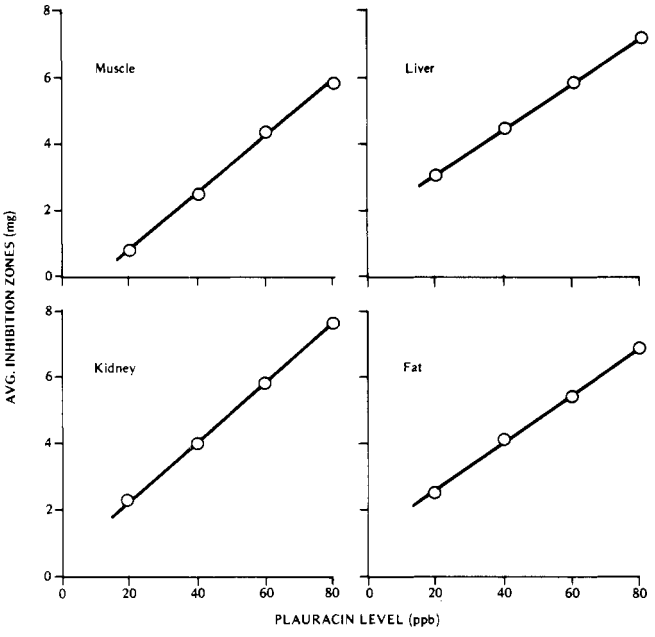


**Figure 1.** Standard curves employed for assay of plauracin in various swine tissues.

**Table I. Percent Recovery of Plauracin from Swine Tissues Fortified at 100 or 50 ppb**

| Swine tissues | % recov[a] | |
|---|---|---|
| | 100 ppb | 50 ppb |
| Liver | 85 ± 3 | 86 ± 9 |
| Muscle | 96 ± 5 | 103 ± 7 |
| Kidney | 87 ± 4 | 91 ± 8 |
| Fat | 91 ± 5 | 96 ± 3 |
| Av | 90 ± 5 | 94 ± 7 |

[a] Average of six replicate samples ± standard deviation.

quantitation, the inhibition zones were measured by the Xerox copying method (Winterlin et al., 1968). In this case the traced zones were copied and each representative zone was cut and weighed on an analytical balance.

## RESULTS AND DISCUSSION

**Feed and Drug Intake.** The average feed consumption was found to be 2.58 kg/pig a day, representing an average daily intake of 3.38 mg of plauracin/kg of body weight. The average weight gain was 19.9 ± 5.3 kg in 29 days.

**Tissue Residues.** The present assay was found to be linear over the range of 20 to 80 ppb (Figure 1). Higher antibiotic levels could be assayed by diluting the final tissue extract. As seen in Table I, the mean recovery of plauracin from various tissues fortified at 50 and 100 ppb ranged from 85 to 103% with standard deviation varying from ±3 to ±9%. These recovery studies indicate that the components of plauracin are carried through the isolation and purification steps quantitatively and reproducibly.

Liver, muscle, kidney and fat from swine maintained for 29 days on plauracin-medicated feed and slaughtered 0, 1, 2, or 3 days after drug withdrawal were assayed according to the method described. Except for fat, no plauracin levels were detected (<20 ppb) in any of the samples analyzed. Fat contained 25 ppb at zero withdrawal but no detectable levels afterward. It could be concluded that the use of plauracin as a feed additive in swine would result in no detectable antibiotic residues in the major edible tissues if animals were subjected to a 1-day withdrawal period.

## ACKNOWLEDGMENT

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# Accumulation and Depletion of Some Organochlorine Pesticides in Broiler Breeder Hens during the Second Laying Cycle

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During the second laying cycle of broiler breeder hens, low levels of organochlorine pesticides (HCB, α-HCH, β-HCH, γ-HCH, heptachlor, *p,p'*-DDT, and dieldrin) were administered via their food. Accumulation ratios (levels in product/levels in the food) were higher than during the first laying cycle and ranged for fat on a fat basis from 25 for β-HCH to 1.8 for α-HCH. In eggs, on a whole egg basis the range was 2.3 for β-HCH to 0.16 for α-HCH, and on a fat basis the figures were 20 for β-HCH to 1.4 for α-HCH. Giving noncontaminated food during 11 weeks resulted only in a drastic decline of α- and γ-HCH residues in eggs and fat. Correlation coefficients of residues within hens in abdominal, intramuscular, and egg fat were found to be high (>+0.9).

