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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.

Table I. Accumulation Ratios for Laying Hens (Concentration in Fat or Egg/Concentration in the Feed)

	Fat (on fat basis)		Eggs (on whole egg basis)	
	Lit. values	Present expt	Lit. values	Present expt
Hexachlorobenzene (HCB)	20–30	17	1–5.5	1.3
α-Hexachlorocyclohexane (α-HCH)		1.8		0.10
β-Hexachlorocyclohexane (β-HCH)		18		1.5
γ-Hexachlorocyclohexane (γ-HCH, lindane)	0.7–3	1.8	0.2–0.5	0.13
Heptachlor → β-heptachlor epoxide (Hepo)	3–5	6	0.5–2.9	0.5
Heptachlor epoxide	11		1.0	
p,p'-DDT → *p,p'*-DDT + *p,p'*-DDE	4–17	14	0.3–1.6	1.2
Dieldrin	8–14	14	0.7–2.5	1.3
Aldrin → dieldrin	11–12		1.2–1.9	
Endrin	9		0.6	

Table II. Intended Concentrations of Organochlorine Insecticides in Experimental Diets (Determined Concentrations of Insecticides Are in Parentheses, Mean of 13 Batches)

	mg/kg for group			
	1	2	3	4
Hexachlorobenzene (HCB)	0 (<0.005)	0.01 (0.010)	0.05 (0.042)	0.1 (0.082)
α-Hexachlorocyclohexane (α-HCH)	0 (<0.01)	0.05 (0.040)	0.25 (0.203)	0.5 (0.393)
β-Hexachlorocyclohexane (β-HCH)	0 (<0.01)	0.10 (0.094)	0.5 (0.435)	1.0 (0.882)
γ-Hexachlorocyclohexane (γ-HCH)	0 (<0.01)	0.05 (0.046)	0.25 (0.211)	0.5 (0.407)
Heptachlor	0 (<0.01)	0.025 (0.025)	0.125 (0.112)	0.25 (0.221)
p,p'-DDT	0 (<0.02)	0.10 (0.104)	0.5 (0.457)	1.0 (0.868)
Dieldrin	0 (<0.01)	0.025 (0.025)	0.125 (0.114)	0.25 (0.217)

Table III. Concentrations of Organochlorine Insecticides in Broiler Breeders Abdominal Fat in mg/kg on Fat Basis (See Text)

Group	HCB	α-HCH	β-HCH	γ-HCH	HEPO[a]	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT	Total DDT	Dieldrin
1	0.13	<0.02	<0.05	<0.02	<0.05	0.2	*b*	0.2	0.4	<0.05
2	0.29	0.075	1.71	0.088	0.15	0.66	*b*	0.93	1.59	0.34
3	0.84	0.36	8.98	0.36	0.71	2.70	*b*	4.17	6.87	1.70
4	1.57	0.70	14.2	0.71	1.36	4.64	*b*	7.27	11.9	3.19

[a] HEPO = β-heptachlor epoxide. [b] *p,p'*-TDE values in the fat were not quantitatively determined (see text).

Table IV. Concentrations of Organochlorine Insecticides in Eggs in mg/kg on a Whole Egg Basis (See Text)

Group	HCB	α-HCH	β-HCH	γ-HCH	HEPO[a]	*p,p'*-DDE	*p,p'*-TDE	*p,p'*-DDT	Total DDT	Dieldrin
1	0.010	<0.001	<0.005	<0.001	<0.005	0.015	*b*	0.015	0.03	<0.005
2	0.020	0.004	0.14	0.006	0.011	0.049	*b*	0.073	0.12	0.028
3	0.074	0.020	0.66	0.027	0.055	0.22	*b*	0.33	0.54	0.15
4	0.14	0.040	1.27	0.056	0.11	0.40	*b*	0.64	1.03	0.29

[a] HEPO = β-heptachlor epoxide. [b] *p,p'*-TDE values in the eggs were not quantitatively determined (see text).

Mixed samples of 20 eggs per group laid at the age of 24, 26, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, and 68 weeks were also analyzed for residues of organochlorine insecticides. The averages during the period 28–68 weeks are shown in Table IV. Feces were collected under the slatted floors during 1 day at the age of 40 and 68 weeks.

Determination of Organochlorine Insecticides. *Extraction and Cleanup of Pesticides from Animal Feedstuff, Fat, Eggs, Straw, Wood Shavings, Etc.* Extraction and cleanup using aluminum oxide were performed according to Greve and Grevenstuk (1975).

Extraction and cleanup of pesticides from feces were performed according to Zweig (1963).

Gas Chromatographic Determination. Use was made of a Tracor MT 220 and a Varian 1740 Model gas chromatograph using ^{63}Ni and ^{3}H electron capture detectors. The stationary phase employed was a mixture of OV 210 and OV 17 and a DEGS–H_3PO_4 mixture.

