if there are two or more isozymes of chorismic mutase, one containing prephenate dehydratase and another prephenate dehydrogenase, both could be inhibited. The reversals with a combination of phenylpyruvic and *p*-hydroxyphenylpyruvic acids would suggest that the block is not at the level of the transaminase reactions. This was also supported by comparable reversals with aryllactic acids which were assumed to be oxidized *in vivo* to their corresponding arylpyruvic acids (data not reported). Similar reversals were noted for *Lemna* but to a lesser degree.

Tryptophan was not a reversing agent in *Lemna*, and combinations of tryptophan with phenylalanine or phenylalanine and tyrosine were not absolutely essential for significant reversals. Thus, it is not likely that the block occurs prior to chorismic acid synthesis. It should be noted, however, that the most complete reversals in *Lemna* were usually achieved with a combination of all three aromatic amino acids. Some blockage of any reaction where chorismic acid serves as the substrate could therefore be invoked, including anthranilate synthetase. Another possible explanation for the benefits of tryptophan in conjunction with phenylalanine and tyrosine may reside in its activation of chorismate mutase, as shown in bacteria (Lingens, 1968), fungi (Baker, 1966), yeast (Lingens *et al.*, 1966), green algae (Weber and Boeck, 1969), and higher plants (Cotton and Gibson, 1968; Gilchrist *et al.*, 1972). This type of activation could lead to a conformational change in chorismate mutase and alter the affinity of the inhibitor for the enzyme.

The reversal studies with chorismic and prephenic acids, while encouraging, present some problems in that they or metabolites derived from them are quite inhibitory to growth. Kinetic studies with these substrates and *N*-phosphonomethylglycine may be particularly appropriate at the subcellular level.

It is apparent that further investigations will be required to sort out the specific site(s) and type of interaction for *N*-phosphonomethylglycine. However, based on these studies and current work with *Salmonella typhimurium* auxotrophs for phenylalanine and tyrosine, the model proposing a localization of the effects of *N*-phosphonomethylglycine at chorismate mutase and/or prephenate dehydratase appears to be reasonable.

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Dieldrin and *p,p'*-DDT Effects on Some Microsomal Enzymes of Livers of Chickens and Mallard Ducks

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Two experiments were conducted to determine effects of dieldrin and *p,p'*-DDT on some mixed-function oxidases of hepatic microsomes of White Leghorn chickens and mallard ducks. Aniline hydroxylase and aminopyrine *N*-demethylase activities in control birds were lower in ducks than in chickens, whereas cytochrome P_{450} concentration and estradiol metabolism in microsomes were about equal in both species. Dieldrin at levels of 10 and 20 μg/g of diet and DDT at 100 and 200 μg/g of diet increased cytochrome P_{450} concentration and estradiol metabolism in microsomes of both species, but they increased these parameters more in ducks than in chickens. DDT decreased aniline hydroxylase activity in chicken microsomes and increased aniline hydroxylase activity in duck microsomes, but did not affect aminopyrine *N*-demethylase activity of either species. Dieldrin did not affect aniline hydroxylase activity in chickens or ducks and did not affect aminopyrine *N*-demethylase activity in ducks, but it probably increased *N*-demethylase activity in chickens.

Trace amounts of chlorinated hydrocarbon insecticides are commonly found in our environment and are regularly found in the body fat of domestic and wild animals. Although it is virtually impossible to determine the effects that very low levels of these residues may have on the physiology of any species of animals, or to study these effects by feeding the chemicals causing these residues to all species of animals, it is important to test these chemicals by feeding them to several species. Data so obtained may be used as guidelines for evaluating research where different species of animals are used and for predicting whether or not effects may occur in nonexperimental animals. Also, biological

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experimentation varies greatly, both within and between experiments. To make evaluations among species, one must know whether or not species differences exceed these variations.

A brief review of the literature reveals that activities of hepatic microsomal enzymes are modified by DDT or dieldrin (see reviews by Deichmann, 1970; Kuntzman, 1969; Street, 1969). However, the direction and magnitude of change are subject to considerable variation. Some of this variation appears to be associated with the level of DDT or dieldrin administered, duration of treatment, and age, sex, and species of animal. Species effects became of particular interest to us when we observed that aniline hydroxylase activity of hepatic microsomes from adult chickens fed DDT was reduced as compared to controls (Sell *et al.*, 1971), a response opposite to that indicated by research with rats or other mammals (Conney, 1967; Street *et al.*, 1966). On the other hand, dietary dieldrin increased *N*-demethylase activity and cytochrome P_{450} concentration in the microsomes from these chickens. Among other substances, microsomal enzymes metabolize steroid hormones. DDT has been shown to increase the metabolism of steroid hormones in birds, and this has been discussed as possibly related to reproductive failure and eggshell thinning (Peakall, 1967, 1970).

The objective of this research was to compare effects of DDT and dieldrin on some hepatic microsomal enzymes of chickens and mallard ducks of similar ages that were managed and housed in the same facilities and fed the same diet. Systems studied were those associated with the metabolism of aniline, aminopyrine, and estradiol.

MATERIALS AND METHODS

Animal Facilities and Feeding. The birds were housed in wire cages, 20 or 30 cm wide × 40 cm deep × 40 cm high, of the type normally used for laying hens. They were fed individually 60 g per day of a commercial chick sharter diet (Ralston Purina Co.) and they consumed this amount of feed each day. Dieldrin, DDT, DDD, or DDE were not detected in the feed when it was analyzed for these by electron capture gas chromatography by the method of Johnson (1965). As little as 0.01 μg of dieldrin/g or 0.02 μg of DDT + DDD + DDE/g would have been detected in the feed by this method.

