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Environmental Toxicology and Chemistry, Vol. 29, No. 3, pp. 650–653, 2010

Printed in the USA
DOI: 10.1002/etc.64

ENHANCED REPRODUCTION IN MALLARDS FED A LOW LEVEL OF METHYLMERCURY: AN APPARENT CASE OF HORMESIS

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(*Submitted* 29 *June* 2009; *Returned for Revision* 25 *August* 2009; *Accepted* 21 *September* 2009)

Abstract—Breeding pairs of mallards (*Anas platyrhynchos*) were fed a control diet or a diet containing 0.5 μg/g mercury (Hg) in the form of methylmercury chloride. There were no effects of Hg on adult weights and no overt signs of Hg poisoning in adults. The Hg-containing diet had no effect on fertility of eggs, but hatching success of eggs was significantly higher for females fed 0.5 μg/g Hg (71.8%) than for controls (57.5%). Survival of ducklings through 6 d of age was the same (97.8%) for controls and mallards fed 0.5 μg/g mercury. However, the mean number of ducklings produced per female was significantly higher for the pairs fed 0.5 μg/g Hg (21.4) than for controls (16.8). Although mercury in the parents' diet had no effect on mean duckling weights at hatching, ducklings from parents fed 0.5 μg/g Hg weighed significantly more (mean = 87.2 g) at 6 d of age than did control ducklings (81.0 g). The mean concentration of Hg in eggs laid by parents fed 0.5 μg/g mercury was 0.81 μg/g on a wet-weight basis. At this time, one cannot rule out the possibility that low concentrations of Hg in eggs may be beneficial, and this possibility should be considered when setting regulatory thresholds for methylmercury. Environ. Toxicol. Chem. 2010;29:650–653. © 2009 SETAC

Keywords—Hormesis Mercury Reproduction Mallards *Anas platyrhynchos*

INTRODUCTION

Hormesis is a toxicological phenomenon in which a chemical causes beneficial effects at a low dose, but harmful effects at higher doses [1]. The harmful effects of methylmercury in the diet of birds and in their eggs are well known [2–8], but there are no reports of beneficial effects on avian reproduction. The results from controlled laboratory breeding studies showing harmful effects have been used to quantify the risk of Hg to reproduction in wild birds [9–14]. Harmful effects on reproduction may begin when Hg concentrations in eggs reach a threshold of approximately 0.5 to 1.5 μg/g in captive ring-necked pheasants (*Phasianus colchicus*) [9] and approximately 0.8 to 1.0 μg/g in captive mallards (*Anas platyrhynchos*) [11,12]. The practical application of these laboratory-derived thresholds comes when elevated levels of Hg are found in the eggs of wild birds and these levels are compared with what are considered from the captive breeding studies with pheasants and mallards to be threshold levels of harm [13–18]. If, in addition to the information on what constitutes harmful concentrations of Hg in bird eggs, one had information on a level that seemed to enhance reproductive success, interpreting the consequences of a given Hg concentration found in the eggs of a wild bird would become easier.

MATERIALS AND METHODS

On March 1, 2007, one adult female and one adult male mallard were randomized to each of 50 1-m^2 outdoor breeding pens. On March 14, a control diet or a diet containing 0.5 μg/g Hg was randomly assigned to each pen. The commercial duck diet fed to these pairs contained only approximately 10% water; therefore, the 0.5 μg/g diet was on close to a dry-weight basis. The mercury was in the form of methylmercury chloride (99% pure; Strem Chemicals) dissolved in corn oil. Thirty pairs were fed, ad libitum, the control diet and 20 pairs were fed 0.5 μg/g Hg in their diet. The diet was a commercial duck breeder pellet containing 17% protein, 2.5% crude fat, and 7.5% fiber (Purina Mills). Each pen was provided with a water bath, a feed bowl, and a nest box. The adults were weighed when randomized to pens and when killed at the end of the study.

