Residues in Chickens Given DDT

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Adult chickens were dosed with 10 mg of *p,p'*-DDT per kg of body weight daily for 90 days and then killed. The residues of *p,p'*-DDT and related compounds were higher in the tissues of treated adult males than in tissues of treated hens. The highest residue levels were found in the fats and preen gland, and the lowest levels were in the breast muscle and brain. The residue levels in control chickens were unchanged throughout the study. Yolks of eggs collected prior to the start of the study and at 30-day intervals during the study were analyzed for residues. Peak residues were found at 60 and 90 days. The tissues of chicks hatched from eggs collected during the 13th week of the study were analyzed. Peak residue levels were found at either day 1 or 7 after hatch. Residues had practically disappeared by 21 days after hatch. No residues were found in chicks of control chickens.

A tremendous amount of research has been published since the discovery of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and two of its metabolites, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE). A review article by St. Omer (1970) gives a background on the research of chlorinated hydrocarbon insecticides.

In the area of residues, work has been done in all types of domestic animals, fish, and wildlife, including quail, falcons, eagles, and ducks. These residue studies have been linked directly with metabolism studies. An article by French and Jefferies (1969) gave the metabolism of *o,p'*-DDT in both living and dead avian tissues. The *o,p'*-DDT is broken down to *p,p'*-DDT and then to DDE in living avian tissues. In dead avian tissues, the *o,p'*-DDT is metabolized to DDD. Residues of DDT and metabolites have been determined in eggs and chicken tissues (Ecobichon and Saschenbrecker, 1968; Graves *et al.*, 1969; Herrick *et al.*, 1969) after short-term feeding trials. In other studies, the residues were traced after injections of DDT and metabolites into the avian embryo (Abou-Donia and Menzel, 1968; Guthrie and Donaldson, 1970). Other researchers have attempted to deplete the DDT residues in laying hens (Wesley *et al.*, 1969) and cockerels (Ecobichon and Saschenbrecker, 1969) by various means.

Few studies, in which DDT and metabolite residues were determined in chickens, have been based on trials in which a known daily oral dose rate was given to the chickens. Most analyses have been carried out on tissues of chickens exposed to unknown levels of DDT and metabolites. Therefore, the objectives of this study were: to determine the amount of residues accumulated in 11 different tissues of adult chickens after 90 days of oral dosage, to determine the buildup of DDT and metabolites in the egg yolk of treated birds, and to determine the decrease of residues in tissues of chicks hatched from eggs produced by treated hens.

METHODS

Forty-eight White Leghorn hens and 19 adult White Leghorn males were used in this study. The chickens were placed in individual cages with feed and water supplied. Twenty-eight hens and nine males were in the treated group, and 20 hens and 10 males were in the control group. The control group of chickens was not allowed to come in contact with the treated group. During the study, the semen from both the treated and control males was collected three times a week and was pooled within each group. The hens in both the treated and control groups were then artificially inseminated with the pooled semen from the appropriate group of adult males. Chickens, both hens and males, from the treated and the control group were killed prior to the start of the test. Each remaining chicken of the treated group was given 10 mg of *p,p'*-DDT per kg of body weight by oral capsule daily for 90 days. At the end of the 90 days, all chickens from each of four groups were killed. The following tissues were obtained for analysis: brain, kidney, liver, heart, breast muscle, thigh muscle, gizzard, preen gland, testes or ovaries, subcutaneous fat, and abdominal fat. All tissues were individually packaged, labeled, and stored in a low temperature freezer (−50° C) until analyzed.

All tissues except preen glands from the adult birds were ground in a Latapie tissue grinder. Samples of the preen glands were sliced in thin strips, with care taken to exclude all external fat. Duplicate samples of each individual tissue were analyzed separately. The procedure used to extract the tissues for DDT and metabolites was a modified procedure of Radomski and Fiserova-Bergerova (1967). In general, 0.250 g of tissue was ground in a Thomas tissue grinder with redistilled petroleum ether. The extract was dried with sodium sulfate, concentrated, brought to volume with hexane, and 2-μl portions were injected into a Micro-Tek 220 gas chromatograph equipped with a nickel-63 high temperature electron capture detector. A 6 ft × 0.25 in. stainless steel column packed with 5% QF-1 coated on 80/100 mesh Chromosorb W was used to separate the various components. Figure 1 shows the representative resolution of a standard mixture containing DDE, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDT in hexane at levels of 0.2, 0.5, 0.5, and 0.5 μg/ml, respectively, obtained by the use of this column.

