

*Environmental Toxicology*INTERACTIONS BETWEEN METHYLMERCURY AND SELENOMETHIONINE
INJECTED INTO MALLARD EGGS

JON D. KLIMSTRA,*† JULIE L. YEE,‡ GARY H. HEINZ,§ DAVID J. HOFFMAN,§ and KATHERINE R. STEBBINS§

†U.S. Fish and Wildlife Service, Laurel, Maryland

‡Western Ecological Research Center, U.S. Geological Survey, Sacramento, California

§Patuxent Wildlife Research Center, U.S. Geological Survey, Beltsville, Maryland

(Submitted 9 June 2011; Returned for Revision 28 July 2011; Accepted 29 September 2011)

Abstract—Methylmercury chloride and seleno-L-methionine were injected separately or in combinations into mallard eggs (*Anas platyrhynchos*), and embryo mortality and teratogenic effects (deformities) were modeled using a logistic regression model. Methylmercury was injected at doses that resulted in concentrations of 0, 0.2, 0.4, 0.8, and 1.6 $\mu\text{g/g}$ Hg in the egg on a wet weight basis and selenomethionine at doses that resulted in concentrations of 0, 0.1, 0.2, 0.4, and 0.6 $\mu\text{g/g}$ Se in the egg, also on a wet weight basis. When selenomethionine and methylmercury were injected separately, hatching probability decreased in both cases. However, when methylmercury was injected at 1.6 $\mu\text{g/g}$ in combination with selenomethionine at 0.2 $\mu\text{g/g}$, the presence of the methylmercury resulted in less embryo mortality than had been seen with 0.2 $\mu\text{g/g}$ Se by itself, but it increased the number of deformed embryos and hatchlings. Selenomethionine appeared to be more embryotoxic than equivalent doses of methylmercury when injected into eggs, and both injected methylmercury and selenomethionine were more toxic to mallard embryos than when deposited naturally in the egg by the mother. The underlying mechanisms behind the interactions between methylmercury and selenomethionine and why methylmercury appeared to improve hatching probability of Se-dosed eggs yet increased deformities when the two compounds were combined are unclear. These findings warrant further studies to understand these mechanisms in both laboratory and field settings. *Environ. Toxicol. Chem.* 2012;31:579–584. © 2011 SETAC

Keywords—Methylmercury Selenomethionine Egg injections Embryo mortality Teratogenic effects Interactions

INTRODUCTION

Mercury (Hg) and selenium (Se) sometimes occur together at elevated concentrations in birds [1–4]. Methylmercury has been shown to harm hatching success in bird eggs in laboratory studies, and similar results have been suggested in field studies [5–13]. Teratogenic effects of methylmercury on avian embryos have been demonstrated in laboratory studies [14–16]. Selenium has also been shown to impair reproductive success, including the production of teratogenic effects in both field studies [3,17–19] and laboratory studies [14,20–24].

Although the toxic interactions between Hg and Se are among the most studied of all environmental contaminants, these interactions can be complicated. Cuvin-Aralar and Furness [25] reported that Hg–Se interactions are normally antagonistic but can be additive and synergistic; the authors concluded that “the interactions between different selenium and mercury compounds are extremely complex and not well understood at present.” Studies combining the most toxic and environmentally realistic forms of Hg (methylmercury) and Se (selenomethionine) are especially lacking. Heinz and Hoffman [14] fed methylmercury and selenomethionine alone or in combination to breeding mallards (*Anas platyrhynchos*), and, although each compound by itself caused teratogenic effects in embryos, the combination of the two compounds caused many more deformities. The objective of the present study was to determine whether toxic interactions occurred between methylmercury chloride and seleno-L-methionine when these compounds were injected into mallard eggs.