Some birds began laying eggs in the week before the start of the treated diets, but to allow the breeding females to accumulate Hg in their bodies, we did not begin collecting eggs for this study until April 9, which represented a period of 26 d on the treated diets. Eggs were collected each day through May 24, stored in a Kuhl egg cooler (Kuhl), and incubated at weekly intervals in a Kuhl incubator at 37.5°C. Two days before the expected hatching date, the eggs were transferred to a Kuhl hatching unit maintained at 37.2°C. The 17th egg (which was approximately halfway through the egg laying sequence of most females) was saved from three randomly selected control females and from every Hg-treated female for Hg analysis. In addition, to determine whether any changes in Hg concentrations took place during the course of egg collecting, egg numbers 1, 9, 17, 25, and 33 from an additional randomly selected control and egg numbers 1, 9, 17, 25, and 33 from one randomly selected female in the 0.5 μg/g Hg treatment were saved. Eggs were analyzed for total Hg at the Western Ecological Research Center, U.S. Geological Survey (Davis, CA) following U.S. Environmental Protection Agency method 7473 [19], using a DMA 80 direct Hg analyzer (Milestone).

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%

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Published online 9 November 2009 in Wiley InterScience (www.interscience.wiley.com).

fiber (Purina Mills). Six days after hatching, the ducklings were reweighed and killed.

Two-tailed *t* tests were used to compare controls with mallards fed 0.5 μg/g Hg. We compared beginning and final weights of adults, percentage of eggs that were fertile, percentage of fertile eggs that hatched, percentage survival of ducklings to 6 d of age, number of 6-d-old ducklings produced per female, hatching weights of ducklings, and 6-d-old weights of ducklings. Percentage data were subjected to an angular transformation before analysis. All statistical tests were performed using GraphPad Prism version 5.0 for windows (GraphPad Software). A significance level of $\alpha = 0.05$ was used in all tests.

RESULTS

There were no significant differences in mean adult body weights at the onset of treatment between the control males (1,161 ± 16.7 g; mean ± standard error) and males fed 0.5 μg/g Hg (1,148 ± 14.0 g) or when the birds were killed at the end of the Hg treatment (1,169 ± 21.8 g vs 1,177 ± 26.1 g). Likewise, there were no significant differences in weights between control females and females fed 0.5 μg/g Hg, at either the onset of Hg treatment (1,156 ± 18.7 g vs 1,117 ± 27.5 g) or at the end (1,264 ± 21.6 g vs 1,279 ± 34.5 g). No overt signs that have been associated with methylmercury toxicity (loss of appetite, incoordination, weakness, and tremors) were observed [13,20–22].

Controls and pairs fed 0.5 μg/g Hg did not differ in the percentage of eggs that were fertile, but the percentage of fertile eggs that hatched was significantly higher for pairs fed 0.5 μg/g mercury than for controls (Table 1). Survival of ducklings that hatched was identical for both groups (Table 1). The mean number of 6-d-old ducklings produced per breeding pair, a measurement that represents the summation of all previous measurements, differed significantly between controls and pairs fed 0.5 μg/g Hg; pairs fed 0.5 μg/g Hg produced approximately 27% more ducklings (21.4 vs 16.8) than did controls (Table 1). In addition, although ducklings from control pairs and pairs fed 0.5 μg/g Hg did not differ in their weights at the time of hatching, the ducklings from parents fed Hg weighed significantly more at 6 d of age than did the controls (Table 2). This increased growth had to be the result of Hg deposited in the egg, because ducklings in both groups of hatchlings were fed an uncontaminated diet.

No Hg was detected in the 17th egg of three of the four controls, and only 0.01 μg/g Hg on a wet-weight basis was reported in the fourth egg (Table 3). The 17th eggs of females fed 0.5 μg/g Hg contained a mean of 0.81 μg/g mercury on a wet weight basis (Table 3). Mercury concentrations in the eggs of controls and birds fed 0.5 μg/g Hg remained relatively unchanged over the course of egg collecting (Fig. 1).

DISCUSSION

The results of our current study suggest that a parental diet of 0.5 μg/g Hg can have beneficial effects on mallard reproduction. These beneficial effects seem to be in contradiction to the results of two earlier studies [11,12]. In a 1979 study [11], the same dietary concentration of Hg, 0.5 μg/g on close to a dry-weight basis, was reported to adversely affect mallard reproduction. In the current study, the mean Hg concentration in the 17th egg from females fed 0.5 μg/g Hg was 0.81 μg/g on a wet weight basis, which is almost identical to the approximately 0.8 μg/g on a wet weight basis in the 1979 study [11]. The question arises, how could similar concentrations of Hg in mallard eggs be associated with harmful effects in one study and beneficial effects in another?