RESULTS AND DISCUSSION

(a) Residues in Food Constituents and Concentrations in the Feed. The residues in food constituents were usually impossible to detect or negligible. The only serious difficulty occurred in the vitamin premix. Owing to interfering substances not removed during the cleanup the presence of HCB or α-HCH could not be detected. But as we had a control feed to which no organochlorine insecticides had been added, we were able to determine the concentration in the experimental diets by correcting for interfering peaks. As only 0.5% of this vitamin premix was used in the feed, the corrections were generally small. It can be seen from Table II, where the analytical figures are given in parentheses, that the concentrations of the organochlorine insecticides were almost invariably lower than they should have been in theory. Recovery from the cleanup was ≥95%, except for β-HCH for which it was ≥90%. Despite several attempts, we were unable to reduce the differences or to establish the reason for them. To our knowledge there is no published evidence on similar problems.

(b) Residues in Broken Oyster Shells, Oats, Wood Shavings, Etc. The only very serious difficulty encountered with these supplementary materials was residues of HCB (up to 1.5 mg/kg) in several batches of wood shavings. A similar figure for HCB residues in wood shavings has been published by Gee et al. (1974). These residues probably originate from the use of pentachlorophenol as wood preservative, HCB being one of the possible starting materials in the synthesis of pentachlorophenol.

(c) Residues in Abdominal Fat. The residues in abdominal fat usually went on increasing throughout the rearing period up to the age of 22 weeks at the onset of lay. This continuous increase, instead of the leveling off which might have been expected after prolonged administration, is probably due to the restricted feeding scheme to which the birds were subjected. This could have prevented the balance between uptake and excretion which is needed for a constant level in the fat. From the age of 26 to 68 weeks the residues in abdominal fat were more or less constant. The differences between birds of the same group killed at the same age were often quite considerable (up to 50%). The highest residues were found, of course, in birds fed the highest concentrations. Heptachlor was transformed in the body to heptachlor epoxide. Furthermore, *p,p'*-TDE, which was present in minor quantities, is not quantitatively determined because thermal decomposition of *p,p'*-DDT to *p,p'*-TDE in the injection system is the major reason for appearance of the *p,p'*-TDE peak in the chromatogram. Two observations require further explanation, viz. (1) the occurrence of HCB in the abdominal fat of all groups after transfer to the laying house and its continuous decrease thereafter. Contrary to expectation the wood shavings used in the laying house contained considerable amounts of HCB, as indicated in the previous section. It was evident from the residues in the abdominal fat that the birds have consumed these contaminated wood shavings, probably as a result of the restricted feeding scheme. After the discovery of the HCB in the wood shavings, these contaminated batches were removed. We then noticed a gradual decline in the HCB content in the fat of all groups. This interference makes it very difficult to calculate accumulation ratios for HCB. This problem will be discussed below. The gradual decline enables the calculation of half-life time of HCB in abdominal fat. Assuming that the residues should return to zero, this half-life time is 9 weeks. Considering the possibility of a "natural" background concentration, this half-life time shortens to 5 weeks, when assuming a background of 0.1 mg/kg HCB in the fat.

(2) *p,p'*-DDT and *p,p'*-DDE were always detected in the abdominal fat of the control group. We attribute this to contamination of the feed with *p,p'*-DDT and possibly *p,p'*-DDE below the level of detection. Cross-contamination in the laying house is unlikely as in this case other persistent compounds such as β-HCH and dieldrin would have been found as well.

(d) Residues in Eggs. Initially a high level of residues of organochlorine insecticides in eggs was found at the onset of lay at the age of 24 and 26 weeks. Afterward, probably also due to the use of mixed samples, the concentrations declined to a more or less constant level. There was no clear indication of any increase in concentrations toward the end of the laying period, when egg production dropped considerably. Such an effect of egg production ratio has been demonstrated by Cecil et al. (1973). As might be expected, the highest concentrations were found in eggs of hens, fed the highest concentrations. The observations made in the previous section about heptachlor epoxide and *p,p'*-TDE residues in fat are equally applicable to these residues in eggs.

Despite the use of mixed samples, the differences in concentrations in eggs of different sampling dates were also considerable. We cannot say for certain whether this is due to variations between hens or in the same hen, as not all eggs laid were used for sampling. In eggs we have the same pattern of a continuously decreasing HCB concentration and *p,p'*-DDT and *p,p'*-DDE residues in the control group as described in the previous section for residues in abdominal fat. It is also possible to calculate a half-life time for HCB residues in eggs. Assuming a zero background level this value is 11 weeks. Correcting for a background level of 0.01 mg/kg HCB in eggs the half-life time in eggs becomes 5 weeks.

