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Accumulation and Depletion of Some Organochlorine Pesticides in High-Producing Laying Hens

Cornelis A. Kan* and Jenny C. Jonker-den Rooyen

Addition of low concentrations of organochlorine pesticides to the feed of high-producing laying hens for 16 weeks had no influence on feed consumption, body weight, egg production, egg weight, and deformation of the egg. Accumulation ratios (concentration of the pesticide in the egg or fat to its concentration in feed) on fat basis were: hexachlorobenzene in egg 11, fat 13; α -hexachlorocyclohexane in egg 2, fat 2; β -hexachlorocyclohexane in egg 13, fat 15; γ -hexachlorocyclohexane (lindane) in egg 2, fat 2; heptachlor(epoxide) in egg 5, fat 7; DDT (total) in egg 10, fat 12, dieldrin in egg 11, fat 14. Up to 80% of the pesticides ingested were excreted via eggs and feces. Half-value times of depletion of residues (with uncontaminated feed for 12 weeks) were 1.5–2 weeks for α - and γ -HCH. The other pesticides have half-value times of about 6–8 weeks. Correlations between concentrations of pesticides in abdominal fat and in egg fat within hens are generally very high (r > +0.9). The same holds for correlations between concentrations in abdominal fat and fat of the thigh muscle, breast muscle, liver, and egg.

The effect of egg production on accumulation of organochlorine pesticides in laying hens has been clearly demonstrated (Cecil et al., 1973; Kan and Tuinstra, 1976b; Kan and Jonker-den Rooyen, 1978b). The accumulation ratios in high-producing (laying percentage >90%) hens could, however, not be predicted from those experiments.

After reaching a plateau in residues in eggs, differences in residues between samples of different dates in these experiments still occur (Kan and Jonker-den Rooyen, 1978b; Waldron and Naber, 1974). It has not been es-

tablished whether this variation is due to day-to-day differences in the analytical procedures, to changes for one hen, or to differences between hens, as generally not all eggs laid are used for analysis.

Descending half-value times of several pesticides in laying hens have been reported (e.g., Cummings et al., 1966, 1967; Wesley et al., 1966, 1969). The same holds for several procedures which accelerate depletion such as charcoal (e.g., Waibel et al., 1972), phenobarbital (e.g., Mick et al., 1973) or starvation (e.g., Wesley et al., 1969). However, ascending half-value times have not been reported.

In a previous study with heavy, low-producing broiler breeder hens (Kan and Jonker-den Rooyen, 1978b), a good correlation existed between residues in eggs and abdominal and intramuscular fat within hens, whereas between hens

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Table I. Intended Concentrations of Organochlorine Pesticides in the Experimental Diets (mg/kg)^a

			Groups	
	1	2	3	4
Hexachlorobenzene (HCB) α -Hexachlorocyclohexane (α -HCH) β -Hexachlorocyclohexane (β -HCH) γ -Hexachlorocyclohexane (γ -HCH) Heptachlor p,p' -DDT Dieldrin	0 (<0.02)	0.01 (0.0094 ± 0.0015) 0.05 (0.048 ± 0.010) 0.05 (0.051 ± 0.011) 0.05 (0.046 ± 0.008) 0.025 (0.028 ± 0.004) 0.10 (0.103 ± 0.015) 0.025 (0.022 ± 0.006)	$0.25 (0.230 \pm 0.029)$	$1.0 (1.010 \pm 0.055)$

^a In brackets are the average estimated values with their standard deviation (eight samples/group).

for one substance the correlations were very variable. However, these observations were restricted to the depletion time, and it is not known whether this relation also holds during continuous administration to light, high-producing hens.

Studies on the balance between uptake and excretion of the pesticides by eggs and feces were made on a group basis (Kan and Tuinstra, 1976b; Kan and Jonker-den Rooyen, 1978b), but the figures on individual hens are not available.

Effects of the low levels of pesticides on performance in our broiler breeder experiment were absent or marginal (Kan and Tuinstra, 1976a; Kan and Jonker-den Rooyen, 1978a). Since, however, literature on this matter conflicted (Kan and Tuinstra, 1976a), it could be that high-producing hens react to low levels of organochlorine pesticides incorporated in the feed.

