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EFFECT OF LINDANE ON EGGSHELL CHARACTERISTICS AND CALCIUM LEVEL IN THE DOMESTIC DUCK

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SUMMARY

Adult laying domestic ducks (*Anas platyrhynchos domesticus*) were forced fed lindane (γ-hexachlorocyclohexane) 20 mg/kg body weight daily, thrice/week and twice/week for 8 weeks. Significant shell thinning (18—21%) was observed in ducks with daily and thrice/week lindane feeding. SEM studies of thinned eggshells revealed reduced and tightly clustered mammillae with apparent decrease in intermammillary spaces and signs of inhibition of calcite formation. Such structural changes of thinned shells were associated with a significant reduction in the level of calcium both of the plasma and of shell gland of the ducks. Simultaneously, density of the pore in the shell and pore area/cm^2 of the shell in these birds were drastically reduced, presumably because of total absence of 'large' pores in such shells. Such porosity change is suggestive of reduced conductivity of eggshell implying impaired hatching success. Diethyl stilboestrol post-treatment neutralized all the adverse effects of lindane on duck eggshell, indicating induced estrogen deficiency in such birds by lindane.

Key words: Lindane toxicity; Eggshell thinning; Estrogen deficiency; Hatching success; Scanning electron microscope studies of eggshell; Hypocalcemia

INTRODUCTION

A decline in population of many species of birds of prey in the United States, Britain and elsewhere noted since early 1950 has been generally attributed to eggshell thinning and other reproductive failure induced by organochlorine pesticides, particularly compounds of the DDT group.

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It is well known that in the field not all bird species are susceptible to induced eggshell thinning. Opinion differs as to the sensitivity of mallards and ducks to organochlorines like lindane. At present duck eggs constitute about 5% of commercial poultry egg production in India [1]. Several duck improvement plans have been initiated at the government level to increase egg production. India is one of the largest users of agricultural pesticides [2] and hexachlorocyclohexane (HCH) compounds are the major pesticides used [3]. We report the effect of lindane (γ-HCH) on eggshell structure and quality of domestic duck in order to ascertain whether this pesticide causes shell thinning and other structural changes that may be detrimental to the well being of the developing embryo.

MATERIALS AND METHODS

Sexually mature indigenous domestic ducks (*Anas platyrhynchos domesticus*) procured from local markets were kept in outdoor enclosures provided with shelters and well drained soil, proper ventilation, free access to drinking water and food (with 2.6% calcium). Egg production was recorded regularly. Pure lindane (99.8%) dissolved in olive oil was administered intragastrically after the ducks had laid 3 eggs successively. The experiments were conducted for a period of 8 weeks in order to get a number of egg clutches. On the basis of pilot experiments involving dose-response studies, the following groups were made (five hens plus one drake per group):

Group I:	Control, vehicle only.
Group II:	Lindane 20 mg/kg body weight daily for 8 weeks.
Group III:	Lindane 20 mg/kg body weight thrice weekly for 8 weeks.
Group IV:	Lindane 20 mg/kg body weight twice weekly for 8 weeks.
Group V:	Lindane same as Group II and then stilboestrol 50 mg/kg body weight, one single injection.
Group VI:	Lindane same as Group III and then stilboestrol 50 mg/kg body weight, one single injection.
Group VII:	Stilboestrol, 50 mg/kg body weight, one single injection only.

Glycerine suspension of diethyl stilboestrol (DES) was injected 24 h after completion of an 8-week period.

The following techniques were used to assess eggshell thinning:

Ratcliffe index

Eggshells were opened by a small hole at one end. The contents were removed and the shells were washed with distilled water and allowed to air-dry. The Ratcliffe index or index of eggshell thickness was determined by the following equation [4].

$$\text{Ratcliffe index} = \frac{\text{Weight of eggshell (g)}}{\text{Length} \times \text{breadth (cm}^2\text{)}}$$

Measurement of shell thickness

Small fragments of shell taken from both the equator and the cap region of the eggs were boiled in sodium hydroxide solution (2.5% w/v) for about 5 min to remove the shell membranes, then washed with distilled water and air-dried. The thickness of the shell fragments were measured with a micrometer calibrated to nearest 0.01 mm.

