Pesticide Residues in Eggs Resulting from the Dusting and Short-Time Feeding of Low Levels of Chlorinated Hydrocarbon Insecticides to Hens

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"Working levels," used by the Florida Department of Agriculture prior to issuance of FDA action level guidelines, were used as criteria. For whole eggs these levels were 0.05 p.p.m. for DDT, BHC, lindane, and methoxychlor and 0.01 for aldrin, dieldrin, and chlordane. The dusting of laying hens with "safe" insecticides contaminated with as little as 200 p.p.m. of DDT caused residues in the egg to exceed the working level. Dusts with up to 300 p.p.m. of BHC did not exceed 0.05 p.p.m. in the eggs. A dust contaminated with 2000 p.p.m. of methoxychlor produced no detectable residues. Chlorinated hydrocarbon insecticides were fed at low levels to laying hens for a one-week period. The insecticides, feeding levels, and maximum residue amounts in the whole eggs were: DDT 0.33 p.p.m. in feed, 0.062 p.p.m. in eggs; aldrin 0.114 p.p.m. in feed; dieldrin 0.011 p.p.m. in eggs; lindane 0.26 p.p.m. in feed, 0.033 p.p.m. in eggs; chlordane 0.08 p.p.m. in feed, no detectable residues in eggs.

With the passage in 1954 of the Miller Amendment to the Food, Drug, and Cosmetic Act of 1938, pesticide residues became a serious problem to producers of poultry and food and feedstuffs. No tolerances have been established for chlorinated hydrocarbon residues in poultry, meat, and eggs, and, in the absence of such established tolerances, a zero level has automatically been the tolerance level. Some enforcement agencies, however, have been using so-called "working zeros" or "working levels," above which the product would be considered unacceptable and legal action taken. Action level guides were issued (Food and Drug Administration, 1967) for certain pesticides of concern to the poultry industry. These levels were considerably higher than those previously used by the Florida Department of Agriculture (Table I). For the studies reported in this paper the working levels of the Florida Department of Agriculture were used as levels of unacceptability. The Food and Drug Administration action level guides are not to be considered as predictions of the negligible residue tolerances that may be established at a later date.

Table I. Working Levels[a] (Florida Department of Agriculture) and Action Level Guidelines (Food and Drug Administration) for Pesticide Residues in Whole Eggs

Insecticide	Working Levels, P.P.M.	Action Level Guidelines, P.P.M.
Aldrin	0.01	0.03
Dieldrin	0.01	0.03
Endrin	0.01	0.03
Heptachlor and heptachlor epoxide (individually or in combination)	0.01	0.03
Chlordane	0.01	[b]
BHC	0.05	0.5
Lindane	0.05	0.5
Methoxychlor	0.05	[b]
DDT, DDE, TDE (individually or in combination	0.05	1.5

[a] In use by Florida Department of Agriculture prior to issuance of Food and Drug Administration action level guidelines.
[b] No action level guides established.

Early studies of pesticides and poultry dealt primarily with toxicity of the pesticide to the birds themselves. In recent years there have been a number of published reports on pesticide residues in both poultry and eggs. In many of these studies levels fed resulted in residues much above acceptable levels. One of the most complete and practical studies to date on the feeding of chlorinated hydrocarbon insecticides to laying hens was conducted by Cummings *et al.* (1966). Low levels (0.05, 0.15, and 0.45 p.p.m.) of lindane, DDT, heptachlor epoxide, dieldrin, and endrin were fed to hens and the accumulation and depletion of the pesticides in eggs determined. It is very unlikely, however, that any commercial poultryman would have a source or supply of feed that would have exactly the same pesticide contamination level over the length of time (14 weeks) used in this study.

In the feeding studies reported here, a 7-day treatment period was used to simulate the time a commercial poultry producer might be using one bulk delivery of feed. The dusting studies attempted to simulate the unintentional contamination of "safe" insecticides such as Co-Ral, malathion, and Sevin with chlorinated hydrocarbon insecticides shown to occur in the manufacture, distribution, and use of these insecticides (McCaskill, 1968).