Therefore an experiment was carried out with high-producing hens in which the following aspects were studied: influence of low levels of organochlorine pesticides on body weight, feed consumption, mortality, egg production, egg weight, and deformation of the egg; accumulation ratios (concentration of the pesticide in the fat or egg to its concentration in the feed); ascending and descending half-value times of the pesticides; correlations between residues in abdominal fat and eggs; variance of residues in eggs from one hen and from the group; balance between uptake and excretion of pesticides.

MATERIALS AND METHODS

Animal Experiment. The experiment started in August 1976 with 120, individually housed "Shaver Starcross 288" hens. The hens, 20-weeks old, received uncontaminated feed during a preparatory period of 32 days. Afterwards, units of four adjoining cages were randomly distributed in a block design (seven blocks) over the four treatments (Table I). The remaining eight birds $[120 - (7 \times 4 \times 4) = 8]$ were killed for residue analysis at the start of the main period.

The birds were housed in three-tier battery cages with artificial light for 14 h daily.

The basic diet (composition, Table II) was mixed with pesticides as described previously (Kan and Tuinstra, 1976b). Per group, 400 kg of contaminated feed was prepared.

The experiment was divided into three parts: (1) a preparatory period of 32 days, meant to reach a maximum egg production with uncontaminated feed; (2) a main period of 16 weeks, during which feed was contaminated with organochlorine pesticides; (3) a depletion period of 12 weeks to study the decrease in residues after administration had ceased.

Measurements. (1) Feed consumption for each unit of four neighboring hens was measured over periods of 4 weeks in the main and the depletion periods. At the same time, each hen was weighed.

(2) Laying percentage and number of eggs produced per

Table II. Composition of the Basal Diet (g/100 g) and Calculated Nutrient Composition

Ingredient	%
Corn	64.4
Soybean meal (44% protein)	15.5
Alfalfa meal	6.5
Fish meal	3.0
Soybean oil	1.5
Limestone	6.5
Mineral premix	2.0
Vitamin premix	0.5
Methionine	0.1
(99% pure)	100.0

Crude protein (N × 6,25)	14.8% (analyzed 14.8%)
Metabolizable energy	12.23 MJ/kg (2922 kcal/kg)
Calcium	3.24% 0.48%
Phosphorus Lysine	0.76%
Methionine + cystine	0.64%

Calculated nutrient composition

hen present were calculated every 2 weeks.

(3) At the end of the preparatory period and after 4 and 10 weeks of the main period, egg weight and deformation of the egg were measured over a 5-day period. Deformation under a load of 4.9 N (produced by a weight of 500 g), along the horizontal axis of the egg, was measured in an automatic device based on the principal described by Schoorl and Boersma (1962).

(4) Feed ingredients were analyzed before mixing the feed, while duplicate feed samples were analyzed monthly during the main period.

(5) Per group, seven eggs were taken and analyzed as a mixed sample at the beginning of the main period and after 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, and 16 weeks in the main period. In the depletion period, samples were taken and analyzed after 1, 2, 3, 4, 5, 6, 8, 10, and 12 weeks.

To estimate the variance of residues in eggs for one hen, all eggs laid during 14 days by three hens per group, after 7 weeks of the main period, were analyzed individually. To estimate variance in residues within groups, each egg laid during 2 days after 15 weeks of the main period was analyzed. Variances of residues in eggs for one hen, within groups and days of analysis were estimated for the two sampling periods according to the scheme given in Table III. The contributions of the different variances to the total variance found were estimated along these lines by making the appropriate comparison and subtractions.

(6) Hens were killed for residue analysis in the abdominal fat at the following times: eight hens at the beginning of the main period, two hens per group after 4, 6, 8, 10, 12, and 14 weeks in the main period, and four hens per group at the end of the main period and after 4, 8, and

Table III. Scheme for Estimating Variances of Residues in Eggs: (1) for One Hen, (2) within Groups, and (3) for Days of Analysis

	First sampling period	Second sampling period
	3 hens/group; 10 eggs/hen; all eggs laid by one hen analyzed at the same day	10 hens/group; 2 eggs/hen; all eggs per group laid on one day analyzed on the same day
Variance within groups Variance for one hen	$\sigma^2 = \sigma_{\text{egg}}^2 + 10 \sigma_{\text{hen}}^2 + 10 \sigma_{\text{day of analysis}}^2$ $\sigma^2 = \sigma_{\text{egg}}^2$	$\sigma^2 = \sigma^2_{\text{egg}} + 2 \sigma^2_{\text{hen}}$ $\sigma^2 = \sigma^2_{\text{egg}} + \sigma^2_{\text{day of analysis}}$

