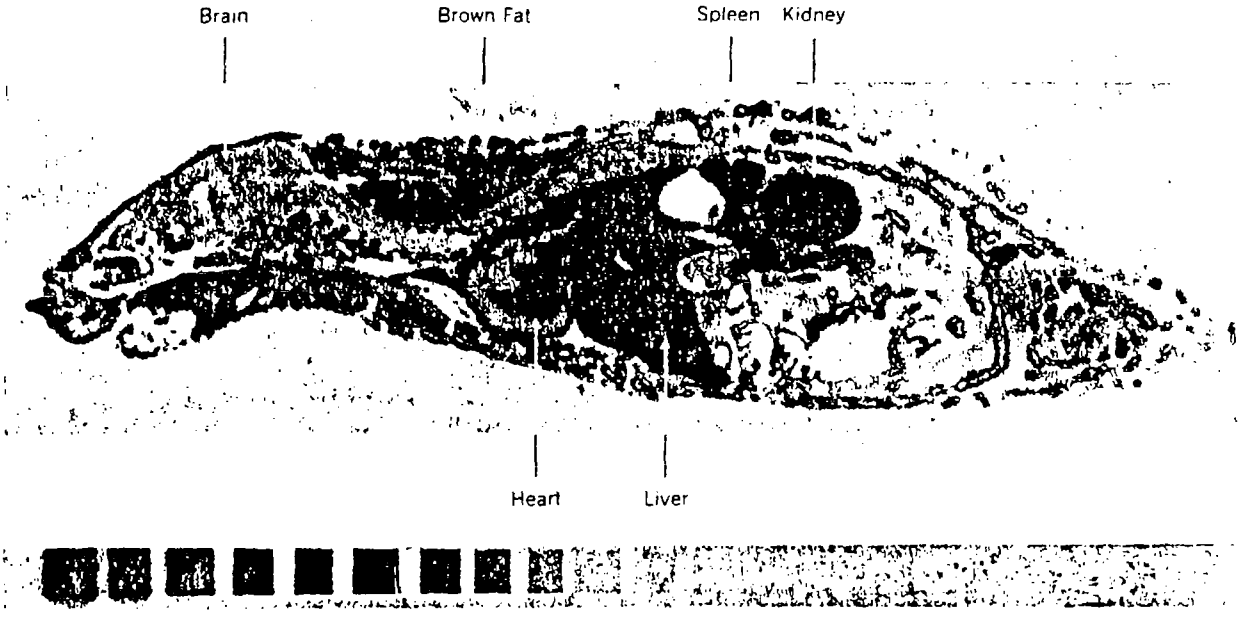
The autoradiogram in Fig 1 shows that 20 minutes after an intravenous injection of the PCB emulsion, a great part of the radioactivity had already left the circulation and entered the tissues. With an intravenous injection of the DMSO solution the proportion in the circulation was rather less at this period, but one hour after administration there was no significant difference between the distribution patterns produced by the two different physical states of the injections. At this time there was little radioactivity in the blood, and most was located in brown fat and the adrenals, with rather less in the liver. Radioactive material was visible in the gallbladder, and in the gastrointestinal tract from the stomach to the transverse colon. At one day no radioactivity could be seen in the blood (Fig 2), and fatty tissues throughout the body were well defined. The distribution pattern at one day after an oral dose of the PCB emulsion was very similar to that in Fig 2. At 8 and 16 days the autoradiographic pattern was dominated by the liver and fatty tissues, but at 32 days (Fig 3) the most clearly defined areas were the lungs, kidneys, and nasal sinuses.

Respiratory Organs.—A preferential concentration of radioactivity in the respiratory organs, particularly in the nasal sinuses and bronchi was seen 20 minutes after injection, and this was maintained throughout the experimental period. At 32 days these tissues were more clearly defined than other organs (Fig 3). The uneven distribution pattern in the lungs, with radioactivity mainly in the bronchial epithelium, is seen clearly in Fig 4.