MATERIALS AND METHODS

Experiment 1

In 2006, 500 fertile mallard eggs were obtained from Clearview Hatchery in Pennsylvania. Eggs were sorted and randomly assigned to 25 groups. The control group received 25 eggs, and 22 of the remaining groups each received 14 eggs. Two groups were assigned 13 eggs because of shortages. Methylmercury chloride and seleno-L-methionine were dissolved in deionized water, and the solutions were kept refrigerated until use. The solutions were injected into the air cell at the rate of 1 μl water per 1 g of egg contents. Controls were injected with untreated water. Doses of methylmercury were injected to create five different concentrations of Hg in the egg (0, 0.2, 0.4, 0.8, and 1.6 $\mu\text{g/g}$ wet wt), and doses of selenomethionine were injected to create five different concentrations of Se in the egg (0, 0.1, 0.2, 0.4, and 0.6 $\mu\text{g/g}$ wet wt). All possible combinations of Hg and Se were injected to create a 5 \times 5 injection matrix (Table 1). Eggs were injected after 4 d of incubation. Previous work has demonstrated that waiting to inject mallard eggs until the embryos were 4 d old permits the removal of infertile or early dead eggs from the study, resulting in good dose–response curves [16]. Any eggs that were cracked, infertile, or dead before being injected were discarded. Eggs were incubated in a Kuhl incubator (Kuhl Corporation) at 37.5°C and 40% relative humidity. They were candled every third day, with any dead eggs being removed. Eggs were incubated on their sides and rolled 180° every hour. On day 25 of incubation (26–28-d incubation period for game-farm mallards), the eggs were transferred from the incubator to a Kuhl hatching unit, which was set to 37.2°C and 70% relative humidity. Eggs were checked periodically throughout the hatching period, and hatchlings were tallied for each treatment group. Hatchlings were

* To whom correspondence may be addressed

(jon_klimstra@fws.gov).

Published online 2 December 2011 in Wiley Online Library
(wileyonlinelibrary.com).

Table 1. Combination matrix of various dosage levels of Hg and Se ($\mu\text{g/g}$ wet wt) in the form of methylmercury and selenomethionine injected into fertile mallard (*Anas platyrhynchos*) eggs in the 2006 experiment^a

Se	Hg				
	0	0.2	0.4	0.8	
0	28	14	14	14	14
0.1	14	14	14	14	14
0.2	14	14	13	13	14
0.4	14	14	14	14	14
0.6	14	14	14	14	14

^a The number at each combination represents the sample size.

removed, visually examined for deformities, and then euthanized according to the Patuxent Wildlife Research Center's Animal Care and Use Committee guidelines. Any dead eggs that had developed to at least the 7-d-old embryo stage were examined for deformities. Prior to the 7-d stage, embryos are small and relatively undeveloped. Furthermore, rapid decomposition often occurs following embryo death in the earlier stages; both of these factors make identifying deformities difficult at earlier stages. Only overt external malformations visible to the naked eye were tallied. No internal examinations were made. Eggs that did not live to be 7 d of age were counted as unhatched. Some of the eggs that did not survive to 7 d of age could have had deformities, so our deformity rates were likely on the low side.

Experiment 2

In 2007, a second experiment was conducted with mallard eggs, again obtained from Clearview Hatchery. Based on the results from 2006, the second experiment focused on four treatment combinations, including the control group (Table 2). Eggs were incubated and hatched in the same manner as in experiment 1.

Data analysis

In SAS Version 9.2 [26], hatching probability for 2006 was estimated using a logistic regression model in which the variables for Hg and Se concentration were treated as continuous effects. In 2007, using the same logistic regression model to estimate hatching probability, Se concentrations were truncated to exclude data at the 0.4 $\mu\text{g/g}$ level and higher, and we focused on only four of the original 25 treatment combinations (0 Hg/0 Se, 0 Hg/0.2 Se, 1.6 Hg/0.2 Se, and 1.6 Hg/0 Se). This truncation was made because, at ≥ 0.4 $\mu\text{g/g}$ Se, no eggs hatched. The 2007 experiment was intended to investigate further some of the findings from 2006 and also to confirm that the 2006 experiment was repeatable. After we had determined that the results from 2006 and 2007 were similar, the four levels analyzed in the 2007 experiment were combined with the corresponding levels from 2006 and refitted to the logistic model. The probability of being

Table 2. Combination matrix for the 2007 experiment focusing on four dosage levels of Hg and Se ($\mu\text{g/g}$ wet wt) in the form of methylmercury and selenomethionine injected into fertile mallard (*Anas platyrhynchos*) eggs^a

Se	Hg		
	0	1.6	
0	45	47	
0.2	45	45	

^a The number at each combination represents the sample size.

deformed was modeled in the same manner; for the sake of brevity, only the 2006 and 2007 combined data are presented.

