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A Congener Analysis of Polychlorinated Biphenyls Accumulating in Rat Pups After Perinatal Exposure

William Shain,[1] Stephen R. Overmann,[2] Lloyd R. Wilson, Jeanne Kostas, and Brian Bush

Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201

Abstract. Rat pups were exposed to polychlorinated biphenyls (PCBs) from conception to weaning. Exposure occurred via feeding dams adulterated certified chow that was designed to contain 0, 3, 30, and 300 μg/gm Aroclor® 1254. Tissue samples from rat pups and dams were analyzed for 67 different PCB congeners at birth and weaning to describe their accumulation during gestation and lactation. Bioaccumulation of PCB congeners was calculated as a function of their concentration in feed or milk. These data indicated that PCB congeners could be divided into three different groups. The "a" group was not avidly bioaccumulated. Congeners in the "b" group accumulated to widely different concentrations. This group contained primarily penta- and hexachlorinated biphenyls. The analysis of two families of pentachlorobiphenyls containing one ring with −2,3,4, or −2,4,5 chlorine substitutions indicated that molecular structure and not physico-chemical properties, *e.g.*, gas chromatographic retention time, determined bioaccumulation. The "c" group were all highly chlorinated congeners and bioaccumulated to nearly equal levels. Most congeners were concentrated in the milk when compared to the feed. When bioaccumulation data from pups at birth and weaning were compared, exposure was much greater during lactation than during gestation. The congener analysis showed that the same congeners were most avidly bioaccumulated during the periods of gestation and lactation, indicating that the increased accumulation during lactation was due to a higher effective dose.

Polychlorinated biphenyls (PCBs) are widespread and persistent environmental contaminants. Human populations have been exposed by both chronic and acute exposures. Chronic exposures have occurred as low-level environmental, as well as, high-level occupational exposures (Kuwabara *et al.* 1978; Fischbein *et al.* 1979; Maroni *et al.* 1981; Smith *et al.* 1982; Wolff *et al.* 1982a, 1982b). Acute exposures have occurred through accidental ingestion (Kuratsune *et al.* 1972; Masuda *et al.* 1982; Drotman *et al.* 1983 Kuratsune and Shapiro, 1984).

Polychlorinated biphenyls are not only a potential health threat to adults, but also to developing fetuses and infants, since they cross the human placenta (Akiyama *et al.* 1975; Nishimura *et al.* 1977; Masuda *et al.* 1978; Umeda *et al.* 1978; Masuda *et al.* 1979) and are found in human milk (Jensen and Sundstrom 1974; US EPA, 1978; Yakushiji *et al.* 1978; Wickizer *et al.* 1981; Baluja *et al.* 1982; Bush *et al.* 1984, 1985; Schwartz *et al.* 1983; Mes *et al.* 1984).

Experimental studies have used either chemical analysis of total PCBs or measurement of a single radiolabelled PCB congener to describe PCB transfer through the placenta or mammary gland in a number of mammalian species including mice (Oberg 1977; Masuda *et al.* 1979), rats (Curley *et al.* 1973; Takagi *et al.* 1976; Baker *et al.* 1977), rabbits (Villeneuve *et al.* 1971), guinea pigs (Brunstrom *et al.* 1982), cows (Platonov and Chen 1973), and nonhuman primates (Allen and Barsotti 1976; Allen *et al.* 1980; Bailey *et al.* 1980; Truelove *et al.* 1982). While these studies provide a general description of PCB transfer, they have assumed that all PCB congeners have similar pharmacokinetic properties.

Recent advances in gas chromatographic analysis of PCBs in biological samples permit reliable and accurate quantification of more than 72 peaks in

[1] To whom correspondence should be addressed.

[2] Present address is Department of Biology, Southeast Missouri State University, Cape Giradeau, Mo.

tissue microsamples (Bush and Barnard 1982; Bush *et al.* 1982). This study was designed to measure the accumulation of individual PCB congeners in the brains of developing rat pups to correlate dose, PCB accumulation, and behavior (Kostas *et al.*, submitted). In order to more clearly describe the pharmacokinetics of PCB congener transfer from maternal animals to fetuses and neonates samples of brain, fat, and liver from pups and dams and dam's milk were analyzed. Tissue samples from pups were collected at the end of gestation (birth, day 0) to describe the transfer of PCB congeners through the placenta and at the end of the period of lactation (weaning, day 21) to describe the transfer of congeners through the mammary gland and dam's milk. Aroclor® 1254 was chosen as the source of PCBs, because this mixture contains congeners with a wide distribution of chlorination numbers and chlorination substitution patterns.

Methods

Animals and PCB Exposure

Wistar rats (Griffin Laboratories, New York State Department of Health) were mated in the laboratory. Sperm positive vaginal smears indicated the beginning of gestation; at which time dams were provided one of four diets in spill-resistant food cups. The diets were prepared to contain 0 μg/g (control, no added PCBs), 3, 30, and 300 μg/g PCBs. The PCBs were added to the diets by spraying a thin layer of powdered, certified Purina Rat Chow (#5002) with one of three different acetone solutions containing an appropriate dilution of Aroclor 1254 (Monsanto). The 0 μg/g diet was sprayed with the same volume of acetone. The acetone was allowed to evaporate and the chow placed in covered stainless steel containers for mixing and storage. Tap water and diets were provided *ad libitum* and a 14:10 light:dark cycle was maintained. At birth (day 0), litters were culled to eight pups, approximately one-half males and one-half females. Representative culled pups from each exposure group were decapitated and tissues removed for analysis. Because of severe postnatal mortality in the 300 ppm exposure group, representative pups and dams were decapitated and tissues collected at day seven. At weaning (day 21), representative dams and pups were removed from the 0, 3, and 30 ppm exposure groups, decapitated, and tissues removed for analysis.

Tissue Samples

Brain, liver, and milk samples were obtained from the day 0 pups. Brain, fat, and liver samples were obtained from the day 7 and 21 pups and dams. Tissues were rapidly dissected, weighed, placed in chemically clean scintillation vials, and stored at −20°C for subsequent preparation and analysis. Milk samples were obtained from the dissected stomachs of newborn pups that had suckled.

Tissue Preparation and PCB Analysis

The procedures for analysis of PCBs in biological microsamples have been previously described (Bush and Barnard 1982). Briefly, the tissues or feed samples were lyopholized, reweighed, and ground to a powder. A 60-250 mg sample was transferred to a 10 ml beaker, 3–4 ml of hexane added, and the contents homogenized with a Tissuemizer (Tekmar Co, Cincinnati, OH). The suspension was allowed to settle for several min and the supernatant was pipetted into a 20 ml test tube. The homogenization was repeated twice and all solids were transferred to the test tube after the third homogenization. The test tube was sealed and the suspension allowed to settle overnight. The next day the supernatant was pipetted onto a 1 cm sodium sulfate layer on a 1 cm diameter glass column of 10 gm of dry 2% deactivated Florisil®. The column was eluted with hexane and 40 ml of the eluate was evaporated in a Kuderna-Danish evaporator to the desired volume. The extract was analyzed on a Hewlett-Packard 5840A chromatograph with a 40 m Apiezon L coated glass capillary column and ^{63}Ni electron capture detector. Chromatographic conditions were: helium linear flow rate of 20–30 cm/sec, initial temperature 70°C for one min, 10°C/min to 130°C, then 3°C/min to 230°C, and held for 10–20 min, make-up gas was argon:methane 95:5 at 40 ml/min. The data from the chromatograph were recorded on cassette tape for transfer to a PDP11/VAX computer for data handling and analysis with the aid of BMDP programs (Dixon 1981). Using this method of analysis and assuming a tissue sample size of 60 mg and clear chromatographic separation, a lower limit of detection for individual peaks was 1 ng/g.

Results

This report describes the accumulation of 67 different PCB congeners assigned to 47 clearly recognized chromatographic peaks. The data indicated that PCB maternal transfer and accumulation in maternal and fetal/neonatal tissues was not simply correlated with physical chemical properties, *i.e.*, partition coefficient or chlorination number, but rather with specific isomer structures.

PCB Congeners in Aroclor 1254 and Experimental Diets

Analysis of samples of the stock Aroclor 1254 and the four prepared diets confirmed that the PCB congener content of the diets was similar to that found in Aroclor® 1254 (Figure 1 and Table 1). The total PCB content of the control diet was 0.021 μg/g. The experimental diets contained PCB levels near the targeted concentrations: 3 μg/g = 2.52 μg/g, 30 μg/g = 25.8 μg/g, and 300 μg/g = 269 μg/g. For convenience, discussions of exposure relate to the 0, 3, 30, and 300 μg/g exposure groups.

The control diet contained low levels of a number of congeners (Table 1). The compounds found at highest concentration in Aroclor 1254 were those found in the control diet, *e.g.*, 2,4,2′,3′,4′, and 2,5,3′,4′. Several congeners that were not detected in the Aroclor 1254 samples were detected in the diets: 2,3,2′,3′ and 2,6,2′,3′,5′.