The strong accumulative properties of some organochlorine pesticides in broiler breeder hens have previously been demonstrated (Kan and Tuinstra, 1976b). The accumulation ratios calculated from that experiment were well in line with literature data, although relatively high figures were found. One of the main factors influencing accumulation—egg production—has been clearly illustrated by Cecil et al. (1973). After feeding a low calcium diet, which resulted in 60% egg production as compared to 90% in the control group, they showed that residues of

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Table I. Intended Concentrations of Organochlorine Pesticides in the Experimental Diets in Milligram/Kilogram (Determined Concentrations Are in Parentheses, Mean of Three Batches)

| | Group | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Hexachlorobenzene (HCB) | 0 (<0.01) | 0.010 (0.010) | 0.050 (0.050) | 0.100 (0.099) |
| α-Hexachlorocyclohexane (α-HCH) | 0 (<0.01) | 0.050 (0.047) | 0.250 (0.212) | 0.500 (0.445) |
| β-Hexachlorocyclohexane (β-HCH) | 0 (<0.01) | 0.100 (0.074) | 0.500 (0.426) | 1.000 (0.858) |
| γ-Hexachlorocyclohexane (γ-HCH, lindane) | 0 (<0.01) | 0.050 (0.045) | 0.250 (0.222) | 0.500 (0.462) |
| Heptachlor | 0 (<0.01) | 0.025 (0.022) | 0.125 (0.131) | 0.250 (0.241) |
| p,p'-DDT | 0 (<0.02) | 0.100 (0.108) | 0.500 (0.496) | 1.000 (1.02) |
| Dieldrin | 0 (<0.02) | 0.025 (0.024) | 0.125 (0.130) | 0.250 (0.252) |

Table II. Concentrations of Organochlorine Pesticides 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 | 0.045 | <0.02 | <0.05 | <0.02 | <0.05 | 0.25 | b | <0.05 | 0.25 | <0.05 |
| 2 | 0.21 | 0.070 | 1.92 | 0.087 | 0.15 | 0.84 | b | 1.10 | 2.07 | 0.38 |
| 3 | 1.06 | 0.40 | 11.7 | 0.44 | 0.87 | 4.06 | b | 5.23 | 9.29 | 2.30 |
| 4 | 1.94 | 0.92 | 20.8 | 1.09 | 1.73 | 7.80 | b | 10.1 | 17.9 | 4.81 |

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

Table III. Concentrations of Organochlorine Pesticides in Eggs in Milligram/Kilogram 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 | 0.005 | <0.005 | <0.01 | <0.005 | <0.005 | 0.027 | b | <0.01 | 0.027 | <0.01 |
| 2 | 0.021 | 0.007 | 0.19 | 0.011 | 0.016 | 0.071 | b | 0.11 | 0.19 | 0.041 |
| 3 | 0.10 | 0.034 | 0.92 | 0.043 | 0.072 | 0.32 | b | 0.45 | 0.77 | 0.21 |
| 4 | 0.23 | 0.076 | 1.91 | 0.097 | 0.17 | 0.69 | b | 0.96 | 1.65 | 0.48 |

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

DDT in abdominal fat and in eggs were approximately 1.5 times higher in the low calcium group (low production group). Therefore, we were interested in ascertaining whether a similar increase in residues would occur in the second laying cycle, when production is generally lower than during the first laying cycle. While keeping the hens for a second laying cycle, we were also able to fulfill some other objectives:

(1) During the first experiment (Kan and Tuinstra, 1976b), the Dutch regulations for residues of organochlorine insecticides in eggs as laid down in the "Residue beschikking van de bestrijdingsmiddelenwet" were amended. Expression of residues on a whole egg basis was changed to residues on a fat basis. During the second laying cycle we therefore determined residues in eggs on a whole egg basis as well as on a fat basis in order to compare the results with those from the previous laying cycle and with legal regulations.

(2) Due to accidental contamination with HCB during the first laying cycle we were able to determine its half-life time (8–10 weeks) in eggs and abdominal fat. In the second laying period we have deliberately included a depletion period to determine half-life times of all pesticides with greater accuracy. We were also interested whether residues in abdominal fat would alter, during this depletion period, in the same way as those in intramuscular fat.

(3) In connection with this comparison between residues in abdominal and intramuscular fat, we studied variation in residues within and between animals in the depletion period by analyzing not only individually all fat samples, but also, if possible, eggs present in the shell gland at the time of killing of the birds. In doing so we can calculate the correlations within animals between residues in abdominal, intramuscular, and egg fat of the separate pesticides administered via the food. We further calculated correlations within pesticides between animals. The aspects of performance during this experiment will be discussed in the accompanying paper (Kan and Jonker-den Rooyen, 1978).

