EFFECTS OF POLYCHLORINATED BIPHENYLS AND TERPHENYLS AND POLYBROMINATED BIPHENYLS ON PENTOBARBITAL SLEEPING TIMES OF JAPANESE QUAIL

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Polychlorinated biphenyls (Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268), polychlorinated terphenyls (Aroclor 5460), a mixture of polychlorinated biphenyls and terphenyls (Aroclor 4465) and polybrominated biphenyls (BP-6) were administered in a single dose, 100 mg/kg body weight injected orally, to mature male and female Japanese quail. Two hr after Aroclor treatment, pentobarbital sleeping times were prolonged (2 to 3 X control) in both male and female quail. Twenty-four hr after treatment, sleeping times were similar to those of controls. However, 48 hr after treatment with Aroclor 1248 through Aroclor 5460 and with BP-6, sleeping times of males were only 1/2 those of controls, whereas after treatment with Aroclor 1221, 1232 and 1242, sleeping times were similar to those of controls. The reduction in sleeping times of male quail 48 hr after administration of PCBs was correlated with the percentage of chlorine in the PCB with greater reductions in male than female quail. The response after three days *ad libitum* feeding of 300 ppm of each substance was the same as the response 48 hr after a single oral injection. Although very few birds died from the toxicity of the Aroclors and BP-6, mortality was greatly increased during anesthesia when the pentobarbital was administered two hr after single oral dosing.

Polychlorinated biphenyls (PCBs) are industrial chemicals that have been widely distributed in our environment. Recently, a mixture of polybrominated biphenyls was introduced for use as a flame retardant in automobile seat covers and could be a potential environmental pollutant. Like DDT, PCBs degrade very slowly and accumulate in the lipid of animal bodies. Much of the research on PCBs indicates similarities in the effects of PCBs and DDT.

Both DDT and PCBs have been shown to induce microsomal enzyme activity in birds (Lincer and Peakall 1970, Nowicki and Norman 1972), rabbit (Villeneuve *et al.* 1971), or rat (Hart and Fouts 1963, Street *et al.* 1969, Bickers *et al.* 1972, Litterst and Van Loon 1972, Litterst *et al.* 1972, and Norback and Allen 1972). Rats fed DDT or PCBs also had reduced pentobarbital sleeping times (Hart and Fouts 1965, Street *et al.* 1969, Bitman *et al.* 1971, Bickers *et al.* 1972, Villeneuve *et al.* 1972) indicating increased metabolism of pentobarbital by the induced liver microsomal enzymes. In quail, we found the opposite effect, DDT and methoxychlor lengthened pentobarbital sleeping times (Bitman *et al.* 1971, Cecil *et al.* 1973).

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In a preliminary study (Bitman *et al.* 1972), we found that the sleeping time pattern elicited by PCBs (Aroclor 1268) or by polychlorinated terpenyls (Aroclor 5460) was in marked contrast to the pattern elicited by the organochlorine pesticide analogs of DDT in quail (Bitman *et al.* 1971, Cecil *et al.* 1973). The present study investigates several PCBs, a mixture of polychlorinated terphenyls (PCT), a mixture of PCBs and PCTs, a mixture of polybrominated biphenyls (PBB) and correlates their effects on liver metabolism of Japanese quail as measured indirectly by pentobarbital anesthesia.

Materials and methods

Mature male and female quail (40-80 days old, 100-125g) were housed individually on a schedule of 14 hr of light and 10 hr of dark. Each test substance was administered as a single feeding in olive oil by stomach tube or by *ad libitum* feeding as a 300 ppm diet for three days. Eight PCBs, a mixture of PCBs and PCTs, a mixture of PCTs, and a mixture of PBBs were used. The PCBs were Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268; the mixture of PCTs was Aroclor 5460; and the mixture of PCBs and PCTs was Aroclor 4465. The last two digits of the Aroclors (Monsanto Company) represent the percentage of chlorine in the formulation. The PBB mixture used was BP-6 (Michigan Chemical Company) containing 75% bromine.[1]

At appropriate times after administration of the test substance, sodium pentobarbital was injected intramuscularly at a dosage rate of 50 mg/kg of the male body weight or 60 mg/kg of the corrected female body weight. Body weights of the females were corrected for the weight of the reproductive tract by subtracting ten g and, if an egg were also present, subtracting an additional ten g. Birds were considered asleep as long as they could not right themselves when placed on their back. Control birds slept for 40 to 60 min. Duration of sleeping time for treated birds is expressed as percentage of control. Statistical comparisons were made with the Student's "t" test, with correction for unequal group size (Snedecor 1956).