One possible explanation is that the mallards came from different sources and one strain could have been more sensitive to Hg poisoning than the other strain. Gardiner et al. [23] fed two strains of 1-d-old chickens (*Gallus gallus*) several concentrations of methylmercury dicyandiamide and reported differences in mortality and growth between the strains. Another difference was that the Hg 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. Hatching success of the controls in the present study was poor (57.5%); just how this could have influenced the outcome of the comparison between controls and the 0.5-μg/g mercury group is uncertain. It is possible that the addition of such a low level of methylmercury to the diet counteracted whatever it was that depressed hatching of control eggs and growth of young, and that under a more normal hatching success of control eggs (70–80%) hatching and growth of ducklings in the 0.5 μg/g Hg group would not have been superior.

A careful examination of the results from the 1979 study [11] reveals one reason why the results from that study showed a Hg-induced impairment of reproduction. Over the three generations of that study, the Hg-treated females produced an average of 18.5% fewer one-week-old ducklings compared with controls [11]. However, of this 18.5% decrease in ducklings, 78.5% of the decrease was attributable to fewer eggs being laid by females fed 0.5 μg/g Hg. In nature, mallards normally lay only eight to 10 eggs per nesting attempt [24]. In the 1979 study, the total number of eggs laid by controls and mallards fed 0.5 μg/g Hg was not listed, but it was reported that the controls produced

Table 1. Reproductive success of mallards fed a control diet or a diet containing 0.5 μg/g mercury as methylmercury chloride (arithmetic mean ± standard error; percentage data were subjected to an angular transformation prior to statistical analyses).

Mercury in the diet (μg/g)	n[a]	% Fertility of eggs	% Hatch of fertile eggs	% Survival of hatchlings	Number of 6-d-old ducklings produced
0	30	98.9 ± 0.31	57.5 ± 3.68	97.8 ± 0.73	16.8 ± 1.42
0.5	20	98.5 ± 0.52	71.8 ± 2.85*	97.8 ± 0.97	21.4 ± 1.30*

[a] Number of breeding pairs of mallards.
* Significantly different from controls at $\alpha = 0.05$ using a two-tailed *t* test.

Table 2. Weights (g) of mallard ducklings whose parents were fed a control diet or a diet containing 0.5 μg/g mercury as methylmercury chloride (arithmetic mean ± standard error).

Mercury in the diet of parents (μg/g)	n[a]	At hatching	At 6 d of age
0	30	35.6 ± 0.55	81.0 ± 1.83
0.5	20	35.3 ± 0.60	87.2 ± 2.14*

[a] Number of breeding pairs of mallards.
* Significantly different from controls at $\alpha = 0.05$ using a two-tailed t test.

an average of 46 one-week-old ducklings per breeding season, compared with 37.7 for the pairs fed 0.5 μg/g Hg [11]; both numbers show that many more than eight to 10 eggs were laid by the females in each group. In a natural setting, a diet of 0.5 μg/g Hg might not cause a reduction in the normal eight to 10 eggs laid by wild mallards, and, consequently, much of the detrimental effect seen in the lab study [11] might not be seen in the wild.

In another study, published in 2003, the lowest concentration of Hg in mallard eggs that was associated with reproductive harm was approximately 1 μg/g on a wet-weight basis [12]. From an experimental design point of view, the 2003 study was more powerful than the 1979 study, and the harmful effects of the approximately 1 μg/g Hg in eggs were seen only rarely; many embryos in eggs that contained 10 or more μg/g Hg hatched and survived.

Perhaps the most likely explanation for the differences between the earlier studies [11,12] and our current study is that a diet containing 0.5 μg/g Hg as methylmercury may fall right at the threshold between causing slight harm to the very most sensitive embryos versus possible benefit to the larger proportion of embryos in the experiment. Adams and Frederick [25] fed juvenile white ibises (*Eudocimus albus*) a control diet or diets containing either 0.05, 0.1, or 0.3 μg/g Hg as methylmercury on a wet weight basis. Assuming that the normal diet of ibises in the wild might contain approximately 80% moisture, if one were to convert the wet-weight ibis diets to a dry-weight basis to compare them with our 0.5 μg/g mercury duck diet, the Hg concentrations fed to the ibises might have been approximately five times higher, with the 0.1 μg/g Hg, wet-weight, diet being approximately equal to our 0.5 μg/g Hg, dry-weight, duck diet. In a controlled experiment, in which the ibises had to prey upon live fathead minnows (*Pimephales promelas*), the groups fed the 0.05 or 0.1 μg/g Hg diets were more efficient feeders than were the controls. Although there was a lack of true replication in the ibis study, the results are at least suggestive of a possible case of hormesis [25].

