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Gas Chromatographic Study of the Rate of Penetration of DDT Into Quail Eggs at Different Stages of Their Development

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Brief immersion of quail and hen eggs in a aqueous suspension of commercial DDT leads to morphological and physiological disorders in the embryo, the chick and the adult bird (DAVID 1973, DAVID 1976, DAVID and LUTZ-OSTERTAG 1976, LUTZ-OSTERTAG and DAVID 1977). Preliminary chromatographic studies (DAVID 1977) have also shown that DDT deposited on the shell passes into the albumin and the vitellus in small but significant amounts.

In the present work the kinetics of this penetration are studied at different stages of the embryonic development of the quail.

MATERIALS AND METHODS

Eggs of laboratory bred quail ("Géromoise" breed of "Coturnix coturnix japonica") were immersed for 30 sec before incubation in a aqueous suspension of commercial DDT "G" containing, according to the manufacturer, 50% of active material. The concentration used (0.5%) was that recommended by the supplier for the control of pests affecting orchards and market gardens. After air drying the contamined eggs were incubated at 38°C and then taken for analysis at regular intervals from 1/2 hr to 14 days after treatment.

After carefully washing the shell, the contents of the egg, i.e. the vitellus and the albumin were taken, weighted (average wet weight 7.9 g for the 249 samples analysed) and then homogenized in the presence of an equal volume of anhydrous sodium sulfate after addition of an internal standard (aldrin).

After initial extraction with acetonitrile the active material was extracted with hexane leaving polar material in the water-acetonitrile phase. Partial elimination of lipids was carried out by adding 1 g of Florisil to the sample.

Extracts of a control batch of eggs which had undergone no direct contamination with DDT were analysed in the same operating conditions for comparison.

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The gas chromatograph used (Packard model 417) was fitted with a Ni-63 electron-capture detector. The temperature of the column (2 m X 3 mm borosilicate glass) was 205°C. It was packed with Chromosorb W AW DMCS 100-120 mesh, impregnated at the top end with 25 cm of 10% "Réoplex 400" and then with 1.5% OV 17 - 2% QF 1. The "Réoplex" lets the organochlorine pesticides through but retains specifically any lipids still present in the biological sample (DEMAIMAY et al. 1972). Injector and detector temperatures were respectively 245° and 295°C. The carrier gas flow rate (Argon U) was 48 ml/min.

In each case aldrin was used as internal standard. Its theoretical concentration was 0.2 ng per 2 µl of injected sample after extraction. In this way any drop in yield due to bad operating conditions could be brought to light and accounted for the statistical calculations.

Chromatographic analysis showed that the commercial product "G" used contained 48% active material, namely 35% p,p'-DDT, 11% o,p'-DDT and only 2% p,p'-DDE. Retention times in min were 3.8 for aldrin, 11 for p,p'-DDE, 15 for o,p'-DDT and 23.2 for p,p'-DDT.

Levels of aldrin, p,p'-DDE, o,p'-DDT, p,p'-DDT and total DDT were measured in each biological sample. The variation of the quantity of residue as a function of the stage of development of the egg was determined by statistical calculation along with the standard deviation and reliability limits.

RESULTS AND DISCUSSION

As far as the control sample is concerned, for the contents of the 19 eggs analysed, average total DDT residues were found to be 105 ng, i.e. 0.013 ppm (30 ng of p,p'-DDE, 5 of o,p'-DDT and 70 of p,p'-DDT). The amount of residual DDT in the shell was even higher (0.022 ppm).

The feed given to the parent quails, made up essentially of cereals, powdered meat extracts, fats, minerals, vitamins and antibiotics, was also found to contain measurable amounts of pesticides, particularly DDT: 0.007 ppm of p,p'-DDE, 0.022 ppm of o,p'-DDT and 0.044 ppm of p,p'-DDT. The contamination of the control eggs was thus due, at least to a large extent, to the presence of these residues in the feed, despite limitations or prohibitions of the use of DDT.

1° - Rate of penetration of DDT into eggs in the course of the first day.

If we look at the histogram obtained by plotting the number of hours of incubation on the x-axis against the amount of DDT contained in eggs on the y-axis, we can see that there is a positive correlation between these two values. The average total DDT value $\bar{y}$ rises to 564 ng, i.e. 0.071 ppm (56 ng for p,p'-DDE, 173 for o,p'-DDT and 335 for p,p'-DDT) for a stage of development corresponding to $\bar{x}$ = 13 hr. This value is clearly significant when compared to that obtained for the control sample (105 ng). In addition, the relative regularity of the regression lines suggests that the relationship sought is a linear one. On this assumption the equations for the total DDT were determined by statistical calculation (LAMOTTE 1957). Then in order to check whether the correlations are significantly different from zero or due to random sampling errors, correlation coefficients "r" and 95% reliability ranges were determined, giving the following results:

total DDT: Y = 17.4x + 338; r = +0.87 (+0.55/+0.97)
p,p'-DDE : Y = 0.5x + 50; r = +0.43 (>0.00/+0.83)
o,p'-DDT : Y = 5.8x + 98; r = +0.69 (+0.20/+0.92)
p,p'-DDT : Y = 11.2x + 189; r = +0.81 (+0.40/+0.95)

