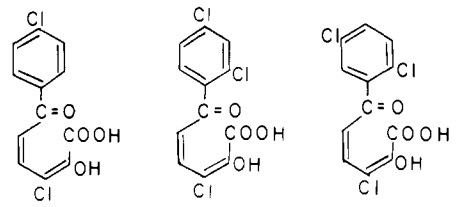
classify: 2,5,2'- and 2,5,3'-trichlorobiphenyl should be included in the first group, but the degradation rate is much slower than for other compounds in this group. Also, a slight yellow color is observed during the incubation period, thus making them a little different from the rest of the components of the first group.

There have been some reports to indicate the chemical nature of the compounds which have a yellow color with absorption maxima around 400 nm in neutral and basic pH and 320 nm in acidic pH. Dagley et al. (1960) reported that the intermediate derived from microbial degradation of catechol has a bright yellow color with absorption maxima at 373 nm in neutral and alkaline pH and 317 nm in acidic pH. The compounds were produced by cleavage of catechol by catechol 2:3-dioxygenase and identified as α-hydroxymuconic semialdehyde. Lunt and Evans (1970) reported that gram-negative bacteria produced α-hydroxy-β-phenylmuconic semialdehyde [mp 122°C, λ_{max} 350 nm (acid) and 430 nm (alkali)] from biphenyl.

Catelani et al. (1973) revealed that an oxidative metabolic process of biphenyl in *Pseudomonas putida* proceeds through formation of catechol-type intermediates on one of the rings and then a derivative of muconic semialdehyde (2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate). There are other excellent works on the metabolic pathways of aromatic compounds (Gibson, 1968) and halogenated aromatics (Smith et al., 1968) via formation of catechols (Dagley and Gibson, 1965) which indicate that the ring opening takes place by the formation of muconic acid analogs which often exhibit yellow color. These facts suggest that yellow colored compounds derived from aromatic compounds with absorption maxima around 400 nm in neutral and alkaline pH are indeed α-hydroxymuconic acid or semialdehyde derivatives. The keto forms have absorption maxima around 320–350 nm. Our mass spectrometric data also support this view inasmuch as the fragmentations corresponding to C_6H_4ClCO or $C_6H_3Cl_2CO$ were derived from the yellow compounds of 4,4'-di- and 2,4,4'- and 2,5,4'-trichlorobiphenyl. The infrared spectra of 2,4,4' and 2,5,4' derived metabolites indicate the presence of ketonic carbonyl, hydroxyl, and carboxylic acid moiety in the molecule. The compounds thus appear to be the chlorinated derivatives of α-hydroxymuconic acid with structures a, b, and c: (a) (from



(a) (b) (c)

4,4'-dichlorobiphenyl) 3-chloro-2-hydroxy-6-oxo-6-(4-chlorophenyl)hexa-2,4-dienoic acid (or sometimes referred to as α-hydroxy-β-chloro-6-(4-chlorophenyl)muconic acid); (b) (from 2,4,4'-trichlorophenyl) 3-chloro-2-hydroxy-6-oxo-6-(2,4-dichlorophenyl)hexa-2,4-dienoic acid; (c) (from 2,5,4'-trichlorophenyl) 3-chloro-2-hydroxy-6-oxo-6-(2,5-dichlorophenyl)hexa-2,4-dienoic acid.

These results clearly indicate that microorganisms are likely to degrade the less chlorinated members of PCBs first, leaving highly chlorinated PCBs in the environment. Among different PCB preparations less chlorinated ones (e.g., Aroclor 1242) degrade faster than the highly chlorinated preparations such as Aroclor 1260. Also, microorganisms appear to preferentially degrade the less chlorinated of the two aromatic rings. It would be of great interest and concern for environmental toxicologists to see whether there will be such a change in the residue composition of PCBs in nature in the next few years eventually leading to accumulation of very stable polychlorobiphenyls.

