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Short-Term Distribution, Metabolism, and Excretion of 2,2′,5-Tri-, 2,2′,4,4′-Tetra-, and 3,3′,4,4′-Tetrachlorobiphenyls in Prepubertal Rats

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Abstract. The excretion and tissue retention of three ^{14}C-labeled lower chlorinated biphenyls were examined in prepubertal male and female Sprague-Dawley rats following *IV* administration. Urine and feces were collected individually at different time intervals up to 72 h for pharmacokinetic analyses. After 72 h, different organs were removed and extracted in acetone: hexane (1:1, v/v) to determine radioactivity. Within the first 10 h after dosing, 2,2′,5-trichlorobiphenyl (PCB 18) was rapidly excreted in urine (8–18% of the administered dose), whereas only 0.6–0.8% of 2,2′,4,4′-tetrachlorobiphenyl (PCB 47) and 0.3–0.8% 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) were found in urine during this time period. The half-life of elimination was shortest for PCB 18 (37.5 to 49.2 h). The half-lives for PCB 47 and PCB 77 were 351 to 672 h and 152 to 186 h, respectively. The cumulative total excretion (urinary + fecal) of PCB 18 within 72 h was 51–62%, of PCB 77 was 22–25%, and of PCB 47 was 7–10%. No parent PCBs were detected in urine. PCB 47 accumulated preferentially in adipose tissues (subcutaneous fat > mesenteric fat); relatively high levels of PCB 47 were also found in adrenals, ovaries, lungs, liver, and skin. The highest concentration of PCB 77 was found in serum, followed by adipose tissues. Very low concentrations of PCB 18 were found in most tissues; the highest being found in serum, followed by ovaries and adrenal glands. This study suggests that prepubertal rats retain higher short-term serum levels and have lower excretion rates than adult rats.

The assessment of hazards associated with polychlorinated biphenyls (PCBs) remains a challenge because of the multiple effects and the multiple components of PCB mixtures (Silkworth and Grabstein 1982; Hansen 1987, 1998; Safe 1994; Li and Hansen 1997). There is a need to develop a broader database concerning the actions, effects, and interactions of PCBs. This is especially critical following the short-term exposures necessary for defining the initial actions for accurate structure: activity relationships. There are important differences between short-term distribution and the equilibria established after 7–14 days and/or chronic exposure (Hansen *et al.* 1977; Lutz and Dedrick 1987). For example, significantly higher serum concentrations of parent PCB are maintained for 48 h and there is a direct relationship between liver and serum residues (Li *et al.* 1994; Soontornchat *et al.* 1994).

Emerging structure:activity relationships from acute exposures can generally be interpreted within the perspectives of PCB pharmacokinetics, including differential PCB metabolism and multiphasic distribution and elimination (Hansen *et al.* 1977; Shimada and Sawabe 1984; Lutz and Dedrick 1987; Sipes and Schnellmann 1987; Saghir *et al.* 1994). For example, disproportionate increases in uterotropic potency by simply dividing the dose over 24 h (Jansen *et al.* 1993) may be caused by an increased generation of bioactive metabolites (Korach *et al.* 1988; Jansen *et al.* 1993) following monooxygenase induction by the first dose (Li *et al.* 1994; Soontornchat *et al.* 1994; Li and Hansen 1997). Decreased serum concentrations at higher doses (Li *et al.* 1994) may be due to enhanced excretion following induction of both phase 1 oxygenases and phase 2 conjugating enzymes.

Immature rats are used in many bioassays to obtain increased sensitivity and because of concerns regarding developmental effects. Events occurring during prepubertal development include nervous system growth and imprinting, hormone receptor and reproductive tract development, and sexual differentiation (Hoar and Monie 1981; Dohler 1986). Drug-metabolizing enzymes (both phase 1 and phase 2) also develop and differentiate postnatally, and prepubertal exposure to PCBs may alter adult expression of these enzyme activities (Lucier 1978; Klinger *et al.* 1981; Poul 1991).

Most pharmacokinetic studies of PCBs have been directed toward long-term equilibrium conditions in adult rats. However, basic information on the short-term tissue distribution and extent of binding, metabolism, and excretion of PCBs is needed to assist in risk assessment following intermittent pulsatic exposure to PCBs, especially in young animals and children, who may behave differently to these chemicals than adults. This study was designed therefore to assist in these interpretations rather than to repeat longer-term toxicokinetic studies. The rapidly metabolized 2,2′,5-trichlorobiphenyl (PCB 18) (Ghiasuddin *et al.* 1976; Hansen *et al.* 1977; Saghir *et al.* 1993) was

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compared to the moderately persistent coplanar 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) and the slowly metabolized 2,2′4,4′-tetrachlorobiphenyl (PCB 47) (Lutz and Dedrick 1987; Sipes and Schnellmann 1987; Saghir *et al.* 1994) in both male and female prepubertal rats. In the environment, PCB 47 is chronically present at low levels; PCB 77 content is highly variable depending on the matrix, but it is generally present at 0.1 to 0.5× the level of PCB 47. PCB 18 occurs at intermittent high levels, primarily as pulses of exposure (Luotamo *et al.* 1993; Hansen 1998). Therefore, the three PCBs used in this study were given doses reflecting their relative environmental exposures: PCB 18 at 10 mg/kg; PCB 47 at 5 mg/kg; and PCB 77 at 2 mg/kg.

