Residues of Polychlorinated Biphenyl in Products from Poultry Fed Aroclor 1254

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White Leghorn chickens were fed diets containing Aroclor 1254 added at concentrations from 0.1 to 10.0 mg/kg, beginning at 3 days of age or at the onset of egg production, to investigate polychlorinated biphenyl (PCB) residues in tissues and eggs at selected periods throughout the life cycle. In 8-week-old birds given Aroclor at 10 mg/kg of diet beginning at 3 days of age, PCB residues in the dissected fat and muscle were 110.6 and 109.0 ppm (extractable fat basis), respectively. Depletion of PCB residues in these birds, monitored at weekly intervals for 3 weeks, indicated a biological half-life of 2.93 weeks. In birds given the diet containing Aroclor at 10 mg/kg for 32 weeks beginning at 3 days of age, PCB residues in the dissectable fat and muscle were 49.5 and 102.8 ppm (extractable fat basis), respectively; in birds given the same diet for 52 days beginning at the onset of egg production, the values were 33.4 and 70.1, respectively. The mean concentration of PCB residues in eggs from hens that had received the diet containing Aroclor at 10 mg/kg beginning at 3 days of age reached a peak of 9.73 ppm approximately 4 weeks after onset of egg production; residues then decreased. A similar pattern was observed in eggs of hens given the same diet beginning at the onset of egg production, but the peak value reached only 2.54 ppm. PCB concentrations in day-old chicks hatched from contaminated eggs closely paralleled the concentration in the eggs. At no time during the study were signs of toxicity exhibited by birds in any treatment group.

Since polychlorinated biphenyls (PCB's) have come into use industrially, they have been utilized extensively in a wide variety of applications. Many of these compounds represent potential sources for contamination of human and animal foodstuffs either directly or *via* environmental contamination. Direct contamination of animal feedstuffs during production, processing, and/or storage has resulted in the presence of PCB residues in meat, milk, and eggs, as well as in losses due to toxic responses in animals or poultry consuming the contaminated feedstuff. Evidence also indicates that the PCB's, like many other chlorinated hydrocarbon chemicals, are concentrated by the food chain. Thus the concentration of PCB residues occurring in food products from animal sources may be greater than the concentration in the feedstuffs that the animal had ingested.

Basic information about the absorption, metabolism, and excretion of PCB's and about the occurrence and incidence of PCB residues in poultry products has been largely lacking. The fact that PCB's, rather than representing a single chemical entity, actually represent a family of chemicals greatly magnifies the complexity of the problem of studying and evaluating their biological effects.

The objective of the study reported here was to evaluate PCB residues in poultry tissues and eggs following the feeding of Aroclor 1254 at graded dietary concentrations.

MATERIALS AND METHODS

Two PCB premixes (10 and 100 mg of PCB per kg) were prepared by adding Aroclor 1254 dispersed in corn oil to a standard carrier ration (Beltsville All-Mash Layer Diet 501, Agricultural Research Center Grainery, Beltsville, Md.). The test diets were then prepared by adding the premixes to the standard basal diet (Beltsville Broiler Diet A, Agricultural Research Center Grainery) to attain the PCB levels of 0, 0.1, 0.5, 1.0, 3.0, 5.0, and 10.0 mg/kg of diet.

A total of 188 3-day-old White Leghorn chicks were used in the study. They were individually identified and allotted to the control group (group 1, 20 chicks) or to one of the six test groups to be given the PCB-containing diets (groups 2–7, 28 chicks each). The test groups were further divided according to type of production; 12 chicks represented broiler production and 16 chicks represented egg production. Of the 16 "layers," eight chicks (subgroup A) were given the PCB-containing diets beginning at 3 days of age and the other eight chicks (subgroup B) were given the basal diet until the onset of egg production, when the test diets were started.

The respective diets and water were available *ad libitum*. At initiation of the feeding period, the birds were housed by groups in standard rack-type incubator-brooder cages, and after 3 weeks, they were transferred to standard rack-type poultry cages. All birds were observed daily throughout the study for overt signs of toxicosis. Feed consumption for each group and individual body weights were recorded weekly, and feed conversion ratios for each group were calculated.