RESULTS

Hatching: Experiment 1

Model estimates from the experiment in 2006 are presented in Figure 1 along with the 95% confidence intervals and the observed hatching success, represented by a circle. All tests were conducted at the 0.05 level and were considered significant when p was below the prespecified level. When selenomethionine doses were held at 0 $\mu\text{g/g}$ and methylmercury doses increased (0–1.6 $\mu\text{g/g}$), hatching probability showed a negative trend and decreased approximately 50% (from ~ 0.82 to ~ 0.43 in Fig. 1). When selenomethionine was held at 0.1 $\mu\text{g/g}$ and methylmercury increased across the full range, hatching probability declined from approximately 40% to about 30%, which was a 25% reduction. An opposite trend was observed, with hatching probability improving by approximately half when methylmercury spanned the full range and selenomethionine was held at 0.2 $\mu\text{g/g}$. When selenomethionine was held at 0.4 $\mu\text{g/g}$ and methylmercury increased, hatching probability was a complete failure at the lower Hg levels but increased slightly when the methylmercury dose increased. This same pattern was observed when selenomethionine was held at 0.6 $\mu\text{g/g}$ and methylmercury spanned the full range of doses, but the effect was less pronounced. The maximum likelihood estimate (Table 3) for the effect of methylmercury alone was only marginally significant in causing a decline in hatching probability ($p = 0.058$). However, selenomethionine alone had a very significant detrimental effect on hatching probability ($p = 0.0001$). When the effects of both selenomethionine and methylmercury were combined, a weakly significant positive interaction seemed to occur ($p = 0.044$), suggesting that the effect of combining the two could be less detrimental than the sum of their parts.

Hatching: Experiment 2

Model estimates for 2007 are presented in Figure 2. The goodness-of-fit test at certain dosage levels from the 2006 experiment showed some unexpected interactions, namely, that the coinjection of methylmercury seemed to improve the hatching success of Se-injected eggs but enhanced teratogenic effects. Therefore, in 2007, attention was focused on the following combinations of methylmercury/selenomethionine: 0/0, 0/0.2, 1.6/0, and 1.6/0.2 $\mu\text{g/g}$. The goodness-of-fit test for the 2007 experiment showed patterns similar to those from 2006 in that both methylmercury and selenomethionine by themselves had a negative effect on hatching probability (Table 4). Furthermore, when the two were combined, the interaction was significantly positive ($p < 0.0001$; Table 4). When selenomethionine levels were held at 0 $\mu\text{g/g}$ and methylmercury increased from 0 to 1.6 $\mu\text{g/g}$, there was a significant negative effect on hatching probability ($p = 0.0001$), which translated into an 81% reduction in hatching compared with the controls (80% for controls vs. 15% for the 1.6/0 Hg/Se group). However, when the level of selenomethionine was held constant at 0.2 $\mu\text{g/g}$ and methylmercury levels increased from 0 to 1.6 $\mu\text{g/g}$, there was a significant ($p = 0.039$) positive effect on hatching probability. This positive effect represented an 89% increase in hatching probability from the 0/0.2 Hg/Se (2%) group to the 1.6/0.2 (18%) Hg/Se group.

Hatching data from experiments 1 and 2 combined

Estimates of hatching probability for the 0/0, 0/0.2, 1.6/0, and 1.6/0.2 $\mu\text{g/g}$ treatments when the results for 2006 were

