Accumulation of Chlorobiphenyls in Chicken Fat and Liver after Feeding Aroclor 1254 Directly or Fat from Swine Fed Aroclor 1254

Larry G. Hansen,*[1] Louis G. M. Th. Tuinstra, Cornelis A. Kan, Johan J. T. W. A. Strik,[2] and Jan H. Koeman

Fat from Aroclor 1254 treated swine was rendered into lard and incorporated into the diets of broiler chickens for 3–4 weeks. The Aroclor 1254 which had been fed to the swine was mixed for comparison also into control lard at dietary concentrations of 0.07–9.0 mg/kg. Concentrations of 18 chlorobiphenyls were determined in dissectable body fat and in liver lipids of the chickens by splitless capillary column gas–liquid chromatography. Accumulation ratios varied among chlorobiphenyls, between swine and chickens, and between chicken fat and chicken liver. Hexachlorobiphenyls substituted in the 2,3,4,2′,4′,5′ and 2,4,5,2′,4′,5′ positions clearly predominated in the fat of chickens fed contaminated swine fat. In chickens fed Aroclor 1254 directly, 2,3,6,2′,5′-, 2,4,5,2′,5′-, and 2,4,5,3′,4′-pentachlorobiphenyls and 2,3,6,2′,4′,5′-hexachlorobiphenyl became considerably more important. Accumulation ratios in liver fat were higher for most chlorobiphenyls, especially 2,3,6,2′,5′-pentachlorobiphenyl, 2,3,5,6,2′,5′-hexachlorobiphenyl, and 2,3,5,6,2′,4′,5′-heptachlorobiphenyl.

Polychlorinated biphenyl (PCB) residues, especially in warm-blooded animals, only vaguely resemble the mixture from which they originate (Cook, 1972; Hutzinger et al., 1972; Kuroki and Masuda, 1977; Hansen, 1979; Ballschmiter and Zell, 1980; Hansen et al., 1981b). Each chlorobiphenyl is an individual compound even though it behaves similarly to other chlorinated hydrocarbons; moreover, subtle differences between chlorobiphenyls are further amplified by species differences in absorption, distribution, biotransformation, and excretion (Borchard et al., 1976; Lutz et al., 1977; Hansen and Welborn, 1977). Major quantitative and qualitative differences in the biochemical and toxic effects of individual chlorobiphenyls makes the variable residue composition an important factor when considering the potential hazards of food chain residues (Ax and Hansen, 1975; McKinney et al., 1976; Goldstein, 1979; Yoshimura et al., 1979; Safe et al., 1981).

Detection and preliminary assessment of total PCB contamination can be accomplished with packed-column gas–liquid chromatography (GLC) and estimation procedures based on selected peaks; however, this method tends to overestimate total PCB (Hansen et al., 1981b). Perchlorination and subsequent determination of decachlorobiphenyl also tends to overestimate total PCB (de Kok et al., 1982; Kerkhoff et al., 1982). In addition and perhaps more important, much valuable descriptive information is lost by both procedures. Several laboratories have developed more accurate and more informative PCB determinations based on capillary column GLC separation and absolute quantitation of individual peaks (Tuinstra and Traag, 1979; Duinker et al., 1980).

Such a determination was necessary to assess residue transmission to complement a study comparing the potency of Aroclor 1254 residues with those of technical Aroclor 1254 (Hansen et al., 1981a). Only a limited number of appropriate chlorobiphenyls were available for quantitative standards, but the species and structural relationships observed emphasize the value of rigorous analysis of PCB residues by means of individual chlorobiphenyls.

In this report, a large portion of such data are presented in various perspectives. It is hoped that researchers in this area will expand the observations appropriately, according to their experiences and perspectives. The calculation of accumulation ratios for individual chlorobiphenyls, as done in this report, thus allows a good balance for tolerances for individual components between animal feed and products of animal origin.

MATERIALS AND METHODS

Chemicals. Aroclor 1254 (lot no. KK 602) was a gift from Monsanto Chemical Co. Commodities used for diet formulation were obtained from commercial sources. Glassware, solvents, and chemicals used for chlorobiphenyl determinations were redistilled or cleaned as is usual for this type of analysis.

Experimental Design. Mesenteric and subcutaneous fat was obtained when boars from a PCB study by the Netherlands Public Health Institute were terminated. The boars had received diets containing approximately 0, 0.25, 2.5, or 25 mg/kg Aroclor 1254 for 6 months. The fat was slowly melted at 98–101 °C until most of the water was driven out; it was then allowed to reach 120 °C for a few minutes and filtered into hexane-cleaned steel cans. Technical Aroclor 1254 was added to melted control lard which was then slowly cooled with gentle shaking. Amounts were based on initial estimates of the total PCB content of lard from treated swine. The lard was stored in sealed cans at −40 °C until the mixing of standard soybean–corn type diets containing 10% of added fat. Diets formulated from contaminated swine fat were designated as "residue diets", while those from control lard seeded with Aroclor 1254 were designated "technical diets" (See also Figure 1).

