Original Scientific Paper
UDC 615.099:632.951

SUBACUTE TOXICITY OF POLYCHLORINATED BIPHENYL (AROCLOR 1242) IN RATS

N. K. Nešković, V. D. Vojinović and M. M. Vukša
INEP, Department of Pesticides, Zemun — Belgrade, Yugoslavia

(Received for publication March 22, 1984)

The distribution, storage and toxic effects o PCB (Aroclor 1242) were examined. The results show that the greatest quantity of Aroclor 1242 administered as a single oral dose is stored in the fat tissues of rats. No statistically significant difference in body weight gain was observed in animals given Aroclor 1242 for a period of 30 days when compared with control animals, but an increase in liver weight was established in test animals of both sexes. Serum alkaline phosphatase activity did not significantly change in animals administered Aroclor 1242, but statistically significant changes in serum glutamic-oxaloacetic (SGOT) and serum glutamic-pyruvic transaminases (SGPT) activities were established. SGPT activity decreased in the test animals of both sexes, whereas SGOT activity increased in males, and decreased in females compared to control animals. At the same time, NADPH-cytochrome *c* reductase activity and cytochrome P-450 content increased significantly in test animals of both sexes. Finally, a statistically significant decrease in the acute toxicity of phorate and propoxur was established in animals given Aroclor 1242 for a period of 30 days.

Polychlorinated biphenyls (PCBs) are widely used as plasticizers in paints and adhesive fire resistants, as hydraulic fluids, lubricants, transformer fluids, and also as protective coatings for wood and metal (1). At the same time, they are ubiquitous and very dangerous environmental pollutants.

PCBs reach the environment in various ways. From there they get into the human body through the food chain. Owing to their high lipid solubility PCBs are stored in the organism where they exert influence for long periods of time (2—4). Industrial PCBs are heterogeneous. They have very different physical and chemical characteristics which influence their metabolism, distribution, excretion and toxicity

level (5, 6). The results of a previous investigation show that PCBs produce various biological effects in the organism such as microsomal enzyme induction, porphyrogenic action, estrogenic activity, and immunosuppression (7—12).

The effect of Aroclor 1242 (with 42% chlorine) on rats was studied in this work. Its distribution and storage in the body, the effect on metabolic and serum enzymes as well as the effects on acute toxicity of the insecticides phorate and propoxur were followed.

MATERIALS AND METHODS

Chemicals

The compounds used in experiments were: phorate (0,0-diethyl-/ethylthio/methyl/phosphorodithionate), purity 83%, produced by Polysciences Corp., USA; propoxur (2-/1-methylethoxy/phenyl methylcarbamate), purity 99.2%, produced by Bayer AG, Leverkusen, W. Germany; Aroclor 1242 (technical product), produced by Monsanto Chem. Comp., St. Louis, USA.

The other chemicals used were of reagent grade.

Animals

Experimental animals were male and female Mill-Hill rats weighing 200—270 g at the beginning of the experiment. The animals were kept in polyethylene cages (two animals in each) at a room temperature of 22—24 °C throughout the experiment (30 days).

Treatment

For the observation of distribution and storage 1200 mg/kg Aroclor 1242 vas administered, as a single oral dose by gavage. Control animals were given sunflower oil only.

After a 30-day period the animals were killed, and samples of blood, fat, muscle (abdominal), liver, kidney, lung, and brain were taken for analysis of Aroclor 1242 content.

In a chronic toxicity experiment, the animals were given Aroclor 1242 (75 mg/kg), daily, for thirty days. The substance was dissolved in sunflower oil and a quantity of 2 ml/kg of the oil was given to the animals by gavage. Control animals received sunflower oil only.

After thirty days the animals were killed, the relevant organs removed, and weighed and the liver and blood were stored for further examinations.

Analysis

Concentrations of Aroclor 1242, in organs and tissues, were determined by gas-chromatography using the methods described by *Mills and co-workers* (13), and *Kadoum* (14). A Varian 3700 gas liquid chromatograph (GLC) with an electron-capture detector (ECD) was used.

Preparation of microsomes

Microsomes were isolated from the liver of the control and experimental animals according to the procedure described by *Cinti and co-workers* (15). Proteins were determined by the method of *Lowry and co-workers* (16), with bovine serum albumin as standard.

