Effects of Low Dietary Levels of Methyl Mercury on Mallard Reproduction

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Contamination of the environment with mercury has caused concern in recent years (GOLDWATER 1971, HAMMOND 1971, PEAKALL and LOVETT 1972). HEINZ (1971) reviewed the literature on the harmful effects of mercury to birds and found that much is still unknown about the effects of long-term, low dietary levels of mercury on birds. The data in this paper were collected in conjunction with a study of the effects of methylmercury dicyandiamide on the behavior of mallard ducklings (*Anas platyrhynchos*); although these data are preliminary, they suggest that 3 ppm mercury (as methylmercury) in the diet has a harmful effect on mallard reproductive success.

Methods

Care of Adults: Breeders were 18-month-old game-farm mallard ducks purchased from Whistling Wings in Hanover, Illinois. Three males and 10 females were randomized to each of three 2.33 m x 3.56 m pens. Flowing water and commercial duck breeder mash were always available. Each pen was equipped with a straw-filled 0.61 m x 1.6 m x 0.61 m high nestbox. On January 27, 1972, the following treatments were randomized to the three breeding pens: controls, 0.5 ppm mercury, and 3 ppm mercury (as methylmercury dicyandiamide, the active ingredient in the fungicide Morsodren). The Morsodren was dissolved in propylene glycol and then mixed into the feed in the ratio of 2 parts propylene glycol to 98 parts of mash; controls received an equal amount of clean propylene glycol. Two samples of control diet and 3 samples each of 0.5 ppm mercury diet and 3 ppm mercury diet were saved for chemical analysis.

Collection and Incubation of Eggs: Eggs were collected each day and were recorded as being sound (in good condition), cracked, or shell-less. I also noted whether each egg was laid inside or outside the nestbox. Eggs were stored at 13° C and were incubated at 2-week intervals in a Petersime incubator at 37.5° C and at about 80% relative humidity. For every 2-week collection period, 4 sound eggs were randomly selected from each treatment; the contents of the 4 eggs were pooled, weighed, and saved in a frozen condition for mercury analysis. Eggshell thickness measurements (air-dried shells with membranes) were made at the equator of each egg.

Bulletin of Environmental Contamination & Toxicology,
Vol. 11, No. 4 © 1974 by Springer-Verlag New York Inc.

Care of Young: Healthy hatchlings were raised in heated Petersime brood-units with water and duck starter mash always available. Ducklings were fed diets containing the same concentration of mercury as their parents had been fed. One-week-old survivors were counted. Ducklings from the fifth egg-collection period were weighed at 1 and 7 days of age to determine percentage weight gain. Of the 1-week survivors from the fourth egg-collection period, 30 randomly selected ducklings from the control group, 30 randomly selected from the group receiving 0.5 ppm mercury, and all of the 28 survivors from the group receiving 3 ppm mercury were raised to 20 weeks of age. The number surviving in each group was recorded.

Mercury Analysis: Feed and egg samples were analyzed for mercury by WARF Institute, Inc. using cold vapor atomic absorption. A 5 g sample of feed or a 10 g sample of the homogenized pool of eggs was digested by refluxing with sulfuric and nitric acids. Hydroxylamine hydrochloride and stannous chloride were added to the digest to reduce the mercury (II) ions to metallic mercury. The sample was aerated, and the mercury was measured in the air stream passing through a gas cell by atomic absorption. The lower limit of detection was about 0.05 ppm mercury. Mercury recoveries using this method were 93 and 94% with spiked feed samples and 96 and 97% with spiked egg samples.

Results

Mercury Content of Feed: Analysis of feed revealed that both samples of control feed contained less than 0.05 ppm mercury. Feed samples from the 0.5 ppm mercury treatment contained 0.49, 0.53, and 0.64 ppm mercury; and feed from the 3 ppm mercury treatment, 3.32, 3.32, and 3.55 ppm mercury; these values seem to be within the range of accuracy of the analytical methods used.

Health of Adults: Only one adult duck died during the 12 months of the experiment. This hen (in the 0.5 ppm mercury treatment group) died on March 3 from what was diagnosed as bacterial valvular endocarditis leading to heart failure. The hen was otherwise in good condition, and her death is not believed to have been related to mercury dosage. All other ducks appeared healthy throughout the experiment.

