Metabolism of a Polychlorinated Biphenyl (Aroclor® 1254) Mixture in the Rat

by D. L. Grant, W. E. J. Phillips, and D. C. Villeneuve
Research Laboratories, Food and Drug Directorate
Department of National Health and Welfare
Tunney's Pasture, Ottawa 3, Ontario

Polychlorinated biphenyls (PCB's) have been used in industry since at least 1930. In 1955, von Oettingen (1) reviewed the literature on the toxicity of these compounds. Industrial workers have developed lesions of chloracne as a result of leaking vapours of a chlorinated biphenyl (Aroclor(R)*) used as a heat-exchange material (2).

Today the problem is not restricted to exposure of industrial workers as polychlorinated biphenyls are widely dispersed in fish and wild life (3-11). Wostoo et al. (11) found low levels of PCB (90%, <0.1 mg per kg fat) in a large number of foodstuffs. They also reported that all 22 samples of human milk analyzed contained PCB residues with 11 samples between 0-0.5 and the other 11 between 0.6-1.0 mg per kg fat. Therefore, there is a need to study the metabolism, distribution, storage, effect on reproduction, etc., of PCB's in mammals. This paper will report on the metabolism and distribution of Aroclor 1254 in normal and carbon tetrachloride-treated rats.

Experimental

Experiment I

The distribution of PCB's in various tissues of the rat and the effect of liver damage on their metabolism, was studied in twenty male Wistar rats randomized into four groups and treated as described in TABLE I.

Experiment II

The effect of Aroclor 1254 on the organ weights, fat content of the liver and on the potentiation of the toxicity of carbon tetrachloride was investigated in thirty eight Wistar rats divided into four groups and treated as described in TABLE II.

In Experiments I and II, the rats were housed in air conditioned rooms and were supplied with food and water ad libitum. The rats were killed by decapitation, blood collected, and brain, liver, heart, spleen, kidneys, testes and omental fat removed, weighed and frozen pending analyses.

*Aroclor(R), Monsanto Company (U.S.A.), registered trade name for polychlorinated biphenyls.

Bulletin of Environmental Contamination & Toxicology, Vol. 6, No. 2, 1971, published by Springer-Verlag New York Inc.

MONS 086639

TABLE I

Dosing regimen for experiment I

Group[a]	1	2	3	4
Day 1	Oil[b]	Oil[b]	CCl_4[c]	CCl_4[c]
Day 2	PCB[d]	PCB[d]	PCB[d]	PCB[d]
Day 3	Oil[e]	Oil	CCl_4[f]	CCl_4[f]
Day 4	Killed	--	Killed	--
Day 8	--	Oil[e]	--	CCl_4[f]
Day 24	--	Killed	--	Killed

[a]Each group contained five male rats, Wistar strain, av. b.w. 375 g.
[b]Corn oil, 2 ml per kg, administered orally.
[c]A 1+1 CCl_4:corn oil solution, 2 ml per kg, administered orally.
[d]A 500 mg per ml corn oil solution of Aroclor 1254, administered orally at 500 mg per kg.
[e]Corn oil, 1 ml per kg, administered orally.
[f]A 1+1 CCl_4:corn oil solution, 1 ml per kg, administered orally.

TABLE II

Dosing regimen for experiment II

Group[a] 1	Nil
Group[b] 2	PCB[c] on day 2
Group[d] 3	CCl_4[e] on days 1, 3, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 47
Group[d] 4	CCl_4[e] on days 1, 3, and 8 and PCB[c] on day 2

[a]Five male rats, Wistar strain, av. b.w. 410 g, killed on day 1.
[b]Fifteen male rats, Wistar strain, av. b.w. 440 g, 5 killed on days 8, 12, and 47.
[c]A 500 mg per ml corn oil solution of Aroclor 1254 administered orally at 500 mg per kg.
[d]Nine male rats, Wistar strain, av. b.w. 442 g.
[e]A 1+1 CCl_4:corn oil solution, 2 ml per kg administered orally on day 1 and 1 ml per kg on other days.