Seven groups of ten broiler-type chickens (1 day old) were started on the diets: one control, three residue, and three technical dose groups. From each group three chickens were terminated after 19 days, four after 20 days, and three after 27 days on treated feed. No detrimental effects due to the PCB were noted, but there were changes in hepatic microsomal oxidase activity (Hansen et al., 1981a).

Department of Toxicology, Agricultural University, Wageningen, State Institute for Quality Control of Agricultural Products, Wageningen, and Spelderholt Institute for Poultry Research, Beekbergen, The Netherlands.

[1]Present address: Department of Veterinary Biosciences, University of Illinois, Urbana, IL 61801.

[2]Present address: Laboratory for General Toxicology, National Institute of Public Health, Bilthoven, The Netherlands.

0021-8561/83/1431-0254$01.50/0

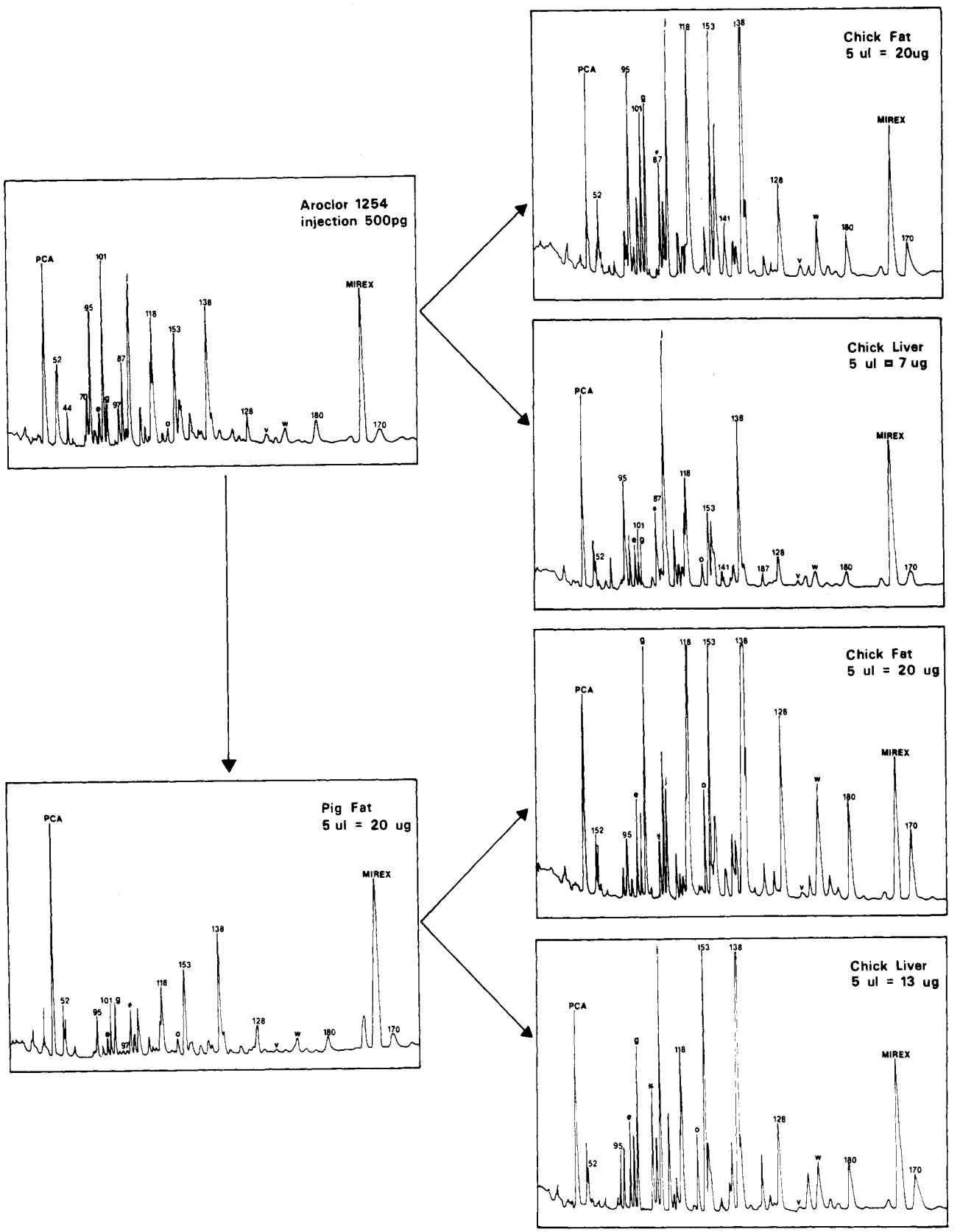


Figure 1. Representative chromatograms of the various sample types compared to that of technical Aroclor 1254. Pentachloroaniline (PCA) and mirex are included as internal standards. See Table I for peak identification.

Fat was removed from the ventral surface of the gizzard, and the 20-day and 27-day samples were pooled separately on an equal weight basis for chlorobiphenyl analysis. Three liver samples from each dosage group were analyzed individually for chlorobiphenyls. In both high-dose groups, fat samples were analyzed individually as well as in a pooled sample.