Pore number and types per unit area in the shell

The number of pores/cm^2 in the shell was determined with the method of Tyler [5]. Pieces of both normal and treated shells taken from the equator region of eggs were dipped quickly in concentrated nitric acid, washed with distilled water, air-dried and viewed under the light microscope. Nitric acid moves through the pore, clears the pathway and makes them distinctly visible under the microscope, though the treatment might have enlarged the pore channels. After acid treatment the number of pores/cm^2 of shell was counted. The pores, as visualised under light microscope, were classified according to their size, as 'large', 'medium' and 'small' pores.

Rate of loss of water

To determine the loss of water vapour through the eggshells, emptied shells were filled with distilled water. Small holes that had previously been made in the shells were carefully covered with adhesive tape. Only sound eggs without any cracks and leaks were used. They were maintained in a dessicator over silica gel at 30 ± 1°C and at 31.824 Torr vapour pressure. Eggs were weighed daily and the rate of water loss was measured for 7 consecutive days. Water vapour conductance (G), which is a convenient measure of water loss, was calculated as follows [6]:

$$G = \frac{\text{Rate of water loss (mg/day)}}{\text{Vapour pressure difference (Torr)}}$$

Ultrastructure of shell by scanning electron microscope (SEM)

Small pieces of shells taken from the equator of eggs were mounted so that the edge and the inner surface with the mammillae and pore openings could be specially studied. Samples were sputter coated with gold and examined with an Hitachi S415A Scanning electron microscope. The shell membranes were predigested in boiling sodium hydroxide (2.5% w/v). The accelerating voltage was 25 kV.

Calcium content of plasma and shell gland mucoa

At the termination of experiment calcium content of plasma and shell gland mucosa was determined both in control and lindane-fed ducks. The shell gland mucosa was scraped out from the remaining tissue and 200 mg of the mucosa was homogenized with 10% trichloroacetic acid solution, centrifuged for 10 min at 1000 *g* and the supernatant was taken. Into 2 ml of this supernatant (or 2 ml of clear serum), 2 ml of distilled water and 1 ml of 4%

ammonium oxalate solution were added and mixed thoroughly. Calcium content was then determined according to Clark-Collip modification of Kramer-Tisdall method [7].

Statistical analyses were done by using Student's *t*-test and the values distributed at least at 0.05 level was considered to be significant.

RESULTS AND DISCUSSION

No ducks fed lindane exhibited any symptoms characteristic of insecticide poisoning even at the highest dose (20 mg/kg daily). Appetite, food intake and water consumption were normal and there was no significant difference in body weight between the control and the treated ducks. However, egg laying was severely affected by lindane. A control duck laid on average 30 eggs over the 8-week study period (frequency = 50%). On the other hand, following lindane administration, group II (lindane daily) and III (lindane thrice weekly) ducks stopped egg laying for the first 3 weeks; they then resumed egg laying, generally 1 egg/week/bird during the next 4 weeks and again stopped egg laying on 8th week until they were given DES. Egg laying in group IV ducks (lindane twice weekly) was not affected as in the other treated groups through the lag phase was slightly lengthened [see ref. 8 for details]. As a result, the frequency of egg laying in groups II, III and IV ducks became 8.3%, 11.7% and 40%, respectively, in contrast to 50% frequency in the control ducks. Also, both the size and weight of eggs of ducks belonging to group II and III became markedly reduced (Fig. 1). However, all the eggs were with formed shell membrane and there was no evidence of premature egg laying.