Eggs were collected prior to the start of the test and at 30-day intervals during the test. These eggs were broken, the yolks and albumen separated, the yolks pooled and mixed, and aliquots withdrawn for analysis. The albumen was not checked for residues since it is only 12% solids with none being fat. Yolk is 52% solids with 33% being fat (Card, 1961). Since DDT and metabolites are fat soluble, the majority of

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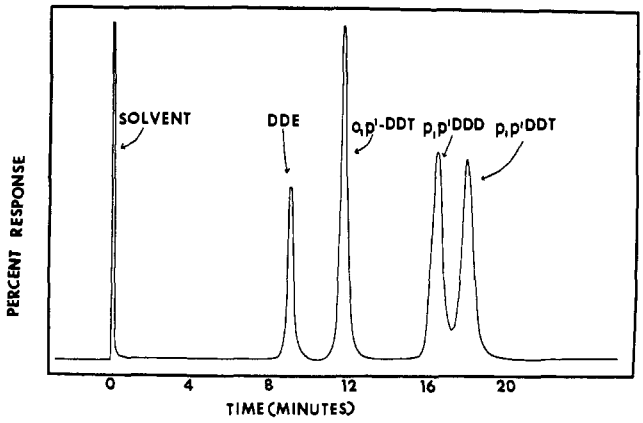

Figure 1. Chromatogram showing resolution of DDT and metabolites on a 5% QF-1 coating on 80/100 mesh Chromosorb W in a 6 ft × 0.25 in. stainless steel column

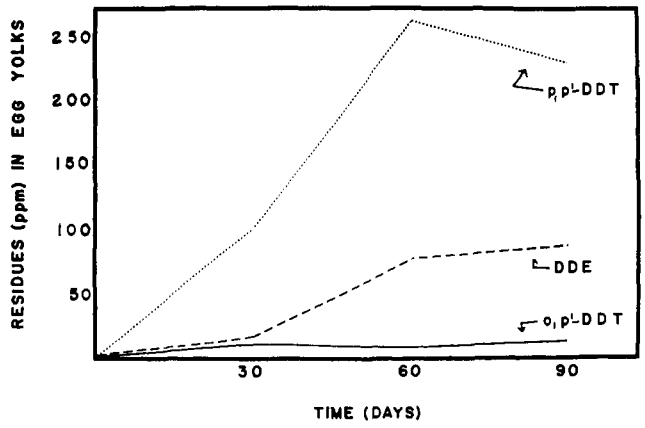

Figure 2. Increase of DDE, *o,p'*-DDT, and *p,p'*-DDT in egg yolks of chickens treated with 10 mg/kg of purified DDT daily for 90 days

Table I. Average Percent Recoveries of *p,p'*-DDT and DDE in Fortified Tissues and Egg Yolks

Tissue[a]	DDE, % recovery	*p,p'*-DDT, % recovery
Brain	86.1	88.9
Fat, abdominal	106.0	82.2
Fat, subcutaneous	105.0	86.0
Gizzard	101.7	98.6
Heart	99.2	95.5
Kidney	98.3	92.4
Liver	99.3	86.5
Muscle, breast	102.3	102.0
Muscle, thigh	108.4	104.0
Ovary	101.3	98.4
Testes	103.6	100.7
Yolk, egg[b]	84.5	88.8

[a] Tissues were fortified with 0.2 and 8.0 ppm of DDE and 0.4 and 16.0 ppm of *p,p'*-DDT. [b] Egg yolks were fortified with 0.25 and 5.0 ppm of DDE and 0.5 and 1.0 ppm of *p,p'*-DDT.

Table II. Average Residues (ppm) in Adult Hens and Adult Male Chickens Dosed with 10 mg/kg/day for 90 Days[a]

Tissue	DDE	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDD
		Hens		
Brain	1.6	2.3	<0.2	<0.2
Fat, abdominal	444.4(0.1)	1037.6	47.4	<28.8
Fat, subcutaneous	426.2(0.1)	1027.0	45.6	<32.4
Gizzard	2.3	5.0	<0.3	<0.3
Heart	7.6	16.3	<0.6	1.1
Kidney	5.6	11.8	0.6	0.8
Liver	7.8	14.0	<0.8	1.7
Muscle, breast	1.4	3.2	<0.2	<0.2
Muscle, thigh	15.1	36.3	<2.1	<1.9
Ovary	13.8	25.9	0.9	1.4
Preen gland	99.6	159.2	<7.4	<5.7
		Adult males		
Brain	15.5	9.2	...	<0.4
Fat, abdominal	2147.5(0.8)	2010.0	...	<95.0
Fat, subcutaneous	1567.5(1.0)	1520.0	...	<102.5
Gizzard	7.2	5.1	...	0.8
Heart	75.5	69.2	...	6.1
Kidney	16.7	10.2	...	2.6
Liver	23.7	8.4	...	7.0
Muscle, breast	4.3	3.6	...	<0.3
Muscle, thigh	29.3	23.2	...	<3.1
Testes	17.5	6.4	...	0.9
Preen gland	614.7(0.1)	537.7	...	<27.3

[a] Figures in parentheses represent residues in control tissues.

the residues should be found in the yolk rather than in the albumen.