Analytical Procedures. Extraction and major cleanup are accomplished simultaneously by saponification of the fat (Tuinstra et al., 1980). After final cleanup on basic alumina, the concentrated extract is splitless injected on a CP-Sil 7 capillary column (Chrompack, the Netherlands): length 25 m; inside diameter 0.25 mm; temperature programming 4 min at 100 °C, heating to 220 °C with rate of

Table I. Concentrations of Chlorobiphenyls in Diets[a] Containing 10% Fat Formulated from Swine Fat and Fed to Broiler Chickens

				concn of individual chlorobiphenyls, mg/kg						
					low		medium		high	
substitution pattern	peak[b]	peak 1254	1254,[d] wt %	control	residue	technical	residue	technical	residue	technical
2,5,2′,5′	52		4.2	<0.0005	0.003	0.002	0.021	0.018	0.21	0.23
2,4,2′,5′	49		1.3	<0.0005	<0.001	<0.001	0.004	0.007	0.037	0.074
2,3,2′,5′	44		1.9	<0.0005	<0.001	0.002	0.007	0.011	0.061	0.14
2,5,3′,4′	70		3.2	<0.0005	<0.001	0.002	0.003	0.014	0.028	0.21
2,3,6,2′,5′	95		7.6	<0.0005	0.005	0.005	0.033	0.043	0.31	0.61
pentachlorobiphenyl		e	1.7							
2,4,5,2′,5′	101		8.8	<0.0005	0.006	0.006	0.039	0.049	0.37	0.74
pentachlorobiphenyl		g	2.0							
2,4,5,2′,3′	97		2.3	<0.0005	<0.001	0.002	0.005	0.013	0.048	0.19
2,3,6,2′,3′,6′	136		1.5	<0.0005	<0.001	<0.001	<0.005	<0.005	<0.01	0.066
pentachlorobiphenyl		j	7.5							
2,3,5,6,2′,5′	151		1.2	<0.0005	0.002	0.001	0.010	0.006	0.076	0.084
hexachlorobiphenyl		l	0.7							
2,3,6,2′,4′,5′	149		6.2	<0.0005	0.005	0.004	0.035	0.031	0.25	0.42
2,4,5,3′,4′[c]	118		9.5	<0.0005	0.011	0.006	0.055	0.043	0.56	0.63
hexachlorobiphenyl		o	0.6							
2,4,5,2′,4′,5′	153		6.1	<0.0005	0.014	0.004	0.065	0.028	0.77	0.37
2,3,4,5,2′,5′	141		1.8	<0.0005	0.001	0.001	0.008	0.010	0.075	0.13
2,3,4,2′,4′,5′	138		7.6	0.001	0.025	0.005	0.10	0.044	1.28	0.62
hexachlorobiphenyl		s	0.2							
2,3,5,6,2′,4′,5′	187		0.4	<0.0005	0.001	<0.001	0.006	0.002	0.051	0.033
2,3,4,2′,3′,4′	128		1.5	<0.0005	0.006	0.002	0.027	0.012	0.31	0.17
heptachlorobiphenyl		v	0.7							
hexa- + heptachlorobiphenyl		w	0.7							
2,3,4,5,2′,4′,5′	180		1.2	<0.0005	0.004	0.001	0.018	0.008	0.20	0.10
2,3,4,5,2′,3′,4′	170		0.7	<0.0005	0.002	0.001	0.009	0.004	0.11	0.060
total identified peaks				<0.0005	0.09	0.04	0.45	0.34	4.75	4.88
total Aroclor					0.16	0.07	0.86	0.58	9.03	8.02

[a] "Residue" source is lard rendered from the fat of boars having received dietary Aroclor 1254 for 6 months; "technical" is the control lard to which the technical Aroclor 1254 mixture was added. [b] According to the system of Ballschmiter and Zell (1980). [c] As a pure standard was not available results are expressed with the response factor of adjacent compound 149. [d] Weight percentage letter codes for peaks in Aroclor 1254 are calculated with the mean response of biphenyls with the same number of chlorine atoms.

40 °C/min; final temperature 220 °C. A Tracor 550 gas chromatograph with electron capture detector was used. For identification purposes, the same column coupled to a Finnigan 4000 mass spectrometer was used.

Peak Designations. Whenever standards were available with matched retention time and molecular weight, the peak is designated by a number corresponding to the chlorobiphenyl classification system of Ballschmiter and Zell (1980); absolute quantition of these standard peaks was performed. Some matched standards were not available; therefore, letter codes for these peaks in Aroclor 1254 are used; although these peaks were not quantitated in an absolute way, comparisons between residues in chicken fat and feed can be made resulting in accumulation ratios. The 2,4,5,3′,4′-pentachlorobiphenyl (peak 118) was not available until after the samples were analyzed, so retrospective identification from the GLC tracings was necessary. As a totally pure standard of 118 was not available, quantitative results are expressed with the response factor of the adjacent compound 149. Peaks 97 and 136 were frequently below the limit of detection. Peak 87 (2,3,4,2′,5′-pentachlorobiphenyl) could not be resolved from the *p,p′*-DDE peak, so no results are reported. A summary of the various peak designation schemes and the dietary concentrations is presented in Table I. Figure 1 further illustrates the relationships between the peaks in the various sample types.