Materials and Methods

Chemicals

Uniformly radiolabeled ^{14}C-2,2′,5-trichlorobiphenyl (PCB 18) was purchased from Mallinckrodt (St. Louis, MO) (MW 261, specific activity 9.9 mCi/m mol; greater than 95% chemically pure) and further purified as reported earlier (Saghir and Hansen 1992; Saghir *et al.* 1993). Briefly, it was separated by preparative thin layer chromatography on 1,000-µm-thick Whatman's silica gel plates using benzene: dioxane:acetic acid (64:34:1.5, v/v). Nonradioactive PCB 18 was added to increase the concentration of the dosing solution (final specific activity was 1.7 mCi/mmol).

Uniformly radiolabeled ^{14}C-2,2′,4,4′-tetrachlorobiphenyl (PCB 47) (MW 296, specific activity 13.8 mCi/m mol) and ^{14}C-3,3′,4,4′-tetrachlorobiphenyl (PCB 77) (MW 296, specific activity 37.1 mCi/m mol) were purchased from Sigma Chemicals (St. Louis, MO). The chemicals were greater than 99% pure and were used without further purification.

Animals—Care and Dosing

Six male (67 ± 16 g) and six female (60 ± 8 g) weanling (22-day-old) Sprague-Dawley rat pups were housed individually in metabolism cages and acclimated to laboratory conditions for 48 h with a 12-h light/12-h dark cycle. Pups were provided with standard Purina rat chow and distilled water *ad libitum.* All animals were fasted for 12 h before dosing. Two pups of each sex were then dosed intravenously into the tail vein with 0.07 µCi/g (10.6 µg/g) of PCB 18 or 0.25 µCi/g of the other two PCBs (5.3 µg/g of PCB 47 or 2.2 µg/g of PCB 77) in dimethylsulfoxide (DMSO). The DMSO dose was 0.72 µg/g of body weight.

Sample Collection

Urine and feces were mechanically separated by the metabolism cages and collected separately every 2 h up to 12 h and then every 6 h up to 72 h. Radioactivity in the urine was quantitated directly by taking a 10% aliquot by weight for liquid scintillation counting (Packard Tri-Carb 1500M scintillation counter) in ScintiVerse BD (Fisher Scientific, Fairlawn, NJ) scintillation cocktail. The scintillation counter was calibrated to correct for quenching by establishing a curve using quench standards. Rate of chemiluminescence was also monitored and samples were counted until chemiluminescence was completely decayed. The counting efficiency of the scintillation counter was > 95%. The total administered doses were corrected for the radioactivity remained at the dosing site. Background radioactivity from a non–^{14}C-treated rat was subtracted from the radioactivity of each sample before analyzing for the concentration in tissues or excreta. The animals were euthanized by decapitation 72 h after dosing and the organs were removed by gross dissection. Instruments were washed in acetone between tissues to avoid cross-contamination. All samples (excreta and tissues) were stored at −10°C immediately after collection until further analysis.

Extraction

Samples were ground separately in a Polytron tissue grinder (Kinematica, Switzerland) in 5 ml acetone, extracted twice with 15 ml acetone:hexane (1:1, v/v) and twice with 15 ml hexane alone. Pooled extracts were passed over sodium sulfate, which was washed with 10 ml hexane at the end of each sample elution. An aliquot was taken from each extract for the quantitation of radioactivity. Residual tissues following extractions were dried and ground, 10% aliquots of which were digested in 1–2 ml Protosol (New Research Products, Boston, MA) at 40°C, pH was neutralized by adding 100–200 µl of acetic acid to eliminate chemiluminescence, and counted in ScintiVerse II (Fisher Scientific) until complete decay of chemiluminescence to determine the unextractable radioactivity.

Kinetic Analysis

Average urinary and fecal excretion data for two males and two females for each PCB were subjected to pharmacokinetic analysis using the SAAM computer program (SAAM Institute, Inc., Seattle, WA). A one-compartment open model with first-order elimination rate constant $K_{el} = K_u + K_f$, where K_u and K_f are first-order rate constants for urinary and fecal excretion, respectively, was fitted simultaneously to the urinary and fecal data expressed as percent of the applied dose versus time with equal weighting.