The cages were located in one continuously lighted area of a large room which was heated to about 20°. The cages were arranged in series of six because of a water trough which carried continuously flowing water in front of each series.

Chickens and ducks were kept in separate series of cages, and pesticide levels were assigned to separate series to avoid cross contamination through spillage of feed in water. Assignment of birds to any series for that species or cage within a series was random. All birds were given 2 to 3 weeks to adjust to changes in housing and diet before pesticides were fed.

DDT. Chemically pure, 99+%, *p,p'*-DDT was purchased from the Aldrich Chemical Co., Inc., Milwaukee, Wis. DDT sufficient for the experiment was dissolved in about 400 ml of acetone and mixed into 2 kg of feed to prepare a premix. The acetone evaporated during mixing. This 2 kg of premix was then mixed into enough feed to last the duration of the experiment. The feed was stored at room temperature in steel cans lined with polyethylene.

Acetone alone was mixed into feed for the control birds in the same manner.

Dieldrin. Technical dieldrin obtained from the Shell Chemical Co., N. Y., was dissolved in acetone and mixed in the feed as above. Dieldrin level referred to below indicates the amount of active ingredient 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene contained per g of diet.

Table I. Experimental Diagram for Study of Some Microsomal Enzymes in Livers of Chickens and Ducks Fed Dieldrin

Species and level of dieldrin fed	Sampling time from beginning of experiment, days[a]			
	0	14, 16	28, 30	56, 58
	Number of birds			
Chickens				
Control diet	5	4	4	4
10 μg of dieldrin/g of diet		4	4	4
20 μg of dieldrin/g of diet		4	4	4
Mallard ducks				
Control diet	5	4	4	4
10 μg of dieldrin/g of diet		4	4	4
20 μg of dieldrin/g of diet		4	4	4

[a] The birds were killed and their livers were removed for assaying after they had consumed dieldrin daily for the number of days indicated. One-half of the birds from each group were killed at times separated by 2 days near the 14th, 28th, and 56th days to spread the work load during the assays.

Experiment 1. Forty-one game-farm strain mallard females, whose parent stock was obtained from the Frost Game Farm, Coloma, Wis., and 41 White Leghorn pullets were assigned to the incomplete 2 × 3 × 4 factorial experiment shown in Table I. Insufficient ducks were available to balance the factorial. At the start of the experiment, the ducks and chickens were 90 and 95 days old, respectively. Before any pesticide was fed, five birds of each species were killed and their livers were removed, weighed, and sampled for the assays described below. The remaining birds were fed the specified levels of dieldrin daily. On days 14, 28, and 56, two birds from each group were killed and their livers were sampled and assayed. Two days after each of the preceding times, the remaining two birds of each group were killed for assay. This 2-day delay was done only to distribute the work load in the laboratory.

Experiment 2. Forty-eight game-farm strain mallard females and 48 White Leghorn pullets were assigned to a balanced 2 × 3 × 4 factorial experiment in which *p,p'*-DDT was studied at levels of 0, 100, and 200 μg/g of diet. Other factors were identical to those of experiment 1. The ducks and chickens were 80 and 73 days old, respectively, when the experiment was begun.

Protein, Aniline Hydroxylase, Aminopyrine *N*-Demethylase, and Cytochrome P_{450} Assays. Microsomes, prepared from 10 g of liver tissue in experiment 1 and from 5 g of liver tissue in experiment 2, were assayed for protein by the method of Lowry *et al.* (1951), for aniline hydroxylase and aminopyrine *N*-demethylase activities by the methods of Schenkman *et al.* (1967), and for cytochrome P_{450} concentration according to the method of Kato (1966), with modifications of these methods as described by Sell *et al.* (1972). The microsomes were prepared immediately after the birds were killed, and all assays except cytochrome P_{450} were conducted that day. Cytochrome P_{450} was assayed the day following preparation of the microsomes.

Estradiol Metabolism. Estradiol-17β-4-^{14}C, 52 mCi/mmol, obtained from New England Nuclear Corp., Boston, Mass., was dissolved in 95% ethanol at a concentration of 10 nmol/ml. Nonradioactive estradiol, obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, was dissolved

Table II. Body and Liver Weights, Microsomal Protein Concentration, Aniline Hydroxylase and Aminopyrine *N*-Demethylase Activities, Cytochrome P_{450} Concentration, and Estradiol Metabolism of Hepatic Microsomes of Chickens and Ducks Fed Dieldrin

