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Distribution and Metabolism of 2,5,2′-Trichlorobiphenyl in Houseflies (*Musca domestica* L.)

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Polychlorinated biphenyls (PCBs) are environmentally persistent and well known global contaminants, still found in the environment two decades after use was banned in almost all developed countries (Hansen, 1987; Safe *et al.*, 1987; Tanabe *et al.*, 1990). Commercially produced PCBs contained mixtures of 209 possible congeners; therefore, it is important to know the persistence, disposition and toxicity of the individual congeners (Barnes *et al.*, 1991). In the past, most of the research concerning biological activity has focused on the dibenzofurans and dibenzo-p-dioxins contaminating PCBs and on TCDD mimicking congeners (Parkinson and Safe, 1987). Information is also needed on other congeners of PCBs in order to assess the hazards associated with various environmental reservoirs (Hansen, 1987; Barnes *et al.*, 1991).

The biological persistence and activity of PCBs are associated with cytochrome P-450 (Safe, 1987; Sipes and Schnellmann, 1987). Microsomal enzymes, beside metabolizing PCBs, can be induced by PCBs, which increases the rate of biotransformation. Metabolites formed can bind to cellular molecules; readily metabolized PCBs (PCBs having vicinyl hydrogens on both of the phenyl rings) can covalently bind to proteins, DNA and RNA (Hansen, 1987).

Studies with insects may provide information regarding the role of insects in the global disposition of PCBs because of their relatively large and mobile biomass. The house fly (*Musca domestica* L.) has an active and well-defined microsomal monooxygenase system (Hodgson, 1985). Also, houseflies have long been used for screening pesticides and they can be used for screening toxicities of different congeners (Tehseen *et al.*, 1992). PCBs are metabolized to polar products (Saghir and Hansen, 1992) and congeners are selectively retained from a commercial mixture by houseflies (Storr-Hansen *et al.*, 1992).

Previous studies have generated questions regarding the sequestration and biotransformation of PCBs by houseflies (Storr-Hansen *et al.*, 1992). This study was undertaken to determine the distribution and elimination of [^{14}C]-2,5,2′-trichlorobiphenyl ([^{14}C]-PCB-18) from different body tissues as well as the patterns

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of metabolites of PCB-18 in the excrement and tissues at different times following topical administration to 5-day old female house flies.

MATERIALS AND METHODS

Radiolabelled PCB-18 [[^{14}C]-2,5,2′-trichlorobiphenyl] was purchased greater than 95% pure from Mallinckrodt, St. Louis, MO. (specific activity 9.9 mCi/mM). The compound was further purified and separated from its degradative product (which was detected in the autoradiographs during pilot experiments) by thin layer chromatography on 1000μ thick Whatman's silica gel plates developed by using benzene:dioxane:acetic acid (64:34:1.5, v/v), a modification of Hansen *et al.*, (1977). Following scanning and autoradiography all the radioactive spots from the plates were scraped and extracted separately in acetone. The fraction containing [^{14}C]-PCB-18 was identified and the concentration determined by high resolution capillary gas chromatography with a ^{63}Ni electron capture detector and a 50 m x 0.25 mm i.d. column coated with 0.25 μm CP-Sil8-CB: carrier gas was hydrogen and temperature was programmed from 180°C to 220°C at 20°C/min, residence times at 180°C and 220°C were 5 and 7 minutes, respectively. Retention time was 8.93 minutes.

Pesticide susceptible houseflies (NAIDM strain) were reared in the Department of Entomology, University of Illinois, Urbana-Champaign. Pupae were kept in 0.3 x 0.3 x 0.3 m wire screen cages for emergence in the Department of Veterinary Biosciences. After emergence adult flies were fed *ad libitum* on sugar cubes, distilled water and 10% powdered milk solution; milk was withdrawn after 2 days to reduce egg laying. At day 5 following emergence, flies were anesthetized with carbon dioxide and batches of 3 females each were used in the experiment.

