Distribution of a Polychlorinated Terphenyl (PCT) (Aroclor® 5460) in Rat Tissues and Effect on Hepatic Microsomal Mixed Function Oxidases

by
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Introduction

Polychlorinated polyphenyls are among the most abundant chlorinated hydrocarbon global pollutants. Aroclor® is a trade name applied to a group of such chemicals used in the manufacture of paints, lacquers and many other products. Recent evidence of the widespread distribution in the ecosystem of polychlorinated biphenyls (PCBs) (JENSEN, 1966; PEAKALL & LINCER, 1970; REYNOLDS, 1971) has stimulated interest into their biochemical and toxicological properties (RISEBROUGH et al, 1968; VOS & KOEMAN, 1970; GRANT et al, 1971).

Polychlorinated terphenyls (PCTs) which are used in similar industrial applications to the PCBs have not received the same widespread attention. (Aroclor series 25 and 24 contain mixtures of PCB and PCT, while the 54 series contains only PCT. The last two digits indicate the chlorine content of the material, e.g. 5460 is a PCT with 60% chlorine. Undistilled PCTs are also available as Aroclor 50 series. The Aroclor 60 series are blends of Aroclor 5460 and PCB Aroclor 1221. The last two digits indicate the percentage of Aroclor 5460 in the blend, e.g. Aroclor 6062 is a blend containing 62% Aroclor 5460 and 38% Aroclor 1221 (MONSANTO CHEMICAL CO., 1962, 1968).)

Toxicity studies have been conducted to investigate the effects of PCTs on insects, both singly and in combination with insecticides(LICHTENSTEIN et al, 1969), their sequential biochemical and ultrastructural alterations within the liver of rats (NORBACK & ALLEN, 1972), and their effect on estrogenic activity and also pentobarbital metabolism in rats and quails (BITMAN et al, 1972).

PCTs have recently been reported in environmental samples (ZITKO et al, 1972). In previous studies we have confirmed their presence in paperboard and food packaging material and have developed an analytical procedure for PCT quantitation (THOMAS & REYNOLDS, 1972). A need was felt to study the metabolism, distribution, and storage of PCTs in mammals.

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Methods and Materials

The distribution of Aroclor® 5460 in various tissues of the rat and its effect on the liver microsomal enzyme systems, were studied in twenty-four male Wistar Albino rats randomized into four groups and treated as described in Table I.

TABLE I

Dosing Regimen of Rats

Group [a]	01	02	03	04
PCT in diet [b] parts per million (ppm)	10	100	1000	control
Duration of Treatment	7 days	7 days	7 days	7 days

[a] Each group contained 6 male rats, Wistar Albino strain, average body weight 300 - 310 g.

[b] PCT administered orally.

The rats were individually caged in air conditioned rooms and were supplied with water and feed ad libitum for seven consecutive days. Food consumption and initial and final body weights were measured. The animals were sacrificed by exsanguination under ether anesthesia, blood collected, and liver, brain, kidney, spleen, testes, heart, and omental fat were removed, weighed, and frozen until analysed.

Polychlorinated Terphenyl Analyses

Tissues were blended (using an Omni mixer) with hexane (100 ml) and anhydrous sodium sulphate (50g) for 10 minutes. The extracts were filtered, concentrated to dryness, and dissolved in hexane (10 ml). 3 ml of acid solution (conc. H_2SO_4 - fuming H_2SO_4 (1:1)) was added to the hexane solution, and the flask stoppered and shaken for 30 min. with the aid of a wrist shaker. The solutions were neutralised with anhydrous sodium carbonate, and the hexane decanted from the residue. The residue was rinsed with hexane (3 x 40 ml) and the rinsings combined with the original hexane decant. The hexane solution was dried with anhydrous sodium sulphate, filtered, and concentrated to dryness. The residue was dissolved in hexane (10 ml). Prior to electron capture gas chromatography (GC-EC) the extracts were treated in

the following manner: group 01, all 10 ml passed through Florisil column; group 02, 1 ml passed through Florisil column; group 03, only appropriate dilution carried out.