Egg yolks were extracted by a modified procedure of Sawyer (1966). In this procedure 2 g of yolk are extracted twice with redistilled acetonitrile, centrifuged, and then partitioned against redistilled petroleum ether and 2% Na_2SO_4 in H_2O. The petroleum ether extract is dried and concentrated to a small volume. A chromatographic column (prewashed with 20% methylene chloride in petroleum ether) containing three layers of Na_2SO_4 and two layers of Florisil was used to clean up the extract. The components of interest were eluted with 20% methylene chloride in petroleum ether, concentrated, and brought to volume with hexane. The gas chromatographic procedures for tissue extracts were also used for the egg yolk extracts.

The eggs collected during the 13th week of the test were incubated and hatched. Five chicks each from the treated group and from the control group were killed at days 1, 7, 14, and 21. The following tissues were obtained and pooled: heart, liver, brain, breast muscle, and thigh muscle. These tissues were treated as those of the adult birds.

RESULTS AND DISCUSSION

The recoveries of *p,p'*-DDT and DDE were checked in tissues and yolks to which known amounts of these compounds were added prior to extraction.

The recoveries of *p,p'*-DDT and DDE from these fortified samples are indicated in Table I. The overall average of the recovery of DDE from tissue samples was 101%, and the average recovery of *p,p'*-DDT from tissue samples was 94.1%. The recovery of *o,p'*-DDT and *p,p'*-DDD from brain and liver averaged 100%. The residue results reported have not been corrected for recovery.

Figure 2 indicates the increase of DDE, *o,p'*-DDT, and *p,p'*-DDT in egg yolks during the treatment period. The *p,p'*-DDT increased up to 60 days and then dropped slightly at 90 days. The DDE increased up to 90 days. The *o,p'*-DDT reached a plateau at 30 days and remained at that level.

Table II shows the average residue levels of DDE, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDD found in five hens given the *p,p'*-DDT daily at 10 mg per kg of body weight for 90 days. The highest levels of DDT and metabolites were in the fats and the preen gland, and the lowest levels were found in the breast muscle. The figures in parentheses represent the residues found in control hens. Only DDE residues were found in

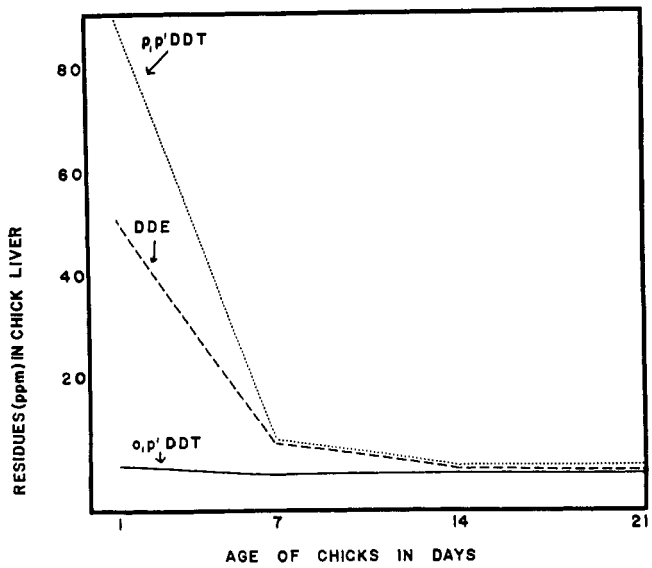

Figure 3. Average residues of DDT and metabolites in pooled liver of chicks hatched from eggs of treated hens collected during the 13th week of the test

the control hens and were in the fat tissues. The levels found were about the same as those determined at the start of the experiment. The "less than" (<) symbol in Table II, which appears before some figures, indicates that these values were calculated from "peaks" which were too small for accurate quantitation due to large dilutions of the extracts.

Table II also shows the average residues of DDE, *p,p'*-DDD, and *p,p'*-DDT found in five treated males. The levels found in control males are indicated in parentheses (only DDE in fats and preen gland). By comparing the levels in treated males to those in treated hens, one can see that no *o,p'*-DDT was found in any male tissues in detectable amounts. Also the levels of DDT and metabolites were much higher in males than those levels found in hens. This is probably due to levels that pass out of the hen *via* eggs. At 90 days, the treated hens were excreting an average of 4.0, 0.2, and 0.6 mg of *p,p'*-DDT, *o,p'*-DDT, and DDE, respectively, in each egg yolk.