RESULTS AND DISCUSSION

Lard and Finished Feed. Samples of solidified lard from the bottom and top of one can and the middle of a second can of both control and high dose lards were analyzed for individual chlorobiphenyls and residues were also expressed as Aroclor 1254. Peaks in the control lard were near or below the detection limit of 0.001 mg/kg. The three samples of high-dose lard contained 91.2 ± 3.3 mg/kg PCB when calculated as total Aroclor 1254; quantitation as the sum of 18 individual peaks yielded a value of 48.9 ± 2.4 mg/kg, this difference being due to the nonavailability of certain chlorobiphenyl standards. In a pilot study, analysis of four separate feed samples (both technical and residue PCB) indicated a variability of about 10%. For the final study, a larger sample of feed containing 10% added lard, was analyzed as Aroclor 1254 and the 9.03 mg/kg for high-dose residue diet agreed well with the predicted 9.12 mg/kg (10% of the 91.2 mg/kg in the lard). Other totals and the concentrations of various peaks are given in Table I.

Chicken Residues. Some contamination of control chickens (e.g., Table II) was expected from feed dust (Hansen et al., 1976) or other factors (Kan et al., 1979), but the magnitude of the contamination (Table III) indicates that the controls may have been mistakenly fed contaminated feed at some point. The "total" concentrations reported in Tables II–IV refer only to chlorobiphenyls quantitated against purified standards. Standards were not available for all chlorobiphenyls. A mean response factor for biphenyls with the same number of chlorine atoms was used to estimate the contents of those compounds for which standards were not available. In this way only about 40–60% of the total liver fat residue is defined by accurately measured peaks (Table IV); on the

Table II. Residues of Chlorobiphenyls in Pooled Samples of Chicken Fat after Receiving Contaminated Diets for 20 (n = 4) or 27 (n = 3) Days

	chlorobiphenyl concn in fat, mg/kg												
		residue						technical					
		0.16[b]		0.86[b]		9.03[b]		0.07[b]		0.58[b]		8.02[b]	
peak[a]	control, 27 days	20 days	27 days	20 days	27 days	20 days	27 days	20 days	27 days	20 days	27 days	20 days	27 days
138	0.035	0.26	0.29	0.92	1.26	10.9	9.49	0.062	0.078	0.55	0.63	5.67	6.36
153	0.036	0.19	0.21	0.57	0.75	7.05	6.80	0.046	0.061	0.36	0.41	3.67	3.96
118[c]	0.020	0.16	0.17	0.67	0.84	6.31	5.23	0.069	0.083	0.73	0.77	7.42	8.60
128	0.004	0.061	0.060	0.24	0.34	2.58	2.13	0.013	0.015	0.13	0.15	1.47	1.64
180	0.008	0.053	0.051	0.14	0.20	1.76	1.45	0.024	0.015	0.092	0.10	0.90	1.04
149	0.027	0.038	0.041	0.18	0.20	1.23	1.07	0.044	0.035	0.20	0.21	2.42	2.75
101	0.011	0.019	0.021	0.11	0.10	1.11	1.08	0.017	0.022	0.16	0.14	2.19	2.33
170	0.003	0.024	0.026	0.078	0.10	0.92	0.79	0.011	0.007	0.045	0.053	0.51	0.56
95	0.026	0.028	0.032	0.10	0.095	0.84	0.75	0.036	0.043	0.24	0.23	2.81	3.15
52	0.005	0.011	0.010	0.056	0.10	0.58	0.57	0.008	0.009	0.059	0.049	0.88	0.94
151	0.008	0.015	0.017	0.056	0.062	0.49	0.44	0.008	0.009	0.044	0.046	0.54	0.81
187	0.005	0.015	0.016	0.038	0.052	0.47	0.39	0.003	0.008	0.027	0.031	0.26	0.30
141	0.006	0.011	0.010	0.040	0.042	0.40	0.32	0.008	0.009	0.065	0.064	0.83	0.93
49	0.006	0.002	0.003	0.010	0.011	0.14	0.10	0.002	0.003	0.017	0.015	0.28	0.31
70	0.004	0.004	0.005	0.010	0.011	0.058	0.073	0.006	0.008	0.10	0.046	0.54	0.63
44	0.007	0.002	0.002	0.010	0.010	0.053	0.071	0.003	0.003	0.011	0.008	0.13	0.15
97	0.026	0.018	0.003	<0.02	<0.02	<0.1	<0.1	0.015	0.025	<0.02	<0.02	0.17	0.19
136	<0.001	<0.001	<0.001	<0.01	<0.01	<0.1	<0.1	<0.001	<0.001	<0.01	<0.01	<0.1	<0.1
total[d]	0.24	0.91	0.97	3.2	4.2	35.0	30.8	0.38	0.43	2.8	3.0	30.7	34.7

[a] According to the system of Ballschmiter and Zell (1980). [b] Mean dietary concentration relative to total Aroclor 1254 (mg/kg). [c] See remark in Table I. [d] Summation of identified peaks.