Cardiovascular and Lymphatic Systems.—The radioactivity in the myocardium was not significantly different from that in the skeletal muscles, and there was no increased concentration in the walls of the blood vessels. There was no specific concentration apparent in the bone marrow or lymph nodes after one day, though this might have been masked by the high concentration in fat. At one day after injection there was a relatively high concentration in the spleen.

Digestive System.—Radioactive material appeared in the stomach mucosa and contents 20 minutes after intravenous injection, and thereafter rapidly spread through the whole of the gastrointestinal tract. A concentration in the gallbladder was clearly visible four hours after intravenous injection (Fig 5). From one day onward, when the high radioactivity in the liver had diminished to some extent, this organ showed a marbled distribution pattern with the highest

Fig 1.—Autoradiogram from mouse 20 minutes after a 10 microcurie intravenous dose of PCB emulsion.



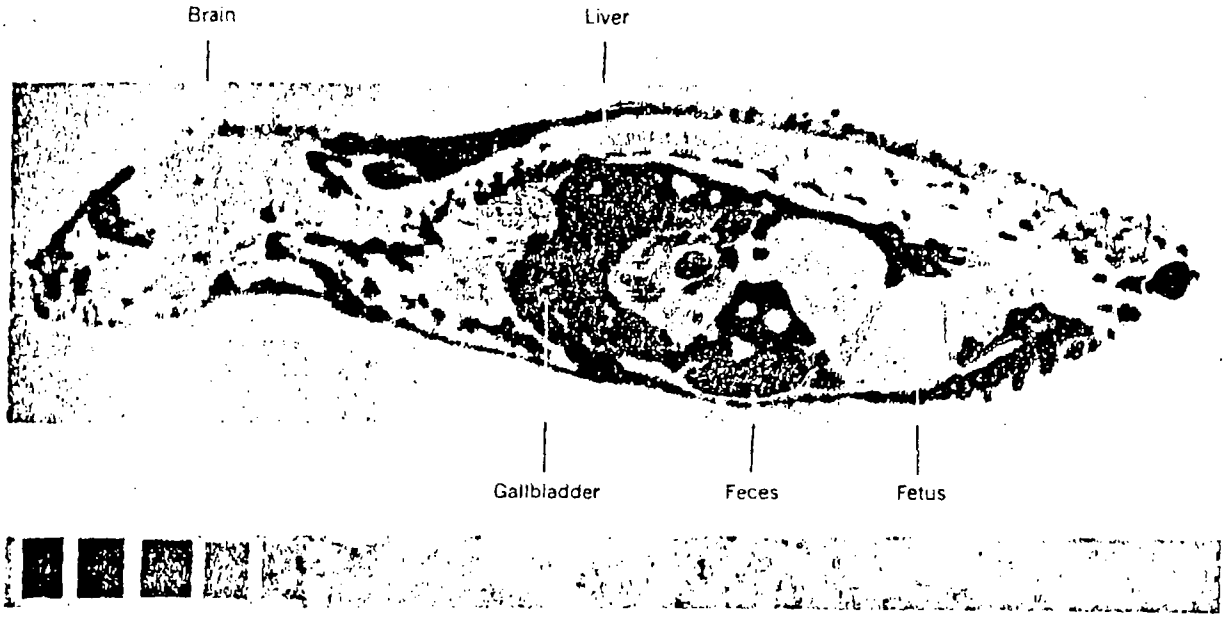


Fig 2.—Autoradiogram from mouse one day after a 10 microcurie intravenous dose of PCB emulsion.