Factors studied and duration	Body weight, g/bird	Liver weight, g/bird	Microsomal protein, mg/ml[e]	Aniline hydroxylase activity[a]	Aminopyrine *N*-demethylase activity[b]	Cytochrome P_{450}[c]	Estradiol assay[d]	
							Extracted, %[f]	Estradiol, %[g]
Chickens								
Control diet								
0 weeks	835	17.4	22.1	5.59	1.76	0.95	61.6	26.9
2 weeks	945	20.8	23.9	4.80	2.10	0.58	79.0	5.7
4 weeks	1085	22.2	22.7	9.76	2.36	0.89	53.1	6.7
8 weeks	1262	27.0	16.8	12.48	3.98	0.67	40.8	31.5
Diet avg	1032	21.9	21.4	8.16	2.52	0.77	58.6	17.7
10 μg of dieldrin/g of diet								
0 weeks	844							
2 weeks	948	19.5	24.8	4.61	2.26	0.77	65.1	22.4
4 weeks	1059	22.2	27.5	10.49	2.76	1.07	39.6	8.8
8 weeks	1199	24.5	18.0	17.78	6.52	0.99	14.0	12.5
Diet avg	1013	22.1	23.4	10.96	3.84	0.94	39.6	14.6
20 μg of dieldrin/g of diet								
0 weeks	776							
2 weeks	910	22.4	25.2	4.14	2.41	0.97	67.6	13.7
4 weeks	981	21.6	25.2	12.31	3.56	1.50	37.6	6.0
8 weeks	1222	26.4	21.8	19.15	6.30	1.24	13.9	16.7
Diet avg	972	23.5	24.1	11.87	4.10	1.24	39.7	12.1
Species avg	1006	22.5	23.0	10.33	3.48	0.98	46.0	14.8
Ducks								
Control diet								
0 weeks	869	17.1	26.2	2.12	1.14	0.95	74.4	19.9
2 weeks	851	13.9	30.0	1.25	1.04	0.70	85.4	30.6
4 weeks	848	16.8	28.8	3.52	1.88	0.81	70.9	24.4
8 weeks	847	13.5	25.4	5.72	3.62	0.94	48.4	38.4
Diet avg	854	15.3	27.6	3.15	1.92	0.85	70.0	28.3
10 μg of dieldrin/g of diet								
0 weeks	927							
2 weeks	885	13.5	26.7	1.34	1.46	0.56	79.3	22.5
4 weeks	913	16.7	28.6	3.82	1.80	1.13	54.5	10.2
8 weeks	889	14.4	30.0	6.24	3.20	1.77	28.8	14.5
Diet avg	904	14.9	28.4	3.80	2.16	1.15	54.2	15.7
20 μg of dieldrin/g of diet								
0 weeks	899							
2 weeks	875	16.9	28.5	1.86	1.52	0.87	78.2	21.2
4 weeks	879	15.8	27.3	6.27	2.26	1.53	49.4	6.4
8 weeks	909	17.9	29.8	7.73	3.78	2.46	20.1	10.2
Diet avg	891	16.9	28.5	5.29	2.52	1.62	49.2	12.6
Species avg	883	15.7	28.2	4.08	2.20	1.21	57.8	18.9

[a] Activity expressed as μg of *p*-aminophenol produced/mg of protein/hr of incubation. [b] Activity expressed as μg of HCHO produced/mg of protein/hr of incubation. [c] Units of optical density change (OD_{450}–OD_{500}) per 100 mg of microsomal protein. [d] One nanomole of estradiol was used in this assay. [e] Mg of protein/ml in 5 ml of microsomal preparation derived from 10 g of fresh liver. [f] Expressed as a percent of the radioactivity that extracted into dichloromethane after radioactive estradiol was incubated 15 min with microsomes. [g] Estradiol is the percent of the material in the extracted radioactivity which had an R_f value by thin-layer chromatography similar to that of the test estradiol-^{14}C.

in 95% ethanol at a concentration of 2 μmol/ml. Either 1 nmol of estradiol, supplied by estradiol-^{14}C, or 200 nmol of estradiol, supplied by 1 nmol of estradiol-^{14}C plus 199 nmol of nonradiative estradiol, were incubated 15 min in a microsomal system identical to that used for the hydroxylase and *N*-demethylase assays.

After incubation, the systems were inactivated by adding 25 ml of dichloromethane to each test tube. The tubes were agitated and let stand 15 min for the estradiol and dichloromethane-soluble metabolites to partition into the dichloromethane. Then, 20 ml of dichloromethane was removed from each tube and placed in another test tube. One milliliter of the 20 ml of dichloromethane was assayed for ^{14}C to determine the amount of ^{14}C from estradiol-^{14}C that extracted into dichloromethane from each test system. The remaining 19 ml of dichloromethane was evaporated and the residue was redissolved in 0.5 ml of dichloromethane preparatory to thin-layer chromatography.

Suitable aliquots of the dissolved residue, depending upon the amount of radioactivity present, were transferred to one end of 5 cm × 20 cm thin-layer plates coated 0.25 mm thick with silica gel G. These plates were developed with a benzene–ethyl acetate (1:1) solvent. When the solvent had progressed 12.5 cm from the point of origin of sample, the plates were dried and scanned for radioactivity. The radioactive areas were scraped from each plate, and these scrapings were assayed for ^{14}C by liquid scintillation methods.

As references for the assays, two blanks were prepared. One contained all ingredients, including microsomes, and was inactivated by adding 25 ml of dichloromethane immediately after the microsomes were added. The other contained all ingredients except microsomes. It was incubated 15 min with the test systems and was then extracted with 25 ml of dichloromethane. The extract from both blanks was processed and chromatographed as described for the test systems. Estradiol-^{14}C was also chromatographed as described to determine its R_f in the chromatographic system. Radioactive areas on the plates with R_f values similar to that for estradiol-^{14}C were assumed to be estradiol for the purposes of these experiments.