EXPERIMENTAL

Analytical Method. All analyses of eggs, dusts, and feeds were made by the Pesticide Residue Laboratory, Florida Department of Agriculture. Each group of

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eggs to be analyzed as a treatment group was broken out and beaten together. A 50-gram aliquot was taken and extracted twice with 150 ml. of acetonitrile (Sawyer, 1966). The acetonitrile extract was partitioned into 100 ml. of petroleum ether, and eluted from a Florisil column with 200 ml. of 6% ethyl ether–hexane, followed by 200 ml. of 20% ethyl ether–hexane (Mills, 1959). The cleaned-up sample was concentrated to 4 ml. and injected into a gas chromatograph, using an electron-capture detector, and calculated relative to standards. The gas chromatographs were Aerograph 680 with 5-foot by ⅛-inch borosilicate glass columns packed with 5% Dow-11 on Chromosorb W, 60- to 80-mesh. The operating parameters were: oven temperature 185° C., and the flow rate of nitrogen 60 ml. per minute (Munks, 1965).

The dusts were analyzed by extracting 25 grams of uniformly mixed dust with 250 ml. of hexane. A 2-ml. aliquot of the extract was eluted from a Florisil column using the procedure of Mills (1959), followed by gas chromatographic analysis. The feeds were analyzed by blending 50 grams of uniformly mixed and ground sample with 35% water-acetonitrile (Bertuzzi *et al.*, 1967). A 70-ml. aliquot of the extract was taken and partitioned into 100 ml. of petroleum ether, followed by Mills' (1959) Florisil elution procedure. The sample was then analyzed by gas chromatography.

Dusting Studies. Four dusting experiments were conducted using DDT and BHC; methoxychlor was also used in the first experiment. The birds used were commercial egg-type hens laying at a rate of approximately 50%. In the first three experiments each bird was dusted individually with a given amount of dust, applied to the abdominal area of each bird with care taken to avoid the vent and not to contaminate feed and water troughs. The dust was rubbed into the skin and feathers in order to limit the amount that fell off after the bird was replaced in the cage. Eggs were collected each day and marked with pen or cage number and date.

Analysis was made of treatment groups combining the eggs for three consecutive days for each sample taken. Pretest analyses were also made on feed and eggs; no pesticide residues of significance were detected in either the basal feed or pretest eggs.

In the first experiment, a 5% Sevin dust was contaminated with 25, 50, 100, and 200 p.p.m. of DDT, 25, 50, 100, and 200 p.p.m. of BHC, and 200, 500, 1000, and 2000 p.p.m. of methoxychlor. Thirty-nine birds were used with three birds on each of the above treatment levels and three birds as a control group. Each bird was dusted with 13 grams of the appropriate dusting compound. Empty cages separated treatment groups. In the second experiment a 5% malathion dust was used which had been contaminated with levels of DDT at 100, 200, and 330 p.p.m. and BHC at levels of 100, 200, and 300 p.p.m. Thirty-five birds were used with five birds per treatment group and one control group. Ten grams of dust were applied to each bird in the manner previously indicated; 13 grams used in the previous study appeared to be somewhat in excess of the amount that would be used under commercial conditions. The third experiment was a replication of the second except for slight variation in the DDT levels and the deletion of the 100-p.p.m. level. The 5% malathion dust was contaminated with 190 and 300 p.p.m. of DDT and 200 and 300 p.p.m. of BHC.

The same dusts were used in the fourth experiment as in the third. The 10 birds used for each treatment were floor-housed in small pens with four metal nests per pen. Each dust was applied to the nesting material (peanut hulls) of each nest at the rate of 25 grams per nest. The total amount of 100 grams per pen is approximately the amount recommended for use in a "dusting box" for this number of birds. The hens had normal access to the nests and nesting material.

