The Metabolism and Distribution of 2,4,5,2',5'-Pentachlorobiphenyl in the Mouse.

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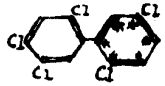
2,4,5,2',5'-Pentachlorobiphenyl is a major constituent of Aroclor 1254, and it forms a minor component of the PCB content of human fat (Jensen, personal communication). A sample labelled with ^{14}C (30 mCi/g) was obtained from Mallinckrodt; it was supplied in benzene solution (0.67 mCi/ml) and gave a single PCB peak by gas-liquid chromatography.



2,4,5,2',5'-Pentachlorobiphenyl
(* ^{14}C-labelled)

This communication is an interim report of an investigation which includes a whole-body autoradiographic study of the distribution of radioactivity in mouse tissues, and the measurement of radioactivity in tissues and excreta after a single oral or intravenous dose of the ^{14}C-PCB.

METHODS

Preparation of the dose

The PCB was administered to CBA mice as a solution in dimethylsulphoxide (DMSO) or in an aqueous emulsion. For the DMSO so-

x) Names are intentionally presented in alphabetical order

lution, a measured volume of the PCB solution was evaporated to dryness and the residue dissolved in sufficient DMSO to give 10 µCi 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 "Intralipid" 20% (Vitrum). 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 a Varian ultrasonic bath maintained at 60°. The droplet size of the emulsion so produced was about the same as that of "Intralipid", most droplets being less than 1 µm diameter.

Whole-body autoradiography

For the whole-body autoradiograms, a dose of 10 µCi of the DMSO solution or of the emulsion was injected into the tail vein, and 10 µCi of the emulsion was administered orally. This dose corresponds to about 15 mg/kg body weight of PCB. Animals were killed 20 min., 1 hr, 4 hr, 1 day and 35 days after the dose, and whole-body autoradiograms were prepared by the method of Ullberg (Ullberg, S., Studies on the Distribution and Fate of S^{35}-Labelled Benzylpenicillin in the Body, Acta Radiol. (Stockh.) Suppl. 118, 1954).

Determination of radioactivity in tissues and excreta

Mice were dosed orally with 5 µCi PCB emulsion and groups of three were killed 1, 4, 8 and 16 days after dosing, and the organs removed for radioactivity measurements. Organs were also included from the mice dosed with 10 µCi for the autoradiographic experiments. Another group of three mice each receiving 5 µCi was maintained in a metabolism cage and the urine and faeces collected separately.

A sample of each tissue was weighed on filter paper, dried and ignited in an oxygen flask containing 1 M sodium hydroxide solu-

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tion. The daily output of faeces 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 Insta-gel and the radioactivity measured with a Packard Series 3000 Scintillation Counter.

RESULTS

Whole-body autoradiography

20 min. After the intravenous DMSO injection, most of the radioactivity had entered the tissues from the blood within 20 min. The activities of fat and of the liver were the highest and about equal. After the emulsion injection the passage into the tissues was slower but the concentration in fat and in the liver was greater than in blood. With both methods of administration the radioactivity in the kidney was localised in specific areas. Radioactivity was clearly present in the stomach contents.

1 hr. After 1 hr there was no difference between the distribution of radioactivity obtained with the DMSO and emulsion intravenous injections. The highest activity was seen in brown fat, with rather less in liver. Radioactivity is clearly present in the contents of the small intestine and of the transverse colon; there was some in the white pulp of the spleen but none in the pancreas.

4 hr. Little radioactivity was left in blood. Most was seen in fat and in the intestinal contents, with the liver as the next most active tissue.

1 day. Fatty tissue was clearly the most radioactive, followed by the contents of the intestines. Some sections including the gall bladder and the bile was seen to contain radioactivity. Other sections showed spots on the liver which may indicate the bile ducts. There was a peculiar pattern in the lung with a concentration in some structure not yet identified. The activity in brain

had decreased. There were radioactive areas in the white pulp of the spleen, in the parotid and lachrymal glands and in the epithelium of the nasal sinuses. There was some radioactivity in the foetal membranes, with a very slight penetration into foetal tissue.

Sections from a mouse killed 1 day after an oral dose of emulsion showed a very similar distribution of radioactivity. There was a similar pattern in the lungs, which may be interpreted as an accumulation in the bronchial and bronchiolar epithelia.

35 days. Most radioactivity had left the animal after this period. The most clearly defined areas were the lungs and the renal cortex; the concentration in fat appeared to be lower, with rather less in brown fat than in white fat.

