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Accumulation and Depletion of Polychlorinated Biphenyl (PCB) Congeners in the Housefly (*Musca domestica*, L.)

Larry G. Hansen[1] and Eva Storr-Hansen[2]

[1]University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln Ave, Urbana, Illinois 61801, USA and [2]Ministry of the Environment, National Environmental Research Institute, Department of Environmental Chemistry, Mørkhøj Bygade 26 H, DK-2860 Søborg, Denmark

Polychlorinated biphenyl (PCB) residues in food chains and in the environment are dynamic complex mixtures (Hansen 1987) and there is considerable interest in the disposition as well as toxicities of individual congeners (USEPA 1988). The persistance and biological activities of PCBs are intimately associated with microsomal monooxygenases (Safe 1987; Sipes and Schnellmann 1987). The house fly, Musca domestica (L.), has long been a standard organism for studies of pesticide toxicology and has an active and well-defined microsomal monooxygenase system. These oxidases in the fly can be induced by PCBs (Rhee and Plapp 1973); they can also be inhibited by PCBs, resulting in a pesticide synergism equivalent to piperonyl butoxide (Fuhremann and Lichtenstein 1972; Plapp 1973). It has recently been shown that ^{14}C-PCB 18 (2,2'5-trichlorobiphenyl) is metabolized to more polar products and excreted by house flies (Saghir and Hansen, 1991) and that different PCB congeners vary in their potency for reducing the life span of house flies (Tehseen et al. 1991).

It was considered of interest to provide data which may indicate whether or not the relatively large and mobile biomass of insects in general may play a role in the global disposition of PCBs. It was also of interest to determine if the house fly could serve as a model for determining the relative accumulation, elimination, and toxicities of individual PCB congeners.

MATERIALS AND METHODS

Pesticide susceptible house flies derived from the NAIDM strain were reared in the Department of Entomology, University of Illinois at Urbana- Champaign. Adults were harvested from emergence cages by carbon dioxide anesthesia, separated by gender, weighed and counted into 125 mL pyrex Ehrlenmeyer flasks for treatment. The flasks were covered with a single layer of surgical gauze secured by a rubber band. Dental wicks (1 x 4 cm) saturated with 20% (w/v) sucrose solution were used as a food and water source; protein (as powdered milk solution) was not provided after treatment in order to diminish egg production by the females.

Treatment was accomplished by slowly wetting the outside of a dry wick with 1.0 mL of an acetone solution of the PCB congener. The acetone was evaporated under a fume hood. The ends of the wick were then slowly treated with

Send reprint requests to Dr. Larry Hansen at the University of Illinois.

0.5 mL sucrose solution; by this method, the inside of the wick became saturated while the outside layer became wet slowly and evenly. Wicks were resaturated with 10% sucrose solution twice daily for the duration of the exposure period. No additional PCB was added.

Glass distilled solvents (Burdick and Jackson) and solvent cleaned reagents were used. Alumina, 80-200 mesh, was activated at 800 C overnight and deactivated with 2% hexane-extracted water. All glassware, including fly flasks, was detergent and solvent cleaned and baked overnight at 230 C as is customary for PCB analyses (Erickson 1986).

PCB congeners were purchased greater than 99% pure from Analabs and then from Ultra Scientific (Hope, RI). Purity was confirmed by GC and by GC/MS. The congeners used are identified in the text by IUPAC number:

PCB 28 2,4,4'-trichlorobiphenyl
PCB 44 2,2',3,5'-tetrachlorobiphenyl
PCB 52 2,2',5,5'-tetrachlorobiphenyl
PCB 95 2,2',3,5',6-pentachlorobiphenyl
PCB 118 2,3',4,4',5-pentachlorobiphenyl
PCB 136 2,2',3,3',6,6'-hexachlorobiphenyl
PCB 153 2,2',4,4',5,5'-hexachlorobiphenyl

Flies were removed after carbon dioxide anesthesia and homogenized (Polytron) in groups of 3-5 in 5 mL acetone:hexane (1:1, v/v)/fly followed by 3 rinses of 10 mL acetone:hexane. Sugar wicks were Soxhlet extracted overnight with acetone:hexane. Pooled extracts were filtered through sodium sulfate and 0.5 mL isooctane added as a keeper along with 50 μL recovery spike solution (3.84 μg PCB 28 + 4.20 μg PCB 118).

Extracts were concentrated, cleaned by alumina column chromatography (3.0 g 2% deactivated alumina eluted with 30 mL hexane), reconcentrated, internal standard added and volume adjusted to 1.0 mL with isooctane. Samples were analyzed with a Hewlett-Packard 5740 gas chromatograph equipped with a 15 m x 0.53 mm megabore capillary column coated with 1 μm DB 1701 (J & W Scientific). Recoveries for 58 samples, including 10 samples that evaporated to dryness, were 96 ± 16 for PCB 28 and 98 ± 18 for PCB 118 (mean ± SD). All data are reported as corrected for recovery. Process blanks indicated no interferring contaminants.

