Effect on Performance of a Low Level Mixture of Organochlorine Insecticides in Broiler Breeder Feed

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A mixture of hexachlorobenzene, α-, β-, and γ-hexachlorocyclohexane, heptachlor, *p,p'*-DDT, and dieldrin was added, at three different levels up to 1 mg/kg, to broiler breeders feed for 68 weeks with a view to studying performance. Body weight, mortality, egg production percentage, and number of eggs produced per hen were unaffected by the treatment. Egg quality as measured by egg weight, specific gravity of the egg, and egg-shell thickness was equally unaffected. Fertility, hatchability, and neonatal mortality did not show that the treatment of the broiler breeders had any detrimental effect on their offspring.

The possible effect on birds and their performance of organochlorine insecticides applied to poultry or present in their feed has been the subject of several experiments. The situation in this field was reviewed some years ago by Vogt (1972a,b) and by Foster (1974).

More recently attention has focused on the low levels of organochlorine insecticides in poultry feed resulting from their application during growth, storage, etc. These low levels (in this paper arbitrarily put around 100 mg/kg or less) have been shown to be beneficial or harmful to poultry or to have no effect at all. A few examples of each kind of reaction will be given.

Lillie et al. (1972), for instance, found increased body weights due to supplementation of the diet with 50 mg/kg *p,p'*-DDT, *o,p'*-DDT, or *p,p'*-DDE. The same workers (Cecil et al., 1973) found increased shell thickness when 5–50 mg/kg DDT was added to pullets feed. Very recently Vogt et al. (1975) found increased egg-shell stability, when up to 0.5 mg/kg hexachlorobenzene (HCB) was added to the diet of laying hens during 329 days. No effects were found, for instance, with 5 mg/kg aldrin, dieldrin, heptachlor, and lindane (Naber, 1974). A very extensive study with up to 200 mg/kg DDT and 20 mg/kg dieldrin was carried out by Davison and Sell (1972). They found no effect on egg production or egg quality.

Smith et al. (1970) found a lower egg production ratio and decreased shell thickness due to 10 mg/kg DDT. The same effects were found by Sauter and Steele (1972) after feeding up to 10 mg/kg DDT or lindane. They also found decreased reproductive efficiency.

Several explanations are possible for the great discrepancies in this published evidence. Firstly, different strains and lines of hens may differ considerably in their susceptibility toward the addition of chemicals in their diet. Secondly, nutrition (e.g., Ca source and level), housing, management (e.g., artificial vs. natural insemination), and other environmental factors may affect this susceptibility. Thirdly, and perhaps most important of all, owing to biological variability the absence or presence of an effect cannot always be statistically proved as a result of an insufficient number of observations. Some of these points have been tackled by other investigators, but some questions remained which led to the present investigation, for which the circumstances were selected to have a reasonable chance to detect effects which may occur in practical poultry keeping.

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(1) Most, if not all, experiments were carried out with laying type hens. These hens were selected for productivity and egg quality during many generations and may be more resistant to foreign chemicals than broiler breeder hens, which were mainly selected for the growth potential of their offspring. (2) The number of birds in each treatment group was usually so small that effects having serious repercussions in practice when thousands of hens are housed together will not be dectected. (3) Moreover, the financial consequences of any effect on productivity or reproductive performance are much more serious in broiler breeder hens.

An experiment was therefore conducted with broiler breeder hens, starting with 150 hens per treatment group, in which a mixture of several organochlorine insecticides was added to the diet at three different levels for the purpose of studying performance. A fourth group received food which was not artificially contaminated. In this way no effect can be attributed to a single component. One may even have a situation in which symptoms are alleviated by counteraction of one (or more) of the components. However, a mixture of several organochlorine insecticides is frequently encountered in the mixed feed used in the poultry industry. The birds were raised and housed under conditions commonly employed in Holland. Accumulation and excretion of the organochlorine insecticides were determined in the same experiment. These aspects will be discussed in the accompanying paper (Kan and Tuinstra, 1976).