MONS 086640

Polychlorinated biphenyl analyses. Tissues were blended with 100 ml hexane and 50 g anhydrous sodium sulfate for 10 minutes. The extracts were filtered, concentrated to 10 ml, 3 ml of conc. H_2SO_4 - fuming H_2SO_4 (1:1) added and shaken. The hexane solutions were dried and neutralized with 4 g of anhydrous sodium sulfate-sodium carbonate (10:1). The solutions were subjected to GLC-EC analyses on a Varian Aerograph Model 600D gas chromatograph, fitted with a coiled 4' x 1/4" O.D. glass column containing Chromosorb W, 80-100 mesh, coated with 4% SE-30 and 6% QF-1. The nitrogen flow rate was 120 ml per minute with column and injection temperatures of 193 and 225°C, respectively. Aroclor 1254 standards and test solutions were chromatographed and the concentration of PCB's in the test solutions determined by comparing the heights of the six major peaks of the standards with corresponding peak heights of the test solutions.

Problems were not encountered with sensitivity during analyses and the lower limit of detection would depend on the tissue being analyzed. Hexane and acetone gave equal recoveries of Aroclor residues. Quadruplicate analyses of a rabbit liver gave values of 431, 438, 464, and 474 ppm. Recoveries of spiked samples were greater than 95%.

Lipid was determined by the method of Bligh and Dyer (12).

Results and Discussion

Experiment I

From day 1 to day 4 the rats dosed with corn oil and Aroclor 1254 (groups 1 and 2) or carbon tetrachloride and Aroclor 1254 (groups 3 and 4) lost an average of 19 and 55 g (b. w.), respectively. On day 8, group 2 rats had regained their lost weight, while group 4 rats continued to lose weight. Four of the five rats in group 4 which received the oral dose of carbon tetrachloride on day 8 died within the next 24 hours. However, by day 24 the 1 remaining rat in group 4 and the five rats in group 2 had gained 20 and an average of 30 g, respectively. The livers of the group 2 rats were enlarged and averaged 5.32±0.30% of their body weight.

Residues of Aroclor 1254 (expressed as ppm wet tissue, TABLE III) were found in all tissues analyzed, with fat and blood having the greatest and least concentration, respectively.

The residues in the blood, testes, liver, kidney and heart were significantly greater in the group 3 rats than in the group 1 rats. This shows that the liver is the main site of Aroclor 1254 metabolism and rats with carbon tetrachloride damaged livers are not able to metabolize this mixture as rapidly as rats with normal livers. The residues in the spleen, brain and fat were similar for both groups. The residues in the brain, liver,

MONS 086641

spleen, blood, testes, heart, kidney and fat of group 2 rats (killed on day 24) were 10, 16, 20, 21, 22, 24, 36 and 67%, respectively, of those of group 1 (killed on day 4). These values show that the PCB residues were being cleared from the various tissues at different rates.

TABLE III

Residues in tissues of rats orally dosed with Aroclor 1254 (500 mg/kg)

	Residue found (ppm, wet tissue)			
Group	1	2	3	4
Blood	1.96[a] ± 0.23[b]	0.42 ± 0.07	3.85 ± 0.46	0.25[c]
Testes	19.22 ± 0.59	4.30 ± 0.44	33.18 ± 1.35	5.62
Heart	24.16 ± 2.84	5.83 ± 0.53	62.40 ± 4.37	6.17
Spleen	29.17 ± 3.44	5.82 ± 1.17	36.60 ± 4.39	--
Kidney	31.14 ± 2.09	11.20 ± 1.76	57.38 ± 3.91	11.08
Brain	39.98 ± 5.91	4.01 ± 0.31	41.91 ± 3.30	5.96
Liver	115.66 ± 10.55	18.85 ± 1.65	796.47 ± 64.96	18.79
Liver[d]	1866.14 ± 166.63	--	6137.64 ± 556.06	--
Fat	996.16 ± 98.58	672.66 ± 155.12	900.46 ± 106.16	1149.05

[a] Mean of five values.
[b] Standard error of the mean.
[c] Single value.
[d] Ppm on a fat basis.