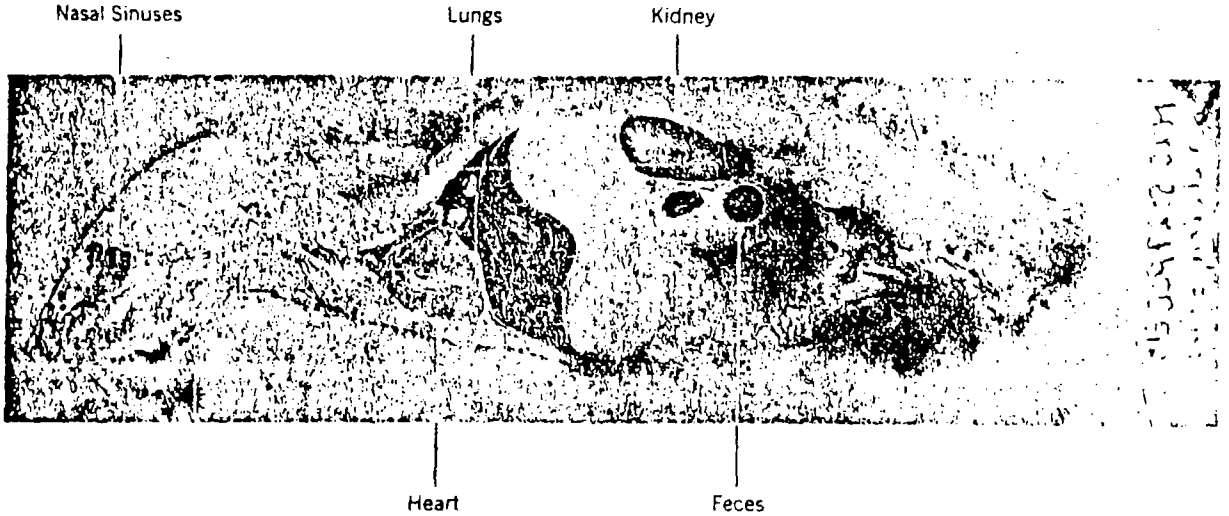


Fig 3.—Autoradiogram from mouse 32 days after a 10 microcurie oral dose of PCB emulsion.

concentrations in the periportal areas (Fig 6). A relatively high concentration was seen in the salivary glands but not in the pancreas.

Endocrine Glands.—The adrenals showed a marked radioactivity 20 minutes after injection (Fig 7), but this decreased rather rapidly and at 24-hours only a trace could be seen in the cortex (Fig 8).

Urogenital Organs.—At the end of the experimental period the kidneys were more clearly defined than most other organs (Fig 9), due to a punctate distribution pattern in the cortex that may have been associated with a special location in the tubular system. Some radioactive material was visible in the bladder at all periods. The ovaries exhibited marked radioactivity up to 24 hours after injection (Fig 10), but this had disappeared at 16 days. There was a slight penetration into the fetus, with no clearly defined distribution pattern. The concentration in testes was low after one day.

Skin and Ectodermal Glands.—A relatively high concentration was observed throughout the experimental period in the hair follicles, particularly in the region of the nose, and to a lesser extent in the lachrymal glands.

Central Nervous System.—The radioactivity in brain decreased as the

Fig 4.—Autoradiogram of mouse lung 16 days after a 10 microcurie oral dose of PCB emulsion.

Fig 5.—Autoradiogram of mouse liver four hours after a 10 microcurie intravenous injection of PCB emulsion.

Fig 6.—Autoradiogram of mouse liver eight days after a 10 microcurie oral dose of PCB emulsion.

Fig 7.—Autoradiogram of mouse kidney with adrenal indicated by arrow, 20 minutes after a 10 microcurie intravenous injection of PCB emulsion.

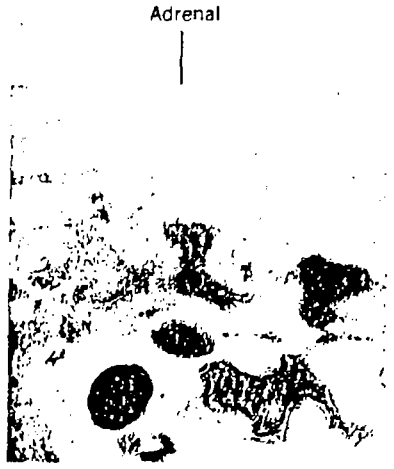


Fig 8.—Autoradiogram of mouse adrenal one day after an intravenous injection of 10 microcuries of PCB emulsion. Black areas indicate feces.