Reproductive Success: Patterns of egg-content weight, shell thickness, mercury residues in eggs, number of sound eggs laid, healthy hatchlings, and survival of hatchlings are illustrated in A, B, C, D, E, and F, respectively, of Figure 1. Each point in A,B, and C is the mean of 4 randomly selected eggs. Each point in D and E represents data from all the eggs that were collected for that 2-week period. Each point in F represents data for only those ducklings which hatched within 26 days of incubation. These data cover the first 21 weeks the adults were on dosage. Since, in a statistical sense, there are no

treatment replicates (the breeding pen is used as the unit of measurement), no statistical analyses were performed on the data in Figure 1; certain trends are apparent, however, in the graphical representation of the data.

During the peak of the egg-laying season (weeks 4 through 9 on dosage), eggs from control hens were heavier than eggs from hens receiving either of the mercury treatments. Differences were greater in comparison with the 3 ppm mercury group. Eggs from hens receiving 3 ppm mercury remained small for the remaining weeks on dosage, but differences from the eggs laid by controls or by ducks receiving 0.5 ppm mercury diminished as time passed.

There were no consistently large differences in eggshell thickness among the three groups. Eggs laid by control birds generally had somewhat thicker shells, but this may only have reflected the fact that the eggs were larger.

Pools of control eggs from the first and last 2-week collection periods contained less than 0.05 ppm mercury. Residues in eggs from the 0.5 ppm mercury dosage plateaued at about 1 ppm mercury after 4 to 5 weeks of dosage; an abnormally high value of 2.34 ppm in the pool for weeks 16 and 17 is believed to represent an error. Residues in eggs from hens receiving the 3 ppm mercury dosage rose gradually until weeks 8 and 9; afterwards residues ranged between 6.46 and 9.19 ppm mercury.

Fewer sound eggs were laid by hens fed 3 ppm mercury than by controls or by hens fed 0.5 ppm mercury. Egg production by ducks receiving 3 ppm mercury stopped after 21 weeks of dosage. Production by ducks receiving 0.5 ppm mercury stopped after 31 weeks, and production by controls stopped after 43 weeks.

There were no significant differences by interaction chi square [$0.70 > P\ (\chi^2_{2df} > 1.008) > 0.50$] among treatments in the proportion of eggs laid outside the nestbox during the first 21 weeks of dosage. Controls laid 5.6% of their eggs outside the nestbox, hens fed 0.5 ppm mercury laid 6.0% outside, and hens fed 3 ppm mercury laid 7.1% outside.

Hatching success was also lower for the eggs laid by the hens fed 3 ppm mercury than for either controls or the group receiving 0.5 ppm mercury. There were no consistent differences in hatching success between eggs laid by control birds and those laid by hens fed 0.5 ppm mercury.

The greatest difference in reproductive success among the groups was the large proportion of ducklings from the 3 ppm mercury group that died within the first week of life. Histological examination revealed that these ducklings died from damage to nervous tissue in the cerebellum; these histological findings will be published in detail in a separate paper.