GLC-EC tracings from 24 ng of Aroclor 1254 and from the residue found in the liver of a group 2 rat, are shown in Figure 1. The amount (%) that each of the six major peaks contribute to the total residue in the tissues is presented in TABLE IV.

MONS 086642

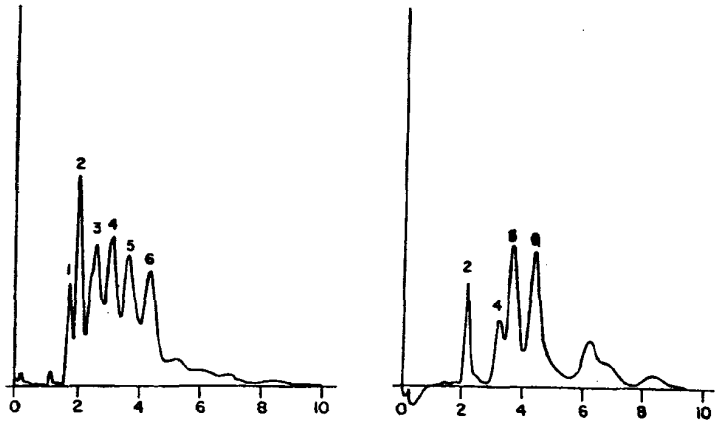

Figure 1 GLC-EC tracings from 24 ng of Aroclor 1254 (left) and from the residue found in the liver of a group 2 rat.

MONS 086643

TABLE IV

The amount (%) that each of the six major peaks of the Aroclor 1254 standard contribute to the total residue in the tissues

Peak number	Percent 1	2	3	4	5	6	Percent 1	2	3	4	5	6
Aroclor 1254	12	25	18	17	15	13	12	25	18	17	15	13
	Group 1						Group 2					
Spleen	6	21	9	22	21	21	3	17	2	11	34	32
Testes	3	15	6	21	27	28	1	19	2	11	35	32
Liver	2	11	3	30	28	27	0	25	2	18	26	28
Kidney	8	21	11	21	19	19	0	18	2	14	34	32
Fat	7	25	11	25	17	16	1	19	4	20	28	28
Brain	1	13	5	23	30	29	2	19	4	12	34	29
Blood	4	13	6	24	26	26	1	20	3	12	33	31
Heart	4	17	6	21	26	26	1	17	2	8	31	29
	Group 3						Group 4					
Spleen	7	25	14	19	18	17	--	--	--	--	--	--
Testes	6	23	14	19	19	19	1	20	4	12	31	32
Liver	4	21	11	21	22	21	0	19	4	20	26	31
Kidney	8	22	15	18	18	17	1	21	4	8	32	34
Fat	10	29	16	21	13	12	1	20	5	20	26	28
Brain	6	25	13	20	18	18	1	22	4	11	30	31
Blood	5	22	14	22	18	18	2	22	3	11	29	33
Heart	5	27	15	18	18	17	1	20	3	10	32	34

MONS 086644

Figure 1 and TABLE IV show that the components of the Aroclor 1254 mixture with the shorter retention times, peaks 1, 2 and 3, and presumably with the lowest chlorine contents (10) were metabolized to a greater degree than those with the longer retention times. This observation agrees with that reported in studies with Phenochlor DP6* fed to Japanese Quail (7). In group 2, peak 2 accounted for 25, 20, 19 and 19% of the residue in the liver, blood, brain and testes, respectively, whereas in group 1 the percents were 11, 13, 13 and 15%, respectively. Thus, the metabolism of this component (peak 2) was very slow between day 4 and 28 or one or more of the other components of the mixture were metabolized to the peak 2 component. The residue pattern in the fat of animals treated with carbon tetrachloride (Group 3) was similar to that of the standard Aroclor 1254 indicating little metabolism.