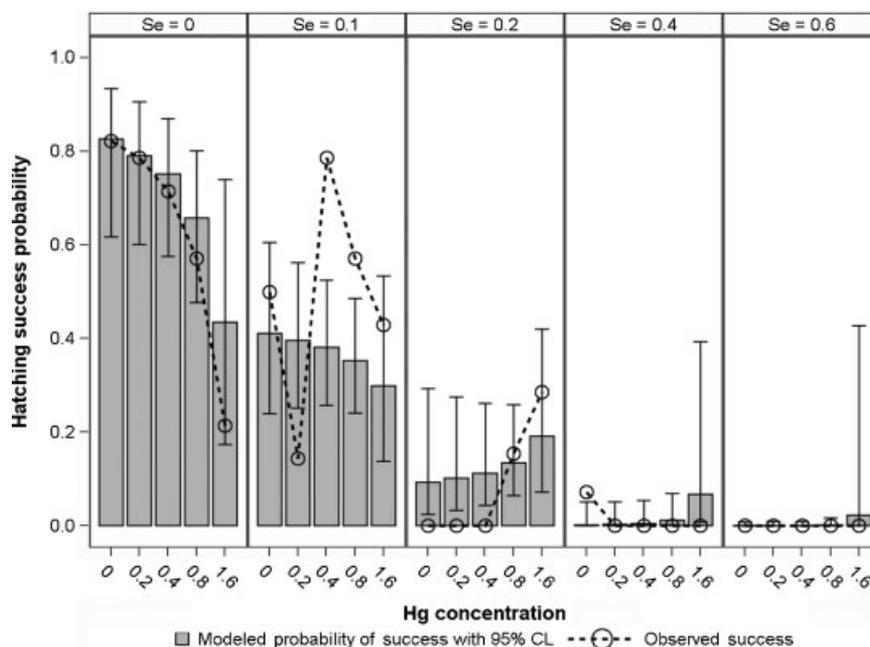


Fig. 1. Probability of hatching estimated using a logistic regression model showing the 95% confidence limits (CL) and observed hatching success represented by the circle for 2006 data.

combined with those from 2007 are presented in Figure 3. Although the *p* values changed slightly with the combined data, the same trends were observed as were seen in the separate analyses of data from experiments 1 and 2. When methylmercury was injected, by itself, at 1.6 μg/g, hatching probability significantly decreased from the controls by 81%, and, when selenomethionine was injected, by itself, at 0.2 μg/g, there was a 97% decrease in hatching probability. The maximum likelihood estimates showed that both methylmercury and selenomethionine had highly significant negative effects on hatching by themselves, with selenomethionine having a slightly greater effect (Table 5). However, when the two chemicals were combined (1.6/0.2), hatching probability increased to 20%. As in the previous two experiments, their interaction had a positive effect on hatching probability (Table 5).

Deformities

With the same logistic model, deformities data were combined from the 2006 and 2007 experiment and were analyzed in the same manner as was hatching probability (Fig. 4). The histograms in Figure 4 illustrate a 17% probability that an embryo would be deformed when methylmercury was injected, by itself, at 1.6 μg/g, and a 50% probability when selenomethionine was injected, by itself, at 0.2 μg/g. Based on maximum likelihood estimates (Table 6), it appears that selenomethionine, by itself, resulted in a higher probability of being deformed than did methylmercury, by itself. When 1.6 μg/g Hg was combined

with 0.2 μg/g Se, the probability of being deformed increased even more, to 66%. This suggests some type of synergy between methylmercury and selenomethionine. This synergy with deformities is the opposite of what was observed with hatching probability, for which the combination of methylmercury and selenomethionine increased the probability of hatching. Although the interaction was not significant (*p* = 0.26), it appears that, when combined, both methylmercury and selenomethionine may increase the probability of an embryo being deformed compared with the case when the two were separate.

DISCUSSION

Interactions between methylmercury and selenomethionine

The most important finding was that, although methylmercury decreased embryo mortality caused by selenomethionine, it acted synergistically to increase the number of deformities.

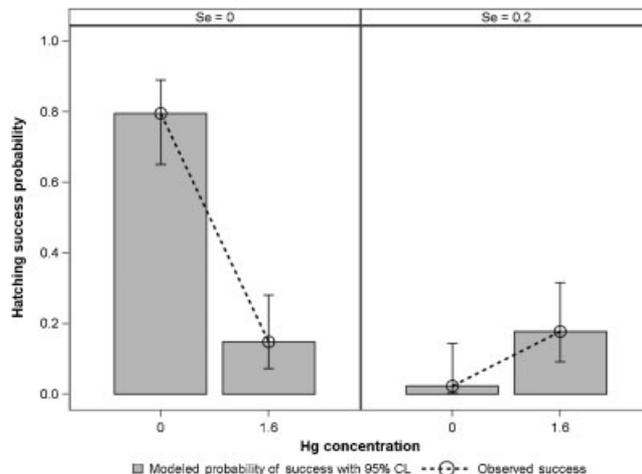


Fig. 2. Probability of hatching estimated using a logistic regression model showing the 95% confidence limits (CL) and observed hatching success represented by the circle for the 2007 data.

