The Metabolism of *p,p'*-DDE in Laying Japanese Quail and Their Incubated Eggs

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INTRODUCTION

DDT has been one of the most widely studied chemicals because of its wide exposure to the environment as a moderately stable agricultural pesticide. Gas chromatographic analyses have shown p,p'-DDE to be the principle compound found in both the terrestrial and marine environment not directly exposed to recent DDT applications. Thus this compound must be relatively stable to metabolism and environmental exposure. Since p,p'-DDE has also caused a thinning of egg shells in some wildlife birds (COOKE, 1973), this has lead to numerous studies in correlating DDE residues in eggs with shell thickness (ANDERSON et al., 1969; CADE et al., 1971; and BLUS et al., 1972). As noted by HAZELTINE (1972), metabolism of DDE during incubation could effect egg residue-shell thickness correlations.

Numerous investigators have attempted to find the metabolic route of p,p'-DDE. PETERSON and ROBINSON (1964) did not find a metabolite by paper chromatography when p,p'-DDE was fed to rats, nor was any urinary metabolite found from human exposure (ROAN et al., 1971). However, ABOU-DONIA and MENZEL (1968) found dichlorobenzophenone when White Leghorn chicks or fertile eggs were exposed to ^{14}C-p,p'-DDE. Eggs were injected with 0.1 ml of peanut oil containing p,p'-DDE, incubated, and the chicks sacrificed. In both sets of chicks 4,4'-dichlorobenzophenone (DBP) was the only metabolite found. DBP represented approximately 13% of the total residue in the chicks. Although ^{14}C-labeled DDT and dieldrin have been shown to distribute into the embryo after injection into the egg (ABOU-DONIA and MENZEL, 1968; GUTHRIE and DONALDSON, 1970), we wanted to determine whether DDE metabolism occurs in the egg during incubation under normal conditions. At the same time the rate of excretion and metabolism of ^{14}C-DDE in laying hens was investigated.

In this experiment two laying Japanese quail hens fed a diet containing DDE were given a single oral dose of ^{14}C-DDE. Frozen and incubated eggs, excretory material, brain, liver and carcasses

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Abbreviations used in this paper are: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (p,p'-DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (p,p'-DDD) and 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p'-DDE) and 4,4-dichlorobenzophenone (DBP), Bis(p-chlorophenyl) acetic acid (DDA).

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of the hens were analyzed for DDE and possible metabolites.

EXPERIMENTAL

Ten, 7 week old, Japanese quail (Coturnix c. japonica), 5 males and 5 females, were placed on a standard O.S.U. corn-soybean meal ration (10% moisture and 15% protein) containing 100 ppm of p,p'-DDE. The DDE was dissolved in corn oil, mixed with the feed, and the p,p'-DDE concentration verified by gas chromatographic analysis. The quail were maintained on this diet for 13 weeks, an exposure period estimated to achieve approximate equilibrium in the storage and excretion of DDE (CECIL et al., 1972). Mating commenced at 8 weeks and the eggs were collected after 9 weeks of age.

Two hens were selected which were found to have regular laying patterns. After 10 weeks on the DDE diet, each of the two hens was given an oral dose of 2 ml of corn oil by a syringe equipped with a 3" x 16 awg teflon tube. Each dose contained 145 µg of ^{14}C-DDE (ring-labeled) with a specific activity of 0.06 µCi/µg (>98.7% p,p'-DDE). The dose was equivalent to 7.3% of the daily intake of a 100 ppm ration based on a 20 g/day feed intake. Each hen weighed approximately 120 g; thus the 100 ppm DDE ration was equivalent to a daily dose of 16.7 mg/kg. Eggs were collected for 18 days after ^{14}C-DDE administration. Alternately the eggs from each hen were frozen or incubated for 15 days. Three frozen and three incubated eggs from the 4th to the 7th day after administration of the ^{14}C-DDE were analyzed for metabolites by thin layer chromatography (radio-metrically) and gas chromatography (e.c. detector). DDE was determined in the remaining eggs. After 18 days the hens were sacrificed and their tissue was analyzed for DDE and metabolites.

The hens were placed in small wire cages 5 days prior to dosage, and the excrement and feed contents in the cage pans were collected daily the first week and every 3 days thereafter. Radioactivity in each collection was determined by liquid scintillation counting methods.