Table IV. Mean Concentrations of Organochlorine Pesticides in Fat of Eggs (mg/kg) in Weeks 4-16 of the Main Period

	НСВ	α-НСН	β-НС Н	γ-НСН	HEPO^a	p_ip' - DDE	p,p' - DDT	DDT total	Dieldrin
Group 1	0,172	<0.02	<0.05	<0.02	<0.02	<0.02	<0.05	<0.05	<0.02
Group 2	0,201	0.090	0.542	0.104	0.136	0.211	0.790	1.01	0.246
Group 3	0,651	0.384	2.60	0.452	0.641	0.99	3.73	4.72	1.16
Group 4	1,18	0.829	5.04	0.899	1.30	2.07	7.61	9.68	2.44

^a HEPO = β -heptachlor epoxide.

12 weeks in the depletion period. If an egg was present in the shell gland of hens when killed, that egg was analyzed too. In the depletion period, analyses in the hens of group 4 (Table I) were extended to include thigh meat, breast meat, and liver.

(7) Air pollution in the battery house was estimated each month by analyzing a glassplate (30 cm \times 30 cm) covered with grease, placed in the house for that month.

(8) Balance between uptake and excretion was estimated by weighing feed, eggs, and feces from each of four hens of group 3 for 1 week. Residues in eggs and feces were measured afterwards in mixed samples per hen.

(9) Egg production, egg weight, and deformation were analyzed statistically, in a block design assuming fixed variables, for variance (Snedecor and Cochran, 1967; Winer, 1970).

Pesticide Analysis. Extraction and cleanup over an aluminum oxide column were by the method of Greve and Grevenstuk (1975). A Packard-Becker Model 419 gas chromatograph with ⁶³Ni electron-capture detector was used, and the stationary phases were a OV-17/OV-210 and a DEGS/H₃PO₄ mixture (Kan et al., 1978).

RESULTS AND DISCUSSION

Body Weight, Feed Consumption, and Mortality. No statistical differences (P>0.05) could be found between groups in body weight or feed consumption. Body weight increased from an average of 1.520 kg at the beginning of the main period to 1.750 kg at the end. Feed consumption per hen increased from 106 g/day during the first 4 weeks to 113 g/day during the last 4 weeks in the main period.

During the whole experiment, no hen died of natural causes, so all the hens could be used for analysis at the predetermined time.

Laying Percentage, Egg Weight, and Egg Shell Quality. Laying percentage reached the very high figure of about 95% during the first 10 weeks of the experiment. It then declined to about 90% after 16 weeks. The number of eggs produced per hen present amounted to 102.3, 102.9, 104.7, and 106.0 for groups 1-4, respectively, over the 16-week period. The differences between groups were not statistically significant (P > 0.05). Mean egg weight increased from 49.3 g in the preparatory period to 58.2 g after 10 weeks of the main period. There were no statistical differences (P > 0.05) between groups.

Deformation of the egg, which is an indicator of shell quality (Oosterwoud, 1977), also revealed no statistical differences (P > 0.05) between groups during the main period.

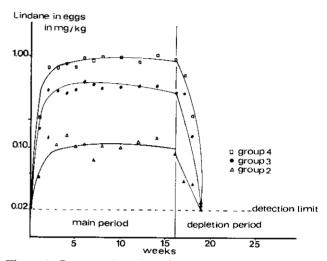


Figure 1. Concentrations of γ -HCH (lindane) in eggs on fat basis in mg/kg.

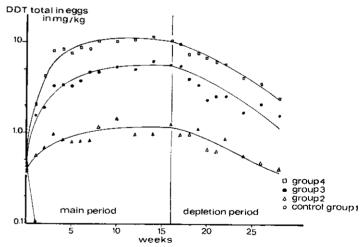


Figure 2. Concentrations of total DDT in eggs on fat basis in mg/kg.

Feed Analysis. Residues of pesticides in feed ingredients were undetectable or negligible. Table I shows that the residues in the experimental diets were once again unaccountably lower than the calculated values.

Residues in Eggs. Figures 1 and 2 show, as an example, the rise, plateau, and fall of concentrations, of γ -HCH (lindane) and total DDT in eggs during the experiment. Similar profiles are found for the other pesticides in the experiment.