(e) Concentration Ratios in Abdominal Fat and Eggs. We have averaged the concentrations of organochlorine insecticides found in fat and eggs for the ages of 26–68 and 28–68 weeks, respectively, during which period these concentrations were almost constant. The accumulation ratios listed in Table I are calculated by comparison with the determined levels of organochlorine insecticides in the feeds. Before comparing these figures with published figures on accumulation ratios a number of points should be borne in mind. Firstly, we have corrected the HCB content in the experimental groups by subtracting the control group values before calculating the accumulation ratios. This appears to be necessary in order to eliminate the HCB contamination from the wood shavings. In adopting this procedure we assume that the contamination was evenly distributed over the treatment groups (there is no evidence to the contrary). We have not corrected for *p,p'*-DDT and *p,p'*-DDE values in the control group as we assume that these residues originate from feed residues, included in the determined levels in the feed. Secondly, owing to the conversion of heptachlor to β-heptachlor epoxide and of *p,p'*-DDT to *p,p'*-DDE, we calculate the accumulation of heptachlor as heptachlor–β-heptachlor epoxide and of *p,p'*-DDT as *p,p'*-DDT–*p,p'*-DDT + *p,p'*-DDE. Thirdly, we assume that no interactions occur during residue formation, when the organochlorine insecticides are fed as a mixture (as in this experiment) instead of in the form of separate components. De Vos et al. (1972) have shown this assumption to be correct in the case of broilers.

Comparison of our corrected accumulation ratios for HCB in fat and eggs reveals a discrepancy, especially for eggs, with the results of Avrahami and Steele (1972). They did not use pure HCB, but a commercial powder to obtain the concentrations mentioned in their paper. Our figures for relatively low producing hens are much more in line with those for eggs from high producing hens recently found by Vogt et al. (1975). They found an average accumulation ratio for HCB in eggs on a whole egg basis of about 1.0. Apparently no recent figures are available on the accumulation of α- and β-HCH in poultry. However, the much more persistent character of the β isomer, in which all Cl atoms are in the equatorial position of the chair conformation of the cyclohexane ring, is well known. It may not be merely fortuitous that the β isomer, which of all HCH isomers has a spatial configuration most ressembling that of HCB, also has a related pattern of physiological behavior. Our accumulation ratios for γ-HCH (lindane) compare very well with those found by Cummings et al. (1966, 1967) and Waldron and Naber (1974). The relatively low accumulation ratios for heptachlor, unlike the high figures found when heptachlor epoxide itself is administered, show that the conversion to the epoxide considerably reduces residue formation.

In this respect our figures agree with those found by Waldron and Naber. Combs and Brewer's (1975) figure for accumulation of heptachlor is much higher. As they used very low levels of heptachlor this might be a departure from the general rule enunciated above, viz. that the accumulation ratios are independent of the concentrations

Table V. Excretion of Organochlorine Insecticides via Eggs and Feces as a Percentage of the Daily Intake via the Feed

	%	
	Via eggs	Via feces
Hexachlorobenzene	35	10
α-Hexachlorocyclohexane	2	±0.5
β-Hexachlorocyclohexane	35	10
γ-Hexachlorocyclohexane	3	±0.5
Heptachlor epoxide	10	2
DDT (total)	25	5
Dieldrin	25	7

used. Our accumulation ratios for DDT in the relatively low producing hens compare very well with those cited in the literature and particularly when one remembers the marked effect of laying percentage on DDT accumulation found by Cecil et al. (1973). The accumulation ratios for dieldrin also compare rather well with the figures cited in the literature. Although Combs and Brewer's (1975) experiment yielded higher values, the low levels they used may have affected the accumulation. As their experiment is not described in detail further interfering complications cannot be located.

(f) Excretion of Organochlorine Insecticides via Eggs and Feces. Besides the question of accumulation in eggs, it may also be asked what percentage of the daily intake is excreted via feces and eggs. We therefore collected feces at the age of 40 and 68 weeks. Using average daily intake of feed, known concentrations of organochlorine insecticides in the feed, estimated output of feces (based on a dry matter basis), known concentrations in the dry feces, known production of egg mass per day, and known concentrations in the egg, it it possible to calculate the excretion via eggs and feces. The average of the estimates made at the age of 40 and 68 weeks is shown in Table V.

Since the concentrations in the fat do not increase after the age of 26 weeks and the birds have a constant body weight (envisaged by restricted feeding) the rest of the daily intake must be excreted by skin, feathers, or exhalation or metabolized into water-soluble products which are lost during the cleanup. Figures of the same order of magnitude for feces and eggs were found for *p,p'*-DDT and *p,p'*-DDE by Cecil et al. (1972). As these figures are rather variable, probably owing to incorrect estimates, the order of magnitude of the figures is much more important than their absolute value. The present figures may seem to contradict those of Davison (1973), who found an almost 100% recovery of [^{14}C]dieldrin, fed to chickens, in eggs and feces after a steady-state situation had been reached in residues. However, as he only measured ^{14}C activity and not dieldrin concentrations, this might explain the discrepancies.

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Supplementary Material Available: A listing of concentrations of organochlorine insecticides in feed, fat, eggs, and feces and of published accumulation ratios (10 pages). Ordering information is given on any current masthead page.

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