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METHYLMERCURY: REPRODUCTIVE AND BEHAVIORAL EFFECTS ON THREE GENERATIONS OF MALLARD DUCKS

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Abstract: Three generations of mallard ducks (*Anas platyrhynchos*) were fed either a control diet or a diet containing 0.5 ppm mercury in the form of methylmercury. The levels of mercury in adult tissues and eggs remained about the same over 3 generations. The methylmercury diet had no effect on adult weights or weight changes during the reproductive season. Females fed a diet containing 0.5 ppm mercury laid a greater percentage of their eggs outside their nestboxes than did controls, and also laid fewer eggs and produced fewer ducklings. Methylmercury in the diet appeared to result in a small amount of eggshell thinning. Ducklings from parents fed methylmercury were less responsive than controls to tape-recorded maternal calls, but were hyper-responsive to a frightening stimulus in avoidance tests; there were no significant differences in locomotor activity in an open-field test.

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The purpose of this paper is to describe reproductive and behavioral effects of methylmercury on 3 generations of mallard ducks. Findings for the 1st and 2nd generations were reported earlier (Heinz 1974, 1975, 1976*b*, 1976*c*, Heinz and Locke 1976). Here, I have combined the data for all 3 generations to determine changes from generation to generation and overall effects that might not have been statistically significant in the analysis of single generations.

To my knowledge, there have been no reported multiple generation studies on the effects of mercury or any other environmental pollutant on waterfowl. Such studies have been reported for Japanese quail (*Coturnix coturnix japonica*) (Carnio and McQueen 1973), ring-necked pheasants (*Phasianus colchicus*) (Dahlgren and Linder 1974), and ring doves (*Streptopelia risoria*) (Peakall et al. 1972). I thank the following people for their help in the study: C. S. Cruger, R. B. Frederick, D. C. Gray, R. G. Heath, P. A. Heinz, G. Hensler, and N. C. Miller. J. B. Elder provided the Morsodren used in this study and others.

METHODS

An abbreviated description of the care of adults, care of eggs and young, and behavioral tests is given below. Additional details were reported previously (Heinz 1974, 1976*b*, 1976*c*). A 1-way analysis of variance was used to check for changes in mercury levels in tissues from generation to generation. Reproductive and behavioral comparisons involved 2 factors (treatment and generation); these comparisons were made with a 2-way analysis of variance. A significance level of 0.05 was used. Generation effects are not discussed because significant differences from 1 year to another, if any, would not be related to mercury treatment but to other factors such as weather.

Care of Adults

The game-farm mallards used as breeders in the 1st generation were 18-month-old males and 30-month-old females. Breeders in the 2nd and 3rd generations were randomly selected offspring from designated hatches of control eggs and eggs of parents fed mercury. Offspring

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were paired randomly, except that brother-sister pairs were excluded. Mercury treatment in the 1st generation began when the breeders were adults (Heinz 1976c:83). In the 2nd and 3rd generations mercury treatment began at 9 days of age. The mercury was in the form of methylmercury dicyandiamide, the active ingredient in the fungicide Morsodren. The dietary concentration was set at 0.5 ppm mercury in dry duck mash, equivalent to about 0.1 ppm in a natural succulent duck diet (Heinz 1975:554–555). In the 1st generation there were 10 pairs of controls and 9 pairs of ducks fed 0.5 ppm mercury. In the 2nd and 3rd generations there were 14 pairs of each treatment. Each pair of breeders was randomized to a 1-m^2 pen and provided with a nestbox and ad libitum food and water. Samples of feed were saved to confirm the mercury level.

For the 2nd and 3rd generations adults were weighed in January and May to check for differences in weights or weight changes during the reproductive season, and food consumption and wastage were measured over a 4-day period in May. Adults were sacrificed each year in July. Liver samples (the tip of the right posterior lobe) were saved for analysis from 3 randomly selected control males and females. Liver samples were saved from all females and 3 randomly selected males fed the treated diet. Samples of kidney, breast muscle, brain, ovary, and primary feathers also were analyzed from 3 randomly selected females in the mercury treatment. All samples were frozen and analyzed for total mercury using cold vapor atomic absorption (Joint Mercury Residues Panel 1961) at WARF Institute, Inc., Madison, Wisconsin. The lower limit of detection was about 0.05 ppm mercury. All mercury residues are reported on a wet-weight basis.