Table III. Mean Residues of Chlorobiphenyls in Chicken Liver Fat after Receiving Contaminated Diets for 19 and 20 Days ($n = 3$)

	chlorobiphenyl concn, mg/kg of liver fat						
		residue			technical		
peak[a]	control	0.16[b]	0.86[b]	9.03[b]	0.07[b]	0.58[b]	8.02[b]
138	0.16	1.01	3.31	18.95	0.25	2.95	6.75
153	0.09	0.36	1.37	7.91	0.12	1.28	4.22
118[c]	0.12	0.24	1.28	6.36	0.12	1.45	7.75
128	0.015	0.097	0.49	2.82	0.034	0.44	1.63
180	0.023	0.073	0.34	1.89	0.024	0.33	1.04
149	0.14	0.21	0.98	2.67	0.14	1.18	6.26
101	0.028	0.066	0.44	2.59	0.045	0.42	3.08
170	0.010	0.027	0.19	1.09	0.019	0.20	0.73
95	0.090	0.082	0.42	2.10	0.012	1.03	7.10
52	0.038	0.066	0.34	1.17	0.055	0.35	1.83
151	0.088	0.27	0.92	3.24	0.094	0.83	3.08
187	0.060	0.26	0.55	2.43	0.051	0.53	1.46
141	0.020	<0.01	0.13	0.45	0.015	0.14	1.06
49	0.012	<0.01	0.060	<0.1	<0.01	0.062	0.42
70	<0.01	<0.01	0.073	<0.1	0.018	0.092	0.70
44	<0.01	<0.01	<0.02	<0.1	<0.01	<0.03	<0.1
97	<0.01	<0.01	<0.02	<0.1	<0.01	<0.03	<0.1
136	<0.01	<0.01	<0.02	<0.1	<0.01	<0.03	<0.1
total[d]	0.90	2.8	10.9	54.0	1.02	11.4	47.3

[a] According to the system of Ballschmiter and Zell (1980). [b] Mean dietary concentration relative to total Aroclor 1254. [c] See remark in Table I. [d] Summation of quantified peaks (mg/kg); peaks below the limit of reliable quantitation were treated as $^1/_2$ the stated value.

Table IV. Mean (±SD for $n = 3$) Residues of Chlorobiphenyls in Chick Body Fat and Liver Fat after 20 Days

	chlorobiphenyl concn, mg/kg			
	residue (9.03 mg/kg)		technical (8.02 mg/kg)	
peak[a]	liver fat	body fat	liver fat	body fat
138	19.67 ± 3.25	10.05 ± 1.31	8.60 ± 1.83	5.31 ± 1.12
153	8.33 ± 1.86	5.90 ± 1.71	3.80 ± 0.97	3.33 ± 0.86
118[b]	6.75 ± 1.78	5.64 ± 0.70	6.73 ± 0.79	7.12 ± 1.89
128	2.92 ± 0.63	2.39 ± 0.35	1.50 ± 0.30	1.47 ± 0.39
180	2.03 ± 0.47	1.52 ± 0.28	0.96 ± 0.32	1.07 ± 0.21
149	2.73 ± 0.75	1.23 ± 0.18	5.39 ± 2.03	2.58 ± 0.15
101	2.67 ± 0.25	1.20 ± 0.20	2.55 ± 0.94	2.33 ± 0.18
170	1.17 ± 0.20	0.91 ± 0.06	0.67 ± 0.29	0.50 ± 0.13
95	2.21 ± 0.52	0.93 ± 0.14	6.44 ± 1.72	3.02 ± 0.45
52	1.30 ± 0.22	0.70 ± 0.13	1.52 ± 0.78	0.98 ± 0.07
151	3.30 ± 1.11	0.48 ± 0.06	3.09 ± 1.18	0.53 ± 0.12
187	2.41 ± 0.92	0.40 ± 0.06	1.51 ± 0.63	0.27 ± 0.08
141	0.48 ± 0.08	0.39 ± 0.04	0.91 ± 0.28	0.86 ± 0.18
49	<0.19	0.14 ± 0.06	<0.25	0.30 ± 0.04
70	<0.10	0.10 ± 0.03	0.58 ± 0.14	0.64 ± 0.14
44	<0.15	0.12 ± 0.03	<0.10	0.17 ± 0.02
97	<0.10	0.06 ± 0.01	<0.10	0.17 ± 0.04
136	<0.10	<0.10	<0.10	<0.10
total identified[c]	56.3 ± 11.1	32.2 ± 4.3	44.5 ± 11.5	30.7 ± 5.8
total[d] as Aroclor 1254	106 ± 16	53 ± 7	102 ± 21	50 ± 9

[a] According to the system of Ballschmiter and Zell (1980). [b] See remark in Table I. [c] Summation of identified peaks; values below the limit of reliable quantitation were treated as $^1/_2$ the stated value. [d] Total residue estimated by quantitating unknown peaks with the mean response of biphenyls with the same number of chlorine atoms.

other hand, 60–75% of the total residue in diets is defined by the measured peaks (Table I).