Table V. Mean Concentrations of Organochlorine Pesticides in Abdominal Fat (mg/kg) in Weeks 6-16

of the Main 101	НСВ	α-НСН	β-НCH	ү-НСН	HEPOα	p,p'- DDE	p,p'- DDT	DDT total	Dieldrin
Group 1	0.136	<0.02	<0.05	<0.02	<0.02	<0.02	<0.05	<0.05	$ < 0.02 \\ 0.288 \\ 1.64 \\ 3.18 $
Group 2	0.210	0.084	0.606	0.089	0.152	0.289	0.902	1.19	
Group 3	0.848	0.509	3.57	0.526	0.863	1.62	4.06	5.68	
Group 4	1.38	0.936	6.29	0.922	1.58	2.49	9.32	11.81	

 α HEPO = β -heptachlorepoxide.

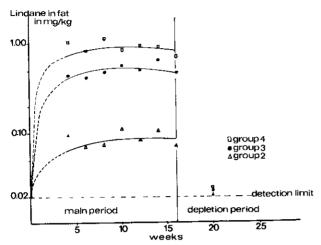


Figure 3. Concentrations of γ -HCH (lindane) in abdominal fat on fat basis in mg/kg.

Average residues during weeks 4-16 are given in Table IV. At the beginning of the main period, residues of HCB and DDT were found in eggs of all groups, indicating contamination during the preparatory period. Afterwards DDT residues in the control group (group 1) rapidly declined below the limit of detection (Figure 2). The HCB residues were found throughout the experiment. This contamination was probably caused by a previous experiment in the same room where large amounts of HCB were used. It was also found in the air pollution samples.

The variance of residues between eggs of each hen was estimated by analyzing individually on one day all eggs laid during 14 days by 9 hens. The mean coefficient of variation per pesticide calculated from these data was of the order of 10–15% with extremes down to 4.4% and up to 42.6%. By analyzing all eggs from one hen on the same day, the day-to-day variation in the analytical procedure was excluded from the variance. The same holds true for estimation of variance of residues in eggs from one group, for which all eggs laid during 2 days were analyzed individually. The mean coefficient of variation ranged between pesticides and groups 14.5–45.2%, usually 20–30%.

After log transformation of the residue data, meant to equalize the variances between the groups, variance of residues per hen and between hens was estimated for the 14 days and the 2 days (Table III).

Data on HCB, γ -HCH, and DDT, which were considered representative of the pesticides used, indicated that neither variance per hen nor between hens differed between the two sampling periods. In these sets of data, variance due to analyses on different days was negligible.

Thus variance observed of residues in eggs sampled and analyzed on different days is largely attributable to variance between hens.

Residues in Abdominal Fat. Figures 3 and 4 show the concentrations of γ -HCH and DDT in abdominal fat during the experiment. Similar curves can be drawn for the other pesticides. The contamination, during the

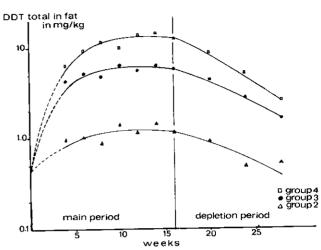


Figure 4. Concentrations of total DDT in abdominal fat on fat basis in mg/kg.

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

	Excretion percentages		
	Via eggs	Via feces	
Hexachlorobenzene (HCB)	55	5	
α-Hexachlorocyclohexane (α-HCH)	8	<1	
β-Hexachlorocyclohexane (β-HCH)	70	9	
γ-Hexachlorocyclohexane (γ-HCH)	9	<1	
Heptachlor $\rightarrow \beta$ -heptachlor epoxide	35	2	
$p, p' ext{-DDT} o p, p' ext{-DDE} + p, p' ext{-DDT}$	50	3.5	
Dieldrin	60	6	

preparatory period, with HCB and DDT was also demonstrable in abdominal fat.

Table V shows average concentrations in weeks 6-16. Residues in fat of one hen that laid no egg and was killed at the end of the main period were about half those in other hens. If excretion via eggs was the main factor in governing accumulation, these residues should be at least as high as in other hens; therefore, metabolism seems to be important too.

Air Pollution. The only pesticide detectable was HCB. The concentrations in fat ranged 0.16-0.25 mg/kg. Concentrations of the same order of magnitude were found in eggs and fat from the control birds. We cannot say whether this was caused by the partition coefficient of HCB between ambient air and a fat phase or by coincidence.