Fig 9.—Autoradiogram of mouse kidney 32 days after a 10 microcurie oral dose of PCB emulsion.

blood concentration decreased. With the shorter experimental periods, a tendency for a preferential retention in white matter was observed.

Radioactivity Measurements in Tissues and Excreta

The radioactivity of pooled urine and feces from three mice, expressed as a percentage of the dose given, is shown in Table 1. The radioactivity in the organs at various periods after dosing is shown in Table 2. The results obtained from the mice used in the autoradiographic experiment are not strictly comparable to the rest, as the dose was twice as large and thus the obtained values have been halved for comparison with the rest of the material. In one pregnant female dosed intravenously with 6 microcuries 0.7 nanocuries/100 mg appeared in the fetus after one day.

Metabolism

The feces from the four mice collected during the seven-day period contained 0.65 microcuries, corresponding to 41% of the dose administered. Figure 11 shows the percentage of this fecal radioactivity that was obtained in the various fractions.

Fraction F1.—The hexane solution was evaporated to small volume and chromatographed on a silica gel column with toluene as the eluant. The radioactive fractions from the column were shown by tlc to contain a major component with R_f 0.28, and a trace of a component with an R_f approximately 0.56. The major component was extracted from the tlc plate with acetone, and by glc it gave a peak with a retention time and shape identical with that of the crystalline me-

Fig 10.—Autoradiogram of ovary from pregnant mouse one day after an intravenous injection of 10 microcuries of PCB emulsion. The fetuses are visible in the lower part of the figure.

tabolite isolated from fraction F3.

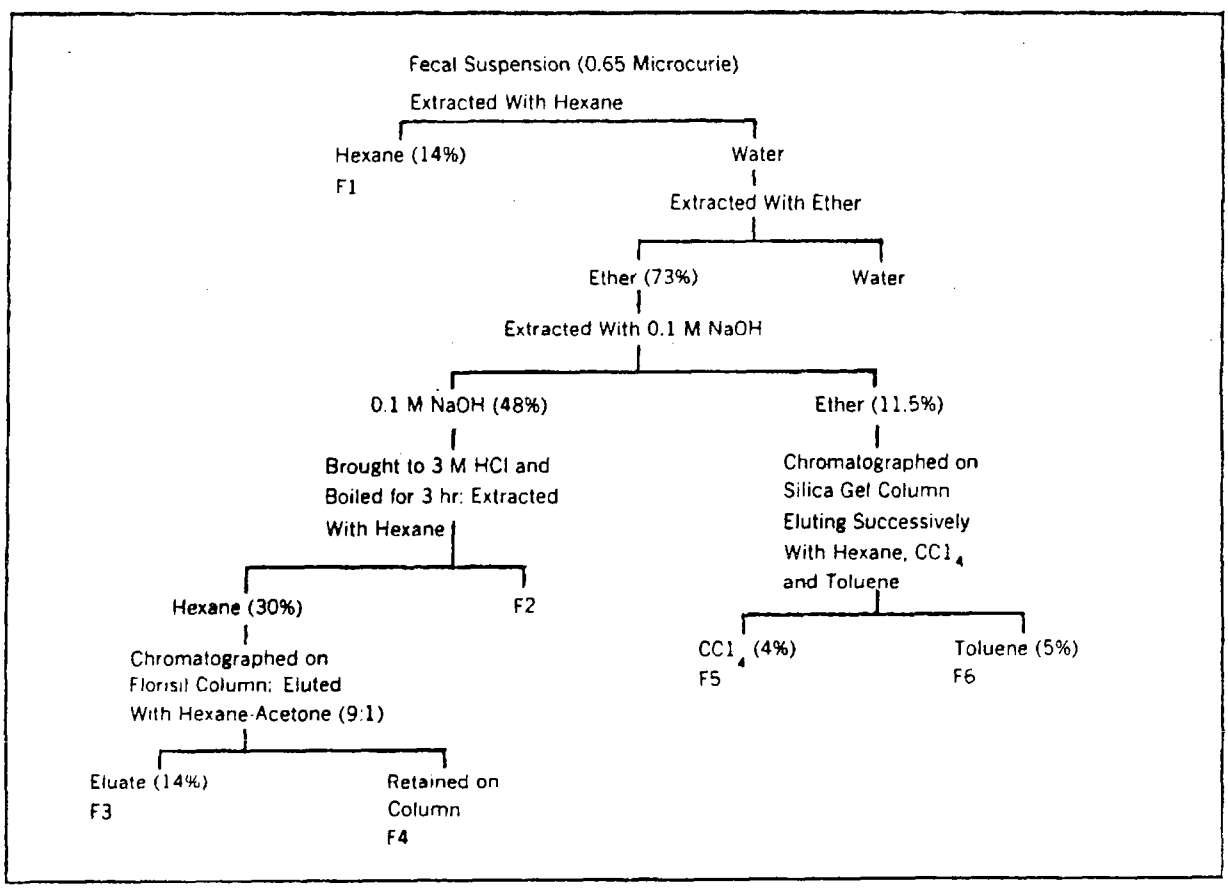
Fraction F2.—Attempts to isolate the components of this fraction from an oil that remained in the aqueous layer after hexane extraction were unsuccessful.

