Bull. Environ. Contam. Toxicol. (1992) 48:101–107

Environmental Contamination and Toxicology

Polychlorinated Biphenyl (PCB) Congener Effects on the Longevity of the Housefly

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Each of the 209 polychlorinated biphenyl congeners has individual properties including differing quantitative and qualitative toxicities and susceptibilities to biotransformation (Hansen 1987). It is important to determine the persistence, biotransformation and biological activity for a large number of congeners in order to more accurately predict the hazards from various PCB residues in the environment and food chains.

The house fly, Musca domestica, has long been used as a standard screening organism for insecticides and may offer a good bioassay organism for PCB congener toxicity comparisons. Life-long experiments are readily conducted (Lu et al. 1978), thus increasing the sensitivity of toxicity tests.

Cytochromes P-450 are pivotal in the toxicity as well as biotransformation of PCBs (Hansen 1987; Parkinson and Safe 1987; Sipes and Schnellmann 1987). The intensity of species-specific effects associated with binding to the Ah receptor can be predicted from agonist potencies as inducers of specific monooxygenases relative to 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) (Safe 1987). The severity of these relatively acute effects frequently precludes detection of more subtle chronic effects associated with exposures to, e.g., PCB residues with lower "dioxin equivalencies" (Hansen 1987).

The microsomal monooxygenase system in the housefly is well-characterized, active, inducible and inhibited by the same types of compounds as in vertebrate systems (Hodgson 1985; Plapp 1972; Rhee and Plapp 1973). Flies metabolize PCBs to polar products (Saghir and Hansen 1991) and retain congeners from a commercial mixture (Storr-Hansen et al. 1989) in proportions consistent with PCB biotransformation and elimination in vertebrates. Although TCDD has been shown to have a high affinity for, and be an effective agonist of the juvenile hormone receptor in insects (Muehleisen et al. 1989), toxic manifestations in adult insects may differ from those in certain highly sensitive vertebrate species. This study was undertaken to determine if the housefly bioassay could effectively compare the net adverse biological activity of a group of PCB congeners independent of Ah receptor effects.

MATERIALS AND METHODS

PCB congeners are identified by IUPAC numbers (Erickson 1986) and struc-

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tures can be determined from summary Table 4. Congeners were purchased greater than 99% pure from Ultra Scientific (Hope, RI) except that PCB 52 was synthesized and purified in this laboratory and PCB 84 was purchased from Cambridge Isotopes (Woburn, MA). PCBs 28, 52, 95 and 136 were further purified by eluting with hexane from a column containing alumina and 1.5% activated charcoal; this procedure would remove contaminating dibenzofurans, dibenzodioxins and coplanar PCBs (Erickson, 1986). Aroclor 1254 was a gift from Monsanto Company.

House flies derived from the NAIDM pesticide susceptible strain were reared in the Department of Entomology and pupae recovered for emergence in 0.3 x 0.3 x 0.3 m wire screen cages. During the first 2 days following emergence, they were offered sugar cubes, distilled water and 10% powdered milk solution ad libitim; milk was withdrawn after 2 days to reduce egg laying. At 3 days of age, flies were anesthetized with carbon dioxide and groups of 25 females were batch weighed and separated for dosing.

PCBs were applied to the dorsal thorax of CO_2 anesthetized flies in 1 μL acetone. The flies were placed in 473 mL (1 pint) cardboard containers fitted with wire screen lids and allowed to recover. Sugar solution (20%) was provided via a dental wick submerged in a 25 mL flask. Flies were re-anesthetized and redosed at 5 days of age. The flies were observed daily and dead flies removed and recorded. The mortality versus time data were analyzed by life table methods.

In order to determine the relative persistence of some of the congeners, female flies topically dosed with 10 μg PCB were placed in groups of 3 in 20 mL vials covered with surgical gauze secured by a rubber band. Flies were fed via dental wicks saturated with 20% sugar solution and, at specified times, were anesthetized, washed with 5 mL acetone and homogenized three times in 8 mL acetone:hexane (1:1). The homogenates were filtered through anhydrous sodium sulfate, concentrated and introduced to a 7 mm i.d. column containing 3.0 g 2% deactivated alumina. The column was eluted with 30 mL hexane; 0.5 mL isooctane was added, the eluate concentrated to 0.2 mL and the volume adjusted to 1.0 mL with isooctane (Storr-Hansen et al. 1989). Samples were analyzed on a Hewlett-Packard 5790A GLC equipped with a Ni-63 ECD and a 50 m x 25 mm i.d. DB-5 capillary column. Temperature was programmed from 150 C to 175 C at 20^{o}/min and then to 275 C at 3^{o}/min to resolve PCBs 84 (retention time = 19.41 min) and 101 (retention time = 19.49 min). Recoveries by this method are 98% for PCB 28 and 96% for PCB 118. Values are not corrected for recovery.