After the diets had been fed for 8 weeks, 6 of the 12 birds representing broiler production in each group were sacrificed. Gross post-mortem examinations were performed on each bird and specimens of dissectable fat, liver, kidney, and muscle (composite sample from breast and thigh) were collected for chemical analysis. The remaining six "broiler" birds in each group were changed to the basal diet and were sacrificed after withdrawal periods of 1, 2, or 3 weeks. Gross post-mortem examinations and collection of tissue specimens were performed as before.

Those birds representing "layers" given the test diets beginning at 3 days of age (subgroup A) continued to receive the basal diet (control) or respective test diets until they were 12 weeks old. The remaining "layers" in each group, those which were not initiated on the respective test diets until after onset of egg production, continued to receive the basal diet. At the end of the 12 weeks, Beltsville All-Mash Layer Diet 501 was substituted for Beltsville Broiler Diet A as the basal diet received by the control group and as the basal diet for those layers not to be initiated on the test diet until after onset of egg production. At this time the birds were transferred to floor pens.

At onset of egg production adult cockerels were introduced into each group and egg hatchability was assessed. After acceptable fertility had been determined, subgroups 2B–7B, which had been receiving the basal diet, were initiated on the respective test diets. Eggs were collected

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from both pretreated and nonpretreated groups at days 0, 6, 12, 24, and 48 after initiation of the nonpretreated birds on the test diets. For sampling days 6 and 12, the collection period was ±1 day, and for sampling days 24 and 48, the collection period was ±2 days so that enough eggs were available for both determination of hatchability and residue analysis. Day-old chicks hatched from eggs collected during the day 24 sampling period were sacrificed and retained for chemical analysis. Eggshell thickness was measured on the wet shells with membranes removed.

The study was terminated at the end of week 32. The remaining birds were sacrificed and gross post-mortem examinations were performed on each. Specimens of the dissectable fat and muscle were collected from three birds in each treatment group.

All chemical analyses were performed "blind" by a commercial laboratory. The samples of feed, tissue, and eggs were processed as described by applicable sections of the Pesticide Analytical Manual (Food and Drug Administration, 1971). For analyses of feed and tissue samples, portions up to 50 g were accurately weighed; for muscle samples, equal portions of breast and thigh tissues were used. Eggs collected at each sampling period from each treatment group (7–10 eggs per group at days 0, 6, and 12; 9–15 eggs per group at days 24 and 48) were composited into three samples; each sample was blended and a 25-g portion was weighed for analysis. Day-old chicks were prepared for analysis by homogenizing the entire chick less beak and feet, and three or more homogenates were then pooled for extraction.

To obtain extractable fat, tissue samples and chick homogenates were extracted with petroleum ether. Feed was extracted with acetonitrile–water and eggs were extracted with acetonitrile. Solvent partitioning was used to remove PCB's from the bulk of lipid and/or other interfering materials. The samples were further purified by Florisil column chromatography (Food and Drug Administration, 1971), employing an elution scheme based on the method of Reynolds (1971). In brief, the column was prewashed with 140 ml of hexane and the eluate discarded. The sample residue obtained after the extraction and partitioning procedures described above was transferred to the column with three 5-ml portions of hexane and eluted with 85 ml of hexane. The eluate, approximately 100 ml, was reduced in volume with a Kuderna-Danish evaporator and analyzed by gas chromatography using suggested analytical parameters (Reynolds, 1971). Recoveries of PCB from tissues, based on spiked samples, were: fat, 83%; liver, 63%; kidney, 71.6%; and muscle, 74.5%.

In agreement with the work of Reynolds (1971), 14 identifiable peaks were observed in the PCB chromatograms. The last four (11–14) were of negligible response and were not used for calculation. Calculations were based on as many peaks as possible that could be identified by comparing the sample chromatograms to those of the standard; peaks 3, 4, and 7–10 were usually used. Peaks 1 and 2 were not used because they were often absent from the sample chromatograms. Peak 5 was omitted because its response was relatively large and out of proportion to other peaks, perhaps because of the presence of DDE (not confirmed) or of sample background interference. The sum of the peak heights was taken as the PCB response, and PCB content of the samples was calculated from the equation

$$\text{ppm} = (S_1A)/(S_2W)$$

where S_1 = PCB response of sample; A = nanograms of PCB standard injected; S_2 = PCB response of standard; and W = weight of sample injected in milligrams. The standard response (S_2) used for each sample was obtained from the standard injected prior to or after injection of the sample. In this way, compensation was made for any changes in column performance during the chromatographic determinations. Such changes were generally of small magnitude.