MATERIALS AND METHODS

The experiment, carried out at Spelderholt Institute for Poultry Research, was begun on Jan 4, 1974 with 600 1-day old "hybro compact" broiler breeder hens and 90 1-day old "hybro compact" cocks. The birds were equally distributed over the four treatment groups. The hens were reared in four separate houses up to the age of 18 weeks, when they were transferred to the laying house for the rest of the experiment. At the age of 6 weeks the hens of each experimental group were divided into two subgroups (a division which was continued for the rest of the experiment). At the same time half the space in the poultry houses was fitted with slatted floors, the other half being covered with wood shavings.

At this age the birds were also subjected to a scheme of restricted feeding (more or less according to the suppliers schedule; see Table I). Restricted feeding, besides saving feeding costs during the rearing period, results in better performance during the laying period of heavy breeds and is therefore widely employed. To obtain an insight into the results of restricted feeding, some of the birds were

Table I. Advised Level of Food Consumption per Hen per Day[a]

Age, weeks	kcal M.E.[b]	Age, weeks	kcal M.E.
0–6		18	225
6–7	190	20	235
8	180	21	265
9	190	22	300
10	190	23	355
11	205	23–24	380
12	205	24–34	435
14	210	34–40	425
16	210		

[a] After 9 weeks of age a supplement of 5 g of oats per hen per day is provided. This supplement is raised to 10 g per hen per day at the age of 11 weeks. [b] M.E., metabolizable energy.

Table II. Composition of the Basic Diet during the Laying Period and Calculated Amounts of Dietary Components

Component	%
Ground corn	71.1
Soybean meal (44–47% protein)	13.7
Fishmeal	2.9
Soybean oil	1.0
Alfalfa meal	5.9
Vitamin premix	0.5
Minerals	1.9
Dicalcium phosphate	0.5
Limestone	2.4
Methionine (99% pure)	0.1
	100.0
Calculated amounts of dietary components	
Metabolizable energy (M.E.)	2850 kcal/kg (11.92 MJ/kg)
Protein	15.3%
Lysine	0.75%
Methionine + cystine	0.64%
Phosphorus	0.58%
Calcium	1.80%

weighed at regular intervals during the rearing period. From 6 to 18 weeks the cocks were reared in a separate house with feed restriction. At 54 weeks the cocks were replaced, as the hatching results showed that the fertility rate was too low.

One hen from each experimental sub-group was killed at regular intervals for residue determination in the abdominal fat. Up to the age of 8 weeks artificial light was given to ensure 24 h of daylight. Thereafter artificial light was supplied to give up to 17 h of daylight. During the laying period the hens had free access to individual nests covered with wood shavings. The experiment was terminated at the age of 68 weeks. All birds were fed the same basic diet consisting of ground corn, soybean meal, fishmeal, alfalfa meal, soybean oil, vitamins, and minerals. The percentages of the components in the diet were adapted to the calculated requirements. The composition of the basic diet during the laying period and the calculated amounts of dietary components are given in Table II. The calcium content of 1.80% was assumed to be half the birds' requirement. The rest of the calcium was supplied in the form of broken oyster shells available ad libitum. Organochlorine insecticides were added to the basic diet to obtain the concentrations shown in Table III.

During the laying period records were kept of the number of eggs laid per pen per day. The average production percentage and average number of eggs produced per hen present can thus be calculated by comparison with the number of hens present during this period.