RESULTS AND DISCUSSION

Protocols for life table analyses consisted of at least 2 replicate studies of 25 flies at each dose level compared to 25 controls. In some cases, two groups of flies were compared against the same group of controls (Table 1). These data illustrate a typical congener study as well as doses adjusted for fly weights. They are also of interest because the PCB 52 preparation contained toxic contaminants which were removed by activated charcoal purification.

Table 2 summarizes the life table analyses for all congeners tested by applying topical doses at 3 and 5 days of age . Congeners are ranked in order of decreasing toxicity based on dose and percent decrease in survival time and

Table 1. Effect of PCB 52 on the longevity of female houseflies.

Dose		Survival Time (mean days)		LT_{50} (days)		
µg/fly	mg/kg	Control	Exper.	Control	Exper.	
10.0	652	23.0	10.7	22	10	**[a]
10.0	697	23.0	10.0	22	7	**
10.0	675	23.0	10.3	22	8	**[b]
10.0	642	28.0	10.7	28	8	**
10.0	537	28.0	13.8	28	11	**
10.0	589	28.0	12.2	28	10	**[b]
10.0[c]	696	26.6	20.1	27	20	*
10.0[c]	648	26.6	19.3	27	18	0
10.0	672	26.6	19.7	27	19	*[b][c]

a) Significance: 0 = NS; * = $P < 0.05$; ** = $P < 0.01$.
b) Combination of 2 tests.
c) PCB further purified by charcoal.

LT_{50}. Aroclor 1254, a mixture of several congeners, was intermediate in toxicity. Among the lower chlorinated congeners, those with 2,4-chlorination on one ring (PCBs 28 and 49) were the most toxic; on the other hand, pentachlorobiphenyls with 3',4'-chlorination (PCBs 118, 126, 105 and 110) were clearly the most toxic. PCBs 84 and 95, with the labile 2,3,6- substitution on one ring, were the least toxic of the pentachlorobiphenyls, but PCB 136 with this same pattern on both rings was the most toxic of the hexachlorobiphenyls. The persistent PCB 153 was moderately toxic, while persistant PCBs 128 and 180 were essentially nontoxic.

PCBs 118 and 126, the best Ah receptor agonists, were the most toxic; however, PCB 126 is several orders of magnitude more potent towards the Ah receptor than is PCB 118 (Safe 1987) while they were equipotent in the housefly. Moreover, lower chlorinated PCBs 28 and 49 were only slightly less toxic to the fly than were PCBs 118 and 126 and were more toxic than PCB 105, which is similar in Ah receptor potency to PCB 118.

PCB 18, which is readily metabolized by mammals (Hansen 1987) and flies (Saghir and Hansen 1991), was not toxic at 10 µg/fly but was highly toxic at 15 µg/fly. PCB 31, another readily metabolized congener (Sipes and Schnellmann 1987), was also less toxic at the low dose and highly toxic at 15 µg/fly. PCB 18 reached the highest levels but was the most quickly eliminated of all 9 congeners tested for persistence (Table 3).

The remaining congeners tested can be generally ranked into four additional groups of relative persistence: 1) PCB 153 is slow to penetrate the cuticle (being removed in the acetone wash), but maintains relatively constant high levels; 2) PCBs 84, 95, 101 and 110 reach peak internal levels at about 24 hr and persist through 120 hr; 3) PCBs 28, 49 and 52 reach peak levels before 24 hr and are below 1 µg /fly by 48 hr; 4) PCB 18. Redosing 48 hr after the ini-

Table 2. PCB congener effects on survival times of houseflies.

IUPAC No.	Rank[a]	Decrease in Survival Parameters[b]					
		Dose 1 (μg/fly)	ST	LT_{50}	Dose 2 (μg/fly)	ST	LT_{50}
		Trichlorobiphenyls					
28P[c]	2	7	11	16	10	54	92**[d]
18	4	10	16	20	15	87	88**
31	5	10	15	12	15	80	92**
		Tetrachlorobiphenyls					
49	2	10	68	84**	-	-	-
52	2	10	56	64**	-	-	-
52P	4	10	26	30*	-	-	-
44	7	15	2	10	-	-	-
70	7	10	0	0	-	-	-
		Pentachlorobiphenyls					
118	1	4.3	26	26**	7.5	33	21**
126	1	7.5	30	21**	-	-	-
105	4	10	27	29**	-	-	-
110	4	10	28	17**	-	-	-
101	5	10	19	24**	-	-	-
95P	5	10	18	17*	-	-	-
95P	6	10	11	24*	15	6	8
84	6	10	13	8*	15	17	8**
		Hexachlorobiphenyls					
136P	3	10	42	52**	-	-	-
153	5	10	19	24**	15	19	36**
128	7	8.5	4	5	-	-	-
		Heptachlorobiphenyl					
180	7	10	5	6	-	-	-
		Aroclor Mixture					
A1254	5	10	14	13*	15	57	13**