Feeding Studies. The feed used for the mixing of the contaminated feed, and the eggs from the hens to be on experiment, were analyzed for initial pesticide residue content and found to be free of contamination. The basis for selection of pesticide feeding levels was an attempt to bracket the Florida Department of Agriculture working levels for each pesticide. One-pound samples of feed were contaminated with high levels of each pesticide to be used. Appropriate dilutions from the premixes were calculated and made with basal feed in a 400-pound capacity horizontal feed mixer. There was some variation in the calculated content and the final analyzed pesticide content of the feed. The following levels were determined by analysis to be present in the feed: DDT, 0.98, 0.61, and 0.33 p.p.m.; lindane, 0.26, 0.21, and 0.14 p.p.m.; aldrin, 0.114 and 0.06 p.p.m.; and chlordane, 0.08 and 0.049 p.p.m.

A total of 144 individually caged commercial egg-type hens with a production rate of approximately 85% were used in this study. Each level of insecticide was fed to 12 birds for one week. Two control groups of 12 birds each were used. Eggs were collected daily and each egg was marked with cage number and date. The eggs were analyzed by groups, by individual days.

RESULTS

Dusting Studies. In the first dusting experiment no methoxychlor residues were found in the eggs, and BHC and DDT from only the highest levels (200 p.p.m.) resulted in significant residues. These are shown in Figure 1; on this and the graphs to follow, the working levels used by the Florida Department of Agriculture prior to issuance of the Food and Drug Administration action level guides are shown as the level of unacceptability for each of the insecticides being considered. In all graphs the DDT values represent a combination of DDT and DDE. In both the dusting and feeding studies only purified *p,p'*-DDT was used and no *o,p'*-DDT isomers were present. No DDD was found in the eggs. The values for BHC represent the sum of all the isomers. The greatest amounts of insecticide were found from the seventh to the ninth day for both BHC and DDT. The BHC in the eggs reached a level of 0.036 p.p.m. during this period. The DDT residue in the eggs reached a peak of 0.057 p.p.m., exceeding the working level. At the time the analyses were discontinued residues of both pesticides had tended to plateau.

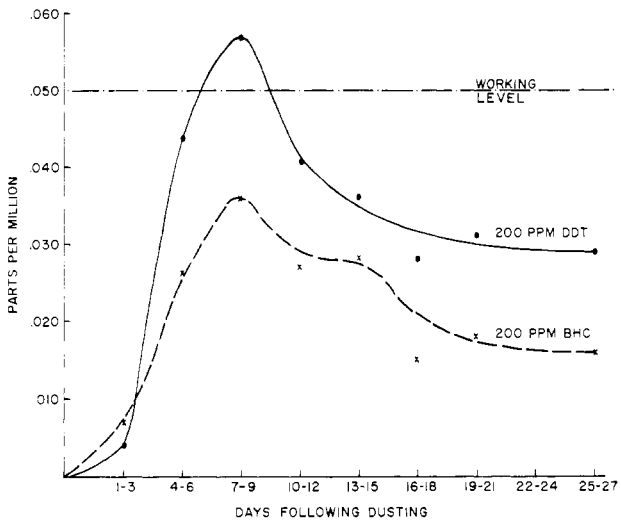

Figure 1. DDT and BHC residues in whole eggs as a result of dusting hens with DDT- and BHC-contaminated safe insecticides (Expt. 1)