Figure 3 shows the residues of DDT and metabolites found in the pooled liver of chicks hatched from eggs of treated hens collected during the 13th week of the test. The residues started out high at 1 day after hatch and then decreased to near zero residues 21 days after hatch. This curve is representative for residues found in breast muscle and brain also.

This is not the case for residues in pooled thigh muscles in these chicks (Figure 4). The residues increased up to 7 days after hatch and then dropped to near zero at 21 days after hatch. This curve is also representative for the heart.

We do not have a positive explanation for the delayed increase and then decrease in the residue levels in the heart and thigh muscles as compared to the steady decrease of residue levels in the brain, breast muscle, and liver of these chicks. One possible explanation is the fact that as much as 30% of

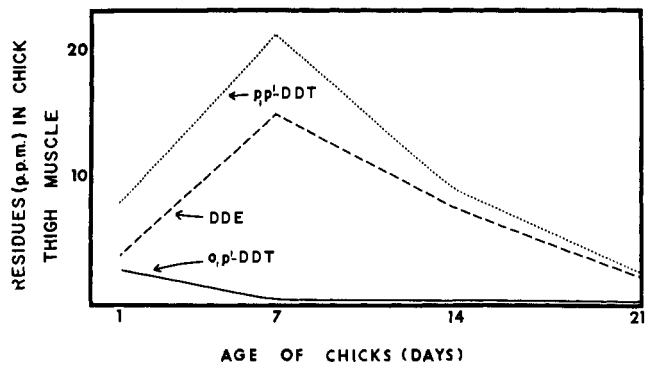

Figure 4. Average residues of DDT and metabolites in pooled thigh muscle of chicks hatched from eggs of treated hens collected during the 13th week of the test

the egg yolk is still unabsorbed at the day of hatch and is absorbed during the first 7 days thereafter (McLaughlin *et al.*, 1963).

No residues were found in any of the pooled tissues of chicks hatched from eggs collected from the control hens.

Reynolds (1969), in his article on polychlorinated biphenyls (PCB), has pointed out that many residues reported as DDT and metabolites may be actually a combination of what was reported and a PCB. To alleviate this possibility, we analyzed our feed, which was a special ration, and found no peaks corresponding to DDT and metabolites. Standards prepared from the purified DDT were compared to those prepared from technical samples. Our purified DDT was analyzed to be about 97 to 98% *p,p'*-DDT with the remainder being *o,p'*-DDT and DDE. No interfering peaks were observed on either a 5% QF-1 column, or a 5% SE-30 column. During the feeding test, there was no increase of DDT and metabolites or other residues in the tissues of the control birds.

LITERATURE CITED

Abou-Donia, M. B., Menzel, D. B., *Biochem. Pharmacol.* **17,** 2143 (1968).
Card, L. E., "Poultry Productions," 9th ed., Chapter 2, Lea & Febiger, Philadelphia, Pa., 1961, p 65.
Ecobichon, D. J., Saschenbrecker, P. W., *Can. J. Physiol. Pharmacol.* **46,** 785 (1968).
Ecobichon, D. J., Saschenbrecker, P. W., *Toxicol. Appl. Pharmacol.* **15,** 420 (1969).
French, M. C., Jefferies, D. J., *Science* **165,** 914 (1969).
Graves, J. B., Bonner, F. L., McKnight, W. F., Watts, A. B., Epps, E. A., *Bull. Environ. Contam. Toxicol.* **4,** 375 (1969).
Guthrie, F. E., Donaldson, W. E., *Toxicol. Appl. Pharmacol.* **16,** 475 (1970).
Herrick, G. M., Fry, J. L., Fong, W. G., Golden, D. C., J. AGR. FOOD CHEM. **17,** 291 (1969).
McLaughlin, Jr., J., Marliac, J. P., Verrett, M. J., Mutchler, M. K., Fitzhugh, O. G., *Toxicol. Appl. Pharmacol.* **5,** 760 (1963).
Radomski, J. L., Fiserova-Bergerova, V., *Ind. Med. Surg.* **36,** 281 (1967).
Reynolds, L. M., *Bull. Environ. Contam. Toxicol.* **4,** 128 (1969).
St. Omer, V. V., *Can. Vet. J.* **11,** 215 (1970).
Sawyer, A. D., *J.A.O.A.C.* **49,** 643 (1966).
Wesley, R. L., Stemp, A. R., Harrington, R. B., Liska, B. J., Adams, R. L., Stadelman, W. J., *Poultry Sci.* **48,** 1269 (1969).

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