Richmond for help in tissue analyses.

Supplementary Material Available: Complete tabulation of each excreted product separately for each labeled preparation and for urine and feces at 0–2, 2–5, 5–12 (or 13), and 0–12 (or 13) days after initiating the treatment schedule (3 pages). Table III of this paper is derived from this supplementary material. Ordering information is given on any current masthead page.

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Possible Influence of Sex and Embryonic Content on Accumulation of Some Organochlorine Pesticides in Broilers

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Low levels of hexachlorobenzene (HCB), α-, β-, and γ-hexachlorocyclohexane (HCH), heptachlor, p,p′-DDT, and dieldrin have been administered via the feed to broilers for 6 weeks. The treatments did not influence growth rate or feed conversion. The mean accumulation ratios (concentration of pesticide in the fat to its concentration in the diet) found were: HCB = 11, α-HCH = 3, β-HCH = 14, γ-HCH = 2, heptachlor(epoxide) = 5, DDT (total) = 10, dieldrin = 11. Male broilers had significantly higher accumulation ratios than females. Organochlorine pesticides carried over from parents through the egg did not influence the accumulation ratios. At the end of rearing (6 weeks), loss in feces as a proportion of daily intake was: HCB = 12%, α-HCH = 5%, β-HCH = 12%, γ-HCH = 4%, heptachlor (epoxide) = 1.5%, DDT (total) = 7%, and dieldrin = 14%.

The accumulation of organochlorine pesticides in broilers was studied rather extensively by de Vos et al. (1972) and by Onley et al. (1975). In these studies no attention was paid to possible sex differences in accumulation. However, recently Siegel et al. (1976) reported a difference in accumulation pattern of dieldrin between male and female broilers.

As a continuation of a trial on accumulation of broiler breeders (Kan and Tuinstra, 1976a,b), we investigated whether or not organochlorine pesticides transferred to the embryo would alter accumulation during rearing. A large amount in the embryo could alter the capacity of the drug-metabolizing enzymes in the liver and thus the accumulation rate.

In the published work, few broilers were used, so that effects of low levels of organochlorine pesticides on growth, feed conversion, and mortality were not demonstrated. An effect on feed conversion was detected during rearing of our broiler breeders (Kan and Tuinstra, 1976a).

We therefore set up a trial with over 1000 broilers hatched from broiler breeders of the control group and that group which received the highest dosage of organochlorine pesticides (Kan and Tuinstra, 1976b). The pesticides and concentrations used there were identical with those in the present experiment (Table I). We studied accumulation ratios (concentration of pesticide in tissue to its concentration in the diet) and possible influences of sex and amount of pesticides present in the embryo on these ratios.

Spelderholt Institute for Poultry Research, Ministry of Agriculture and Fisheries, Beekbergen, Netherlands (C.A.K., J.C.J.D.R.) and Government Dairy Station, Leyden, Netherlands (L.G.M.T.T., A.H.R., W.T.).

In the same trial growth feed conversion and mortality were recorded. Preliminary results have already been published (Kan and Tuinstra, 1975).

The occurrence of interactions during residue formation was not investigated, as de Vos et al. (1972) did not find these interactions in their broiler study.

MATERIALS AND METHODS

Animal Experiment. The trial was factorial with three factors: four treatments (Table I), two parental treatments, and two sexes. There were four replicate cages in each of the 16 (4 × 2 × 2) experimental groups.

Hatching eggs were collected from the broiler breeders (Kan and Tuinstra, 1976a) for 14 days in August 1974. Of 1600 chickens hatched, 256 male and 256 female day-old chicks from the two parent groups I and IV [control and highest dosed group, respectively, identical with this experiment (Table I)], were taken at random. The 1024 chicks were distributed over 64 cages in groups of 16 chicks/cage. Over the 6 weeks of the trial, average room temperature was lowered from 30 °C during the first week to 20 °C in the last 2 weeks. The broilers were kept in continuous artificial light, and relative humidity was maintained at about 60%. They were all given the basic diet specified in Table I, at 600 kg per treatment. The ingredients were checked for residues and were mixed with organochlorine pesticides as described previously (Kan and Tuinstra, 1976b) to obtain the contents as specified in Table I.

Measurements. Unincubated eggs and hatched chicks were sampled for residue analyses.

For each feeding group, residues in the feed were analyzed in four samples.

At the age of 3 and 6 weeks, each broiler was weighed.