Results

Single oral injection of PCBs. Two hr after PCB administration, pentobarbital sleeping times of quail were two to three times longer than sleeping times of control birds (Figure 1, $p < 0.001$), and five hr after PCB administration sleeping times were 1.5 times longer than those of controls ($p < 0.001$). However, the effects of a single dose of PCB were short-lived and when pentobarbital was given 24 hr after PCB, the PCB-treated birds had sleeping times similar to those of controls. PCB had no further effect in female quail; but in males the trend toward shorter sleeping times continued, and at 48 hr some male groups had significantly shorter sleeping times than those of controls. Closer examination of the 48-hr data revealed that the shorter sleeping times of male quail were related to the percentage chlorine in PCBs (Figure 2). The PCBs containing only 21% and 32%

[1]Trade names were used solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the Federal Government or an endorsement by the Government over other products not mentioned.

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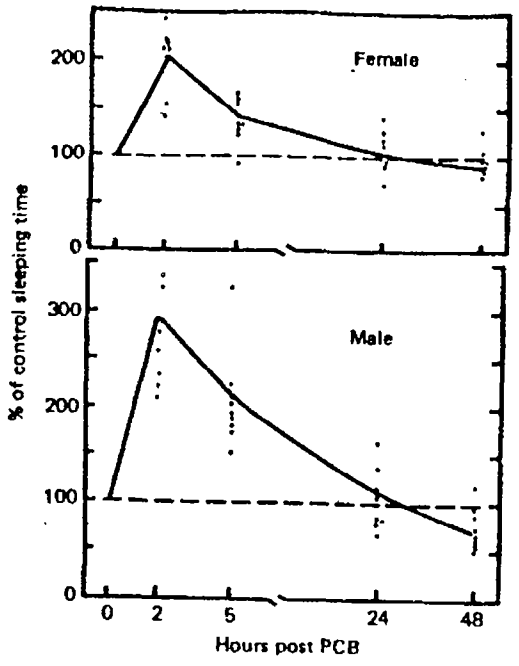


Fig. 1. Pentobarbital sleeping times of Japanese quail after a single oral injection of polychlorinated biphenyls (Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, or 1268). The administered dosage was 100 mg test substance per kg body weight. Each point represents the mean for 4 to 6 animals for each point.

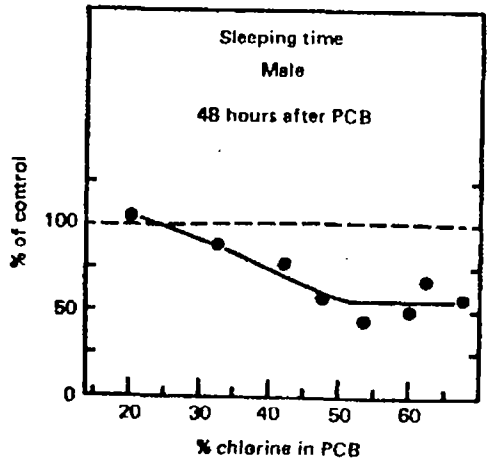


Fig. 2. Pentobarbital sleeping times of male Japanese quail 48 hr after a single oral injection of polychlorinated biphenyls containing 21% to 68% chlorine. The administered dosage was 100 mg test substance per kg body weight. Each point represents the mean for 5 or 6 animals.

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chlorine did not reduce pentobarbital sleeping time; however, as the chlorination increased, sleeping times decreased. With 54% chlorine, the reduction in sleeping time reached a plateau, and male quail treated with the PCBs containing 54, 60, 62 and 68% chlorine had sleeping times that were only half as long as those of controls ($p < 0.001$).

Comparison of polychlorinated biphenyls and terphenyls and a polybrominated biphenyl mixture (Figures 3 and 4). A mixture of PCBs and PCTs containing 65% chlorine had the same effect as PCBs of comparable chlorination, with sleeping times twice that of controls two hr after treatment ($p < 0.001$), while the PCT mixture (60% chlorine) and the PBB mixture (75% bromine) only slightly lengthened sleeping times two hr after treatment (males, PCT, $p < 0.001$; PBB, $p < 0.005$; females, PCT, p = n.s.; PBB, $p < 0.001$). However, at later times the PCT and PBB had more effect than the PCBs. Twenty-four hr after PCT or PBB treatment, sleeping times of treated male quail were half as long as those of control males (Figure 3, $p < 0.001$) and at 48 hr after treatment, all the higher halogenated compounds reduced sleeping times in male quail ($p < 0.001$). In female quail, the polybrominated compound (PBB) was the only compound which reduced sleeping times (Figure 4, $p < 0.001$).