Regardless of the PCB source, liver lipids tend to accumulate higher total concentrations than do lipids in body fat reservoirs. It should be noted that liver lipid content varied from 4 to 6% (fresh weight) so that the net concentration of PCB in the total liver would generally be about 5% of that reported for liver lipids. In a previous study (Hansen et al., 1976), liver lipids also accumulated somewhat higher concentrations of Aroclor 1254 than did body fat, but an inverse relationship was seen for the less persistent Aroclor 1242. When the contents given in Tables I–III are converted to percentage of total identified peaks or total Aroclor, the resulting percentages of compounds 118, 149, 101, 95, 141, 49, and 70 are higher in the technical diet than in the residue diet (Table I), indicating that swine selectively degrade and/or eliminate these chlorobiphenyls; likewise, their relative concentration is higher in chicken body fat (Table II) and chicken liver fat (Table III) when the dietary source was technical Aroclor 1254. Marked differences in residues of other chlorobiphenyls are readily apparent in Tables II and III and in Figure 1. The sequence of chlorobiphenyls in these and subsequent tables is based on the amount of residues accumulated in the fat of the highest residue dose group (9.03 mg/kg). This aids in detecting pattern deviations. For example, chickens accumulate mainly biphenyls 138, 153, and 118 in their body fat and liver fat when fed diets formulated from swine residues; however, biphenyls 118 and 95 become more important when technical Acoclor

Table V. Comparison of Accumulation Ratios of Various Chlorobiphenyls in Swine Fat and Chicken Body Fat

	accumulation ratios (fat concn/dietary concn)								
				chicken fat					
	swine fat			residue			technical		
peak[a]	0.25[b]	2.5[b]	25[b]	0.16[b]	0.86[b]	9.03[b]	0.07[b]	0.58[b]	8.02[b]
138	12	6	7	11	11	8	14	13	10
153	11	5	6	14	10	9	13	14	10
118	10	4	3	15	14	10	13	17	13
128	18	14	8	10	11	8	7	12	9
180	17	8	7	13	21	8	14	12	10
149	3	2	1	8	5	5	10	7	6
101	3	2	2	3	3	3	3	3	3
170	13	12	7	13	10	8	6	12	9
95	3	2	2	6	3	3	8	5	5
52	4	4	3	3	4	3	4	3	4
151	6	3	3	8	6	6	8	7	8
187	12	6	5	16	8	8	>5	14	8
141	3	2	1	10	5	5	8	6	7
49	2	2	1	2	2	3	2	2	4
70	0.3	0.4	0.4	>5	4	3	3	4	3
44	1	2	1	>2	1	1	2	1	1
e	9	4	2	6	6	6	4	6	6
g	13	6	3	8	10	7	7	11	8
j	18	12	11	5	4	4	4	5	5
l	6	16	2	6	4	4	>5	5	3
o	10	6	1	13	9	8	>8	14	9
s	10	5	3	13	10	10	>10	14	10
v	12	8	4	>2	5	4	>2	7	6
w	11	11	13	7	5	4	8	6	5

[a] According to Ballschmiter and Zell (numbers) or by letter if no standard was available. [b] Dietary concentration (mg/kg) fed for 6 months to swine or 20 days (n = 4) and 27 days (n = 3) to chicks.

1254 is administered (Tables II and III). Some chlorobiphenyls such as 153, 128, and 180 tend to attain concentrations in body fat almost as high as those in liver; on the other hand, compounds 151 and 187 accumulate in the liver well in excess of the ratio for other chlorobiphenyls (Table IV). Continued and more extensive evaluation of these data on the basis of structural characteristics should help to clarify species and tissue differences in chlorobiphenyl distribution and degradation.

Most analyses were conducted on pooled samples; however, Table IV compares the liver lipid and body fat residues for individual chickens terminated at 20 days of age, illustrating considerable individual variation in chlorobiphenyl accumulation.

Accumulation Ratios (Levels in Fat of Liver/Level in Feed). The swine were fed the contaminated diets much longer than the chicks, but, except for biphenyl 128 and peaks j, v, and w, the chickens were more efficient in accumulating chlorobiphenyls from the diets (Table V). Accumulation ratios, not depending on the PCB source, generally declined with increasing dietary concentrations (Table V), reflecting a pattern similar to that seen with chickens fed hexachlorobenzene (HCB) in excess of 1 mg/kg (Hansen et al., 1979). Below 1 mg/kg, equilibrium is reached rapidly in broilers and accumulation of chlorinated hydrocarbons is generally proportional to dietary concentration (de Vos et al., 1972; Kan et al., 1978).

If one allows for decreased accumulation factors above 1 mg/kg, biphenyls 70, 141, and 149 were accumulated much more effectively by chickens than by swine. Accumulation of 153, 118, 95, 187, and peak o was slightly more efficient in the chicken. Chlorobiphenyls 97 and 136 are readily metabolized and/or eliminated by both species while 70 is apparently handled effectively only by swine. Chlorobiphenyls 44 and 49 (tetrachlorobiphenyls) do not accumulate appreciably above dietary concentrations in either species. The accumulation factors in chickens do not appear to be greatly influenced by the PCB source; therefore, these values may be the most valuable in comparing analogues and species. The ultimate tissue concentrations, of course, depend on three factors: swine or chicken accumulation and Aroclor 1254 composition—so a large accumulation ratio for a minor component (e.g., 187) can result in lower residues than a lesser accumulation ratio for a major component (e.g., 149).