At the age of 31, 52, and 66 weeks, weight, specific gravity, and shell thickness of the eggs were determined. Specific gravity is calculated by comparing weight in air with the weight in water; shell thickness is measured at two points on the waist of the egg. To study possible effects on hatchability hatching eggs were collected three times for a fortnight. These eggs were artificially incubated and the hatchability was determined. After the first hatch some of the chicks were kept for 6 weeks and after the second and third hatch all chicks were kept for a fortnight to study mortality during early life. All birds which died during these experiments (including the main experiment) were autopsied to determine the cause of death. Statistical analyses were made by analysis of variance according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

(a) Body Weight and Mortality. At the age of 6, 8, 10, 12, 14, and 16 weeks half the hens in groups 1 and 4 were weighed to check the restricted feeding scheme. The hens in group 4 were usually heavier than those in group 1 (the control group). The difference was significant ($P < 0.05$) at 8, 14, and 16 weeks. At the age of 18 weeks, when the birds were transferred to the laying house, they were all weighed. The average weights were 1954, 1993, 2028, and 2073 g, respectively, for groups 1–4. As each group contained about 130 hens and the coefficient of variation of body weight was 11%, the differences between groups 1 and 3–4 and between groups 2 and 4 were statistically significant ($P < 0.05$).

At the age of 20, 24, and 35 weeks when the hens were weighed again no differences in body weight were noticed. There is no obvious explanation of the clear dose–response relationship in body weight during the period of severe food restriction up to the age of 18 weeks. Although Lillie et al. (1972) found increased body weights due to DDT, there was no dose–response relationship in their experiment. The disappearance of the differences in body weight after 18 weeks is probably due to the rapid increase in food supply (see Table I), which may have obscured the differences in food conversion ratio (kilograms of feed/kilograms of growth) apparent during the rearing period, owing to the onset of lay during this period. In a similar

Table III. Intended Concentrations (mg/kg) of Organochlorine Insecticides in the Experimental Diets and Maximum Allowable Concentrations (mg/kg) in Dutch Poultry Feed for Laying Hens and Broilers (Regulations of the Commodity Board of Animal Feed)

Insecticide	Group 1	Group 2	Group 3	Group 4	Max allowable concns: Laying hens	Max allowable concns: Broilers
Hexachlorobenzene (HCB)	0	0.01	0.05	0.1	0.025	0.025
α-Hexachlorocyclohexane (α-HCH)	0	0.05	0.25	0.25	0.05 } 0.3 (α-, β-, γ-HCH)	0.08 } 0.5 (α-, β-, γ-HCH)
β-Hexachlorocyclohexane (β-HCH)	0	0.10	0.5	1.0		
γ-Hexachlorocyclohexane (γ-HCH)	0	0.05	0.25	0.5		
Heptachlor	0	0.025	0.125	0.25	0.03	0.03 (incl. epoxide)
p,p′-DDT	0	0.10	0.5	1.0	0.2	0.2 (total DDT)
Dieldrin	0	0.025	0.125	0.25	0.03	0.03 (incl. aldrin)

Table IV. Egg Weight, Specific Gravity, and Shell Thickness

	Egg wt, g	Sp gravity	Shell thickness, mm
31 weeks			
Group 1	58.93	1.085	0.364
Group 2	59.34	1.087	0.366
Group 3	58.40	1.087	0.367
Group 4	58.54	1.087	0.369
52 weeks			
Group 1	67.73	1.080	0.339
Group 2	67.60	1.081	0.337
Group 3	67.44	1.081	0.337
Group 4	67.97	1.081	0.341
66 weeks			
Group 1	68.39	1.078	0.340
Group 2	66.65	1.079	0.341
Group 3	67.81	1.080	0.346
Group 4	68.42	1.079	0.344

experiment with approximately 750 broilers we found no effects on body weight, food consumption, or food conversion ratio (C. A. Kan and L. G. M. Th. Tuinstra, unpublished observations). At the age of 20.5 weeks six hens per treatment group were weighed and slaughtered. After slaughter the liver weight was determined. There were no differences in liver weight or in liver/body weight ratio between treatment groups. The livers of hens in groups 1 and 4 were microscopically examined by Professor Koeman of Wageningen Agricultural University. He found no indication of intoxication by organochlorine insecticides.

The mortality due to diseases was 11, 6, 15, and 15 hens, respectively, for groups 1–4. There were also some missexed chicks, which were removed, and some accidental deaths. A total of 30 hens per group were killed for analysis of organochlorine insecticides in abdominal fat. Neither the number of deaths nor the causes revealed increased mortality owing to the supplementation of the basic diet with increasing concentrations of organochlorine insecticides.