## MATERIALS AND METHODS

**Animal Experiment.** Details have been given on housing, rearing, and feeding of the animals and the preparation of the contaminated food (Kan and Tuinstra, 1976a,b; Kan and Jonker-den Rooyen, 1978). Three batches of contaminated food were prepared during the second laying cycle for each feeding group. Starting at the age of 97 weeks control (uncontaminated) food was provided to all groups in order to study diminution of the residues. The results of the determinations in the food are given in Table I. Two hens from each experimental group were killed for residue determination in the abdominal fat at the age of 81, 89, 93, and 95 weeks. The individual figures are given in the Supplementary Material. At the age of 97, 98, 98.5, 99, 100, 101, 102, 103, 104, 106, and 108 weeks, six animals, respectively, the rest of the animals were killed. In the Supplementary Material the average residues of the animals of both subgroups (replicate pens) per experimental group, killed at the same time, are given. The average residues during the period of 81–97 weeks are shown in Table II. Mixed samples of 20 eggs per group (10 eggs/day) laid at the age of 77, 81, 85, 89, 93, 95, 97, 97.5, 98, 98.5, 99, 99.5, 100, 100.5, 101, 102, 103, 104, 106, and 108 weeks were also analyzed for residues of organochlorine pesticides. Figures per subgroup (replicate pens) are given in the Supplementary Material. The averages during the period 81–97 weeks are shown in Table III. Feces were collected under the slatted floors for 1 day at the age of 82 and 97 weeks.

**Determinations of Organochlorine Pesticides.** Extraction and cleanup, using $Al_2O_3$, were performed according to Greve and Grevenstuk (1975).

