ACKNOWLEDGMENTS AND ADDRESSES

Received March 27, 1973, from the *College of Pharmacy, University of Oklahoma, Norman, OK 73069.*

Accepted for publication May 28, 1976.

Abstracted from a dissertation submitted by Loyd V. Allen, Jr., to the graduate school, University of Texas, in partial fulfillment of the Doctor of Philosophy degree requirements.

The authors thank The Upjohn Co. and Schering Co. for the corticosteroids.

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Distribution, Dilution, and Elimination of Polychlorinated Biphenyl Analogs in Growing Swine

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Abstract □ Growing swine were given 15 mg/kg po of purified polychlorinated biphenyl analogs and a commercial mixture. Backfat biopsies for 16–18 weeks exhibited two-compartment elimination kinetics when based on concentration. Total body fat was estimated from potassium-40 abundance, and the calculated total fat load indicated that most of the decline in residue concentrations was due to dilution by growth and expansion of the fat compartment. Redistribution in the fat was apparent for various analogs at different times, and tissue distribution varied significantly among analogs.

Keyphrases □ Biphenyls, polychlorinated—tissue distribution, dilution by growth, and elimination, swine □ Polychlorinated biphenyls—tissue distribution, dilution by growth, and elimination, swine □ Distribution, tissue—various polychlorinated biphenyls, swine □ Elimination—various polychlorinated biphenyls, swine

Stable compounds of low water solubility and high lipid affinity such as chlorinated pesticides and polychlorinated biphenyls comprise a special class of pharmacokinetically distinct xenobiotics. Recent studies involving intravenous and oral administrations of a polychlorinated biphenyl mixture containing 54% chlorine to swine and sheep showed that component peaks were distributed at different rates (1, 2). The smaller isomers of low chlorine content generally distributed at a faster rate than those of higher chlorination and were metabolized to a greater extent (1–4).

The high fat–blood concentration ratios of polychlorinated biphenyls (PCB) make sampling of the fat more meaningful for pharmacokinetic modeling. Because the apparent $t_{1/2}$ for elimination is on the order of weeks when fat concentration alone is presented (5), dilution due to growth is a required parameter for studies of rapidly growing animals such as young swine. In addition, the fat compartment is neither static nor a constant proportion of body weight.

This paper presents methods for evaluating the total amount of three pure polychlorinated biphenyl analogs and a commercial mixture[1] in the fat of growing swine. The "total fat load," L_t, is defined as the weight of chlorinated biphenyl present in the fat compartment. The pharmacokinetic parameters defined from these calculations demonstrate that traditional models must be reconstructed for persistent chemicals in food-producing animals.

EXPERIMENTAL

Fifteen matched weanling pigs of mixed Yorkshire/Hampshire breeding were divided into four experimental and one control group of three animals each. The pigs were fed a wet mash containing 100 ppm of 2,5,2′,5′-tetrachlorobiphenyl (I), 2,4,5,3′,4′-pentachlorobiphenyl (II), 2,4,5,2′,4′,5′-hexachlorobiphenyl (III), and a commercial mixture[1] (IV) for 1 week. The mash was offered every morning and left until totally consumed in quantities sufficient to produce a 2.14-mg/kg/day or 15-mg/kg/week dosing regimen.