In the experiment with large amounts of HCB in the same house, an average HCB concentration in air of 1.8 $\mu g/m^3$ was measured (Kan and Jonker-den Rooyen, 1976).

Balance Studies. Table VI shows that generally the larger part of daily intake was excreted via eggs and feces. These measurements were made after residues had reached plateau. This implies that, because body weight is rather constant, the rest of daily intake must have been metabolized or excreted by other routes. Breakdown of most substances administered was demonstrated in the domestic

Table VII. Accumulation Ratios on Fat Basis (Concentration of the Pesticide in Product to Its Concentration in Feed)a

		Laying Broiler hens breeders		
	Eggs	Fat	Eggs	Fat
Hexachlorobenzene (HCB)	11	13	16	19
α-Hexachlorocyclohexane (α-HCH)	2	2	1.4	1.8
3-Hexachlorocyclohexane (β-HCH)	13	15	20	25
-Hexachlorocyclohexane (γ-HCH)	2	2	1.8	2
Heptachlor $\rightarrow \beta$ -heptachlor epoxide	5	7	6	7
$p, p' \cdot DDT \rightarrow p, p' \cdot DDE + p, p' \cdot DDT$ Dieldrin	10	12	14	18
Dietutiii	11	14	15	17

^a Results for broiler breeders from Kan and Jonker-den Rooyen (1978b).

fowl (e.g., HCB metabolism by Koss et al., 1977). In comparison with studies on broiler breeders (Kan and Tuinstra, 1976b; Kan and Jonker-den Rooyen, 1978b), excretion percentages are higher. The lower rate of breakdown resulting from or caused by this higher excretion is also demonstrated in the higher $\operatorname{DDT}/\operatorname{DDE}$ ratio of 5/1 in the present experiment compared to 3/1 in the broiler breeders.

Also the ratio between excretion via eggs and via feces differs considerably (approximately 5 in broiler breeders compared to 10 in this study). This might be partly due to the higher laying percentage of the hens in the present experiment. Some of the differences found may, however, be caused by the use of individual hens and the longer sampling period in this study: in the previous study groups of broiler breeders were used.

Accumulation Ratios. Table VII shows the mean accumulation ratios (concentration of pesticide in product to its concentration in feed) calculated from the present experiment. For comparison data from the second laying period of our broiler breeders (Kan and Jonker-den Rooyen, 1978b) are included. HCB data have been corrected for contamination in the control group (group 1). Especially for the strongly accumulating substances, differences in accumulation ratios from the previous trial, probably due to differences, in laying percentage are clear.

The difference in accumulation ratios between egg fat and abdominal fat found in broiler breeders (Kan and Jonker-den Rooyen, 1978b) was also found in laying hens. We still cannot explain this difference. In general, there are no large differences from data published by others.

Half-Value Times. The rising curve of residues to the plateau and the falling curve during depletion can both be described by first-order kinetics ($C_{\rm t}$ = Ae^{-kt}). After log transformation, the data can be fitted to a straight line (lin $C_{\rm t} = \ln A - kt$), which allows calculation of the time at which half the plateau was reached or during which the concentration had fallen to half of its original value ($t_{1/2}$ $= \ln 2/k$).

We could not calculate the rising curve for fat residues because of limited data. Only the egg residues of group

4 gave reliable data (coefficient of correlation >+0.7). From these limited data, we calculated rising half-value times of 1.5 week for α - and γ -HCH and 2–3.5 weeks for HCB, β -HCH, β -heptachlor epoxide, total DDT, and dieldrin. From figures of Avrahami and Steele (1972), a rising half-value time of HCB in eggs of 4-5 weeks can be deduced. The data of Cummings et al. (1966) and Waldron and Naber (1974) give a value of approximately 1.5-2 weeks for γ -HCH. Their data, as well as those of Driver et al. (1976), give half-value times of about 3-6 weeks for heptachlor epoxide, total DDT, and dieldrin. So, in general, the limited data on rising half-value times do not

During depletion more reliable half-value times in eggs and fat could be calculated. Table VIII shows the data calculated for HCB, β -HCH, β -heptachlor epoxide, total DDT, and dieldrin. Because of the rapid decline of α - and γ -HCH (Figures 1 and 3), no half-value times could be calculated. From the figures a half-value time for both compounds of 1.5 weeks in eggs can be estimated.

