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Polychlorinated Biphenyls: Effect of Diet Level on ATPase Activity in Rats*

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INTRODUCTION

The polychlorinated biphenyls (PCB's) have been used for diverse industrial purposes for the past 40 years. Although their use has recently restricted in the U. S. and in France (decret July 1975 8th) global environmental contamination and accumulation in food chains has been demonstrated (PEAKALL, 1972). The public significance of the PCB's became acutely apparent in 1968 with Yusho poisoning caused by ingestion of rice oil contaminated with this product (KURATSUNE et al. 1972). Several workers have reported the toxicity of these chemicals to several organisms but little is known about the physiological action of these materials. Some of the major findings to date for the rat include :renal and hepatic injury (BRUCKNER et al. 1974), hepatic microsomal enzyme induction (LITTERST et al. 1972) and lipid metabolic alterations (NAGAI et al. 1971).

Many pesticides are known to influence ATPase activity in mammal tissues (KOCH 1969, MATSUMURA and PATIL 1969). Because of the similarity of PCB's to some of the organochlorine insecticide and the evidence of PCB effect in fish tissues in vivo and in vitro (KOCH et al. 1972) it was interesting to determine the effect of PCB administration on ATPase activity in the rat. In order to know more upon the toxic nature of French PCB's, a representative commercial product, Phenoclor DP_6 **, was administered to rats. We have examined the activity of the ATPase enzyme system in liver, kidney and brain microsomal fractions.

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- METHODS -

Male Sprague Dawley rats, weighing 150 g were divided into groups of 8 rats each and were fed diets containing 0,10 100 or 500 ppm (wet weight) of Phenoclor DP_6 incorporated into arachid oil. After the rats had been fed on experimental diets for 4 weeks, they were sacrified. Livers, kidneys and brains were excised and immediately placed into chilled sucrose solution 0,25 M, pH 7,3 (containing bovine serum albumine 0,5 g/l). All subsequent manipulations were done at 4°C. Each organ is homogenized with 15 ml of the later sucrose solution in a Potter-Elvehjeim homogenizer with teflon pestle. The homogenate is centrifuged at 3000 g for 15 minutes and the supernatant is centrifuged at 105 000 g for 30 minutes. The 105 000 g pellet (microsomal fraction + heavy mitochondria) is resuspended in 3 x 3 ml of the sucrose solution and stored in liquid nitrogen. ATPase activity were determined according to the automatical enzymatic method previously described (GALLIS and BOURDICHON 1976). The composition of incubation medium (final volume 2 ml) is 4 mM ATP, 3 mM $MgCl_2$, 0,5 mM ethylene glycol-bis (βamino ethylene ether) N, N'-tetra acetic acid, 20 mM KCl, 100 mM NaCl and 0,05 to 0,4 ml of enzyme solution (medium type I). For Mg^{++} ATPase or "residual" ATPase incubation medium, there is in addition 10^{-3}M ouabain (medium type II).

Proteins were determined by the method of LOWRY et al. (1951).

- RESULTS AND DISCUSSION -

Table I presents the DP_6 ingested dose in each group. 50 p. cent mortality was found after 8 days in 500 ppm group (4/8) in agreement with a preliminary experiment (NARBONNE and GILLET 1977). The 4 rats left were sacrified immediately.

Table II shows the effect of Phenoclor DP_6 treatment on ATPase activity in liver microsomal fraction : although Na^+K^+ and Mg^{++} specific activity decreases, total liver activity remains constant. It has been reported that PCB's given to rats caused elevated liver weight and protein content. Increased microsomal protein (table IV) "dilute" ATPase enzyme but total liver activity was unaffected. The results given in table III indicate that inhibition and stimulation reponses were observed for Mg^{++} activity, respectively, in brain and kidney. KOCH et al. (1972) demonstrated that fishes treated with Aroclor 1254 showed inhibition in brain Mg^{++} activity and stimulation in kidney Mg^{++} activity. Our results confirmed a similar effect for Phenoclor DP6 in rat. There appears to be a difference in the response of the Na^+ K^+ ATPase activity to Phenoclore DP_6 between lower dose and LD_{50} dose. In brain Na^+K^+ activity remains stable at lower dose. In kidney 10 ppm determine a consistent decrease in Na^+K^+ activity.

TABLE I - PCB INGESTED DURING INTOXICATION TIME

Groups	DP_6 dietary level	Administration time	Bodyweight at sacrifice	Ingested PCB (mg)	Ingested dose (mg/kg bodyweight)
A	10 ppm	30 days	279 ± 10 *	12,6 ± 1,43	45
B	100 ppm	30 days	258 ± 18	119 ± 22	460
C	500 ppm	8 days	120 ± 7	84 ± 17	700