Urine Analysis

Total urine of each sex and PCB was pooled separately after taking aliquots and concentrated in a freeze dryer. Aliquots of the concentrated samples were acid hydrolyzed in 2 N HCl overnight at 60°C. Aliquots of the acid-hydrolyzed and non–acid hydrolyzed urine samples were spotted directly on 20 × 20 cm, 250-µm-thick Whatman's LK5F linear-K silica gel plates and developed with benzene:dioxane:acetic acid (64:34:1.5, v/v). Radioactive spots on the TLC plates were determined by exposing the plates for 25–30 days to a phosphor screen and detected by a phosphor imager (Molecular Dynamics, Sunnyvale, CA).

Results and Discussion

Most of the data presented in this paper are expressed as cumulative percent of the total administered dose excreted from the body or percentage accumulated by different organs. Total radioactivity does not differentiate between the parent PCBs and their metabolites; however, reported relative rates of metabolism in different species are PCB 18 > PCB 77 ≫ PCB 47 (Ghiasuddin *et al.* 1976; Hansen *et al.* 1977; Shimada and Sawabe 1984; Saghir *et al.* 1993, 1994). Parent PCBs are not excreted in urine, and relative urinary excretion rates were consistent with reported relative rates of metabolism. Qualitative thin layer chromatography of urine with and without acid

Table 1. Pharmacokinetic parameters for the total radioactivity associated with three ^{14}C-labeled PCBs in prepubertal rats following IV injection

Congener	% Dose Excreted Within 72 h (Observed)		Fractional Route of Excretion in 72 h[a]		Rate Constants of Elimination K_u, K_f (h^{-1})[b]		$T_{1/2}$ Elimination[c] (h)
	Urine	Feces	Urine	Feces	Urine	Feces	
Male							
PCB 18	44.36	17.24	0.72	0.28	0.01350	0.00499	37.5
PCB 47	1.55	5.08	0.23	0.77	0.00026	0.00077	672.2
PCB 77	3.13	18.86	0.14	0.86	0.00057	0.00316	185.7
Female							
PCB 18	26.80	24.00	0.53	0.47	0.00761	0.00647	49.2
PCB 47	3.07	7.28	0.30	0.70	0.00050	0.00148	350.5
PCB 77	2.61	21.78	0.11	0.89	0.00053	0.00404	151.5

[a] Fraction of the excreted dose recovered in urine or feces

[b] K_{el}-first-order rate constant of elimination from one-compartment open model; $K_{el} = K_u + K_f$, where K_u and K_f are first-order rate constants for urinary and fecal excretion, respectively

[c] Elimination half-life $= 0.693/K_{el}$

hydrolysis for metabolites more polar than parent PCB and radioactivity unextractable from tissues support the putative relative rates of metabolism for these PCBs.

Urinary and Fecal Excretion

A large fraction of the administered dose of PCB 18 (51–62%) was excreted within 72 h, whereas a smaller fraction of PCB 77 (22–24%) and a very small fraction of PCB 47 (7–10%) were excreted during the same time period (Table 1). The major route of excretion for PCB 18 was urine, whereas fecal excretion predominated for PCB 47 and PCB 77.

The cumulative urinary recoveries of ^{14}C-PCBs for 72 h following single IV injections are shown in Figure 1. The figure reemphasizes the dramatic effect of the biphenyl chlorination patterns on the fraction of the total radioactive dose excreted in the urine. Although most PCBs are preferentially excreted in feces (Matthews and Anderson 1975; Lutz *et al.* 1984; Klasson-Wehler *et al.* 1989), some of the lower chlorinated congeners are excreted predominantly as metabolites in urine (Matthews and Anderson 1975; Lutz *et al.* 1984; Lutz and Dedrick 1987). Most of the administered PCB 18 (27–44%) was rapidly excreted from the body into urine (Figure 1). PCB 18 is rapidly metabolized to a large number of different polar metabolites by rat liver (Ghiasuddin *et al.* 1976) and sheep liver microsomes (Hansen *et al.* 1977) and is very rapidly metabolized and excreted by houseflies (Saghir and Hansen 1992; Saghir *et al.* 1993). Urinary excretion of PCB 47 and PCB 77 was much slower (Figure 1), with most of the excretion, again, occurring within the first 24 h. Total urinary excretion of the two tetrachlorobiphenyls was around 3% (Figure 1 and Table 1).

Fecal excretion was an important route of elimination for all three PCBs (Figure 2, Table 1). The slowly metabolized PCB 47 was the least excreted, although the total amounts in feces were 2–3-fold higher than in urine (Table 1). More than 85% of the total elimination of PCB 77 was into the feces of both sexes. The predominance of the fecal route of excretion of PCB 77 has also been reported by Abdel-Hamid *et al.* (1981). Very high biliary and fecal excretion of PCB 77 has been reported in mice and adult rats (Klasson-Wehler *et al.* 1989; McKinley *et al.* 1993).