Table 3. Concentrations of mercury in eggs of mallards fed a control diet or a diet containing 0.5 μg/g mercury as methylmercury chloride (mean ± standard error, with extremes shown in parentheses).

Mercury in the diet (μg/g)	n[a]	Mercury in egg (μg/g, wet wt)
0	4	0.0 ± 0.0025 (0.0–0.01)
0.5	20	0.81 ± 0.027 (0.64–1.2)

[a] Number of breeding pairs of mallards from which an egg was sampled for mercury.

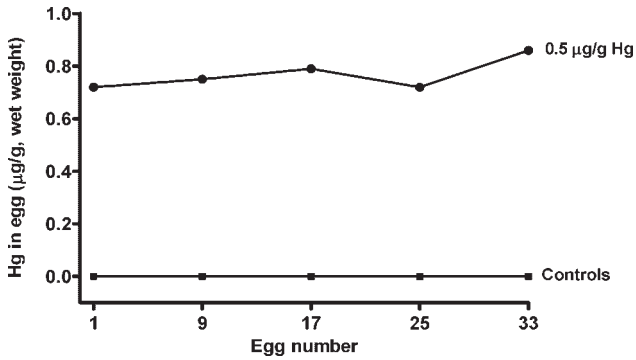

Fig. 1. Concentrations of mercury in the 1st, 9th, 17th, 25th, and 33rd eggs laid by mallards fed a control diet or a diet containing 0.5 μg/g mercury as methylmercury chloride.

Borg et al. [20] used chi-square analyses to compare hatching of eggs in a group of control ring-necked pheasants to hatching in a group fed wheat containing 15 to 20 μg/g Hg as methylmercury dicyandiamide. Eighty-two percent of the eggs laid 3 d after the start of the Hg diet hatched compared with 74% for controls (a statistically significant chi-square difference), and eggs laid after 6 d on the Hg diet had a hatching success of 81% (no statistical comparison was run with this group). After 9 d on the Hg-contaminated diet, only 55% of the eggs hatched in the Hg-fed group, which was significantly lower than the value of 74% for controls [20]. The mean Hg concentrations in eggs of pheasants fed the diet of wheat containing 15 to 20 μg/g Hg were 0.3 μg/g on a wet-weight basis after 2 d of feeding, 0.7 μg/g after 4 d, and 0.9 μg/g after 7 d (up to 7 d was roughly the period when hatching was better than controls), whereas after 9 d on the Hg diet, Hg in eggs ranged from 1.3 to 1.4 μg/g (when hatching was worse than controls). In a separate study with ring-necked pheasants, Fimreite [9] reported that a concentration of 0.5 to 1.5 μg/g Hg on a wet-weight basis was associated with reproductive impairment, which is, at least at the lower end of the 0.5 to 1.5 μg/g Hg range, opposite the beneficial effect produced by the 0.3 to 0.9 μg/g Hg in eggs reported by Borg et al. [20]. As with our mallard data, the pheasant data illustrate the puzzling finding that one may observe detrimental effects of methylmercury on egg hatching in one study as opposed to apparent beneficial effects in another study, even though the Hg concentrations in eggs were very similar in both studies.

Prati et al. [26] reported a hormetic effect of methylmercury for the larval life stage of the frog, *Xenopus laevis*. When larvae were placed in solutions containing less than 0.25 μM methylmercury chloride, mortality was reduced compared with controls, whereas higher concentrations resulted in increased mortality [26].

The practical importance of findings in the present study is that a dietary concentration of 0.5 μg/g Hg as methylmercury and the resulting egg concentration of approximately 0.8 μg/g Hg should be viewed as something either very close to a lowest-observed-adverse effect level (LOAEL), or as dietary and egg concentrations that may, in fact, be somewhat beneficial, at least under certain circumstances. At least for mallards, if approximately 0.8 to 1.0 μg/g Hg in eggs on a wet-weight basis is used

as a LOAEL, it would seem to provide an adequate degree of protection.

Acknowledgement—This research was funded by the CALFED Bay-Delta Program's Ecosystem Restoration Program (grant ERP-02D-C12) with additional support from the U.S. Geological Survey Patuxent Wildlife Research Center. We thank Nelson Beyer and Nimish Vyas for their reviews of an early draft of this manuscript.

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