Care of Eggs and Young

Eggs were collected each day for several weeks during the peak of the reproductive season. Each egg was recorded as being sound, cracked, or shell-less, and whether laid inside or outside the nestbox. Eggs were incubated at 2-week intervals. One egg was randomly selected from each hen during a designated 2-week collection period. Control eggs were pooled for analysis, but eggs from hens fed mercury were analyzed individually. Shell thickness measurements were made at the equator of each egg; thickness index was calculated according to Ratcliffe (1967). Ducklings were held in brooders for 1 week. Water and untreated feed were available at all times. In the 2nd and 3rd generations, ducklings were weighed shortly after hatching and again 1 week later.

Controls and ducks fed mercury were compared for (1) percentage of eggs cracked or shell-less, (2) percentage of eggs laid outside the nestbox, (3) sound eggs laid per hen-day, (4) hatching success, (5) duckling survival, (6) ducklings produced per hen, (7) eggshell thickness, and (8) duckling weight and weight gain. Angular transformations were made on all percentage data, except percentage weight gain. For statistical comparisons in which data for more than 1 egg or duckling were collected per hen, I computed a mean value for each hen, based on all the eggs laid by that hen or ducklings hatched from her eggs.

Behavior Tests

Approach responses of ducklings to maternal calls were measured shortly after hatching. Each duckling was randomly assigned to a runway in a test apparatus and its responses to a tape-recorded call of a female mallard were measured. Ducklings from parents fed mercury

Table 1. Residues of mercury (ppm wet-weight, mean ± SE) in eggs and tissues of 3 generations of mallard hens fed a diet containing 0.5 ppm mercury.

Tissue[a]	Ppm mercury in generation: 1	2	3
Egg	0.79 ± 0.041	0.86 ± 0.098	0.84 ± 0.055
Liver	1.62 ± 0.160	0.89 ± 0.112[b]	1.49 ± 0.141
Kidney	1.82 ± 0.399	1.52 ± 0.364	1.60 ± 0.240
Breast muscle	0.82 ± 0.053	0.67 ± 0.062	0.83 ± 0.070
Brain	0.50 ± 0.057	0.44 ± 0.042	0.59 ± 0.044
Ovary	0.65 ± 0.100	0.51 ± 0.132	0.58 ± 0.095
Primary feathers	11.17 ± 0.555	9.03 ± 0.396	9.07 ± 1.268

[a] There were 9 samples of egg and liver in generation 1, and 14 in generations 2 and 3; sample size was 3 for all other tissues, for all generations.
[b] Different from generations 1 and 3 ($P = 0.05$).

were compared to controls for percentage of ducklings that approached the call and, for those ducklings that did approach, for time taken to approach.

Avoidance responses to a frightening stimulus were also measured by testing each duckling in a runway in a test apparatus. A revolving axle that produced noise and a flashing pattern frightened the ducklings, and the distance each bird retreated from the stimulus was measured.

The behavior of a randomly selected subsample of ducklings in an open field was also measured in the 2nd and 3rd generations. A bird was placed in a sound-attenuated wooden box equipped with photoelectric sensors, and the locomotor activity of the bird, as expressed by the number of light-beam interruptions in 5 minutes, was measured.

For approach, avoidance, and open-field tests I computed a mean for each hen, based on the measurements of behavior of all of her young tested. The number of ducklings per hen tested for open-field behavior varied from 4 to 9. For the approach and avoidance tests, the means for hens were based on a variable number of young; except for 1 hen that provided only 2 ducklings, the number ranged from 8 to 94. During the course of the 3 generations, a total of 3.160 ducklings was tested, or an average of about 42 per hen. Angular transformations were made on percentage data.

RESULTS AND DISCUSSION

Mercury Residues

Residues are reported as means ± 1 standard error. Feed samples from the 0.5-ppm mercury treatment contained 0.53 ± 0.006, 0.47 ± 0.021, and 0.43 ± 0.037 ppm mercury for the 1st, 2nd, and 3rd generations, respectively. All samples of control feed and eggs for 3 generations contained less than 0.05 ppm mercury. One control male during the 1st generation had 0.14 ppm mercury in its liver. All other control samples of liver contained less than 0.1 ppm mercury.

