

Reproduction in mallards exposed to dietary concentrations of methylmercury

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Abstract The purpose of this experiment was to use mallards (*Anas platyrhynchos*) tested under controlled conditions to determine how much harm to reproduction resulted from various concentrations of mercury in eggs. Breeding pairs of mallards were fed a control diet or diets containing 1, 2, 4, or 8 $\mu\text{g/g}$ mercury, as methylmercury chloride. Mean concentrations of mercury in eggs laid by parents fed 0, 1, 2, 4, or 8 $\mu\text{g/g}$ mercury were 0.0, 1.6, 3.7, 5.9, and 14 $\mu\text{g/g}$ mercury on a wet-weight basis. There were no signs of mercury poisoning in the adults, and fertility and hatching success of eggs were not affected by mercury. Survival of ducklings and the number of ducklings produced per female were reduced by the 4 and 8- $\mu\text{g/g}$ dietary mercury treatments (that resulted in 5.9 and 14 $\mu\text{g/g}$ mercury in their eggs, respectively). Ducklings from parents fed the various mercury diets were just as heavy as controls at hatching, but by 6 days of age ducklings whose parents had been fed 4 or 8 $\mu\text{g/g}$ mercury weighed less than controls. Because we do not know if absorption of mercury from our diets would be the same as absorption from natural foods, the mercury concentrations we report in eggs may be more useful in extrapolating to possible harmful effects in nature than are the dietary levels we fed. We conclude that mallard reproduction does not appear to be particularly sensitive to methylmercury.

Keywords Methylmercury · Eggs · Toxicity · Reproduction · Mallards · *Anas platyrhynchos*

Introduction

Contamination of the environment with mercury continues to be a serious environmental problem in many parts of the United States (Alpers and Hujnerlach 2000; Henny et al. 2007; Meyer et al. 1998; Scheuhammer et al. 2007; Spalding et al. 1994). High mercury concentrations in fish were responsible for 76% of all fish consumption advisories in the United States in 2004, with 44 states issuing such advisories due to mercury (USEPA 2005). Avian reproduction has been shown to be a sensitive indicator of mercury contamination (Burgess and Meyer 2008; Evers et al. 2008; Fimreite 1974; Schwarzbach et al. 2006). When elevated concentrations of mercury are found in the eggs of wild birds, these concentrations are compared to what are considered to be threshold concentrations of harm. What are considered to be harmful threshold levels of mercury in bird eggs have been determined largely through controlled feeding studies, generally with game farm birds such as ring-necked pheasants (*Phasianus colchicus*) and mallards (*Anas platyrhynchos*), and with chickens (*Gallus gallus*) (Fimreite 1971; Heinz 1974, 1979; Heinz and Hoffman 2003; Tejning 1967), but also with captive colonies of wild species such as black ducks (*Anas rubripes*) (Finley and Stendell 1978) and American kestrels (*Falco sparverius*) (Albers et al. 2007). Despite the findings of these earlier controlled studies, there remains much uncertainty about the degree of reproductive harm caused by various concentrations of mercury in eggs. For example, results from captive breeding studies have shown that harmful effects on reproduction may begin when mercury concentrations in eggs reach about 0.5–1.5 $\mu\text{g/g}$ in ring-necked pheasants (Fimreite 1971) and about 0.8–1.0 $\mu\text{g/g}$ in mallards (Heinz 1979; Heinz and Hoffman 2003). However, the degree of harm at these low mercury concentrations in eggs appears

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to be small and difficult to measure. In addition, the same low concentrations of mercury in mallard eggs shown to cause possible harm in the earlier studies (Heinz 1979; Heinz and Hoffman 2003) have recently been shown to actually have a possible beneficial (or hormetic) effect on hatching and duckling growth (Heinz et al. 2010). Therefore, there are apparent contradictory findings that need to be resolved. Also, because earlier studies were conducted with relatively low dietary concentrations of mercury, it is of interest to learn how much harm might be caused by higher concentrations of mercury in the adult female's diet and in her eggs. The purpose of our current study was to measure the effects of a wide range of dietary methylmercury concentrations on the health and reproductive success of breeding mallards and relate the concentrations of mercury in eggs to the degree of reproductive harm. Our specific null hypothesis was that concentrations of mercury in mallard eggs in a range that encompasses the concentrations found in wild bird eggs would have no harmful effects on reproduction.