Radioactivity measurements in tissues and excreta

The radioactivity in the pooled urine and faeces of 3 mice each dosed with 5 μCi, expressed as a percentage of the dose, is shown in Table 1. From these results it can be calculated that the half-time of the PCB in the body is about 7 days.

Table 2 shows the radioactivity in the organs at various periods after dosing. The results obtained from mice used in the autoradiographic experiments are not strictly comparable, as the dose was twice as large. At present, the results on only one animal from each group are available. One pregnant female dosed intravenously with 6 μCi showed 0,7 nCi/100 mg in the foetus after 1 day..

DISCUSSION

The results show that the uptake of PCB by the tissues from the circulation is rapid and not much delayed if the PCB is administered intravenously 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.

Throughout the experiment, measurements of radioactivity show the highest concentration to be in fat. The radioactivity in fat rises to a maximum between 4 and 24 hr after dosing, at a time when the radioactivity in blood is low and the concentration in tissues is decreasing rapidly. It must be assumed, therefore, that the PCB that is initially deposited in the tissues subsequently migrates to the fat and is stored there. Fat is confirmed as the major reservoir by the autoradiograms, although at 35 days the lungs and renal cortex appear to be more clearly defined, probably because the radioactivity is located in discrete areas. Radioactivity is lost fairly rapidly from the body with a half-time of about 7 days, and appears almost entirely in faeces. Autoradiograms show radioactivity in the contents of the intestines and gall bladder, so it seems likely that the major route of excretion is through the bile.

No information is yet available on whether the measured radioactivity is due to PCB or to a metabolite thereof; experiments are in progress to investigate this. It seems likely that the distribution seen at 20 min. after injection is due to unchanged PCB, and PCB may be stored as such in fat. However, it seems unlikely that PCB will be excreted in bile without undergoing some metabolic transformation, and the distribution in tissues other than fat at longer periods after injection may be due mainly to the metabolites. This may account for the sustained high concentration in liver and for the appearance of radioactivity in glandular secreting tissue and in certain areas of the lungs. The presence of radioactivity in the stomach contents may be due to excretion through the stomach wall or salivary glands, or to reflux of the duodenal contents.

No evidence of localisation of radioactivity in specific areas of the brain can be observed in the autoradiograms. Analysis of

brain tissue shows a low level of radioactivity which falls off during the experimental period, but a trace still remains after 35 days.

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Table 1. Excretion of radioactivity in urine and faeces

The results were obtained from pooled excreta of three mice, and are expressed as percentages of the dose administered (5 μCi/mouse).

Days after dosing	Faeces	Urine	Total	Cumulative total
1	20.3	0.23	20.53	20.5
2	9.9	0.11	10.01	30.5
3	4.1	0.12	4.22	34.8
4	7.0	0.13	7.13	41.9
5	3.6	0.17	3.77	45.7
6-7	5.4	0.16	5.56	51.2
8	3.6	0.13	3.73	55.0
9-10	6.4	0.11	6.51	61.5
11	2.3	0.07	2.37	63.8
12	1.8	0.10	1.90	65.7
13	2.5	0.08	2.58	68.3
14	2.2	0.09	2.29	70.6
15	2.0	0.09	2.09	72.7
16	2.3	0.06	2.36	75.1
17-18	2.4	0.12	2.52	77.6
19	1.6	0.06	1.66	79.2
Total	77.4	1.8	79.2	

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Table 2. Distribution of radioactivity in tissues after a single dose of ^{14}C-PCB.

Figures in Table represent /uCi/100 mg tissue.

Dose, /uCi/mouse	10^{x}	10^{x}	10^{x}	5	5		5	5	10^{x}
Route	iv	iv	iv	oral	oral		oral	oral	oral
Time after dosing	20 min	1 hr	4 hr	1 day	4 days		8 days	16 days	35 days
Sex	M	F	M	M	M	F	M	M	F
Blood	7.90	3.50	3.60				0.43	0.18	
red cells				0.40	0.16				
plasma				1.30	2.76	0.45			
Heart	12.50	9.90	13.00	2.18	0.50	0.37	0.41	0.57	
Lungs	27.00	14.80	5.70		5.10	3.15	1.90	0.22	2.28
Liver	50.90	33.40	12.20	6.50	2.80	1.80	3.60	0.78	8.64
Kidneys	19.30	9.80	8.40	8.60	1.70	1.50	1.00	0.50	8.76
Fat	4.30	13.30	11.90	27.90	18.70	14.80	13.40	8.20	3.30
Muscle	7.80	5.60	1.73		1.95	0.94	1.51	2.65	8.29
Brain	5.83	6.52	3.80	0.97	0.18	0.23	0.21	0.11	0.08

x Results have been halved to bring in line with 5 /uCi experiments

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