Metabolites in Urine

Thin layer chromatography and autoradiography of the urinary samples revealed that all of the radioactivity of the three PCBs was associated with metabolites more polar than parent PCB; no parent PCB was excreted in urine (Figure 3). Two or three different radioactive spots appeared in the autoradiograms, with most of the radioactivity remaining at or near the origin (Figure 3). Almost all the radioactivity at the origin of the PCB 47 chromatogram disappeared following acid hydrolysis and 2–3 new spots appeared, indicating the presence of acid-labile conjugated polar metabolites (Figure 3). Very little of the PCB 18 and PCB 77 radioactivity was found in the autoradiograms of the acid-hydrolyzed urine, but was recovered in the precipitates that were formed during acid hydrolysis. The method of acid hydrolysis used during these experiments, therefore, was appropriate for PCB 47 but not for PCB 18 and PCB 77. However, the disappearance of most of the radioactivity and its appearance in the acid-hydrolyzed urine as precipitates suggested the presence of conjugated polar metabolites in urine. No attempts were made to quantitate or identify any of the radioactive spots in the autoradiograms. Klasson-Wehler *et al.* (1989) found no parent PCB 77 in mouse urine prior to acid hydrolysis and a total of seven phenolic PCB 77–related products afterward, suggesting the presence of acid-labile conjugates of phenolic metabolites.

Kinetics of Excretion

A major goal was to compare tissue distribution shortly after dosing and the compressed time-limited kinetic interpretation. Both one- and two-compartment open models of disposition were fitted to the urinary and fecal excretion data. The two-compartment model gave a slightly better fit to the first and the last 12 h of the observed data; however, the lack of plasma levels to better define distribution and the availability of only 72 h of excretion data (at which time approximately 50% or more of the doses remained in the body) resulted in unrealistically long estimates (approaching infinite time) of the biological half-lives. Therefore, the one-compartment model was

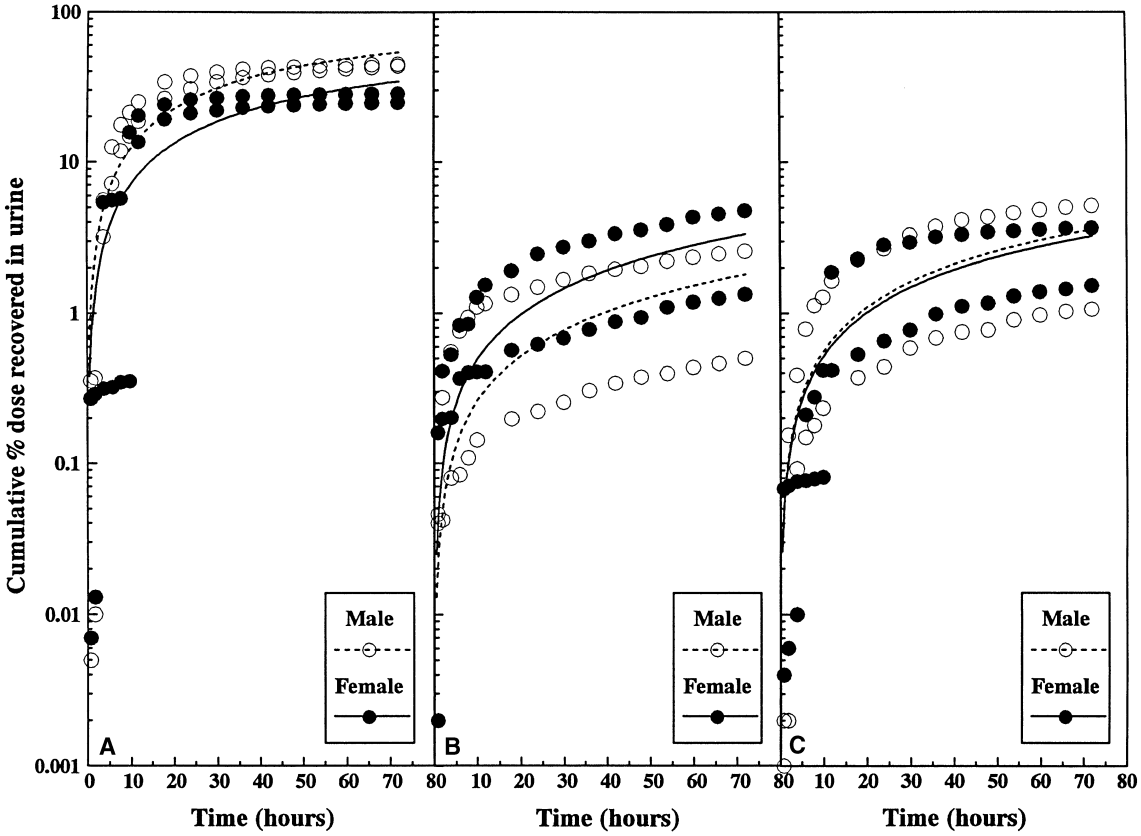

Fig. 1. Percent of the administered radioactivity excreted in urine. Symbols represent observed data points of individual rats, lines were generated from fitted pharmacokinetic parameters of averaged values. (A), PCB 18; (B), PCB 47; (C), PCB 77

chosen to compare elimination rates. The fits of the model to the data are shown in Figures 1 and 2.