Two criteria were used as evidence for metabolism of estradiol. The first criterion was a decline in the amount of

Table III. Least-Squares Analysis of Variance for Data Obtained Weeks 2, 4, and 8 of Table II

Source	Degrees of freedom	Mean squares: Liver weight	Microsomal protein	Aniline hydroxylase	Aminopyrine N-demethylase	Cytochrome P_{450}	Estradiol assays: Extracted	Estradiol
Total	71							
MU	1	7.6	1.5	8.6	1.92	0.04	412	65
Species	1	984.7[a]	525.8[a]	285.2[a]	16.44[a]	0.86[a]	2346[a]	703[a]
Dieldrin level	2	17.6	19.5	11.6	1.10	2.73[a]	2390[a]	741[a]
Time (age)	2	45.1[a]	66.5[a]	166.5[a]	26.64[a]	2.35[a]	13306[a]	697[a]
Assay/time	1	1.7	107.9[a]	36.5[a]	4.52[a]	0.03	3634[a]	492[a]
Species × dieldrin	2	6.4	10.7	1.6	1.16	0.09	47	458[a]
Species × time	2	46.0[a]	70.4[a]	26.0[a]	1.28	1.10[a]	50	124
Dieldrin × time	4	10.1	20.5	4.1	0.40	0.34	176	392[a]
Remainder	56	7.2	8.8	4.8	0.48	0.12	133	69

[a] Indicates significance at a probability of 1%.

^{14}C which extracted from the test systems, compared with that which extracted from the blank containing microsomes. The second criterion was a decline in the amount of ^{14}C in the extract which chromatographed as estradiol-^{14}C, compared with the total ^{14}C in the extract.

Recovery by extraction of ^{14}C from blanks that contained microsomes compared with the recovery from blanks that did not contain microsomes was 96.7% when 1 nmol of estradiol was used for the assays, and was 95.6% when 200 nmol of estradiol was used. Recovery of ^{14}C from the blanks that did not contain microsomes was essentially equal to the ^{14}C added. The R_f for estradiol-^{14}C on the chromatograms was 0.77. When 1 nmol of estradiol was used, 88% of the radioactivity on the chromatograms of the extracts of blanks containing microsomes was recovered in the area with an R_f similar to that of estradiol-^{14}C, and 93% of the radioactivity from blanks that did not contain microsomes was recovered as estradiol. When 200 nmol of estradiol was used, 98% of the radioactivity on the chromatograms of the extracts of either blank system was recovered as estradiol. Two of the more obvious radioactive areas assumed to be metabolites on the chromatograms of extracts from the test systems had R_f values of about 0.20 and 0.50.

Analysis of Microsomes for DDT, DDD, and DDE. One milliliter of the microsomal preparations was extracted with 1 ml of hexane. DDT, DDD, and DDE present in this extract were determined without further clean-up by electron capture gas chromatography.

Statistical Analysis. The data were analyzed by analysis of variance, using least-squares analysis where unequal subclasses occurred. The "0" time of experiment 1 was not included in the statistical analysis because of the incompleteness of this portion of the experiment (see Table I). The data obtained from estradiol assays in experiment 2 were analyzed covariant with protein concentrations in the microsomal preparations. Significance was assessed at probabilities of 1% or less.

RESULTS

Experiment 1. Data obtained from experiment 1 are shown in Table II. The analysis of variance for the microsomal assays done at 2, 4, and 8 weeks is given in Table III.

The chickens increased in body weight through the eighth week, while the ducks maintained body weight. Dieldrin in the diet did not measurably affect body weight of either species.

One death attributable to dieldrin poisoning occurred during the sixth week in the group of chickens fed 20 μg of dieldrin/g of diet. Four chickens, two control and one from each group fed dieldrin, laid eggs during the eighth week, and one duck showed slight ovarian stimulation preparatory to lay when killed at the end of 8 weeks.

Liver weight increased considerably with time (age) in the chickens, but it did not increase in ducks. A sizable increase in liver weight occurred in chickens between the fourth and eighth weeks. This increase was attributed to the accumulation of fat in the livers. The high fat content of the livers was obvious when the livers were removed from the chickens. This fat was removed from the liver homogenates as a thick layer after the homogenates were centrifuged during separation of the microsomes. Fat normally accumulates in livers of chickens with onset of lay. Liver weight was not affected by dieldrin consumption.

Microsomal protein concentration remained constant in the ducks throughout the experiment, but it declined after the fourth week in chickens, with the development of fatty livers. Microsomal protein concentration was not affected by feeding dieldrin.

Both aniline hydroxylase and aminopyrine N-demethylase activities were lower by 60 and 40%, respectively, in microsomes from control ducks than in microsomes from control chickens. Hydroxylase and N-demethylase activities increased with time in both species. However, hydroxylase activity increased proportionately more from the second to the eighth week in ducks than in chickens. This was indicated by the significant species × time interaction for aniline hydroxylase activity (Table III), and can be seen when the data are summarized by species and time period, ignoring the dieldrin level. No such interaction occurred for N-demethylase activity. Dieldrin in the diet did not affect aniline hydroxylase activity of either species, but there was some indication that it might have increased aminopyrine N-demethylase activity in chickens ($0.01 < p < 0.05$).

Cytochrome P_{450} concentration was higher in duck microsomes than in chicken microsomes and was higher in birds fed dieldrin than in controls. In both species, cytochrome P_{450} concentration was higher initially than at the second week. Cytochrome P_{450} in duck microsomes increased with time from the second to the eighth week, while that in chicken microsomes increased from the second to the fourth week but did not increase from the fourth to the eighth week.

