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Eggshell Thickness in Mallards Fed Methylmercury

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Eggshell thinning has been linked to impaired reproduction in many wild birds (HICKEY and ANDERSON 1968, RATCLIFFE 1967, KEITH et al. 1970, ANDERSON and HICKEY 1972, BLUS et al. 1974). Both field and laboratory studies have implicated DDE as the primary cause of shell thinning (BLUS et al. 1972, LINCER 1975, HEATH et al. 1969, LONGCORE et al. 1971, WIEMEYER and PORTER 1970, MCLANE and HALL 1972, HAEGELE and HUDSON 1974).

Previous work of my own and others led me to believe that methylmercury may cause some eggshell thinning in birds. When I fed mallards (Anas platyrhynchos) 0.5 ppm mercury in the form of methylmercury dicyandiamide, eggshells were, on the average, 4.4% thinner than controls, a difference that was statistically significant (HEINZ 1979). In another study, I found the eggshells of mallards fed the same level of methylmercury to be 3.8% thinner than controls (unpublished data); however, sample sizes were small and the differences were not statistically significant. Black ducks (Anas rubripes) fed 3 ppm mercury as methylmercury laid eggs whose shells were thinner by 7.4% at the equator, 9.3% at the cap, and 11.9% at the apex than were the shells of controls (FINLEY and STENDELL 1978); these differences were not statistically significant and differences were considerably less in the second breeding season.

Because of the somewhat uncertain findings on the ability of methylmercury to thin eggshells, I designed the present study to determine whether methylmercury in the diet of mallards would thin their eggshells and whether it would add to eggshell thinning caused by DDE.

METHODS

On 15 December 1976, one 18-month-old female game-farm mallard was randomized to each of 141 one-m^2 pens provided with flowing water and ad libitum food. One hundred of these pens were indoors and 41 were outdoors. On 5 January, all birds were switched from untreated feed to the following diets: 23 were fed a control diet, 23 were fed a diet containing 1 ppm methylmercury chloride (MMC), 24 were fed 5 ppm MMC, 23 were fed 5 ppm DDE, 23 were fed 1 ppm MMC + 5 ppm DDE, and 25

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were fed 5 ppm MMC + 5 ppm DDE. Methylmercury chloride and DDE were first dissolved in a small amount of acetone and then into propylene glycol. The propylene glycol was then added to the feed to comprise 1% by weight. The feed was commercial duck developer mash until 3 March when all birds were switched to duck breeder mash.

Lighting in the indoor pens was adjusted periodically to match natural outdoor day length. There was no heat in the sheds holding the indoor pens and, therefore, temperatures in these sheds resembled those outdoors.

Eggs were collected each day. The length and width of each egg were measured and then the eggs were opened by cutting through the shell around the equator (the widest part of the egg). The shells were gently rinsed out, air-dried, and weighed. The thickness index was calculated as follows: thickness index = weight of shell (mg)/[length of shell (mm) x width of shell (mm)] (RATCLIFFE 1967). After each shell was weighed, two 5-mm holes were drilled -- one at the apex (pointed end of the egg) and one as close to the cap (blunt end) as possible without hitting the aircell. At each location on the egg (equator, cap, and apex) I recorded the average of three shell thickness measurements (shell plus shell membrane) using a micrometer accurate to the nearest 0.005 mm.

Data were collected from the first 12 eggs laid by each hen. This was done to approximate a normal clutch of eggs. For the sake of consistency, if a hen laid fewer than 12 eggs, the data from her eggs were not used. Further, to more nearly approximate a natural situation, data on eggs were used only if the clutch of 12 eggs was laid within a 30-day period. In instances where birds laid 12 eggs but some were unsuitable for shell measurements because they were broken in the pens, the data from the remaining intact eggs were used.