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Residues of Polychlorinated Biphenyl (PCB) Components in Broiler Cockerels Receiving Two Aroclors in Three Dietary Variations

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Broiler chickens were fed three dietary variations containing 20 ppm of the polychlorinated biphenyl mixtures Aroclor 1242 or Aroclor 1254 for an 8-week growth period. The time course of residue accumulation in fat and blood was determined. Fat accumulation reached a maximum of 98 ppm for Aroclor 1242 and 161 ppm for Aroclor 1254. Highest residue levels were found in fat followed by liver, kidney, spleen, muscle, brain, and blood. Differences between tissues in component composition of total residue were examined.

The presence and persistence of polychlorinated biphenyls (PCB's) in biological systems throughout the world have been well documented (Jensen, 1966; Risebrough et al., 1968). Several incidents have been reported where this group of compounds has caused human disease (Kuratsune et al., 1972) and residues leading to condemnation of food producing animals (Pichirallo, 1971; Maugh, 1972).

PCB's have been reported to adversely affect hatchability through embryotoxicity (Britton and Huston, 1973;

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Table I. Total PCB Residues (ppm) in Blood and Fat of Broiler Cockerels Fed 20 ppm of Aroclor 1242 or 1254 for Eight Weeks

	Total PCB concentration, ppm												
	Blood at week							Fat at week					
	1	3	5	7	8[a]	10	12	1	3	5	8[a]	10	12
1242													
Normal	0.31	0.15	0.16	0.17	0.30	0.09	0.07	NS[b]	26.59	17.80	56.92	NS	64.94
Lo pro	0.36	0.13	0.30	0.36	0.40	0.05	0.09	13.20	98.00	19.69	53.30	135.43	109.05
High fat	0.32	0.11	0.19	0.20	0.25	0.09	NS	NS	53.40	20.60	39.10	99.10	165.93
1254													
Normal	0.28	0.39	0.41	0.95	0.59	0.26	NS	58.03	143.30	160.94	59.30	368.78	NS
Lo pro	0.37	0.41	0.69	0.84	0.51	0.15	NS	65.95	106.16	132.52	95.14	75.59	106.40
High fat	0.32	0.23	0.30	0.45	0.41	0.16	0.13	58.29	93.76	121.36	108.19	177.06	19.33
Control													
Normal	NS	0.02	0.03	0.04	0.01	0.02	NS	NS	0.70	0.57	1.05	NS	2.42
Lo pro	NS	0.01	0.09	0.04	0.00	0.04	0.01	NS	1.03	2.70	1.10	NS	4.25
High fat	NS	0.00	0.04	0.02	0.01	NS	NS	NS	0.69	0.55	1.07	11.25	NS

[a] Treated feed discontinued at the end of week 8. [b] NS = no sample.

Cecil et al., 1974; Ax and Hansen, 1975). Other pathologic effects reported are generalized edema and liver necrosis (Flick et al., 1965; Vos, 1972). PCB's have also been shown to induce hepatic drug metabolizing enzyme activity in birds (Cecil et al., 1975).

Several papers have dealt with PCB residues in wild and domestic fowl (Risebrough et al., 1968; Dahlgren et al., 1972; Platonow and Funnell, 1973; Britton and Huston, 1973; Cecil et al., 1974; Teske et al., 1974; Collier et al., 1976). While elimination of residues has been studied (Cecil et al., 1974; Teske et al., 1974), little data are available on the accumulation or changes in relative component concentration of PCB's during oral exposure.

This study was undertaken to more thoroughly examine the accumulation of PCB's resulting from a long-term oral exposure. Compositional changes were also examined to determine the relative importance of single PCB isomers as residues in human food. The broiler-type chicken is markedly different in its body composition and growth patterns from the layer-type birds used in most other studies. We have used broiler-type birds in our study to determine if these differences are of consequence in studying the accumulation and distribution of environmental contaminants.