The extracts were subjected to GC-EC analyses on a Varian Model 1200 series chromatograph fitted with a coiled 6' x 1/8" I.D. glass column containing Chromosorb W, DMCS, 60/80 mesh, coated with a mixture of 3% OV-210 and 3% SE 30 (ultraphase). The nitrogen flow rate was 25 ml per minute with column temperature 255°C, injection temperature 275°C, and detector temperature 225°C (block 275°C). Aroclor 5460 standard was used for quantitation purposes using a disc integrator to measure the complete area under the curves.

Hepatic Drug Metabolism Studies

Microsomes were isolated from the liver portions and enzyme assays carried out following reported procedures (FEUER et al, 1971; SOSA-LUCERO et al 1972).

The following biochemical parameters were examined: N-dealkylation of aminopyrine was estimated by the rate of formation of 4-amino antipyrine according to LA DU et al (1955). The Aromatic ring p-hydroxylation of aniline was measured by the formation of p-aminophenol (BRODIE & AXELROD, 1953). Recordings were made in a Unicam SP 800 spectrophotometer. All activities were expressed as mμmoles of substrate disappeared or metabolite formed per milligram of microsomal protein. Protein was measured by the method of LOWRY et al, (1951) with the automated procedure of GIBBS and BRIGHT (1968). Microsomal phospholipids were extracted according to FOLCH et al, (1957) and phospholipid-phosphorous was determined by the method of MARTIN and DOTY (1949). Cytochrome P_{-450} was measured as described by OMURA and SATO (1964) using a value of 91 $cm^{-1}mm^{-1}$ for the molar extinction coefficient. Glucose-6-phosphate was determined by following the procedure described by HARPER (1965).

Statistical analysis of data was carried out by means of the Student's t-test (SNEDECOR & COCHRAN, 1967).

Results and Discussion

No macroscopic manifestation of texicity was observed in any of the groups receiving the Aroclor 5460. Table II shows the effect of PCTs on body and liver weight.

TABLE II

Relation of intake of Aroclor 5460 to change in body weight and liver weight

Groups	Body Weights	Liver Weight g	
	g	Absolute	Relative
Controls	316 ± 10.7	14.47 ± 0.77	4.58 ± 0.24
10 ppm	307 ± 12.7	14.52 ± 1.07	4.71 ± 0.21
100 ppm	309 ± 8.0	14.80 ± 0.99	4.80 ± 0.25
1000 ppm	309 ± 6.1	16.62* ± 0.57	5.39* ± 0.14

Results are expressed as mean ± SE, six animals per group.

* Significantly different from controls $p < 0.05$.

No significant differences were found in the body weight in the different groups. A significant increase in liver weight, absolute and relative was observed after one week in the rats fed 1,000 ppm PCT.

Residues of PCT (expressed as ppm wet tissue, Table III) were found in all tissues analyzed, with liver and blood having the greatest and least, concentration respectively.

GC-EC profiles obtained from 3 ng of Aroclor 5460 and from residue found in the liver of a group 02 rat are shown in figure 1.

The contribution of the early peaks in the PCT profile pattern to the total residue is changed in the liver profile compared to the standard. This change in profile pattern was consistent throughout the experiment with all liver samples showing essentially the same pattern. This GC-EC profile change suggests that the components of the Aroclor 5460 formulation with the shorter retention times, (peaks 1 to 4), and presumably which contain the lowest chlorine content were metabolized to a greater degree than those with the longer retention times. This result is in agreement with the general conclusions of other workers that the rate of metabolism or excretion of chlorinated aromatics (PCBs in particular) is dependent on the amount of chlorine in the molecule and that the lower chlorinated isomers are metabolized extremely rapidly (GRANT et al, 1971; BAILEY & BUNYAN, 1972).

Table IV indicates the effect of PCTs on microsomal protein and phospholipids.