Table I. PCB Concentrations (ppm), on an Extractable Fat Basis, in Dissectable Fat, Liver, Kidney, and Muscle of 8-Week-Old White Leghorn Hens Given Diets Containing Aroclor 1254 for 8 Weeks[a]

Group	Dietary level, mg/kg	Dissectable fat	Liver	Kidney	Muscle[b]
1	0	0.48	1.30	2.09	1.09
2	0.1	1.89	4.20	3.53	2.15
3	0.5	11.3	28.3	18.8	12.8
4	1.0	9.52	18.5	11.5	10.9
5	3.0	25.1	36.8	21.8	30.7
6	5.0	66.4	61.8	49.3	69.8
7	10.0	110.6	98.1	87.4	109.0

[a] Values are means of six hens. [b] Composited from breast and thigh.

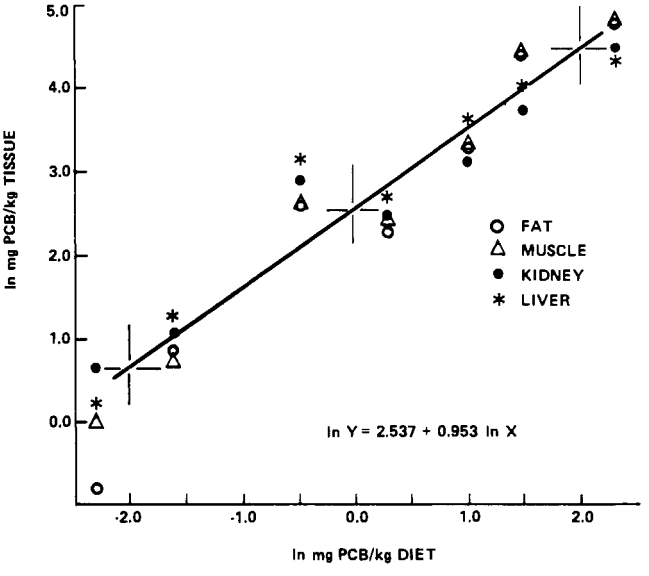

Figure 1. Linear relationship between the concentration of Aroclor 1254 added to the diet and the concentration of PCB in the tissues of 8-week-old White Leghorn chickens. Natural logarithms of the PCB concentrations were used to maintain homogeneity of error variances.

The data were analyzed statistically by using the method of least squares. In order to maintain homogeneity of error variances, natural logarithms of the tissue PCB concentration were used in the analysis, and natural logarithms of the PCB levels in the diet were also used to retain the original linear relationship between dietary and tissue PCB concentrations.

RESULTS

Toxicity. No signs of toxicity were observed among birds given diets containing Aroclor 1254 at concentrations of 0.1–10.0 mg/kg of diet for either 8 or 32 weeks. No significant differences between groups were noted in feed consumption, growth, or feed efficiency, and no lesions that could be related to Aroclor feeding were observed during necropsy examinations. Liver weights and liver-to-body weight ratios were variable, but differences could not be related to the graded dietary concentrations of Aroclor 1254. In addition, hatchability and eggshell thickness did not appear to be significantly affected by exposure of the layers to Aroclor 1254 under the conditions of this study.

Table II. PCB Concentrations (ppm), on an Extractable Fat Basis, in the Dissectable Fat, Liver, Kidney, and Muscle of White Leghorn Hens Given Diets Containing Aroclor 1254 for 8 Weeks Followed by Withdrawal Periods of 1, 2, or 3 Weeks[a]