Fraction F3.—This fraction contained the hydrolysis products of conjugated metabolites in feces. There was much oily contamination and the fraction was further purified by chromatography on a florisil column, eluting with hexane:acetone (9:1 v/v). A further clean-up was effected by tlc, and the single radioactive component was extracted with acetone and again subjected to tlc. The metabolite had an R_F 0.28; it was extracted from the plate with hexane and on evaporation the solution yielded a trace of solid residue with no oily contamination. By glc the solution showed a single component with a tailing peak at a retention time of 15.1 minutes.

Table 1.—Excretion of Radioactivity in Urine and Feces Expressed as Percentages of the Dose Administered (5 Microcuries per Mouse).

Days After Dosing	Feces	Urine	Total
1	20.3	0.23	20.53
2	9.9	0.11	10.01
3	4.1	0.12	4.22
4	7.0	0.13	7.13
5	3.6	0.17	3.77
6-7	5.4	0.16	5.56
8	3.6	0.13	3.73
9-10	6.4	0.11	6.51
11	2.3	0.07	2.37
12	1.8	0.10	1.90
13	2.5	0.08	2.58
14	2.2	0.09	2.29
15	2.0	0.09	2.09
16	2.3	0.06	2.36
17-18	2.4	0.12	2.52
19	1.6	0.06	1.66
Totals	77.4	1.8	79.2

Fig 11.—Fractionation of feces. Figures in parentheses indicate percentage of initial radioactivity in feces.



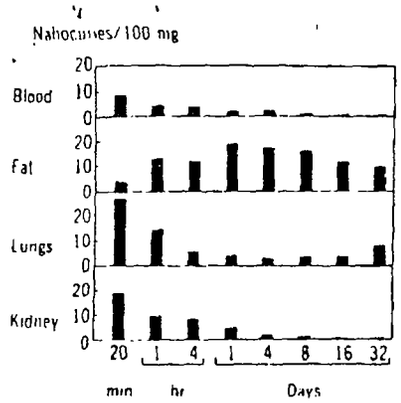


Fig 12.—Mean tissue radioactivity (nanocuries/100 mg) from groups of mice in Table 2.

The metabolite was examined by mass spectroscopy and the spectrum showed the characteristic isotope cluster for Cl_5 at m/e (mass to charge ratio) 340 to 348, corresponding to a molecular formula $C_{12}H_4OCl_5$. There was a clearly defined Cl_4 cluster at 270 to 276, and another Cl_3 at 241, attributable to a loss of CHO.

Fraction F4.—This fraction remained on the florisil column and all attempts to remove it were unsuccessful.

Fraction F5.—The radioactive fractions were evaporated to small bulk and subjected to tlc. A single radioactive component with R_f 0.59 was obtained.

Fraction F6.—This was evaporated to small volume and on tlc showed a radioactive component remaining at the origin. This was extracted with acetone and hydrolyzed by boiling for three hours with 3 M HCl. All of the radioactivity could then be extracted into hexane. The extract on glc showed a peak with a shape and retention time identical with that of the metabolite from F3.