Enzyme assays

NADPH-cytochrome c reductase activity was estimated spectrophotometrically at 550 nm by following cytochrome c reduction, as previously described (17). Cytochrome P-450 was determined by the method of *Omura and Sato* (18), serum alkaline phosphatase according to the procedure described by *Kaplan and Carmen* (19), and serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) with the method of *Reitman and Frankel* (20).

Acute toxicity

The acute intraperitoneal toxicity of phorate and propoxur was determined in the controls and in animals that had been given Aroclor 1242 (75 mg/kg/day) for thirty days. LD_{50} was determined by the method of *Litchfield and Wilcoxon* (21).

Statistical analysis

Statistical analysis was carried out by means of the usual procedure (22). Differences between the mean values of control and test groups were analysed using Student's t-test.

RESULTS

Distribution and storage of Aroclor 1242 in different organs and tissues are presented in Table 1. The presence of Aroclor 1242 was ascertained in all examined organs and tissues sixty minutes after administration. The largest quantity (14.3 mg/kg) was found in the lungs, and in the brain (13.5 mg/kg). After 24 hours the largest quantity of Aroclor 1242 was found in the brain (27.1 mg/kg), and in the lungs (24.1 mg/kg). Thirty days after administration a larger amount was found only in the fat tissue (21.6 mg/kg). In the other organs and tissues a very small quantity was found: from 0.50 in the plasma to 2.06 mg/kg in the brain.

After 90 days 7.50 mg/kg of Aroclor 1242 was found in fat, while in the other organs and tissues it was present in a much lesser quantity.

No statistically significant differences were found in the body weight gain between the control animals and the animals that had received Aroclor 1242 (75 mg/kg/day) for a 30-day period (Table 2). However, there was a significant increase in liver weight in test animals. At the

Table 1

Distribution and storage of Aroclor 1242 in different tissues of rats[a]

Tissue	Aroclor 1242[b] (Dosage level — 1200 mg/kg)			
	60 minutes	24 hours	30 days	90 days
Brain	13.5 ± 0.5	27.1 ± 2.3	2.06 ± 0.07	0.25 ± 0.05
Liver	12.7 ± 1.2	16.3 ± 0.9	1.10 ± 0.02	0.20 ± 0.02
Kidneys	13.2 ± 0.3	19.7 ± 1.3	1.90 ± 0.05	0.09 ± 0.01
Lungs	14.3 ± 1.7	23.1 ± 2.0	1.75 ± 0.05	0.15 ± 0.02
Fat	5.4 ± 0.2	16.1 ± 1.1	21.65 ± 2.50	7.50 ± 1.05
Muscle	7.4 ± 1.1	17.8 ± 0.2	1.05 ± 0.09	0.20 ± 0.05
Plasma	5.3 ± 0.4	1.8 ± 0.2	0.50 ± 0.02	0.05 ± 0.00

[a] Oral ingestion, by gavage.
[b] Values are means ± S.D. expressed as mg/kg fresh tissue weight or ml plasma for 8—10 samples per group.

Table 2

Body weight gain in rats administered Aroclor 1242 for a period of 30 days[a]

Sex	Treatment	Dose (mg/kg)	Initial weight		Terminal weight	
			g	%	g	%
Males	Control	0.0	258.6 ± 5.2	100.0	299.9 ± 10.7	116.0
	Aroclor 1242	75.0	273.4 ± 4.9	100.0	309.6 ± 7.3	113.2
Females	Control	0.0	201.2 ± 4.2	100.0	221.8 ± 4.5	110.2
	Aroclor 1242	75.0	199.1 ± 3.5	100.0	222.4 ± 5.2	111.7

[a] Mean ± S. D. There were 10 animals in each group.

same time, no statistically significant differences were noticed in the weight of other organs (kidneys, lungs, heart, spleen and adrenals) between control and test animals (Table 3).

Table 4 shows the activities of serum alkaline phosphatase, SGOT and SGPT at the end of the experiment.

It is evident that Aroclor 1242 did not affect the activity of alkaline phosphatase, but SGPT was decreased in male rats by 6.3 and in female by 14.6%. The difference is statistically significant ($P < 0.05$). SGOT in the male rats which had been administered Aroclor 1242 increased by 22.5, and in female it decreased by 17.6%. The differences are statistically significant ($P < 0.01$).