Residue Composition. As accumulation ratios and Aroclor composition interact, it is easy to see that hexachlorobiphenyls 138 and 153 predominate in both the fat and liver (Tables IV and V). Chlorobiphenyls 149 and 95, both containing a ring with the labile 2,3,6 substitution, are effectively removed by swine but are accumulated by the chickens (Table V).

The highly toxic (Ax and Hansen, 1975; Hansen, 1979) 2,4,5,3′,4′-pentachlorobiphenyl (118) is also a very significant contributor to the total residue. When the contents given in Table IV are converted to percentage of total identified peaks, it can be seen from the percentage distribution that the symmetrical hexachlorobiphenyls 153 and 128 do not contribute as much to liver lipid residues as to body fat residues; on the other hand, the asymmetrical 95, 151, and 187 make relatively greater contributions to the liver lipid residue than to the body fat residue.

ACKNOWLEDGMENT

We thank Dr. H. van Haeringen, J. W. M. Haas, Ing. J. B. Schutte, J. H. M. van den Berg, and Ellen Harmsen for their assistance with the animals. A. J. van Munsteren and A. H. Roos executed the PCB analyses and Drs. F. L. van Velsen and J. G. Vos (National Institute for Public Health, Bilthoven) provided the swine fat.

Registry No. 2,5,2′,5′-Tetrachlorobiphenyl, 35693-99-3; 2,4,2′,5′-tetrachlorobiphenyl, 41464-40-8; 2,3,2′,5′-tetrachlorobiphenyl, 41464-39-5; 2,5,3′,4′-tetrachlorobiphenyl, 32598-11-1; 2,3,6,2′,5′-pentachlorobiphenyl, 38379-99-6; 2,4,5,2′,5′-pentachlorobiphenyl, 37680-73-2; 2,4,5,2′,3′-pentachlorobiphenyl, 41464-51-1; 2,3,6,2′,3′,6′-hexachlorobiphenyl, 38411-22-2; 2,3,5,6,2′,5′-hexachlorobiphenyl, 52663-63-5; 2,3,6,2′,4′,5′-hexa-

chlorobiphenyl, 38380-04-0; 2,4,5,3′,4′-pentachlorobiphenyl, 31508-00-6; 2,4,5,2′,4′,5′-hexachlorobiphenyl, 35065-27-1; 2,3,4,5,2′,5′-hexachlorobiphenyl, 52712-04-6; 2,3,4,2′,4′,5′-hexachlorobiphenyl, 35065-28-2; 2,3,5,6,2′,4′,5′-heptachlorobiphenyl, 52663-68-0; 2,3,4,2′,3′,4′-hexachlorobiphenyl, 38380-07-3; 2,3,4,5,2′,4′,5′-heptachlorobiphenyl, 35065-29-3; 2,3,4,5,2′,3′,4′-heptachlorobiphenyl, 35065-30-6; Aroclor 1254, 11097-69-1.

LITERATURE CITED

Ax, R. L.; Hansen, L. G. *Poult. Sci.* **1975**, *54*, 894.
Ballschmiter, K.; Zell, M. *Fresenius' Z. Anal. Chem.* **1980**, *302*, 20.
Borchard, R. E.; Welborn, M. E.; Hansen, L. G.; Link, R. P.; Teske, R. H. *Arch. Environ. Contam. Toxicol.* **1976**, *4*, 226.
Cook, J. W. *EHP, Environ. Health Perspect.* **1972**, *1*, 3.
de Kok, A.; Geerdink, R. B.; Frei, R. W.; Brinkman, U. A. Th. *Int. J. Environ. Anal. Chem.* **1982**, *11*, 17.
de Vos, R. H.; Bouman, J.; Engel, A. B. *Pestic. Sci.* **1972**, *3*, 421.
Duinker, J. C.; Hillebrand, M. T. J.; Palmork, K. H.; Wilhelmsen, S. *Bull. Environ. Contam. Toxicol.* **1980**, *25*, 956.
Goldstein, J. A. *Ann. N.Y. Acad. Sci.* **1979**, *320*, 164.
Hansen, L. G. *Ann. N.Y. Acad. Sci.* **1979**, *320*, 238.
Hansen, L. G.; Dorn, S. B.; Beamer, P. D. *Poult. Sci.* **1979**, *58*, 1.
Hansen, L. G.; Strik, J. J. T. W. A.; Koeman, J. H.; Kan, C. A. *Toxicology* **1981a**, *21*, 203.
Hansen, L. G.; Washko, P. W.; Tuinstra, L. G. M. Th.; Dorn, S. B.; Hinesly, T. D. *J. Agric. Food Chem.* **1981b**, *29*, 1012.
Hansen, L. G.; Welborn, M. E. *J. Pharm. Sci.* **1977**, *66*, 497.
Hansen, L. G.; Wilson, D. W.; Metcalf, R. L.; Welborn, M. E. *J. Agric. Food Chem.* **1976**, *24*, 256.
Hutzinger, O.; Safe, S.; Zitko, V. *Analabs, Res. Notes* **1972**, *12*, 1.
Kan, C. A.; Jonker-den Rooyen, J.; Tuinstra, L. G. M. Th.; Roos, A. H.; Traag, W. *J. Agric. Food Chem.* **1978**, *26*, 618.
Kan, C. A.; Strik, J. J. T. W. A.; Koeman, J. H. *Meded. Fac. Landbouwwet., Rijksuniv. Gent* **1979**, *44*, 965.
Kerkhoff, M. A. T.; de Vries, A.; Wegman, R. C. C.; Hofstee, A. W. M. *Chemosphere* **1982**, *11*, 165.
Kuroki, H.; Masuda, Y. *Chemosphere* **1977**, *6*, 469.
Lutz, R. J.; Dedrick, R. L.; Matthews, H. B.; Eling, T. E.; Anderson, M. W. *Drug Metab. Dispos.* **1977**, *5*, 386.
McKinney, J. D.; Chae, K.; Gupta, B. N.; Moore, J. A.; Goldstein, J. A. *Toxicol. Appl. Pharmacol.* **1976**, *36*, 65.
Safe, S.; Parkinson, A.; Robertson, L.; Cockerline, R.; Safe, L. In "Toxicology of Halogenated Hydrocarbons"; Khan, M. A. Q.; Stanton, R. H., Eds.; Pergamon Press: New York, 1981; pp 106–119.
Tuinstra, L. G. M. Th.; Traag, W. A. *HRC CC, J. High Resolut. Chromatogr. Commun.* **1979**, *2*, 723.
Tuinstra, L. G. M. Th.; Traag, W. A.; Keukens, H. J. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 952.
Yoshimura, H.; Yoshihara, S.; Oazwa, N.; Miki, M. *Ann. N.Y. Acad. Sci.* **1979**, *320*, 179.