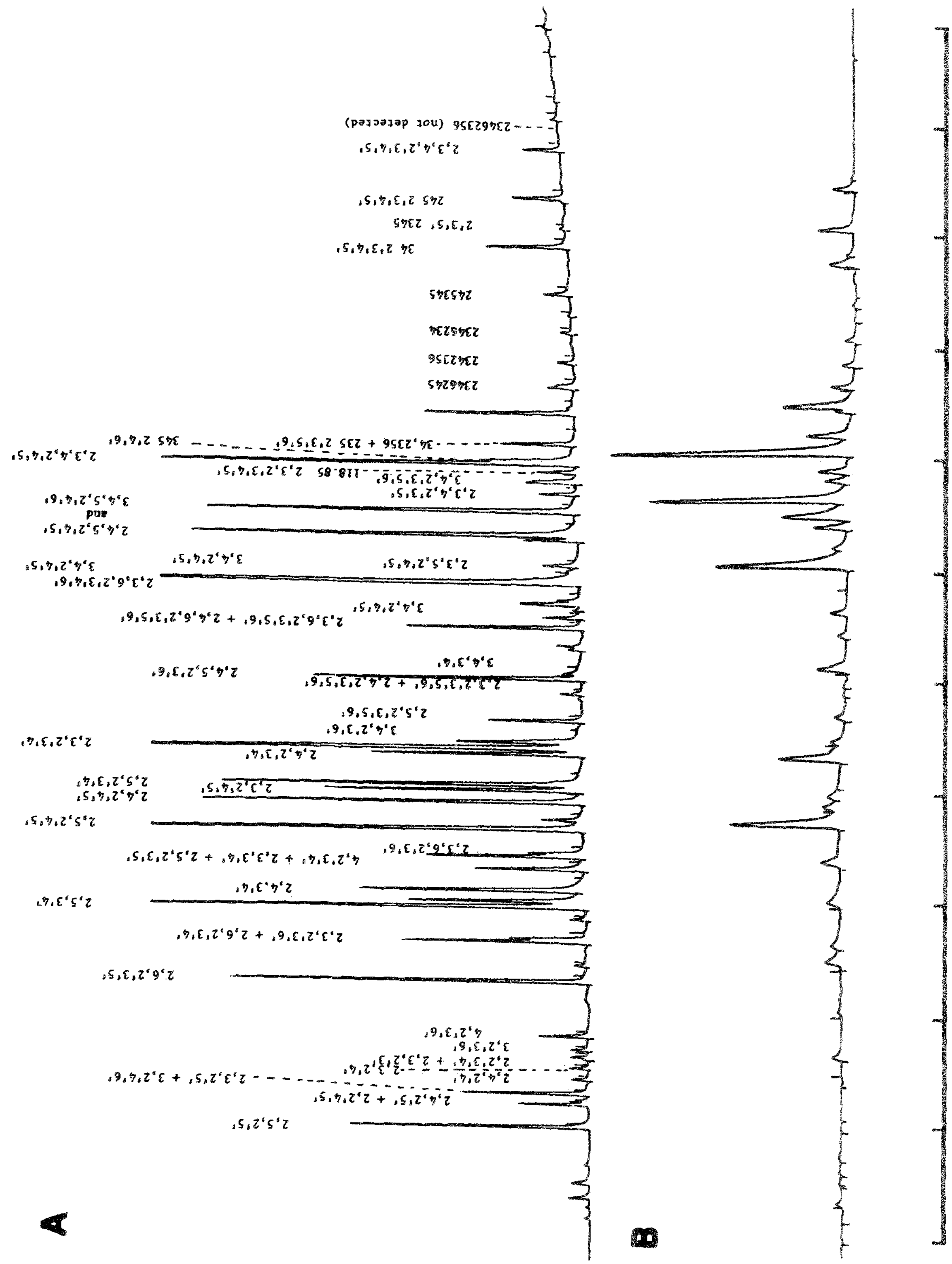


Fig. 1. Representative gas chromatograms from a 200 ng/g sample of Aroclor® 1254 (A) and adulterated rat chow fed to the 30 μg/gm exposure group (B). Chromatographic conditions are described in Materials and Methods

Table 1. PCB congener analysis of diets for the control, 3, 30, and 300 μg/g exposure groups[a]

Congener	Aroclor mix	Retention time	Aroclor® 1254		0 μg/g		3 μg/g		30 μg/g		300 μg/g	
			ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%
4,2′4′	16	24.25	*	—	*	—	*	—	163	0.63	*	—
25,2′5′	16,54	26.80	5	2.43	1	3.41	66	2.61	611	2.36	5684	2.12
24,2′5′	16,54											
2,2′4′5′	16,54	27.72	1	0.65	*	—	17	0.67	201	0.78	1674	0.62
23,2′5′	16,54	28.12	2	1.14	*	—	32	1.26	338	1.31	3048	1.14
3,2′4′6′												
24,2′4′	16,54	28.74	*	—	*	—	*	—	1561	6.04	374	0.14
23,2′4′	16,54	29.15	*	—	*	—	*	—	22	0.09	334	0.12
23,2′3′	16,54	29.58	*	—	3	13.66	13	0.50	*	—	1132	0.42
2,2′3′4′												
3,2′3′6′	16,54	29.91	*	—	*	—	*	—	*	—	957	0.36
4,2′3′6′	16,54	30.65	4	1.94	*	—	22	0.89	291	1.12	2709	1.01
26,2′3′5′	54,60	33.34	*	—	2	11.71	*	—	29	0.11	*	—
23,2′3′6′	54,60	35.33	4	1.99	*	—	56	2.22	654	2.53	6976	2.60
26,2′3′4′	54,60											
25,3′4′	54,60	37.47	17	8.54	1	5.37	178	7.04	1821	7.05	20072	7.47
24,3′4′	54,60	38.52	9	4.22	*	1.46	80	3.19	603	2.33	6294	2.34
23,3′4′	54,60											
25,2′3′5′	54,60											
236,2′3′6′	54,60	40.77	*	—	*	—	*	—	194	0.75	1801	0.67
25,2′4′5′	54,60	42.07	10	5.01	1	4.88	125	4.94	1141	4.41	11743	4.37
24,2′3′5′	54,60											
24,2′4′5′	54,60	44.02	8	4.02	1	5.85	90	3.57	857	3.32	8847	3.29
23,2′4′5′	54,60	44.39	8	3.92	1	2.44	92	3.64	843	3.26	9591	3.57
25,2′3′4′	54,60	44.78	18	8.98	1	4.88	208	8.27	1930	7.47	23877	8.89
24,2′3′4′	54,60	46.13	13	6.25	*	—	129	5.11	1285	4.97	16372	6.10
23,2′3′4′	54,60	46.41	23	11.36	3	12.20	258	10.24	2488	9.63	28521	10.62
34,2′3′6′	54,60											
25,2′3′5′6′	54,60	47.49	1	0.60	*	—	19	0.74	178	0.69	1845	0.69
23,2′3′5′6′	54,60	48.67	*	—	*	—	*	—	17	0.07	662	0.25
24,2′3′5′6′	54,60											
245,2′3′6′	54,60	49.31	4	1.94	1	2.93	54	2.14	493	1.91	5326	1.98
34,3′4′	48	49.19	*	—	*	—	*	—	*	—	490	0.18
23,2′3′4′6′	54,60	50.46	*	—	*	—	*	—	*	—	161	0.06

234,2′3′6′	54,60	51,38	3	1.69	*	—	43	1.72	387	1.50	4583	1.71
236,2′3′5′6′												
246,2′3′5′6′												
34,2′4′5′	54,60	53.28	*	—	*	—	3	0.10	70	0.27	2306	0.86
236,2′3′4′6′	54,60	53.79	19	9.43	1	6.34	219	8.70	1996	7.72	23319	8.68
235,2′4′5′	54,60	54.86	1	0.45	*	—	11	0.43	45	0.17	1716	0.64
34,2′3′4′	54,60	55.06	13	6.40	1	3.41	290	11.51	2564	9.92	22319	8.31
245,2′4′5′	54,60	55.94	8	4.12	1	4.39	98	3.89	1013	3.92	11138	4.15
25,2′3′4′5′												
234,2′3′5′	54,60	56.60	1	0.69	*	—	18	0.71	158	0.61	2351	0.88
34,2′3′5′6′		57.03										
23,2′3′4′5′	54,60	57.38	2	0.89	*	—	51	2.03	238	0.92	2261	0.84
234,2′4′5′	54,60	57.72	9	4.67	2	7.32	123	4.89	1200	4.64	14709	5.48
345,2′4′6′												
235,2′3′5′6′												
34,2′3′4′6′	54,60	58.41	3	1.34	*	—	34	1.35	291	1.12	4003	1.49
245,2′3′5′6′	60	58.93	*	—	*	—	*	—	*	—	42	0.02
234,2′3′4′												
235,2′3′4′6′	54,60	59.46	3	1.44	1	3.90	39	1.56	481	1.86	4627	1.72
245,2′3′4′6′	54,60	60.34	1	0.30	*	—	8	0.34	107	0.41	1006	0.37
246,2′3′4′5′												
2356,2′3′5′6′												
236,2′3′4′5′												
234,2′3′5′6′	54,60	61.19	*	—	*	—	2	0.07	31	0.12	423	0.16
234,2′3′4′6′	54,60	62.18	*	—	*	—	*	—	*	—	442	0.16
245,3′4′5′	54,60	63.49	*	—	*	—	*	—	*	—	1420	0.53
2345,2′3′5′6′												
2346,2′3′4′6′												
34,2′3′4′5′	54,60	65.02	4	1.94	*	—	50	1.97	415	1.61	5737	2.14
235,2′3′4′5′	54,60	65.52	*	—	*	—	*	—	*	—	239	0.09
245,2′3′4′5′	54,60	66.53	1	0.50	1	4.39	19	0.74	213	0.83	1705	0.63
234,2′3′4′5′	54,60	68.08	1	0.45	*	1.46	14	0.54	133	0.51	1518	0.57
2346,2′3′5′6′	54,60	68.56	*	—	*	—	*	—	*	—	26	0.01
Other congeners			*	—	*	—	*	—	455	1.76	577	0.21
Total			202	100.00	21	100.00	2520	100.00	25842	100.00	268521	100.00

[a]Each detected peak is indicated by the retention time. The congeners assigned to the peaks are indicated as well as the Aroclor® mixture in which they are usually found. Data for each peak is presented as the concentration in the sample (ng/g) as well as the relative concentration, e.g. contribution of a particular peak to total PCBs in sample (%). The asterisk (*) indicates that the congener was not detected

Table 2. PCB congener analysis of brain and liver samples of newborn pups in the 30 μg/g exposure group[a]