Total body fat was estimated by a variation of the potassium-40

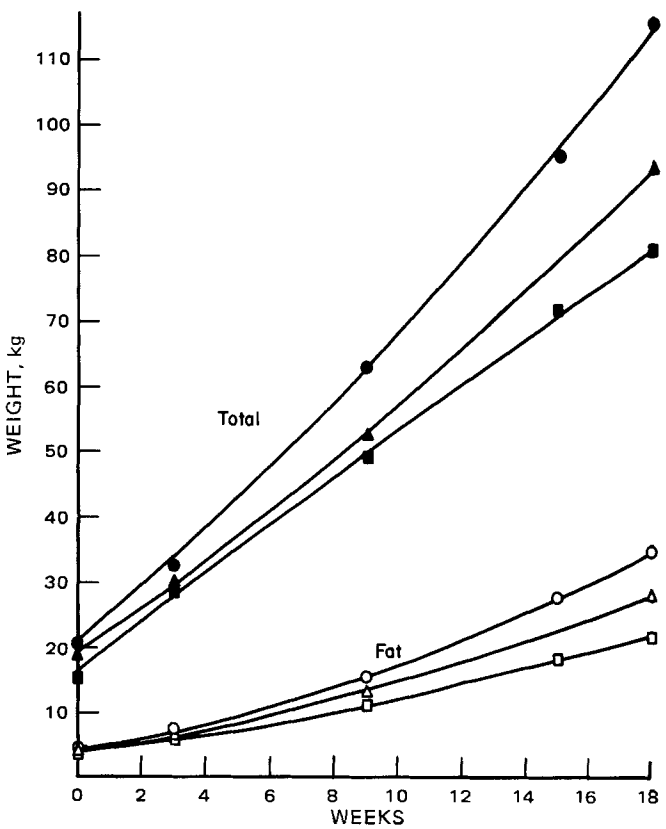

Figure 1—*Comparison of body weight (solid symbols) and body fat (open symbols) in single pigs receiving control (circles), hexachlorobiphenyl (triangles), and 54% chlorine mixture (squares) feed.*

[1] Aroclor 1254, Monsanto electrical grade, lot KB-05-612.

Table I—Polynomial Coefficients for Total Pig Weight *versus* Time[a]

Pig	A_0	A_1	A_2 (× 10^3)	D_{max}, kg
1	17.04	4.91	0.62	0.76
2	22.40	2.74	109.57	0.90
3	11.44	4.11	−24.70	1.56
4	17.76	2.74	39.81	2.16
5	18.65	3.07	53.70	0.65
6	17.85	3.26	57.41	1.85
7	18.38	3.93	28.70	0.58
8	21.21	4.07	63.33	1.01
9	17.58	3.04	82.41	1.08
10	18.95	3.41	40.74	0.45
11	14.60	3.79	48.15	0.40
12	15.92	2.58	101.85	0.42
13	18.97	3.89	43.21	0.03
14	18.73	3.66	−4.32	0.33
15	16.43	3.82	−12.96	1.23

[a] $Y_{(kg)} = A_0 + A_1X_{weeks} + A_2X^2_{weeks}$.

Table II—Polynomial Coefficients for Potassium-40 *versus* Time[a]

Pig	B_0	B_1	B_2	D_{max}, kg
1	3.283	1.250	0.03148	0.317
2	4.731	0.481	0.06636	0.431
3	2.128	0.994	0.00679	0.472
4	3.603	0.636	0.02068	0.503
5	5.535	0.843	0.02241	0.065
6	4.225	0.631	0.03981	0.225
7	3.580	1.020	0.02000	0.020
8	4.199	0.882	0.04522	0.199
9	3.995	0.333	0.06800	0.695
10	3.951	0.732	0.03344	0.251
11	3.040	0.804	0.03407	0.040
12	3.436	0.319	0.06327	0.336
13	3.844	0.794	0.04753	0.044
14	3.928	0.744	0.02346	0.228
15	3.317	0.783	0.01296	0.317

[a] $Y_{(fat\ weight)} = B_0 + B_1X_{weeks} + B_2X^2_{weeks\ kg}$.

method of Lohman *et al.* (6). The equation describing the conversion of potassium-40 to total potassium and finally to body fat is:

$$\text{potassium (g)} = \frac{[(A_1 - A_2) + (0.0583B) - (A_3)][C]}{(0.2028 - 0.000174B)(\gamma)} \quad \text{(Eq. 1)}$$

where:

A_1 = total potassium-40 counts per second
A_2 = background potassium-40 counts per second
A_3 = net counting capsule counts per second
B = weight of subject (pounds); $0.0583B$ is background depression factor, correcting for background absorbed by mass of subject

$$C = \frac{\text{net cps of standard on date of calibration}}{\text{net cps of standard on date of subject count}} = \frac{10^5 \text{ cps}}{\text{std cps}}$$

γ = gamma ray disintegrations/sec/g, $K = 3.0$

The $(0.2028 - 0.000174B)$ function was determined experimentally by Lohman *et al.* (6).