Of the two criteria selected as indications of estradiol metabolism by the microsomes and shown in Table II, the first criterion would include unmetabolized estradiol-^{14}C and dichloromethane-soluble metabolites produced from estradiol-^{14}C by the microsomes during incubation. Radioactive materials that did not extract would include metabolites of

Table IV. Metabolism of Estradiol-^{14}C by Hepatic Microsomes Obtained from Chickens and Ducks Fed Dieldrin Daily for 8 Weeks

Species and dieldrin level	1 nmol of estradiol/assay		200 nmol of estradiol/assay	
	Extracted, %[a]	Estradiol, %[b]	Extracted, %[a]	Estradiol, %[b]
Chickens				
Control diet	40.8	31.5	101.4	78.5
10 μg of dieldrin/g of diet	14.0	12.5	88.4	40.4
20 μg of dieldrin/g of diet	13.9	16.7	89.3	46.0
Species avg	23.0	19.2	92.5	54.5
Ducks				
Control diet	49.4	38.4	97.2	71.9
10 μg of dieldrin/g of diet	28.8	14.5	85.2	25.5
20 μg of dieldrin/g of diet	20.1	10.2	81.0	15.4
Species avg	32.7	21.0	87.7	37.5

[a] Expressed as a percent of the radioactivity that extracted into dichloromethane after radioactive estradiol was incubated 15 min with microsomes. Outcome significantly affected by level of dieldrin fed and level of estradiol in the assay ($p < 0.01$). Species × level of estradiol in the assay also significant ($p < 0.01$). [b] Estradiol is the percent of the material in the extracted radioactivity which gave an R_f value by thin-layer chromatography similar to that of the test estradiol-^{14}C. Outcomes significantly affected by level of dieldrin fed and by level of estradiol in the assay ($p < 0.01$).

estradiol-^{14}C that were water soluble and estradiol-^{14}C conjugated with or bound to the microsomal protein. For the second criterion, it could be that some dichloromethane-soluble metabolites of estradiol would chromatograph in the system described with the same R_f as estradiol. However, with this knowledge of the limits of these criteria, they were selected as indicative of metabolism of estradiol by the microsomes, but identification of the metabolites was not attempted.

Microsomes from chickens metabolized more estradiol than microsomes from ducks. Estradiol metabolism was increased in both species when dieldrin was fed and was increased in both species with time (age). Significant species × dieldrin and species × time interactions occurred in the amount of estradiol in the extract, but the metabolism of estradiol was so great that it was impossible to make physiologically meaningful conclusions regarding these interactions. Because metabolism of the 1 nmol of estradiol was so extensive by microsomes collected at the second and fourth weeks, microsomes prepared at the eighth week were assayed with both 1 and 200 nmol of estradiol used as the substrate. These results are given in Table IV.

The metabolism of either level of estradiol was increased in microsomes from both chickens and ducks when dieldrin was fed. However, microsomes from chickens fed 10 μg of dieldrin/g of diet metabolized estradiol to the same extent as did microsomes from chickens fed 20 μg of dieldrin/g of diet, whereas in ducks, microsomes from those fed 20 μg of dieldrin/g of diet metabolized estradiol to a greater extent than did microsomes from ducks fed 10 μg of dieldrin/g of diet. A smaller percent of the estradiol was metabolized when 200 nmol was used in the assays than when 1 nmol was used.

Residues were measured in the carcasses of both species and in the few eggs laid. These residues were proportional to the amount of dieldrin fed and increased in the carcasses with length of time that dieldrin was fed. Residues were not measured in the microsomal preparations. Eggs from treated chickens were of similar weight and shell thickness as eggs from control chickens. Further details on residue or shell thickness measurements for this experiment or for experiment 2 are available from the authors.

Experiment 2. Data obtained from experiment 2 are shown in Table V, and the analysis of variance on these data is shown in Table VI.

Body weight was not significantly affected by DDT in the diet. Liver weight increased in both species with each level of DDT fed. Effects of time (age) and species on body and liver weights were the same as those observed in experiment 1.

The concentration of hepatic microsomal protein was higher at the fourth week in both species at all levels of DDT fed than at the initial, second, or eighth week time periods. No reasonable explanation is available for this other than to suggest that something peculiar to the harvesting of the microsomes occurred at this time period. The concentration of microsomal protein was higher from duck livers than from chicken livers, but DDT did not measurably affect the concentration of microsomal protein in either species.

Aniline hydroxylase activity was lower in microsomes from ducks than in those from chickens. Activity of aniline hydroxylase was lower in livers of chickens fed DDT than in controls, while in contrast, activity of aniline hydroxylase was higher in livers of ducks fed DDT than in control ducks. Hydroxylase activity increased in both species with time (age). This effect can be seen when the data are averaged across time periods within species, but it is less obvious than the effects of DDT levels within species mentioned previously.

Aminopyrine *N*-demethylase was not significantly affected by time (age) or by feeding DDT, and it was not significantly different between species in this experiment.

Cytochrome P_{450} concentration was initially higher in microsomes from ducks than in microsomes from chickens. However, by the end of the experiment, the concentration of P_{450} in microsomes from control chickens had increased to approximately the same level as in control ducks. Cytochrome P_{450} concentration was increased in both species by feeding DDT; but cytochrome P_{450} was higher at all time periods in chickens fed 200 μg of DDT/g of diet than in chickens fed 100 μg of DDT/g of diet, while cytochrome P_{450} was equally as high at the fourth and eighth weeks in ducks fed 100 μg of DDT/g of diet as in ducks fed 200 μg of DDT/g of diet.