Ad libitum feeding. The effects 48 hr after a single oral injection were compared with those of *ad libitum* feeding for three days (Figure 5). The single oral injection was 100 mg/kg of body weight, which is comparable to the daily intake of birds eating feed

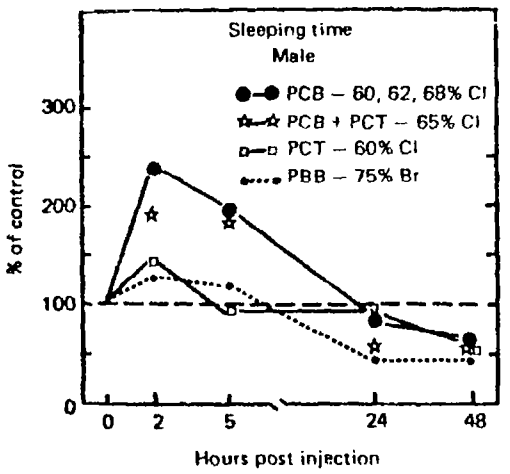


Fig. 3. Pentobarbital sleeping times of male Japanese quail after a single oral injection of higher chlorinated polychlorinated biphenyls (Aroclor 1260, 1262 or 1268), a mixture of polychlorinated biphenyls and terphenyls (Aroclor 4465), a polychlorinated terphenyl (Aroclor 5460) and a polybrominated biphenyl (BP-6). The administered dosage was 100 mg test substance per kg body weight. Each point represents the mean for 4 to 6 animals.

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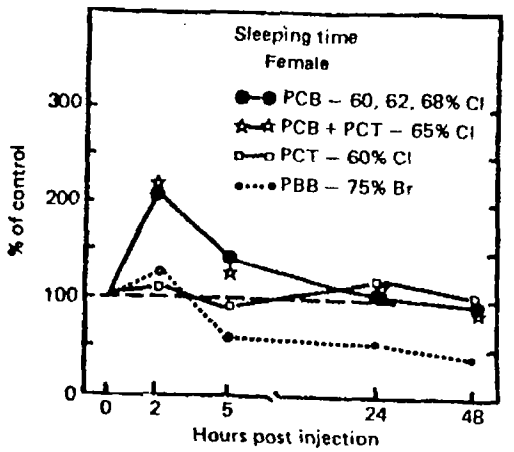


Fig. 4. Pentobarbital sleeping times of female Japanese quail after a single oral injection of higher chlorinated polychlorinated biphenyls (Aroclor 1260, 1262 or 1268), a mixture of polychlorinated biphenyls and terphenyls (Aroclor 4465), a polychlorinated terphenyl (Aroclor 5460) and a polybrominated biphenyl (BP-6). The administered dosage was 100 mg test substance per kg body weight. Each point represents the mean for 4 to 6 animals.

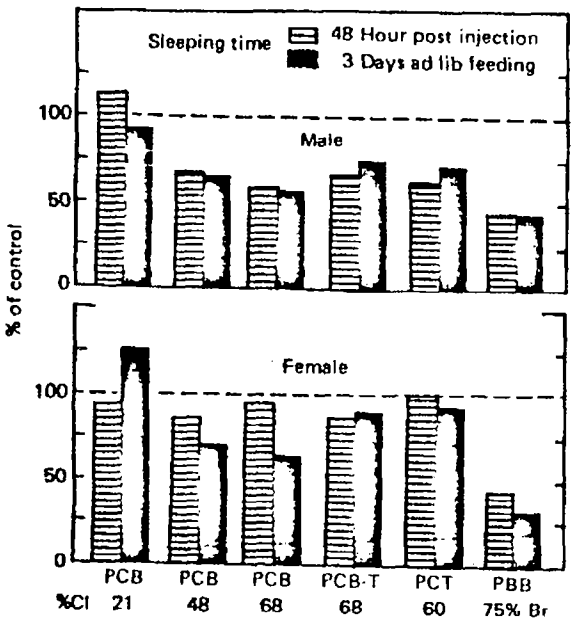


Fig. 5. Pentobarbital sleeping times of Japanese quail 48 hr after a single oral injection or 3 days *ad libitum* feeding of polychlorinated biphenyls, a mixture of polychlorinated biphenyls and terphenyls, polychlorinated terphenyls and a polybrominated biphenyl. The administered dosage for the oral injection was 100 mg test substance per kg body weight; and the dosage for feeding *ad libitum* was 300 ppm of the test substance in the diet. Each bar represents the mean of 4 to 6 animals.