Table 3

Absolute organ weight of rats administered Aroclor 1242 for a period of 30 days[a]

	Liver (g)	Kidney (g)	Lungs (g)	Heart (g)	Spleen (g)	Adrenals (mg)
Males						
Control	8.2±0.4	2.4±0.1	1.7±0.1	1.02±0.04	0.74±0.04	67.7±1.9
Aroclor 1242	13.1±0.5[b]	2.6±0.1	1.8±0.1	1.04±0.03	0.80±0.03	63.4±2.0
Females						
Control	6.5±0.1	1.9±0.1	1.6±0.1	0.85±0.02	0.60±0.02	88.4±2.3
Aroclor 1242	9.5±0.2[b]	1.8±0.0	1.4±0.1	0.80±0.02	0.66±0.02	84.3±1.7

[a] Aroclor 1242 (75 mg/kg/day) was given orally (by gavage). The results are means ± S. D. There were 10 animals in each group.
[b] Significantly different from control group, $P < 0.001$.

Table 5 shows microsomal NADPH-cytochrome c reductase activity and cytochrome P-450 content in rats treated with Aroclor 1242.

Compared to control values NADPH-cytochrome c reductase activity increased by 34.8% in male rats, and by 47% in female and cytochrome P-450 content also increased by 407.5% in females and 434% in males. The differences are statistically significant ($P<0.01$ and $P<0.001$, respectively).

Table 6 shows the effect of Aroclor 1242 on acute toxicity of propoxur and phorate.

The LD_{50} values for propoxur in rats administered Aroclor 1242 were higher than the control values (Table 6). The increase in males was 43.3%, and in females about 20%. Both differences are statistically significant ($P < 0.01$ and $P < 0.05$, respectively).

The LD_{50} values for phorate in rats administered Aroclor 1242, were also higher in relation to the control values (Table 6). The increase in males was 93.7%, and in females about 36.4%. The differences are statistically significant ($P < 0.001$ and $P < 0.01$, respectively).

At the same time, both examined compounds, were more toxic to female than to male rats.

DISCUSSION

Investigations on the effects of PCBs have shown that, depending on the type of the chemical compound (higher or lower chlorine content), the dose and the time of administration, PCBs have different retention times in the organism and provoke different changes. There are many controversial data concerning this matter and the investigations still arouse great interest.

Table 4

Effect of Aroclor 1242 treatment (30 days) on serum enzymes in rats[a]

Sex	Treatment	Dose (mg/kg)	Alkaline phosphatase		SGOT		SGPT	
			Bodansky units	% of control	mU	% of control	mU	% of control
Males	Control	0.0	11.4±0.4	100.0	27.6±2.5	100.0	20.8±2.2	100.0
	Aroclor 1242	75.0	11.5±0.3	100.9	33.8±2.1	122.5[c]	19.5±1.9	93.7
Females	Control	0.0	9.2±0.6	100.0	30.7±2.8	100.0	19.2±1.2	100.0
	Aroclor 1242	75.0	9.5±0.9	103.3	25.3±1.5	82.4[b]	16.4±1.0	85.4[b]

[a] Means ±S.D. There were 10 animals in each group.
[b] Significantly different from control group, $P < 0.05$.
[c] Significantly different from control group, $P < 0.01$.

Table 5

Effect of Aroclor 1242 treatment (30 days) on NADPH-cytochrome c reductase activity and cytochrome P—450 content in rats[a]

Sex	Treatment	Dose (mg/kg)	NADPH-cytochrome *c* reductase		Cytochrome P-450	
			nmoles cytochrome *c* reduced/min/mg protein	% of control	nmoles/mg protein	% of control
Males	Control	0	64.6±3.5	100.0	1.03±0.02	100.0
	Aroclor 1242	75	**87.1**±5.2	134.8[b]	5.50±0.25	534.0[c]
Females	Control	0	66.0±3.9	100.0	1.33±0.05	100.0
	Aroclor 1242	75	**97.4**±9.3[b]	147.6[b]	6.75±0.45	507.5[c]

[a] Means ±S.D. There were 10 animals in each group.
[b] Significantly different from control group, $P < 0.01$.
[c] Significantly different from control group, $P < 0.001$.