In all cases the correlative coefficients are positive. They are significantly different from zero except for p,p'-DDE. Thus we conclude that during the first day after treatment, the rates of penetration of total DDT, p,p'-DDT and o,p'-DDT increase in direct proportion to the stage of developement.

2° - Rate of penetration of DDT into eggs from the 1st to the 14th day.

A statistical study carried out as above shows an average total DDT level $\bar{y}$ = 1200 ng, i.e. 0.152 ppm (103 ng of p,p'-DDE, 354 of o,p'-DDT and 743 of p,p'-DDT) corresponding to an average development time $\bar{x}$ = 7.5 days. The equations of the regression lines, the correlation coefficients and their reliability ranges are:

total DDT: Y = 77.5x + 619; r = +0.89 (+0.66/+0.97)
p,p'-DDE : Y = 4.5x + 69; r = +0.58 (+0.01/+0.86)
o,p'-DDT : Y = 9.5x + 283; r = +0.43 (>0.00/+0.80)
p,p'-DDT : Y = 63.3x + 268; r = +0.85 (+0.54/+0.96)

These results show that the rates of penetration of total DDT and of p,p'-DDT increase with incubation time. However, the correlation coefficient determined

TABLE 1

Average levels of DDT (in nanograms, with the standard deviation) inside eggs during the 1st day of incubation (n: number of samples analysed)

Time(hr)	n	p,p'-DDE	o,p'-DDT	p,p'-DDT	tot. DDT
Controls	19	30 ± 3	5 ± 3	70 ± 14	105 ± 19
0.5/1/2	20	47 ± 3	74 ± 7	195 ± 17	316 ± 22
03/04	14	48 ± 3	127 ± 28	191 ± 18	366 ± 39
05/06	12	55 ± 5	127 ± 10	204 ± 15	386 ± 24
07/08	9	62 ± 4	226 ± 42	259 ± 36	547 ± 65
09/10	8	58 ± 2	161 ± 21	445 ± 37	664 ± 48
11/12	8	47 ± 2	149 ± 12	303 ± 18	499 ± 26
13/14	8	51 ± 5	207 ± 41	399 ± 50	657 ± 77
15/16	8	75 ± 2	175 ± 6	408 ± 27	658 ± 28
17/18	8	54 ± 3	168 ± 13	327 ± 34	549 ± 40
19/20	8	55 ± 2	134 ± 7	464 ± 32	653 ± 33
21/22	8	58 ± 4	231 ± 33	411 ± 41	700 ± 62
23/24	13	61 ± 4	299 ± 30	409 ± 39	769 ± 58
Average	13	56 ± 2	173 ± 10	335 ± 15	564 ± 27

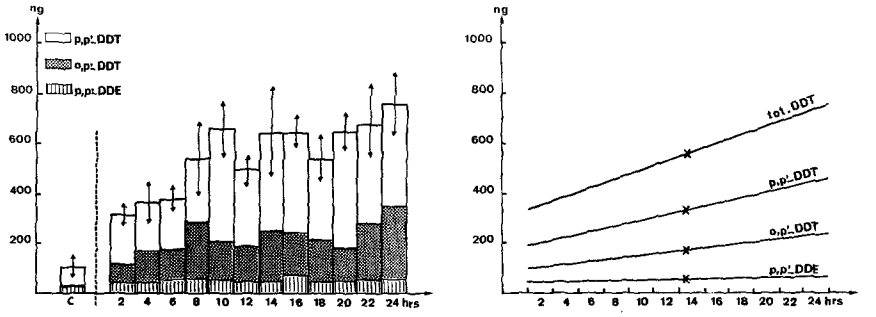

Figure 1 - a) Histogram showing average total DDT values (with reliability range) for p,p'-DDE, o,p'-DDT and p,p'-DDT inside eggs during the 1st day after treatment.

b) Regression lines and average points (X) determined for the 3 compounds and for the total DDT.

TABLE 2

Average levels of DDT inside eggs during the first 14 days of development.