Table 3. Coefficients of the logistic regression on the hatching probability of mallard (*Anas platyrhynchos*) eggs injected with various doses (μg/g) of Hg and Se during the 2006 experiment

Parameter	Degrees of freedom	Estimate	Standard error	Wald χ^2	<i>p</i>
Intercept	1	1.5607	0.5519	7.9976	0.0047
Hg	1	-1.1371	0.5993	3.5996	0.0578
Se	1	-19.2239	4.9467	15.1028	0.0001
Hg × Se	1	8.3221	4.1219	4.0764	0.0435

Table 4. Coefficients of the logistic regression on the hatching probability of mallard (*Anas platyrhynchos*) eggs injected with various doses ($\mu\text{g/g}$) of Hg and Se during the 2007 experiment

Parameter	Degrees of freedom	Estimate	Standard error	Wald χ^2	<i>p</i>
Intercept	1	1.3581	0.3737	13.2049	0.0003
Hg	1	-3.1011	0.5546	31.2700	<0.0001
Se	1	-5.1192	1.0783	22.5366	<0.0001
Hg \times Se	1	5.3307	1.2177	19.1651	<0.0001

At the dose of 1.6 $\mu\text{g/g}$ Hg combined with 0.2 $\mu\text{g/g}$ Se, there was a protective effect of methylmercury, which allowed embryos to hatch that would not have hatched had they been exposed only to Se. However, at this same 1.6 Hg/0.2 Se $\mu\text{g/g}$ combination, there was a higher probability that an embryo or hatchling would be deformed. This finding of antagonism and synergism occurring at the same combination of injected Hg and Se was paradoxical in that one might expect an antagonistic reduction in embryo mortality to be associated with a similar reduction in the rate of deformed embryos and hatchlings.

In another study, in which mallard eggs were injected with various doses of methylmercury, but no Se, the lowest dose (0.05 $\mu\text{g/g}$ Hg on a wet wt basis) was associated with a hormetic (beneficial) effect on hatching [27], and, in a feeding study that also did not involve Se, a concentration of 0.5 $\mu\text{g/g}$ Hg, as methylmercury chloride, fed to breeding mallards seemed to have a hormetic effect on the hatching success of their eggs and growth of their young [28]. At present, we cannot offer insights into the biochemical mechanisms responsible for the synergy between the Hg and Se with regard to the creation of deformities and the beneficial effects Hg had on hatching of Se dosed eggs.

Although the *p* value of 0.26 does not, in a statistical sense, conclusively prove that a combined dose of methylmercury and selenomethionine resulted in a greater rate of deformed embryos than either compound alone, other evidence supports this conclusion. Detailed analyses of the various deformities caused by methylmercury and selenomethionine in the current experiment are presented in a separate paper [29]. Most of the deformities caused by Se by itself or Se combined with methylmercury were to the eyes, bill, wings, and legs. However, some deformities such as spina bifida (an incomplete closure of the backbone and spinal cord) and craniorachischisis (a fissure

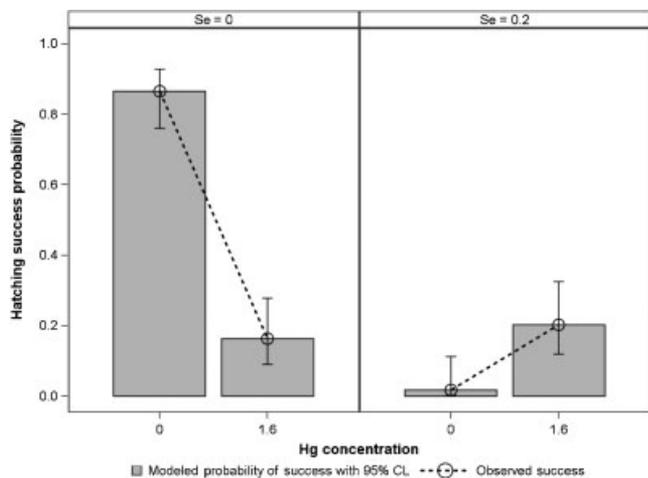


Fig. 3. Probability of hatching estimated using a logistic regression model showing the 95% confidence limits (CL) and observed hatching success represented by the circle for 2006 and 2007 data combined.

Table 5. Coefficients of the logistic regression on the hatching probability of mallard (*Anas platyrhynchos*) eggs injected with various doses ($\mu\text{g/g}$) of Hg and Se for both the 2006 and 2007 experiments combined

Parameter	Degrees of freedom	Estimate	Standard error	Wald χ^2	<i>p</i>
Intercept	1	1.8632	0.3583	27.0473	<0.0001
Hg	1	-3.4925	0.4980	49.1904	<0.0001
Se	1	-5.9063	1.0705	30.4426	<0.0001
Hg \times Se	1	6.1703	1.1705	27.7876	<0.0001

of the cranium that continues down the spine, exposing part of the brain and spinal cord) were present in mallard embryos only when selenomethionine and methylmercury were combined [29]. In a separate study, when breeding mallards were fed diets containing selenomethionine, methylmercury, or a combination of the two, spina bifida again occurred only in embryos from eggs containing both compounds [14].