**Gas Chromatographic Determination.** Use was made of a Packard Becker Model 419 gas chromatograph using

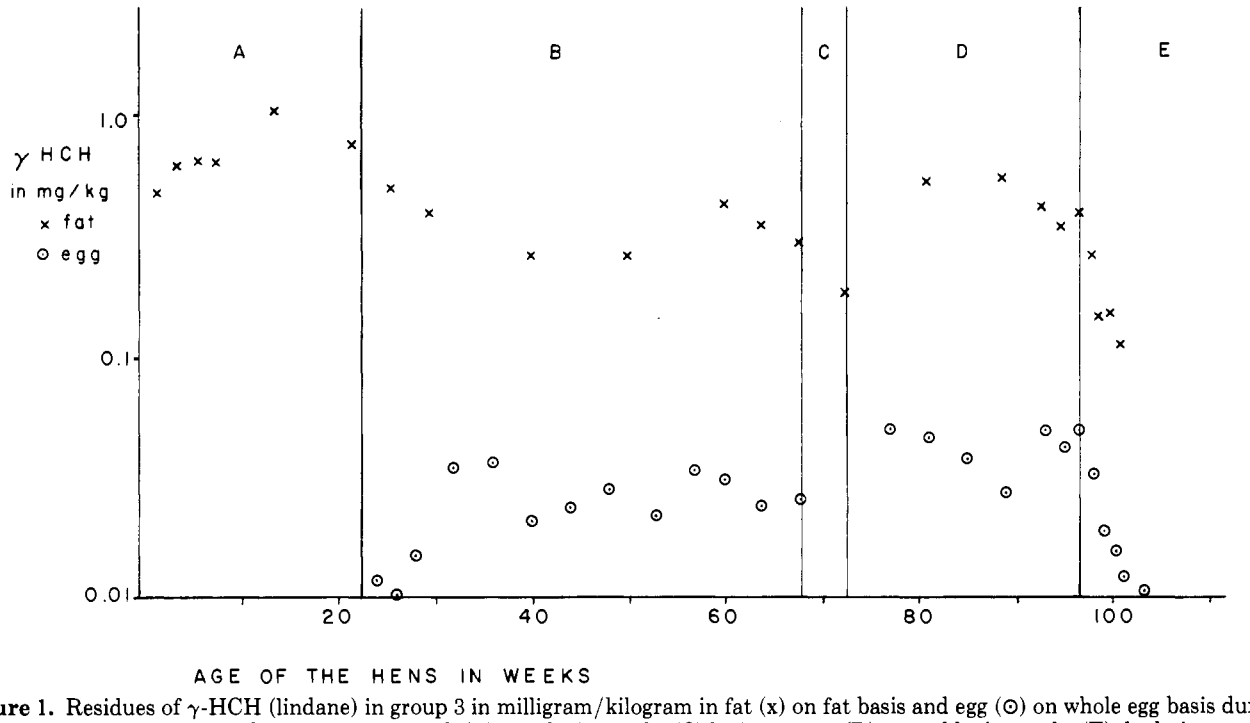


**Figure 1.** Residues of γ-HCH (lindane) in group 3 in milligram/kilogram in fat (x) on fat basis and egg (⊙) on whole egg basis during the entire experimental period: (A) rearing period, (B) first laying cycle, (C) laying pause, (D) second laying cycle, (E) depletion period.

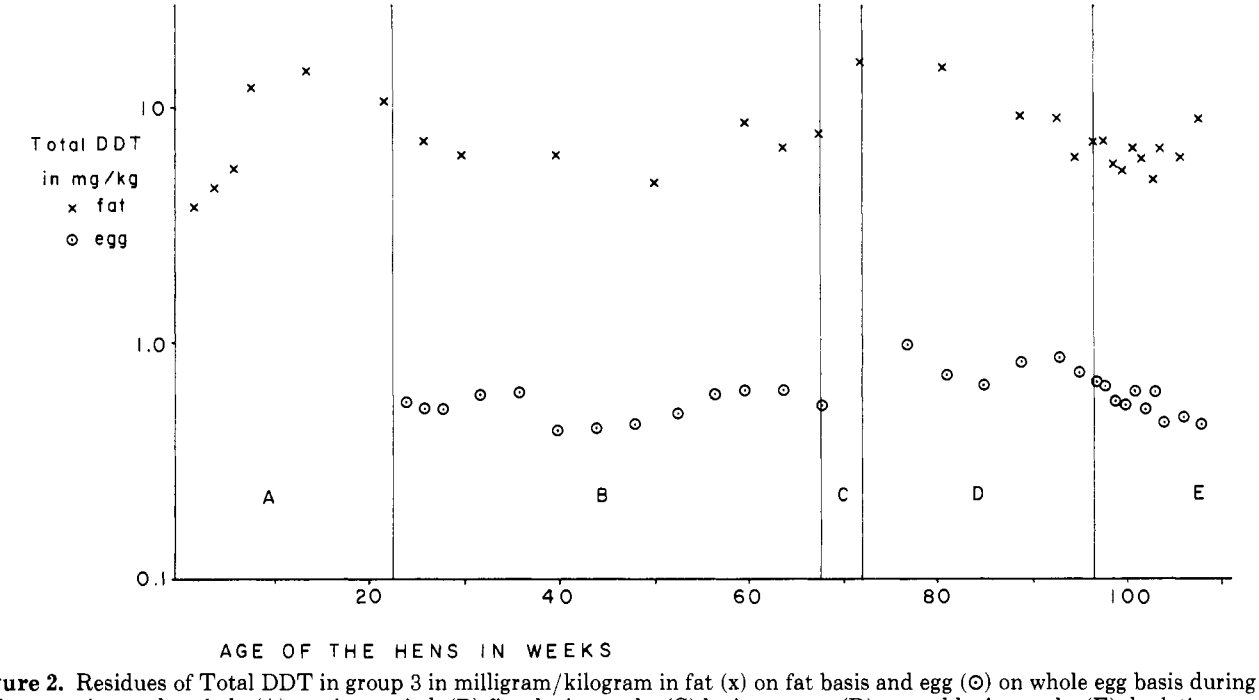


**Figure 2.** Residues of Total DDT in group 3 in milligram/kilogram in fat (x) on fat basis and egg (⊙) on whole egg basis during the entire experimental period: (A) rearing period, (B) first laying cycle, (C) laying pause, (D) second laying cycle, (E) depletion period.

$^{63}Ni$ electron-capture detectors. The stationary phase employed was a mixture of OV-17 and OV-210, and a DEGS–$H_3PO_4$ mixture.

## RESULTS AND DISCUSSION

**A. Residues in Food Constituents and Concentrations in the Food.** The residues in food constituents were usually below the limit of detection or negligible. The determined levels of the organochlorine pesticides in the experimental diets (see Table I) were somewhat lower than they should have been in theory, as has been found during the first laying cycle.

**B. Residues in Broken Oyster Shells, Oats, Wood Shavings, etc.** During the second laying cycle we encountered no problems with unexpected residues in these supplementary materials.