MATERIALS AND METHODS

Experimental Design. Day-old hybrid broiler cockerels were obtained from a commercial source, divided into groups of 50, and reared for 4 weeks in a Petersime brooder unit. Chicks were then transferred to larger cages and reared for 8 additional weeks. Diets were formulated from ingredients determined to be free from detectable organochlorine contamination as normal (23% protein, 4.5% fat), low in protein (17% protein, 4.9% fat), and high in fat (23% protein, 8.3% fat). Each diet was contaminated via a premix to a level of 20 ppm of either Aroclor 1242 or Aroclor 1254. Chickens were fed treated diet from 1 through 8 weeks of age and were then fed untreated diets from 9 to 12 weeks of age to examine elimination of residues. Every 2 weeks, three birds from each group were sacrificed, and blood and mesenteric fat taken for residue analysis. At 8 weeks, liver, kidney, muscle, brain, spleen, mesenteric fat, and blood were taken for residue analysis. Blood was collected by cardiac puncture prior to sacrifice.

Analytical Methods. Tissues were homogenized with dry ice in a high speed Waring blender and the CO_2 allowed to sublime overnight at −20 °C. A 1.0-g sample was then ground with 35 g of Na_2SO_4 with a mortar and pestle and packed into a 19 mm (i.d.) × 300 mm chromatography column. Small tissues (<5 g) were weighed and ground without homogenization. The column was eluted with 175 ml of hexane-saturated acetonitrile into a 300-ml boiling flask. The solvent was evaporated on a rotary evaporator to 5 ml and transferred to an alumina column for cleanup and analysis by gas chromatography as previously described (Welborn et al., 1974). Components are identified by their retention times relative to DDE (RRT).

Quantitation of individual components is performed by comparison of peak heights to standards of Aroclors 1242 and 1254 using linear regression analysis and expressing concentration as parts per million relative to Aroclor 1242 or 1254 as previously described (Borchard et al., 1974). Total PCB concentration is calculated by multiplying each relative component concentration by the fraction of this component determined to be present in Aroclor mixtures (Hirwe et al., 1974) and summing the resulting concentrations as follows: total PCB = Σ (relative component concentration) × (percent in mixture). The percent of the total PCB residue contributed by components is then readily calculated by the following: percent contributed = {[(relative concentration) × (percent in mixture)]/total PCB} × 100.

RESULTS AND DISCUSSION

Chickens gained an average of 1.70 kg during the 8-week treatment period(2667%). This gain was affected by diet, but not by PCB (Hansen et al., 1976). Chickens consumed an average total of 3555 g of feed each during this period for a dose level of 71.1 mg/bird per 8 weeks.

Accumulation of PCB's in Fat and Blood. After 3 weeks PCB residues in fat of chickens fed 20 ppm of Aroclor 1242 or Aroclor 1254 accumulated to levels threefold over the dietary level for Aroclor 1242 and 5.7-fold for Aroclor 1254 (Table I). During the remainder of the feeding period, residue levels ranged from 1 to 2.5 times the dietary level for Aroclor 1242 and from 4.4 to 6.9 times for Aroclor 1254. Aroclor 1254 total residues were consistently higher than Aroclor 1242 total residues in all diets. This is to be expected since the principal constituents of Aroclor 1254 have a higher metabolic stability (Borchard et al., 1974). The greater lipid solubility of Aroclor 1254 could also decrease elimination by making less of the total residue available in the blood for elimination and causing greater resorption from the kidney tubules.

For Aroclor 1242, blood levels in each dietary group were initially high and declined between 1 and 3 weeks. Thereafter, blood levels rose throughout the feeding period.

Table II. Total PCB Residues in Tissues of Broiler Chicken Fed Diets Containing 20 ppm of Aroclor 1242 or Aroclor 1254 for 8 Weeks

	Total PCB concentration, ppm						
	Fat	Liver	Kidney	Muscle	Brain	Spleen	Blood
1242							
Normal	56.29	2.53	2.85	2.17	0.84	1.96	0.30
Low protein	53.30	4.29	3.45	1.39	2.37	2.27	0.40
High fat	99.10	2.63	3.53	1.64	1.29	2.54	0.25
1254							
Normal	59.30	4.91	6.16	1.46	0.86	ND[a]	0.59
Low protein	95.14	5.46	1.81	0.86	0.93	1.11	0.51
High fat	108.79	6.06	9.44	1.23	1.04	1.41	0.41

[a] ND = not determined.