The only significant difference among residues of mercury in the eggs and tissues of females fed 0.5 ppm mercury for 3 generations was a lower level of mercury in the livers of females in the 2nd generation than in the 1st or 3rd (Table 1); the reason for this difference is not known. There were no significant differences from generation to generation in levels of mercury in the livers of males fed 0.5 ppm mercury; levels were 2.75 ± 0.281, 3.90 ± 0.805, and 6.44 ± 2.524 ppm for the 1st, 2nd, and 3rd gen-

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Table 2. Food consumption and wastage (mean ± SE) by mallards fed either a diet containing 0.5 ppm mercury or a control diet.

Generation	Number of pairs	Food eaten (g/kg of body weight)		Food wasted (g/kg of body weight)	
		Control	Mercury	Control	Mercury
2	14	119 ± 6.7	174 ± 7.1‡	6 ± 1.1	13 ± 3.2
3	14	137 ± 4.7	139 ± 9.8	15 ± 1.3	17 ± 3.1
Combined (2 & 3)	28	128 ± 4.4	156 ± 6.8‡	11 ± 1.2	15 ± 2.2

‡ Different from controls ($P = 0.001$).

erations, respectively. The significantly lower levels of mercury in the livers of females than in those of males may have resulted from elimination of mercury through continuous egg laying.

A previous paper (Heinz 1976c) reviewed the levels of mercury found in the tissues and eggs of birds collected in the wild. More recently published literature supports the conclusion that ducks and other birds collected from contaminated areas in the wild sometimes contained more mercury than my experimental mallards did. Baskett (1975) found isolated examples of mercury levels in the breast muscle of dabbling ducks that exceeded the levels in my mallards; diving and sea ducks generally had higher levels of mercury than did dabbling ducks, but usually the levels were below 0.5 ppm. Common mergansers (*Mergus merganser*), hooded mergansers (*Lophodytes cucullatus*), and common goldeneyes (*Bucephala clangula*) from Ball Lake, Ontario, Canada, had mercury levels in liver and breast muscle that generally far exceeded the levels in my mallards (Annett et al. 1975). Additional literature (Drobney 1973, Hesse et al. 1975, Benson et al. 1976) confirms that fish-eating birds from contaminated areas often contain mercury levels far in excess of levels in the mallards I fed 0.5 ppm mercury.

Weight and Food Consumption

There were no significant differences in weights or weight changes during the reproductive season between controls and adults fed mercury. No adults died during the 3 generations and no birds appeared sick or weak during the study. During the 2nd generation, adults fed 0.5 ppm mercury ate significantly more feed

Table 3. Mean measurements of reproduction for 3 generations of mallards fed either a diet containing 0.5 ppm mercury or a control diet.

Generation[a]	% eggs laid outside nestbox		% egg cracked or shell-less		Sound eggs laid/hen-day[b]		% incubated eggs producing normal hatchlings		% normal hatchlings surviving 1 week		1-week-old ducklings produced[b]	
	Control	Hg	Control	Hg	Control	Hg	Control	Hg	Control	Hg	Control	Hg
1	6.3	8.8	1.7	2.2	0.63	0.60	64.9	69.3	99.5	99.3	25.4	30.1
2	5.4	13.6*	2.1	3.7	0.65	0.55	68.8	59.8	98.7	97.8	50.9	36.3*
3	2.3	6.8	1.5	3.5	0.76	0.62†	63.8	60.6	99.2	99.6	55.9	43.5
Combined (1, 2, & 3)	4.3	9.7*	1.8	3.3	0.69	0.59*	65.9	62.4	99.1	99.0	46.0	37.5*

[a] There were 10 control females and 9 females fed mercury in generation 1; in generations 2 and 3, there were 14 females in each treatment.
[b] The means for "sound eggs laid/hen-day" and "1-week-old ducklings produced" are arithmetic means; all other means were retransformed from the angular transformation mean.
* Different from controls ($P = 0.05$).
† Different from controls ($P = 0.01$).