**C. Residues in Abdominal Fat.** Immediately after the forced moulting at the beginning of the second laying period, a number of birds were killed at random to equalize the numbers in the experimental groups. In these birds the residues of the relatively persistent substances like HCB, β-HCH, and DDT were higher than at the end of the first laying period, while the levels of α- and γ-HCH had somewhat declined. These changes are demonstrated for γ-HCH and DDT in Figures 1 and 2, where residues during the entire experimental period are shown.

The increase in residues must be ascribed to the loss of weight (mainly fat) during the forced moulting and resting

Table IV. Concentrations of Organochlorine Pesticides in Eggs in Milligram/Kilogram 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 | 0.046 | <0.02 | <0.05 | <0.02 | <0.02 | 0.23 | b | <0.05 | 0.23 | <0.05 |
| 2 | 0.18 | 0.061 | 1.69 | 0.080 | 0.14 | 0.62 | b | 0.97 | 1.58 | 0.35 |
| 3 | 0.87 | 0.30 | 8.04 | 0.37 | 0.63 | 2.81 | b | 3.92 | 6.65 | 1.80 |
| 4 | 1.96 | 0.66 | 16.5 | 0.84 | 1.43 | 5.92 | b | 8.33 | 14.3 | 4.16 |

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

period. From 81 to 97 weeks of age, the residues were more or less constant. Also during this laying cycle the differences in residues between birds killed at the same time were often quite considerable. Some attention will be paid to this problem in the discussion on correlation coefficients in a following section. The remarks in our previous paper (Kan and Tuinstra, 1976b) on the conversion of heptachlor into β-heptachlor epoxide, the nonquantification of p,p'-TDE, and the occurence of HCB and p,p'-DDE in the control group have still to be kept in mind. The residues during depletion will be discussed below.

**D. Residues in Eggs.** At the age of 77 weeks, shortly after the laying pause, the residues were relatively high (see, for example, Figures 1 and 2). Afterwards, the concentrations declined to a more or less constant level. Although the levels were consistently higher than those in the first laying period (probably as a result of a lower egg production), there was no indication of an increase in residues toward the end of the laying period, when egg production dropped to a very low level of ca. 40%.

The problem of variation in concentrations in eggs between animals, resulting in a variation between sampling dates, will be discussed along with the correlation coefficients in a following section. The remarks on heptachlor epoxide, p,p'-TDE, p,p'-DDE, and HCB made in the previous section also apply to these residues in eggs.

We have also compared residues in eggs on a whole egg and on a fat basis, as the Dutch regulations for expressing residues have been changed in this way. The residues on a fat basis, during the period of 81–97 weeks are summarized in Table IV. In general, the difference between both ways of expressing residues is a factor 9. This implies that the fat content of eggs of our broiler breeder hens was approximately 11%. This figure is somewhat higher than the figure of 10%, used in changing the official regulations, but especially in the borderline cases the difference might be important. Residues which might be not acceptable on a whole egg basis may become acceptable on a fat basis. The reverse is perhaps true for high-producing laying hens where we found a fat content of ca. 9% (Kan and Jonker-den Rooyen, unpublished observations). The residues during depletion will be discussed below.

**E. Concentration Ratios in Abdominal Fat and Eggs.** We have averaged the concentrations of organochlorine pesticides found in fat and eggs at the age of 81–97 weeks, during which these concentrations were almost constant. The accumulation ratios listed in Table V are calculated by comparison with the determined levels of organochlorine pesticides in the food.

Like during the first laying period (Kan and Tuinstra, 1976b), we have corrected for HCB contamination originating from the contaminated woodshavings, but not for the p,p'-DDE values found in the control group. Once again accumulation of heptachlor was calculated as heptachlor → β-heptachlor epoxide and p,p'-DDT as p,p'-DDT → p,p'-DDT + p,p'-DDE. We have further maintained the assumption, that no interaction in residue formation took place.