Congener	Retention time	Brain		Liver	
		ng/g	%	ng/g	%
4,2′4′	24.45	3	0.38	*	—
25,2′5′	26.80	4	0.51	*	—
24,2′5′					
2,2′4′5′	27.72	2	0.25	*	—
23,2′5′	28.12	4	0.51	62	0.53
3,2′4′6′					
24,2′4′	28.74	1	0.13	331	2.81
23,2′4′	29.15	*	—	*	—
23,2′3′	29.58	2	0.25	10	0.09
2,2′3′4′					
3,2′3′6′	29.91	8	1.02	125	1.06
4,2′3′6′	30.65	2	0.25	*	—
26,2′3′5′	33.34	*	—	*	—
23,2′3′6′	35.33	2	0.25	*	—
26,2′3′4′					
25,3′4′	37.47	1	0.13	8	0.07
24,3′4′	38.52	6	0.76	54	0.46
4,2′3′4′	40.55	5	0.63	20	0.17
23,3′4′					
25,2′3′5′					
236,2′3′6′	40.77	*	—	29	0.25
25,2′4′5′	42.07	7	0.89	68	0.58
24,2′3′5′					
24,2′4′5′	44.02	53	6.73	520	4.42
23,2′4′5′	44.39	6	0.76	259	2.20
25,2′3′4′	44.78	6	0.76	185	1.57
24,2′3′4′	46.13	129	16.37	1900	16.16
23,2′3′4′	46.41	6	0.76	831	7.07
34,2′3′6′					
25,2′3′5′6′	47.49	2	0.25	395	3.36
23,2′3′5′6′	48.67	1	0.13	299	2.54
24,2′3′5′6′					
245,2′3′6′	49.31	8	1.02	422	3.59
34,3′4′	50.19	*	—	*	—
23,2′3′4′6′	50.46	1	0.13	*	—
234,2′3′6′	51.38	1	0.13	*	—
236,2′3′5′6′	52.13	104	13.20	906	7.70
246,2′3′5′6′					
34,2′4′5′	53.28	9	1.14	92	0.78
236,2′3′4′6′	53.79	*	—	*	—
235,2′4′5′	54.86	10	1.27	153	1.30
34,2′3′4′	55.06	103	13.07	556	4.73
245,2′4′5′	55.94	58	7.36	490	4.17
25,2′3′4′5′					
234,2′3′5′	56.60	9	1.14	290	2.47
34,2′3′5′6′	57.03	—	—	—	—
23,2′3′4′5′	57.38	13	1.65	231	1.96
234,2′4′5′	57.72	99	12.56	1303	11.08
345,2′4′6′					
235,2′3′5′6′					
34,2′3′4′6′	58.41	30	3.81	407	3.46
245,2′3′5′6′	58.93	*	—	199	1.69
234,2′3′4′					
235,2′3′4′6′	59.46	28	3.55	446	3.79
245,2′3′4′6′	60.34	2	0.25	415	3.53
246,2′3′4′5′					
2356,2′3′5′6′					
236,2′3′4′5′					
234,2′3′5′6′	61.19	1	0.13	273	2.32

Table 2. (cont'd)

Congener	Retention time	Brain ng/g	%	Liver ng/g	%
234,2'3'4'6'	62.18	1	0.13	*	—
245,3'4'5'	63.49	*	—	*	—
2345,2'3'5'6'					
2346,2'3'4'6'					
34,2'3'4'5'	65.02	39	4.95	112	0.95
235,2'3'4'5'	65.52	*	—	173	1.47
245,2'3'4'5'	66.53	11	1.40	114	0.97
234,2'3'4'5'	68.08	11	1.40	83	0.71
2346,2'3'5'6'	68.56	*	—	*	—
Others		*	—	*	—
Total		788	100.00	11761	100.00

[a] Data is presented as described in Table 1

Transplacental Transfer of PCBs

Detectable levels of PCBs were found in all rat pups at birth, including those born to dams in the control group. A congener by congener analysis was performed in order to more precisely and comprehensively describe the accumulation of PCBs in tissue samples. The data were analyzed by first comparing accumulation of congeners in different tissues after exposure to a fixed concentration of PCBs and second by determining dose-dependent accumulation of congeners in brain tissue.

The comparison of congener accumulation in different tissues was made by using the tissue and feed concentrations of each congener to calculate an index of bioaccumulation determined as the logarithm of the fraction ((tissue concentration)/(feed concentration)) (log T/F). This method of analysis was used to determine the accumulation of each congener as a function of dose, *e.g.*, the concentration in the feed. The relative concentration of each congener has also been calculated as the percent of total detected PCBs (see Tables 2–4).

At least three observations can be made from the description of bioaccumulation of PCB congeners in dam's tissue samples (Figure 2A). First, bioaccumulation of individual PCB congeners did not correlate with their chromatographic retention time. While there may have been a general increase in the bioaccumulation of congeners in fat with respect to chromatographic retention time (Figure 2A,△—△), this relationship did not occur for brain and liver. A more careful inspection of the data from fat samples illustrated several series of sequentially eluting congeners with nearly the same variation in bioaccumulation as observed over the entire elution profile, *i.e.*, the 100-fold difference in bioaccumulation of [2,4,2',3',4'], [2,3,2',3',4' and 3,4,2',3',6'], and [2,5,2',3',5',6'] eluting between 46.13 and 47.49 min compared with the similar variation observed over the 35 min between the elution of [4,2',4'] and [2,3,5,2',3',4',6']. (In order to make the presentation of the data easier to read the congener(s) assigned to a single chromatographic peak are indicated by a pair of brackets).

A second observation is that different tissues accumulate PCB congeners to different degrees. In dams, congeners bioaccumulated to greater concentrations in fat than liver or brain (Figure 2A and Table 4). For those congeners detected in both brain and liver, bioaccumulation was similar. In fat samples most congeners were detected at concentrations near the feed concentrations, *e.g.*, log (T/F) = 0, six peaks with 10 congener assignments accumulated to tissue concentrations ≥ 10-fold greater than the feed concentrations: [2,3,6,2',3',4',6'], [2,3,5,2',4',5'], [2,3,4,2',4',5',3,4,5,2',4',6'] and [2,3,5,2',3',5',6'], [3,4,2',3',4',6'], [2,3,4,2',4',5',3,4,5,2',4',6'] and [2,3,5,2',3',5',6'], and [2,3,4,2',3',5',6']. Twenty of the 44 chromatographic peaks detected in brain and fat samples were not detected in liver samples. Of those congeners observed in liver most bioaccumulated to values 10 to 100 fold less than those for fat. In brain samples the number of congeners detected was similar to that observed with fat samples; however, bioaccumulation was 10-to 250-fold less.

Finally, eight peaks with 12 congener assignments were observed in tissue samples and not detected in feed samples: [2,3,2',3' and 2,2',3',4'], [3,2',3',6'], [2,4,3',4'], [2,3,2',3',4',6'], [2,4,5,2',3',5'6' and 2,3,4,2',3',4'], [2,3,4,2',3',4',6'], [2,4,5,3',4',5',2,3,4,5,2',3',5',6' and 2,3,4,6,2',3',4',6'], and [2,3,5,2',3',4',5'].

While the general pattern of congener bioaccumulation in newborn rat pups (Figure 2B) was similar to that observed in the dams (Figure 2A), some differences were apparent. (No comparison was made between fat tissue samples since newborn rat pups had little or no peritoneal fat). Bioaccumula-

Table 3. PCB congener analysis of brain, fat, and liver of 21 day old pups in dam's milk on the day of birth from the 30 μg/g exposure group[a]

Congener	Retention time	Brain		Fat		Liver		Milk	
		ng/g	%	ng/g	%	ng/g	%	ng/g	%
4,2′4′	24.45	*	—	*	—	*	—	*	—
25,2′5′	26.80	*	—	12	0.02	8	0.02	184	0.40
24,2′5′									
2,2′4′5′	27.72	5	0.17	*	—	3	0.01	19	0.04
23,2′5′	48.12	3	0.10	6	0.01	8	0.02	43	0.09
3,2′4′6′									
24,2′4′	28.74	10	0.33	24	0.04	101	0.22	151	0.33
23,2′4′	29.15	0	—	*	—	*	—	*	—
23,2′3′	29.58	4	0.13	*	—	*	—	*	—
2,2′3′4′									
3,2′3′6′	29.91	11	0.37	*	—	*	—	*	—
4,2′3′6′	30.65	5	0.17	*	—	*	—	*	—
26,2′3′5′	33.34	*	—	*	—	*	—	111	0.24
23,2′3′6′	35.33	*	—	*	—	*	—	12	0.03
26,2′3′4′									
25,3′4′	37.47	7	0.23	*	—	*	—	46	0.10
24,3′4′	38.52	30	1.00	125	0.19	313	0.686	1042	2.28
4,2′3′4′	40.55	29	0.97	106	0.16	251	0.55	873	1.91
23,3′4′									
25,2′3′5′									
236,2′3′6′	40.77	*	—	*	—	8	0.02	*	—
25,2′4′5′	42.07	13	0.43	64	0.10	173	0.38	559	1.22
24,2′3′5′									
24,2′4′5′	44.02	301	10.03	6864	10.56	4725	10.27	3947	8.63
23,2′4′5′	44.39	13	0.43	91	0.14	238	0.52	333	0.73
25,2′3′4′	44.78	11	0.37	63	0.10	168	0.37	476	1.04
24,2′3′4′	46.13	351	11.70	13448	20.69	4560	9.91	8383	18.34
23,2′3′4′	46.41	*	—	0	—	13	0.03	*	—
34,2′3′6′									
25,2′3′5′6′	47.49	1	0.03	1728	2.66	60	0.13	124	0.27
23,2′3′5′6′	48.67	*	—	8	0.01	3	0.01	42	0.09
24,2′3′5′6′									
245,2′3′6′	49.31	3	0.10	26	0.04	78	0.17	480	1.05
34,3′4′	49.19	1	0.03	*	—	38	0.08	*	—
23,2′3′4′6′	50.46	4	0.13	4594	7.07	149	0.32	221	0.48
234,2′3′6′	51.38	2	0.07	13	0.02	2	—	312	0.68
236,2′3′5′6′	52.13	415	13.83	8411	12.94	7616	16.56	5314	11.62
246,2′3′5′6′									
34,2′4′5′	53.28	12	0.40	103	0.16	569	1.24	452	0.99
236,2′3′4′6′	53.79	*	—	*	—	*	—	*	—
235,2′4′5′	54.86	49	1.63	1132	1.74	763	1.66	553	1.21
34,2′3′4′	55.06	377	12.57	6261	9.63	4781	10.39	4561	9.98
245,2′4′5′	55.94	258	8.60	5229	8.04	3940	8.57	3560	7.79
25,2′3′4′5′									
234,2′3′5′	56.60	39	1.30	153	0.24	705	1.53	653	1.43
34,2′3′5′6′									
23,2′3′4′5′	57.38	53	1.77	198	0.30	925	2.01	816	1.78
234,2′4′5′	57.72	404	13.47	8144	12.53	6016	13.08	5346	11.69
345,2′4′6′									
235,2′3′5′6′									
34,2′3′4′6′	58.41	98	3.27	1025	1.58	1637	3.56	1438	3.15
245,2′3′5′6′	58.93	1	0.03	*	—	65	0.14	29	0.06
234,2′3′4′									
235,2′3′4′6′	59.46	107	3.57	4972	7.65	1567	3.41	1788	3.91
245,2′3′4′6′	60.34	11	0.37	40	0.06	230	0.50	229	0.50
246,2′3′4′5′									
2356,2′3′5′6′									
234,2′3′5′6′	61.19	12	0.40	35	0.05	258	0.56	136	0.30