Other studies using combinations of inorganic Se and methylmercury have demonstrated protective effects of these two elements. El-Begearmi et al. [30] found that sodium selenite, by itself, at 6 or 12 $\mu\text{g/g}$ Se in the diet and methylmercury, by itself, at 15 $\mu\text{g/g}$ Hg reduced hatching of Japanese quail (*Coturnix japonica*) eggs, but together the effects were not as severe. The same study reported that the addition of 3 or 6 $\mu\text{g/g}$ Se, as sodium selenite, reduced the toxicity of 20 $\mu\text{g/g}$ Hg, as methylmercury hydroxide, to young Japanese quail, even though brain levels of Hg were higher when sodium selenite was included in the diet. Selenium levels in the brain also were higher when methylmercury was fed, suggesting that Se was somehow protecting the brain from methylmercury damage [30]. Stoewsand et al. [31] reported similar protective effects in Japanese quail fed 5 $\mu\text{g/g}$ Se as sodium selenite and 20 $\mu\text{g/g}$ Hg as methylmercury chloride, and Sell and Horani [32] found that 8 $\mu\text{g/g}$ Se as sodium selenite protected young chickens from the toxicity of 20 $\mu\text{g/g}$ Hg as methylmercury chloride.

The results of the present study support the generally accepted findings that these two contaminants protect against each other's toxicity, at least relative to hatching probability. However, in looking at the probability of being deformed, the results of the present study are counter to previous findings of mutually protective effects. It might have been the particular combination of the two specific forms of these elements

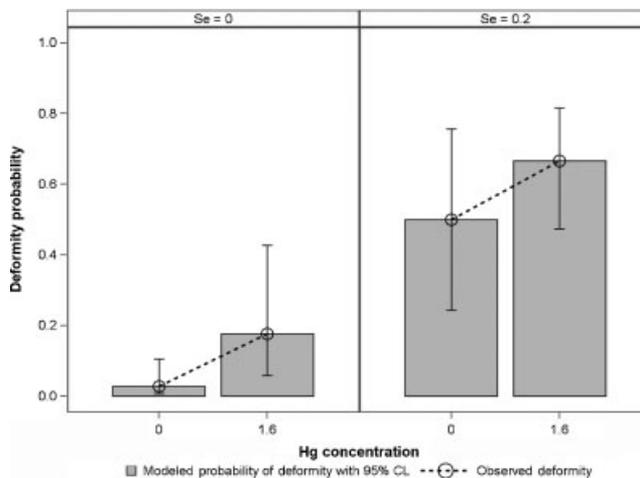


Fig. 4. Probability of being deformed estimated using a logistic regression model showing the 95% confidence limits (CL) and observed deformities represented by the circle for 2006 and 2007 data combined.

Table 6. Coefficients of the logistic regression on the probability of chick deformities in mallard (*Anas platyrhynchos*) eggs injected with various doses ($\mu\text{g/g}$) of Hg and Se during the 2006 and 2007 experiments combined

Parameter	Degrees of freedom	Estimate	Standard error	Wald χ^2	<i>p</i>
Intercept	1	-3.5553	0.7171	24.5788	<0.0001
Hg	1	2.0149	0.9587	4.4174	0.0356
Se	1	3.5553	0.9207	14.9129	0.0001
Hg \times Se	1	-1.3218	1.1912	1.2311	0.2672

(methylmercury and selenomethionine) in the present study that caused the unexpected results. Other studies with birds, such as those mentioned above, have used methylmercury in combination with Se, but the form of the Se was not selenomethionine. As with the methylmercury form of Hg, the selenomethionine form of Se is the most appropriate to use in toxicity studies with birds. Hamilton et al. [33] reported that selenomethionine is a good laboratory model for Se toxicity in fish, and Heinz et al. [34] and Heinz and Hoffman [35] concluded that it is a good model for Se poisoning in birds. In a study with rats, Magos et al. [36] concluded that selenomethionine might not protect as well as sodium selenite against inorganic Hg, and perhaps methylmercury, poisoning.