Ten micrograms of [^{14}C]-PCB-18 was topically applied to the dorsal thorax of each CO_2 anesthetized fly in 1 μL acetone using a micro-syringe fitted to a manually operated micro-applicator. Flies were kept in groups of three in 25 mL scintillation vials. Vials were lined internally with filter paper (Whatman, No. 50) to trap the excrement and reduce reabsorption of PCB by contact. Vials were covered on top with muslin cloth held in place with rubber bands. Small filter paper discs were inserted between cloth layers to supply food and water by wetting the disc with sugar solution (20%).

At specified times, groups of three flies were anesthetized with CO_2, removed from the vial, rinsed with 5 mL acetone, bled by using a capillary tube, dissected and different organs were removed; the pooled tissues were ground and extracted three times (24 hours each time with frequent agitation) in 8 mL acetone:hexane (1:1). The filter paper lining of the vials was also extracted thrice in order to recover the PCB and its metabolites from excrement. Counts of all the extractable fractions were adjusted for the analytical loss which was 18.2$\pm$11% (determined by topical application of [^{14}C]-PCB-18 to six group of flies; half of them were extracted as described above, whereas the other half were digested in protosol, without extraction, and counted to calculate the analytical loss).

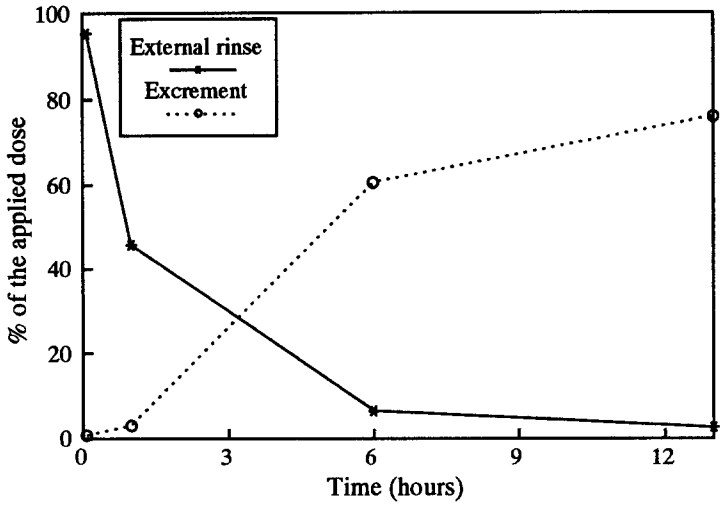

Figure 1. Disappearance of radioactivity from the surface of flies (external rinse) and appearance in the excrement following topical administration of [^{14}C]-PCB-18. Each point represents a pooled sample from 3 flies.

The extracts were dried, 25% of the total counted for radioactivity (Packard Tri-Carb 300M Scintillation counter) and the rest spotted on 20 x 20 cm, 250μ thick Whatman's LK5F linear-K silica gel plates for thin layer chromatography. The plates were developed as described earlier. Radioactive spots on the TLC plates were determined by exposing the plates for 20 days for autoradiography.

Some of the radioactivity in the filter paper lining could not be extracted even after three 24-hour extractions with acetone:hexane (1:1) (unextractable fraction). Filter papers were then extracted in toluene, acetone:hexane (24-hour soxhlet extraction), chloroform:methanol (2:1, v/v), and then water to determine the extractability of the filter paper bound radioactivity.

RESULTS AND DISCUSSION

The applied dose was absorbed into the body relatively rapidly with more than 93% disappearing from the surface within 6 hours (Figure 1). Applied PCB which could not be removed by the acetone wash accumulated in the deeper layers of the thoracic cuticle (Figure 2). It was apparently distributed first to the thoracic muscles since the concentrations were parallel to those in the thoracic cuticle (Figure 3). Around 13% of the applied dose was recovered from the thoracic cuticle and 3% from the thoracic muscles after 1 hour post-treatment; this declined with time, indicating distribution to different organs (Figures 2, 3). This is consistent with the mechanism of pesticide penetration as described by Brooks (1976) and Baillie and Wright (1985).