Group	Dietary level, mg/kg	Withdrawal period, weeks	Fat	Liver	Kidney	Muscle[b]
1	0	1	0.63	1.49	1.65	0.89
		2				1.02
		3	0.61	1.48	1.88	1.01
2	0.1	1	2.76	3.75	3.92	2.56
		2	1.60	1.75	2.15	2.15
		3	0.88	1.94	1.79	1.29
3	0.5	1	7.39	9.22	6.86	6.95
		2	9.51	9.57	7.42	
		3	7.46	9.65	1.40	8.55
4	1.0	1	9.95	14.8	8.55	10.8
		2	8.54	10.4	5.74	8.08
		3	7.35	11.5	4.64	8.08
5	3.0	1	18.3	33.4	15.0	19.5
		2	14.1	19.8	16.8	14.1
		3	15.5	18.0	15.1	17.8
6	5.0	1	30.1	40.0	42.0	35.3
		2	24.2	36.1	28.9	26.2
		3	20.6	33.2	19.4	27.4
7	10.0	1	48.0	87.4	55.0	87.8
		2	55.5	56.5	56.3	41.3
		3	35.9	56.1	41.1	46.0

[a] Control diet was given during the withdrawal period. Values are the means of two hens. [b] Composited from breast and thigh.

Table III. PCB Concentrations (ppm), on an Extractable Fat Basis, in Dissectable Fat and Muscle of White Leghorn Hens Given Diets Containing Aroclor 1254 Starting at 3 Days of Age (Subgroup A) or after Onset of Egg Production (Subgroup B)[a]

Group	Dietary level, mg/kg	Muscle[b]	Fat
1	0	1.85	0.83
2A	0.1	4.30	1.98
2B	0.1	2.55	2.35
3A	0.5	9.07	4.80
3B	0.5	6.44	4.88
4A	1.0	12.6	7.11
4B	1.0	9.52	6.65
5A	3.0	33.6	24.5
5B	3.0	18.0	20.2
6A	5.0	56.3	27.4
6B	5.0	21.1	24.1
7A	10.0	102.8	49.5
7B	10.0	70.1	33.4

[a] At the time of sampling, test diets had been given to hens of subgroup A for 224 days and to hens of subgroup B for 52 days. Values are the means of three hens. [b] Composited from breast and thigh.

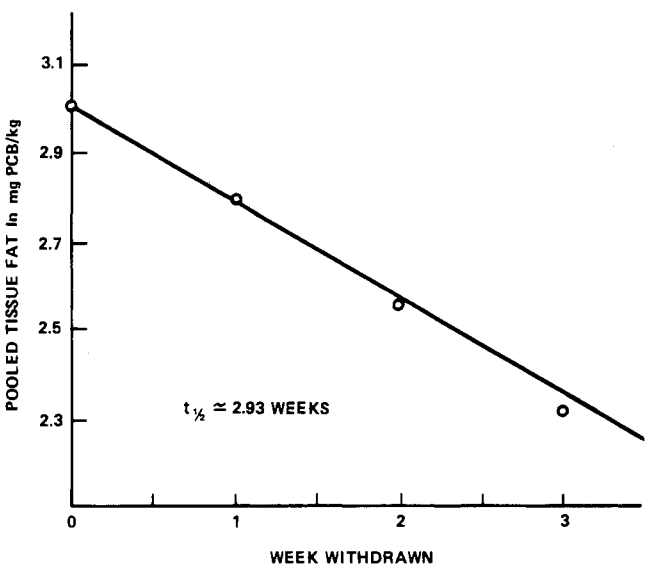

Figure 2. Depletion rate of PCB residues from tissues of White Leghorn chickens beginning at 8 weeks of age. Residue concentrations of each withdrawal period were pooled and plotted as the natural logarithm against time.

Diet Analysis. Analysis of samples collected at random from the basal and test diets revealed that the basal diet employed was contaminated with PCB at a level of approximately 0.1 mg/kg of diet. The source of this contamination was not determined.

Residue Analysis. *Residues in Tissues from Broiler Phase Birds.* Table I gives the mean PCB residue concentrations, reported on an extractable fat basis, for the dissectable fat, liver, kidney, and skeletal muscle of the birds sacrificed without a withdrawal period. Linear regression accounted for over 90% of the variation due to dietary level or tissue involved. There were small but significant differences in the concentration of PCB in extractable fat of the various tissues analyzed, reflecting variation in the accretion rate in different tissues. However, since these differences were small, the data were pooled in order to illustrate the overall linear relationship between concentration of PCB in the tissues and that in the diet (Figure 1). The equation defining this relationship is

$$\ln Y = 2.537 + 0.953 \times \ln X$$

where Y = PCB concentration in the extractable fat and X = PCB concentration in the diet.