a) Based on dose and decrease in mean survival time:
1 = dose < 10 μg/fly; MST decrease > 25%
2 = ST decrease > 50% at 10 μg/fly
3 = 40-50%;
4 = 25-39%;
5 = 14-24%;
6 = ambiguous or higher dose required;
7 = not significant at doses tested.

b) ST = Mean Survival Time in days;
LT_{50} = time to 50% mortality in days.

c) P = further purified by activated charcoal.

d) Significance: * P < .05, ** P < .01 (n = 25 or 50).

Table 3. PCB residues in 3-day female flies at various times (hr) following a topical dose of 10 μg.

PCB	μg/fly for n = 3				
	8	24	48	72	120
18	7.3	1.2	0.4	0.1	0.02
28	4.3	3.6	0.7	ns[a]	ns
52	3.8	3.4	0.8	0.3	0.03
49	4.2	2.4	0.7	0.4	0.05
95	1.6	3.2	1.1	1.0	0.4
84	1.8	2.0	2.4	1.6	1.2
101	2.4	2.4	2.3	1.2	ns
110	4.2	5.2	2.3	0.9	0.8
153	2.2	5.9	5.4	5.9	2.6

a) No reliable sample.

Table 4. Effect of chlorine substitution on the toxicity and persistence of PCB congeners.

IUPAC	Rank		Positions of Chlorination									
No.	T[a]	P[b]	2	3	4	5	6	2'	3'	4'	5'	6'
118	1	(2)[b]	x		x	x			x	x		
126	1	(2)		x	x	x			x	x		
28	2	5	x		x					x		
49	2	6	x		x			x			x	
136	3	(4)	x	x			x	x	x			x
18	4	7	x			x		x				
52	4	5	x			x		x			x	
105	4	(2)	x	x	x				x	x		
110	4	2	x	x			x		x	x		
31	5	(7)	x			x				x		
101	5	3	x		x	x		x			x	
153	5	1	x		x	x		x		x	x	
A1254	5	(3)										
84	6	3	x	x			x	x	x			
95	6	4	x	x			x	x			x	
44	7	(7)	x	x				x			x	
70	7	(5)	x			x			x	x		
128	7	(1)	x	x	x			x	x	x		
180	7	(1)	x	x	x	x		x		x	x	
			2	3	4	5	6	2'	3'	4'	5'	6'

a) T = Toxicity Rank from Table 2.
b) P = Persistence Rank from Table 3; parenthetic numbers are estimates based on the literature.

tial dose would result in relatively higher body residues of congeners in the first two groups.

It was expected that the 2,3,6-substituted congeners (PCBs 84, 95 and 110) would be less persistent in the housefly, but vertebrate species differ also in relative persistence. Domestic sheep rapidly clear PCB 110, for example, but swine generally retain significant residues while catfish and chickens retain higher proportions (Hansen 1987). PCB 110 is difficult to resolve from PCB 77 (Erickson 1986) and is generally not quantitated in environmental samples.

Ranking congener residues at 24, 48 and 72 hr and/or ranking based on 24 + 48 + 72 hr residues results in 7 clear ranks for comparison to the 7 toxicity ranks (Table 4).

Pentachlorobiphenyls appear to be less toxic as their relative susceptibility to biotransformation increases while the opposite is true for higher chlorinated congeners. For lower chlorinated congeners which are readily metabolized, there is a dramatic increase in toxicity between 10 and 15 μg/fly which may represent a saturation of the detoxication enzymes and/or an accumulation of toxic metabolites.

Although some coplanar and mono-ortho congeners are quite toxic to the housefly, the relationships do not correlate with relative potency as Ah receptor agonists. The juvenile hormone receptor in insects will bind Ah receptor agonists (Muehleisen et al. 1989), but it does not appear to be associated with the same constellation of effects as seen in mammals and birds; thus, the housefly may be very useful in detecting adverse biological activities not associated with the Ah receptor.

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Received September 21, 1991; accepted October 30, 1991.