The metabolism of estradiol by microsomes from control chickens and control ducks was approximately equal. Microsomes from DDT-fed chickens or ducks metabolized more estradiol than did microsomes from control birds, and microsomes from birds fed 200 μg of DDT/g of diet metabolized more estradiol than microsomes from birds fed 100 μg of DDT/g of diet. Also, the increased capacity of microsomes to metabolize estradiol was greater in ducks fed DDT than in chickens, and estradiol metabolism increased with time or age of the bird in both species.

Residues of DDT, DDE, and DDD present in the microsomal preparations are given in Table VII. Microsomal preparations from chickens contained greater total residues and greater residues of DDE and DDT than preparations from ducks. DDD was the least abundant residue in chicken microsomes, but it was the most abundant residue in duck microsomes.

One duck fed 200 μg of DDT and 1 g of diet laid four small eggs during the last 5 days of the experiment. No other birds laid eggs and no deaths occurred.

DISCUSSION

Chickens and ducks of about the same chronological age were selected for this study because physiological age could

Table V. Body and Liver Weights, Microsomal Protein Concentration, Aniline Hydroxylase and Aminopyrine *N*-Demethylase Activities, Cytochrome P_{450} Concentration, and Estradiol Metabolism of Hepatic Microsomes of Chickens and Ducks Fed *p,p'*-DDT

Factors studied and duration	Body weight, g/bird	Liver weight, g/bird	Microsomal protein, mg/ml[e]	Aniline hydroxylase activity[a]	Aminopyrine *N*-demethylase activity[b]	Cytochrome P_{450}[c]	Estradiol assay[d]	
							Extracted, %[f]	Estradiol, %[g]
Chickens								
Control diet								
0 weeks	750	15.8	20.2	8.41	2.88	0.69	106.0	88.0
2 weeks	840	20.1	19.7	10.98	3.66	0.90	96.7	74.3
4 weeks	901	20.5	25.9	8.42	2.44	0.72	90.3	75.8
8 weeks	1189	20.0	17.1	12.61	3.52	1.04	86.9	74.8
Diet avg	920	19.1	20.7	10.11	3.12	0.84	95.0	78.2
100 μg of *p,p'*-DDT/g of diet								
0 weeks	735	17.3	20.6	8.51	2.90	0.79	102.5	89.0
2 weeks	801	19.9	19.4	8.69	3.96	0.92	85.8	69.7
4 weeks	857	19.6	22.4	8.41	3.08	1.09	79.9	67.2
8 weeks	1182	23.2	15.3	10.47	4.96	1.39	85.8	72.8
Diet avg	894	20.0	19.4	9.02	3.72	1.05	88.5	74.7
200 μg of *p,p'*-DDT/g of diet								
0 weeks	803	19.6	19.9	8.35	2.84	0.62	109.8	92.2
2 weeks	861	19.7	21.3	8.84	3.58	1.45	73.7	56.8
4 weeks	941	21.4	22.4	7.78	2.92	1.75	72.9	55.0
8 weeks	1219	24.5	18.2	9.65	4.32	1.80	70.2	63.4
Diet avg	956	21.3	20.5	8.66	3.42	1.41	81.7	66.9
Species avg	923	20.1	20.2	9.26	3.42	1.10	81.6	73.2
Ducks								
Control diet								
0 weeks	912	14.1	21.0	3.74	2.32	0.86	100.3	90.6
2 weeks	945	16.8	19.6	4.51	3.08	0.90	92.8	79.9
4 weeks	939	14.5	24.7	3.83	2.22	0.75	91.8	77.7
8 weeks	993	15.8	18.7	4.20	2.76	0.95	82.0	77.8
Diet avg	947	15.3	21.0	4.07	2.60	0.87	91.7	81.5
100 μg of *p,p'*-DDT/g of diet								
0 weeks	897	14.3	21.0	3.99	2.78	0.91	105.1	89.8
2 weeks	902	16.1	20.7	6.14	2.80	1.19	90.6	58.5
4 weeks	914	17.5	22.8	6.67	2.64	1.99	80.6	48.4
8 weeks	900	18.2	19.9	7.61	2.74	2.86	71.4	36.3
Diet avg	903	16.5	21.1	6.10	2.74	1.74	86.9	58.3
200 μg of *p,p'*-DDT/g of diet								
0 weeks	895	15.2	21.5	3.67	2.42	0.70	98.4	90.1
2 weeks	906	16.4	20.8	6.82	2.80	1.79	83.7	46.5
4 weeks	901	20.6	25.2	6.85	2.72	1.75	71.0	45.8
8 weeks	884	19.6	24.0	7.24	2.74	2.85	74.9	37.4
Diet avg	896	18.0	22.9	6.15	2.68	1.77	82.0	55.0
Species avg	915	16.6	21.6	5.43	2.66	1.46	86.9	64.9

[a] Activity expressed as μg of *p*-aminophenol produced/mg of protein/hr of incubation. [b] Activity expressed as μg of HCHO produced/mg of protein/hr of incubation. [c] Units of optical density change (OD_{450}–OD_{500}) per 100 mg of microsomal protein. [d] 200 nmol of estradiol was used in each assay. [e] Mg of protein/ml in 5 ml of microsomal preparation derived from 5 g of fresh liver. [f] Expressed as a percent of the radioactivity that extracted with dichloromethane after radioactive estradiol was incubated 15 min with microsomes. [g] Estradiol is the percent of the material in the extracted radioactivity which had an R_f value by thin-layer chromatography similar to that of the test estradiol-^{14}C.

not be determined. The changes in body weight during both experiments suggest that the two species differed in physiological age with respect to growth. Ducks had reached a stable body weight for the experimental conditions, but the chickens had not; they were still growing. However, by the eighth week of the experiments, it appeared that some of both species had reached a physiological condition or age necessary for egg production.