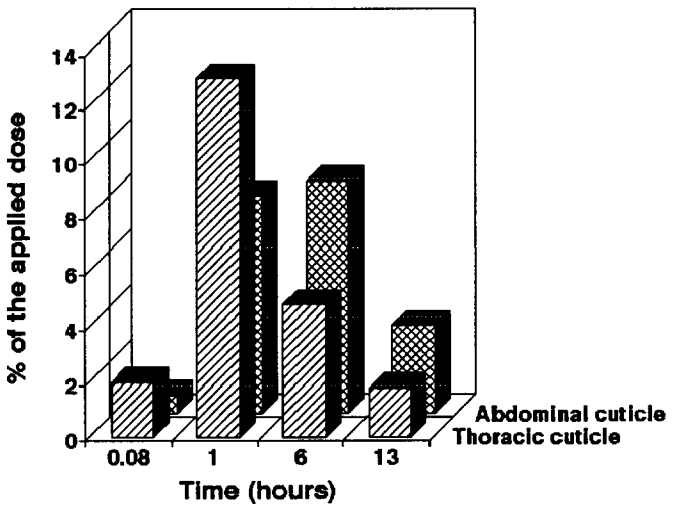

Figure 2. Fate of radioactivity in the deeper (acetone rinsed) cuticle of pooled samples from 3 flies after topical dosing with [^{14}C]-PCB-18.

The PCB also spread to the abdominal cuticle and head, probably by lateral diffusion over the entire body surface via the wax layer (Brooks, 1976; Hollingworth, 1976). A maximum of 4% of the applied dose was found in the head and 8% spread to the abdominal cuticle. The highest level of radioactivity in the abdominal cuticle was found at 6 hours (Figure 2) and in the head at 1 hour post-treatment (Figure 3).

The highest concentration in hemolymph was found at 1 hour (Figure 3) and then declined with time. A relationship was found in the declining concentration of radioactivity in hemolymph with that of increasing concentration in the ovaries and alimentary canal (Figure 4). This indicated that some of the PCB was transported to different organs via hemolymph, which is similar to Brooks (1976).

Peak accumulation in the alimentary canal and ovaries occurred at 6 hours post-treatment (Figure 4). The amount in the gut was higher than in the ovaries at 1 hour, probably due to the larger size of the gut. The 6 hour higher concentration in the ovaries than gut is probably due to excretion from the gut and the higher lipid content of the ovaries.

Most of the applied PCB was rapidly eliminated from the body. About 60 and 76% of the applied dose was collected from the excrement at 6 and 13 hours, respectively (Figure 1). The figure includes total (extracted + residual) radioactivity, but the hexane:acetone unextractable fraction on the filter paper increased with time to 20% and 50% of the applied dose at 6 and 13 hours, respectively, whereas the extractable fraction from the filter paper peaked at 6

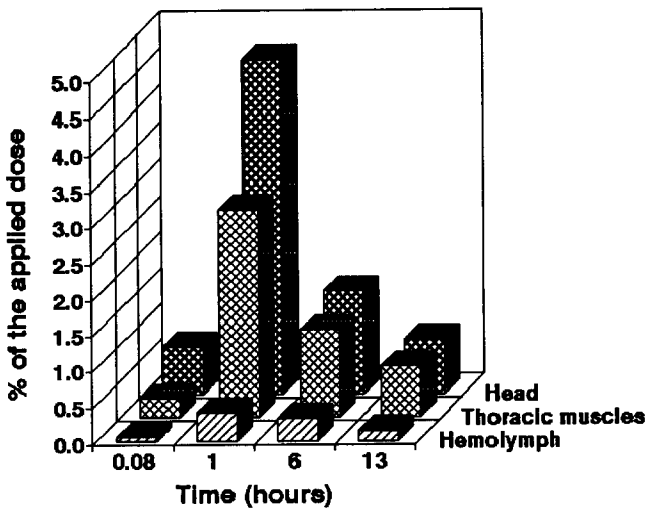

Figure 3. Accumulation and depletion of radioactivity in pooled hemolymph, head and thoracic muscles from 3 flies topically dosed with [^{14}C]-PCB-18.