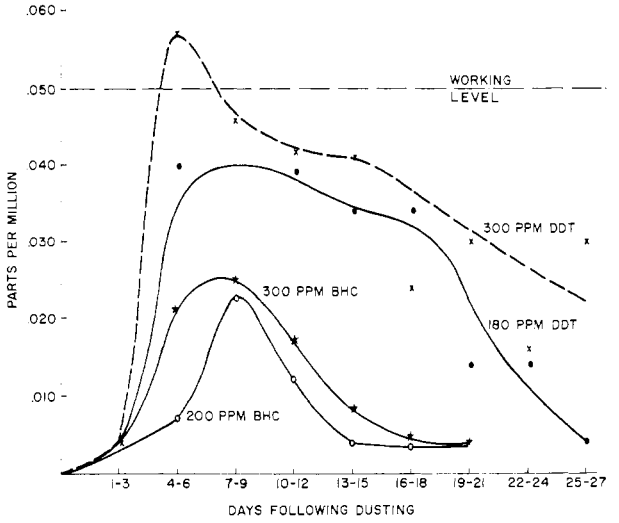

Figure 3. DDT and BHC residues in whole eggs as a result of dusting hens with DDT- and BHC-contaminated safe insecticides (Expt. 3)

DDT resulted in much the same type of response in the second study (Figure 2) when a higher contamination level but smaller amount of dust was used. The residue peaks approached the working level for both levels of contamination but the lower level depleted more rapidly. Analyses were continued until the residues were depleted to trace amounts; the plateau observed in Figure 1 continued for approximately 15 days before more rapid depletion began again.

BHC (Figure 2) reached its highest peak at 7 to 9 days and accumulated to a much lesser extent than DDT. The rates of accumulation and of depletion were not as rapid as in the first experiment and lower maximum levels were reached.

In the third dusting study the 300-p.p.m. level of DDT resulted in an earlier peaking than in previous trials and exceeded the working level. Depletion of both the 200- and the 300-p.p.m. levels appeared to be more gradual than in the previous trials, with less evidence of the plateaus previously noted. The BHC curves were more similar to those of the first study than the second and were well below the working level, again showing a lower BHC deposition in the eggs than of DDT even though dusting levels were the same (Figure 3).

When only the nests were treated with contaminated dusts, no detectable levels of BHC or DDT were found in the eggs examined for a 15-day period following treatment of the nests. The dilution of the dusts in the nesting material and the protection offered by the feathers apparently limited significant contact with the skin and

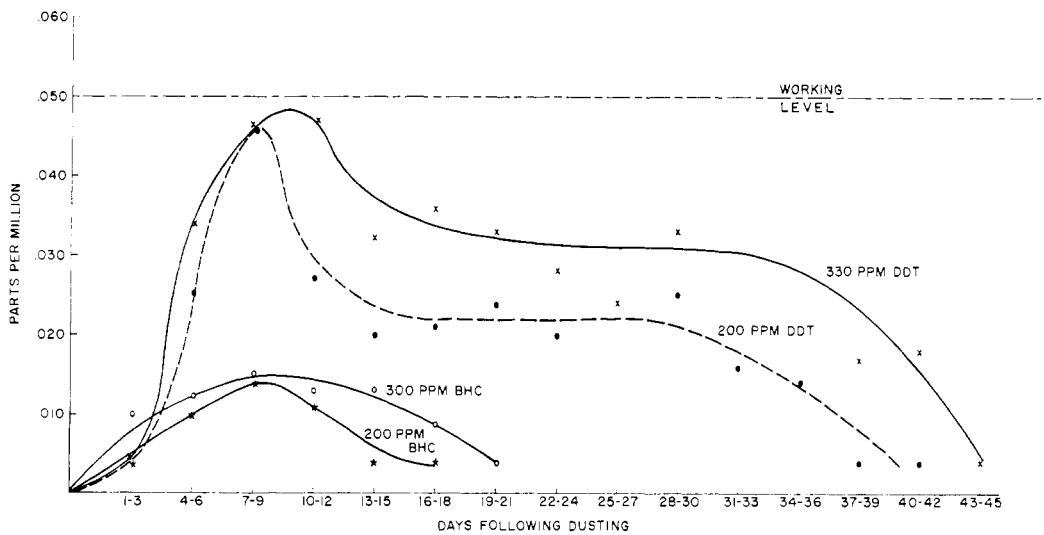

Figure 2. DDT and BHC residues in whole eggs as a result of dusting hens with DDT- and BHC-contaminated safe insecticides (Expt. 2)

subsequent absorption into the body of the bird. Ingestion of the nesting material likewise was apparently not a significant factor.