Table 3. (cont'd)

Congener	Retention time	Brain		Fat		Liver		Milk	
		ng/g	%	ng/g	%	ng/g	%	ng/g	%
234,2'3'4'6'	62.18	9	0.30	32	0.05	142	0.31	154	0.34
245,3'4'5'	63.49	9	0.30	38	0.06	290	0.63	187	0.41
2345,2'3'5'6'									
2346,2'3'4'6'									
34,2'3'4'5'	65.02	152	5.07	1523	2.34	3396	7.38	1783	3.90
235,2'3'4'5'	65.52	5	0.17	14	0.02	74	0.16	126	0.28
245,2'3'4'5'	66.53	49	1.63	135	0.21	851	1.85	615	1.35
234,2'3'4'5'	68.08	47	1.57	135	0.21	749	1.63	588	1.29
2346,2'3'5'6'	68.56	*	—	*	—	*	—	27	0.06
Others		89	2.97	248	0.38	527	1.15	4	0.01
Totals		3000	100.00	65000	100.00	46000	100.00	45717	100.00

[a] Data is presented as described in Table 1

tion of PCB congeners in the newborn pup liver samples was different; only 11 peaks were not detected and bioaccumulation was greater. Ten peaks with 16 congener assignments were present at concentrations ⩾ than those measured in the feed: [2,4,2',3',4'], [2,5,2',3',5',6'], [2,3,2',3',5',6' and 2,4,2',3',5',6'], [2,3,6,2',3',4',6'], [2,3,5,2',4',5'], [2,3,4,2',3',5' and 3,4,2',3',5',6'], [2,3,4,2',4',5', 3,4,5,2',4',6' and 2,3,5,2',3',5',6'], [3,4,2',3',4',6'], [2,4,5,2',3',4',6',2,4,6,2',3',4',5' and 2,3,5,6,2',3',5',6'], and [2,3,4,2',3',5',6'].

In contrast to liver, the pattern of bioaccumulation in newborn rat pup brains was very similar to that observed in the dams (Figure 2A and B (o—o)). The maximum bioaccumulation was similar log T/F = −1, and the bioaccumulation of individual congeners was nearly identical.

Transmammary Transfer of PCB Congeners and Further Accumulation in Preweaning Rats

Transmammary accumulation of PCBs was determined by measuring the PCB content of tissues from rat pups at weaning (day 21). The PCB content of tissues at this time represented the total accumulation during both prenatal and the first 21 days of postnatal life, not just accumulation during the period of postnatal life.

In order to more clearly determine differences between transplacental and transmammary routes of exposure, bioaccumulation of congeners was calculated as a function of both feed and milk PCB congener concentrations. The difference between the feed bioaccumulation index, log (T/F), and milk bioaccumulation index, log ((tissue concentration)/(milk concentration)) (T/M), should be a function of both PCB accumulation in the milk and subsequent accumulation in the tissues of the young animals.

Panel A, Figure 3 illustrates the bioaccumulation of PCB congeners in the dams' milk. Bioaccumulation of congeners in the milk most closely resembled that observed in dams' fat samples and not liver or brain. Twenty-one peaks were detected at concentrations in the milk ⩾ than feed concentrations. Three peaks with four congener assignments were not detected in the feed and the milk: [2,3,2',3' and 2,2',3',4'], [3,2',3',6'], and [3,2',3',4']. Another five peaks with six congener assignments were not detected in the milk samples: [4,2',4'], [2,3,2',4'], [4,2',3',6'], [2,3,6,2',3',6'], [2,3,2',3',4', and 3,4,2',3',6']. Six peaks with nine congener assignments were detected in milk samples and not in the feed: [2,3,2',3',4',6'], [2,4,5,2',3',5',6' and 2,3,4,2',3',4'], [2,3,4,2',3',4',6'], [2,4,5,3',4',5', 2,3,4,5,2',3',5',6', and 2,3,4,6,2',3',4',6'], [2,3,5,2',3',4',5'], and [2,3,4,6,2',3',5',6']. There was no clear correlation of PCB congener bioaccumulation and retention time.

Bioaccumulation of PCB congeners in weaning rat pup liver samples (Figure 3B) permitted PCB congeners to be assigned to three groups. The first group, "a", had retention times of 24.00–38.00 min. Of the possible 12 peaks with 16 congener assignments eluting in this group only four peaks were detected having six congener assignments: [2,5,2',5' and 2,4,2',5'], [2,2',4',5'], [2,3,2',5' and 3,2',4',6'], and [2,4,2',4']. Each of these peaks had a larger index of bioaccumulation when calculated as a function of milk concentrations rather than feed concentrations. Thus all of the congeners in this group were either not found in milk or the dose was reduced.

The second group of congeners, designated "b" in Figure 3B, had retention times of 38.00 to 52.00 min. When the bioaccumulation of these congeners was calculated as a function of feed concentrations,

Table 4. PCB congener analysis of brain, fat and liver samples of dams exposure at the time of pup weaning (day 21) in the 30 μg/g exposure group[a]

Congener	Retention time	Brain		Fat		Liver	
		ng/g	%	ng/g	%	ng/g	%
4,2′4′	24.45	5	0.31	45	0.05	*	—
25,2′5′	26.80	12	0.74	541	0.55	*	—
24,2′5′							
2,2′4′5′	27.72	3	0.18	93	0.09	*	—
23,2′5′	28.12	4	0.25	174	0.18	*	—
3,2′4′6′							
24,2′4′	28.74	5	0.31	242	0.24	22	0.57
23,2′4′	29.15	*	—	40	0.04	*	—
23,2′3′	29.58	2	0.12	*	—	*	—
2,2′3′4′							
3,2′3′6′	29.91	3	0.18	119	0.12	*	—
4,2′3′6′	30.65	1	0.06	*	—	*	—
26,2′3′5′	33.34	*	—	*	—	*	—
23,2′3′6′	35.33	2	0.12	196	0.20	*	—
26,2′3′4′							
25,3′4′	37.47	3	0.18	925	0.94	*	—
24,3′4′	38.52	32	1.97	1548	1.57	*	—
4,2′3′4′	40.55	51	3.13	1051	1.06	22	0.57
23,3′4′							
25,2′3′5′							
236,2′3′6′	40.77	*	—	447	0.45	*	—
25,2′4′5′	42.07	19	1.17	1487	1.51	9	0.23
24,2′3′5′							
24,2′4′5′	44.02	120	7.38	7105	7.19	218	5.65
23,2′4′5′	44.39	26	1.60	1572	1.59	217	5.62
25,2′3′4′	44.78	17	1.04	2671	2.70	44	1.14
24,2′3′4′	46.13	182	11.19	9116	9.23	758	19.64
23,2′3′4′	46.41	12	0.74	1623	1.64	*	—
34,2′3′6′							
25,2′3′5′6′	47.49	6	0.37	595	0.60	76	1.97
23,2′3′5′6′	48.67	3	0.18	356	0.36	*	—
24,2′3′5′6′							
245,2′3′6′	49.31	26	1.60	1950	1.97	83	2.15
34,3′4′	50.19	*	—	136	0.14	*	—
23,2′3′4′6′	50.46	6	0.37	730	0.74	154	3.99
234,2′3′6′	51.38	13	0.80	777	0.79	*	—
236,2′3′5′6′	52.13	221	13.58	10918	11.05	558	14.46
246,2′3′5′6′							
34,2′4′5′	53.28	27	1.66	824	0.83	30	0.78
236,2′3′4′6′	53.79	*	—	*	—	*	—
235,2′4′5′	54.86	23	1.41	1715	1.74	24	0.62
34,2′3′4′	55.06	137	8.42	7524	7.62	284	7.36
245,2′4′5′	55.94	122	7.50	9982	10.10	156	4.04
25,2′3′4′5′							
234,2′3′5′	56.60	18	1.11	1498	1.52	20	0.52
34,2′3′5′6′	57.03	*	—	*	—	—	—
23,2′3′4′5′	57.38	29	1.78	1183	1.20	47	1.22
234,2′4′5′	57.72	165	10.14	12495	12.65	333	8.63
345,2′4′6′							
235,2′3′5′6′							
34,2′3′4′6′	58.42	68	4.18	3043	3.08	153	3.96
245,2′3′5′6′	58.93	2	0.12	140	0.14	*	—
234,2′3′4′							
235,2′3′4′6′	59.46	43	2.64	7949	8.05	119	3.08
245,2′3′4′6′	60.34	11	0.68	801	0.81	15	0.39
246,2′3′4′5′							
2356,2′3′5′6′							
234,2′3′5′6′	61.19	7	0.43	513	0.52	*	—

Table 4. (cont'd)

Congener	Retention time	Brain		Fat		Liver	
		ng/g	%	ng/g	%	ng/g	%
234,2'3'4'6'	62.18	14	0.86	296	0.30	*	—
245,3'4'5'	63.49	6	0.37	277	0.28	24	0.62
2345,2'3'5'6'							
2346,2'3'4'6'	65.02	64	3.93	159	0.16	270	7.00
235,2'3'4'5'	65.52	3	0.18	2282	2.31	*	—
245,2'3'4'5'	66.53	28	1.72	1721	1.74	21	0.54
234,2'3'4'5'	68.08	25	1.54	92	0.09	91	2.36
2346,2'3'4'6'	68.56	1	0.06	*	—	*	—
Others		60	3.69	1835	1.86	111	2.88
Totals		1627	100.00	98786	100.00	3859	100.00

[a] Data is presented as described in Table 1

there were nearly 100-fold differences among the indices of bioaccumulation for a number of these congeners. The largest variations included peaks with congener assignments of two pentachlorobiphenyl families. The first family included three peaks: [2,5,2',4',5' and 2,4,2',3',5'], [2,4,2',4',5'], and [2,3,2',4',5']. These peaks represented 173, 4725, and 238 ng/gm tissue sample, respectively. The second family also included three peaks with four congener assignments: [2,5,2',3',4'], [2,4,2',3',4'], and [2,3,2',3',4' and 3,4,2',3',6']. These latter peaks were detected at concentrations of 168, 4560, and 13 ng/g tissue sample, respectively.