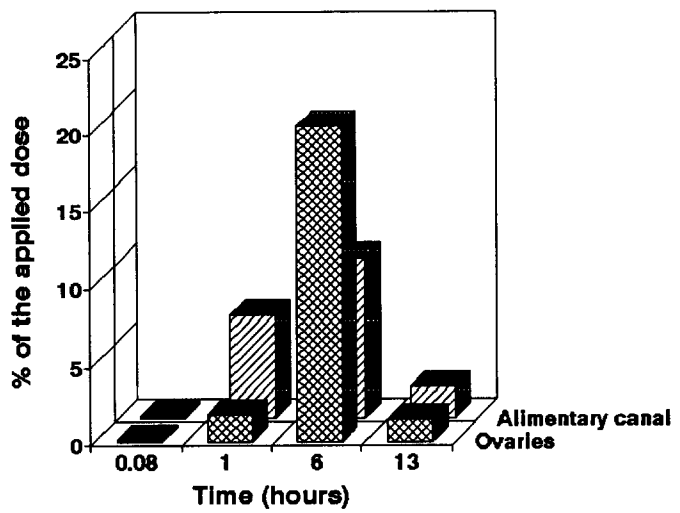

Figure 4. Accumulation and depletion of radioactivity in the pooled gut and ovaries from 3 flies dosed topically with [^{14}C]-PCB-18.

hours. Extractability of the filter paper-bound fraction in different solvents was: 0, <10, 46, 66, and 28% in acetone:hexane (washing); toluene; acetone:hexane (soxhlet extraction); chloroform:methanol (2:1); and deionized water (pH 7.8), respectively. The increased extractability with more polar solvents suggests the hexane:acetone unextractable fraction was primarily polar metabolites.

Table 1. Comparative disposition of the metabolites found in fly excrement*.

Time♣ (hours)	Total % Dose	% metabolites at different time and their Rf values						
		0.00 (Polar)	0.02	0.03	0.16	0.29	0.7 (parent)	unext♠
6	60.67	0.87	0.61	0.56	3.94	0.61	3.85	89.57
13	76.03	0.81	1.19	0.53	1.72	0.97	3.08	91.69

* % of the dose found in the excrement as metabolites.

♣ Only one metabolite (Rf 0.29) was found at 0.08 (0.25%) and 1 (0.12%) hour.

♠ Was not extracted from the filter paper lining of the vials.

The cumulative highest level of radioactivity in flies was found at 6 hours post-treatment. An earlier peak body burden of PCB-18 was reported in flies topically dosed with Aroclor 1242 (Storr-Hansen *et al.*, 1992), but this time period was not sampled; furthermore, determination of the body burden was based only on the parent PCB whereas, in the present study, radioactivity represents parent PCB as well as metabolites present in the tissues.

Metabolism of PCBs, mainly to mono- and di-hydroxy biphenyls (Sipes and Schnellmann, 1987), depends on the level of microsomal enzymes. At least five different metabolites were separated by thin layer chromatography and detected by autoradiography in the excrement extract (Figure 5). Metabolites were found even after 5 minutes post-treatment and the concentration of metabolites increased with time. The proportion of parent PCB decreased with time due to biotransformation. More polar metabolites, which remain at or near to the origin, appeared late after treatment and are very apparent at 13 hours (Table 1 and Figure 5). Since the radioactivity initially unextracted from the excrement was more polar, it is likely that the proportion of metabolites is much higher than represented (Table 1). PCB-18 was metabolized to at least 3-4 and 6-7 different metabolites by sheep liver microsomes after 1 and 5 minutes of incubation, respectively, whereas, higher chlorinated PCBs yielded fewer metabolites (Hansen *et al.*, 1977). Experiments to generate large amounts of metabolites for identification are underway. The solvent system used in this study would not resolve less polar metabolites; however, some indication for the production of a metabolite less polar than PCB-18 has been reported (Metcalf *et al.*, 1975).