Blood samples and bilateral backfat biopsies were taken on Days 3, 7 (last day of treatment), 14, 28, and every 28 days thereafter for 16 or 18 weeks. A pilot study showed that backfat concentrations accurately reflected concentrations of polychlorinated biphenyl in other dissectible fat stores of swine (e.g., mesenteric and perirenal); concentrations within the same pig did not vary anteriorly-posteriorly, but each side varied consistently from the other. Bilateral biopsies were analyzed independently, and the results are reported as an average of the two. Blood and tissues were analyzed by electron-capture GLC as described previously (1, 7).

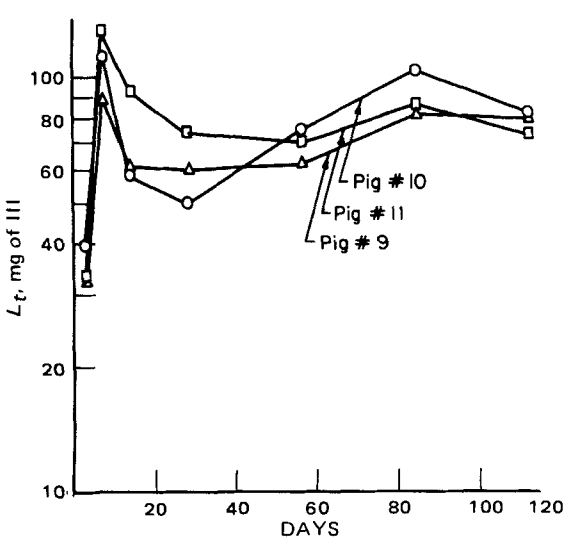

Figure 2—*Total fat content of III in three pigs receiving 2.14 mg/kg/day for 7 days.*

The animals were weighed and counted in a whole body counter for potassium-40 at 0, 3, 9, and 15 or 18 weeks. Fat content was calculated, and both the gross weight and fat compartment weight were plotted against time. The data were fit to a second-degree polynomial using the Chebyshev method where $\max|P(X_i - Y_i)|$ is a minimum for all A_i.

The program was run on a computer[2] and produced the polynomial parameters A_0, A_1, A_2, and D_{max} (maximum deviation from fit) so that:

$$Y \text{ (total weight)} = A_0 + A_1t + A_2t^2 \quad \text{(Eq. 2)}$$

where t is weeks after dosing, and:

$$Y \text{ (fat weight)} = B_0 + B_1t + B_2t^2 \quad \text{(Eq. 3)}$$

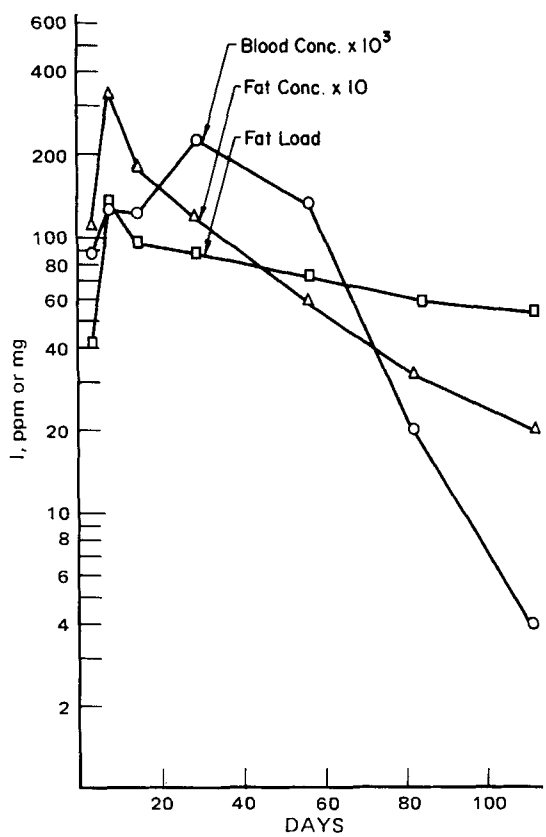

Figure 3—*Mean blood and fat concentrations (parts per million) and total fat content (milligrams) in pigs receiving 2.14 mg/kg/day of I for 7 days* (n = 3).

[2] IBM 360/65.

Table III—Apparent Pharmacokinetic Parameters for Fat Elimination

Treatment	Concentration Basis		Total Load Basis	
	$t_{1/2}$ Elimination, weeks	K_{el}, week^{-1}	$t_{1/2}$ Elimination, weeks	K_{el}, week^{-1}
I[a]	3.43	0.202	15.4	0.045
II[a]	3.57	0.194	38.3	0.018
III[b]	9.00	0.077	DI[c]	DI[c]
IV[b]	6.00	0.116	40.5	0.017
Peak 127[d]	5.36	0.129	24.2	0.028
Peak 149[e]	6.00	0.116	23.3	0.030

[a] Start of elimination phase at 2 weeks. [b] Start of elimination phase at 4 weeks. [c] Data were inadequate for estimate; a longer sample time was required. [d] 16.7% of polychlorinated biphenyl mixture; predominantly II. [e] 13.7% of polychlorinated biphenyl mixture; III is a significant, but not predominant, component.

The total fat load, L_t, is calculated by:

$$L_t \text{ (mg PCB)} = \text{(concentration PCB)}(t)(B_0 + B_1t + B_2t^2) \quad \text{(Eq. 4)}$$

RESULTS AND DISCUSSION

Computer Design—The computer-generated coefficients and D_{max} values for total weight and fat weight are presented in Tables I and II. Three sample curves are given in Fig. 1: for a control animal, for one treated with III, and for one treated with IV. The computer-fitted curves were drawn with the raw data points to permit visual comparison of fit to the polynomial. The trends in growth shown are consistent with the means and indicative of possible effects of polychlorinated biphenyls on swine performance. Percent weight gains (n = 3) were: control, 555 ± 105; III, 482 ± 62; II, 471 ± 52; I, 410 ± 67; and IV, 372 ± 42.

Pharmacokinetics—Figure 2 shows the total fat load data for pigs given the hexachlorobiphenyl ration[3]. In each case, a rise in total load occurred at 60–84 days after dosing due to the possible redistribution of residues (8). If the means of each group are examined for total fat load, fat concentration, and blood levels (Figs. 3–6), it becomes apparent that a redistributive phase existed in the pigs receiving II and III between 56 and 84 days after dosing. Although redistribution also may have occurred with I, its lower molecular weight and size made it easier to mobilize in the animal and the redistribution phase may have been missed in the sampling.

Generally, the amplitude and time shift of this redistribution are directly proportional to lipid solubility and degree of chlorination and inversely proportional to mobility and water solubility. The large time lag before redistribution discounts the more common factors responsible for nonlinearity such as enterohepatic recirculation and saturated tubular secretion. Changes in plasma protein binding characteristics might be suspect, but the volume of the blood compartment is less than fat and the concentration in blood is 10^{-3} that of fat so that the amounts in blood are inadequate.