Extraction. The eggs were extracted by mixing the contents with 130 g Na_2SO_4 and extracting overnight with 300 ml of $CHCl_3$:MeOH (2:1, v/v) in a Soxhlet extractor. A 0.5 ml aliquot was taken for ^{14}C counting. The solvent was then evaporated to dryness with a stream of air. The residue was dissolved in hexane and an aliquot taken for column chromatography.

Each brain and liver was extracted twice with 25 ml of acetone using a PT20-ST Polytron^{R} mechanical, ultrasonic homogenizer (Brinkman Instruments). The extracts were filtered through Whatman #1 filter paper which was rinsed with acetone. The acetone was evaporated to 2-3 ml on the steam bath using an air jet; then 10 ml of trimethylpentane was added as a keeper while the remaining acetone was removed. The samples were then counted and loaded onto an adsorbent column for lipid separation.

The remaining carcass, including feathers and bones, was extracted twice with 750 ml and a third time with 500 ml of a hexane: acetone (2:1, v/v) mixture in a 1 gallon Waring blender. The extract was evaporated to 150 ml and filtered through Na_2SO_4 before counting.

The excretory material was extracted by blending it with 200 ml of 20% H_2O in MeOH (v/v) for 30 seconds in the polytron homogenizer. After filtering, the paper and residue were re-extracted with 150 ml of distilled MeOH and filtered. The filter cake was then soaked and rinsed with an additional 50 ml of MeOH.

Column Chromatography. An aliquot equivalent to 1.5 grams of liver or brain, or 4 grams of egg was cleaned up for gas chromatographic analysis on a 25 g alumina column (CLAEYS and INMAN, 1974). DDE and DBP were eluted with 150 ml of η-hexane. Separation of DDE and DBP was achieved on a 15 g activated Florisil^R (100-200 mesh) column. DDE was eluted with 125 ml of 5% benzene in hexane (v/v) and DBP was eluted 200 ml of 25% ethyl ether and 0.25% acetone in hexane (v/v). Both of the columns were eluted with 100 ml of acetone to remove any other polar metabolites that might be present.

Thin Layer Chromatography. Thin layer chromatography was performed using 250 μ layer of Silica gel H (Merck) developed with 5% (v/v) ethyl ether in pentane. The compounds on the plates were visualized with the silver nitrate reagent method of KOVACS (1963) or scanned using a Packard model 7200 Radiochromatogram scanner. The most sensitive settings for this instrument at a 5.0 mm slit width were used for DBP counting.

Gas Chromatography. The cleaned-up extracts were analyzed by electron capture gas chromatography employing a 122 cm (4 ft) x 2 mm id pyrex column filled with a mixed packing of 100/120 mesh Chromosorb W-HP coated with 7% QF-1 and 7% DC-11 (2:1). The retention times of DBP and p,p'-DDE were 4 and 6 min, respectively, at a column temperature of 195°C and a nitrogen flow rate of 20 ml/min.

RESULTS

The DBP metabolite of DDE could not be detected in the eggs (incubated or nonincubated), tissues, or excrement of the quail hens. No other metabolite of DDE was found either by gas chromatography or by thin-layer radiochromatography. Analyses by thin layer radiochromatographic methods showed that any DBP present in egg extract was less than 2% of the radioactive DDE found, while analyses of the various eluant fractions from the alumina and Florisil columns showed that all but 0.08% of the total activity in the egg was eluted in the 5% benzene (DDE) fraction from the Florisil column. The acetone fraction from the alumina column contained the 0.08% activity. This fraction was found to contain only DDE when analyzed by gas chromatography. Gas chromatographic analysis of the 25% ethyl ether -0.25% acetone (DBP) fraction did not show any DBP present at a minimum detection level of 0.06 μg DBP. This

amount represents less than 0.015% of the 100 ppm DDE found in the eggs. A statistical comparison, the analysis of variance of unweighted means, showed no significant difference among the means of the total amount of DDE/egg in the incubated and nonincubated eggs or the eggs laid by the two quails.

The DDE concentration in the eggs averaged 100 ppm wet weight (Table I). The total amount of ^{14}C excreted in the eggs was 39.6% of the recovered activity. Figure 1 shows the daily excretion of the ^{14}C-DDE dose in the egg and in the excrement. The first 24-hour excretion probably represents the unabsorbed dose. Omitting the first 24 hours, 6-9% of the recovered radioactivity was excreted in the excrement during the 18 days following administration. As with the eggs, no DBP was found by gas chromatography in the excrement at a sensitivity of 0.003 ppm in a three-day excrement sample.