Comparing eggs and fat of groups 2-4, no large differences can be found. From concentrations in different tissues within group 4, half-value times in thigh muscle were the shortest except for β -heptachlor epoxide. However, the differences were not impressive. We cannot explain why ascending half-value times were, in general, much shorter than descending. A similar phenomenon has been described by Rauws (1975) for bromide accumulation in the growing rat. However, the explanation given there is probably not applicable to our experiment. Compared to the half-value times of HCB in eggs of 11 weeks (Avrahami and Steele, 1972) and 9–11 weeks (Kan and Tuinstra, 1976b), the present value of 5–8 weeks is rather short. For α - and β -HCH, no literature data are available, but the curves are in line with those expected from their accumulation pattern.

Several authors have reported half-value times of γ -HCH. Cummings et al. (1966, 1967) and Waldron and Naber (1974) reported a rapid decline as in the present experiment. Heptachlor epoxide was also studied by several authors. Rather long half-value times (more than 8 weeks) were found in eggs (Cummings et al., 1966; Driver et al., 1976), and eggs and fat (Waldron and Naber, 1974). However, in fat, Cummings et al. (1967) found a rather rapid decline more in line with the present data.

DDT depletion has been studied rather extensively. Half-value times ranged from 6-12 weeks (Cummings et al., 1966, 1967; Lillard and Noles, 1973; Waldron and Naber, 1974; Wesley et al., 1969). Wesley et al. (1969) found no influence of protein content in the diet, androgen injection, or starvation on half-value times, although the half-value time in eggs was about half that in abdominal fat. Lillard and Noles (1973), however, shortened the half-value time of DDT by starvation or induced hyperthyroidism. Our data are found in the lower range of reported half-value times for DDT. The same is so for the half-value time of dieldrin (Cummings et al., 1966, 1967;

Table VIII. Declining Half-Value Times (Weeks)

	Group	НСВ	β-НСН	HEPO^a	Total DDT	Dieldrin
Egg Abdominal fat Egg Abdominal fat Thigh muscle Breast muscle Liver PO = β-heptachlor epo	2-4 2-4 4 4 4 4	8.0 6.8 8.0 6.6 4.9 6.4 6.6	7.0 6.7 7.0 7.3 4.4 6.3 6.5	6.5 4.8 5.2 4.8 5.0 6.0 4.6	6.7 6.0 5.9 5.2 4.8 6.4 5.8	6.1 6.0 4.7 6.7 3.2 4.8 3.6

Driver et al., 1976; Waldron and Naber, 1974).

In conclusion, it seems that for α - and γ -HCH metabolism is the main factor in determining disappearance of residues as half-value times in both broiler breeders (Kan and Jonker-den Rooyen, 1978b) and laying hens are almost identical. In view of the discrepancy for the other pesticides between broiler breeders and laying hens, excretion via eggs seems to determine half-value time of HCB, β -HCH, β -heptachlor epoxide, total DDT, and dieldrin.

Correlations. The correlations between the concentrations of pesticides between egg fat and abdominal fat per hen were high (r > +0.85) both in main and depletion period. Per pesticide within groups of hens, correlation coefficients were above +0.65. They were much lower in the broiler breeders (Kan and Jonker-den Rooyen, 1978b).

The correlation coefficients of residues of group 4 in abdominal fat and fat in thigh muscle, breast muscle, liver, and eggs (matrix of correlations) during depletion were also very high (> +0.93). The correlations with concentrations in liver were generally the lowest. The lower correlations with liver concentrations can probably be explained as follows: due to metabolism of the pesticides in the liver, changes in concentrations are first demonstrable there. Remarkable were the correlation coefficients in the hen that laid no eggs. In this hen all coefficients were above +0.988. Probably the absence of excretion via eggs gives rise to a more even distribution.

In conclusion, low levels of pesticides do not have an adverse effect on performance of high-producing laying hens. The pesticides accumulate to a similar extent in both abdominal and egg fat. Depletion of residues, when the treatment ceased, is dependent on the pesticide considered, and there are no large differences in depletion between egg, abdominal fat, thigh muscle, breast muscle, or liver.

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Supplementary Material Available: A listing of body weights, feed consumption, egg production, egg weight, deformation of the eggs, concentrations of organochlorine pesticides in eggs and fat, variance in residues per hen and in groups and balance data (12 pages). Ordering information is given on any current masthead page.

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