Induction of microsomal oxidases by polychlorinated biphenyls (4) may account for the more rapid elimination rate at early times, followed

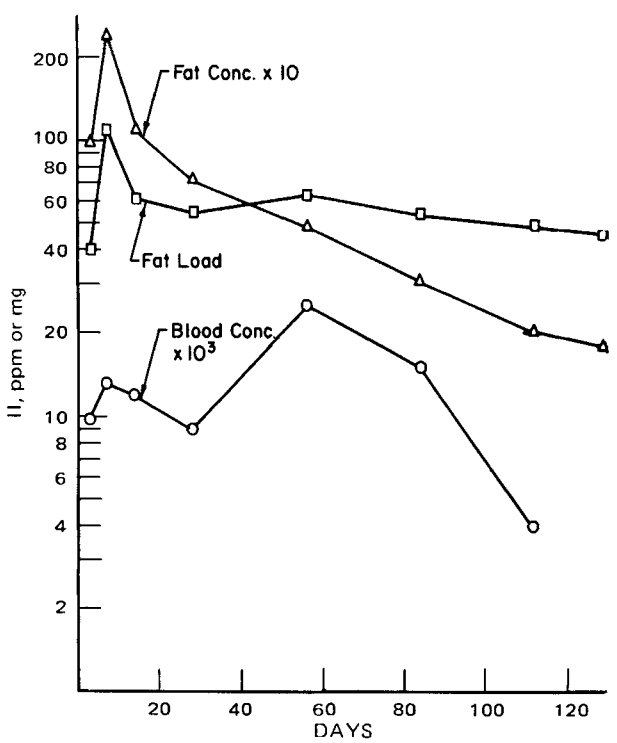

Figure 4—*Mean blood and fat concentrations and total fat content in pigs receiving II.*

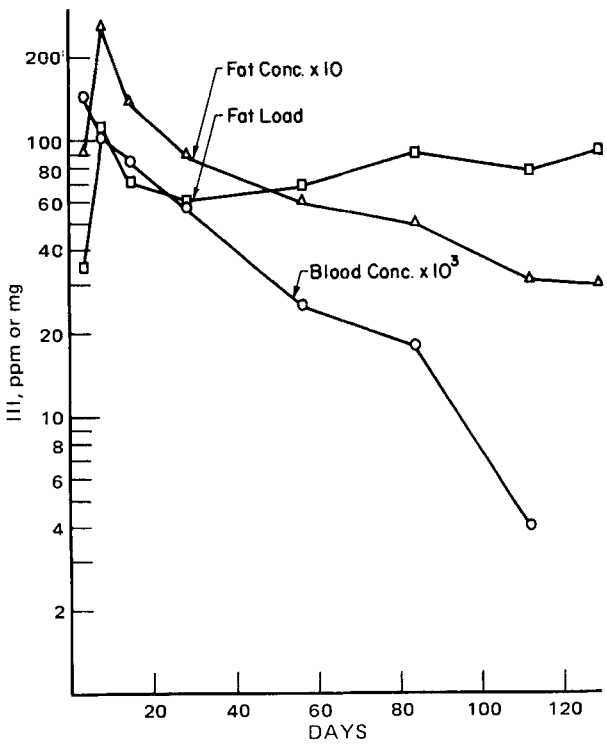

Figure 5—*Mean blood and fat concentrations and total fat content in pigs receiving III.*

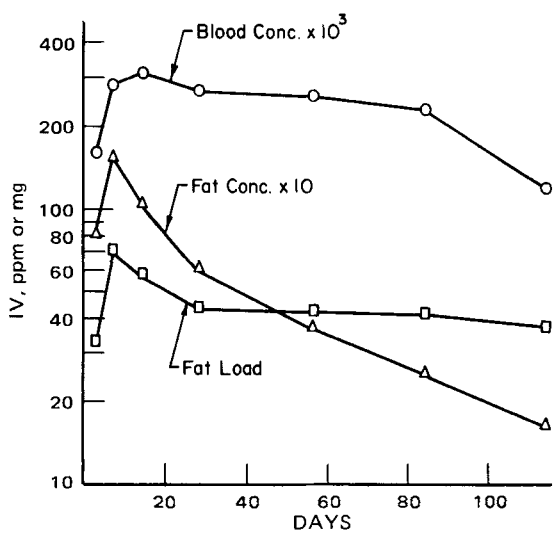

Figure 6—*Mean blood and fat concentrations and total fat content in pigs receiving a commercial mixture containing 54% chlorine.*

[3] Lines connected to raw data points were drawn by hand.