(b) Productivity. The first birds began laying at the age of 21 weeks. A maximum egg production of 75% was reached during weeks 31–33. Afterward there was a gradual decline to about 50% at the age of 58–64 weeks. The total number of eggs laid per hen present throughout the laying period was 187.9, 189.2, 186.2, and 177.8 eggs for groups 1–4, respectively. Although these figures would seem to indicate that the performance of group 4 (with the highest dose of organochlorine insecticides) was inferior to that of the other groups, actually the difference is due to the fact that one of the pens in group 4 gave a bad performance. Birds in the other pen put up the same performance as the other groups. Hence there is no evidence that these levels of organochlorine insecticides have any effect on productivity.

(c) Egg Weight, Specific Gravity, and Shell Thickness. The egg weight, specific gravity of the egg, and egg-shell thickness of some 160 eggs in each treatment group were determined at the age of 31, 52, and 66 weeks. The results are listed in Table IV. The only statistical difference ($P < 0.05$) found was in the specific gravity of the egg at the age of 31 weeks between group 1 and groups 2–4.

(d) Fertility, Hatchability, and Neonatal Mortality. The results of the three hatches are summarized in Table V. The results of the second hatch were greatly influenced by the large (up to 30%) and very variable percentage of unfertilized eggs, which was unrelated to the treatment. The old cocks were therefore replaced and in the third hatch fertility was in the normal range. As there were considerable differences in hatchability between pens within groups no statistical differences could be found between groups. It may therefore be concluded that under the conditions obtained the treatment had no effect on fertility or hatchability. Although there are some differences between groups and sexes, the number of deaths during early life was so small (about 2% during the first 2 weeks of life) that no statistical differences due to treatment of the parents could be observed.

Table V. Fertility and Hatchability

Group	No. of eggs set	No. of unfertilized eggs	No. of chicks hatched	No. of chicks hatched: As % of eggs set	No. of chicks hatched: As % of fertile eggs
1	2727	351	2097	76.9	88.3
2	2770	432	2075	74.9	88.8
3	2697	212	2208	81.9	88.9
4	2367	311	1815	76.7	88.3

(e) General. In contradiction to the findings of Smith et al. (1970) and Sauter and Steele (1972) in the present experiment it was not found that low levels of organochlorine insecticides had any detrimental effect on performance. The same conclusion was reached by Vogt et al. (1975) after feeding up to 0.5 mg/kg HCB. In both experiments the number of observations and birds were large enough to permit detection of possible effects of the order of magnitude found by Smith et al. (1970) and Sauter and Steele (1972). It must therefore be concluded that in these two experiments some unresolved factors (genetics, feed, management, or other environmental—perhaps non-treatment related—factors) were responsible for the effects observed.

Several of these possibilities, e.g., age of the hens and calcium level in the feed, have been investigated by Lillie et al. (1973). Some other factors may be worth investigating such as the practice of artificial insemination twice a week used by Sauter and Steele (1972). They also used commercially wettable powders to disperse the insecticides in the feed. The additives contained in these powders may have been a further factor. In conclusion, organochlorine insecticides in concentrations which are (at least partly) allowed (see Table III) and encountered in Dutch poultry feeds could not be shown to have any detrimental effect on the performance of broiler breeders under the conditions of this experiment.

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Supplementary Material Available: A listing of sub-group figures on egg production, egg shell quality, egg weight, and hatchability (4 pages). Ordering information is given on any current masthead page.