Aniline hydroxylase activity increased with time or age in control birds of both species in both experiments. However, the relative magnitude of these increases was greater in experiment 1 than in experiment 2. We felt that our efficiency in processing and assaying the microsomes improved as the experiments progressed, and that this improvement might have influenced some of the results of the assays. Since measurements taken with increasing age of the birds and with increasing length of time that the insecticides were fed were also taken later in the experiments, it is obvious that these measurements could be confounded with an improvement in our ability to process and assay the microsomes. If present, this confounding factor was probably of greater importance in experiment 1 and probably accounts for some of the discrepancy indicated between experiments. A relatively smooth rhythm was established in processing the microsomes by the end of experiment 1, and this carried through experiment 2.

The confounding mentioned above would probably not alter cytochrome P_{450} measurements as noticeably, since this assay was a direct chemical determination.

In both experiments, aniline hydroxylase and aminopyrine *N*-demethylase activities were lower in microsomes from ducks than in those from chickens. This was interpreted as a true species difference. Consumption of dieldrin did not induce aniline hydroxylase activity in either species. Consumption of dieldrin did appear to stimulate aminopyrine *N*-demethylase activity in chickens, but not in ducks. These observations with chickens are consistent with previous observations with chickens fed dieldrin (Sell *et al.*, 1971).

Consumption of DDT suppressed aniline hydroxylase activity in chickens, as previously reported for chickens and quail (Sell *et al.*, 1971, 1972), but it induced aniline hydroxylase activity in ducks. To our knowledge, these differences have not been reported previously in studies with avian species.

Table VI. Analysis of Variance for the Data Shown in Table V

Source	Degrees of freedom	Mean squares: Liver weight	Microsomal protein	Aniline hydroxylase	Aminopyrine *N*-demethylase	Cytochrome P_{450}	Estradiol assays: Extracted[a]	Estradiol[a]
Total	95							
Species	1	298.9[b]	50.5[b]	350.8[b]	13.64	3.14[b]	3952[b]	7855[b]
DDT level	2	46.5[b]	15.8	1.9	1.12	4.67[b]	245	862[b]
Species × DDT	2	0.4	10.0	29.7[b]	0.44	0.88[b]	1469[b]	789[b]
Time (age)	3	73.6[b]	107.8[b]	27.2[b]	4.44	4.52[b]	1504[b]	2620[b]
Species × time	3	4.0	18.1	6.4	1.92	0.58[b]	70	11
DDT × time	6	9.2	11.1	1.4	0.28	0.98[b]	150	178
Species × DDT × time	6	6.0	3.4	4.0	0.32	0.34	465[b]	247
Assay/time	1	1.9	176.0[b]	69.8[b]	2.40	0.28	2	<1
Remainder	71[a]	4.2	6.5	4.3	5.08	0.12	68	93

[a] The estradiol assays were analyzed with microsomal protein concentration as a covariant. Degrees of freedom for the remainder would be 70 in this case. [b] Indicates significance at a probability of 1% or less.

Table VII. Residues of DDT, DDE, and DDD in Microsomes from Chickens and Ducks Fed DDT[a]

Factors studied and duration	ng/mg of microsomal protein: DDE	DDD	DDT	Total
Chickens				
Control diet				
All time periods	N.D.[b]	N.D.	N.D.	N.D.
100 μg of *p,p'*-DDT/g of diet				
2 weeks	2.1	0.7	1.2	4.0
4 weeks	4.1	1.7	6.2	12.0
200 μg of *p,p'*-DDT/g of diet				
2 weeks	4.2	1.6	3.8	9.6
4 weeks	9.0	3.9	17.2	30.2
Ducks				
Control diet				
All time periods	N.D.	N.D.	N.D.	N.D.
100 μg of *p,p'*-DDT/g of diet				
2 weeks	0.4	1.4	N.D.	1.8
4 weeks	1.1	4.2	0.9	6.1
200 μg of *p,p'*-DDT/g of diet				
2 weeks	0.8	3.1	0.8	4.7
4 weeks	2.7	11.0	6.2	20.0

[a] Data for the 8-week time period lost through analytical error. [b] None detected. As little as 0.25 ng/mg of microsomal protein would have been detected.

However, Bitman *et al.* (1971) reported that dietary DDT increased phenobarbital-induced sleeping time in quail, while it decreased this sleeping time in rats, presumably by imposing opposite effects on some mixed-function oxidases. Ducks may have responded to DDT in a fashion similar to that of rats.

Sell *et al.* (1972) suggested that the decreased aniline hydroxylase activity of microsomes from quail fed DDT was caused by competitive inhibition from the DDT present in the microsomal preparations. However, aniline hydroxylase was decreased proportionately more in the quail than in the chickens. The total residues of DDT, DDE, and DDD in the microsomes from chickens in this study were only about $^1/_{10}$ of those in the microsomes from quail, which could account for the somewhat greater effect of a similar level of dietary DDT when fed to quail. *In vitro* DDT additions to preparations of duck microsomes did not show competitive inhibition of aniline hydroxylase, as was shown for quail microsomes. Also, the concentration of DDD was greater and the concentration of DDT was lower in duck microsomes compared with chicken microsomes. These observations provide some evidence that the rate or extent of metabolism of DDT may differ between chickens and ducks, and that this should be explored further.