In Table II, the mean PCB concentrations, also reported on an extractable fat basis, are given for the dissectable fat, liver, kidney, and skeletal muscle of birds sacrificed after withdrawal periods of 1, 2, or 3 weeks. As expected, time of withdrawal accounted for most of the variation in depletion. Variation due to tissues was again small but significant. Pooling the data for each withdrawal period and plotting them as the logarithm against time gave a depletion rate with a biological half-life of 2.93 weeks (Figure 2).

Table IV. PCB Concentrations (ppm) in Day-Old Chicks Hatched from Eggs of White Leghorn Hens Given Diets Containing Aroclor 1254 Starting at 3 Days of Age (Subgroup A) or after Onset of Egg Production (Subgroup B)[a]

Group	Dietary level, mg/kg	Tissue[b]	Fat[c]
1	0	0.07	2.20
2A	0.1	0.09	3.46
2B	0.1	0.05	1.58
3A	0.5	0.36	9.49
3B	0.5	0.05	1.70
4A	1.0	0.38	11.2
4B	1.0	0.04	1.07
5A	3.0	1.49	46.4
5B	3.0	0.05	1.56
6A	5.0	2.23	77.3
6B	5.0	0.11	3.23
7A	10.0	4.13	120.4
7B	10.0	0.42	10.8

[a] At the time eggs were collected, test diets had been given to hens of subgroup A for 196 days and to hens of subgroup B for 24 days. Three or more chick homogenates were pooled for each subgroup at each dose level. [b] Whole chick less beak and feet. [c] Extractable fat basis.

Table V. PCB Concentration (ppm) in Eggs from White Leghorn Hens Given Diets Containing Aroclor 1254 Starting at 3 Days of Age (Subgroup A) or after Onset of Egg Production (Subgroup B)[a]

Group	Dietary level, mg/kg	Sampling day 0	6	12	24	48
1	0	0.04	0.06	0.04	0.03	0.01
2A	0.1	0.13	0.09	0.07	0.09	0.12
2B	0.1	0.04	0.04	0.04	0.04	0.07
3A	0.05	0.52	0.27	0.52	0.33	0.43
3B	0.05	0.09	0.09	0.09	0.15	0.15
4A	1.0	0.88	0.58	0.69	0.51	0.54
4B	1.0	0.03	0.10	0.25	0.27	0.33
5A	3.0	2.24	1.55	2.09	1.76	1.09
5B	3.0	0.04	0.27	0.87	0.48	0.58
6A	5.0	2.04	2.87	2.97	2.24	0.93
6B	5.0	0.04	0.49	1.07	0.91	0.66
7A	10.0	2.76	9.73	4.40	4.49	2.28
7B	10.0	0.25	1.03	2.54	2.36	1.13

[a] At day 0, subgroup A had been fed the test diets for 172 days. Values are the means of three samples containing from three to five eggs.

Residues in Tissues from Layer Phase Birds. Table III shows the mean PCB residue concentrations on an extractable fat basis for the dissectable fat and muscle of hens from both the pretreated and nonpretreated layer groups (subgroups A and B). A dose-related, linear increase in tissue PCB concentrations occurred in both the dissectable fat and muscle from both layer groups. The concentration of PCB residues in the extractable fat or skeletal muscle from pretreated layers was significantly greater ($p < 0.001$) than the concentration in either the dissectable fat or muscle from nonpretreated layers or the dissectable fat of pretreated layers.

Residues in Day-Old Chicks. Residue concentrations reported on an extractable fat basis for day-old chicks hatched from eggs collected during the day 24 sampling period are presented in Table IV. Residue concentrations ranged from 3.46 and 1.58 ppm, respectively, for the pretreated and nonpretreated groups given the diet containing Aroclor 1254 at 0.1 mg/kg to 120.4 and 10.8 ppm, respectively, for the pretreated and nonpretreated groups given the diet containing 10.0 mg/kg.