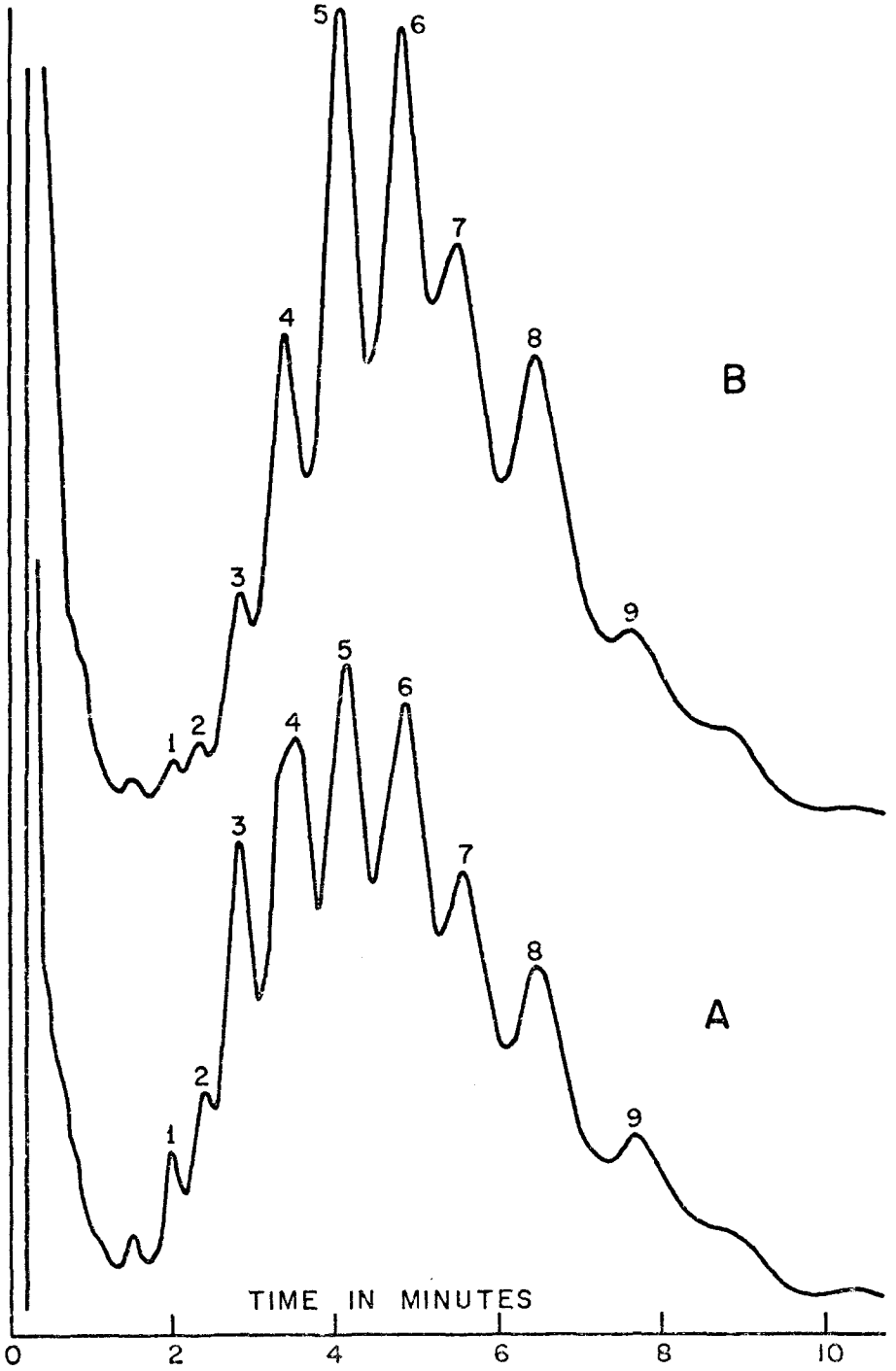

FIGURE 1 GC-EC profiles of "A" Aroclor 5460 (3 ng) and "B" hexane extract of liver from group 02 rat.

TABLE III

Residues found in tissues of
rats orally dosed with Aroclor 5460

	Residue found (ppm, wet tissue)			
Group	10 ppm	100 ppm	1000 ppm	Control
Blood	1.24[a]	1.32[a]	5.85[a]	
Brain	1.68 ± 0.12	5.11** ± 0.80	16.13* ± 3.43	N.D.
Testes	1.75 ± 0.56	4.90** ± 0.52	30.58* ± 4.76	"
Kidney	2.25 ± 0.33	15.12** ± 0.96	89.60* ± 10.90	"
Spleen	4.35 ± 0.52	19.67** ± 1.62	96.32* ± 8.05	"
Heart	4.00 ± 0.50	21.45** ± 2.03	106.68* ± 13.90	"
Fat	4.90[b] ± 0.60	--	148.42* ± 9.45	"
Liver	6.03 ± 0.23	46.55** ± 4.43	610.96* ± 47.00	"

All values are expressed as mean ± SE, six animals per group unless otherwise stated.