Hepatic microsomal cytochrome P_{450} concentrations were approximately equal in control chickens and ducks, and cytochrome P_{450} concentrations increased in both species when either dieldrin or DDT was fed. However, the ducks responded more in this respect than did the chickens. The decline in cytochrome P_{450} between the fourth and eighth weeks for chickens in experiment 1 may have been caused by onset of lay, since it has been observed previously that cytochrome P_{450} concentration in liver microsomes was inversely related to rate of egg production (Sell *et al.*, 1971).

Both dieldrin and DDT feeding increased the capacity of the microsomal enzymes to metabolize estradiol. When the amount of estradiol used in the assay was increased to 200 nmol at the end of experiment 1 and throughout experiment 2, it was easier to conduct the assays because the amount of radiotracer being extracted from the test systems was of a magnitude great enough to quantitate easily on the thin-layer chromatograms. This larger amount of estradiol also made it possible to determine that microsomes from the ducks fed DDT could metabolize estradiol more rapidly than microsomes from chickens fed DDT. The microsomes collected at the eighth week of experiment 2 were also assayed with 1 nmol of estradiol, but this amount of estradiol was virtually all metabolized by the controls, as was observed in experiment 1. Estradiol apparently ranges from 50 to 1000 pg per ml of plasma in the White Leghorn hen during the laying cycle (Arcos, 1972). Also, Peakall (1970) has reported that estradiol in blood of ring doves ranges from 1.2 to 18 ng per ml. If data from either of these sources can be extrapolated to the chickens and ducks studied here, the birds would have 0.3 to 10 nmol of estradiol in their blood.

There is no doubt that the amounts of dieldrin or DDT fed here induced some microsomal enzymes associated with the metabolism of estradiol. It is also apparent that these microsomal enzymes possessed the potential for metabolizing estradiol rapidly without induction by DDT or dieldrin. To make further interpretation of the physiological significance of this induction to estradiol metabolism in the living animal as a unit, one should have data on the rate of production and release of estradiol into the blood, and on the rate of transport of estradiol from the blood, across the cell membrane, to the microsomal enzymes.

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Organochlorine Insecticide Residues in Quail, Rabbits, and Deer from Selected Alabama Soybean Fields

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Samples of edible meat were collected from bobwhite quail (*Colinus virginianus*), swamp and cottontail rabbits (*Sylvilagus aquaticus* and *S. floridanus*), and white-tailed deer (*Odocoileus virginianus*) found in or adjacent to selected soybean fields in Alabama and from areas with little or no history of insecticide use. Samples were analyzed for residues of organochlorine and organophosphate insecticides. DDT and its metabolites (DDT + DDE + DDD) were the only insecticides occurring consistently in the samples tested. Toxaphene, heptachlor epoxide, and dieldrin were detected in a small percentage of the animals. Total DDT residues (DDT + DDE + DDD reported on a lipid basis) averaged 17.08±, 3.00±, and 2.47± ppm, respectively, for bobwhite quail, white-tailed deer, and rabbits collected from treated soybean fields. Total DDT residues averaged 1.68±, 0.10±, and 0.05± ppm, respectively, for bobwhite quail, white-tailed deer, and rabbits collected from areas with little or no history of insecticide application.

During the past few years much has been written about the presence of various insecticide residues in our environment. It has been fairly well established that most components of the ecosphere contain detectable amounts of one or more of the organochlorine insecticides and the addition of these chemicals to the environment continues each year (Dustman and Stickel, 1969).

An increasing demand for soybean products has stimulated an expansion of acreages devoted to this legume in Alabama and other southeastern states. Various insect pests sometimes necessitate the use of chemical control agents for the successful production of a soybean crop. During the 1968 and 1969 growing seasons, such chemicals as DDT, toxaphene, carbaryl, methyl parathion, and parathion were applied to soybean fields in Alabama to control insect pests.

Many of these fields are located in areas supporting high populations of such game species as bobwhite quail, cottontail and swamp rabbits, and white-tailed deer. It was suspected that these animals, living in close association with these fields, would come in contact with some of the insecticides in their normal movements and feeding habits.

A study was initiated to determine the occurrence and magnitude of insecticide contamination in the above game species found in or adjacent to selected insecticide-treated soybean fields, since these species are important game animals and are eaten by many people who hunt them.

METHODS AND MATERIALS

Collection and Preparation of Samples. During the summers and autumns of 1968 and 1969, landowners in various Alabama counties were interviewed to determine if any insecticides had been applied to their soybean fields during the growing season. The kinds and amounts were determined as accurately as possible. Only those fields treated with one or more of the organochlorine insecticides and located in good game habitat were selected as study areas. The insecticide formulation most often used was a mixture of DDT, toxaphene, and methyl parathion (an organophosphate). Many fields had not been treated, according to the owners, and many others had been treated with carbaryl or methyl parathion, which present little or no persistent residue problem. Areas were also located that had little or no history of organochlorine insecticide treatment and these areas were designated as control areas.

Bobwhite quail from treated and control areas were hunted with dogs and collected by shooting with shotguns. Quail taken in this manner were kept whole and placed in ice-filled coolers for transporting to the Auburn campus.

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