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Accumulation and Excretion of Certain Organochlorine Insecticides in Broiler Breeder Hens

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The accumulation of hexachlorobenzene, α-, β-, and γ-hexachlorocyclohexane, heptachlor, *p,p'*-DDT, and dieldrin in abdominal fat and eggs was determined in an experiment with broiler breeder type laying hens. The accumulation ratios (level in fat or egg/level in the feed) in fat on a fat basis were 17, 1.8, 18, 1.8, 6, 14, and 14, respectively. The values in eggs on a whole egg basis were 1.3, 0.10, 1.5, 0.13, 0.5, 1.2, and 1.3, respectively. Calculations were made on excretion of the total daily intake via eggs and feces. The values ranged from 25 to 35% for HCB, β-HCH, DDT, and dieldrin, to 10% for heptachlor, to 2–3% for α- and γ-HCH via the eggs. The excretion via feces was 5–10% for HCB, β-HCH, DDT, and dieldrin, 2% for heptachlor, and for α- and γ-HCH ±0.5% of the daily intake.

Following the discovery that certain organochlorine insecticides can accumulate in the fatty tissues of several animal species (including man), Cummings et al. (1966, 1967) were the first to demonstrate that after prolonged feeding of the same concentration a relatively constant level is reached in the concentration of organochlorine insecticides in the eggs and fat of laying hens. The phenomenon of a constant level after prolonged administration shows that an equilibrium must have been reached between uptake and excretion. This constant level enables us to calculate the ratio of the concentration in the animal fat or product to the concentration in the feed. This accumulation (or storage) ratio is apparently independent of the concentration in the feed over a fairly wide concentration range. Several experiments have been carried out from which accumulation ratios have been or can be calculated. These figures are summarized in Table I. The most extensive experiments were those of Cummings et al. (1966, 1967), who used lindane, dieldrin, heptachlor epoxide, DDT, and endrin, and Waldron and Naber (1974), who used lindane, dieldrin, heptachlor, DDT, methoxychlor, and aldrin. Most accumulation ratios are of the same order of magnitude, more or less depending on the type of bird and the egg production ratio.

Very high accumulation ratios in fat and eggs for hexachlorobenzene (HCB) were found by Avrahami and Steele (1972). Very recently Combs and Brewer (1975) found very high accumulation ratios in eggs for dieldrin, heptachlor, and DDT. As Cecil et al. (1973) have shown that the egg production ratio has a marked effect on accumulation ratios of DDT in fat and eggs, we were interested in ascertaining whether broiler breeders might have higher accumulation ratios. These birds have a lower egg production than the laying type hens generally used in accumulation experiments. An experimental design was therefore set up in which four experimental groups were placed. In addition to a control group, which was given no added organochlorine insecticides in the diet, we formed three treatment groups, which were given increasing amounts of a mixture of organochlorine insecticides added to the diet. The intended concentrations, together with the analytical values in parentheses, are shown in Table II. A preliminary account of a part of this work has already been given (Kan and Tuinstra, 1975). The possible effects on viability, egg production, shell quality, and hatchability are discussed in an accompanying paper (Kan and Tuinstra, 1976).

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MATERIALS AND METHODS

Animal Experiment. The animal experiment was carried out at Spelderholt Institute for Poultry Research and the residue determinations were conducted at the Government Dairy Station. Details on housing, rearing, and feeding of the birds are given in the accompanying paper (Kan and Tuinstra, 1976). All groups were fed the same basic diet to which organochlorine insecticides were added in concentrations as specified in Table II. The components of the basic diet had been previously checked for pesticide residues. Standard solutions of the insecticides in acetone were prepared in advance for 600-kg batches. The standard solution for each batch was thoroughly mixed with 3 l. of soybean oil. This oil was then blended to a premix with 50 kg of ground corn and the premix added to the rest of the diet to form a 600-kg batch. In all 13 batches were prepared for each group. All batches were checked at least twice for their content of the respective organochlorine insecticides. The results of these determinations are shown in Table II. The oats and broken oyster shells used as dietary supplements as well as the woodshavings used to cover the floor and nests were also checked for their residue content. Two hens from each experimental group were killed for residue determination in the abdominal fat at the age of 2, 4, 6, 8, 14, 22, 26, 30, 40, 50, 60, 64, and 68 weeks, respectively. The averages during the period 26–68 weeks are shown in Table III.