Table IV—Mean Terminal Tissue Residues 16 or 18 Weeks after 2.14 mg/kg/day of Polychlorinated Biphenyl for 7 Days

Treatment	Concentration, ppb					
	Fat	Blood	Liver	Kidney	Muscle	Spleen
I[a]	1987 ± 732	4 ± 3	330 ± 51	535 ± 71	689 ± 55	321 ± 14
II[b]	1807 ± 631	4 ± 1	140 ± 14	31 ± 2	71 ± 1	59 ± 22
III[b]	3117 ± 445	4 ± 2	358 ± 49	59 ± 10	81 ± 11	64 ± 16
IV[a]	1660 ± 360	120 ± 10	1593 ± 208	340 ± 76	780 ± 280	577 ± 217
Peak 127	354 ± 65	3.3 ± 0	127 ± 28	12 ± 7	27 ± 10	20 ± 10
Peak 149	283 ± 42	2.7 ± 0	ND[c]	11 ± 7	23 ± 5	26 ± 16

[a] Sixteen weeks. [b] Eighteen weeks. [c] Not determined due to interference.

by reduced activity as the blood concentration drops and microsomal parameters return to near normal; however, this should result in a flattening in the decline of the fat load rather than a rise. The redistribution must result from, or be partially dependent on, changes in concentration throughout the sampled fat subcompartment due to nonparallel changes in this and other fat subcompartments. Further delineation of the redistribution requires more extensive (destructive or more traumatically invasive) sampling of several subcompartments.

Fat concentration alone provides pharmacokinetic curves indicative of the fast distribution and slow elimination phases of a two-compartment model (9). The elimination from a single compartment and the β-elimination phase are calculated exactly the same as long as both processes exhibit a first-order decline.

Elimination constants (K_{el} in Table III) were calculated on the basis of first-order elimination from the fat compartment using:

$$K_{el} = 0.693/t_{1/2} \qquad \text{(Eq. 5)}$$

The values based on concentration half-lives are not indicative of elimination only but rather a combination of elimination and dilution due to compartment growth. Elimination constants based on total fat load, however, can be used as a precise measure of true elimination from fat. Compared to fat concentrations, blood concentrations indicate a faster elimination rate for all compounds (Figs. 3–6); however, the actual elimination phase for blood is delayed beyond that for fat. In addition, blood concentrations tend to increase (Fig. 4) or deviate from linearity (Fig. 5) at the times the total fat load indicates a redistribution of residues. The smaller, more mobile tetrachlorobiphenyl (I) maintains relatively higher blood concentrations than the heavier analogs and implicates a redistribution at about 28 days (Fig. 3).

Total fat load curves show that the true elimination half-lives based on total content are much longer than indicated by concentration (Figs. 2–6). In no case did the total fat load curve ever reach a half-life due to true elimination (starting at 2–4 weeks, depending on the isomer). In Table III, elimination half-lives based on total fat load are compared to those computed from more familiar concentration methods. It is necessary to use the apex of the redistributive phase as the initial start of elimination, since growth at this time is linear and a decrease in total load seems due to metabolism and elimination. It is possible that the total body load of polychlorinated biphenyls does not decrease at all in swine (10); however, the declines seen after redistribution is complete (Figs. 3 and 4) indicate that, although slow, some elimination does occur.

The commercial mixture (IV) used in this study contained 54% chlorine by weight and was composed of several components identified by GLC retention times (11, 12). Two predominant peaks produced by GLC separation occurred at retention times of 127 and 149 with respect to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane = 100. Peak compositions were confirmed by mass spectrometry, and studies of individual components were conducted previously (4, 11, 12).

