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DEGRADATION OF SOME PESTICIDES IN AVIAN EMBRYOS

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On day 9 or 12 of the hatching period different pesticides (parathion, methyl-parathion, carbendazim, 2,4-D-amine Na, phosmethylane) were applied in ecotoxicological trials. The formulations were either injected into the air space of pheasant, quail or hen eggs or hen eggs were treated by the immersion technique. The residues of pesticides were measured in samples on days 13, 14 and 16 of incubation of chicken and pheasant embryos, while the Japanese quail embryos were analysed on days 10–14 of incubation. Analytical chemistry data showed a varying degradation rate of the compounds in avian embryos of the same species. The residues directly affect the embryos, disturbing their normal development and causing pathophysiological and morphological changes.

Key words: Teratology, pesticide, chicken, pheasant, quail, embryo, degradation, residue

Agrochemicals, including pesticides, are being used in increasing amounts in agriculture and are therefore potential environmental contaminants which may affect a variety of biological systems. It may be true to say that of all the toxicological hazards arising from chemicals in industry and agriculture, the risk of embryotoxicity in avian species is the least known.

In recent years numerous trials have been performed to evaluate the teratogenic potential of pesticides. Ecotoxicological examinations of these compounds have also been very important (Fusi et al., 1977). These are aimed at detecting the toxic effects exerted on non-target animals. Complementary testing for teratogenic effect to birds is indispensable for assessing the impact of pesticides on wildlife, in the interest of a more efficient protection of the environment by preventive measures against pesticide contamination.

The aim of this study was to investigate the degradation of some pesticides applied in plant protection practice in different avian embryos during the hatching period. The duration of the presence of an active ingredient may be an important indicator of its effect exerted on the embryo.

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Materials and methods

Fresh fertile pheasant (*Phasianus colchicus mongolicus et torquatus*), hen (*Gallus domesticus*, Shaver Starcross 288) and Japanese quail (*Coturnix coturnix japonica*) eggs were used. The applied pesticides included Parathion 20 WP (20% parathion), Methylparathion 18 WP (18% methylparathion), Nevifosz 50 EC (50% phosmethylan), Kulfugo 25 FW (25% carbendazim), and Dikamin D (40% 2,4-D-amine Na). Each test material was used in aqueous suspension or emulsion at different concentrations and doses, respectively. Treatment was performed on day 9 (quail) or 12 (chicken, pheasant) of incubation.

Volumes of suspensions or emulsions (0.1 ml in pheasant and chicken, 0.05 ml in quail) were administered by a single injection into the air chamber. Control eggs were injected similarly with distilled water. Before the treatment the calcic egg-shell was bored through, then, after injection, it was sealed with paraffin (Clegg, 1964). In one case the immersion technique was applied. On day 12 of incubation the hen's eggs were immersed in the appropriate suspensions, while the control eggs in distilled water, for 30 min at 37 °C, and then they were put back into the incubator (Ragus, Wien).

The incubation temperature was 37.0–37.5 °C, the relative humidity 65–75% and the eggs were turned three times a day. The eggs were opened for examination on various days of the hatching period.

Pesticide residues were determined analytically from pooled samples, avoiding contamination of the embryos with pesticides from the egg chamber or egg surface. Parathion, methylparathion, phosmethylane and 2,4-D-amine Na were measured using a Packard 421 or 428 type GLC apparatus (Marquardt et al., 1964; Pápa, 1976; Schlenk and Gellerman, 1960; Somlyay et al., 1989; Analytical methods of determination of pesticide residues I–IV, 1976). The following detectors were used: thermoionic detector with KCl application – (parathion and methylparathion), ECD (Ni^{63}) – (2,4-D-amine Na), selective phosphorus-sensitive thermoionic glass beads – (phosmethylane). An Apecord UV VIS type instrument was applied for the determination of carbendazim at 282 nm (Bleidner et al., 1975; Pease et al., 1973).

Results

The data of analytical chemistry are summarised in Tables 1–6. Table 1 shows the quite fast degradation of parathion and methylparathion, respectively. On post-treatment days 4 or 3 the active substance could not be measured analytically in the Japanese quail embryos.