Materials and methods

On March 1, 2007 we randomized one adult female and one adult male mallard to each of 80 1-m² breeding pens. On March 14 we randomly assigned one of five dietary concentrations of mercury (0, 1, 2, 4, or 8 µg/g) to each pen. We chose these dietary concentrations as ones we expected to result in mercury concentrations in eggs that encompassed the concentrations reported in the eggs of wild birds. Because the commercial duck diet fed to the pairs in the current study, and in our previous mallard studies (Heinz 1974, 1979; Heinz and Hoffman 1998, 2003), contained only about 10% water, the dietary concentrations were on close to a dry-weight basis and will be referred to hereafter in all of these studies as dry-weight. The mercury was in the form of methylmercury chloride (99% pure, Strem Chemicals, Inc., Newburyport, MA, USA) dissolved in corn oil. We have successfully used corn oil as a solvent for methylmercury chloride in past studies and found that methylmercury in corn oil is readily absorbed by the female and transferred to her eggs (Heinz and Hoffman 2003; Heinz et al. 2009b), and that the mercury deposited in eggs is almost exclusively methylmercury in the albumen, presumably bound to proteins (Heinz and Hoffman 2004). Thirty pairs were fed, ad libitum, the control diet, 15 pairs were fed the 1 µg/g diet, 15 pairs the 2 µg/g diet, 10 pairs the 4 µg/g diet, and 10 pairs the 8 µg/g diet. The diet was a commercial duck breeder pellet containing 17% protein, 2.5% crude fat, and 7.5% fiber (Purina Mills, LLC, St. Louis, MO, USA). Three samples of each diet were saved for mercury analysis. The

samples of control diet were reported to contain a mean of 0.01 µg/g mercury, and the four mercury-containing diets were confirmed to contain from 73 to 130% of the calculated values, with a mean recovery of 94% of the calculated values. Each pen was provided with a water bath, a feed bowl, and a nest box. The adults were weighed when randomized to pens and at the end of the study.

Some birds began laying eggs in the week prior to the start of the treated diets, but to allow the females to accumulate mercury in their bodies we did not begin collecting eggs for this study until April 9, which represented a period of 26 days on the treated diets. Eggs were collected each day through May 24, stored in a Kuhl egg cooler (Kuhl, Inc., Flemington, NJ, USA), and incubated at weekly intervals in a Kuhl incubator at 37.5°C. Two days prior to the expected hatching date the eggs were transferred to a Kuhl hatching unit maintained at 37.2°C. Starting on April 9, the first egg laid by each female was numbered as number 1. In order to measure any changes in mercury concentrations during the course of egg collecting we saved egg numbers 1, 9, 17, 25, and 33 from one randomly selected female in each treatment. In addition, we saved for mercury analysis the 17th egg from three additional randomly selected control females and from every mercury-treated female. Eggs and feed samples were analyzed for total mercury at the Western Ecological Research Center, US Geological Survey, Davis, CA following EPA method 7473 (U.S. EPA 2000), using a DMA 80 Direct Mercury Analyzer (Milestone Inc., Monroe, CT, USA).

Ducklings that hatched were banded, weighed, and kept in heated pens provided with flowing water and untreated game bird starter diet containing 18% protein, 3% crude fat, and 5% fiber (Purina Mills, St. Louis, MO, USA). Six days after hatching the ducklings were reweighed and killed.

To determine if any of the mercury treatments differed from controls on measures of adult health or reproductive success we used analysis of variance, followed by a Dunnett's test. Groups were compared with regard to adult weights at the beginning of treatment and at sacrifice, percentage of eggs that were fertile, percentage of fertile eggs that hatched, percentage survival of ducklings to 6 days of age, number of 6-day-old ducklings produced per female, hatching weights of ducklings, and 6-day-old weights of ducklings. Percentage data were subjected to an angular transformation prior to analysis; if this transformation failed to make the variances homogeneous, as tested by a Bartlett's test, we used a Kruskal–Wallis test to compare controls and treated groups. In addition to the analysis of variance test we performed a post test for linear trend, which is a regression analysis, to determine if there was a trend toward declining reproductive success as mercury concentrations in the diet increased. All statistical tests were performed using GraphPad Prism version 5.0 for

windows (GraphPad Software, San Diego, CA, USA). A significance level of $\alpha = 0.05$ was used in all tests.