When components 127 and 149 of IV are compared to their respective analogs (Figs. 7 and 8), the pentachlorobiphenyl analog (II) more closely matches the course of absorption and redistribution than the hexachlorobiphenyl analog (III). This result must be considered primarily due to the fact that II is a major component of peak 127 while III is only a minor component of peak 149 (11, 12). Therefore, conclusions concerning the behavior of the purified analogs compared with their behavior in a mixture cannot be drawn from these data alone.

Tissue Residues—According to the data in Table IV, II is the least persistent in all tissues analyzed, including blood. The fat load curves in Figs. 3–6 show, however, that fat content remains high. This finding indicates an increased vulnerability to metabolism rather than low lipophilicity. Compound II is the only isomer present that can assume two adjacent *ortho*-positions which are open to attack. This singularity would also produce a more planar molecule, which would be susceptible to dibenzofuran formation or hydroxylation.

The tetrachlorobiphenyl (I) maintained its presence in the blood and in the highly vascularized liver and kidney to a greater degree than did II and III, indicating a low level of biotransformation. It was the only analog significantly eliminated from the fat compartment, but this elimination was probably due to mobility rather than biotransformation.

The components of the mixture were generally distributed in the tissues according to degree of chlorination (Table V). Peaks 85 and 105, tetra- and pentachlorobiphenyl(s) (12), were eliminated from most tissues after 16 weeks. Peak 85 was apparently highly mobile and present in high levels in blood and liver only. Peaks 83 and 99 were concentrated in all

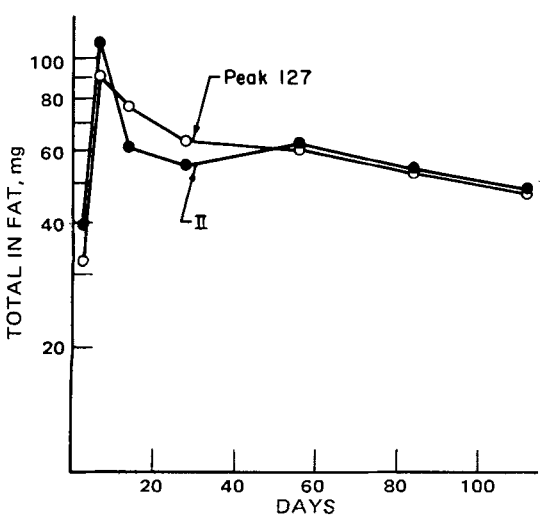

Figure 7—*Comparative fat dynamics of II and peak 127 of the commercial mixture.*

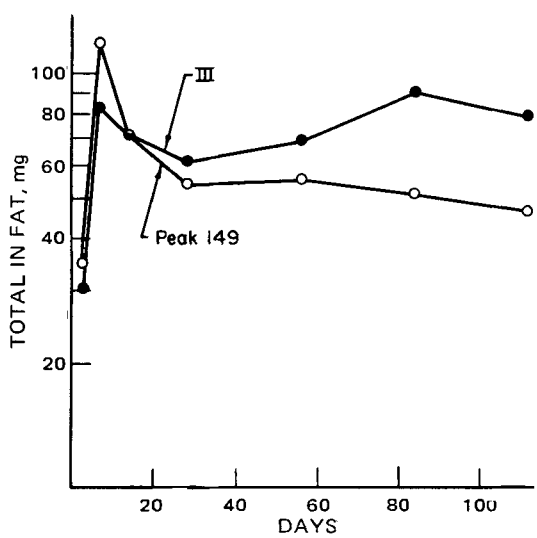

Figure 8—*Comparative fat dynamics of III and peak 149 of the commercial mixture.*

Table V—Distribution of Polychlorinated Biphenyl Mixture Components in Swine Tissues