Toxicities of injected methylmercury and selenomethionine

In other laboratory studies with methylmercury and selenomethionine, a small percentage of the control embryos exhibited deformities [14,21,37], but the low rate of deformities in controls in the present study suggests that it was the methylmercury and selenomethionine that caused the majority of the deformities observed. Seleno-L-methionine injected into mallard eggs was clearly a more potent teratogenic agent than was methylmercury chloride; the maximum likelihood estimates indicated that selenomethionine when injected alone caused the probability of being deformed to increase more than when Hg was injected alone. The greater effect of Se in the present study is supported by a study in which mallards were fed diets containing 10 $\mu\text{g/g}$ Hg as methylmercury chloride or 10 $\mu\text{g/g}$ Se as selenomethionine; the Se treatment resulted in more deformities than did the Hg treatment [14].

Toxicity of injected versus maternally deposited Hg and Se

Although egg injections are an effective way to compare the types and frequency of deformities that Hg and Se can cause and their possible interactions, it is important to recognize that the toxicities of both methylmercury chloride and selenomethionine are greater when these compounds are injected into eggs than when the same concentrations are achieved by feeding the Hg or Se to the parents and having the female deposit the Hg or Se naturally into her eggs. For example, in the present study, the injection of selenomethionine that resulted in 0.2 $\mu\text{g/g}$ Se on a wet weight basis in eggs caused many deformities, but 0.2 $\mu\text{g/g}$ Se, naturally deposited in the egg by the female, would not be expected to cause deformities [20]. Likewise, the 1.6 $\mu\text{g/g}$ Hg injected into eggs caused deformities, but this concentration of Hg would be expected to cause very few deformities if the mother deposited the methylmercury into the egg [15].

When compounds such as methylmercury chloride or selenomethionine are injected into the air cell of an egg, they pass through the inner shell membrane and into the albumen of the egg [38]. If dissolved in the aqueous matrix of the albumen, these compounds may be able to come in contact with the membranes covering the embryo and readily cause deformities

and mortality. In addition, embryos float just under the air cell when an egg is held vertically for the injection. Because the injected eggs were held vertically for 30 min after the injection, the toxicity of the methylmercury and selenomethionine might have been enhanced as the concentrated solutions of Hg and Se passed through the inner shell membrane and came in close proximity to the embryos. In contrast, methylmercury and selenomethionine that are deposited in the egg by the mother become bound to proteins that have to be metabolized before the Hg and Se are available to the developing embryo [39,40].

CONCLUSIONS

The present study supports the idea that seleno-L-methionine is a more potent teratogenic compound than is methylmercury chloride and that both compounds are more teratogenic when injected into eggs than when the mother deposits them naturally into her eggs. The unexpected finding of methylmercury enhancing embryo survival, but worsening Se-induced deformities, warrants additional study to understand not only the specific combinations of Hg and Se at which the phenomenon occurs but also the underlying biochemical mechanisms. Because Hg and Se can occur together in avian tissues and eggs in the wild, field studies are needed to determine whether these paradoxical interactions occur in nature.

Acknowledgement—This research was funded by the CALFED Bay-Delta Program's Ecosystem Restoration Program (grant ERP-02D-C12) with additional support from the USGS Patuxent Wildlife Research Center. We thank K. Kenow and J. Ackerman for early reviews of the manuscript. Use of trade, product, or firm names does not imply endorsement by the U.S. Government.

REFERENCES

- Eagles-Smith CA, Ackerman JT, Yee J, Adelsbach TL. 2009. Mercury demethylation in livers of four waterbird species: Evidence for dose-response thresholds with liver total mercury. *Environ Toxicol Chem* 28:568-577.
- Norheim G. 1987. Levels and interactions of heavy metals in sea birds from Svalbard and the Antarctic. *Environ Pollut* 47:83-94.
- Ohlendorf HM, Fleming WJ. 1988. Birds and environmental contaminants in San Francisco and Chesapeake Bay. *Mar Pollut Bull* 19:487-495.
- Ohlendorf HM, Lowe RW, Kelly PR, Harvey TE. 1986. Selenium and heavy metals in San Francisco Bay diving ducks. *J Wildl Manag* 50:64-71.
- Albers PH, Koterba MT, Rossmann R, Link WA, French JB, Bennett RS, Bauer WC. 2007. Effects of methylmercury on reproduction in American kestrels. *Environ Toxicol Chem* 26:1856-1866.
- Evers DC, Savoy LJ, DeSorbo CR, Yates DE, Hanson W, Taylor KM, Siegel LS, Cooley JH Jr, Bank MS, Major A, Munney K, Mower BF, Vogel HS, Schoch N, Pokras M, Goodale MW, Fair J. 2008. Adverse effects from environmental mercury loads on common loons. *Ecotoxicology* 17:69-81.
- Fimreite N. 1971. Effects of dietary methylmercury on ring-necked pheasants. Occasional Paper 9. Canadian Wildlife Service, Ottawa, Ontario, Canada.
- Fimreite N. 1974. Mercury contamination of aquatic birds in Northwestern Ontario. *J Wildl Manag* 38:120-131.
- Finley MT, Stendell RC. 1978. Survival and reproductive success of black ducks fed methyl mercury. *Environ Pollut* 16:51-64.
- Heinz GH. 1979. Methylmercury: Reproductive and behavioral effects on three generations of mallard ducks. *J Wildl Manag* 43:394-401.
- Hill EF, Henny CJ, Grove RA. 2008. Mercury and drought along the lower Carson River, Nevada: II. Snowy egret and black-crowned night-heron reproduction on Lahontan Reservoir, 1997-2006. *Ecotoxicology* 17:117-131.
- Schwarzbach SE, Albertson JD, Thomas CM. 2006. Effects of predation, flooding, and contamination on reproductive success of California clapper rails (*Rallus longirostris obsoletus*) in San Francisco Bay. *Auk* 123:45-60.