Table III. Percent of Total PCB Residue Contributed by Individual Components: Normal Diet, Aroclor 1242 at 20 ppm

RRT[a]	1242	Fat	Liver	Kidney	Muscle	Brain	Spleen	Blood
21	0.5	0.0	0.0	0.0	0.0	0.1	0.1	0.0
26	3.4	0.2	0.2	0.0	0.0	0.8	0.5	0.0
31	8.8	0.2	0.1	0.0	0.0	2.5	0.9	0.0
37	15.5	29.8	16.2	22.4	27.2	18.4	23.9	25.0
41	5.7	0.0	0.0	0.0	0.0	2.5	1.7	0.0
48	27.4	33.9	25.4	33.5	32.5	20.6	28.3	37.8
53	11.0	1.1	0.0	4.2	4.2	5.8	3.6	1.1
57	11.5	3.1	26.6	1.8	1.8	6.5	6.8	5.0
70	9.0	16.2	9.9	14.6	14.6	10.4	11.5	15.8
83	3.5	16.8	9.4	10.7	10.7	7.4	10.2	6.6
85	0.7	1.0	0.0	0.0	0.0	0.3	0.6	1.2
99	1.1	2.3	5.3	3.5	3.5	1.8	1.7	2.7
105	1.2	1.1	3.7	1.7	1.7	1.2	1.2	1.5
127	0.5	2.0	1.2	2.2	2.2	18.2	5.0	1.8
149	0.3	1.8	1.4	1.2	1.2	3.1	3.4	1.1
176	0.05	0.3	0.4	0.4	0.4	0.3	0.8	0.3

[a] Component relative retention time (p,p-DDE = 100).

Fat concentrations were comparatively high at 3 weeks, were lowest at 5 weeks, and increased thereafter. This suggests that the differential growth rates of tissues important in accumulation of PCB's (such as fat) and those involved in elimination (such as blood and liver) have a significant effect on actual tissue concentrations. Development of metabolic capabilities could also be of importance here; however, since differing metabolic rates for each component would be expected and ratios of components remained fairly constant throughout the experiment, metabolic development is probably not a significant factor.

For Aroclor 1254, residues in blood and fat in all dietary groups increased during the first 5 weeks and then decreased at 8 weeks. Changes in body composition could account for these changes also. It is unclear what the differences between 1242 and 1254 are in this aspect, but perhaps the greater effects of 1254 on performance are involved here. Weight gains were depressed at 7 weeks in our study but were higher between 7 and 8 weeks while feed consumption remained relatively constant. The increased weight gain (most likely due to alleviating the stress of crowding) without greater feed exposure would correlate with the residue changes during these times.

When treated feed was replaced with control feed at 8 weeks, very little elimination of PCB residues from fat occurred during the next 4 weeks (Table I). In some diets, increased PCB residues in fat were observed. Since the birds were maintained in the same environment, continued exposure through dust (which contained 2–3 times more PCB than the coarser components) and other vehicles may have occurred. Merely removing contaminated feed in a practical situation may not, then, eliminate residue accumulation. Redistribution from tissue compartments not examined (e.g., skin, alimentary tract) may also effect elimination or further accumulation in fat after the dietary source is removed.

PCB Residues in Broiler Chicken Tissues. *Aroclor 1242.* The information in Table II shows the residue of total PCB in broiler chicken fat, liver, kidney, muscle, brain, and spleen and blood when fed normal, low protein, and high fat diets containing 20 ppm of PCB over a period of 8 weeks. Total Aroclor 1242 residues in fat after 8 weeks averaged 69.56 for the three diets. This was approximately 22× greater than the average in liver (3.15 ppm) and kidney (3.28 ppm). Spleen averaged slightly less (2.24) while residues in muscle average 1.73 ppm, about one-half those of liver and kidney. The lowest residues were found in brain (1.50 ppm) and blood (0.32 ppm).