The statistical analysis was a mixed model, nested analysis of variance (SCHEFFÉ 1959), which allowed the effect of pen location (indoors or outdoors) to be separated from the effect of dietary treatment. Separation of means was done by using Duncan's multiple range test (DUNCAN 1955).

RESULTS AND DISCUSSION

There were no statistically significant effects of methylmercury on eggshell thickness or thickness index (Table 1).

There were no significant differences in shell thickness or thickness index between eggs laid by hens indoors or outdoors, and there were no significant interactions between diet and pen location. Hens fed 5 ppm DDE laid thinner-shelled eggs than did controls or, in most instances, birds fed 1 or 5 ppm MMC. The shells of ducks fed 1 ppm MMC + 5 ppm DDE were significantly thicker than those of birds fed only DDE. This result is somewhat puzzling because I know of no studies indicating that methylmercury can reduce the shell-thinning effect of DDE. Because this is the only reported occurrence of such an antagonistic effect and because 5 ppm MMC did not produce the effect, I am uncertain whether it is a real effect or just a chance occurrence.

TABLE 1

Shell thickness and thickness index of eggs laid by control mallards and mallards fed methylmercury chloride and DDE.

Dietary Treatment (ppm)		Number of Hens	Mean Eggshell Thickness (mm)			Mean Thickness Index[a]
MMC	DDE		Equator	Cap	Apex	
0	0	15	0.370a[b]	0.360a	0.325a	2.11a
1	0	14	0.360a	0.345ab	0.320a	2.03a
5	0	13	0.370a	0.355a	0.325a	2.08a
1	5	12	0.360a	0.345ab	0.320a	2.01a
5	5	17	0.340b	0.330bc	0.300b	1.89b
0	5	18	0.340b	0.325c	0.295b	1.88b

[a] Thickness index = $\frac{\text{Eggshell weight (mg)}}{\text{Egg length (mm)} \times \text{Egg width (mm)}}$

[b] Means that do not share a letter in common were significantly different at $\alpha = 0.05$.

This study gives no evidence that low dietary levels of methylmercury cause eggshell thinning. The statistical design of this study was fairly strong, in that it involved measuring up to 12 eggs from many different hens on each treatment. Yet the results are somewhat incompatible with those from earlier studies I ran using birds from the same source (HEINZ 1979, and unpublished data). These earlier studies showed a few percentage points of eggshell thinning in mallards fed methylmercury. One possible explanation is that these earlier experiments were conducted at different times, using different groups of birds, slightly different test conditions, and a different formulation of methylmercury. These differences could account for the different results. For example, the present study was started during an exceptionally severe winter in Maryland. The mallards were under considerable stress, especially in the indoor pens where at times birds were deprived of water when pipes froze, splashed water froze on the floors making regular cleaning impossible, and the absence of nest box shelters and large water pans forced birds to spend all of their time on the vinyl-coated wire floors.

None of the birds in outdoor pens died, but 13 in indoor pens died during the course of the experiment. Eight of these birds were fed a diet containing 5 ppm MMC, alone or in combination with DDE. Two were controls, one of which likely died from head injuries caused by banging into its cage. Three were fed 1 ppm MMC, alone or with DDE. It is possible that, under conditions of stress, some birds died of methylmercury poisoning, at least those fed 5 ppm MMC. In another study, mallard drakes I had fed 3 ppm mercury as methylmercury dicyandiamide died after prolonged dietary treatment (unpublished data).

Under conditions of stress, eggshell thicknesses, including those of controls, could have been affected. By eliminating data from all but those birds that laid 12 eggs within a 30-day period, I feel I am looking at results from at least fairly healthy birds. Controls in the present study, however, did lay eggs with thinner shells than did controls in other studies using the same source of mallards (HEINZ 1979, and unpublished data). For this reason, I do not feel it is proper to say that results from the present study conclusively show that methylmercury causes no eggshell thinning whatsoever in ducks, but I do feel it is fair to conclude that methylmercury is, at best, a weak eggshell-thinning agent.

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