The biological half-life of elimination ($t_{1/2}$ of K_{el}) was shortest for PCB 18 (38 h in male and 49 h in female pups) and longest for PCB 47 (672 h in male and 351 h in female pups). The half-life of elimination for PCB 77 was intermediate at 186 and 152 h in male and female pups, respectively (Table 1). Adult rats were much more efficient in eliminating PCBs (Abdel-Hamid *et al.* 1981; McKinley *et al.* 1993) than the immature pups in this study suggesting an age effect on the rates of elimination of PCBs.

Tissue Distribution

The concentrations of PCBs in different tissues of male and female pups are shown in Tables 2 and 3. Some radioactivity was so firmly bound to tissues that it could not be recovered, even after rigorous acetone:hexane extraction, and was reported as unextractable. These unextractable fractions are probably covalently bound polar metabolite(s), rather than the parent PCBs, which are easily extracted from tissues with acetone: hexane (more than 95% of the spiked radioactivity was extracted using this procedure). Covalent binding of PCB metabolites to blood, serum, and tissues has been reported by several investigators (Matthews and Anderson 1975; Abdel-Hamid *et al.* 1981; Shimada and Sawabe 1984; Sipes and Schnellmann 1987; Klasson-Wehler *et al.* 1989; Saghir *et al.* 1993, 1994; Bergman *et al.* 1994).

Since more than half of the PCB 18 was excreted within 72 h, very low concentrations (<2% dose/g of tissue) were found in tissues. The highest of these concentrations were in serum followed by ovaries and adrenal glands (Table 2). Concentrations in adipose tissues were less than two fold higher than those in the liver in both sexes. The higher blood concentration of PCB 18 is probably due to the formation of hydroxylated polar metabolites reportedly restricted to extracellular fluid and bound to serum proteins (Bergman *et al.* 1994).

Although lower concentrations of PCB 18 remained in tissues after 72 h, much of that was tightly or covalently bound (unextractable) (Table 3) and was assumed to be polar metabolites (Matthews and Anderson 1975; Lutz and Dedrick 1987; Saghir *et al.* 1993, 1994; Bergman *et al.* 1994). Parent PCB 18 is known to be rapidly metabolized and generally does not accumulate in tissues (Hansen *et al.* 1977; Saghir and Hansen 1992; Saghir *et al.* 1993). Rapid metabolism of PCB 18 is also apparent from the presence of very low parent PCB in serum (Table 4) as reported by Li and Hansen (1995). The relatively high accumulation of both orthochlorinated congeners in adrenal glands is noteworthy since this is a target tissue for PCBs (see Hansen 1998).

The tissue burden of PCB 47 was the highest among the three PCBs, whereas that of PCB 77 was intermediate. Marked differences in the tissue distribution of the two tetrachlorobiphe-