A perusal of Table I reveals that lindane has significantly reduced shell thickness in both group II and III birds as soon as they started laying after

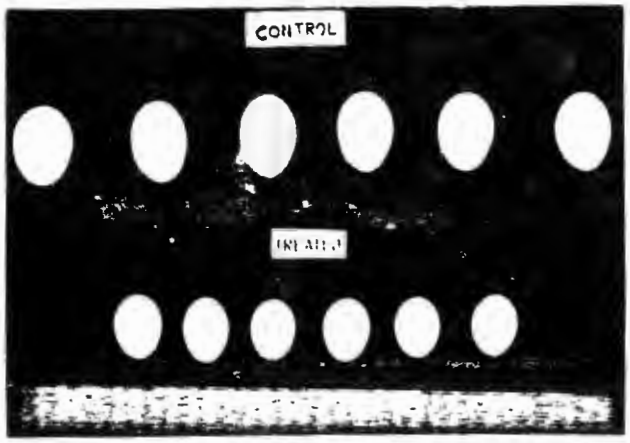

Fig. 1. Photograph of eggs of normal (upper) and lindane treated (20 mg/kg body wt, daily) ducks at 7th week. Note significant reduction in size.

TABLE I

THICKNESS, SIZE AND WEIGHT OF EGGSHELLS OF DUCKS AT 4th (A) AND 7th WEEK (B) OF LINDANE TREATMENT (5 EGGSHELLS/EACH GROUP)

Group			Means, standard errors and percentage differences between dosed and undosed groups				
			Eggshell thickness measurement (mm)		Weight of shell (g)	Size of shell (cm^2)	Ratcliffe index (g/cm^2)
			Equator	Cap			
Control	Group I		0.503 ± 0.03 (100%)	0.516 ± 0.01 (100%)	5.91 ± 0.26 (100%)	23.67 ± 0.56 (100%)	0.250 ± 0.002 (100%)
γ-HCH, daily	Group II	A	0.433 ± 0.003[a] (−14%)	0.442 ± 0.02[a] (−13.25%)	4.19 ± 0.32[a] (−29.03%)	19.60 ± 1.60[a] (−17.19%)	0.214 ± 0.012[a] (−14.40%)
		B	0.396 ± 0.01[a] (−21.27%)	0.409 ± 0.02[a] (−20.74%)	3.96 ± 0.40[a] (−32.99%)	19.41 ± 1.50[a] (−17.99%)	0.204 ± 0.014[a] (−18.40%)
γ-HCH, 3/week	Group III	A	0.442 ± 0.005[a] (−12.13%)	0.454 ± 0.02[a] (−12.01%)	4.34 ± 0.36[a] (−26.56%)	20.12 ± 1.11[a] (−14.99%)	0.216 ± 0.010[a] (−13.60%)
		B	0.410 ± 0.01[a] (−18.48%)	0.423 ± 0.03[a] (−18.02%)	4.17 ± 0.40[a] (−29.44%)	19.86 ± 1.30[a] (−16.10%)	0.209 ± 0.016[a] (−16.40%)
γ-HCH, 2/week	Group IV	A	0.495 ± 0.01 (−2%)	0.507 ± 0.01 (−1.74%)	5.72 ± 0.30 (−3.21%)	23.60 ± 1.40 (−0.29%)	0.242 ± 0.001 (−3.2%)
		B	0.475 ± 0.02 (−5.57%)	0.489 ± 0.03 (−5.23%)	5.60 ± 0.25 (−5.24%)	23.53 ± 0.75 (−0.59%)	0.238 ± 0.006 (−4.8%)

[a]Significant.