Table 6

The effect of Aroclor 1242 (30 days) on acute toxicity of propoxur and phorate in rats

Sex	Treatment	Dose (mg/kg)	LD_{50} (mg/kg)	
			Propoxur	Phorate
Males	Control	0	120.0 (68.6—210.0)[a]	4.8 (4.3—5.3)
	Aroclor 1242	75	172.0[c] (132.3—223.6)	9.3[d] (6.0—12.5)
Females	Control	0	110.0 81.5—148.5)	2.2 (1.9—2.6)
	Aroclor 1242	75	132.0[b] (94.3—164.8)	3.0[c] (2.7—3.4)

[a] 95% confidence limits.
[b] Significantly different from control group, $P < 0.05$.
[c] Significantly different from control group, $P < 0.01$.
[d] Significantly different from control group, $P < 0.001$.

Our results show that Aroclor 1242 is retained for a longest period in fat tissues (Table 1). This was to be expected considering its lipid solubility. The results also show (Table 3), that Aroclor 1242 markedly increases liver weight but as far as the weight of other organs is concerned there seems to be no difference from control animals.

Many other authors have come to the conclusion that PCBs induce an increase in liver weight (10, 25, 26), and that this effect depends on the type of chemical compound. They offer different explanations of this PCBs effects, but the most acceptable is the one attributing it to the increase in the amount of phospholipids and in smooth endoplasmic reticulum.

This is no doubt due to the fact that PCBs provoke the induction of liver microsomal enzymes which has been confirmed in our investigations with Aroclor 1242 (Table 5) and is in agreement with the results of other authors (2, 4, 10, 26—29).

Inoue and co-workers (29) have shown that NADPH-cytochrome c reductase activity and the content of the cytochrome P-450 increase in fetal liver of rats that had been exposed to PCBs.

The results of the experiments of *Litterst and co-workers* (7) on animals suggest that chronic exposure to small doses of PCBs affects the enzyme activity of the human liver as well.

The increased activity of these enzymes is connected with the microsomal oxidative capacity and this leads us to conclude that PCBs may play an important role in the biological response of mammals to toxic effects of different chemicals. This relation reflects itself in an increase of metabolic enzyme activity which changes the rate of metabolism and consequently the toxicity of different substances (26).

Our results (Table 6) show that the intraperitoneal toxicity of phorate and propoxur in rats which were given Aroclor 1242 during 30 days is significantly reduced. They also show (Table 4) that Aroclor 1242 induces changes in the activity of functional liver enzymes (SGOT and SGPT). This response was probably a result of Aroclor 1242 induced damage in the liver.

ACKNOWLEDGEMENTS

This investigation was supported by research grants from the Scientific Research Council of Serbia. The authors thank Mrs Zora Žagar for her technical assistance.

References

1. *Broadhurst, M. G.:* Environ. Health Perspect., *2* (1972) 81.
2. *Villeneuve, D. C., Grant, D. L., Philips, W. E. J., Clark, M. L., Clegg, D. J.:* Bull. Environ. Contam. Toxicol., *6* (1971) 120.
3. *Dikshith, T. S., Rockwood, W., Abraham, R., Coulston, F.:* Exp. Mol. Pathol., *22* (1975) 376.