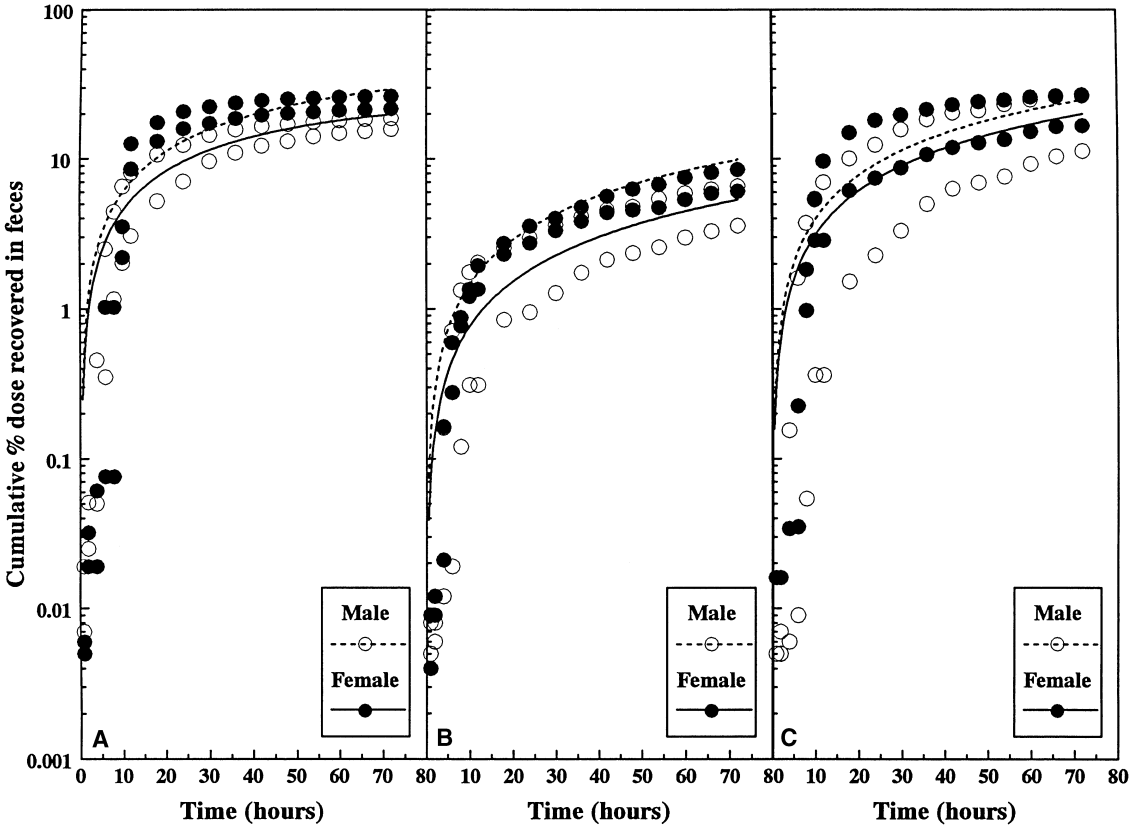

Fig. 2. Percent of the administered radioactivity excreted in feces. Symbols represent observed data points of individual rats, lines were generated from fitted pharmacokinetic parameters of averaged values. (A), PCB 18; (B), PCB 47; (C), PCB 77

nyls were noted (Tables 2 and 3). The maximum concentration of PCB 47 was observed in adipose tissues. The blood concentration of PCB 47 was lower than most of the tissues, whereas that of PCB 77 was the highest of all the tissues examined. Shimada and Sawabe (1984) have reported a similar pattern of distribution of PCB 47 and PCB 77 between fat and blood 24 h after a single oral dose in 80-g rats. A similar relationship of PCB 47 and PCB 77 between blood and adipose tissues was also found following multiple oral doses (Shimada and Sawabe 1984) (Table 4); however, in that case the higher concentration of PCB 77 in fat was due to the longer equilibration time. Abdel-Hamid *et al.* (1981) have also reported a similar ratio of PCB 77 in blood and adipose tissues of adult rats (210–270 g) (see Table 4). Although residues of both of the tetrachlorobiphenyls in liver and lungs were about equal in this study (Tables 2 and 3), other investigators have found that concentrations of both PCBs were higher in liver than lung (Shimada and Sawabe 1984). The body burden of PCB 47 was also found to be significantly higher than that of PCB 77 in mice (Mizutani *et al.* 1977).

Most of the radioactivity (>90%) associated with PCB 47 was extractable from tissues with organic solvents (Tables 2 and 3) representing a higher concentration of parent PCB 47 than of polar metabolites. This is also apparent from the high parent PCB 47 in serum (Table 4) as reported by Soontornchat *et al.* (1994). Significantly higher tissue burdens but lower binding of

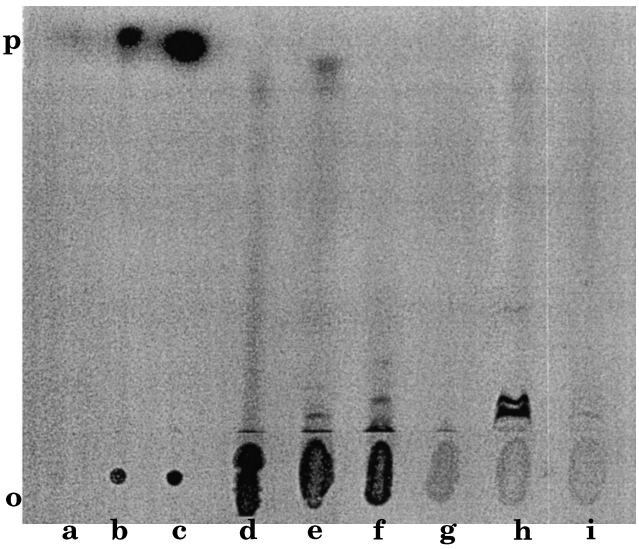

Fig. 3. Autoradiogram of a thin layer chromatographic plate showing appearance and disappearance of different ^{14}C-PCB metabolites in the urine of prepubertal rats following IV injection. Concentrated acid-hydrolyzed and non–acid hydrolyzed urine samples were spotted directly on the chromatographic plate. a–c are standard PCBs; d–f are without acid hydrolysis; and g–i are after acid hydrolysis of urine samples from rats treated with PCB 18, PCB 47, and PCB 77, respectively; o is origin and p is parent PCBs

Table 2. Concentrations of extractable radioactivity associated with three ^{14}C-labeled PCBs in the prepubertal male and female rat tissues 72 h after receiving a single IV dose