Metabolites began appearing in different tissues as early as 1 hour post-treatment. Figure 6 shows metabolites in different tissues 13 hour post-treatment; 2-3 metabolites can easily be seen in alimentary canal, ovaries, and abdominal cuticle.

PCBs chlorinated in the 2- or 2,5- position are relatively less persistent in house flies than are other substitution patterns (Storr-Hansen *et al.*, 1992). We have demonstrated that PCB-18 is metabolized by the house fly to more polar products

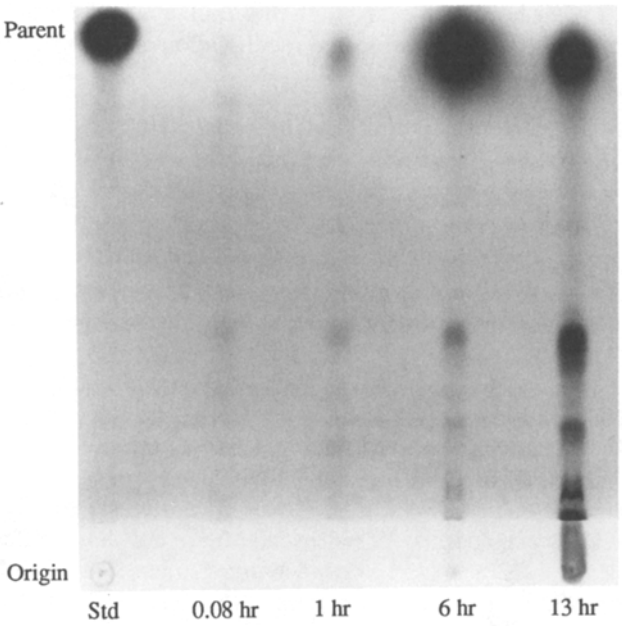

Figure 5. Autoradiograph of house fly excrement extracts chromatographed by TLC (benzene:dioxane:acetic acid, 64:34:1.5) at different times after topical administration of [^{14}C]-PCB-18.

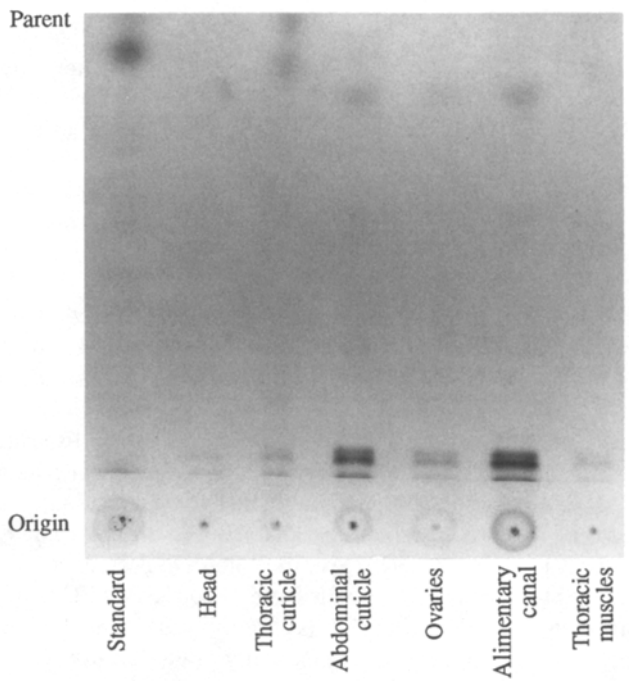

Figure 6. Autoradiograph of house fly tissue extracts at 13 hours after topical dosing with [^{14}C]-PCB-18, chromatographed by TLC.

and that insects, by virtue of their large mobile biomass and biotransformation potential, may play a significant role in the modification and global dispersion of PCB reservoirs.

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