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# Effect of Age and Sex on Liver Response to Phenoclor $DP_6$, a Polychlorinated Biphenyl, in the Rat*

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Many metabolic adaptations are necessary for the survival of an animal when administered PCBs in diet (DAUBEZE 1977). In PCB treated rats, increase in liver weight (LITTERST et al. 1972, BRUCKNER et al. 1973, KIMURA and BABA 1973) and microsomal protein content (BRUCKNER et al. 1973, TURNER and GREEN 1974), liver smooth endoplasmic reticulum proliferation (NISHIZUMI 1970), enhanced microsomal oxygenase activity (BRUCKNER et al 1974, LITTERST and VAN LOON 1974), and fatty infiltration of the liver (NISHIZUMI 1970, KIMURA and BABA 1973) were promptly ovserved. When rats were fed diets containing Phenoclor $DP_6$ (French PCB PRODELEC-FRANCE) the same effects were noted (DAUBEZE 1977, NARBONNE and GILLET 1977), FURNER et al. (1969) showed that age and sex influence the action of drug treatment on the induction of drug metabolism enzymes. Moreover ALLEN et al. (1974) indicate that proteosynthesis was enhanced in young rats.

Previous work (NARBONNE 1976) demonstrated that the principal response of liver to $DP_6$ treatment consisted in increased proteosynthesis. This observation suggested a difference in reactions between male and female rats and between young and adult rats to PCB treatment. However, GRANT and PHILLIPS (1974) showed that sex and age had very little effect on the toxicity of Aroclor 1254 in rats. Because of the difference in the toxicity between Aroclor and Phenoclor (PEAKALL 1972), studies were undertaken to determinate the effect of age and sex on rat liver response to Phenoclor $DP_6$ treatment.

## METHODS

Effect of sex on liver response to $DP_6$ : male and female Sprague Dawley rats weighing 300 g were divided into 4 groups of 6 animals each. Groups of males and of females were fed diets containing 100 ppm (wet weight) of Phenoclor $DP_6$ incorporated into arachid oil. Control groups were fed standard diet. Rats were fed on experimental diets for 8 days. 33 ± 1 mg of $DP_6$ were ingested during intoxication time by treated rats.

On the 7th day all rats were injected with sodium phenobarbital (100 mg/kg body weight). Sleeping times were measured as the interval from injection of the barbiturate to the regaining of the righting ability (ZEPP et al. 1974).

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On the 8 th day the rats were killed by decapitation and liver removed and weighed. 0.5 g liver samples were solubilized in KOH 0,66 N for total protein estimation. 2 g liver samples were homogenized in chilled sucrose solution (0.25 M, pH 7.4). The liver fractions were separated by differential centrifugation (NARBONNE 1978). All fractionation was carried out at 4°. Nucleus and mitochondrial fractions were discarded after centrifugation at 9.000 x g for 15 min. The 9.000 x g supernatant was centrifuged at 105.000 x g for 60 min. The remaining microsomal pellet was solubilized in 2 ml 0.66 N KOH. Proteins were determined by the method of LOWRY et al. (1951).

Effect of age on liver response to $DP_6$ : adult Sprague Dawley rats weighing 300 to 350 and 200 days old, and young rats weighing 90 g and 35 days old were divided into 4 groups of 6 rats each after one week of adaptation to standard diet. Groups of young rats and of adult rats were fed diet containing 100 ppm (wet weight) of Phenoclor $DP_6$.

The doses ingested daily were 24 mg/kg in young rats and 13 mg/kg in adult rats. Treated rats were fed on experimental diets for 8 days and killed by decapitation. Control groups were killed at zero time. Total and microsomal liver proteins were determined as described above. Liver fat content was determined by an automatic method (CANAL et al. 1973).

## RESULTS AND DISCUSSION

Effect of sex on liver response to $DP_6$ : the effect of dietary $DP_6$ treatment on phenobarbital sleeping times, relative liver weight, total and microsomal liver proteins contents on both male and female group are summarized in table 1. In male group fed $DP_6$, sleeping time was significantly reduced, liver weight and total protein contents were significantly increased (- 18 %, + 77 %, + 165 %, respectively). Female rats treated with $DP_6$ exhibited a small increase in liver weight and protein content (+ 12 % and + 13 % respectively). Sleeping time remains little affected by $DP_6$ treatment. Barbiturate sleeping time is commonly used for indirectly measuring hepatic microsomal enzyme induction (VILLENEUVE et al. 1972). Our results suggest an inverse correlation between microsomal proteins and sleeping time.