Table V. Accumulation Ratios (Concentration in Fat or Egg/Concentration in the Feed)

| | First laying period (Kan and Tuinstra, 1976b) | | Second laying period | | |
|---|---|---|---|---|---|
| | Eggs (whole egg basis) | Fat (fat basis) | Eggs (whole egg basis) | Eggs (fat basis) | Fat (fat basis) |
| Hexachlorobenzene (HCB) | 1.3 | 17 | 1.9 | 16 | 19 |
| α-Hexachlorocyclohexane (α-HCH) | 0.10 | 1.8 | 0.16 | 1.4 | 1.8 |
| β-Hexachlorocyclohexane (β-HCH) | 1.5 | 18 | 2.3 | 20 | 25 |
| γ-Hexachlorocyclohexane (γ-HCH, lindane) | 0.13 | 1.8 | 0.2 | 1.8 | 2 |
| Heptachlor → β-heptachlor epoxide | 0.5 | 6 | 0.7 | 6 | 7 |
| p,p'-DDT → p,p'-DDT + p,p'-DDE | 1.2 | 14 | 1.6 | 14 | 18 |
| Dieldrin | 1.3 | 14 | 1.7 | 15 | 17 |

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

| | % via eggs | % via feces |
|---|---|---|
| Hexachlorobenzene | 50 | 10 |
| α-Hexachlorocyclohexane | 4 | |
| β-Hexachlorocyclohexane | 60 | 15 |
| γ-Hexachlorocyclohexane | 5 | 2 |
| Heptachlor epoxide | 15 | 4 |
| DDT (total) | 40 | 10 |
| Dieldrin | 40 | 10 |

A comparison of accumulation ratios for the first and second laying periods reveals that during the second laying period the accumulation ratios were higher. This is probably a result of the lower egg production (Kan and Jonker-den Rooyen, 1978) during this period. Such an effect of egg production percentage has been demonstrated by Cecil et al. (1973). As noted before, the difference between residues and thus in concentrations ratios on a whole egg basis and on a fat basis in eggs is approximately a factor 9 in our broiler breeder hens.

The accumulation ratios for eggs on a fat basis are consistently lower than those for fat on a fat basis. This difference might be the result of the two different types of fat concerned (mainly phospholipids vs. mainly triglycerides) leading to different partition coefficients of the lipophilic organochlorine pesticides between the aqueous and lipid phase. The possibility of two independent compartments (ovary vs. abdominal fat) as an explanation for this difference is more or less ruled out by the results during the depletion period which will be discussed below.

Table VII. Residues of Organochlorine Pesticides at the Beginning and End of the Depletion Period in Eggs and Fat on Fat Basis in Milligram/Kilogram

| | Time in Weeks | HCB | α-HCH | β-HCH | γ-HCH | HEPO[a] | p,p'-DDE | p,p'-DDT | Total DDT[b] | Dieldrin |
|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | | | | | | | | | | |
| Eggs | 0 | 0.030 | <0.02 | <0.05 | <0.02 | <0.02 | 0.19 | <0.05 | 0.19 | <0.02 |
| | 11 | 0.038 | <0.02 | <0.05 | <0.02 | <0.02 | 0.21 | <0.05 | 0.21 | <0.02 |
| Abdominal fat | 0 | 0.028 | <0.02 | <0.02 | <0.02 | <0.02 | 0.19 | <0.05 | 0.19 | <0.02 |
| | 11 | 0.045 | <0.02 | <0.02 | <0.02 | <0.02 | 0.27 | <0.05 | 0.27 | <0.02 |
| Intramuscular fat | 0 | 0.023 | <0.02 | <0.05 | <0.02 | <0.02 | 0.15 | <0.05 | 0.15 | <0.05 |
| | 11 | 0.055 | <0.02 | <0.05 | <0.02 | <0.02 | 0.21 | <0.05 | 0.21 | <0.05 |
| Group 2 | | | | | | | | | | |
| Eggs | 0 | 0.23 | 0.071 | 1.98 | 0.11 | 0.13 | 0.61 | 1.01 | 1.62 | 0.32 |
| | 11 | 0.12 | <0.01 | 1.20 | <0.01 | 0.11 | 0.60 | 0.43 | 1.03 | 0.23 |
| Abdominal fat | 0 | 0.19 | 0.075 | 2.70 | 0.091 | 0.11 | 0.73 | 1.48 | 2.21 | 0.37 |
| | 11 | 0.20 | <0.01 | 2.10 | <0.01 | 0.16 | 0.92 | 1.07 | 1.99 | 0.40 |
| Intramuscular fat | 0 | 0.19 | 0.078 | 2.07 | 0.093 | 0.16 | 0.71 | 1.05 | 1.76 | 0.39 |
| | 11 | 0.17 | <0.02 | 1.53 | <0.02 | 0.16 | 0.79 | 0.96 | 1.75 | 0.32 |
| Group 3 | | | | | | | | | | |
| Eggs | 0 | 0.87 | 0.36 | 8.17 | 0.45 | 0.57 | 2.40 | 3.86 | 6.26 | 1.74 |
| | 11 | 0.54 | <0.02 | 6.73 | <0.02 | 0.45 | 2.46 | 1.70 | 4.16 | 1.06 |
| Abdominal fat | 0 | 0.98 | 0.36 | 10.4 | 0.40 | 0.72 | 2.80 | 4.38 | 7.18 | 1.90 |
| | 11 | 1.22 | <0.02 | 12.1 | <0.02 | 0.93 | 5.32 | 3.70 | 9.02 | 2.44 |
| Intramuscular fat | 0 | 0.97 | 0.44 | 12.1 | 0.49 | 0.91 | 2.95 | 5.40 | 8.35 | 2.14 |
| | 11 | 1.12 | <0.02 | 10.6 | <0.02 | 1.01 | 5.86 | 3.63 | 9.49 | 2.14 |
| Group 4 | | | | | | | | | | |
| Eggs | 0 | 2.06 | 0.77 | 16.8 | 0.85 | 1.38 | 5.08 | 9.54 | 14.6 | 4.46 |
| | 11 | 1.26 | <0.02 | 9.53 | <0.02 | 0.93 | 4.55 | 3.62 | 8.17 | 2.53 |
| Abdominal fat | 0 | 2.45 | 1.12 | 30.4 | 1.08 | 1.86 | 8.36 | 10.6 | 19.0 | 5.14 |
| | 11 | 2.04 | <0.02 | 20.6 | <0.02 | 1.60 | 7.58 | 6.78 | 14.4 | 3.79 |
| Intramuscular fat | 0 | 2.00 | 1.05 | 26.5 | 1.22 | 1.97 | 8.03 | 14.4 | 22.4 | 5.64 |
| | 11 | 2.02 | <0.02 | 18.7 | <0.02 | 1.63 | 8.70 | 6.29 | 15.0 | 3.90 |