	Percent of the Administered Dose per g or ml of Tissue					
	Male			Female		
Tissue	PCB 18	PCB 47	PCB 77	PCB 18	PCB 47	PCB 77
Serum[a]	0.369	0.078	0.738	0.498	0.453	2.419
Clotted fraction[b]	0.013	0.022	0.119	0.010	0.030	0.052
Adrenal	0.204	1.564	0.133	0.104	0.944	0.210
Lung	0.058	0.325	0.165	0.019	0.564	0.110
Spinal cord	0.036	0.103	0.035	0.025	0.158	0.029
Thymus	0.038	0.117	0.046	0.016	0.197	0.183
Muscle	0.032	0.262	0.055	0.009	0.367	0.049
Heart	0.044	0.119	0.080	0.016	0.171	0.082
Testis/ovary	0.033	0.244	0.080	0.240	1.220	0.158
Spleen	0.061	0.057	0.032	0.052	0.228	0.046
Kidney	0.061	0.135	0.073	0.011	0.243	0.067
Intestinal fat	0.040	1.006	0.155	0.021	3.651	0.171
Subcutaneous fat	0.162	4.943	0.392	0.063	6.372	0.441
Skin	0.035	0.597	0.143	0.042	1.663	0.115
Liver	0.089	0.356	0.209	0.044	0.518	0.121
Stomach[c]	0.016	0.078	0.068	0.009	0.138	0.045
Duodenum[c]	0.020	0.085	0.052	0.009	0.183	0.041
Cecum[c]	0.082	0.069	0.286	0.008	0.226	0.286
Colon[c]	0.093	0.329	0.224	0.031	0.268	0.135
Brain	0.011	0.316	0.050	0.003	0.112	0.009

[a] Represents total radioactivity/ml of sample, serum was not extracted
[b] Includes RBC, WBC, and clotting factors
[c] With contents

Table 3. Concentrations of unextractable radioactivity associated with three ^{14}C-labeled PCBs in the prepubertal male and female rat tissues 72 h after receiving a single IV dose

	Percent of the Administered Dose per g or ml of Tissue					
	Male			Female		
Tissue	PCB 18	PCB 47	PCB 77	PCB 18	PCB 47	PCB 77
Serum[a]	—	—	—	—	—	—
Clotted fraction[b]	0.035	0.007	0.018	0.015	0.008	0.012
Adrenal	0.039	0.003	0.007	0.020	0.004	0.011
Lung	0.040	0.013	0.015	0.021	0.010	0.013
Spinal cord	0.014	0.001	0.007	0.005	0.002	0.004
Thymus	0.017	0.006	0.007	0.014	0.003	0.011
Muscle	0.024	0.007	0.008	0.017	0.013	0.009
Heart	0.026	0.011	0.008	0.014	0.009	0.003
Testis/ovary	0.028	0.004	0.022	0.007	0.012	0.012
Spleen	0.017	0.008	0.005	0.152	0.006	0.004
Kidney	0.047	0.011	0.019	0.029	0.020	0.010
Intestinal fat	0.039	0.024	0.015	0.017	0.053	0.031
Subcutaneous fat	0.045	0.034	0.019	0.027	0.506	0.056
Skin	0.044	0.102	0.025	0.204	0.384	0.060
Liver	0.033	0.013	0.019	0.031	0.045	0.028
Stomach[c]	0.015	0.004	0.008	0.018	0.007	0.007
Duodenum[c]	0.018	0.007	0.019	0.017	0.011	0.012
Cecum[c]	0.017	0.007	0.034	0.025	0.087	0.043
Colon[c]	0.027	0.016	0.024	0.015	0.056	0.032
Brain	0.005	ND[d]	ND	0.011	0.003	0.003

[a] Represents total radioactivity/ml of sample, serum was not extracted
[b] Includes RBC, WBC, and clotting factors
[c] With contents
[d] Not determined

PCB 47 versus PCB 77 have been reported by Shimada and Sawabe (1984). Lower tissue burdens and higher unextractable binding of PCB 18 and PCB 77 (Tables 2 and 3) may result from chlorine substitution patterns that favor rapid formation of polar metabolites capable of binding to tissues. PCB 77 can easily transform to 4-hydroxylated polar metabolites by rearrangement of chlorines on the ring and is reported to be retained in plasma or blood (Bergman *et al.* 1994). Mizutani *et al.* (1977) have also suggested rapid metabolism of PCB 77, but not PCB 47. Conjugated as well as unconjugated polar metabolites of PCB 77 were found to be retained in the adipose tissues and livers of mice (Klasson-Wehler *et al.* 1989).

Lower concentrations of all three PCBs entered the brain in the order of PCB 47 $>$ PCB 77 $>$ PCB 18 (Table 2). The autoradiographic analysis of the brain sections taken from these rats has been reported elsewhere (Ness *et al.* 1994). PCB 47 was evenly distributed throughout the brain with no specific region of binding, whereas PCB 77 remained within the vasculature and did not pass the blood-brain barrier. No autoradiographic analyses were made of PCB 18 in rat brains.