Feeding Studies. The feeding levels of DDT, aldrin, lindane, and chlordane were selected in an attempt to bracket the working levels for each. Only the feeding level for each insecticide that peaked nearest the working level is graphed and discussed. Chlordane, fed at levels of 0.049 and 0.080 p.p.m. in the feed, resulted in no detectable residues in the eggs. (In a subsequent study, chlordane, at higher levels in the feed, resulted in significant residues in eggs and a very slow depletion rate.)

Aldrin, at 0.114 p.p.m. in the feed, produced dieldrin in the eggs exceeding the working level of 0.01 p.p.m. (Figure 4). The maximum level occurred on the ninth day after feeding began. There was then a short but rapid decline in residue level, followed by a plateau for approximately 10 days, after which decline was again more rapid. The residue levels during depletion were more erratic for dieldrin, as well as for DDT and lindane, than during the time the residues were increasing.

The feeding of 0.26 p.p.m. of lindane produced a maximum level of 0.033 p.p.m. of lindane in the eggs on the eighth day after feeding began (Figure 5). A slight reduction occurred following the eighth day and a plateau was observed until a rapid decline to trace amounts on the 18th day.

The residues of DDT and its metabolite DDE produced a curve (Figure 6) very similar to those found for the dusting studies. At 0.33 p.p.m. in the feed the working level was exceeded on the tenth day after feeding was begun. The plateau was much shorter than that observed for the dusting experiment in which analyses were continued until trace amounts were reached (Figure 2).

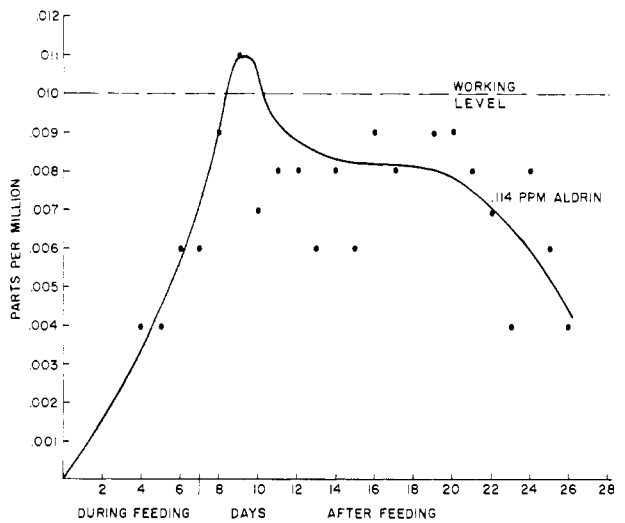

Figure 4. Dieldrin residues in whole eggs during and after feeding 0.114 p.p.m. of aldrin for 7 days

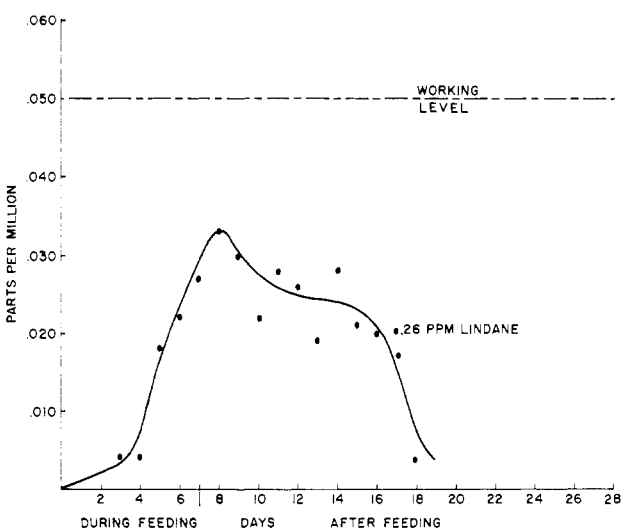

Figure 5. Lindane residues in whole eggs during and after feeding 0.26 p.p.m. of lindane for 7 days

DISCUSSION

The dusting studies of this report point out the necessity of quality control and adequate cleanup procedures in the manufacture and distribution of insecticides that are recommended for use with poultry. There is considerable variation in the degree of absorption of various insecticides by laying hens. The amounts of residues in the eggs are further affected by the amount of the insecticide applied, the level of contamination, and the method of application.