RESULTS AND DISCUSSION

Flies (8 days of age; n = 25/congener) were allowed to feed on PCB-treated sugar wicks for 24 hr. After 24 hr, 3 were analyzed, 9 were removed to clean flasks with untreated wicks and 13 retained in the original flasks. Three flies from each flask were removed and analyzed at subsequent time periods, and the results (Figure 1) indicated marked differences in PCB recovered from the flies among the congeners and between the sexes. Maximum body burdens are reached between 24 and 48 hours and, except for PCB 153, there is a marked reduction in the body burden between 48 and 72 hours, even in the continued presence of the treated sugar wicks.

These 8-day females accumulated higher residues at later times and generally had greater rates of elimination than did 2-day, 9-day, 10-day and 17-day old

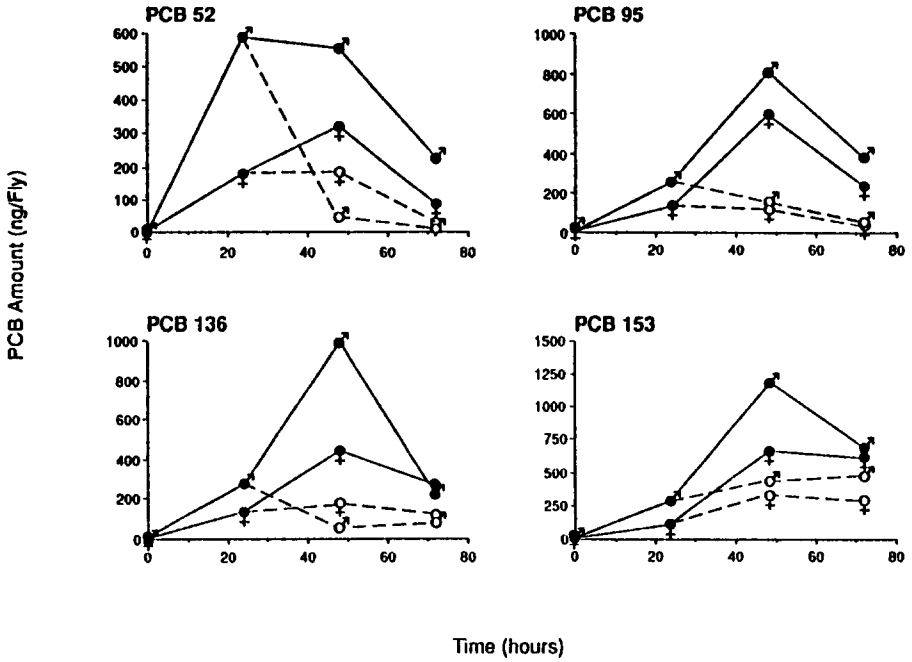

Figure 1. Accumulation and elimination of PCB congeners from 8-day male and female houseflies fed via treated sugar wicks. (Open symbols and broken lines indicate flies removed from PCB exposure.)

females. This may have been due to early (24 hr) removal of a substantial number of flies or to variability; most likely, these two factors interacted with insufficient time points to detect actual peak residues. Data for male files were inadequate for comparisons.

It is likely that the flies accumulate some PCB from contact exposure. Excretion of unmetabolized PCB onto the vessel walls as well as the sugar wicks would increase the likelihood of this route contributing to body burdens. Thus, differences in apparent rates of decrease in total body PCB might be exagerated if biotransformation preceeding excretion were more rapid for some congeners than for others.

Table 1 estimates the PCB remaining in the flies and in the sugar wicks after 120 hours. The recoveries of PCBs 52, 95, 136 and 153 (55, 67, 85 and 112%, respectively, in males and 39, 54, 76 and 75% in females) are also consistent with their relative biotransformation rates by microsomal monooxygenases. Female flies should have been at or near their peak monooxygenase activity at 8 days of age while the male flies would be in a declining phase of activity (Hansen and Hodgson 1971); thus, the lower recovery of PCB from treatment vessels populated with female flies is further evidence of biotransformation-mediated decline in PCBs. Attempts to clarify the age relationship, however, were rather ambiguous, perhaps because age also affects other factors such as feeding rates and mobility.

In order to determine residue variability and effects of variations in weight,

flies were weighed and analyzed individually and the results presented as concentrations rather than average amounts in groups of flies. The results (Table 2) indicate considerable variation in PCB concentrations (μg/g or PPM).

Table 1. Amounts of PCBs in flies and sugar wicks after 120 hours.

