Modification of Pentobarbital Sleeping Times in Rats Following Chronic PCB Ingestion

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Polychlorinated biphenyl (PCB) is a term applied to a group of chemicals used in the manufacture of paints, resins, lacquers and many other products. Recent evidence of their widespread distribution in the ecosystem (1, 2) has stimulated interest into their biochemical and toxicological properties. Although there is a considerable amount of data on the effect of PCB administration following acute or short term administration (1) no reports to our knowledge have been made of long term PCB ingestion on microsomal enzyme activity. The present paper reports on the modification of sleeping times in male and female rats following the ingestion of 4 PCB's and 2 organochlorine insecticides.

Materials and Methods

Male and female Wistar rats (30-40 g) were fed diets of ground fox cubes (Master Feeds, Toronto) containing an additional 4% corn oil with or without organohalogens. The compounds used in this study were the Aroclors(R) 1221, 1242, 1254, 1260 (Monsanto Co., St. Louis) p,p-methoxychlor (E.I. Dupont de Nemours and Co., Wilmington) and α-chlordane (Velsicol Chemical Corp., Chicago). Control groups received ground cubes containing only the 4% additional corn oil.

Sodium pentobarbital ("Somnipentyl", Pitman-Moore Co., Don Mills, Ontario) was injected intraperitoneally at a dose of 25 mg/kg for female rats and 40 mg/kg for male rats. Sleeping time was defined as the interval between injection of the barbiturate and restoration of the righting reflex and was determined on 5 animals from each group after 30, 78, 240 and 350 days from initiation of the feeding regime. Tissues from 3 male rats (350 days on test) per group were analyzed by gas liquid chromatography for residues of the Aroclors, oxychlordane and methoxychlor as described previously (Grant et al., 1971).

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Results

The effects of a 20 ppm dietary regime of one of 4 PCB's and 2 organochlorine insecticides on pentobarbital sleeping times are shown in Table 1.

TABLE 1

Pentobarbital Sleeping Times in Rats Fed Various Aroclors and Organochlorine Insecticides at 20 ppm in the Diet

Days on Test[1]	Compound	Sleeping Time[2] (Minutes) ± S.E.M. 0	0
30	---	53.6 ± 3.8	85.2 ± 7.1
	Aroclor 1221	53.8 ± 1.7	76.0 ± 3.2
	Aroclor 1242	34.2*± 1.9	64.5 ±21.4
	Aroclor 1254	17.0*± 2.4	31.2*± 6.3
	Aroclor 1260	22.0*± 1.6	DID NOT SLEEP
	Methoxychlor	55.2 ± 0.7	73.8 ± 8.0
	Chlordane	32.4*± 4.1	51.2*± 3.4
78	---	80.4 ± 7.8	81.6 ±12.2
	Aroclor 1221	69.4 ± 9.0	85.4 ± 4.8
	Aroclor 1242	46.6*± 2.9	67.6 ±18.8
	Aroclor 1254	26.4*± 3.8	47.0*± 4.9
	Aroclor 1260	25.2*± 3.3	35.6*± 5.9
	Methoxychlor	69.6*± 4.1	72.6 ± 2.5
	Chlordane	50.2*± 2.9	47.0 ± 5.7
240	---	116 ± 2.9	---
	Aroclor 1221	74.8*± 9.2	---
	Aroclor 1242	67.2*± 4.6	---
	Aroclor 1254	55.8*± 9.2	---
	Aroclor 1260	25.4*± 1.8	---
	Methoxychlor	119.2 ± 7.2	---
	Chlordane	70.6*± 7.3	---
350	---	---	115.6 ± 9.1
	Aroclor 1221	---	111.3 ±13.6
	Aroclor 1242	---	97.4 ±13.2
	Aroclor 1254	---	81.7*± 9.4
	Aroclor 1260	---	37.5*± 3.5
	Methoxychlor	---	100.9 ±16.5
	Chlordane	---	63.3*± 5.2

[1] Animals started on their diets at 21 days of age.

[2] Pentobarbital sleeping times determined using levels of 40 and 25 mg/kg for 0 and 0 respectively. Figures represent the mean value of 5 animals.

* Indicates significant difference from controls (P = 0.05).