Aroclor 1254. The information in Table II shows the residues of total PCB in broiler chicken fat, liver, kidney, muscle, brain, spleen, and blood when fed normal, low protein, and high fat diets containing 20 ppm of Aroclor 1254 over a period of 8 weeks. Leghorn cockerels receiving 10 ppm of Aroclor 1254 had comparable fat and muscle levels, but kidney and liver residues were about one-third those seen in this study (R. H. Teske, FDA, Beltsville, Md., personal communication). Aroclor 1254 residues in fat averaged 87.74 ppm (Table I) and were approximately 16× greater than the average in liver (5.48 ppm) and kidney (5.80 ppm). Residues in spleen averaged 2.24 ppm, about one-half of the liver and kidney concentrations while muscle (1.13 ppm), brain (0.94 ppm), and blood (0.50 ppm) were much lower. The leghorn cockerel data show residues in fat (61.67 ppm) and muscle (1.42 ppm) approaching the levels seen in broilers on the 20-ppm diet but much lower residues in liver and kidney. The residues in kidney and liver were nearly equal in broiler cockerels but were higher in kidney than in liver in the leghorn cockerels. Since broiler chickens have a higher proportion of fat and muscle

Table IV. Percent of Total PCB Residue Contributed by Individual Components: Low Protein Diet, Aroclor 1242 20 ppm

RRT[a]	1242	Fat	Liver	Kidney	Muscle	Brain	Spleen	Blood
21	0.5	0.0	0.0	0.0	0.0	0.4	0.1	0.0
26	3.4	0.2	0.9	0.0	0.0	2.6	0.8	0.2
31	8.8	0.2	2.9	0.0	0.0	7.4	1.7	0.7
37	15.5	29.1	23.4	23.4	18.8	16.5	12.8	18.5
41	5.7	0.0	0.0	0.0	0.0	3.9	2.0	0.1
48	27.4	35.6	31.4	33.3	30.1	20.9	13.6	38.8
53	11.0	2.4	0.0	5.1	10.7	6.7	3.4	2.2
57	11.5	4.6	10.5	14.2	2.7	8.7	49.1	10.9
70	9.0	15.0	12.4	11.3	13.8	7.0	6.5	13.2
83	3.5	5.9	10.6	7.1	13.9	3.2	3.1	5.4
85	0.7	0.9	0.0	0.0	0.0	0.4	0.5	1.1
99	1.1	2.2	3.0	1.9	4.7	1.3	1.3	3.7
105	1.2	1.2	1.9	1.5	2.2	1.0	0.8	2.0
127	0.5	1.5	1.6	1.2	1.8	16.3	2.2	1.5
149	0.3	1.0	1.0	0.7	0.9	3.3	1.8	1.1
176	0.05	0.2	0.5	0.4	0.4	0.5	0.3	0.6

[a] Component relative retention time (p,p-DDE = 100).

Table V. Percent of Total PCB Residue Contributed by Individual Components: High Fat, Aroclor 1242 at 20 ppm

RRT[a]	1242	Fat	Liver	Kidney	Muscle	Brain	Spleen	Blood
21	0.5	0.1	0.0	0.0	0.0	0.4	0.1	0.0
26	3.4	0.4	0.1	0.0	0.0	2.8	0.8	0.0
31	8.8	1.0	0.7	0.0	0.0	8.2	1.8	0.7
37	15.5	31.9	13.4	25.0	14.4	18.9	24.4	47.0
41	5.7	0.0	0.0	0.0	0.0	4.6	2.0	0.0
48	27.4	33.3	24.6	33.4	25.5	22.5	28.4	21.9
53	11.0	1.1	0.0	4.8	12.6	7.7	4.8	0.0
57	11.5	3.4	22.3	10.2	3.5	10.3	7.9	6.4
70	9.0	15.8	10.5	11.7	14.0	8.7	13.5	11.1
83	3.5	6.2	13.9	7.5	17.5	4.9	7.8	5.0
85	0.7	0.8	0.0	0.0	0.0	0.3	0.6	1.4
99	1.1	2.2	7.3	2.5	6.7	1.6	2.1	2.5
105	1.2	0.5	4.0	0.9	2.7	1.2	2.5	1.1
127	0.5	2.0	1.4	2.1	1.6	4.2	0.5	1.4
149	0.3	1.2	0.9	1.3	0.8	3.7	2.4	1.0
176	0.05	0.2	0.8	0.6	0.6	0.03	0.4	0.04