Received for review March 29, 1982. Revised manuscript received September 29, 1982. Accepted November 6, 1982.

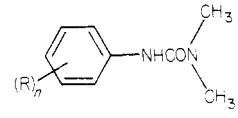
The Design of Postemergence Phenylurea Herbicides Using Physicochemical Parameters and Structure–Activity Analyses

Barrington Cross,* Peggy P. Hoffman, George T. Santora, David M. Spatz,[1] and Andrew R. Templeton

A series of herbicidal 3-[*p*-(benzyloxy)phenyl]-1,1-dimethylureas was prepared from an assessment of a published Hill inhibition correlation equation on 1,1-dimethyl-3-phenylureas. By use of herbicidal data for our compounds, the correlation equation obtained was $\log (1/C) = 1.3 + 0.42\pi - 5.4E_R$, where $r^2 = 0.89$ and $s = 0.30$. In this expression C is a molar concentration obtained from ED_{85} herbicidal data, where ED_{85} is the effective dose of compound to cause 85% kill of wild mustard, π refers to the hydrophobic substituent parameter, and E_R is a free radical parameter. Critical analysis of this equation led to the discovery of new herbicidal compounds of which a series of 1,1-dimethyl-3-[*m*- (or *p*-) (phenylalkoxy)phenyl]ureas were particularly effective postemergence herbicides.

Over 300 patents have been issued on herbicidal phenylureas since the discovery of monuron [1,1-dimethyl-3-(*p*-chlorophenyl)urea]. In spite of this seemingly insurmountable patent domination of this structural class, we questioned whether the most potent phenylurea herbicides have already been made, and if not, how they might be identified.

It is known that phenylureas exert their effect by blocking an electron-transfer step in the oxygen-evolving photochemical reaction of plants, and this light-catalyzed activity of isolated chloroplasts is known as the Hill reaction (1937). In 1956, Wessels and Van der Veen (1956) reported on the 50% inhibition of the Hill reaction (pI_{50}) for twelve 3- and 4-substituted 1,1-dimethyl-3-phenylureas (I).



I

Where R is H, 3-Cl, 4-Cl, 4-Cl, 3,4-Cl_2, 3-NO_2, 4-NO_2, 3-CF_3, 4-CF_3, 4-CH_3, 4-$N(CH_3)_2$, 4-OCH_3, and 4-$NHCOCH_3$. Ten years later Hansch and Deutsch analyzed these data by regression and correlation analyses using the physicochemical parameters σ, the Hammett electronic parameter, and π, the free energy related lipophilicity substituent constant. The best-fit eq 1 suggested that the lipophilic nature of the substituent plays a major role and its degree of electron withdrawal a lesser role in the inhibition of the Hill reaction.

$$pI_{50} = 0.54\sigma + 1.29\pi + 4.18 \quad (1)$$

$$n = 12 \qquad r^2 = 0.94 \qquad s = 0.37$$

$pI_{50} = -\log I_{50}$, where I_{50} is the molar concentration of

Agricultural Research Division, American Cyanamid Company, Princeton, New Jersey 08540.
[1]Present address: Chevron Chemical Co., Richmond, CA 94804.

0021-8561/83/1431-0260$01.50/0