Parameter	PCB in Wick or Estimated (a) in Flies (μg)			
	PCB 52	PCB 95	PCB 136	PCB 153
Dose in Wicks	100.5	112.3	102.3	105.4
Male Flies	23.6	18.7	20.2	19.3
Wicks	31.7	56.2	66.7	99.2
Total μg	55.3	74.8	86.9	118.4
Recovery, %	55	67	85	112
Female Flies	6.7	11.6	8.2	10.2
Wicks	32.1	48.5	69.5	69.1
Total μg	38.8	60.1	77.7	79.3
Recovery, %	39	54	76	75

a) Total of (μg/fly at end of time period - μg/fly at end of elimination period) X number of flies.

Table 2. PCB concentrations in flies fed with sugar wicks containing 100 μg PCB.

Age or Time	PCB Concentration (a)				
	PCB 52	PCB 44	PCB 95	PCB 136	PCB 153
2-DAY	**Female**	**Male**	**Male**	**Female**	**Male**
24 hr	11±8	17±12	34±13	19±17	21±4
10-DAY	**Female**	**Female**	**Female**	**Female**	**Female**
24 hr	23±12	12.5±6	38±12	35±6	14.5±3
48 hr	13.5±4	5±2.5	22±8	25±4	20±4
72 hr	9±4	4±1.5	19.5±5	20±5	24±5.5

a) Mean ± standard deviation μg/g for n = 9.

The variability was about the same as when the results were expressed as ng/fly (Table 3). As would be expected, the greatest variation occurs with the more rapidly metabolized PCB congeners because of the increased importance of the biotransformation variable; in addition, the 2-day flies, with rapidly developing but not fully expressed monooxygenases, are relatively more variable than are 10-day flies.

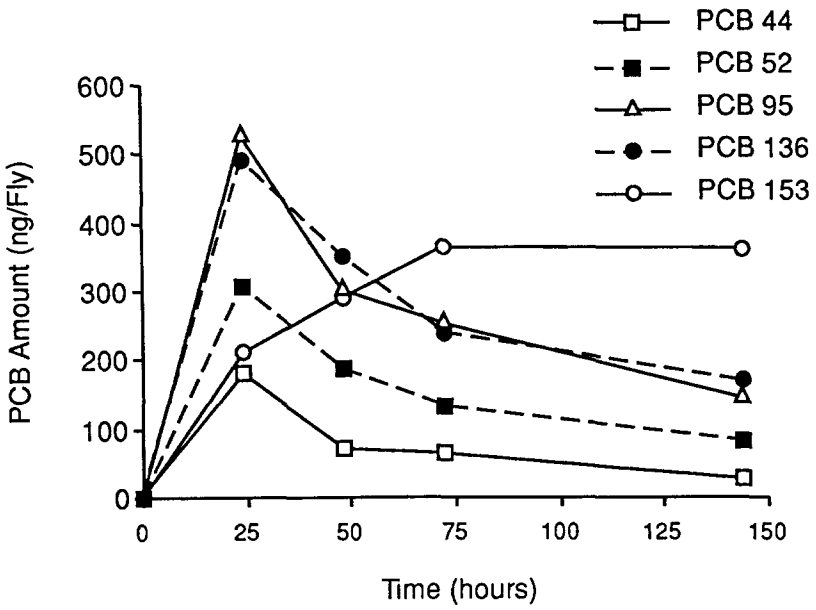

Figure 2. Comparative accumulation and depletion of PCB congeners in 10-day old female houseflies.

The 10 remaining flies for each congener were maintained for 3 more days and the additional time point was added to the data from Table 2 at 141 hours (the average of 5 flies batch-analyzed at 138 hours and 144 hours) (Figure 2). The results were plotted as ng/fly ("amount" as in Figure 1).

Table 3. Percent standard deviations for PCB concentrations (μg/g) and amounts (ng/fly).

Time	Percent SD for PCB Residues				
(hr)	PCB 52	PCB 44	PCB 95	PCB 136	PCB 153
2-DAY					
24 μg/g	77	69	39	93	21
ng/fly	71	72	38	97	29
10-DAY					
24 μg/g	55	44	31	18	20
ng/fly	53	34	33	20	21
48 μg/g	33	46	36	16	20
ng/fly	33	53	42	17	13
72 μg/g	48	36	27	23	23
ng/fly	49	37	39	21	20

Residues at 48 hours were significantly different from residues at 24 hours (ANOVA, $P < .05$), but the differences between 48 and 72 hours were significant only for PCB 136. Since ANCOVA indicated highly significant ($P < .001$) negative slopes for PCBs 44, 52, 95 and 136, the 48-72 hour comparisons were masked by the large variations. The highly persistent PCB 153 was slow to accumulate and had not entered a declining phase even by 141 hours. These trends are consistent with the previous observations that the congeners most rapidly metabolized by mammalian systems (Sipes and Schnellmann 1987) disappear most rapidly from the flies (Figure 2).

Thus, house flies may be used economically to determine differences in persistance among PCB congeners; however, individual variability indicates that a more precise method of dose delivery is desirable.

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