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Table 4. Shell thickness and thickness index (mean ± SE) of eggs laid by mallards fed either a diet containing 0.5 ppm mercury or a control diet.

Generation[a]	Eggshell thickness (mm)		Thickness index[b]	
	Control	Mercury	Control	Mercury
1	0.379 ± 0.011	0.376 ± 0.012	2.13 ± 0.045	2.11 ± 0.073
2	0.380 ± 0.005	0.364 ± 0.010	2.21 ± 0.031	2.11 ± 0.055
3	0.393 ± 0.005	0.367 ± 0.010[c]	2.29 ± 0.028	2.10 ± 0.063[c]
Combined (1, 2, & 3)	0.385 ± 0.004	0.368 ± 0.006[c]	2.22 ± 0.022	2.10 ± 0.035[†]

[a] One randomly selected egg was measured from each of 10 control females and 9 females fed mercury in generation 1; in generations 2 and 3, one egg was measured from each of 14 females in each treatment.
[b] Thickness index = {shell weight (mg)}/{shell length (mm) × shell width (mm)}.
[c] Different from controls ($P = 0.05$).
[†] Different from controls ($P = 0.01$).

(g/kg of body weight) than did controls (Heinz 1976*b*); however, there were no differences during the 3rd generation (Table 2). Food consumption of birds fed mercury differed significantly from that of controls when the data for the 2nd and 3rd generations were combined into a 2-way analysis of variance; there was also a highly significant treatment × generation interaction. Because the results were inconsistent between generations, they are difficult to interpret and should be looked at with caution. There were no significant differences in feed wasted.

Reproduction

Methylmercury had no significant effects on reproduction during the 1st generation (Table 3). There were no significant differences in the incidence of cracked or shell-less eggs, hatching success, or duckling survival during any of the 3 generations. In each generation, hens fed mercury laid a greater percentage of eggs outside their nestboxes than did controls; in the 2nd generation and the combined data for all generations, the differences were statistically significant. Tejning (1967) found that chickens fed 4.4 ppm mercury as methylmercury also laid an above normal number of eggs outside the nestbox.

During all 3 generations, hens fed mercury laid fewer sound eggs than did the controls, although the differences were statistically significant only in the 3rd generation and combined data for all generations. During the 2nd generation and for the combined data for all generations, hens in the mercury treatment produced significantly fewer ducklings than did the

Table 5. Weight and growth (mean ± SE) of ducklings from parents fed either a diet containing 0.5 ppm mercury or a control diet.

Generation[a]	Hatching weight (g)		% weight gain in 1 week	
	Control	Mercury	Control	Mercury
2	32 ± 0.8	33 ± 0.8	173 ± 4.3	158 ± 6.1[c]
3	38 ± 0.6	36 ± 0.7	159 ± 5.4	159 ± 2.9
Combined (2 & 3)	35 ± 0.8	34 ± 0.6	166 ± 3.6	158 ± 3.3

[a] There were 14 hens that provided ducklings in each generation; many ducklings from each hen were weighed.
[c] Different from controls ($P = 0.05$).

is fed either a diet containing 0.5 ppm

Thickness index[a]	
Control	Mercury
13 ± 0.045	2.11 ± 0.073
21 ± 0.031	2.11 ± 0.055
29 ± 0.028	2.10 ± 0.063*
22 ± 0.022	2.10 ± 0.035†

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Control	Mercury
173 ± 4.3	158 ± 6.1*
159 ± 5.4	159 ± 2.9
166 ± 3.6	158 ± 3.3

hen were weighed.

Table 6. Mean behavioral responses of mallard ducklings from the mercury or control treatments: approach, avoidance, and open-field tests during 3 generations.