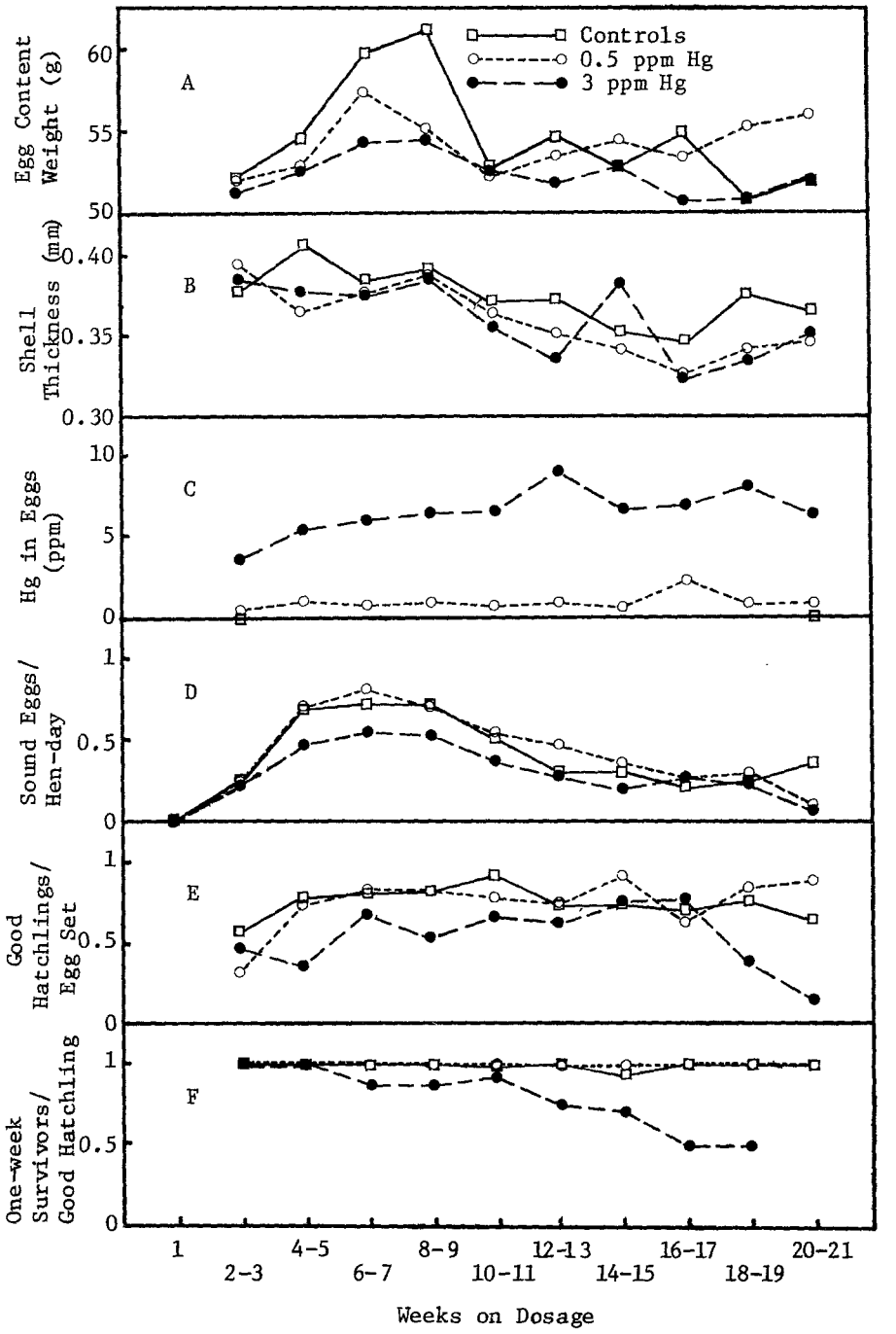

Figure 1. Reproductive success of mallard ducks.

On the assumption that percentage weight gain is independent of parentage, ducklings from weeks 10 and 11 on dosage differed significantly ($F = 6.814$; $d f = 2 \cdot 118$; $0.005 > P > 0.001$) during the first week of life. Controls, which averaged a 123.30% weight gain, did not differ from ducklings from 3 ppm mercury dosage (120.62% gain); however, both of these groups gained significantly less than ducklings from the 0.5 ppm mercury dosage (138.82% gain). Means were separated by DUNCAN's (1955) New Multiple Range Test with modifications for unequal sample sizes (KRAMER 1956).

Of the 1-week survivors from the hatch of weeks 8 and 9, only 2 ducklings did not survive to 20 weeks of age; one of 30 controls and one of 30 fed 0.5 ppm mercury were injured in handling and were killed. No signs of mercury poisoning appeared in any birds.

Discussion

A continuous dietary dosage as high as 3 ppm mercury (as methylmercury) apparently is not lethal to adult mallards over a 12-month period. HILL (personal communication) calculated the LC_{50} for mallard ducklings to be 60 ppm methylmercury dicyandiamide (about 41.7 ppm mercury); 10-day-old ducklings were fed the toxic diet for 5 days followed by 3 days of untreated feed. In other studies, hens (*Gallus gallus*) died when fed seed dressed with 15-20 ppm mercury (as methylmercury) (BORG et al. 1969), and goshawks (*Accipiter gentilis*) died after 30 to 47 days on a diet of chicken muscle and liver containing about 13 ppm mercury (BORG et al. 1970).

Although the adult mallards fed dietary dosages of either 0.5 or 3 ppm mercury remained healthy, there were serious effects on reproduction. Mallards receiving mercury laid smaller eggs than did controls; the significance of this finding is not known. FIMREITE (1971) also noted that ring-necked pheasant (*Phasianus colchicus*) hens fed a diet containing methylmercury laid eggs that were smaller than control eggs; diets containing as little as 0.92 ppm mercury produced this effect (FIMREITE, personal communication).