TABLE I

Summary of ^{14}C-DDE and DDE Residues Found in the Tissues, Eggs, and Excrements of Two Japanese Quail Hens

	Bird A		Bird B	
	% Recovered Dose (^{14}C)	Ave. ppm[2] (Range)	% Recovered Dose (^{14}C)	Ave. ppm[2] (Range)
Eggs[1]	39.80	89.8 (81.2-93.7)	35.82	111.7 (103.5-123.1)
Liver	0.43	108 (90-130)	1.58	215 (195-250)
Brain	0.009	10.5	0.007	11.0
Excrement (Unadsorbed)	17.36	*	20.42	*
Excrement (Days 2-18)	8.50		6.62	
Carcass	33.91	*	35.56	*
% Dose Recovered	79.30		72.30	

* Not determined.

[1] Incubated eggs corrected for weight lost during incubation.

[2] Wet weight.

The brain, liver and carcass of the sacrificed hens were analyzed by gas chromatography or for radioactivity as indicated in Table I. No metabolites of p,p'-DDE were found in the liver (neither DBP nor more polar metabolites). The minimum detectable amount of DBP in the liver by gas chromatography was <0.01 ppm or 0.007% of the DDE present.

The recovery of DDE and DBP in tissue and egg samples spiked just prior to extraction was between 90 and 97% for both compounds. Re-extraction of the excrement using a 24-hour $CHCl_3$:MeOH (2:1, v/v) Soxhlet extraction system yielded only 1.7% additional radioactivity.

The total recovery of radioactivity from the birds was 79.6 and 72.3% of the theoretical dose.

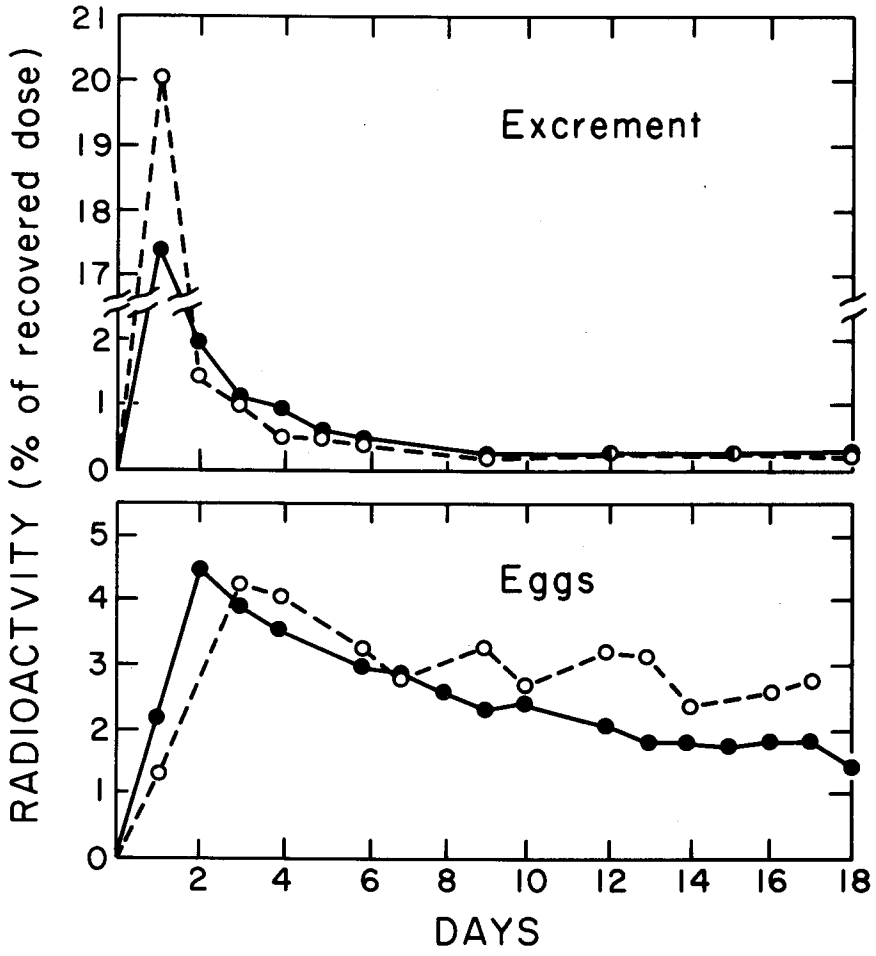

Figure 1. Excretion of ^{14}C-DDE from quail via eggs and excrement. Cage A ●—●; Cage B O—O.