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Table I. Intended Concentrations of Organochlorine Pesticides in Experimental Diets in Milligram/Kilogram (Determined Concentrations of Pesticides Are in Parentheses, Mean of Four Samples ± Standard Deviation)[a]

	1		2		3		4	
Hexachlorobenzene (HCB)	0	(<0.005)	0.010	(0.010 ± 0.0015)	0.05	(0.046 ± 0.002)	0.10	(0.083 ± 0.009)
α-Hexachlorocyclohexane (α-HCH)	0	(<0.01)	0.050	(0.034 ± 0.007)	0.25	(0.21 ± 0.042)	0.50	(0.37 ± 0.033)
β-Hexachlorocyclohexane (β-HCH)	0	(<0.01)	0.100	(0.093 ± 0.017)	0.50	(0.50 ± 0.024)	1.00	(0.93 ± 0.12)
γ-Hexachlorocyclohexane (γ-HCH)	0	(<0.01)	0.050	(0.038 ± 0.007)	0.25	(0.21 ± 0.030)	0.50	(0.37 ± 0.029)
Heptachlor	0	(<0.01)	0.025	(0.022 ± 0.002)	0.125	(0.11 ± 0.006)	0.25	(0.20 ± 0.026)
p,p'-DDT	0	(<0.04)	0.100	(0.079 ± 0.010)	0.50	(0.44 ± 0.024)	1.00	(0.87 ± 0.170)
Dieldrin	0	(<0.01)	0.025	(0.024 ± 0.004)	0.125	(0.11 ± 0.010)	0.25	(0.21 ± 0.030)

[a] Composition of the basic diet and calculated amounts of dietary components: ground corn 58.5%, soybean meal (>48% protein) 30.0%, fishmeal 3.5%, soybean oil 4.2%, vitamin premix 0.5%, minerals 2.0%, dicalcium phosphate 0.9%, calcium propionate 0.3%, methionine (99% pure) 0.1%. Metabolizable energy (M.E.) 3140 kcal/kg (13.13 MJ/kg), protein 22.1%, lysine 1.22%, methionine + cystine 0.85%.

In that interval feed consumption per cage was estimated.

At 2 and 4 weeks, two broilers per cage were taken at random and the abdominal fat was analyzed as a mixed sample per cage.

At 6 weeks, the remaining broilers in each cage were divided at random into two groups. The abdominal fat of each group was analyzed as a mixed sample.

During week 6 feces were collected from all cages for 24 h. The residues in dried feces (70 °C) were estimated in mixed samples per treatment group.

The broilers and feed were weighed at Spelderholt Institute for Poultry Research.

Residues in unincubated eggs, day-old chicks, feed, and feces samples were estimated at the Government Dairy Station, which also analyzed abdominal fat from 16 cages (one cage per treatment) after 4 weeks of treatment. All mixed samples of abdominal fat from half the broilers per cage of group 4 and half the cages of groups 1, 2, and 3 were also analyzed there. The rest of the estimates of residues was carried out at Spelderholt Institute for Poultry Research.

Determinations of Organochlorine Pesticides. Extraction and cleanup with an aluminum oxide column were by the method of Greve and Grevenstuk (1975).

Gas Chromatography. The Government Dairy Station used as a Tracor MT 220 and a Varian 1740 Model Gaschromatograph with ^{63}Ni and ^{3}H electron-capture detectors. Spelderholt Institute for Poultry Research used a Packard-Becker 419 Model Gaschromatograph with ^{63}Ni electron-capture detectors.

Two stationary phases were used: The first was a 3% OV-17 + OV-210 (mixed 1:4) on Gas Chrom Q (100–120 mesh). With an argon–methane (90:10) flow of 60 mL/min the retention time of p,p'-DDE was 8.1 min. The elution order and relative retention times (p,p'-DDE = 1.00) were on this column: HCB (0.20), α-HCH (0.24), γ-HCH (0.31), β-HCH (0.36), heptachlor (0.36), β-heptachlor epoxide (0.75), p,p'-DDE (1.00), dieldrin (1.18), o,p'-DDT (1.42), p,p'-TDE (1.64), p,p'-DDT (1.91). The second phase was 3% DEGS + 1% H_3PO_4 on Gas Chrom Q (100–120 mesh). With a nitrogen flow of 35 mL/min the retention time of p,p'-DDE was 3.7 min. The elution order and relative retention times (p,p'-DDE = 1.00) were in this case: HCB (0.11), heptachlor (0.26), α-HCH (0.31) γ-HCH (0.50), β-heptachlor epoxide (0.74), p,p'-DDE (1.00), dieldrin (1.14), o,p'-DDT (1.40), β-HCH (1.64), p,p'-DDT (2.64), p,p'-TDE (3.22).