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containing 300 ppm. The response after a single oral injection was similar to the response after three-day *ad libitum* feeding for each substance. The PCB containing 21% chlorine did not affect sleeping times, whereas higher chlorinated or brominated compounds reduced sleeping times of male quail. In female quail, the mode of administration of the test substance made very little difference in the response to pentobarbital. The only exception was the PCB containing 68% chlorine, which reduced sleeping times in females more after *ad libitum* feeding than after a single oral dose.

Mortality. Although very few birds died from the toxicity of the PCBs alone, a significant number of birds died when pentobarbital was administered (Table 1). Mortality during anesthesia was greatest two hr after a single oral injection of PCB. This is also the time when sleeping times were increased two to three times those of controls. The greatest mortality during anesthesia was in birds who were fed the PCBs containing 32% to 48% chlorine by stomach tube. PCBs containing 21% chlorine and 58% chlorine had little effect on mortality of male quail during anesthesia. Female quail were more susceptible to these compounds, and 20% to 36% of the treated females died during anesthesia.

Table I. *Mortality of male and female Japanese quail during pentobarbital anesthesia after a single oral feeding of polychlorinated biphenyls, polychlorinated terphenyls or polybrominated biphenyl (100 mg per kg body weight)*[a]

Test substance[b]	Mortality (%) after feeding test substance for							
	2 hr		5 hr		24 hr		48 hr	
	M	F	M	F	M	F	M	F
PCB 1221	0	29	0	8	0	20	0	0
PCB 1232	45	55	17	0	20	0	0	17
PCB 1242	25	58	0	0	0	20	0	0
PCB 1248	83	41	20	8	0	0	0	17
PCB 1254	0	33	60	33	0	0	0	0
PCB 1260	17	33	17	0	0	0	0	0
PCB 1262	0	36	0	0	0	0	0	0
PCB 1268	17	20	0	33	0	33	0	0
PCB-PCT 4465	33	0	10	0	0	0	0	0
PCT 5460	0	18	17	17	8	9	10	17
PBB BP-6	0	0	50	17	0	18	0	9

[a] Control mortality = 4% for males, 8% for females.
[b] 2 and 5 hr PCB treatment: Males, n=6-14; females, n=10-17.
24 and 48 hr PCB treatment: Males n=5-6; females, n=5-6.
2, 5, 24 and 48 hr PCT and PCB treatment: Males, n=11-12; females, n=11-12.

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Because administration of the PCBs containing 32% to 48% chlorine at a dosage of 100 mg/kg of body weight greatly increased mortality during anesthesia, these compounds were administered at lower levels (Table II). When the dose was reduced to 50 mg/kg of body weight, the number of deaths during anesthesia was greatly reduced; however, sleeping times of the treated quail were still two to three times those of controls. At 25 mg/kg of body weight, mortality was reduced, and sleeping times approached those of controls.

Neither *ad libitum* feeding of 300 ppm of PCBs for three days increased mortality, nor did *ad libitum* feeding of PCT or PBB.

Table II. *Duration of pentobarbital anesthesia and mortality during anesthesia of male and female Japanese quail 2 hours after a single oral feeding of polychlorinated biphenyls containing 32 to 54% chlorine*

Test substance	Sleeping time % control		Mortality %	
	M	F	M	F
Aroclor 1232				
100 mg/kg BW	256	213	45	55
50	337	183	0	33
25	153	114	20	0
Aroclor 1242				
100 mg/kg BW	330	340	36	58
50	320	107	27	0
25	184	169	27	20
Aroclor 1248				
100 mg/kg BW	218	222	83	41
50	205	217	33	0
25	159	123	20	0
Aroclor 1254				
100 mg/kg BW	330	224	0	33
50	295	214	33	40
25	147	104	25	0

Discussion

The biphasic sleeping times induced by chlorinated hydrocarbon industrial pollutants in quail are extremely interesting. Initially, they appear to exert a depressing effect upon liver metabolizing enzymes and respiratory systems. At later times after administration