Results

At the time of sacrifice we inadvertently failed to weigh one male on the 8 µg/g mercury diet and, therefore, this bird was excluded from the analysis of adult male weights. There were no significant differences between the controls and any of the mercury-treated groups in the mean weights of either adult males or adult females at the onset of mercury treatments or at sacrifice (Table 1). Post tests for linear trend also were not significant, suggesting there were no trends in either sex toward a difference in body weight as the dietary mercury treatments increased. In addition, we observed none of the overt signs of methylmercury toxicity (loss of appetite, incoordination, weakness, tremors) that have been associated with methylmercury toxicity in birds (Borg et al. 1969; Tejning 1967; Heinz 1996; Thompson 1996).

One female fed 4 µg/g mercury did not lay any fertile eggs, and was excluded from all statistical analyses of reproductive success. Even when the fertility data were subjected to an angular transformation, the variances were not homogeneous; therefore, we compared the means with a Kruskal–Wallis test, and there were no significant differences among groups in the percentage of eggs that were fertile (Table 2). The fertility of eggs of one female fed 8 µg/g mercury was only 57.5%. The next lowest percentage fertility in the 8-µg/g group was 87.5%; all other females fed this diet had between 94.7 and 100% fertile eggs. There are two possible explanations for the low value of 57.5% fertility and for the next lowest value (87.5%) in this group. One is that, when an egg is candled after a few days of incubation and no living embryo is observed, it is sometimes difficult to distinguish between an egg that is truly infertile versus one in which the embryo was killed at a very early stage. The other explanation is that for some

reason the male in the pen was not fertilizing the eggs properly, and this could be unrelated to the mercury diet. When this outlier of 57.5% was removed from the mean, the mean for this treatment rose from 93.4 to 97.4%, which is just slightly below the means for the other groups. Because of the lack of homogeneity of variances, we did not run a post test for linear trend, but an examination of the means after excluding the one outlier value for the 8-µg/g group suggests there was no trend toward decreasing fertility as the dietary concentration of mercury was increased.

Although females fed 8 µg/g mercury had the lowest hatching success of any group, there were no statistically significant differences among any of the groups, and a post test for linear trend also was not significant (Table 2). Analysis of variance revealed no significant differences among treatment groups in percentage survival of ducklings, but $P = 0.06$ for the post test for linear trend, suggesting an effect (Table 2). An examination of the survival values in Table 2 indicates there was a downward trend in duckling survival for the 4 and 8 µg/g mercury groups. Analysis of variance showed no significant differences among treatments in terms of the number of 6-day-old ducklings produced per female (Table 2). However, the post test for linear trend was significant ($P = 0.05$), and an examination of the means suggests that the 8 µg/g mercury group, and perhaps the 4 µg/g mercury group, exhibited a downward trend.

The analysis of variance test for duckling weights was not significant for the weights at the time of hatching, but was significant for the weights at 6 days of age (Table 3). The Dunnett’s test at 6 days of age showed that controls weighed more than ducklings from parents fed 4 µg/g mercury. Likewise, the post test for linear trend also was significant for the 6-day-old weights. An examination of the means suggests that both the 4 and 8 µg/g mercury treatments may have impaired growth.