Statistical Procedures. Growth, feed conversion, and feed consumption were examined by variance analysis (Snedecor and Cochran, 1967).

Accumulation ratios (concentration of pesticide in fat to its concentration in the diet), based on feed analyses by the Government Dairy Station, were treated as a factorial design. Group 1, receiving uncontaminated feed, was not included in the analysis for accumulation ratios. The following statistical tests, assuming fixed variables, were made by analysis of variance for each pesticide: (1) results of the Government Dairy Station after 6 weeks with three factors: three treatments, two parentages, and two sexes; (2) results of the government Dairy Station and Spelderholt comparing only those cages which had been analyzed by both institutes with four factors: three treatments, two parentages, two sexes, and two laboratories.

RESULTS AND DISCUSSION

Unincubated Eggs and Hatched Chicks. Differences in residues between unincubated eggs and day-old chicks indicated that α-HCH had almost disappeared (probably metabolized) during embryonic development. Only 20% of γ-HCH and 35% of β-heptachlor epoxide found in unincubated eggs was recovered in the day-old chicks. A decrease in the tissue level in the other substances (HCB, β-HCH, DDT, and dieldrin) could not be reliably detected because of large variations between samples of different groups. Recently, Driver et al. (1976) published similar figures but ascribed the decrease to dilution in the fat. As the day-old chick has a fat content of 4–5% compared to 10–11% in the unincubated eggs, this explanation is unlikely. In our opinion metabolism by the embryo, probably the liver, is more likely. Embryonic metabolism of p,p'-DDE was demonstrated by Abou-Donia and Menzel (1968).

Residues in Feed and Feed Ingredients. Residues in ingredients were not detected or were negligible. Residues in the experimental diets (Table I) were unaccountably almost invariably lower than predicted.

Growth Rate, Feed Consumption, Feed Conversion, and Causes of Death and Culling. Growth rate, feed consumption, and feed conversion were not influenced by treatment. Parentage had no effect either.

Cocks consumed 2680 g of feed over the 6-week period and reached a body weight of about 1550 g (feed conversion = 1.79). Hens consumed 2305 g of feed and reached a bodyweight about 1300 g (feed conversion = 1.90). The predictable differences in these parameters between sexes were not statistically analyzed.

On average, 9.8% of the broilers were removed during the 6 weeks. The main causes were skeletal disorders (mainly slipped tendon) frequently observed in broilers (Haye and Simons, 1978). Cocks were more affected than hens, but once again, predictable sex differences were not statistically analyzed. No differences in mortality or causes of mortality could be attributed to treatment or parentage.

Residues after 2, 4, and 6 Weeks. Residues in abdominal fat after 2, 4, and 6 weeks indicated that the chickens from parentage IV in groups 1 and 2 (control and lowest treatment group) had much larger residues of the

Table II. Residues in Feces (70 °C) in Milligram/Kilogram and Calculated Proportions of Daily Intake Lost in Feces

Group	HCB	α-HCH	β-HCH	γ-HCH	HEPO[a]	p,p′-DDE	p,p′-TDE	p,p′-DDT	DDT (total)	Dieldrin
1	<0.005	<0.005	<0.010	<0.005	<0.005	<0.010	<0.010	<0.010	<0.04	<0.010
2	0.005	0.013	0.043	0.008	<0.005	<0.010	0.024	<0.010	<0.06	0.013
3	0.022	0.046	0.23	0.024	<0.005	0.010	0.090	0.026	0.13	0.065
4	0.042	0.085	0.50	0.064	0.012	0.17	0.18	0.040	0.24	0.11
Loss %	12	5	12	4	±1.5				7	14

[a] HEPO = heptachlor → β-heptachlor epoxide.

Table III. Accumulation Ratios

		HCB[a]	α-HCH	β-HCH	γ-HCH	Heptachlor (epoxide)	DDT (total)	Dieldrin
Treatment	2	10.2^{a}	2.5^{a}	14.9^{a}	1.9^{a}	4.2^{a}	10.4^{a}	9.7^{a}
	3	9.6^{a}	2.4^{a}	11.5^{b}	2.1^{a}	3.6^{a}	8.1^{b}	9.3^{a}
	4	14.0^{b}	3.8^{b}	16.1^{a}	2.8^{b}	6.1^{b}	10.3^{a}	12.7^{b}
Parentage	I	11.1^{c}	2.9^{c}	13.8^{c}	2.2^{c}	4.4^{c}	9.5^{c}	10.4^{c}
	IV	11.4^{c}	2.9^{c}	14.3^{c}	2.3^{c}	4.7^{c}	9.8^{c}	10.7^{c}
Sex	♂	12.6^{d}	3.2^{d}	15.8^{d}	2.5^{d}	4.8^{d}	10.0^{d}	11.5^{d}
	♀	10.0^{e}	2.5^{e}	12.4^{e}	2.1^{e}	4.3^{e}	9.3^{d}	9.6^{e}