[a] Component relative retention time (p,p-DDE = 100).

Table VI. Percent of Total PCB Contributed by Individual Components in Broiler Chicken Tissues: Normal Diet, Aroclor 1254 at 20 ppm

RRT[a]	1254	Fat	Liver	Kidney	Muscle	Brain	Blood
70	16.1	10.9	8.5	10.2	12.7	13.5	9.3
84	16.4	14.4	9.3	12.7	14.3	11.4	13.0
99	10.1	9.6	15.5	9.6	10.4	1.5	9.9
105	10.1	5.7	19.2	8.9	0.0	5.4	6.3
127	16.7	22.3	15.7	22.0	23.9	25.8	20.6
149	13.7	19.1	13.8	18.6	19.9	22.5	20.2
176	8.2	11.2	12.3	11.8	11.6	12.2	11.9
208	2.1	2.9	3.2	3.1	3.2	3.4	5.4
253	1.9	2.9	2.1	2.2	2.8	3.1	2.4
286	0.6	0.7	0.6	0.8	1.2	1.1	0.8
332	0.1	0.1	0.1	0.2	0.2	0.2	0.2

[a] Component relative retention time (p,p-DDE = 100).

than the leghorn birds, one would expect the relatively lower residues in muscle and fat seen in broiler chickens. The differences in body composition between these birds are, then, important in predicting residue accumulation.

Tissue Differences in Component Composition of Total PCB Residues. Striking differences between tissues are apparent when the component composition of tissue residues is examined (Tables III–IX). Differences due to dietary influences were few. In general, the high fat diet appeared to cause less deviation from the composition of both standard Aroclor mixtures.

As exemplified by the fat, kidney, muscle, and blood residue composition compared with the percent composition of Aroclor 1242, broiler chickens concentrated the components with the following RRT: 37, 48, 57, 83, 99, 127, 149, and 176. Components with RRT of 21, 26, 31, 41, 53, and 57 were in general eliminated more rapidly. Components 70, 85, and 105 generally remained unchanged and may be useful as "indicator peaks" to estimate total Aroclor residues. More rapid elimination of lower chlorinated compounds is evident with the exception of peaks 37 and 48. Component 37 is primarily 2,4,4'-trichlorobiphenyl and component 48 is primarily 2,5,2',5'-tetrachlorobiphenyl (Webb and McCall, 1972). It appears that substitution in these key positions effectively blocks metabolism or elimination. This is in contrast to com-

Table VII. Percent of Total PCB Contributed by Individual Components in Broiler Chicken Tissues: Low Protein Diet, Aroclor 1254 at 20 ppm

RRT[a]	1254	Fat	Liver	Kidney	Muscle	Brain	Spleen	Blood
70	16.1	10.8	9.3	9.4	18.4	16.7	10.6	11.8
84	16.4	13.9	14.4	12.6	19.9	11.0	12.1	13.0
99	10.1	9.4	15.9	13.3	12.5	1.6	1.5	9.5
105	10.1	6.2	17.9	12.6	0.0	6.9	6.1	7.1
127	16.7	22.7	13.9	18.4	18.5	25.4	24.7	20.2
149	13.7	18.6	12.6	16.5	15.5	21.1	22.7	19.7
176	8.2	11.4	10.9	11.4	9.6	10.7	13.4	11.5
208	2.1	3.2	2.6	2.7	2.4	3.0	3.8	4.3
253	1.9	3.0	1.7	2.2	2.4	2.6	3.9	2.2
286	0.6	0.8	0.7	0.8	1.0	1.0	1.0	0.7
332	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1

[a] Component relative retention time (p,p-DDE = 100).