Expression of the Aroclor 1254 residues in the tissues relative to those in blood (TABLE V) shows that the ratios depend on the length of time following the oral dose (kidney, brain, liver and fat, group 1 vs 2) and the metabolic activity of the liver (liver and fat, group 1 vs 3).

TABLE V

Concentrations of the Aroclor 1254 residues in tissues relative to those in blood

Group	1	2	3	4
Blood	1.00	1.00	1.00	1.00
Testes	9.81	10.24	8.62	22.48
Heart	12.33	13.88	16.21	24.68
Spleen	14.80	13.86	9.51	--
Kidney	15.89	26.67	14.90	48.32
Brain	20.40	9.55	10.89	23.84
Liver	59.17	44.88	206.88	75.16
Fat	508.24	1601.57	233.89	4596.20

*Phenochlor DP6, Prodelec's (France) registered trade name for polychlorinated biphenyls.

MONS 086645

However, since blood samples are relatively easy to obtain they could be used for estimating the residue in other tissues, providing the investigator is aware of the limitations of the estimates.

Experiment II

In Experiment I no rats received carbon tetrachloride alone, therefore experiment II was conducted to determine whether Aroclor 1254 (a) potentiates the toxicity of carbon tetrachloride and (b) alters the weights of other organs besides the liver.

Eight of the 9 rats in group 4 died within 24 hours of receiving the carbon tetrachloride on day 8, while none of the rats in group 3 died up to day 47, when they were killed. Thus, it is concluded that Aroclor 1254 potentiated the toxicity of carbon tetrachloride in a manner similar to that reported for phenobarbital (13-15) and for DDT (13, 15).

Treatment with Aroclor 1254 evoked a number of significant changes in organ weights (TABLE VI), the most consistent being an increase in liver weight.

TABLE VI

Significant differences (Student Fisher's t test) found in organs weights from rats treated with Aroclor 1254 and untreated rats

Day killed	8	12	47
Organ		Probability	
Spleen	nil	nil	<0.01[a]
Liver	<0.005[a,b]	<0.005[a,b]	<0.005[a,b]
Heart	nil	<0.05[b]	<0.05[b]
Kidney	-	<0.05[a]	-
Testes	-	<0.05[a]	-

[a] Expressed as % body weight.
[b] Expressed as actual organ weight.

MONS 086646

This agrees with results from experiments in this laboratory on rabbits treated with PCB's, which will be reported elsewhere. More differences were observed on day 12 than on days 8 and 47. The only organ to significantly decrease in weight was the spleen on day 47.

The results of the lipid analyses of some livers from experiments I and II are presented in TABLE VII.

TABLE VII

Percent lipid of rat livers from experiments I and II

Treatment	Lipid (%)
Nil, killed on day 1[a]	4.45[b] ±0.008[c]
Aroclor 1254 on day 2, killed on day 4[d]	6.19 ±0.23
Carbon tetrachloride on day 1, killed on day 4[e]	13.22 ±1.17
Aroclor 1254 on day 2, killed on day 8[f]	8.35 ±0.99
Aroclor 1254 on day 2, killed on day 12[f]	7.78 ± 0.38

[a] Experiment II, group 1.
[b] Mean of five values.
[c] Standard error of the mean.
[d] Experiment I, group 1.
[e] Experiment I, group 3.
[f] Experiment II, group 2.

In experiment II, the lipid content of livers from rats killed 6 and 10 days after receiving the Aroclor were significantly higher than the controls ($P<0.005$, student Fisher's t test). Tanaka et al. (16) reported that chlorobiphenyls given orally (0.1 g per kg per day) to rats for four weeks caused loss of body weight, hepatomegaly and marked increase in serum lipid components. As expected, livers from rats which received the carbon tetrachloride and Aroclor 1254 had a very high content of lipid.