It is widely accepted that PCBs accumulate in fatty tissues and their affinity for blood is very low (Matthews and Anderson 1975; Hansen *et al.* 1977). In the present studies, PCB 47 was shown to be typical in this regard. However, the other two PCBs did not fit this criterion; higher concentrations of total radioactivity in blood than that of adipose tissues were observed (Tables 2 and 3). High affinity of PCB 77 for blood in adult rats and monkeys has also been reported (Abdel-Hamid *et al.* 1981; Shimada and Sawabe 1984). Higher concentrations of the readily metabolized PCBs (PCB 18 and PCB 77) in blood are, thus, probably due to a greater proportion of polar metabolites which distributed more to aqueous serum than to fatty tissues.

In conclusion, the present study has attempted to determine the excretion and tissue distribution of three structurally distinct PCBs in prepubertal rats within 72 h following a single IV injection. The trichlorobiphenyl (PCB 18) was very rapidly excreted from the body, consistent with its putative role as a pulsatile environmental contaminant. Most of the injected PCB 18 was recovered from urine, whereas the two tetrachlorobiphenyls were predominantly excreted in feces. The biological fate of the two tetrachlorobiphenyls was very distinct. The excretion pattern of the three PCB congeners showed that the metabolism was in the order of PCB 18 $\gg$ PCB 77 $>$ PCB 47. The concentration of PCB 47 was higher in most tissues than the other two PCB congeners. In addition, most of the PCB 47 was easily extracted into the organic solvent (extractable fraction), whereas a larger fraction of PCB 18 and PCB 77 remained unextractable.

Accurate definition of the net toxic effect of a chemical requires achieving equilibrium exposure and careful consideration of the element of time as well as dose (Rozman and Doull 1998). On the other hand, pulsatile exposures to some environmental toxicants may cause significant effects, especially in developing animals, which are not manifest until the record of exposure no longer exists. PCBs 18 and 77 are examples of developmental toxicants to which animals may be briefly

Table 4. Distribution of the three PCBs to different tissues in various conditions

Congener	Animal	No. of Doses	% Dose per ml Blood/Serum or g Tissue (mean ± SD)				Reference
			Blood	Serum	Liver	Fat	
PCB 18	Prepubertal female rat	2	—	0.005 ± 0.001	—	—	[a]
PCB 47	Prepubertal female rat	2	—	0.37 ± 0.01	—	—	[b]
	Prepubertal male rat	4	0.097 ± 0.01	—	0.613 ± 0.053	10.19 ± 2.23	[c]
PCB 77	Prepubertal male rat	4	0.493 ± 0.04	—	0.663 ± 0.063	2.77 ± 0.76	[c]
	Adult male rat	1	0.31 ± 0.04	—	0.09 ± 0.01	1.28 ± 0.12	[d]
	Adult female rat	1	0.35 ± 0.15	—	0.08 ± 0.01	0.67 ± 0.17	[d]
	Adult female mouse	1	—	0.36 ± 0.05	0.23 ± 0.05	1.61 ± 0.89	[e]

[a] Li and Hansen (1995). Female Sprague-Dawley rats (44.4 ± 1.3 g) were dosed (IP) with 8 µg/g of PCB 18 on day 20 and 21 (total of 16 µg/g) and killed on day 22. Concentrations represent parent PCB 18 only

[b] Soontornchat *et al.* (1994). Female Sprague-Dawley rats (46.1 ± 2.2 g) were dosed (IP) with 35 µg/g of PCB 47 on day 21 and 22 (total of 69.9 ± 3.4 µg/g) and killed on day 23. Concentrations represent parent PCB 47 only

[c] Shimada and Sawabe (1984). Male Sprague-Dawley rats (~80 g) were dosed (oral) with 0.54 µg/g of PCB 47 or 0.51 µg/g of PCB 77 (twice daily for 2 days), totaling 2.16 µg/g of PCB 47 or 2.04 µg/g of PCB 77. Concentrations represent total radioactivity (parent + metabolites)

[d] Abdel-Hamid *et al.* (1981). Concentrations 24 h following a single IV dose of 0.6 µg/g of PCB 77 to young adult male rats (CD strain) (210–270 g). Concentrations represent total radioactivity (parent + metabolites)

[e] Klasson-Wehler *et al.* (1989). Concentrations 5 days following a single oral dose of 10 µg/g of PCB 77 to adult female mice (20 ± 1 g). Concentrations represent total radioactivity (parent + metabolites)

exposed at doses exceeding ambient contamination levels (Hansen 1998). This brief study is an initial step in describing the transient distribution of labile toxicants which may be relevant in defining events leading to developmental changes related to pulsatile exposures.

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