When the "b" group of congeners was analyzed as a function of PCB congener concentrations in milk samples, an attenuation of the extremes in bioaccumulation was observed. Thus, for those congeners found at highest concentration there was a reduction in bioaccumulation while for those found at lower concentrations there was an increased bioaccumulation.

The third group of congeners had retention times between 52.00 and 72.00 min. These "c" group congeners had nearly equal bioaccumulation indices. When bioaccumulation was calculated as a function of feed *vs* milk congener concentrations, there was somewhat more variability, similar to the "b" group.

Bioaccumulation of PCB congeners in fat (Figure 3C) was similar to that observed in liver in several ways. First, congeners in the "a" group had nearly the same bioaccumulation pattern. One additional congener was not detected in the fat samples: [2,2',4',5']. In the "b" group greater than 100-fold differences in bioaccumulation were observed for the 2',4',5' and 2',3',4' pentachlorobiphenyl families described for the liver samples. When bioaccumulation was calculated as a function of milk congener concentrations, there was an attenuation of this variability. In the "c" group of congeners, there was somewhat greater variability in the bioaccumulation of congeners when compared to liver samples but, similar to liver, the milk bioaccumulation indices were less than those calculated from feed. The bioaccumulation of congeners was generally similar in liver and fat with bioaccumulation indices similar to those calculated for milk.

Bioaccumulation of congeners in brain samples from weanling animals was less than that observed in liver and fat. The pattern of bioaccumulation was most similar to that described for liver. Congeners in the "a" group were present with similar indices of bioaccumulation. Congeners in the "b" group showed the same variation; however, the highest values of bioaccumulation were approximately 10-fold lower than those observed with the liver samples. When bioaccumulation was calculated as a function of congener concentrations in milk, an attenuation of the variability was observed. In the "c" group, the pattern of bioaccumulation of congeners was nearly the same as that seen in liver; however, the indices of bioaccumulation were again approximately 10 fold lower.

PCB Accumulation in Brain

Detectable levels of PCBs were found in brain samples from all rat pups, including those born to dams in the control group. The total PCB content of brains increased as a function of dose in both newborn (day 0) and weanling (day 21) tissue samples; (however, even in the 300 μg/g exposure group) there was no indication of brain tissues reaching a saturating concentration of PCBs. The dose-dependent accumulation of total PCBs in the weanling brain samples was nearly parallel to newborn samples, but was shifted to lower concentrations indicating a significantly greater total PCB ac-

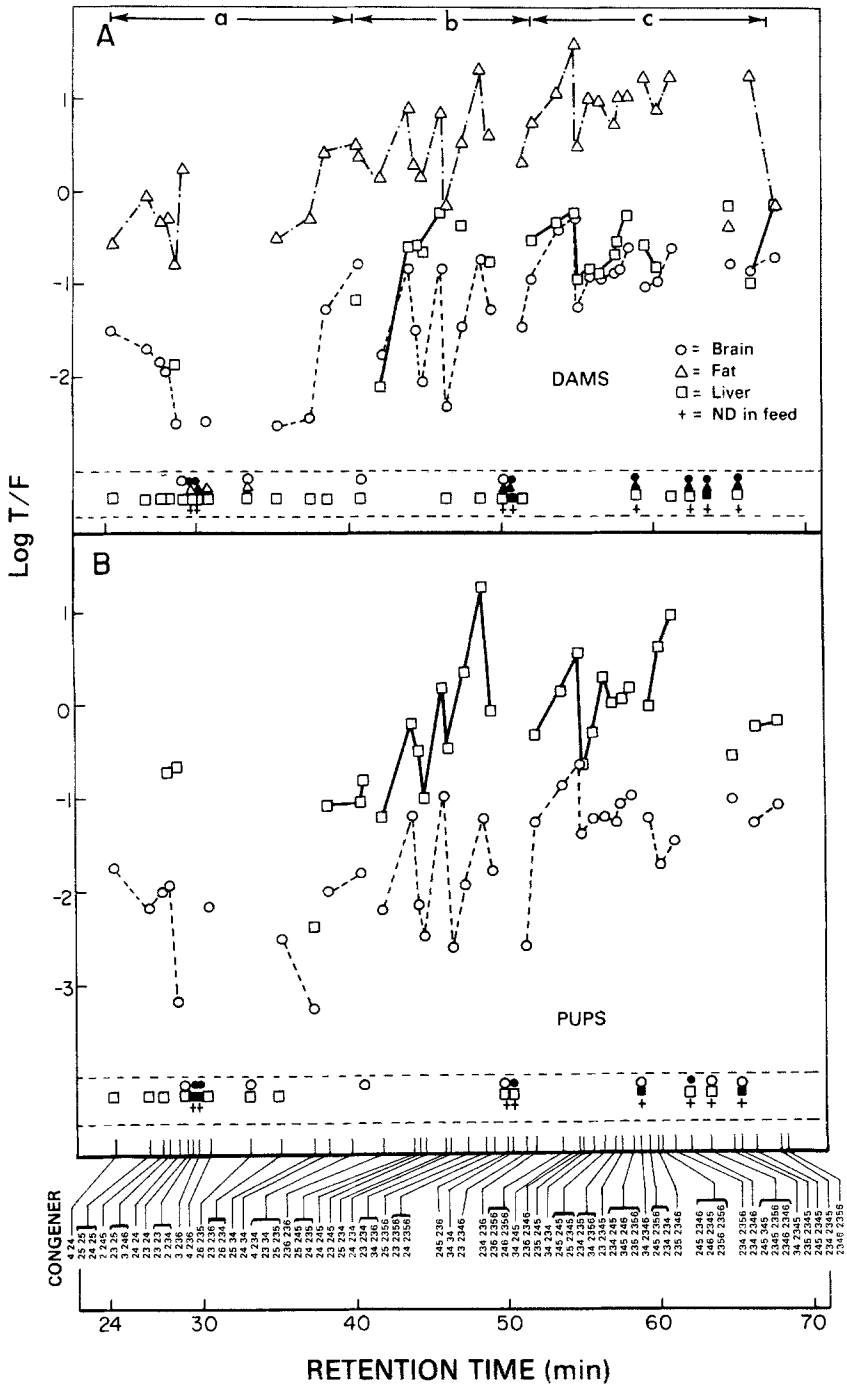


Fig. 2. Bioaccumulation of PCB congeners in tissue samples from dams at weaning (A) and pups at birth (day 0) (B). The data are plotted as the feed index of bioaccumulation, log (tissue concentration/feed concentration) vs chromatographic retention time (min). The structural assignments of the chromatographic peaks are given. Brain (o), fat (△), and liver (□) samples are presented for dams and brain and liver for pups. An open symbol or "+" appearing between the broken lines at the bottom of each panel indicate that the congener was not detected in the tissue or feed, respectively. A filled symbol indicates the congener was detected in the tissue sample. See Tables 1 and 2 for the data used to compute the bioaccumulation indices. Congeners were assigned to one of three subgroups, a, b, or c, as indicated at the top of panel A and described for Figure 3 in the text

cumulation at the same exposure level. For instance, in samples from the 30 μg/g exposure group, the mean brain PCB concentration was more than 350% greater at day 21 than at day 0. The increased accumulation may reflect the additional 21 days of exposure, an increased transfer of PCBs through the mammary gland and milk, or a combination of both.

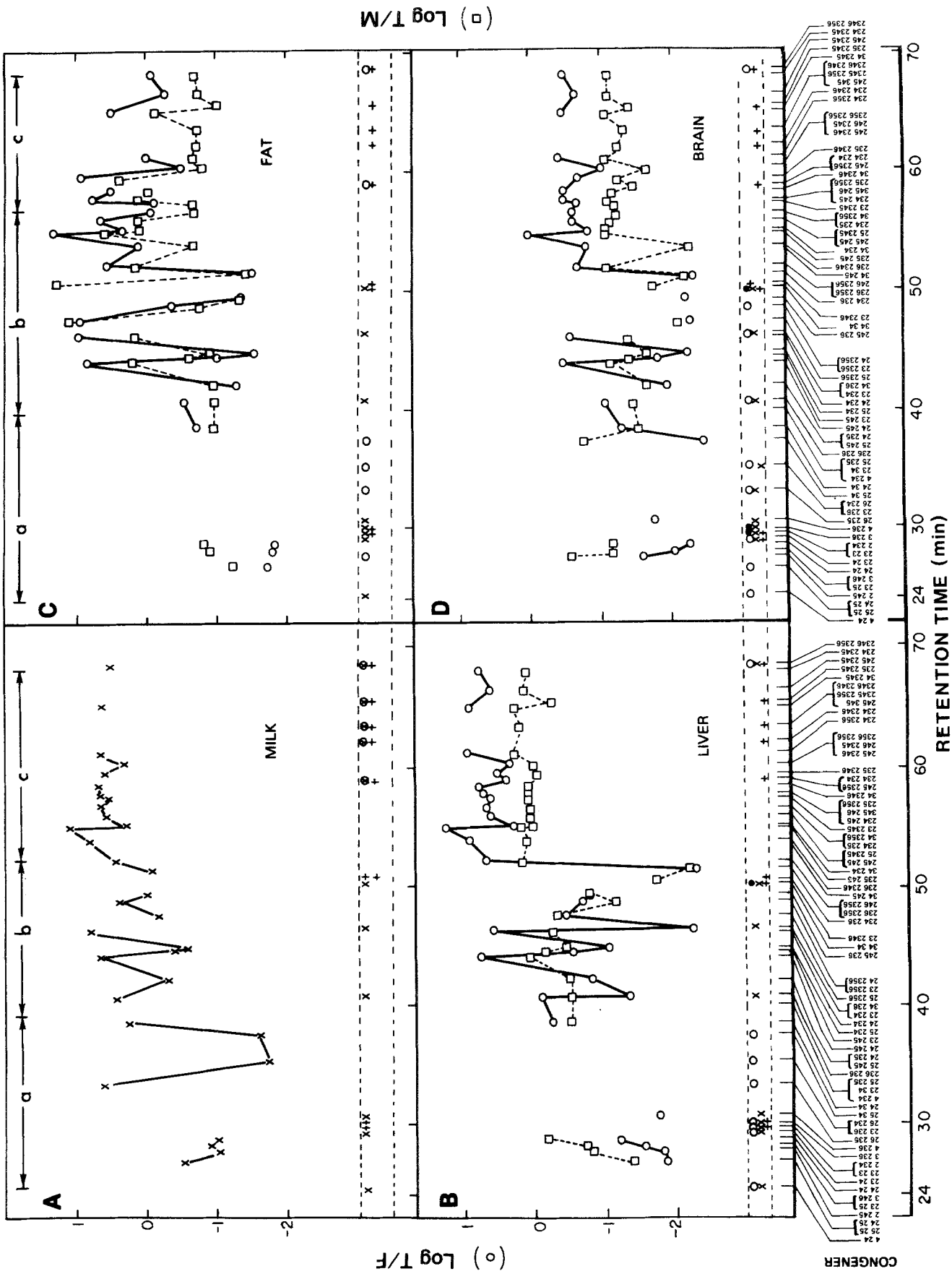
The dose-dependent accumulation of individual PCB congeners from newborn and weanling brain samples is presented in Figures 4 and 5, respectively. For the purpose of presentation, the data are shown using the congener groupings introduced when describing bioaccumulation (see Figures 2 and 3). The data were plotted on three-dimensional axes to permit correlation of tissue concentration (log tissue concentration; y-axis), dose (log feed concentration; x-axis), and chromatographic retention time (z-axis). Data from the newborn samples were obtained from all exposure groups. With one