A number of the tissues from the rats killed on day 47 were analyzed for PCB residues (TABLE VIII).

MONS 086647

TABLE VIII

Residues in tissues of rats 45 days after being orally administered a single dose of Aroclor 1254 (500 mg/kg)

Residue Group[a]	ppm, wet tissue 2	Relative to Blood 2
Blood	0.18[b] ±0.05[c]	1.00
Heart	2.71 ±0.61	15.06
Kidney	3.39 ±0.29	18.83
Brain	4.19 ±0.34	23.28
Liver	16.04 ±3.79	89.11
Fat	397.30 ±41.86	2207.22

[a]Group 2 of Experiment II killed on day 47.
[b]Mean of five values.
[c]Standard error of the mean.

The residues in the blood, heart, kidney, brain, liver and fat were 9, 11, 11, 10, 14 and 40% respectively, of those found in group 1, Experiment I rats (TABLE I). The tissue-blood residue ratios again show these values depend on the length of time following dosing. The uneven rate of metabolism of the individual Aroclor components was again noted. For example, the amount (%) that each of [redacted] six major peaks contributed to the total residue in the fat w[redacted] 15, 1, 12, 40 and 34, compared to 12, 25, 18, 17, 15, and 13 for Aroclor 1254 standard.

Summary

Male rats were orally dosed with Aroclor 1254 and residues were found in all tissues analyzed, 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, spleen, blood, testes, heart, kidney and fat were reduced by 90, 88, 80, 79, 78, 76, 69 and 33% respectively in 20 days. Aroclor 1254 significantly increased the size of the liver and also the percent lipid in the liver. Aroclor 1254 was found to potentiate the toxicity of carbon tetrachloride in the rat.

MONS 086648

Acknowledgment

We acknowledge C.R. Westerby for technical assistance and B.B. Coldwell and K.A. McCully for review of this manuscript.

References

1. von Oettingen, W.F., Public Health Service Publication No. 414, 306 (1955).

2. Meigs, J.W., Albom, J.J., and Kartin, B.L., J. Am. Med. Assoc. 156, 1417 (1954).

3. Risebrough, R.W., Reiche, P., Peakall, D.B., Herman, S.G., and Kirven, M.N., Nature 220, 1098 (1968).

4. Jensan, New Scientist 32, 612 (1966).

5. Holmes, D.C., Simmons, J.H., Tatton, J.O'G., Nature 216, 227 (1967).

6. Holden A.V., and Marsden, K., Nature 216, 1274 (1967).

7. Koeman, J.H., Tan Noever De Brauw, M.C., and DeVos, R.H., Nature 221, 1126 (1969).

8. Jensen, S., Johnels, A.G., Olsson, M., and Otterlind, G., Nature 224, 247 (1969).

9. Duke, T.W., Lowe, J.I., and Wilson, A.J., Jr., Bulletin Environmental Contamination and Toxicology 5, 171 (1970).

10. Bagley, G. E., Reichel, W. L., and Cromartie, E., J. Assoc. Offic. Anal. Chem. 53, 251 (1970).

11. Wastoo, G., Norén, K., and Andersson, M., Varfoda 22, 9 (1970).

12. Bligh, E.G., and Dyer, W.J., Can. J. Biochem. and Physiol. 37, 911 (1959).

13. McLean A.E.M. and McLean, E.K., Biochem. J. 100, 564 (1966).

14. Farber, T.M., Heider, A., Paters, E.L., Ritter, D.L., Disraely, M., and Van Loon, E.J., Ninth Annual Meeting, Society of Toxicology, Atlanta, Georgia, March 15-19 (1970).

15. Cawthorne, M.A., Bunyan, J., Sennitt, M.V., and Green, J., Br. J. Nutr. 24, 357 (1970).

16. Tanaka, K., Fujita, E., Komatsu, F., and Tamura, N., Fukuoka-Igaku-Zasshi 60, 548 (1969).

MONS 086649