[a] HEPO = β-Heptachlor epoxide. [b] p,p'-TDE values were not quantitatively determined (see text).

**F. Excretion of Organochlorine Pesticides via Eggs and Feces.** Similar to the first laying period we calculated excretion by eggs and feces after collecting feces at the age of 82 and 97 weeks. Maintaining the same determinations and assumptions, we calculated the excretion percentages given in Table VI. Excretion percentages are somewhat higher, especially via eggs, than in the first laying cycle. The increase is mainly due to higher residues in the eggs and heavier eggs, which more than compensated the lower production percentage during this cycle.

The general picture of a considerable amount of daily intake which cannot be accounted for by the determined compounds and excretion routes is still valid.

**G. Depletion Study.** In order to study depletion of residues in eggs and fat, the animals were provided with uncontaminated food during 11 weeks, starting at 97 weeks of age. The residues at the beginning and end of this period can be seen from Table VII. The compounds which totally disappeared during this period from fat as well as from eggs are α- and γ-HCH.

From the data obtained, half-life times of approximately 2 weeks can be calculated for both pesticides in fat as well as in eggs. For γ-HCH this is well in line with the data from, for example, Cummings et al. (1966, 1967). In eggs most other residues are decreasing also, but as the figures are highly variable (as can be seen in the Supplementary Material and in Figures 1 and 2), reliable half-life times cannot be calculated. The only exception is p,p'-DDE, but this is of course the result of the continuous metabolism of p,p'-DDT to p,p'-DDE, also demonstrated in the changing DDT/DDE ratio during this period.

Most residues in abdominal fat tend to indicate that this is a closed pool from which no substances disappear during this period. However, as α- and γ-HCH disappear from abdominal fat completely, this idea must be rejected. In this respect there is no difference between intramuscular and abdominal fat. One might have anticipated that the intramuscular fat was a more mobile pool, but this suggestion was not found to be true in our experiment. The difference between fat and eggs might be explained in the following way. The levels in eggs (and thus in the ovary) are a reflection of the circulating levels in the blood, which is probably also true for the fat near to the blood vessels. The fat taken at autopsy represents the bulk in which changes in concentrations of organochlorine pesticides are not observable due to the large amounts of fat present and the low excretion by eggs (approximately 30% laying percentage during this period). Another explanation might be the fact that the depletion period coincided with wintertime. The amount of food provided daily might have been less than the requirement, resulting in mobilization of body fat. The increase in residues caused by this fat mobilization might have obscured the decrease to be found during depletion. The difference between α- and γ-HCH and the other pesticides is probably not a result of increased excretion, but of relatively easy metabolism of these compounds.