exception, the "a" group congeners were not detected in samples from the control or 3 μg/g exposure group: [4,2',4'] (Figure 4A). All but two congeners were detected in samples from the 30 μg/g exposure group: [2,4,2',4'] and [2,6,2',3',5',6']. All the congeners in the "a" group of samples from the 30 μg/g exposure group were detected at low concentrations, <10 ng/g, and were not detected in samples from the 300 μg/g exposure group. Congeners in this group represented <4% of the total PCB content in brain samples (see Table 2).

The "b" group congeners had significant differences in dose-dependent accumulation. One congener was detected in samples from the control group: [2,4,2',4',5']. Two congeners were not detected: [2,3,6,2',3',6'] and [3,4,3',4']. Three peaks with five congener assignments had accumulation patterns similar to that observed in the "a" group: [2,3,2',3',4' and 3,4,2',3',6'], [2,3,2',3',5',6' and 2,4,2',3',5',6'], and [2,3,2',3',4',6']. The other congeners all had dose-dependent increases in tissue concentration. No correlation between retention time and the dose-dependent accumulation of congeners in this group was observed. This was seen most clearly when accumulation of the congeners in the 2',4',5'- and 2',3',4'-pentachlorobiphenyl families previously described above was observed. In the 2',3',4' family, 2,5,2',3',4', retention time = 44.78, was not detected in samples from the control and 3 μg/g exposure group and only accumulated to concentrations of 6 and 10 ng/g in the 30 and 300 μg/g exposure groups, respectively. The next eluting congener, 2,4,2',3',4', retention time = 46.13, was not detected in control samples but showed a nearly linear dose-response relationship reaching a concentration of 624 ng/g in the 300 μg/g exposure group. The next eluting peak, [2,3,2',3',4' and 3,4,2',3',6'], retention time = 46.41, was only detected in the 30 μg/g exposure group similar to the "a" group congeners. The corresponding congeners of the 2',4',5' family of pentachlorobiphenyls showed a similar pattern of accumulation. In general, the congeners in the "b" groups were also detected at low concentrations, <10 ng/g; however, two congeners, [2,4,2',4',5'] and [2,4,2',3',4'], were detected at high concentrations, 53 and 129 ng/g, respectively. These two congeners account for approximately 23% of the total PCBs detected in the brain samples from the 30 μg/g exposure group. The other congeners in this group accounted for 6% of the total (see Table 2). The congeners in the "c" group generally accumulated in a dose-dependent fashion. There were four obvious exceptions. Three congeners were not detected: [3,4,2',3',4',6'], [2,3,4,2',3',4',6'], and [2,3,4,6,2',3',4',5']. One congener was detected in the control group, found in lower concentrations as the dose increased, and not detected in samples from the 300 μg/g exposure group: [2,3,6,2',3',4',6']. Three peaks with seven congener assignments were detected in samples from the control group and accumulated to increasing concentrations with dose: [2,3,6,2',3',5',6' and 2,4,6,2',3',5',6'], [2,4,5,2',4',5' and 2,5,2',3',4',5'], and [2,3,4,2',4',5',3,4,5,2',4',6' and 2,3,5,2',3',5',6']. One congener was detected only in the 300 μg/g exposure group: [2,3,4,2',3',4',6']. One congener, [3,4,2',3',4'], was detected in samples from the 3 μg/g exposure group and had a nearly linear dose-response relationship with a final concentration of 389 ng/g. The remaining 11 peaks with 19 congener assignments were all detected in samples from the 30 μg/g exposure group and with increasing concentrations in the 300 μg/g exposure group. The congeners in the "c" group were, in general, detected at higher concentrations, ≥10 ng/g, and accumulated in a dose-dependent fashion. Four peaks representing nine congeners were detected at concentrations ≥50 ng/g: [2,3,6,2',3',5',6' and 2,4,6,2',3',5',6'], [3,4,2',3',4'], [2,4,5,2',4',5' and 2,5,2',3',4',5'], and [2,3,4,2',4',5',3,4,5,2',4',6' and 2,3,5,2',3',5',6']. The congeners found in these four peaks accounted for approximately 46% of the brain PCB content in the 30 μg/g exposure group. The remaining congeners accounted for approximately 21%. Thus, this group constituted the majority of PCB congeners detected in brains at birth.

The dose-dependent accumulation of PCB congeners in brain samples from weanling (day 21) rat pups was obtained only from the control, 3, and 30 μg/g exposure groups due to the severe mortality of pups in the 300 μg/g exposure group. The "a" group congeners had a pattern of accumulation similar to the newborn samples. One congener was detected in samples from the control group: [2,2',4',5']. One congener was detected in samples from the 3 μg/g exposure group: [2,4,2',4']. Four peaks representing 6 congeners were not detected at any dose: [2,5,2',5' and 2,4,2',5'], [2,3,2',4'], [2,6,2',3',5'] and [2,3,2',3',6' and 2,6,2',3',4']. The other congeners in this group were all detected in samples from the 30 μg/g group. All the congeners in the "a" group were detected at low concentrations, ≤11 ng/g and only represented 1.5% of the total PCB content in the brain samples in the 30 μg/g exposure group (see Table 3).

The "b" group of congeners showed one striking difference when compared to the accumulation pattern observed with samples from newborn animals. Six peaks with 11 congener assignments were detected in brain samples from the control group:


(□) Log T/M
(○) Log T/F
A
MILK
B
LIVER
C
FAT
D
BRAIN
a
b
c
CONGENER
RETENTION TIME (min)

[2,4,3',4'], [4,2',3',4',2,3,3',4' and 2,5,2',3',5'], [2,5,2',4',5' and 2,4,2',3',5'], [2,4,2',4',5'], [2,4,2',3',4'], and [2,4,2',3',4',6', 2,5,2',3',4',6' and 2,4,5,2',3',6']. The congeners represented by this last peak did not accumulate to a greater concentration at the 3 μg/g dose. The other congeners detected in the control samples all had dose-dependent increases in brain concentration. Three peaks representing five congeners were not detected: [2,3,6,2',3',6'], [2,3,2',3',4' and 3,4,2',3',6'], and [2,3,2',3',5',6' and 2,4,2',3',5',6']. All of the other congeners in this group had increased in brain concentration between 3 and 30 μg/g. Again, at this age, there was no correlation between brain accumulation and chromatographic retention time for congeners in this group. The same relationship for the two families of pentachlorobiphenyls observed with the newborn samples was observed with these samples from weanling animals; 2,4,2',3',4' and 2,4,2',4',5' were found at higher concentrations than their 2,3 or 2,5 congeners. Nearly one-half of the congeners in this group were accumulated to intermediate concentrations, 10 to 30 ng/g, and one-half were either undetected or detected at low concentrations. Two congeners were detected at high concentrations, ≥300 ng/g: [2,4,2',3',4'] and [2,4,2',4',5']. These two congeners accounted for approximately 20% of the total brain congeners in samples from the 30 μg/g exposure group. The other congeners only account for 3.5% of the total.

The "c" group of congeners had similar dose-dependent accumulation. Only one congener was not detected at any dose: [2,3,4,5,2',3',5',6']. Three peaks with six congener assignments were only detected in the 30 μg/g exposure group: [2,4,5,2',3',5',6' and 2,3,4,2',3',4'], [2,4,5,3',4',5', 2,3,4,5,2',3',5',6' and 2,3,4,6,2',3',4',6'], and [2,3,5,2',3',4',5']. Ten peaks with 17 congener assignments were detected in samples from the control animals. Six peaks with 10 congener assignments were first detected in samples from the 3 μg/g exposure group. All of the congeners in these 16 peaks had nearly linear dose-dependent accumulation. It is interesting to note that 2,3,6,2',3',4',6', the congener that was found at decreasing concentrations with dose in the newborn samples, was in this latter group. Most of the congeners in this group were detected at concentrations ≥9 ng/g. Six peaks with 10 congener assignments were found at concentrations >100 ng/g: [2,3,6, 2',3',5',6' and 2,4,6,2',3',5',6'], [3,4,2',3',4'], [2,4,5,2',4',5' and 2,5,2',3',4',5'], [2,3,4,2',4',5',3,4,5,2',4',6' and 2,3,5,2',3',5',6'], [2,3,5,2',3',4',6'], and [3,4,2',3',4',5']. These 10 congeners represented more than 57% of the total brain content in the 30 μg/g exposure group. The remaining congeners represented 13% of the total. Thus, as with the newborn samples, this group represents the majority of the PCB congeners found in the brain.