Table VIII. Percent of Total PCB Contributed by Individual Components in Broiler Chicken Tissues: High Fat Diet, Aroclor 1254 at 20 ppm

RRT[a]	1254	Fat	Liver	Kidney	Muscle	Brain	Spleen	Blood
70	16.1	12.1	10.2	9.5	27.3	16.2	14.0	9.4
84	16.4	16.0	13.7	12.2	23.1	10.1	11.4	12.4
99	10.1	9.0	15.0	7.9	14.3	1.6	1.6	9.8
105	10.1	6.4	18.0	6.6	0.0	6.6	6.8	6.9
127	16.7	13.6	13.2	23.1	11.6	25.7	22.5	22.4
149	13.7	21.5	11.7	19.2	9.6	19.1	20.4	20.7
176	8.2	12.8	10.5	11.6	6.0	10.6	11.6	11.6
208	2.1	3.5	2.5	2.8	1.6	2.7	3.3	4.0
253	1.9	3.1	1.7	2.4	1.3	2.5	3.3	2.1
286	0.6	1.0	0.6	0.7	1.0	0.8	1.0	0.7
332	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1

[a] Component relative retention time (p,p-DDE = 100).

Table IX. Representative Component and Total PCB Residues in Control and Treated Feed and Fat

	Concentration, ppm as Aroclor 1242			
Component	20 ppm of treated feed	Control feed	Treated fat	Control fat
21	19.5	0.05	0.00	0.00
26	20.7	0.05	0.00	0.00
31	20.0	0.10	0.00	0.00
37	20.0	0.09	74.95	0.30
41	20.0	0.46	0.00	0.28
48	23.4	0.09	53.96	1.86
53	20.4	0.00	5.68	1.59
57	18.3	0.00	8.15	0.00
70	20.2	0.24	65.03	0.44
83	20.8	0.40	73.02	3.00
85	24.7	0.75	78.27	8.00
99	20.4	0.69	61.69	4.72
105	20.0	0.78	28.70	0.00
127	22.2	1.99	132.97	5.37
149	26.5	3.11	135.63	9.16
176	26.9	3.45	234.83	32.00
Total PCB	20.9	0.15	39.10	1.07

ponent 31 which is primarily 4,4'-dichlorobiphenyl and is rapidly eliminated. Together components 37 and 48 comprise 42.9% of Aroclor 1242 (Hirwe et al., 1974) and 63.5% of the total residue in chicken fat at 8 weeks (normal diet).

Liver was distinguished from other tissues in that it did not concentrate components 37 and 48 while it did concentrate components 57 and 105. Brain did not eliminate components 21, 26, 31, and highly concentrated component 127. The brain's apparent affinity for lower chlorinated peaks combined with its low total PCB residue suggests that it does not act in vivo as a highly lipophilic tissue. Stereochemical configuration as well as lipid solubility may be important in determining which components accumulate in brain. Both brain and spleen failed to concentrate 99 and were relatively low in component 48.

Changes in Aroclor 1254 component composition in tissues were less apparent than in Aroclor 1242. In general, as shown by residues in fat, kidney, and blood, components 70, 84, and 105 were in lower concentrations than in the standard while components 127, 149, and 176 were concentrated by most tissues. The remaining components were relatively unchanged from the composition of standard Aroclor 1254. Liver concentrated components 99 and 105. Brain and spleen both had relatively low concentrations of component 99 as it did in Aroclor 1242 residues, while muscle was low in component 105. Muscle was also the only tissue to retain or concentrate components 70 and 84. The most constant components in all tissues for Aroclor 1254 were 208 and 253.

These data point out potential problems in examining only fat or blood for tissue residues for PCB's. Screening methods for the detection of certain of the more constant components (such as 70, 85, and 105 in Aroclor 1242 and 208 and 253 for Aroclor 1254) may be possible. Abdominal fat is the best tissue for residue detection because of the 15–22 times higher residues; however, descriptions and identification of residues should employ data from several key tissues.

