Effect of Aroclor 1016 and 1242 on Selected Enzyme Systems in the Rat

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Introduction

Concern over the presence of polychlorinated biphenyls (PCB's) in the environment, their entry into the food chain, and the documented cases of human toxicity, has prompted an intensive assessment of these complex mixtures. It now appears that the residues found in the environment, and in potential food sources are derived from the mixtures containing the penta-chlorobiphenyl and higher molecular weight isomers. These isomers are apparently not as susceptible to biotransformation as the isomers of lower molecular weight.

Recent studies, among them the investigations of BRUCKNER et al. (1973); CHEN and DUBOIS (1973) ALLEN and ABRAHAMSON (1973), LITTERST et al. (1972) and BICKERS et al. (1972) have attempted to define the effects of the degree of chlorination on a range of biochemical parameters, including the microsomal enzyme system of the rat. While it again appears that the mixtures with the highest percent chlorination in general have the greatest effect, the choice of parameters measured, and the development of toxicity in the animal, may be such that the significant effects are observed with PCB of lower percent chlorination (i.e.) estrogenic activity; BITMAN and CECIL (1970) and body weight gain (ALLEN and ABRAHAMSON, 1973).

Reports are also appearing on the biochemical effects noted with the individual isomers comprising the PCB mixture. At least one investigation (CHEN et al. 1973) noted that the lack of response may indicate that the PCB effects may arise from interaction between the individual components of the mixture.

In view of the preceding work, it seemed that an indication of the effects of the high molecular weight isomers, within a mixture, might be revealed by a comparison of effects produced by Aroclor 1016 and 1242. 1016 is a relatively new Aroclor where the content of the penta-, hexa-, and hepta-chlorine

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isomers is reduced approximately 10-fold relative to amounts in 1242. The chlorination remains about 42% for both compounds.

Methods

Sprague Dawley rats, 190-210 g were obtained from BioBreeding, Ottawa, Canada. Aroclors 1016 or 1242 were given po (0.5 ml/100 g body weight) in corn oil at levels of 0, 0.01, 0.1, 1, 10 and 100 mg/kg. After 21 days the animals were sacrificed with the guillotine, the livers removed, weighed and packed in crushed ice. Within 30 minutes, the livers were minced and homogenized 2.5:1 with TRIS-HCl buffer, pH 7.4, 0.1 M. A 0.5 ml aliquot was removed for determination of total liver porphyrin by the method of ABBRITTI and DEMATTEIS (1972). The homogenates were centrifuged at 17,500 x g for 20 min and an aliquot of the supernatant used for the determination of aniline hydroxylase (SCHENKMAN et al. 1967) and aminopyrine demethylase (VAN PETTEN et al. 1968). The remaining supernatant was diluted 1:1 with 0.1 M phosphate buffer, pH 7.0 and centrifuged at 105,000 x g for 1 hr. The microsomal pellet was washed, resuspended in phosphate buffer and sedimented again. The washed microsomes were used for the determination of cytochrome P_{450} by the method of OMURA and SATO (1964). Protein was measured in the microsomal suspension previously clarified by the addition of 10% sodium desoxycholate, by the method of GORNALL et al (1948). Levels of statistical significance were determined by Students "t" test. There were six animals at each dose level.

Results

The effects of Aroclor 1016 on the male rat are listed in Table 1. This Aroclor produced a significant increase in body weight gain at 1 and 100 mg/kg. Liver, as a percent of body weight, aniline hydroxylase and aminopyrine demethylase were significantly elevated, beginning at 1 mg/kg. P_{450} levels were raised at a dose level of 10 mg/kg and above while liver porphyrins were raised at the highest dose level. Since there were no significant effects noted with either Aroclor at 0.01 or 0.1 mg/kg, these data have been deleted from the tables.

Table 2 lists the results obtained with Aroclor 1242 and male rats. In contrast to 1016, this mixture had no effect on body weight gain at the dose levels given, an effect also noted by BRUCKNER et al. (1973). With the exception of the demethylase activity, 1242 and 1016 produce significant effects at equivalent

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TABLE 1

Effect of Aroclor 1016 on male rats after daily oral dosing for 21 days

	DOSE 1016				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	74.0±4.6	90.7±2.8 **	83.1±4.1	90.4±4.4 **	122
LIVER % BODY WEIGHT	4.11±0.08	4.48±0.04 **	4.66±0.07 **	6.04±0.10 **	147
P_{450} nm/mg protein	0.92±0.04	0.90±0.05	1.43±0.11 **	1.76±0.17 **	181
ANILINE HYDROXYLASE µm/hr/g	2.09±0.10	2.45±0.13 **	4.50±0.35 **	5.60±0.28 **	269
AMINOPYRINE N-DEMETHYLASE µm/hr/g	1.04±0.11	1.44±0.10 **	2.39±0.19 **	2.56±0.20 **	246
LIVER PORPHYRINS µm/g liver	0.18±0.01	0.20±0.03	0.16±0.02	0.44±0.04 **	244