No mercury was detected in the 17th egg of three of the four controls, and 0.01 µg/g mercury on a wet-weight basis

Table 1 Weights (g) of breeding pairs of mallards fed a control diet or diets containing 1, 2, 4, or 8 µg/g mercury as methylmercury chloride

Mercury in the diet (µg/g)	Males			Females		
	<i>n</i> ^a	At onset of treatment	At sacrifice	<i>n</i>	At onset of treatment	At sacrifice
0	30	1161 ± 16.8	1169 ± 21.8	30	1156 ± 18.7	1264 ± 21.6
1	15	1156 ± 25.8	1141 ± 27.6	15	1149 ± 23.3	1287 ± 26.4
2	15	1109 ± 28.0	1058 ± 73.1	15	1111 ± 18.7	1278 ± 30.6
4	10	1190 ± 24.7	1170 ± 20.5	10	1133 ± 34.7	1265 ± 30.0
8	9	1136 ± 28.5	1146 ± 34.2	10	1133 ± 25.9	1221 ± 27.2

There were no statistically significant differences among any of the treatment groups for males and females at the onset of treatment or at sacrifice (analysis of variance; $\alpha = 0.05$)

^a Number of males or females weighed

Table 2 Reproductive success of mallards fed a control diet or diets containing 1, 2, 4, or 8 $\mu\text{g/g}$ mercury as methylmercury chloride (arithmetic means \pm SE)

Mercury in the diet ($\mu\text{g/g}$)	n^a	% Fertility of eggs	% Hatch of fertile eggs	% Survival of hatchlings	Number of 6-day-old ducklings produced
0	30	98.9 \pm 0.31 A ^b	57.5 \pm 3.68 A	97.8 \pm 0.73 A	16.8 \pm 1.42 A
1	15	99.7 \pm 0.22 A	61.2 \pm 3.65 A	97.5 \pm 1.17 A	17.9 \pm 1.39 A
2	15	98.7 \pm 0.46 A	57.0 \pm 5.45 A	95.1 \pm 1.61 A	15.3 \pm 1.80 A
4	9	98.4 \pm 0.93 A	59.8 \pm 7.12 A	93.8 \pm 2.97 A	14.6 \pm 2.50 A
8	10	93.4 \pm 4.18 A	51.9 \pm 6.99 A	94.5 \pm 1.98 A	12.5 \pm 2.40 A

^a Number of breeding pairs of mallards

^b Means in the same column that share a letter in common were not significantly different by an analysis of variance at $\alpha = 0.05$. Percentage data were subjected to an angular transformation prior to running the analysis of variance. Because the variances among the groups for % fertility of eggs were not homogeneous, even when transformed (Bartlett's test; $P < 0.0001$), we used a Kruskal–Wallis test on this measurement

Table 3 Weights of mallard ducklings whose parents were fed a control diet or diets containing 1, 2, 4, or 8 $\mu\text{g/g}$ mercury as methylmercury chloride

Mercury in the diet of parents ($\mu\text{g/g}$)	n^a	At hatching	At 6 days of age
0	30	35.6 \pm 0.55 A ^b	81.0 \pm 1.80 A
1	15	35.5 \pm 0.71 A	83.4 \pm 2.68 A
2	15	36.4 \pm 0.65 A	78.9 \pm 2.66 A
4	9	35.5 \pm 0.52 A	72.0 \pm 1.65 B
8	10	35.8 \pm 0.72 A	74.5 \pm 1.93 A

^a Number of breeding pairs of mallards

^b Means in the same column that do not share a letter in common with the controls were significantly different from controls by an analysis of variance, followed by a Dunnett's test (both at $\alpha = 0.05$)

was reported in the fourth egg (Table 4). The 17th eggs of females fed 1, 2, 4, or 8 $\mu\text{g/g}$ mercury contained means of 1.6, 3.7, 5.9, and 14 $\mu\text{g/g}$ mercury on a wet-weight basis, respectively (Table 4). These mercury concentrations in eggs from our study more than encompassed the range encountered in wild bird eggs from mercury-contaminated areas (Burgess and Meyer 2008; Evers et al. 2008; Hill et al. 2008; Schwarzbach et al. 2006; Thompson 1996;

Table 4 Concentrations of mercury in the 17th egg laid by mallards fed a control diet or diets containing 1, 2, 4, or 8 $\mu\text{g/g}$ mercury as methylmercury chloride (mean \pm SE with extremes shown in parentheses)