TABLE II

POROSITY CHANGES OF EGGSHELLS OF DUCK AT 4th (A) AND 7th WEEK (B) OF LINDANE TREATMENT (5 EGGSHELLS/ EACH GROUP)

Group		Number of pores/ cm^2 of shell	Type of pore and distribution (%)	Size of pore (mm^2)	Pore area per surface area of shell (mm^2/cm^2)
Group I		117.70 ± 6.10 (100%)	Large (53%)	0.018	1.80 ± 0.06 (100%)
			Medium (30%)	0.003	
			Small (17%)	0.001	
Group II	A	72.00 ± 5.60[a] (−38.83%)	Medium (30%)	0.003	0.34 ± 0.002[a] (−81.11%)
			Small (70%)	0.001	
	B	59.00 ± 4.50[a] (−50.13%)	Medium (10%)	0.004	0.25 ± 0.004[a] (−86.11%)
			Small (90%)	0.001	
Group III	A	80.13 ± 5.10[a] (−32.03%)	Large (80%)	0.018	0.40 ± 0.004[a] (−77.77%)
			Medium (20%)	0.003	
	B	67.20 ± 5.60[a] (−42.91%)	Medium (18%)	0.005	0.30 ± 0.005[a] (−83.33%)
			Small (82%)	0.001	
Group IV	A	112.60 ± 10.20 (−4.33%)	Large (51.9%)	0.018	1.72 ± 0.04 (−4.44%)
			Medium (27.3%)	0.003	
			Small (20.8%)	0.001	
	B	107.80 ± 11.50 (−8.41%)	Large (50%)	0.018	1.68 ± 0.04 (−6.67%)
			Medium (22%)	0.003	
			Small (28%)	0.001	

[a]Significant.

pesticide dosing (i.e. from 4th week onwards). The reduction in thickness reached maximum on the 7th week (18—21% at equator and 18—20% at cap) though the trend was set from the 4th week as well (12—14% at equator, 12—13% at cap). In group IV ducks, where egg laying was not interrupted, shell thinning was quite marginal even at the 7th week of pesticide dosing (Table I).

In the normal shells 3 types of pores were identified viz., 'large pores' (0.018 mm^2), 'medium pores' (0.003 mm^2) and 'small pores' (0.001 mm^2). Distribution of such pores in shells of normal ducks was 53%, 30% and 17%, respectively (Table II). Lindane administration caused remarkable alteration of the types of pores in both group II and III ducks. Thus the large pores became altogether absent from the 4th week in group II and from the 5th week in group III ducks. In the latter group, only 8% large pores were found on the 4th week. The absence of large pores in such shells induced proliferation of small pores (which were minimal in normal shells) and eventually they became the dominant type of pores (80—90%) with medium sized pores reducing to only 10—20%. The type of pore system in group IV birds

TABLE III

WATER VAPOUR CONDUCTANCE AND AVERAGE RATE OF WATER LOSS OF EGGSHELLS AT 4th (A) AND 7th WEEK (B) OF LINDANE TREATMENT (5 EGGSHELLS/EACH GROUP)

Group		Means, standard errors and percentage difference between dosed and undosed groups	
		Average rate of water loss (mg/day)	Water vapour conductance (mg/day/Torr)
Group I		220.20 ± 7.90 (100%)	6.92 ± 0.54 (100%)
Group II	A.	142.90 ± 6.50[a] (−35.10%)	4.49 ± 0.23[a] (−35.26%)
	B.	120.20 ± 5.70[a] (−45.41%)	3.78 ± 0.05[a] (−45.38%)
Group III	A.	157.20 ± 9.80[a] (−27.00%)	4.94 ± 0.18[a] (−27.17%)
	B.	130.50 ± 4.30[a] (−40.74%)	4.10 ± 0.10[a] (−40.75%)
Group IV	A.	214.90 ± 10.00 (−2.41%)	6.75 ± 0.50 (−3.18%)
	B.	200.00 ± 6.50 (−9.17%)	6.28 ± 0.22 (−9.25%)

[a]Significant.

remained almost unaffected. The mean number of pores in a given area of shells, i.e. mean pore density and mean area of pores per surface area of shells were also significantly reduced in groups II and III (Table II).