	% ducklings approaching calls[b]		Latency of approach (seconds)		Distance traveled in avoidance (cm)		No. of photo-cell interruptions in open field	
Generation[a]	Control	Hg	Control	Hg	Control	Hg	Control	Hg
1	96	95	44	31	26	29		
2	98	93	33	41	37	44	178	176
3	96	93†	24	42†	32	37	186	181
Combined (1, 2, & 3)	97	94†	32	39	32	38*	182	179

[a] Ten control and 9 mercury-treated females provided ducklings in generation 1; in generations 2 and 3, there were 14 females in each treatment.
[b] The mean for "% ducklings approaching calls" was retransformed from the angular transformation mean; all other means are arithmetic means.
* Different from controls ($P = 0.05$).
† Different from controls ($P = 0.01$).

controls. Methylmercury has been shown to impair reproduction in experimental studies with chickens (Tejning 1967) and ring-necked pheasants (Borg et al. 1969, Fimreite 1971).

The shells of eggs laid by hens fed mercury were significantly thinner than the shells of those laid by controls during the 3rd generation and for the combined data of 3 generations (Table 4); eggshell thickness as measured by thickness index also indicated that shells were of poorer quality. Mercury generally is not regarded as an eggshell thinning agent (see review by Heinz 1976c). Only a few percentage points of thinning appeared to be caused by methylmercury in my study. Additional study with large sample sizes is needed to examine the ability of dietary concentrations of methylmercury to thin eggshells.

There were no significant differences in hatching weight between ducklings from parents fed methylmercury and control ducklings (Table 5). In the 2nd generation, ducklings from parents fed mercury gained significantly less weight during the 1st week of life than controls did. However, there were no significant differences in weight gain during the 3rd generation, or for the combined data of generations 2 and 3. The feeding of methylmercury to domestic chicks has been shown to reduce their growth (Fimreite 1970, Gardiner et al. 1971, Sell and Horani 1976). Because the results in my 2nd and 3rd generations were not consistent, I believe it is questionable whether methylmercury fed to the parents causes reduced growth of mallard ducklings.

Duckling Behavior

A smaller percentage of ducklings in the mercury treatment than control ducklings approached the tape-recorded maternal call (Table 6); the difference in the 3rd generation and the overall difference when data for the 3 generations were combined were highly significant. In the 3rd generation, ducklings in the mercury treatment also had a longer approach time to the call; differences between controls and ducklings in the mercury treatment were nearly significant ($0.1 > P > 0.05$) when data for the 3 generations were combined. In avoidance behavior tests, ducklings from parents fed mercury ran greater distances than did controls in all generations, although results were not statistically significant until the overall treatment effects were examined in the 2-way analysis of variance. Mallard duck-

lings from parents fed 3 ppm DDE differed significantly from controls in their approach to tape-recorded maternal calls and in avoidance tests (Heinz 1976*a*). The effects of DDE, however, were the reverse of those caused by methylmercury; ducklings in the DDE treatment were more responsive to maternal calls than were control ducklings, but less responsive in avoidance tests. In another study (Heinz 1975), ducklings from parents fed 0.5 or 3 ppm mercury responded differently than did control ducklings to tape-recorded maternal calls and were hyper-responsive in avoidance tests.

In the present study, open-field activity of ducklings from parents fed mercury was not significantly different from that of the controls. Because open-field behavior was not affected, it appears that the methylmercury had a specific effect on approach behavior to maternal calls and avoidance behavior, and not a generalized effect on activity level or exploratory behavior. Methylmercury has been shown in other studies to affect the behavior of young birds. Detour learning in domestic chicks was impaired when eggs were injected with 0.5 or 5.0 mg/kg methylmercury dicyandiamide (Rosenthal and Sparber 1972, Hughes et al. 1976); the injection of 0.5 mg/kg was less than the concentration of mercury in my mallard eggs. Evans et al. (1975) reported abnormal conditioned behavior of pigeons receiving 20 mg/kg mercury in the form of methylmercury.

CONCLUSIONS

The dietary concentration of 0.5 ppm mercury, in the form of methylmercury, decreased reproductive success of game-farm mallard ducks and altered the behavior of their young. The tissues and eggs of ducks and other species of birds collected in the wild have sometimes contained levels of mercury equal to or far exceeding the level I found to be associated with reproductive and behavioral aberrations. Therefore, it is possible that reproduction and behavior of wild birds has been affected by methylmercury contamination. Although all statistically significant differences between the mercury treatment and controls occurred in the 2nd and 3rd generations, there was no conclusive evidence that the effects of methylmercury became progressively more severe through the 3 generations.

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