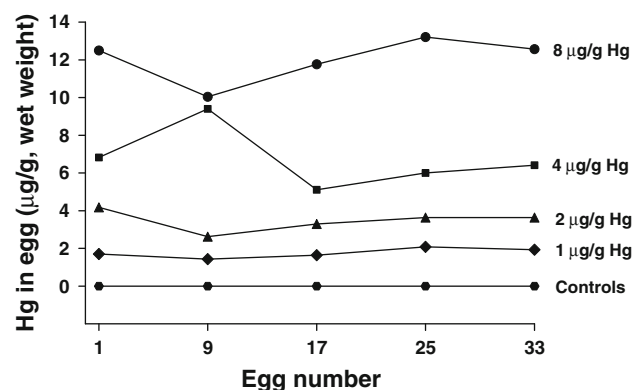
Mercury in the diet ($\mu\text{g/g}$)	n^a	Mercury in egg ($\mu\text{g/g}$, wet weight)
0	4	0.0 \pm 0.0025 (0.0–0.01)
1	15	1.6 \pm 0.04 (1.4–1.9)
2	15	3.7 \pm 0.21 (2.5–5.6)
4	9	5.9 \pm 0.25 (4.7–6.9)
8	10	14 \pm 0.95 (11–20)

^a Number of females from which a 17th egg was collected for mercury analysis

Scheuhammer et al. 2007). Unlike our experimental diets where methylmercury chloride was dissolved in corn oil, the methylmercury in the natural foods a wild bird might eat is likely bound to the sulfhydryl groups of proteins (Wiener et al. 2003). Because absorption of mercury from our experimental diets might not have been the same as absorption from natural foods, the mercury concentrations we report in eggs are probably more useful in extrapolating to possible harmful effects in nature than are the dietary levels we fed. Within each dietary treatment there were no apparent changes in mercury concentrations in eggs over the course of egg collecting (Fig. 1).

Discussion

Mallards have been the subject of several feeding studies with methylmercury and do not seem to be particularly sensitive to its effects on reproduction. In one earlier study, female mallards fed a diet containing a dry-weight concentration of 0.5 $\mu\text{g/g}$ mercury as methylmercury dicyandiamide laid eggs

**Fig. 1** Concentrations of mercury in the 1st, 9th, 17th, 25th, and 33rd eggs laid by mallards fed a control diet or diets containing 1, 2, 4, or 8 $\mu\text{g/g}$ mercury as methylmercury chloride. One female was tracked for each mercury treatment

containing an average of about 0.8 $\mu\text{g/g}$ mercury on a wet-weight basis, and hatching of eggs was unaffected (Heinz 1979). However, in a separate study, female mallards were fed a control diet or diets containing 5, 10, or 20 $\mu\text{g/g}$ mercury as methylmercury chloride, and in the earliest eggs laid by these females as little as about 1 $\mu\text{g/g}$ mercury in eggs did, on rare occasions, cause embryo mortality, deformities, or early death of the hatchlings (Heinz and Hoffman 2003). However, in this same study, other eggs containing 30 or more $\mu\text{g/g}$ mercury hatched and the ducklings appeared normal and survived, suggesting that there are considerable individual differences in sensitivity. In a third study, mallards were fed a dry-weight dietary concentration of 3 $\mu\text{g/g}$ mercury, as methylmercury dicyandiamide, and laid eggs that contained from about 3.8 $\mu\text{g/g}$ mercury (wet-weight) after 2–3 weeks on the diet up to about 9.2 $\mu\text{g/g}$ after several weeks on the diet (Heinz 1974). Over the course of the 21-week-long feeding study, the reduction in hatching success caused by the 3.8–9.2 $\mu\text{g/g}$ mercury in eggs was highly variable, ranging from no reduction (during weeks 14–17 when the mercury concentration in eggs was about 7 $\mu\text{g/g}$) to about a 55% reduction (during weeks 4–5 when only about 5.5 $\mu\text{g/g}$ mercury was in eggs). In a fourth study, female mallards fed 10 $\mu\text{g/g}$ mercury as methylmercury chloride laid eggs containing an average of 16 $\mu\text{g/g}$ mercury, which reduced the percentage hatch of fertile eggs by about 75% of what it was in the control group (Heinz and Hoffman 1998).