Information on the rate of water loss from the eggshells is given in Table III. Compared to the normal ducks the rate of loss of water was significantly reduced in eggs of group II and III birds from the 4th week after pesticide dosing, with maximum loss being recorded on the 7th week. In group IV ducks again, there was no marked change in water vapour conductance even on the 7th week of lindane treatment.

Within 24 h of DES administration, group V and VI ducks resumed egg laying regularly. DES treatment also neutralised the adverse effects of lindane on thickness, porosity and vapour conductance of eggshells (Tables IV—VI). The large pores which were obliterated in such eggshells reappeared (Table V) and the thickness, porosity and vapour conductance potential of the egg shells surpassed even the control ducks. Also the size and weight of such eggs became normal (Table IV) and egg laying was resumed within 24 h of DES post-treatment.

Scanning electron microscope (SEM) studies of the inner surface of eggshells revealed interesting structural changes in lindane fed ducks of groups II and III. In the control ducks, the mammillary layers have characteristic protruding mammillae. The knobs look like flowers with varied oriented crystalline flakes as petals (Fig. 2a). In shells of groups II and III ducks, this layer was particularly affected. The mammillae became smaller and tightly clustered (Figs. 2b and 2c) with apparent decrease in inter-mammillary space and signs of inhibition of calcite formation. Such a decrease in size of mammillae also indicates a reduction of the mammillary layer. Further, in the normal shells the mammillae were relatively broader at the base than in the thinned shells, where the apices of the mammillae became more pointed compared to the normal shells. Such structural changes of the shells were not seen in the group IV ducks (Fig. 2d). Examination of the fractured radial face of the thinned shells also reveals considerable reduction in total thickness and height of mammillae in groups II and III ducks (Fig. 3b and 3c) in contrast to control (Fig. 3a) or group IV birds (Fig. 3d).

Lindane treatment also reduced calcium content of both plasma and shell gland in group II and III ducks (Table VII); with DES treatment hypocalcemia was corrected.

In the past it has been often debated whether lindane induces eggshell thinning in birds either under controlled laboratory condition [9] or in the field [10]. We found that at doses relatively higher but not unrealistic with regard to the field situation, lindane significantly induced eggshell thinning in the domestic duck to the extent of 21% on the 7th week of treatment. It is known that thinning of more than 10% over a period of 1 year is sufficient to cause a general decline of a population [11]. As judged by our findings the effective dose for shell thinning appears to be around 700 mg; ingestion of about half of this dose induced shell thinning to about 5% only (Table I). Such marginal thinning is not uncommon among the

TABLE IV

EFFECT OF STILBOESTEROL (DES) POST-TREATMENT ON THICKNESS, SIZE AND WEIGHT OF EGGSHELLS OF LINDANE FED DUCK (5 EGGSHELLS/EACH GROUP)

	Group	Means, standard errors and percentage differences between dosed and undosed groups				
		Eggshell thickness measurement (mm)		Weight of shell (g)	Size of shell (cm^2)	Ratcliffe index (g/cm^2)
		Equator	Cap			
Control	Group I	0.503 ± 0.03 (100%)	0.516 ± 0.01 (100%)	5.91 ± 0.26 (100%)	23.67 ± 0.56 (100%)	0.250 ± 0.002 (100%)
γ-HCH, daily + DES	Group V	0.491 ± 0.01 (−2.38%)	0.502 ± 0.02 (−2.71%)	5.80 ± 0.32 (−1.86%)	23.50 ± 0.61 (−0.72%)	0.247 ± 0.001 (−1.20%)
γ-HCH, 3/week + DES	Group VI	0.495 ± 0.02 (−1.59%)	0.508 ± 0.02 (−1.55%)	5.85 ± 0.33 (−1.00%)	23.59 ± 0.63 (−0.34%)	0.248 ± 0.002 (−0.80%)

TABLE V

EFFECT OF STILBESTEROL (DES) POST TREATMENT ON POROSITY CHANGES OF LINDANE FED DUCK EGGSHELLS (5 EGGSHELLS/EACH GROUP)