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they induce the well-known stimulatory effects upon liver enzymes which have been characterized as microsomal enzyme induction. The initial increases in sleeping times two hr after PCB treatment indicate suppression of pentobarbital metabolism by the liver. Respiratory and central nervous system depression undoubtedly occurred as evidenced by the increased mortality during anesthesia. Unfortunately, we could not establish a dose-response relationship. Two hr after a dosage of 100 mg/kg of body weight of Aroclors containing 32% to 48% Cl, sleeping times lengthened and mortality was greatly increased during anesthesia, whereas 25 and 50 mg/kg had much less effect. A graded response may exist between 50 and 100 mg.

The initial increases in sleeping times two hr after PCB treatment were similar to the increases after a single oral injection of DDT (Cecil *et al.* 1973). Three PCBs, Aroclors, 1232, 1242, and 1248, increased mortality during anesthesia to a greater extent than DDT or other Aroclors with lower or higher chlorination. We also found these three Aroclors to be more embryotoxic when fed to White Leghorn chickens (Cecil *et al.* 1974). Although all of the PCBs studied increased sleeping times two hr after a single dose, PCT and PBB did not. The lack of action by the PCT and PBB two hr after a single dose could be due to their larger molecule size. As larger molecules there may be less absorption from the gastrointestinal tract. Also, penetration into the liver or brain may not have occurred as readily as penetration by the smaller PCBs.

Although the action of the PCBs resembled that of DDT two hr after a single dose, DDT and PCB did not have comparable effects 48 hr after a single dose or after *ad libitum* feeding. In this study, we found that the Aroclors had no effect on sleeping times of females after 48 hr, whereas, males fed the higher chlorinated Aroclors had reduced sleeping times. The PBB had an even more marked effect and reduced sleeping times in both males and females. These reductions in sleeping times contrast with the lengthened sleeping times elicited by DDT feeding (Bitman *et al.* 1971). Here, too, we find the action of the PCBs, PCT and PBB appearing to be dependent on the degree of halogenation and consequently, molecule size. It appears that the larger or heavier molecule, either the brominated biphenyl, which is twice as heavy as a comparable chlorinated biphenyl, or a terphenyl, which has three phenyl rings, exerts less immediate effect on sleeping time but has a greater effect at later times (48-hr or three-day continuous feeding).

This observed reduction in sleeping time elicited by the polyhalogenated polyphenyls is probably the result of induction of liver microsomal enzyme systems capable of metabolizing pentobarbital. Street (1969) reported that the higher chlorinated PCBs fed to rats for 15 days reduced sleeping times and increased liver microsomal activity. Villeneuve *et al.* (1972) also found that only the higher chlorinated Aroclors reduced sleeping times in rats. Litterst *et al.* (1972) studied the effect of the chlorine content of PCBs on the production of hydroxylated metabolites of pentobarbital by rat liver. He found that hydroxylation was greater after Aroclor 1254 and 1260 treatment than after Aroclor 1242 or 1248 treatment.

The commercial Aroclors and BP-6 used in this study are complex mixtures of many chlorinated biphenyls. Chen *et al.* (1973) studied the effects of two isomeric tetra-

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chlorobiphenyls (2, 3, 4, 5, and 3, 5, 3′, 5′-tetrachlorobiphenyl) and compared their results with those of Vos (1972) who used a hexachlorobiphenyl. As a result of their study, Chen and coworkers concluded that "the effects of individual isomeric PCBs on rats is quite different from commercial PCBs. Thus, the total effect of Aroclors is probably the result of interactions between each component in them and is not merely the cumulative effect of each individual component." Our report suggests that the action of PCB on the liver metabolism of birds is similar to the effect of PCBs in rats. However, the response of Japanese quail to a short exposure of PCBs is quite different from the response after a short exposure to DDT.

The differential effects of the PCBs, which are probably related to the extent of metabolism and permeability (relative ease or difficulty in crossing biological membranes), appear to be correlated with their differing degrees of chlorination. In experiments in which PCBs and PBB were administered to White Leghorn chickens, Fries *et al.* (1973a, b) found that the lower chlorinated isomers present in the PCB mixtures were readily metabolized and the residues in fat contained only the chlorinated PCBs of longer GLC retention times. These residue studies also indicated that the more highly chlorinated PCBs or the heavier PBB were resistant to transfer across the biological membrane (Fries *et al.* 1973a, b).

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