Component	Mean Concentration, ppm Relative to Standard						Percent Total Polychlorinated Biphenyl						
	Fat	Liver	Kidney	Muscle	Spleen	Blood	Fat	Liver	Kidney	Muscle	Spleen	Blood	Standard
70	0.96	2.57	0.26	0.99	0.34	0.04	7.9	24.8	11.5	20.3	8.2	5.05	16.1
83	3.81	8.08	2.26	4.93	4.27	0.73	15.9	39.6	51.0	51.6	52.2	47.0	8.2
85	0.00	2.63	0.00	0.00	0.00	0.31	0.0	12.9	0.0	0.0	0.0	20.0	8.2
99	3.11	1.27	0.82	0.98	1.49	0.20	16.0	7.7	22.8	12.6	22.4	15.9	10.1
105	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	10.1
127	2.12	0.76	0.07	0.16	0.12	0.02	18.0	7.6	3.2	3.4	3.0	2.6	16.7
149	2.06	ND	0.08	0.17	0.19	0.02	14.4	ND[a]	3.0	3.0	3.9	2.2	13.7
176	3.61	0.35	0.11	0.19	0.15	0.04	15.1	1.7	2.5	2.0	1.8	2.6	8.2
208	3.56	0.23	0.13	0.27	0.30	0.02	3.8	0.3	0.8	0.7	0.9	0.3	2.1
253	3.47	0.16	0.09	0.23	0.32	0.02	3.4	0.2	0.5	0.6	0.9	0.3	1.9
286	4.79	1.44	0.43	2.43	1.63	0.04	1.5	0.5	0.7	1.9	1.5	0.2	0.6
332	5.36	12.93	ND	ND	8.04	0.03	0.3	0.8	ND	ND	1.2	Trace	0.1
Total	1.66	1.59	0.34	0.78	0.58	0.12							

[a] Not determined due to interference.

tissues above the relative levels present in the mixture administered. Otherwise, the peaks of longer relative retention time (more highly chlorinated) accumulated in fat while blood carried the peaks of shorter relative retention time. Other tissues were intermediate, except that the liver maintained high levels of component 70, which is rapidly distributed or eliminated from swine and sheep fat and blood (1, 2, 4, 5); the level of peak 253, which is rapidly eliminated by fish (13), was lower in the liver than in any other tissue.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 16, 1975, from the *Comparative Environmental Pharmacology and Toxicology Program, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801.*

Accepted for publication June 11, 1976.

Supported by Research Contract FDA 72-116 from the Bureau of Veterinary Medicine, Food and Drug Administration.

The authors thank Monsanto Chemical Co. for supplying Aroclor 1254, S. B. Dorn and S. N. Hirwe for the synthesis and purification of the analogs, and R. E. Borchard, T. G. Lohman, R. H. Teske, and D. W. Wilson for their advice and assistance.

[x] To whom inquiries should be directed.

Renal Function Testing: Differentiation between a Nephrotoxic Agent and Diuretic Drugs

E. E. VOGIN

Abstract □ Preliminary studies indicate that it may be possible to differentiate the effects of a nephrotoxic substance from those of diuretic agents by the measurement of both urine and plasma osmolality. The nephrotoxic substance, mercuric chloride, decreases urinary osmolality and increases plasma or serum osmolality. The diuretic agents, at exceedingly high dosages, may show a dose-related decrease in urine osmolality. However, serum osmolality either remains unchanged or is only slightly lowered. This difference in the serum response of animals treated with a nephrotoxin or diuretic agents may allow for the differentiation in toxicological studies.

Keyphrases □ Osmolality, urine and plasma—effects of nephrotoxic and diuretic agents compared, dogs □ Renal function testing—differentiation between nephrotoxic and diuretic agents by urine and plasma osmolality measurements, dogs □ Nephrotoxic agents—mercuric chloride, effect on urine and plasma osmolality compared to various diuretic agents, dogs □ Diuretics, various—effects on urine and plasma osmolality compared to nephrotoxic agent, dogs □ Mercuric chloride—effect on urine and plasma osmolality compared to various diuretic agents, dogs

The evaluation of nephrotoxicity in safety evaluation studies has always posed a problem to the toxicologist. The need to determine histopathological changes in the urinary bladder prohibits catheterization because of possible irritant effects. The clinical evaluation of nephrotoxicity evolves about the determination of blood (serum) urea