Group	Number of pores/ cm^2 of shell	Type of pore and distribution (%)	Size of pore (mm^2)	Pore area/ surface area of shell (mm^2/cm^2)
Group I	117.70 ± 6.10 (100%)	Large (53%) Medium (30%) Small (17%)	0.018 0.003 0.001	1.80 ± 0.06 (100%)
Group V	110.20 ± 12.70 (−6.37%)	Large (48.5%) Medium (28.5%) Small (23%)	0.018 0.003 0.001	1.68 ± 0.09 (−6.67%)
Group VI	115.40 ± 13.00 (−1.96%)	Large (50.5%) Medium (29%) Small (20.5%)	0.018 0.003 0.001	1.76 ± 0.07 (−2.20%)

natural population and also does not adversely affect fledging success [12]. It was reported earlier that concentration of pesticide approaching rather toxic doses is needed to induce gross blockage of the reproductive system in birds [13]. Subtle changes of the breeding cycle may be caused by comparatively lower concentrations of pesticide [14].

Since ducks did not lay eggs prematurely (as evidenced by the presence of shell membranes in the shells), we can exclude premature termination of

TABLE VI

EFFECT OF STILBOESTEROL (DES) POST TREATMENT ON WATER VAPOUR CONDUCTANCE AND RATE OF WATER LOSS OF WHOLE EGG OF LINDANE FED DUCKS AT 30 ± 1°C AND 31.824 TORR (5 EGGSHELLS/EACH GROUP)

Group	Mean, standard errors and percentage differences between dosed and undosed groups	
	Average rate of water loss (mg/day)	Water vapour conductance (mg/day/Torr)
Group I	220.20 ± 17.90 (100%)	6.92 ± 0.54 (100%)
Group V	200.20 ± 18.50 (−9.10%)	6.29 ± 0.25 (−9.10%)
Group VI	210.45 ± 17.50 (−4.43%)	6.69 ± 0.23 (−3.32%)