DISCUSSION

The metabolite 4,4'-dichlorobenzophenone was not detected in our investigation as has been previously reported by ABOU-DONIA and MENZEL (1968). These investigators exposed White Leghorn chicks to DDE either by injection during incubation or by feeding a 100 ppm DDE diet 5 days. The chick livers contained respectively 44 and 81 ppm dry weight of DBP or 14 and 27% of the total chlorinated hydrocarbon residue (DDE+DBP). Their carcass analyses showed that 15 and 10%, respectively, of the residue was DBP. In our experiments any DBP present in the liver was less than 0.007% of the DDE residue. White Leghorn chicks apparently have a metabolic route not found in Japanese quail.

BAILEY et al. (1969) fed pigeons 1000 ppm DDE for 21 days. The birds were sacrificed at various intervals up to 288 days. The half-life was estimated at 250 days and no metabolites were found. Polar metabolites such as DBP apparently were not determined.

CECIL et al. (1972) have reported the accumulation of DDE in the eggs and body fat of White Leghorn pullets after feeding 5, 25, and 50 ppm of DDE for 28 weeks. The concentration of DDE in the eggs plateaued in 10 to 12 weeks. The concentration of DDE in the egg approximated the dietary level while the body fat concentration was 13 times the dietary concentration. About 42% of the ingested DDE was excreted via the eggs and 7% was found in the feces. These results compare favorably with those reported here; i.e., 40 and 7.5% of the recovered ^{14}C-DDE was excreted by way of the eggs and feces, respectively. Likewise the concentration in the eggs was nearly equal to the dietary level.

Figure 1 shows the daily excretion rate of a single dose of ^{14}C-DDE from the two hens previously equilibrated with 100 ppm feed. Of this dose, about 2% daily is eliminated by way of the eggs and about 0.3% via the excretory material. The observed differences in p,p'-DDE residues in the liver and eggs of the two birds appear to be the result of dissimilar egg laying habits, even though an attempt had been made to select birds with similar laying patterns. Bird A (Table 1) maintained a daily egg production rate of 85% while bird B declined to 67% after dosing. Bird B with the lower egg production maintained a higher DDE level within her eggs and liver.

A comparison of our DDE residues with other avian feeding experiments is given in Table II. The ratio of the concentration of DDE in the liver to the concentration in the brain was 10 to 20. This compares favorably with the experiments of STICKEL et al. (1970) and BAILEY et al. (1969) for high levels of exposure. Where the dose is much lower, ratios from 1 to 3 are found, as seen in the data of PORTER and WIEMEYER (1972) and the environmental levels in peregrine falcons (RISEBROUGH et al., 1968). A ratio of 2.2 was observed in Japanese quail and pigeons given 10 to 50 ppm of dieldrin (ROBINSON et al., 1967).

The ratio of concentration of DDE in the brain to the concentration in the feed was 0.1 which also agrees with the other high level dose experiments in Table II and with the dieldrin experiments of ROBINSON et al. (1967). Note that this ratio with American kestrels is 15 times higher (Table II). This would imply that these birds had little storage capacity and had higher blood levels.

The 20% loss of radioactivity may have occurred at several points in the experiment. Some losses may have occurred with the evaporation of the solvents; however, this would be contrary to previous experience. Some of the corn oil dose may not have been swallowed. Some losses by codistillation of DDE with water from the excrement in the collection pans may have occurred.

TABLE II

Summary of p,p'-DDE Residues in Birds and Eggs
In Controlled Feeding Experiments

Species Tissue	Sex	No.	DDE in Diet[a]	Days on Diet	ppm DDE Wet wt.	Concentration ratio of: Liver to Tissue Brain	to Feed	Ref.
J. Quail	F	2	110	91				This Exp.
Brain					10.8		0.1	
Liver					108-215	10-20	1-2	
Eggs					100		1.9	
Cowbirds	M	19	1500	15-27				Stickel
Brain					500		0.3	et al.
Liver					3900	8	1.6	1970
Carcass					1300			
Pigeons	M&F	9	1000	21				Bailey
Brain					113		0.1	et al.
Liver					919	8.1	0.9	1969
American Kestrel	M	11	10	368-472				Porter &
Brain					15		1.5	Wiemeyer
Liver					24	1.6	2.4	1970 &
Carcass					70			1972
Eggs	F				32		3.2	

[a] ppm, dry weight.

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