Residues in Eggs. Table V gives the mean PCB concentrations in eggs collected at various intervals from the pretreated and nonpretreated layers. Each of the primary effects, *i.e.*, dietary level of Aroclor 1254, duration of feeding, and time of sampling, were statistically significant. At the initial sampling period (day 0), eggs from pretreated layers contained PCB residues at levels ranging from 0.13 ppm (group 2A, 0.1 mg/kg of diet) to 2.76 ppm (group 7A, 10 mg/kg of diet). Effects were somewhat variable during the 48-day sampling period; residues in eggs from hens in groups 6A and 7A increased through sampling days 6 and 12 and then decreased through day 48.

Eggs from nonpretreated layers (subgroup B) had a residue pattern over the 48-day sampling period similar to that of eggs from pretreated layers. Because of PCB contamination of the basal diet, eggs collected at day 0 (before the test diets were started) contained PCB residues at a mean concentration of approximately 0.05 ppm. The PCB residue concentration of eggs from birds in groups 5B, 6B, and 7B increased, reaching levels ranging from 0.87 (group 5B) to 2.54 ppm (group 7B) at day 12 of the sampling period, and then decreased. Residues in eggs from birds in groups 2B, 3B, and 4B reached a state of apparent equilibrium at residue concentrations of 0.04, 0.15, and 0.27 ppm, respectively, midway through the 48-day sampling period.

DISCUSSION

Residue concentrations resulting from lifetime exposure to diets containing Aroclor 1254 added at graded levels were followed in tissues and eggs from White Leghorn chickens during selected periods representing the complete life cycle. The differences in residue concentrations, expressed on an extractable fat basis, between tissues from birds given the same dietary concentrations for 8 weeks were very small. Therefore, the level of PCB residue present in a given tissue was related to the extractable fat content of the tissue. However, this relationship between residue concentration and extractable fat content did not exist in tissue from hens given the test diets for 32 weeks.

It seems logical to assume that a complex series of interrelating mechanisms exists, and involves mobilization and redistribution of existing body compartment loads as well as absorption and distribution of dietary PCB's. The pattern, with time, of PCB residues in eggs from both pretreated and nonpretreated hens is further evidence of the complexity of the overall picture. The fluctuations and changes occurring in the endocrine system of the hens with the initiation of egg production might also play an important role, particularly from the standpoint of an interrelationship with energy metabolism. The pattern of time shown here differs from the results of Scott *et al.* (1971), who reported that after 4 weeks PCB residues reached a plateau in eggs from White Leghorn hens that were in active egg production at the time that diets containing Aroclor 1248 at 10.0 and 20.0 mg/kg were initiated. However, in our study, the PCB residue concentrations in eggs from both pretreated and nonpretreated hens receiving diets containing up to 1.0 mg of added Aroclor 1254 per kg of diet did reach an apparent plateau after approximately 4 weeks; these results are consistent with the plateauing effects reported by Scott *et al.* (1971) for similar dietary concentrations of Aroclor 1248.

Cecil *et al.* (1972) reported that a series of PCB compounds, including Aroclor 1254, fed to White Leghorn layers at dietary concentrations of 2 and 20 ppm for 9 weeks had no effect on feed consumption or body weight gain, mortality, egg weight, eggshell thickness, or fertility. Dietary concentrations of 20 ppm of Aroclor 1232, 1242, 1248, and 1254 did cause reductions in egg production, hatchability, body weight, and weight gain of progeny, and also produced teratogenic effects.

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Excreted Metabolites of 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane in the Mouse and Hamster

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Swiss mice and Syrian golden hamsters were fed 250 μg/g of dietary DDT for up to 4 months and urinary and fecal metabolites were investigated. Both species excreted the base labile glucuronide of bis(*p*-chlorophenyl)acetic acid (DDA) as the principal urinary metabolite. The more stable glycine and alanine conjugates of DDA were also found. The mouse differed from the hamster in excreting DDE in the urine. The excretion of DDE increased during the course of the experiment until in the fourth month it was nearly as prominent as DDA. Endogenously formed cinnamoylglycine was also excreted by the mouse in amounts that increased upon DDT administration. The feces of both species contained DDD and DDT. Fecal excretion was not an important route for the elimination of polar DDT metabolites in these animals.