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Diphenadione Residues in Tissues of Cattle

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Blood and tissue of cattle were tested for residues of diphenadione (2-(diphenylacetyl)-1,3-indandione), an anticoagulant livestock systemic intended for use in vampire bat (*Desmodus rotundus*) control. Liver and kidney samples from heifers given single 1 mg/kg intraruminal injections contained 0.15 ppm or less up to 90 days posttreatment. Detectable levels (>0.01 ppm) could not be found by gas–liquid chromatography in blood, brain, heart, fat, and muscle tissue samples taken at 30, 60, and 90 days posttreatment. Adult Sprague Dawley rats were fed the liver from test cattle in a 14-day secondary hazard feeding study. No rats died, treated and control rats did not differ in prothrombin clotting time, and diphenadione was not detected in the liver or blood of treated rats. Calculations based on residue levels in this study indicate that humans may safely eat the meat, including liver and kidney, of treated cattle.

Vampire bats (*Desmodus rotundus*) are reported to cost the Latin American cattle industry up to $250 million annually (Greenhall, 1970). They are important carriers of paralytic rabies, which claims an estimated 1 million head of cattle every year. In addition, there is widespread belief that severe debilitating effects are caused through malnutrition, myiasis, and loss of blood resulting from vampire bat attacks. In 1960, the Mexican Agriculture and Livestock Ministry's National Institute of Livestock Research and the U.S. Fish and Wildlife Service (sponsored by the U.S. Agency for International Development) cooperated in a program to develop methods of controlling vampire bat populations in areas where bat-borne rabies was a problem. The livestock systemic method of control was developed under this program.

In this method, cattle are injected with an anticoagulant, diphenadione (2-diphenylacetyl)-1,3-indandione). A single intraruminal dose of about 1 mg/kg is capable of killing vampire bats that feed on the blood of a treated animal within 3 days after dosing (Thompson et al., 1972). The cattle apparently do not suffer any ill effects. However, it was not known whether this control method results in significant residues in their tissues.

The residue levels of diphenadione in the tissues of treated animals, and the effects of these levels, must be considered before the method can be adopted. Therefore, we determined tissue residue levels at 30, 60, and 90 days following a 1 mg/kg injection and investigated the secondary hazard to rats that ate the liver of treated cattle.

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EXPERIMENTAL SECTION

Treatment of Animals and Collection of Samples. Six Hereford heifers weighing approximately 230 kg each were dosed with 1 mg/kg of diphenadione by injecting a Carbopol 941 suspension into the rumen. A pistol-grip automatic syringe (Vaco HL 013700) fitted with a 14 gauge, 1.5-in. disposable needle was used for the injection.

At 30, 60, and 90 days posttreatment, two animals were randomly selected and killed. Samples of blood plasma, liver, heart, kidney, brain, muscle from the hindquarter, and fat (1:1 mixture visceral and subcutaneous) were collected from each animal. Pretreatment blood samples and samples of other tissues from a local butcher shop served as untreated controls. All samples were stored at −12 °C until analyzed or used in feeding tests.

Residue Analysis. A gas–liquid chromatographic (GLC) procedure developed earlier in our laboratory (Bullard et al., 1975) was used for all analyses. In this procedure, diphenadione is oxidized to benzophenone, which chromatographs well on silicone columns and is sensitive to electron-capture detection. An oxidation step following sample cleanup provides constant yields of benzophenone under standardized conditions.

An Aerograph 1520B gas chromatograph equipped with a tritium foil electron-capture detector was used for all analyses. The 5 ft × 1/8 in. glass column was packed with 3% XE-60 on 100–120 mesh Gas-Chrom Q. The operating parameters were: injection port, 225 °C; column, 115 °C; and nitrogen flow rate, 35 ml/min. Under these conditions, benzophenone had a retention time of 14.2 min. The