Discussion

The present study was designed to determine the accumulation of PCB isomers and congeners in rat pups that had been continuously exposed to PCBs from conception to birth or weaning. Analysis of this data has been focused on two issues: (1) correlation of PCB congener and isomer structures and accumulation and (2) differences in transport of PCBs via the placenta and mammary gland in milk.

Correlation of Structure and Bioaccumulation of PCB Congeners and Isomers

The chemical data are sufficient to make several observations concerning PCB congener accumulation. First, PCB congeners can be separated into three groups according to their accumulation in pups' and dams' tissues. Such a classification could be related to either chromatographic retention time or molecular structure (Gage and Holm 1976; Goldstein *et al.* 1977). Correlation with retention time implies that accumulation is related to physicochemical properties associated with partition coefficient (Bush *et al.* 1985), while correlation with molecular structure implies a more specific molecular interaction, *e.g.*, substrate-enzyme. The "a" group of congeners had retention times <38 min and, except for two peaks that had structural assignments of pentachlorobiphenyls: [2,6,2',3',5' and 2,3,2',3',6'] and [2,6,2',3',6'], all had lower numbers of chlorine substitutions. The "b" group of congeners had retention times between 38 and 52 min. This group consisted primarily of tetra-, penta-, and hexachlorobiphenyls. The "c" group of congeners had retention times longer than 52 min. Two peaks had structure assignments of penta-

Fig. 3. Bioaccumulation of PCB congeners in samples of dams' milk (A) and tissue samples from weanling pups' liver (B), fat (C), and brain (D). The data are presented as a function of feed (o—o) and milk (□—□) congener concentrations. An open symbol or "x" between the lines at the bottom of each panel indicates the congener was not detected in tissue or milk samples. A filled symbol and "+" indicates the peak was detected. See Tables 1 and 3 for the data used to compute the bioaccumulation indices. Congeners were assigned to one of three subgroups, a, b, or c, as indicated at the top of panels A and C and described in the text

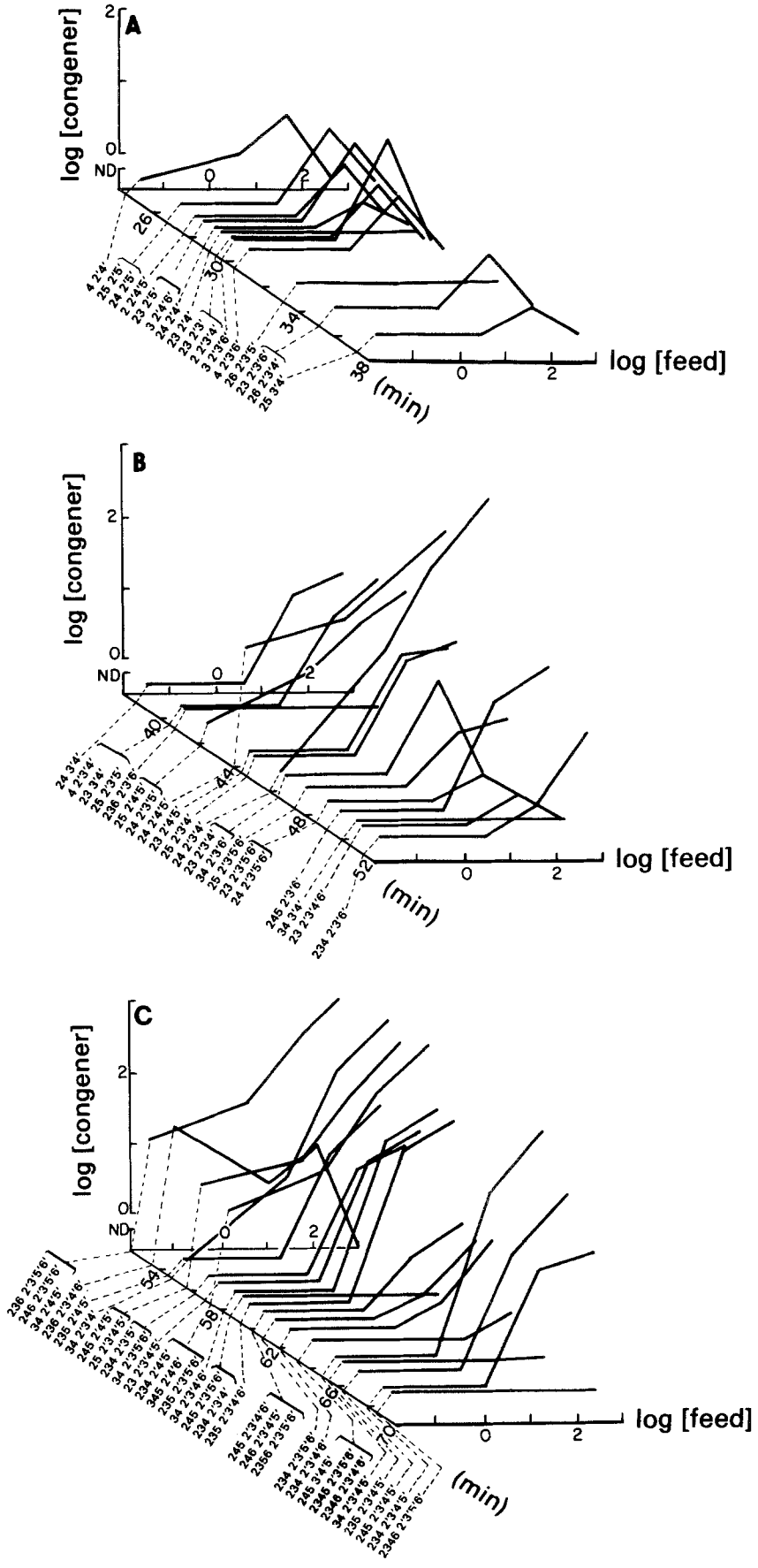


Fig. 4. Dose-response curves for accumulation of PCB congeners in brain tissue samples from weanling pups (day 0). Data are plotted as log tissue concentration (y-axis), log feed concentration (x-axis), and retention time (z-axis). The data presented on each panel have been grouped according to the designations indicated in Figures 2 and 3

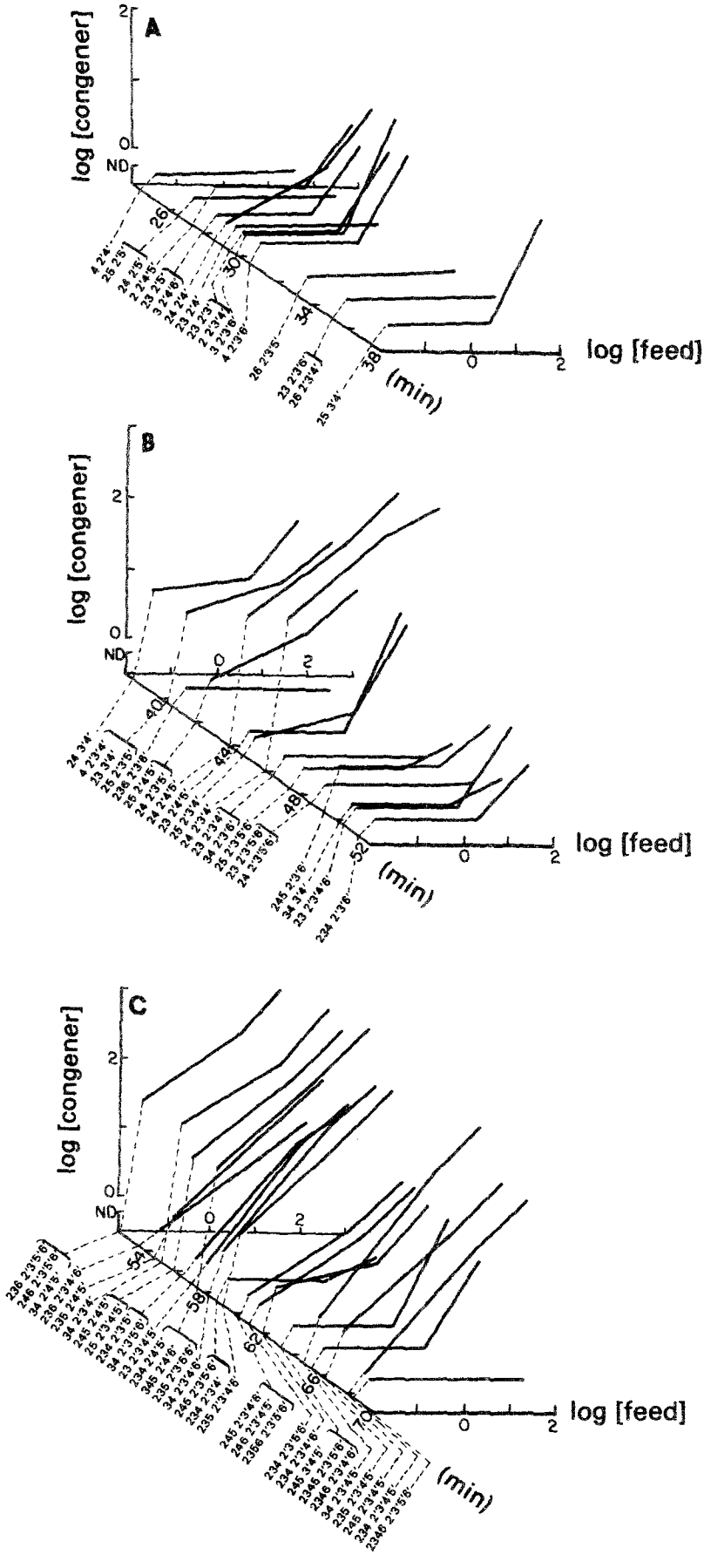


Fig. 5. Dose-response curves for accumulation of PCB congeners in brain tissue samples from weanling pups (day 21). The data are presented as described for Figure 4

chlorobiphenyls: [3,4,2′,4′,5′] and [3,4,2′,3′,4′]. These were, respectively, the first and fourth eluting peaks in this group. The rest of the peaks were assigned hexa-, hepta-, and octochlorinated biphenyls.