A comparative study of the urinary and fecal metabolites of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) in the hamster and the mouse is of interest since these species respond differently to both acute and chronic administration of the compound. The LD_{50} in Swiss mice is about 300 mg/kg while in hamsters it is greater than 2000 mg/kg, solubility limitations making an accurate assessment difficult (Gingell and Wallcave, 1974; Agthe *et al.*, 1970). In chronic feeding studies DDT was a liver tumorigen in several mouse strains (reviewed by Terracini *et al.*, 1973) but the Syrian golden hamster was resistant (Agthe *et al.*, 1970).

We have attempted to identify urinary and fecal DDT metabolites in these species and, in long-term feeding studies, to determine whether there were qualitative or quantitative time-dependent changes in the metabolites. These studies were continued for 16 days in animals fed 100 μg/g of dietary DDT and for about 4 months in mice and 3 months in hamsters fed 250 μg/g. Additional information concerning the total number of conjugated forms of excreted metabolites was obtained from acute oral administration of ^{14}C-labeled DDT.

There are no reports to our knowledge on the identification of urinary or fecal DDT metabolites in the hamster. Mouse urine was investigated in some detail by Apple (1968) who reported bis(*p*-chlorophenyl)acetic acid (DDA) as the principal excreted metabolite.

EXPERIMENTAL SECTION

Chemicals. DDT of >99.0% purity (glc check) was obtained from Geigy Agricultural Chemicals, Ardsley, N. Y. Randomly ring-^{14}C-labeled DDT (64 μCi/mg) was obtained from the Radiochemical Centre, Amersham, Bucks, England. DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane], and DDA were purchased from Pfaltz and Bauer, Flushing, N. Y. Bovine liver β-glucuronidase was purchased from Sigma Chemical Co., St. Louis, Mo.

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The glycine and alanine conjugates of DDA were made in about 40% yields by the following procedure. Crude bis(*p*-chlorophenyl)acetyl chloride was prepared by refluxing 0.005 mol (1.4 g) of DDA and 3 ml of thionyl chloride for 2 hr and removing excess of the latter *in vacuo* at 50°. The acid chloride was added in three portions with vigorous shaking to an ice-cold solution of 0.006 mol of the amino acid in 10 ml of 7% (w/v) NaOH. After acidification of the solution with concentrated HCl the precipitated DDA conjugate was dried on filter paper, extracted with small portions of ether to remove unreacted DDA, and recrystallized from benzene–ethyl acetate (9:1). Methyl esters of the conjugates were made by reaction with ethereal diazomethane and recrystallized from benzene.

These conjugates have not previously been reported. They had the following characteristics (analyses by Micro-Tech Labs., Inc., Skokie, Ill.): *N*-bis(*p*-chlorophenyl)acetylglycine (DDA-Gly), colorless needles, mp 153° (*Anal.* Calcd for $C_{16}H_{13}O_3NCl_2$: C, 56.82; H, 3.88; N, 4.14; Cl, 20.96. Found: C, 56.88; H, 3.85; N, 3.96; Cl, 21.06); methyl ester, mp 159°; *N*-bis(*p*-chlorophenyl)-acetyl-DL-alanine (DDA-Ala), colorless platelets, mp 163° (*Anal.* Calcd for $C_{17}H_{15}O_3NCl_2$: C, 57.98; H, 4.29; N, 3.98; Cl, 20.14. Found: C, 58.10; H, 4.27; N, 3.89; Cl, 19.92); methyl ester, mp 135°.

Cinnamoylglycine was prepared similarly, mp 191° (lit. 193°). It has been described by Dakin (1909). The methyl ester, made by reaction with diazomethane, had mp 89°.

Preparation of Diets. Stick food was required for mice and pellets for hamsters. Sticks containing DDT were prepared by spraying a 1% acetone solution of DDT onto 1 kg of powdered diet (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.). After mixing on a roller, a hot 5% gelatin solution was added, a paste formed, and the mixture extruded into sticks using a cake decorating implement. The sticks were dried at room temperature and had a gelatin content of about 4%. Sticks not containing DDT were made for control purposes. DDT was added to the hamster diet by dropping the acetone solution onto Wayne Lab-Blox pellets and allowing the solvent to evaporate.