	HCB[b]		α-HCH		β-HCH		γ-HCH		HEPO		DDT		Dieldrin	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
I	13.1^{ap}	9.1^{br}	3.4^{ap}	2.3^{br}	16.3^{ap}	11.3^{br}	2.6^{ap}	1.8^{br}	5.1^{ap}	3.7^{br}	10.7^{ap}	8.3^{bs}	12.2^{ap}	8.7^{br}
IV	12.0^{cp}	10.9^{cs}	3.1^{cp}	2.7^{cr}	15.3^{cp}	13.6^{cr}	2.4^{cp}	2.3^{cs}	4.5^{cp}	4.9^{cs}	9.3^{cr}	10.2^{ct}	10.9^{cp}	10.4^{cs}

[a] Within each pesticide, and main effect, figures in the same column with a common letter do not differ significantly ($P > 0.05$). [b] Within each pesticide, figures in the same column or the same row with a common letter do not differ significantly ($P > 0.05$).

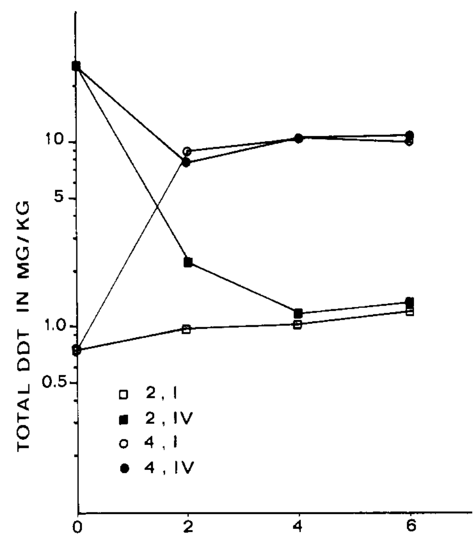

Figure 1. Residues of DDT (total) in abdominal fat of broilers from treatments 2 and 4 and parentages I and IV after 2, 4, and 6 weeks on fat basis in milligram/kilogram.

persistent substances like DDT, especially after 2 weeks. This effect is not present for less persistent substances like lindane. The effect of parentage was masked by the dosage given to group 3 and 4. Residues of lindane and DDT in groups 2 and 4 are as an example given in Figures 1 and 2. Plateauing of residues seems to have been reached after 2 weeks.

Losses with Feces. Residues in feces, sampled for 24 h at the end of the trial, are given in Table II. Assuming an average intake of 100 g a day and an average output of 25 g of fecal dry matter per day, the proportion daily lost in feces could be calculated. Retention of the substances was not complete as was suggested for HCB in broilers by Reed et al. (1977).

The proportion of HCB, β-HCH, DDT, and dieldrin lost were in line with those found in broiler breeders (Kan and

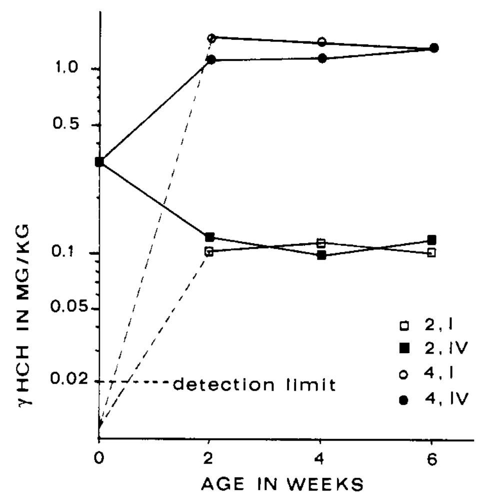

Figure 2. Residues of γ-HCH (lindane) in abdominal fat of broilers from treatment 2 and 4 and parentages I and IV after 2, 4, and 6 weeks on fat basis in milligram/kilogram.

Tuinstra, 1976b; Kan and Jonker-den Rooyen, 1978). Remarkable are the higher proportions for α-HCH and γ-HCH and the lower figure for heptachlor epoxide than in the earlier trial. The proportion of heptachlor epoxide lost by broiler breeders was always twice that figure for α-HCH and γ-HCH.