Table 1

Degradation of parathion and methylparathion in Japanese quail embryos after injection of Parathion 20 WP (P) and Methylparathion 18 WP (MP)

Doses at treatment (µg active ingredient/ embryo)	Days of incubation									
	10		11		12		13		14	
	No. of embryos	Active agent (µg/g)	No. of embryos	Active agent (µg/g)	No. of embryos	Active agent (µg/g)	No. of embryos	Active agent (µg/g)	No. of embryos	Active agent (µg/g)
0.0 C	10	0.000	9	0.000	9	0.000	10	0.000	11	0.000
30.0 P	11	0.075	9	0.020	9	0.015	8	–[a]	10	–[a]
0.0 C	11	0.000	10	0.000	10	0.000	11	0.000	9	0.000
45.0 MP	10	0.050	12	0.008	11	–[a]	10	–[a]	10	–[a]

C = Control; [a] = Under detectable limit; Detection limits: P = 0.01 mg/kg, MP = 0.003 mg/kg

Table 2 demonstrates the phosmethylane residues found in pheasant embryos after treatment, on days 13, 14 and 16 of incubation. The degradation of this compound was dose dependent, as indicated by a moderate tendency observed. Carbendazim is not a persistent agent: it was practically undetectable after the treatment of pheasant embryos (Table 3).

Table 2

Degradation of phosmethylan in pheasant embryos after injection of Nevifosz 50 EC

Doses at treatment (µg active ingredient/ embryo)	Days of incubation					
	13		14		16	
	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)
0	10	0.00	12	0.00	11	0.00
15	11	0.01	14	–[a]	21	–[a]
150	16	0.14	16	0.06	16	0.03
1500	10	0.90	14	0.37	16	0.21

[a] = Under detectable limit; Detection limit = 0.001 mg/kg

The degradation rate of 2,4-D-amine Na in pheasant embryos is dilatory (Table 4), although the data show a dose-dependent decrease in the amount of active substance during the hatching period.

Table 3

Degradation of carbendazim in pheasant embryos after injection of Kolfugo 25 FW

Doses at treatment	Days of incubation					
	13		14		16	
(µg active ingredient/ embryo)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)
0.0	10	0.00	12	0.00	11	0.00
7.5	15	–[a]	14	–[a]	15	–[a]
75.0	16	0.22	16	–[a]	13	–[a]
750.0	15	–[a]	14	–[a]	16	–[a]

[a] = Under detectable limit; Detection limit = 0.02 mg/kg

Table 4

Degradation of 2,4-D amine Na in pheasant embryos after injection of Dikamin D

Doses at treatment	Days of incubation					
	13		14		16	
(µg active ingredient/ embryo)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)
0	8	0.00	9	0.00	9	0.00
40	6	0.16	8	0.06	11	–[a]
400	7	1.47	4	0.87	12	0.47
4000	6	13.57	5	11.71	10	1.91

[a] = Under detectable limit; Detection limit = 0.001 mg/kg

Tables 5 and 6 demonstrate the degradation dynamics of 2,4-D-amine Na in chicken embryos. The data indicate a dilatory speed of degradation after the injection of Dikamin D, while the decay rate of the active ingredient was found to be faster if the immersion treatment technique was used.

Discussion

Different pesticides (insecticides, fungicide, herbicide) were used in our experiments to determine the degradation dynamics. The data of analytical chemistry showed a fast degradation rate of parathion, methylparathion and carbendazim after injection treatment, and of 2,4-D-amine Na after administration by immersion

technique on day 12 of incubation. In pheasant embryos, residues of 2,4-D-amine Na were detected even on day 4 after the injection of test material during the hatching period.

Table 5

Degradation of 2,4-D amine Na in chicken embryos after injection of Dikamin D

Doses at treatment	Days of incubation					
	13		14		16	
(µg active ingredient/ embryo)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)
0	12	0.00	11	0.00	11	0.00
40	15	0.08	10	0.08	14	0.03
400	10	0.79	11	0.29	12	0.04
4000	8	12.27	7	5.90	9	0.55

Detection limit = 0.001 mg/kg

Table 6

Degradation of 2,4-D amine Na in chicken embryos after treatment with Dikamin D (immersion technique)

Concentration (%) at treatment	Days of incubation					
	13		14		16	
	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)	No. of embryos	Active agent in sample (µg/g)
0.0	10	0.00	10	0.00	11	0.00
0.1	11	0.02	8	–[a]	14	–[a]
1.0	6	0.15	9	0.09	12	–[a]
10.0	12	0.70	9	0.39	8	0.08

[a] = Under detectable limit; Detection limit = 0.001 mg/kg

These active ingredients and formulations had been studied in teratogenicity tests, and the teratological effect of the experimental agents was proved (Fáncsi and Várnagy, 1986; Somlyay et al., 1988, 1992; Somlyay and Várnagy, 1986, 1988; Várnagy, 1981, 1995; Várnagy et al., 1981, 1982).

These teratological trials can be complemented by analytical chemistry measurements made after treatment with various pesticides, and the obtained data facilitate the evaluation of relationships between morphological and chemical results (Várnagy and Füzesi, 1979). On the basis of previous and the present experiments we can establish a direct relationship between the short- or long-term presence of experimental agents in developing embryos and the pathomorphological consequences.

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