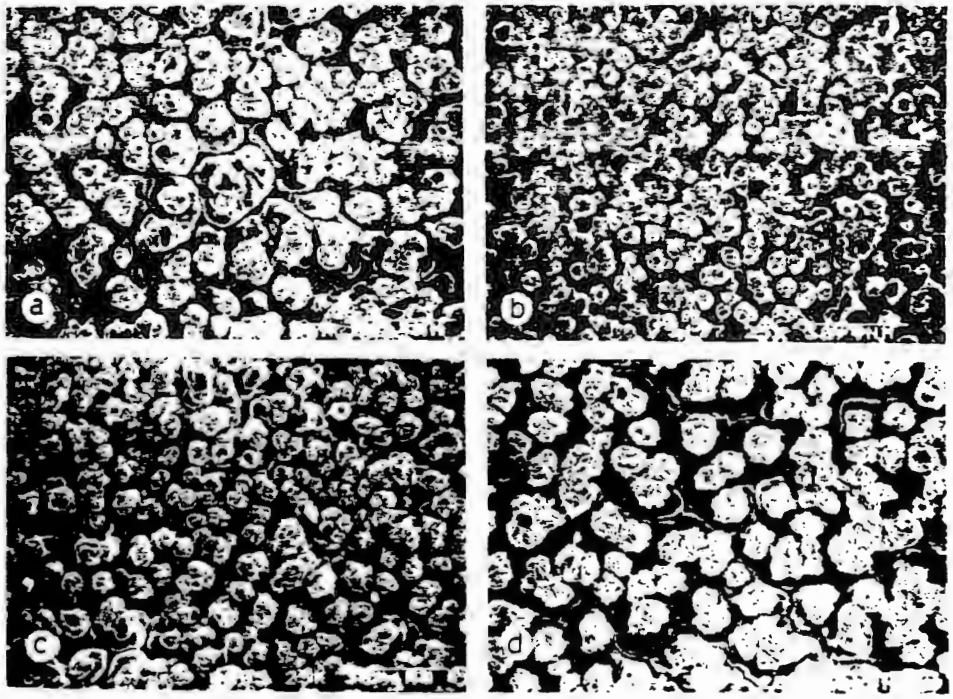

Fig. 2. Scanning electron micrographs of inner surface of eggshells of ducks. Bar = 100 μm. (a) Normal shell showing mammillary layer with typical mammillae and inter-mamillary spaces. (b) and (c) Treated shells at 7th week of daily and thrice per week lindane fed ducks respectively, showing small, clustered mammillae with signs of inhibition of calcite formation. (d) Treated shells at 7th week of twice per week lindane fed ducks. Note marginal changes compared to the normal (Fig. 2a).

shell growth (i.e. shorter period of shell formation) as a cause of shell thinning. The structural changes of eggshells, a reduction of the mammillary layer, merit some comments. It is known that when an egg enters the shell gland region, the only components present are fibrous membranes and the spherulite initiation sites which will be developed into mammillae [15]. Since the shell gland provides the essential shell components (i.e., calcium carbonate), the reduction of mammillae seems to be an indication of either a decrease in the rate of shell formation or a deficiency in the availability of shell materials in the uterine lumen or both. We have found a significant reduction of calcium level in both plasma and shell gland in such ducks (Table VII). Since DES administration rectified structural and other defects of eggshells as well as calcium deficiency inflicted by lindane, it appears that the reduction of calcium in the shell gland are due to possible estrogen deficiency per se caused by lindane.

Reduction of the density of pore and pore area per unit area of the shell (Table II) can be attributed to the observed absence of large pores in shells of such birds. Why the large pores were specially affected by lindane treat-