In the present experiment, female rats were little affected by short time $DP_6$ treatment. GRANT and PHILLIPS (1974) indicated, in female rats 120 days old, dosed orally with 10 mg/kg of Aroclor 1254 for 7 consecutive days, that the PCBs has a less effect on relative liver weight (+ 19 %) than in male rats treated in same conditions (+ 26 %). ZEPP et al. (1974) showed an influence of sex on reduction of sleeping time induced by pentobarbital in rabbits fed for 10 weeks a

TABLE 1 - EFFECT OF SEX ON RAT LIVER RESPONSE TO DP6 TREATMENT

| Diet [a] | Sex | Liver weight [c] % body weight | Total Liver Proteins [bc] | | Microsomal Liver Proteins [bc] | | Sleeping times [c] (minutes ± SEM) |
|---|---|---|---|---|---|---|---|
| | | | A | B | A | B | |
| CONTROL | ♂ | 3.57 ± 0.24 | 138 ± 2 | 492 ± 17 | 9.4 ± 0.9 | 33.6 ± 0.9 | 100 ± 4 |
| CONTROL | ♀ | 3.32 ± 0.04 | 254 ± 5 | 844 ± 17 | 11.2 ± 0.8 | 42 ± 2 | 124 ± 6 |
| DP6 | ♂ | 6.12 ± 0.26 | 213 ± 5 | 1306 ± 66 | 13.0 ± 0.2 | 79 ± 2 | 82 ± 3 |
| DP6 | ♀ | 3.78 ± 0.19 | 255 ± 5 | 961 ± 52 | 14.8 ± 0.7 | 48 ± 2 | 133 ± 3 |
| Compared groups | | Variation and significance [d] | | | | | |
| T ⟶ I | % | + 71 | + 54 | + 165 | + 38 | + 136 | - 18 |
| ♂ | P | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| T ⟶ I | % | + 12 | + 0.4 | + 13 | + 32 | + 15 | + 7 |
| ♀ | P | < 0.001 | < 0.90 | < 0.001 | < 0.001 | < 0.001 | < 0.02 |

a Each group comprised 6 rats

b mg of protein A per g liver (wet weight) B per whole liver per 100 g body weight

c Mean ± standard error of the mean

d Significance was judged by the Student t test

TABLE 2 - EFFECT OF AGE ON RAT LIVER RESPONSE TO DP6 TREATMENT

| Diet [a] | Age days | Body weight | Liver weight [ce] | Total Liver Proteins [bc] | | Microsomal Liver proteins [bc] | | Liver Fat [bc] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | B | A | B | A | B |
| CONTROL | 200 | 343 ± 12 | 3.5 ± 0,2 | 138 ± 2 | 492 ± 17 | 9.4 ± 0.9 | 34 ± 1 | 43 ± 2 | 148 ± 14 |
| CONTROL | 35 | 90 2 | 3.7 ± 0.1 | 143 ± 10 | 529 ± 27 | 7.6 ± 0.1 | 27 ± 1 | 38 ± 3 | 136 ± 28 |
| DP6 | 208 | 329 ± 7 | 6.1 ± 0.3 | 213 ± 6 | 1306 ± 66 | 13.0 ± 0.2 | 79 ± 2 | 55 ± 2 | 340 ± 25 |
| DP6 | 43 | 93 ± 2 | 6.6 ± 0.2 | 131 ± 7 | 869 ± 36 | 8.6 ± 0.2 | 57 ± 1 | 29 ± 4 | 196 ± 30 |
| Compared groups | | Variation and significance [d] | | | | | | | |
| T → I | % | + 3 | + 77 | - 8 | + 64 | + 12 | + 112 | - 23 | + 44 |
| Youngs | P | <0.05 | <0.001 | <0.10 | <0.001 | <0.001 | <0.001 | <0.01 | <0.01 |
| T → I | % | - 4 | + 71 | + 54 | + 165 | + 38 | + 136 | + 25 | + 129 |
| Adults | P | <0.10 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

a Each group comprised 6 rats

b mg A per g liver (wet weight) B per whole liver per 100 g body weight

c Mean ± standard error of the mean

d Significance was judged by the student t test

e % body weight

diet containing 100 ppm Aroclor 1254 (♂ - 28 %, ♀ - 11 %). This result indicate that female mammals were less sensible to PCB actions than male mammals.

Effect of age on liver response to $DP_6$ : table 2 summarizes the effect of $DP_6$ treatment on liver weight, total and microsomal liver proteins and liver fat content in young and adult rats. $DP_6$ treatment had a same effect on liver weight in both groups. However, liver composition was significantly different. Total liver protein was more increased in adult group (+ 165 %) than in young group (+ 65 %) although microsomal fractions were increased similarly in both groups (+ 136 % and + 112 %, respectively). Liver fat content was higher in adult rats than young ones, after $DP_6$ treatment (+ 129 % and + 44 %, respectively).

The results show that microsomal response to $DP_6$ treatment in young rats was unaffected by the difference in apparent half life of microsomal protein observed in young and adult rats (DALLMAN and MANIES 1973).

The present study demonstrates that female rats were less affected by $DP_6$ treatment than male rats. Age had little effect on liver response to $DP_6$ although liver fat content was significantly increased in adult rats.

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