[a] Single value

[b] Mean of four values.

* Significantly different from the other groups $p < 0.05$.

**Significantly different from the other groups $p < 0.05$.

N.D. Denotes none detected.

TABLE IV

Effect of Aroclor 5460 on Microsomal protein and phospholipids

Groups	Microsomal	
	Protein mg/g wet liver	Phospholipids mg/g wet liver
Control	25.48 ± 0.94	6.66 ± 0.57
10 ppm	27.36 ± 0.93	6.72 ± 0.33
100 ppm	30.30 ± 1.96	7.00 ± 0.55
1000 ppm	34.64* ± 1.86	8.52* ± 0.67

Results are expressed as mean ± SE, six animals per group.

* Significantly different from controls $p < 0.05$.

Both parameters show the same pattern, which is a dose response type with the highest content in microsomal protein and phospholipids being observed in the 03 group rats.

Table V indicates the effect of PCTs on cytochrome P_{-450} and glucose-6-phosphatase.

TABLE V

Effects of Aroclor 5460 on Cytochrome P_{-450} and Glucose-6-Phosphatase

Groups	Glucose-6-Phosphatase µmoles P/g liver/i	Cytochrome P_{-450} mµmoles/g liver
Control	13.53 ± 0.84	21.98 ± 0.68
10 ppm	12.85 ± 0.76	22.88 ± 0.74
100 ppm	12.39 ± 1.24	25.32 ± 0.34
1000 ppm	10.04 ± 1.43	37.78* ± 2.61

Results are expressed as mean ± SE, six animals per group.

* Significantly different from controls $p < 0.05$.

Cytochrome P_{-450}, the terminal oxidase in the metabolism of many drugs, pesticides and foreign compounds is shown to be readily increased with the increasing concentrations of PCTs used in the feeding experiments. Glucose-6-phosphatase shows a slight diminution in the mean values obtained but they were not statistically significant.

The results for liver aniline hydroxylase type II and aminopyrine n-demethylase type I enzyme activities are shown in Table VI.

TABLE VI

Effect of Aroclor 5460 on Microsomal enzyme activities

Groups	Aniline Hydroxylase mµmoles/mg microsomal protein	Aminopyrine N-demethylase /30 min.
Control	15.36 ± 1.53	13.77 ± 0.33
10 ppm	12.03 ± 0.71	12.00 ± 1.04
100 ppm	10.47 ± 0.79	9.27 ± 0.88
1000 ppm	17.25 ± 1.54*	22.49* ± 2.36

Results are expressed as mean ± SE, six animals per group.

* Significantly different from controls $p < 0.05$.

A slight but significant diminution on aniline hydroxylation was observed in the 02 group rats, whereas an induction phenomenon was observed in the 03 group rats which was a significant increase compared to the controls.

Aminopyrine n-demethylase shows a similar behaviour pattern to the aniline hydroxylase.

SUMMARY

Male rats were orally dosed with Aroclor 5460 for 7 days and residues were found in all tissues analysed, with the greatest concentration in the liver. The GC-EC pattern of the residue in the liver was different from the standard mixture administered indicating that all components are not metabolised at the same rate.

Administration of Aroclor 5460 at 10 ppm level produced no observable changes in the microsomal enzyme systems, however, at the 1000 ppm level a significant increase in liver size was observed and an inductive effect is clearly evidenced. Aminopyrine n-demethylase (Type I) and aniline hydroxylase (Type II) activities were significantly raised in the microsomal fractions of the livers of this group.

The increased levels in drug metabolising enzyme activities and the parallel increase in the content of microsomal protein, phospholipids and Cytochrome P-450 would indicate a truly inductive effect of Aroclor 5460 on the hepatic microsomal enzyme systems in the male rat.

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