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After 30 days on test only two compounds Aroclor 1221 and methoxychlor did not reduce pentobarbital sleeping times in male rats. In female rats only Aroclor 1254, Aroclor 1260 and chlordane decreased sleeping times. After 78 days on test, Aroclors 1242, 1254 and 1260, methoxychlor and chlordane significantly reduced sleeping times in males, but only Aroclor 1254, 1260 and chlordane had this effect in females. After 240 days the only compound that did not reduce sleeping time in male rats was methoxychlor. Sleeping times were not determined at 240 days in females. After 350 days on test only Aroclor 1254, 1260 and chlordane reduced pentobarbital sleeping times in female rats. No sleeping times were determined on male rats at this time.

The residues of the various compounds in different tissues of male rats (350 days) are shown in Table 2. As would be expected the fat contained much higher residues of all compounds (except methoxychlor) than liver, heart or brain. The degree of storage tended to increase with the chlorine content of the compound. No significant methoxychlor residues were found in any of the tissues examined.

Discussion

Considerable information is available on the ability of PCB's to induce microsomal enzymes and has recently been reviewed by Peakall and Lincer (1) as well as by Gustafson (2). The present study reports on the effects of PCB and organochlorine insecticide ingestion on an indirect parameter of pentobarbital metabolism i.e., pentobarbital sleeping time. Of six compounds only Aroclor 1254, Aroclor 1260 and chlordane consistently reduced sleeping times in both males and females at all time intervals. Methoxychlor and Aroclors 1221 and 1242 showed no effect in female rats at any time interval while Aroclor 1242 reduced sleeping times in male rats. The reason why methoxychlor and Aroclor 1221 reduced sleeping times at only one time period is unclear and perhaps fortuitous.

The residue data are in general consistent with the results published by other workers. Grant et al. (3) reported that following oral administration of Aroclor 1254 residues were found in all tissues

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TABLE 2

Residues in Tissues of Rats Fed Various Aroclors and Organochlorine Insecticides at 20 ppm in their Diet

Compound	Fat	Liver	Heart	Brain
Aroclor 1221	4.89[a] ± 0.28[b]	0.06 ± 0.00	<0.2	<0.2
1242	11.38 ± 0.80	0.56 ± 0.00	<0.2	<0.2
1254	121.99 ± 18.43	5.40 ± 0.59	1.98[c]	1.69 ± 0.37
1260	94.25 ± 24.95	6.45 ± 0.53	2.30 ± 0.18	1.14 ± 0.13
Chlordane[d]	20.40 ± 4.49	0.72 ± 0.08	0.20 ± 0.03	0.12 ± 0.00
Methoxychlor	Nil	Nil	Nil	Nil

[a] Average of 3 values (ppm).

[b] Standard error of mean.

[c] Average of 2 values.

[d] Residue calculated as oxychlordane.

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analyzed (blood, brain, liver, heart, spleen, kidneys, testes and omental fat) with the highest residues in the fat. Kunze et al. (4) found that methoxychlor fed to rats at 25 ppm in the diet was not stored in their adipose tissue. Schwemmer et al. (5) have recently identified oxychlordane as a metabolite of α and γ chlordane and have shown that it is stored in adipose tissue. Polen et al. (6) have shown that male and female rats fed a dietary regime of 15 ppm α-chlordane for 1 year contained between 7-13 ppm oxychlordane in their fat. Our residue data are in agreement with their results considering that 20 ppm α-chlordane was fed. In the present study there seemed to be a direct relationship between the storage of the compound in adipose tissue and the compound's ability to reduce sleeping times. The amount of residues in the tissues and the reduction of sleeping times are summarized as follows:

Aroclor 1260 > Aroclor 1254 > Chlordane >

Aroclor 1242 > Aroclor 1221 > Methoxychlor

As previously reported for other enzymes the activity of barbiturate metabolizing enzymes decreases with age. In the present study the age of the male rats did not influence the degree of enzyme induction by a particular compound. With female rats chlordane had the same quantitative effect on sleeping times at 30 and 350 days, but Aroclor 1254 decreased sleeping times 69.3% at 30 days and only 29.3% at 350 days. This type of sleeping time adaptation had been observed in quail fed a dietary regime of p,p'-DDT and other DDT metabolites (8).

The most likely explanation for the modified sleeping times observed in this study is enzyme induction. However one must also be aware that these compounds could elicit changes in central nervous system activity or in the pharmacokinetic parameters in the central nervous system (9).

In summary, data have been presented to show the effects of long term ingestion of 4 PCB's and 2 organochlorine insecticides on pentobarbital sleeping times in rats. Tissues from male rats were analyzed for residues of these compounds after 350 days on test.

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