In the present study, females fed 1 or 2 $\mu\text{g/g}$ mercury suffered no reproductive impairment and their eggs contained means of 1.9 and 3.5 $\mu\text{g/g}$ mercury on a wet-weight basis. Females fed the diets containing 4 or 8 $\mu\text{g/g}$ mercury laid eggs containing means of 5.9 and 14 $\mu\text{g/g}$ mercury on a wet-weight basis. One might have expected these two higher concentrations of mercury (5.9 and 14 $\mu\text{g/g}$) to have produced the degree of harm seen in the earlier studies (Heinz 1974, Heinz and Hoffman 1998), but they did not; this leads to the question, how could similar concentrations of mercury in mallard eggs be associated with serious harmful effects in one study and only modest effects in another. One possible explanation is that the mallards came from different sources and one strain could have been more sensitive to mercury poisoning than the other strain. Gardiner et al. (1971) fed two strains of one-day-old chickens several concentrations of methylmercury dicyandiamide and reported differences in mortality and growth between the strains. Another difference was that the mercury in the 1979 study was in the form of methylmercury dicyandiamide, a form used in early applications as a fungicide for seeds, whereas we used methylmercury chloride in the current study. We know of no reports comparing the toxicity of these two forms, so we cannot say if the chemical form may have been a factor in the different results.

In contrast to our mallard data, chickens fed a control diet or diets containing either 4.6 or 9.2 $\mu\text{g/g}$ mercury, as methylmercury dicyandiamide, deposited about 5 and 10 $\mu\text{g/g}$ mercury in their eggs, respectively (Tejning 1967). Hatching success of eggs was reduced about 72% compared to controls for the eggs containing about 5 $\mu\text{g/g}$ mercury and by about 83% for the eggs containing about 10 $\mu\text{g/g}$ mercury. Some caution needs to be used when comparing the results of the mallard and chicken feeding studies because the experimental methods, endpoints, and mercury analyses were different, but reproduction in chickens seems to be more sensitive to methylmercury than in mallards. For example, based on the results discussed above, it takes about 16 $\mu\text{g/g}$ mercury maternally deposited in mallard eggs to cause a 75% reduction in hatching (Heinz and Hoffman 1998), whereas about 10 $\mu\text{g/g}$ in chicken eggs caused an 83% reduction (Tejning 1967).

In experimental studies, ring-necked pheasants also seem to be more sensitive to methylmercury than are mallards. Fimreite (1971) fed breeding ring-necked pheasants a diet containing about 3.7 $\mu\text{g/g}$ mercury, as methylmercury dicyandiamide, on a dry-weight basis and, after 10 weeks on the diet, their eggs contained about 1.5 $\mu\text{g/g}$ mercury on a wet-weight basis, which resulted in about a 46% decrease in hatching compared to controls. In another study, Borg et al. (1969) fed methylmercury dicyandiamide to ring-necked pheasants at a concentration of 15–20 $\mu\text{g/g}$ mercury for 9 days, resulting in an egg mercury concentration of about 1.35 $\mu\text{g/g}$ on a wet-weight basis. Hatching success of eggs laid by these females was decreased about 26% below that of controls. When compared to results from mallards, where about 1 $\mu\text{g/g}$ caused very little effect on hatching (Heinz and Hoffman 2003) and about 5.5 $\mu\text{g/g}$ mercury in eggs was needed to cause about a 55% decrease (Heinz 1974), pheasant embryos are clearly more sensitive than are mallard embryos. In a recent field study, Hill et al. (2008) reported that snowy egrets (*Egretta thula*) can suffer serious reproductive failure when concentrations of mercury in eggs reached 0.8 $\mu\text{g/g}$ on a wet-weight basis, suggesting that they also are more sensitive to the reproductive effects of methylmercury than are mallards.

In a study involving 26 species of birds in which methylmercury chloride was injected into the eggs, there was only one species, the double-crested cormorant (*Phalacrocorax auritus*), that seemed to be less sensitive than the mallard (Heinz et al. 2009a). Because the mallard is not an especially sensitive species to the reproductive effects of methylmercury, less reliance should be placed on using threshold values of mercury in eggs derived from mallard studies. In its place, controlled feeding studies and field studies are needed with additional species of birds to establish the degree of reproductive harm caused by various concentrations of mercury in eggs.

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