This report has verified the findings of Stadelman *et al.* (1965), Cummings *et al.* (1966), and others that the laying hen is capable of putting into the egg small amounts of chlorinated hydrocarbon insecticides present in the feed. As in the dusting studies, there were variations in the degree of deposition in the egg as related to levels in the feed. The p.p.m. level of DDT in the whole eggs was 18.8% of that in the feed. For lindane

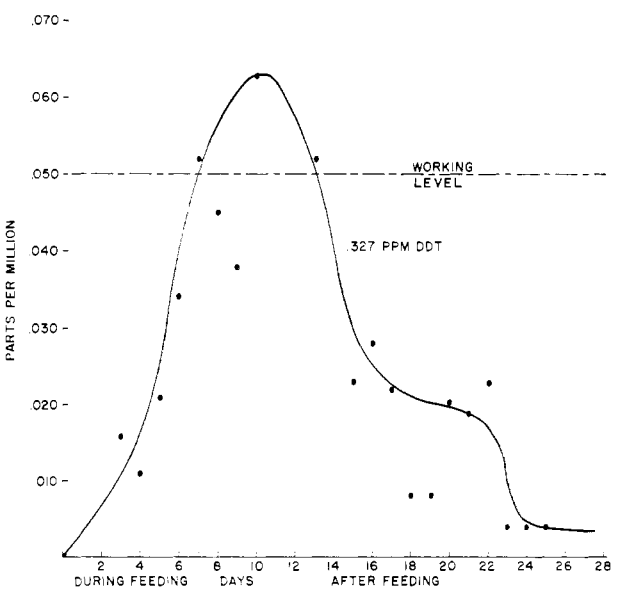

Figure 6. DDT residues in whole eggs during and after feeding 0.33 p.p.m. of DDT for 7 days

and aldrin the conversion was 12.7 and 9.6%, respectively. These percentages may not hold true for higher or lower feeding levels and would certainly be affected by differences in feed intake per day, rate of lay, and composition of the diet.

In the graphs presented for both the dusting and feeding studies the maximum residue levels in the eggs were reached, in most cases, at approximately 9 days after dusting or after feeding began. This is related to the fact that it takes the hen approximately 9 days to produce a mature yolk. The yolk produced on the ninth day after the 7-day feeding period began would have equal distribution of residues throughout the various yolk layers. After the peak is reached a rapid decline is noted, followed by the plateau previously mentioned. These plateaus for the feeding trials were a duration of 7 to 10 days. This indicates that deposition of residues in the yolks that had begun to develop during the feeding period would have greater residue concentrations in the yolk centers than the outside. The outermost layers no doubt have small amounts of residues and thus keep the plateau level relatively constant for a 9- to 10-day period. The DDT plateau is much longer in the dusted birds (Figure 2) than in the feeding studies (Figure 6); this is probably due to a greater deposition of residues in the fat of the bird. Depletion rates are affected by the rate of egg production and the dynamic state of the body fat. If mobilization of fat is made more rapid, depletion rates are increased. This was the case with force-molting laying hens that had high residues of pesticides in the body fat (Wesley *et al.*, 1966).

The criteria for these studies have been the working levels used by the Florida Department of Agriculture prior to issuance of the action level guidelines by the Food and Drug Administration. There is no assurance that these levels set by the Food and Drug Administration will be the same as those that may be set as negligible residue tolerances at a later date. However, studies are under way to determine the amounts of certain chlorinated hydrocarbon insecticides that can be in the feed of laying hens for a 7-day feeding period and not exceed the Food and Drug Administration action level guidelines.

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