Time(d)	n	p,p'-DDE	o,p'-DDT	p,p'-DDT	tot. DDT
01	13	61 ± 4	299 ± 30	409 ± 39	769 ± 58
02	12	99 ± 21	253 ± 53	584 ± 74	936 ± 118
03	12	112 ± 37	333 ± 67	489 ± 60	934 ± 132
04	13	133 ± 52	440 ± 31	530 ± 97	1104 ± 188
05/06	23	74 ± 6	285 ± 49	574 ± 55	933 ± 88
07/08	16	85 ± 12	312 ± 57	654 ± 54	1052 ± 99
09/10	16	90 ± 7	346 ± 53	715 ± 92	1151 ± 122
11/12	18	111 ± 12	548 ± 58	832 ± 106	1491 ± 140
13/14	15	162 ± 18	325 ± 32	1423 ± 160	1910 ± 168
Mean	7.5	103 ± 9	354 ± 26	743 ± 86	1200 ± 100

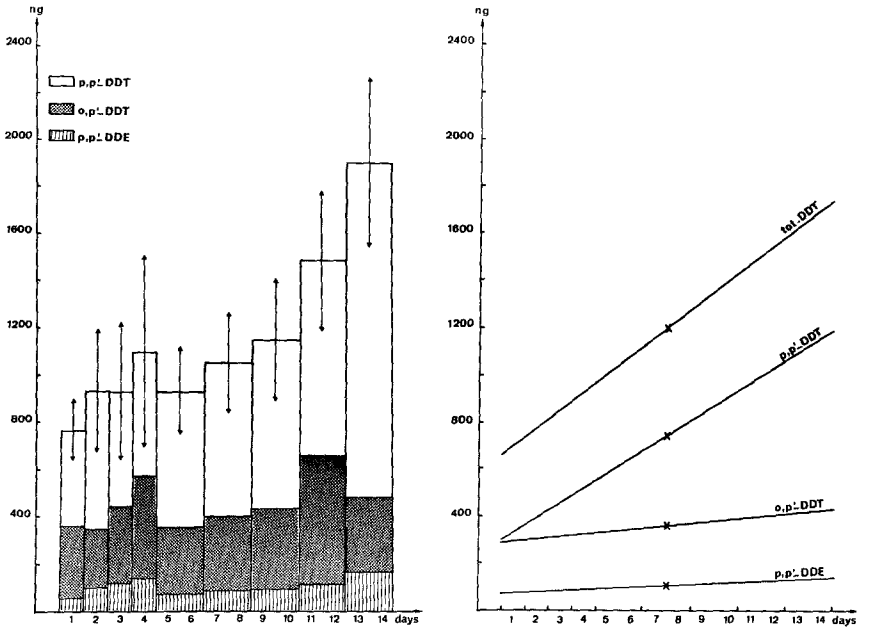

Figure 2 - a) Histogram showing DDT levels in eggs during the first 14 days of development.

b) Graphical representation of regressive lines.

for o,p'-DDT is not significant, and that determined for p,p'-DDE is only very slightly so.

Thus the amount of DDT which passes through the shell and into the vitellus and albumin increases in direct proportion to the incubation time. One half-hour after treatment contamined eggs already showed a significant DDT level compared with the control sample (244 ng ± 39). Between the 2nd and the 8th hour, the take-up of pesticide increases distinctly, then slows down up to the 10th day, only to pick up again after the 11th day.

Nonetheless, the proportion of the product "G" able to enter eggs remains low; only 0.242 ppm 14 days after treatment.

Previous analysis (DAVID 1977) concerning the amount of DDT left on the shell after treatment with the aqueous suspension of "G" (0.5%), gave an average total DDT value of 7231 ng ± 1060, i.e. 1.780 ppm: 534 ng ± 71 for p,p'-DDE (7.4%), 619 ng ± 116 for o,p'-DDT (8.6%) and 6078 ng ± 982 for p,p'-DDT (84.1%). Thus only 10.6% of the DDT on the shell penetrates into the egg after 1 day, and only 23.5% between the 11th and 14th days.

If the respective levels of the 3 organochlorine compounds in the eggs are determined as a function of their levels on the shells, we obtain, after 1 day of incubation: 11.4% of p,p'-DDE, 48.3% of o,p'-DDT and 6.7% of p,p'-DDT; and between the 11th and 14th days, respectively 25.9%, 70.5% and 18.6%.

The comparison of the two sets of values, one concerning the shell and the other the contents of the egg, shows that o,p'-DDT penetrates faster and more easily, and that the p,p'-DDE level increases to a lesser extent. This may be due, in the main, to the metabolisation of part of the p,p'-DDT to give p,p'-DDE.

It is disturbing to find that even though the amounts of pesticide able to pass through the shell and contaminate the vitellus and the albumin are small, the pathological effects on the development and reproduction of the quail nevertheless reach a high level. These results suggest that residual levels of pesticides of the DDT family, still present in control eggs and in industrial feed, may not be entirely harmless.

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