* mean ± SD

TABLE II - EFFECT OF DP_6 DIETARY LEVEL ON LIVER ATPase ACTIVITY

Groups	DP_6 dietary level	Administration time	ATPase activity			
			Na^+K^+ µmol/mg prot./h	Na^+K^+ ⁂ µmol/100 g/h	Mg^{++} µmol/mg prot./h	Mg^{++} ⁂ µmol/100 g/h
Control	0	30 days	1,64 ± 0,20 ⁎ (8)	167 ± 48 (8)	1,25 ± 2,1 (8)	1225 ± 145 (8)
PCB treated						
A	10 ppm	30 days	0,58 ± 0,104 (8)	125 ± 24 (8)	5,30 ± 1,1 (8)	1152 ± 252 (8)
B	100 ppm	30 days	0,52 ± 0,158 (8)	149 ± 46 (8)	4,70 ± 0,74 (8)	1368 ± 247 (8)
C	500 ppm	8 days	0,41 ± 0,13 (4)	205 ± 35 (4)	3,60 ± 0,94 (4)	1372 ± 323 (4)
Compared groups			Variation and signification ⁂			
T ⟶ A			-64 % P < 0,001	NS	-57 % P < 0,001	NS
T ⟶ B			-68 % P < 0,001	NS	-62 % P < 0,001	NS
T ⟶ C			-75 % P < 0,001	NS	-71 % P < 0,001	NS

⁎ mean ± SD ( ) animal number

⁂ signification was juged by the Student test t

⁂⁂ µmol in total liver/100 g of body weight/h

TABLE III - EFFECT OF DP_6 DIETARY LEVEL ON KIDNEY AND BRAIN ATPase ACTIVITY

Groups	DP_6 dietary level	Administration time	KIDNEY		BRAIN	
			Na^+ K^+ µmol/mg prot./h	Mg^{++} µmol/mg prot./h	Na^+ K^+ µmol/mg prot./h	Mg^{++} µmol/mg prot./h
Control	0	30 days	11,90 ± 5,10* (8)	39,40 ± 5,01 (8)	44,30 ± 9,68 (8)	26,70 ± 1,55 (8)
A	10 ppm	30 days	2,72 ± 0,79 (8)	45,70 ± 1,91 (8)	35,60 ± 3,67 (8)	17,90 ± 0,74 (8)
B	100 ppm	30 days	7,15 ± 1,56 (8)	52,60 ± 5,37 (8)	43,70 ± 4,48 (8)	14,90 ± 1,53 (8)
C	500 ppm	8 days	18,02 ± 0,49 (4)	51,20 ± 1,92 (4)	59;0 ± 4,85 (4)	26,50 ± 2,90 (4)
Compared groups			Variation and signification**			
T ⟶ A			- 77% P < 0,001	+ 16% P < 0,05	NS	- 33% P < 0,001
T ⟶ B			NS	+ 33% P < 0,01	NS	- 44% P < 0,001
T ⟶ C			+ 51% P < 0,01	+ 30% P < 0,001	+ 33% P < 0,05	NS

* mean ± SD ( ) animal number

** signification was juged by the Student test t

TABLE IV - EFFECT OF DP_6 DIETARY LEVEL ON LIVER KIDNEY AND BRAIN MICROSOMAL FRACTION

Groups	DP_6 dietary level	Administration time	Protein microsomal fraction			
			LIVER mg/g	LIVER mg/100 g ⁂⁂	KIDNEY mg/g	BRAIN mg/g
Control	0	30 days	29,3 ± 1,89 ⁂ (8)	100 ± 21 (8)	17,7 ± 1,99 (8)	18,5 ± 1,50 (8)
A	10 ppm	30 days	41,5 ± 3,94 (8)	215 ± 17 (8)	21,1 ± 1,22 (8)	18,6 ± 1,56 (8)
B	100 ppm	30 days	40,0 ± 4,09 (8)	300 ± 48 (8)	18,9 ± 0,66 (8)	16,2 ± 2,99 (8)
C	500 ppm	8 days	52,6 ± 4,06 (4)	383 ± 48,7 (4)	18,2 ± 0,60 (4)	13,1 ± 0,95 (4)
Compared groups			Variation and signification ⁂⁂			
T ⟶ A			+ 41% P < 0,001	+ 115% P < 0,001	+ 19% P < 0,01	NS
T ⟶ B			+ 36% P < 0,001	+ 200% P < 0,001	NS	NS
T ⟶ C			+ 79% P < 0,001	+ 283% P < 0,001	NS	- 29% P < 0,001

⁂ mean ± SD ( ) animal number

⁂⁂ signification was juged by the Student test t

⁂⁂⁂ mg prot. in total liver/100 g body weight

500 ppm of Phenoclor DP_6 in the diet caused stimulation of Na^+K^+ ATPase activity in brain and kidney. A preliminary report indicates that 500 ppm determine increased nervous system response (NARBONNE and GILLET 1977).

Table IV presents the effect of Phenoclor DP_6 on microsomal protein content of liver, kidney and brain. A large increase in liver microsomal protein content was seen with increasing dose of DP_6. A preceding report showed the same effect on both liver microsomal protein and total liver protein (NARBONNE and DAUBEZE 1977). In brain and kidney, no significant effect was observed at lower dose tested (10-100 ppm). 500 ppm decrease microsomal proteins in brain.

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