Although I noticed no appreciable eggshell thinning in eggs laid by ducks fed diets containing methylmercury, STOEWSAND et al. (1971) reported eggshell thinning in Japanese quail (*Coturnix coturnix japonica*) fed 8 ppm mercury (as mercuric chloride). SPANN et al. (1972) found no significant differences in eggshell thickness between control pheasants and those fed a diet containing 4.2 ppm mercury (as ethylmercury). PEAKALL and LINCER (1972) found no eggshell thinning in ring doves (*Streptopelia risoria*) given a 10 mg/kg i. m. injection of dimethyl mercury, in ring doves given a 10 mg/kg oral or i. m. injection dose of monomethyl mercury chloride, or in American kestrels (*Falco sparverius*) fed a diet containing 10 ppm dimethyl mercury.

Impaired reproduction of ducks fed 3 ppm mercury in the diet resulted from the combined effects of reduced egg laying, poorer hatching success, and increased mortality of ducklings; the net result of these 3 factors was that ducks fed 3 ppm mercury in the diet produced only 46.5% as many 1-week-old ducklings as did the control ducks.

Methylmercury-induced reproductive impairment has been reported for chickens (BORG et al. 1969, TEJNING 1967) and pheasants (FIMREITE 1971) in experimental studies.

I did not find excessive numbers of eggs laid outside the nestbox by ducks on mercury treatment as TEJNING (1967) discovered with chickens fed diets containing about 4.4 ppm mercury (as methylmercury).

The increased growth rate of ducklings from the 0.5 ppm mercury dosage group may indicate a stimulatory effect of low levels of methylmercury. FIMREITE (1971) found that methylmercury stimulated egg laying in pheasants; a dosage of about 3.66 ppm mercury in the diet for 2 weeks caused this effect (FIMREITE, personal communication).

For both mercury treatments, residues of mercury in the eggs reached levels about twice those in the feed. Even a level of 1 ppm mercury, which occurred in the eggs from the ducks fed 0.5 ppm mercury, is rarely encountered in wild duck eggs. Mallard eggs gathered in the Canadian prairie provinces contained less than 0.1 ppm mercury; gadwall (*Anas strepera*) and lesser scaup (*Aythya affinis*) eggs contained 0.1 to 0.3 ppm mercury (VERMEER 1971). Even in the Lake St. Clair area, which appears to be heavily polluted with mercury, mallard eggs generally contained less than 1 ppm mercury; the highest value was 2.7 ppm (DUSTMAN et al. 1972), still well below the 6 ppm level at which reproduction suffered in my experimental ducks fed 3 ppm mercury in the diet. Eggs of certain other species of birds have contained higher levels of mercury than have been found in mallard eggs. A pied-billed grebe (*Podilymbus podiceps*) egg from Lake St. Clair contained 4.0 ppm mercury as did the egg of one common tern (*Sterna hirundo*); a second tern egg contained 6.25 ppm mercury (DUSTMAN et al. 1972).

Mallards may be a convenient experimental species to test for methylmercury-induced reproductive effects even though this species is not likely to carry high burdens of mercury in the wild.

Summary

Mallard ducks were fed a control diet or a diet containing 0.5 ppm or 3 ppm mercury (as methylmercury dicyandiamide). Health of adults and reproductive success were studied. The dietary level of 3 ppm mercury had harmful effects on reproduction, although it did not appear to affect the health of the

adults during the 12 months of dosage. Ducks that were fed the diet containing 0.5 ppm mercury reproduced as well as controls, and ducklings from parents fed 0.5 ppm mercury grew faster in the first week of life than did controls.

The greatest harm to reproduction associated with the diet containing 3 ppm mercury was an increase in duckling mortality, but reduced egg laying and increased embryonic mortality also occurred.

During the peak of egg laying, eggs laid by controls tended to be heavier than eggs laid by ducks fed either level of mercury; however, there seemed to be no eggshell thinning associated with mercury treatment.

Levels of mercury reached about 1 ppm in eggs from ducks fed a dietary dosage of 0.5 ppm mercury and between 6 and 9 ppm in the eggs from ducks fed 3 ppm mercury.

Acknowledgments

I thank the following people for contributing to the study and manuscript preparation: R. Heath, P. Heinz, W. Jones, W. Kramer , and L. Stickel.

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