** P<0.05

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TABLE 2

Effect of Aroclor 1242 on male rats after daily oral dosing for 21 days

	DOSE 1242				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	74.7±5.2	67.0±8.7	7.50±3.4	74.0±6.6	100
LIVER % BODY WEIGHT	3.97±0.06	4.26±0.06 **	4.64±0.11 **	6.83±0.18 **	172
P_{450} nm/mg protein	0.92±0.04	10.30±0.05	1.29±0.05 **	1.77±0.09 **	192
ANILINE HYDROXYLASE µm/hr/g	1.81±0.07	2.11±0.11 **	3.81±0.17 **	5.75±0.39 **	318
AMINOPYRINE N-DEMETHYLASE µm/hr/g	1.71±0.21	2.11±0.16	3.37±0.28 **	3.85±0.21 **	225
LIVER PORPHYRINS µm/g liver	0.21±0.03	0.19±0.07	0.30±0.05	1.07±0.11 **	510

** P<0.05.

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TABLE 3

Effect of Aroclor 1016 on female rats after daily oral dosing for 21 days

	DOSE 1016				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	29.2±2.7	30.5±1.8	35.5±2.4	33.6±1.9	115
LIVER % BODY WEIGHT	3.98±0.23	4.05±0.08	4.45±0.08	5.73±0.14 **	144
P_{450} nm/mg protein	0.62±0.08	0.77±0.05	0.78±0.04	1.10±0.03 **	177
ANILINE HYDROXYLASE µm/hr/g	1.10±0.06	1.13±0.09	1.23±0.13	2.48±0.19 **	226
AMINOPYRINE N-DEMETHYLASE µm/hr/g	0.40±0.02	0.44±0.02	0.38±0.04	0.70±0.05 **	175
LIVER PORPHYRINS µm/g liver	0.21±0.02	0.14±0.01	0.24±0.05	1.14±0.20 **	543

** P≤0.05

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TABLE 4

Effect of Aroclor 1242 on female rats after daily oral dosing for 21 days

	DOSE 1242				
	CONTROL	1 mg/kg	10 mg/kg	100 mg/kg	100 mg/kg (% control)
BODY WEIGHT GAIN (g)	28.7±2.3	28.0±3.7	35.3±3.7	18.3±5.5 **	64
LIVER % BODY WEIGHT	3.63±0.13	3.71±0.11	4.19±0.09 **	6.18±0.22 **	170
P_{450} nM/mg protein	0.85±0.05	0.82±0.03	1.14±0.04 **	1.50±0.05 **	177
ANILINE HYDROXYLASE µm/hr/g	1.01±0.02	1.08±0.03	1.84±0.10 **	3.01±0.23 **	298
AMINOPYRINE N-DEMETHYLASE µm/hr/g	0.45±0.01	0.48±0.05	0.81±0.02 **	1.12±0.12 **	249
LIVER PORPHYRINS µm/g liver	0.20±0.04	0.23±0.02	0.44±0.06 **	1.76±0.30 **	871

** $P \leq 0.05$

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dose levels with the remaining parameters.

The effect of Aroclors 1016 and 1242 on female rats is listed in Tables 3 and 4 respectively. With 1016, there was no effect on weight gain and the other parameters were increased only at the 100 mg/kg dose level. With 1242 all parameters, except body weight gain, were increased at 10 mg/kg and above while the weight gain was reduced at the highest dose level.

Discussion

The approximate 10-fold reduction in penta-, hexa-, and hepta-chlorine containing isomers in Aroclor 1016 as compared to 1242 did not appear to have a great effect on changes in biochemical parameters of male rats as determined under our experimental design. The major difference occurred with body weight gain where 1016 produced a weight increase while 1242 had no effect. 1016 also appeared to enhance N-demethylase activity at a dose level of 1 mg/kg while 1242 produced significant effects at 10 mg/kg. Although not indicated in Table 2, 1242 does produce a significant increase at 1 mg/kg for N-demethylase, but at the 90% confidence level, rather than the 95% level chosen here for all parameters. While both Aroclors increased liver porphyrin levels, the percent increase over control levels was more than 2-fold greater with the highest dose of 1242 compared to an equivalent dose of 1016.

With female rats, changes in the parameters occurred at higher dose levels than in males, however 1242 consistently elicited effects at a dose level 10-fold lower than 1016. This distinction was not evident with the males. The females also seemed more susceptible to effects on body weight since 1016 produced no change in weight gain and 1242 actually caused a sharp decrease in weight gain, at the 100 mg/kg level. Finally, porphyrin levels were higher in female than male rats and increased to greater levels than in males under treatment with either Aroclor. Again 1242 was more effective on a percent basis, raising levels 871%, compared to 543% for 1016.

CHEN and DUBOIS (1973) indicated that maximal induction of enzyme activity occurred after 3 weeks administration of PCB's. This would suggest that the levels of P_{450}, aniline hydroxylase and N-demethylase, in the present study, are near their maximal values. However, this may not be the case for the porphyrin levels, since GRANT et al. (1974) have shown with hexachlorobenzene that an increase in liver porphyrin levels is sex dependent and that porphyrin levels in females may increase more than 1000-fold over control

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values. While an enhanced porphyrogenic effect of 1242 compared to 1016 is evident in the present study, it seems probable that continued dosing would have significantly increased female porphyrin levels especially with 1242. Previous work by VOS (1972), and VOS and KOEMAN (1970) would also suggest that the porphyria is associated with the isomers of higher chlorine content.

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