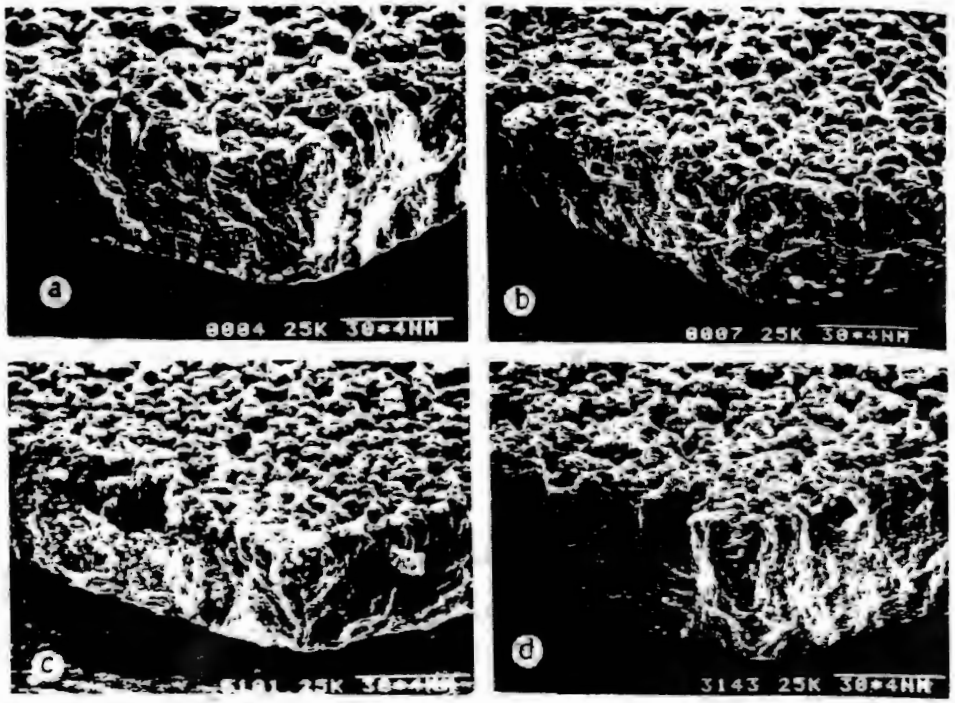

Fig. 3. Scanning electron micrographs of the fractured radial face of eggshells of ducks. Bar = 100 μm. (a) Normal. (b) and (c) Treated shells at 7th week of daily and thrice per week lindane fed ducks respectively, showing considerable reduction in total thickness of the edges and height and size of the mammillae, compared to the normal shells (Fig. 3a). (d) Treated shells at 7th week of twice per week lindane fed ducks. Note marginal changes in total thickness of edge and size and height of mammillae compared to the normal shells (Fig. 3a).

ment is presently not known, though the structural changes of the eggshells as revealed by the SEM studies may be attributed to such an abnormality. Fox [16,17] earlier reported that in the common tern viable eggs have higher shell porosity than those who failed to produce young. In ducks, ring doves or American kestrels DDE also induced both shell thinning and reduced water loss [18]. In the same paper the authors also reported fewer pores in the thin shells laid by the ducks. It is known that porosity of shells not only permits the embryo to exchange respiratory gases with the atmosphere but also facilitates the evaporation of water from the contents of eggs during incubation. Field observations by Drent [19] indicate that on average 16% of the water pressure at oviposition is lost by evaporation during incubation of an egg. Therefore, such porosity changes as induced by lindane is likely to impair the conductivity of the eggshell and might be highly detrimental for embryonic survival. HCH compounds are the most widely used agricultural pesticide in India. It remains to be assessed to what extent this pesticide contributed to the observed population decline of many bird species in India noted in recent past.

TABLE VII

CALCIUM CONTENT OF PLASMA AND SHELL GLAND MUCOSA IN CONTROL, LINDANE FED AND LINDANE + DES FED DUCKS (ON 8th WEEK OF LINDANE TREATMENT)

Group	Plasma (mg/100 ml)	Shell gland (mg/g of tissue)
I (Control)	22.2 ± 0.88 (100%)	1.10 ± 0.06 (100%)
II (γ-HCH, daily)	11.6 ± 0.60[a] (−47.8%)	0.32 ± 0.02[a] (−70.9%)
III (γ-HCH, thrice weekly)	12.7 ± 0.57[a] (−42.7%)	0.42 ± 0.01[a] (−61.8%)
IV (γ-HCH, twice weekly)	20.29 ± 1.00 (−8.6%)	0.98 ± 0.12 (−10.9%)
V (γ-HCH, daily + DES)	109.00 ± 2.60[a] (+909.30%)	1.26 ± 0.03[a] (+293.70%)
VI (γ-HCH, thrice weekly + DES)	109.48 ± 2.80[a] (+746.10%)	1.30 ± 0.01[a] (+209.50%)
VII (DES only)	113.30 ± 5.20[a] (+401.30%)	33.80 ± 2.50[a] (+317.30%)

[a]Significant.

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REFERENCES

1 G.C. Banerjee, Poultry, Oxford and IBH Publishing Co., New Delhi, Bombay, Calcutta, 1982, p. 122.
2 J.R. Allen, W.A. Hargraves, M.T. Stephen Hsia and Francis S.D. Lin, Comparative toxicology of Chlorinated compounds on mammalian species, in F. Matsumura (Ed.), Differential Toxicities of Insecticides and Halogenated Aromatics, Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt, 1984, p. 485.
3 B.L. Wattal, The role and use of pesticides in health practices. Pestic. Inform., 9(3) (1983) 43.
4 D.A. Ratcliffe, Decrease in eggshell weight in certain birds of prey. Nature, 215 (1967) 208.
5 C. Tyler, Studies on eggshells. II. A method for marking and counting pores. J. Sci. Food Agric., 4 (1953) 261.
6 Ar. A.C.V. Paganelli, R.B. Reeves, D.G. Greene and H. Rahn, The avian egg: Water vapour conductance, shell thickness and functional pore area. Condor, 76 (1974) 153.