Analysis of Fat

All of the radioactivity in the collected fat samples analyzed was extracted into hexane. By glc the extract gave a peak with a retention time identical with that of PCB. There was no such peak in an extract of fat from an untreated mouse and no evidence of any dechlorination of PCB.

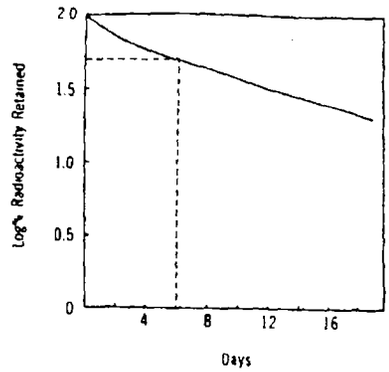


Fig 13.—Log percent radioactivity retained by mice after 5 microcuries of PCB emulsion administered orally. Curve calculated from the results in Table 1.

COMMENT

The results show that the uptake of

Table 2.—Distribution of Radioactivity in Tissues After a Single Dose of ^{14}C-PCB in Nanocurie/100 mg (Equivalent to 0.33 ppm PCB)*

Tissue	Sex	20 min	1 hr	4 hr	1 Day	4 Days	8 Days	16 Days	32 Days
Blood	M	(8.0)		(3.6)	1.7, 1.8	2.8	0.43, 0.27	(0.18)	0.16, 0.32
	F		(3.5)		0.58	1.1	0.41	0.29, 0.25	0.20
Bile	M	(38)			(20)	(8)		(7.6)	(3.2)
	F		(590)		(60)	(25), (21)	(14)	(10), (5)	(2)
Heart	M	12		13	2.2, 2.4	0.50	0.41, 0.44		0.18, 0.24
	F		9.9		0.47	0.37, 0.29	0.45	0.54, 0.41	0.38
Lungs	M	27		5.7	0.88, 9.2	3.1	1.9, 3.0	0.22	9.8, 6.7
	F		15		2.8	3.2, 2.1	4.0	5.2, 4.9	6.3
Liver	M	51		12	6.5, 5.7	2.8	3.6, 1.1	0.78	0.47, 0.49
	F		33		2.6	1.8, 3.2	1.7	1.3, 0.97	1.8
Spleen	M	7.0		2.1	12, 4.1	1.2	0.75, 0.39	(0.19)	0.11, (0.28)
	F				0.33	0.40, 0.63	0.35	0.30, 0.22	(0.14)
Kidneys	M	19		8.4	8.6, 3.4	1.7	0.99, 1.31	0.50	0.86, 0.92
	F		9.8		2.1	1.5, 2.6	1.40	0.10, 0.90	1.2
Adrenals	M				(6.4), (69)	(18)	(9), (1.5)	(3.9)	(2), (2.9)
	F		(54)		(35)	(8.2), (12)	(8.1)	(51), (6.3)	(5.0)
Testes Ovaries	M	0.34		0.44	1.0, 0.57	0.92	0.55, 0.48	0.37	0.14, 0.19
	F		(7)		(4)	(3.4), (7.7)	(6.6)	(1.3), (4.0)	
Fat	M	4.3		12	28, 25	11	13, 12	8.2	5.2, 5.0
	F		13		4.1	11, 21	22	14, 14	19
Skin	M			17	12, 8.9	9.6	4.1, (6.6)	5.0	1.1, 1.5
	F		61		7.1	8.1, 11	10	6.4, 6.8	4.9
Muscle	M	2.8		1.7	15, 9.0	2.0	1.5, 3.5	2, 7	0.54, 0.66
	F		5.8		5.4	0.94, 2.7	6.7	2.6, 2.5	0.35
Thymus	M	(44)		1.1	5.3, 1.6	0.71	2.7, 2.0	0.59	0.23, 0.37
	F		(23)		3.2	4.5, 0.94	3.4	2.2, 1.5	1.1
Brain	M	0.50		3.8	0.97, 0.41	0.18	0.21, 0.14	0.11	(0.06), (0.08)
	F		6.5		(0.14)	0.23, 0.31	0.19	0.21, 0.13	0.21

* Figures in parentheses are of low precision due to lower organ weights or low counts.