4. *Alvares, A. P., Useng, T. H., Eiseman, J. L.:* Life Sci., *30* (1982) 747.
5. *Matthews, H. B., Anderson, M. W.:* Drug Metab. Dispos., *3* (1975) 371.
6. *Kato, S., McKinney, J. D., Matthews, H. B.:* Toxicol. Appl. Pharmacol., *53* (1980) 389.
7. *Litterst, C. L., Farber, T. M., Baker, A. M., Van Loon, E. J.:* Toxicol. Appl. Pharmacol., *23* (1972) 112.
8. *Vos, J. G.:* Environ. Health Perspect., *1* (1972) 105.
9. *Chen, T. S., Du Bois, K. P.:* Toxicol. Appl. Pharmacol., *26* (1973) 504.
10. *Coldstein, J. A., Hickman, P., Burse, V. W., Bergman, H.:* Toxicol. Appl. Pharmacol., *32* (1973) 461.
11. *Ecobiochon, D. J., Comeau, A. M.:* Toxicol. Appl. Pharmacol., *33* (1975) 94.
12. *Allen, J. R., Abrahamson, L. J.:* Arch. Environ. Contam. Toxicol., *8* (1979) 191.
13. *Mills, P. A., Onley, J. H., Gaither, R. A.:* J. Assoc. Off. Anal. Chem., 46 (1963) 186.
14. *Kadoum, A. M.:* Bull. Environ. Contam. Toxicol., *2* (1967) 264.
15. *Cinti, D. L., Moldeus, P., Schenkman, J. B.:* Biochem. Pharmacol., *21* (1972) 3249.
16. *Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, P. J.:* J. Biol. Chem., *193* (1951) 265.
17. *Nešković, N., Vitorović, S., Plesničar, M.:* Biochem. Pharmacol., *22* (1973) 2943.
18. *Omura, T., Sato, R.:* J. Biol. Chem., *239* (1964) 2370.
19. *Kaplan, S. A., Del Carmen, F. T.:* Pediatrics, *17* (1956) 857.
20. *Reitman, S., Frankel, S.:* Am. J. Clin. Pathol., *28* (1957) 56.
21. *Litchfield, J. T., Wilcoxon, F.:* J. Pharmacol. Exp. Ther., *96* (1949) 99.
22. *Snedecor, G. W., Cochran, W. G.:* Statistical Methods, 6th ed., Iowa State Univ. Press, Ames. 1967.
23. *Grant, D. L., Phillips, W. E. J., Villeneuve, D. C.:* Bull. Environ. Contam. Toxicol., *6* (1971) 102.
24. *Burse, V. W., Kimborough, R. D., Villaneuva, E. C., Jennings, R. W., Linder, R. E., Sovocool, G. W.:* Arch. Environ. Health, *29* (1974) 301.
25. *Allen, J. R., Abrahamson, L. J.:* Arch. Environ. Contam. Toxicol., *1* (1973) 265.
26. *Buckner, J. V., Khanna, K. L., Cornish, H. H.:* Toxicol. Appl. Pharmacol., *24* (1973) 434.
27. *Norback, D. H., Allen, J. R.:* Fed. Proc., *29* (1970) 816.
28. *Parkki, M. G., Marniem, J., Vainio, H.:* Toxicol. Environ. Health, *3* (1977) 903.
29. *Inoue, K., Takanaka, A., Mizokami, K., Fujimori, K., Sunouchi, M., Kasuya, Y., Omori, Y.:* Toxicol. Appl. Pharmacol., *59* (1981) 540.

Sažetak

SUBAKUTNA TOKSIČNOST POLIHLOROVANIH BIFENILA (AROCLOR 1242) U PACOVA

Ispitivani su distribucija, nakupljanje i toksični efekti PCBs (Aroclor 1242) u pacova. Rezultati su pokazali da se najveća količina Aroclora 1242, posle njegove jednokratne oralne primjene, nagomilava u masnom tkivu. Nije konstatovana statistički značajna razlika u prirastu telesne mase životinja koje su dobijale Aroclor 1242 u toku 30 dana, u poređenju sa kontrolnim

životinjama. Međutim, konstatovano je statistički značajno povećanje mase jetre u test životinja oba pola. Nisu konstatovane statistički značajne promene aktivnosti alkalne fosfataze u životinja koje su dobijale Aroclor 1242 u toku 30 dana, ali su utvrđene značajne promene u aktivnosti SGOT i SGPT. Aktivnost SGPT je bila smanjena u test životinja oba pola, dok je aktivnost SGOT u mužjaka povećana, a u ženki smanjena u poređenju sa kontrolnim životinjama. Istovremeno, u test životinja oba pola konstatovano je statistički značajno povećanje aktivnosti NADPH-citohrom c reduktaze i sadržaja citohroma P-450.

Najzad, konstatovano je statistički značajno smanjenje akutne toksičnosti insekticida forat i propoksur u životinja koje su prethodno dobijale Aroclor 1242 u toku 30 dana.

Institut za primenu nuklearne energije u poljoprivredi, veterinarstvu i šumarstvu, Zavod za pesticide, Zemun

Primljeno 22. III 1984.