Accumulation Ratios. Statistical evaluation of the accumulation ratios from those cages analyzed by the Government Dairy Station indicated that the treatment had a highly significant effect ($P < 0.01$) on accumulation ratios for all pesticides. The difference is mainly due to the higher accumulation ratio in group 4 than in groups 2 and 3. Only groups 2 and 3 differed for β-HCH and DDT (Table III). These differences are inexplicable.

For all pesticides, parentage had no significant effect on accumulation ratios ($P > 0.25$), and females had significantly lower accumulation ratios ($P < 0.05$) than males. This effect is due mainly to the females from parentage

Table IV. Accumulation Ratios (Content in Fat to Content in Feed)

	Literature values (see Supplementary Material)	Experimental
Hexachlorobenzene (HCB)	11–30	11
α-Hexachlorocyclohexane (α-HCH)		3
β-Hexachlorocyclohexane (β-HCH)		14
γ-Hexachlorocyclohexane (γ-HCH)	2–4.5	2
Heptachlor → β-heptachlor epoxide	8–40	5
Heptachlor epoxide	13–20	
p,p′-DDT → p,p′-DDE + p,p′-DDT	6–30	10
Dieldrin	10–70	11
Aldrin	14	
Endrin	7–10	

I (Table III). This difference between males and females is probably due to a dilution effect of the higher fat content, which is usually found in female broilers. The average accumulation ratios from the trial and from the literature are given in Table IV. Full literature data are given in the Supplementary Material (see Supplementary Material Available paragraph).

The statistical comparison of the data from the Government Dairy Station and Spelderholt revealed a highly significant ($P < 0.01$) laboratory × treatment interaction for all pesticides. As all accumulation ratios from Spelderholt were calculated from data on residues in feed from Government Dairy Station we cannot draw any reliable conclusions from the Spelderholt data. Spelderholt data on all cages after 6 weeks and on cages after 2, 4, and 6 weeks were therefore not assessed statistically. Conclusions were therefore only based on the data from the Government Dairy Station.

Accumulation ratios found in broilers were somewhat lower than those in broiler breeders reported previously (Kan and Tuinstra, 1976b; Kan and Jonker-den Rooyen, 1978). However, Liska et al. (1964) found larger residues of DDT in broilers than in laying hybrids. They ascribed this phenomenom to the rapid growth rate of broilers and the high feed consumption in relation to body weight. Their results are rather dubious, because they did not demonstrate that a plateau in residues had been reached. As Cummings et al. (1966, 1967) and our trial (Kan and Jonker-den Rooyen, unpublished results) have shown that it takes 5–6 weeks before plateauing is reached in laying hybrids, but only 2–4 weeks in broilers (de Vos et al., 1972), the difference might be explained as follows. The measurements by Liska et al. (1964) in broilers were made after reaching a plateau, whereas the residues in the laying hybrids were still accumulating.

Comparing each compound separately, we conclude that our data on HCB on male and female broilers together agree rather well with those on male broilers by de Vos et al. (1972) and Reed et al. (1977).

For α-HCH and β-HCH no literature data exist. Whereas β-HCH had a lower accumulation ratio in broilers than in broiler breeders α-HCH had a higher accumulation ratio in broilers (Kan and Tuinstra, 1976; Kan and Jonker-den Rooyen, 1978).

The value for γ-HCH is in line with the literature, but for heptachlor the data conflict. The value given by Putnam et al. (1974) was much higher. They may have used, however, heptachlor epoxide, which would account for part of the difference. For DDT the value from Putnam et al. (1974) is again much higher than ours and the data of Onley et al. (1975) and de Vos et al. (1972).

The difference between our data and those of Putnam et al. (1974) are largest for dieldrin (11 and 70, respectively). One explanation might be that, at the very low levels they used, the rule that accumulation is independent of concentration used does not hold. The very low levels in the feed, which were used in that study would, however, be very difficult to determine accurately. Another explanation could be that their broilers were housed on the floor, whose contamination level also influenced the residues found. Recently, Siegel et al. (1976) reported a difference in accumulation of dieldrin between broilers kept on the floor and those in cages. The effect was, however, probably mainly due to somewhat larger amounts of dieldrin in the feed during the floor trial and not to the management system. They also found a pronounced sex difference in accumulation of dieldrin, which was similar to that found in our trial.

In conclusion, accumulation ratios found are in good agreement with literature data. Female broilers have lower ratios than males, but embryonic contents of pesticides did not influence accumulation.

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Supplementary Material Available: A listing of published accumulation ratios, residues in unincubated eggs, day-old chicks, residues after 2, 4, and 6 weeks (6 pages). Ordering information is given on any current masthead page.

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