Analysis of the bioaccumulation data indicated that congeners in the "a" group had lower indices of bioaccumulation than other PCB congeners. In the newborn brain samples, these congeners were found at lower concentration in the 300 μg/g exposure group than in the 30 μg/g exposure group. At weaning, all the congeners in this group only accounted for 1.5% of the total brain PCB content from animals in the 30 μg/g exposure group. There are at least two explanations for the low indices of bioaccumulation and the decreases observed: (i) these congeners were competitively eliminated by other more highly chlorinated congeners at transport or absorption sites or (ii) these congeners were more effectively metabolized by the dams, especially when exposed to the higher PCB concentration. Within the "b" group there was a great deal of variability in both brain concentration and bioaccumulation. The two congeners in this group that accumulated to the highest concentrations—[2,4,2′,3′,4′] and [2,4,2′,4′,5′]—also had the greatest indices of bioaccumulation in all tissues studied and together accounted for 20% of total PCB content in the brains of weanling animals in the 30 μg/g exposure group. The peaks eluting immediately preceeding and succeeding these congeners contain the respective 2,3- and 2,5- substituted pentachlorobiphenyls and were detected at much lower concentrations in the brain and had very low indices of bioaccumulation in all tissues. The remaining congeners in this group only accounted for 3.5% of the total weanling brain PCB content in the 30 μg/g exposure group. Several of the "c" group congeners were found at low concentrations in the brain; however the bioaccumulation of all of the congeners in this group was similar. These congeners accounted for 75% of the total weanling brain PCB content.

Even though the general pattern of PCB accumulation in tissues can be divided into three major groups according to gas chromatographic retention time, several lines of evidence indicate that the structure-activity relationship for the bioaccumulation of individual congeners may be related primarily to molecular structure and not retention time. The first observation is made by examining the accumulation of the 2,3,4 and 2,4,5 families of pentachlorobiphenyls in the "b" group. When the 2 and 4 positions of the second ring are chlorinated, these congeners were highly accumulated, while the 2,3 and 2,5 congeners were not. Further substitution of either ring does not increase accumulation. Substitution at the 4 position is associated with accumulation. Peaks containing 3,4- substitution pentachlorobiphenyls in the second ring were also accumulated as were "a" group congeners with 4, or 2,4 chlorinated rings.

Additional evidence consistent with this interpretation has been obtained by measuring congener content of rat samples after either an acute exposure to Aroclor 1254 (Baker *et al.* 1977) and a mixture of Aroclor 1254 and 1260 (Seegal *et al.* 1985) or loss of congeners after perinatal exposure to Aroclor 1254. In these latter studies, those congeners detected at the end of the experiment or with the longest half-lives were penta- or hexachlorobiphenyls with −2,3,4 or −2,4,5 substitutions in one ring. Further evidence for the significance of these molecular structures comes from examination of human milk and blood samples (Bush *et al.* 1984, 1985) and adipose tissue samples (Jensen and Sundstrom 1974; Wolff *et al.* 1982a, 1982b). In whole milk samples, 88% of the congeners detected at concentrations $=>1$ ng/g were penta- or hexachlorobiphenyl congeners with 2,3,4 or 2,4,5 substitutions on one ring (Bush *et al.* 1985). Twenty of the 37 PCB congeners reported in adipose tissue by Wolff *et al.* (1982a) accounting for 83% of the reported PCB content were members of this family of congeners. Jensen and Sundstrom (1974) reported that 2,4,5,2′,4′,5′ and 2,3,4,2′,4′,5′ accounted for significant amounts of total tissue PCBs, i.e. >35%. Wolff *et al.* (1982a) hypothesized that chlorination of at least the 4,4′ positions gives PCB congeners molecular characteristics resulting in their accumulation.

The structure activity relationship of several PCB congeners has been studied as a function of interaction with the cytosolic tetra-chlorodibenzo-*p*-dioxin (TCDD) receptor (Poland and Glover 1977) and enzyme induction in the liver (Bradlaw and Casterline 1979). The most active PCB congener, 3,4,3′,4′, was not detected in our feed samples. It was, however, found in milk samples and at a low concentration in brain samples from weanling animals in the 30 μg/g exposure group. 2,4,5,2′,4′,5′ has also been used in studies of TCDD receptor function activity and related enzyme induction and found to have very weak activity, i.e., 10,000-fold less than TCDD (Bradlaw and Casterline 1979). If toxicity is related to tissue concentration of toxicant, then the neurotoxic effects observed after perinatal exposure of rats to Aroclor® 1254, *e.g.* 2,4,2′,4′,5′ and 2,4,2′,3′,4′, must result from a structure-activity relationship and mode of action different from that described for TCDD receptor function and hepatic toxicity.

PCB Transfer via the Placenta and Mammary Gland

Several observations suggest that the level of exposure across the placenta and via the mammary gland and milk may differ. The first observation can be made by comparing the dose-dependent accumulation of PCBs in brain samples for both newborn and weanling animals (Figures 4 and 5). For example, total PCB accumulation in the weanling animals was significantly greater than that observed in newborn animals. In the 30 μg/g exposure group there was a greater than 350% increase in accumulation of total PCBs. While this increase may simply be due to the additional time of exposure, and therefore greater accumulation, it may also be a result of the different route of exposure during the suckling period, *i.e.*, mammary and milk vs placental transfer.

The second observation can be made by comparing the bioaccumulation of PCB congeners in dams' milk (Figure 2A) and liver (Figure 3A). A vast majority of congeners were found at three- to ten-fold greater concentrations in the milk than in the feed, while the concentrations found in the dams' liver samples were less than those found in the feed. In fact, nearly one-half of the congeners detected in the milk samples were undetected in the liver samples. Thus, the concentration of PCBs in dams' milk were significantly greater than those observed in liver samples.

A third observation can be made by comparing the bioaccumulation of PCB congeners in brain and liver from newborn and weanling pups (see Figures 2B and 3B and D). In Figure 2B, bioaccumulation was calculated as a function of feed concentration. In Figure 3B and D, bioaccumulation is calculated as a function of both feed and milk concentrations. Several features of these data indicate that exposure during suckling was different from that observed during the prenatal period. First, while the general patterns of congener bioaccumulation were similar in tissues from animals of both ages, the level of bioaccumulation was greater in the weanling animals. Second, the variability in bioaccumulation observed, especially in the "b" group of congeners, was greatly attenuated when bioaccumulation was calculated as a function of milk rather than feed concentrations. If the variability is a function of metabolism by the dam, then these data suggest that PCBs transferred to the pups via the milk avoid metabolism by some means. Third, most of the bioaccumulation values calculated as a function of milk concentrations are lower than those determined when using feed. Since the tissue concentration was constant in the calculation of bioaccumulation and is the numerator in this calculation, the lower values indicate that congener concentrations were higher in milk. All three of these observations suggest that exposure during suckling was greater than that during the prenatal period. Comparison of bioaccumulation in dams' tissues and milk suggests that the effective exposure may be ten-fold or even greater.

Additional observations are also consistent with this interpretation and indicate that the effective exposure through the mammary system may be even larger. If placental transfer of PCBs is directly related to circulating PCB concentrations, then there may be an even greater difference between placental vs mammary transfer of PCBs, since blood levels of total PCBs (Kurschi and Mio 1983) or 2,4,2′,4′ (Shimada and Sawabe 1984) are nearly ten-fold lower than liver levels. Thus, there might be as much as 100 times greater transfer of PCBs through the mammary glands and milk than across the placenta in rats. Consistent with this conclusion is the observation in humans that there is almost a 25-fold greater concentration of PCBs in milk than in blood (Mes *et al.* 1984; Bush *et al.* 1984, 1985).

While these suggest that increased transfer of PCBs through the milk may significantly contribute to the increase in PCBs observed in weanling vs newborn brain samples, the contribution of continued exposure is difficult to determine. While one might expect that the concentration of PCBs observed in the newborn samples has come to steady-state with regard to dose, there is no data to support this hypothesis. However, data does exist that is consistent with this hypothesis—in human milk and blood samples collected over a 98-day period to environmentally exposed women there was no observed time-dependent change in tissue total PCB concentration (Mes *et al.* 1984) suggesting that, at least, blood and milk come to steady-state with regard to PCB exposure. Additional data consistent with our general hypothesis of greater bioaccumulation during lactation was observed in experiments where pregnant rat dams were given a single dose of DDT and pesticide levels determined in pups for 24 hours, (Fang *et al.* 1977). In this report, it was observed that DDT levels in livers from pups that had suckled was five times greater than litter mates of the same age that had not, indicated a rapid increase in toxicant transfer after suckling began. Thus the increased concentrations of PCB congeners and isomers that we observed in weanling animals may principally be a function of an increased exposure due to greater transfer of PCBs through the mammary gland and milk vs the placenta.

The mechanism(s) responsible for greater accu-

mulation during lactation and differences in tissue levels are not known. PCB bioaccumulation in milk was striking in that almost all congeners were detected at concentrations greater than those observed in the feed. Similarly, PCB bioaccumulation in fat of dams and weanling animals is also higher than liver and brain. Finally, PCB concentrations in newborn liver samples were also high. Since at birth the liver may contain large amounts of non-polar lipids (Entenman *et al.* 1940; Lorenzo *et al.* 1981) and the brain contains mostly predominantly highly charged lipids (Czisa *et al.* 1982), the generalized accumulation of PCBs in tissues may be a function of their high concentrations of non-polar lipids. The high concentration of PCBs in milk may reflect the metabolism of lipids during lactation. Lipids in the circulating blood are compartmentalized into large complexes, chylomicrons (Scow *et al.* 1977; Redgrave 1983) that during lactation are rapidly absorbed by cells in the mammary gland. Thus, the high accumulation of PCBs in the milk may be a function of PCB congener partitioning into chylomicrons formed at the time of ingestion of the PCBs and their rapid transfer to the mammary gland before passing to the liver for metabolism.

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