**H. Correlation between Concentration in Eggs, Abdominal, and Intramuscular Fat.** During the depletion period we analyzed individually, when present, the yolks from the hens killed for residue determination in the fat. The residues in these eggs were generally well in line with those in the abdominal fat. Calculations of the correlations of these concentrations *within* hens revealed generally very high coefficients. At the beginning of the depletion period all correlation coefficients were over +0.9. Later on, somewhat lower values down to +0.83, with one exception of +0.62, were found. The coefficients between residues in abdominal fat and intramuscular fat were of the same high magnitude both at the beginning and at the end of the depletion period. The correlation within the treatment group within substances and *between* hens for residues in eggs, abdominal and intramuscular fat, were very variable. The coefficients of correlation ranged from −0.82 to +0.98. Our conclusion from these data is that each single hen has her own characteristic way of handling these

foreign substances; or, in other words, within hens all substances are handled the same way, but between hens there can be very large variations in metabolism, storage, etc. This is probably one of the main reasons for finding such large variations in residues between hens killed at the same age and the large variations between eggs sampled at different dates and probably laid by different hens.

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**Supplementary Material Available:** A listing of concentrations of organochlorine pesticides in fat, eggs (on a whole egg and on a fat basis), and feces (12 pages). Ordering information is given on any current masthead page.

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# Second Laying Cycle Effects of a Mixture of Organochlorine Insecticides on Broiler Breeder Hens

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During the second laying cycle of broiler breeder hens, a mixture of hexachlorobenzene, $\alpha$-, $\beta$-, and $\gamma$-hexachlorocyclohexane, heptachlor, $p,p'$-DDT, and dieldrin was added at three different levels between 0.01 and 1 mg/kg to the food with a view to study performance, following a similar treatment during the first laying cycle. Mortality and number of eggs produced per hen present were unaffected even when combined over both laying periods. Egg weight, specific gravity of the egg, and shell thickness did not differ due to the treatments. Fertility and neonatal mortality showed no influence of the treatments, but the number of good quality chicks hatched from fertile eggs was lowered in the highest treatment group.

In a previous publication we have reported that low levels of organochlorine insecticides had no influence on the performance of broiler breeders during their first laying cycle (Kan and Tuinstra, 1976). There were, however, two reasons for extending the experiment to a second laying cycle after forced moulting: (1) Davison et al. (1970) have shown that during severe dietary restriction (which in practical situations is the causative factor for the forced moulting) levels of dieldrin in the food, which are normally harmless to laying hens, will have an adverse effect. (2) Cecil et al. (1973) demonstrated that pullets fed DDT had thicker shells than the control hens, while hens in their second laying cycle laid eggs which had significantly thinner shells. Therefore, at the end of the first laying cycle, the hens were subjected to a forced moulting procedure and after a production stop of 3 weeks kept for a second laying cycle of 30 weeks. The experimental conditions were identical with those in the first laying cycle (Kan and Tuinstra, 1976). Accumulation and depletion in fat and eggs will be discussed in the accompanying paper (Kan and Jonker-den Rooyen, 1978).

## MATERIALS AND METHODS

The experimental scheme of four treatment groups, each consisting of two replicate pens, was maintained. Housing and management were also identical with those described for the first laying period (Kan and Tuinstra, 1976). The forced moulting (production stop) was induced at 69 weeks of age by food withdrawal during 6 days. Thereafter the hens received ground oats during 3 weeks (1 week ad libitum and 2 weeks 100 g per hen per day). Following the laying stop, the amount of food provided was gradually increased as can be seen from Table I.

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

| Age, weeks | M.E.,[b] kcal | Age, weeks | M.E., Kcal |
|---|---|---|---|
| 69-70 | | 77-78 | 400 |
| 70-71 | 290 | 78-81 | 430 |
| 71-73 | 240 | 81-84 | 400 |
| 73-74 | 290 | 84-86 | 370 |
| 74-75 | 315 | 86-101 | 355 |
| 75-76 | 340 | 101-108 | 370 |
| 76-77 | 370 | | |

[a] Food consumption in weeks 70-73 consisted of ground oats (ad libitum or restricted). Starting week 73-74, normal food was provided. After 97 weeks all hens received the control food. [b] M.E. = metabolizable energy.

At the beginning of the second laying cycle, the number of hens in each group was reduced by random selection to 84 in order to equalize the number of hens in each group. During the laying pause the cocks were housed separately (not moulted) and fed the same (restricted) amount of food. After 97 weeks of age all the groups received the

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