PCB by the tissues from the circulation after intravenous injection is rapid, and not much delayed if the PCB is administered in the lipid phase of an aqueous emulsion. This method of administration is free from some of the disadvantages associated with the use of organic liquids as solvents for intravenous injections.

The tissue analyses in Table 2 are in general agreement with the conclusions drawn from the autoradiograms. During the first 20 minutes after intravenous injection most of the radioactivity leaves the circulation and is taken up mainly by liver, kidneys, and brown fat. Thereafter the radioactivity increases in the general body fat, rising to a maximum between 4 and 24 hours, at a time when the amount in other tissues is rapidly decreasing. Thus, it must be assumed that the radioactivity is initially stored in the tissues and subsequently migrates to fat and is stored there. The results are too few to give any clear indication of a sex difference, thought there is a suggestion that the radioactivity is retained longer in the fat of female mice. The mean values for all mice in each group are presented diagrammatically in Fig 12.

Analysis of fat has shown that PCB is stored mainly unchanged in the body, so it is likely that the early tissue distribution is due to unchanged PCB. However, at the dose administered, nearly all the PCB is excreted as a metabolite. Table 1 shows that radioactivity is lost fairly rapidly from the body with a half-time of about six days, and is excreted almost entirely in the feces. The results in Table 1 have been transformed in Fig 13 to show the rate of change of log residual radioactivity in the body. The graph shows a two-phase excretion, an initial more rapid loss that is probably due to metabolism and excretion of PCB while it is concentrated in the liver, and a later linear portion that can be attributed to a release from fat. Extrapolation of the graph shows that a reduction of the total body burden to 1% of its original value would require about 65 days. The presence of radioactive material in bile 20 minutes after intravenous injection indicates that this is the major route of excretion of the metabolite. Radioactivity in the stomach contents after intravenous injection may be due to excretion through the stomach wall or salivary glands, or to a reflux of duodenal contents.

The adrenals initially have a high affinity for PCB, but like most other tissues their radioactivity decreases rapidly as the blood concentration falls. Kuratsume[15] has reported that the pattern of urinary 17-ketosteroid excretion in patients suffering from Yusho disease indicates adrenocortical hyperfunction, and it is possible that the changes in steroid metabolism associated with PCB may in part be due to a direct action of PCB or its metabolites on the adrenals,[8] in addition to the induction of liver enzymes reported by several investigators.

It is possible that the initial higher concentration of PCB in brown fat than in yellow fat is due to the greater vascularity of the former, leading to a more rapid attainment of an equilibrium with PCB in the blood. This would result in a slower rise in concentration in yellow fat but a more prolonged retention as PCB is lost from the body. The accumulation of radioactivity in the bronchial epithelium leading to a late rise in the lung concentration, and the sustained concentration in mucous membranes, may be due to a high affinity of these tissues for a metabolite produced in the lungs or elsewhere. Reid et al[16] have observed that metabolites of certain halogenated hydrocarbons are firmly bound to lung tissue.

This investigation into the metabolism of a PCB leads to the conclusion that earlier investigations into the fecal excretion of unlabeled PCBs are likely to have given incomplete results when only unchanged PCB was measured,[7] or when only the metabolites in a hexane extract of feces were investigated.[5]

Stalling and Mayer[17] have shown that the pentachlorinated biphenyls are a major component of the PCB residues in fish, and are, therefore, likely to constitute an important part of the PCB intake in food. The rather low proportion of 2,4,5,2',5'-pentachlorobiphenyl in the PCB content of human fat suggests that man, like the mouse, is able to metabolize and excrete this PCB more rapidly than lower members of the food chain.

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Rolf Serwin, Department of Organic Chemistry, University of Lund, prepared and interpreted the mass spectrograms.

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