13. Tejning S. 1967. Biological effects of methylmercury dicyandiamide-treated grain in the domestic fowl *Gallus gallus* L. *Oikos* 8:1–116.
14. Heinz GH, Hoffman DJ. 1998. Methylmercury chloride and selenomethionine interactions on health and reproduction in mallards. *Environ Toxicol Chem* 17:139–145.
15. Heinz GH, Hoffman DJ. 2003. Embryotoxic thresholds of mercury: Estimates from individual mallard eggs. *Arch Environ Contam Toxicol* 44:257–264.
16. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA. 2009. Species differences in sensitivity of avian embryos to methylmercury. *Arch Environ Contam Toxicol* 56:129–138.
17. Ohlendorf HM. 1989. Bioaccumulation and effects of selenium in wildlife. In Jacobs LW, ed, *Selenium in Agriculture and the Environment*. Special Publication 23. American Society of Agronomy and Soil Science Society of America, Madison, WI, pp 133–177.
18. Ohlendorf HM, Hoffman DJ, Saiki MK, Aldrich TW. 1986. Embryonic mortality and abnormalities of aquatic birds: Apparent impacts of selenium from irrigation drainwater. *Sci Total Environ* 52:49–63.
19. Ohlendorf HM, Hothem RL. 1995. Agricultural drainwater effects on wildlife in central California. In Hoffman DJ, Rattner BA, Burton GA Jr, Cairns J Jr, eds, *Handbook of Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 577–595.
20. Heinz GH, Hoffman DJ, Gold LG. 1989. Impaired reproduction of mallards fed an organic form of selenium. *J Wildl Manag* 53:418–428.
21. Hoffman DJ, Heinz GH. 1988. Embryotoxic and teratogenic effects of selenium in the diet of mallards. *J Toxicol Environ Health* 24:477–490.
22. Santolo GM, Yamamoto JT, Pisenti JM, Wilson BW. 1999. Selenium accumulation and effects on reproduction in captive American kestrels fed selenomethionine. *J Wildl Manag* 63:502–511.
23. Smith GJ, Heinz GH, Hoffman DJ, Spann JW, Krynsky AJ. 1988. Reproduction in black-crowned night-herons fed selenium. *Lake Reservoir Manag* 4:175–180.
24. Wiemeyer SJ, Hoffman DJ. 1996. Reproduction in eastern screech-owls fed selenium. *J Wildl Manag* 60:332–341.
25. Cuvin-Aralar MLA, Furness RW. 1991. Mercury and selenium interaction: A review. *Ecotoxicol Environ Saf* 21:348–364.
26. SAS Institute. 2007. SAS OnlineDoc[®] 9.2. SAS Institute Inc., Cary, NC, USA.
27. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA. 2012. Hormesis associated with a low dose of methylmercury injected into mallard eggs. *Arch Environ Contam Toxicol* 62:141–144.
28. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR. 2010. Enhanced reproduction in mallards fed a low level of methylmercury: An apparent case of hormesis. *Environ Toxicol Chem* 29:650–653.
29. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR. 2011. A comparison of the teratogenicity of methylmercury and selenomethionine injected into bird eggs. *Arch Environ Contam Toxicol* DOI: 10.1007/s00244-011-9717-4.
30. El-Begearmi MM, Sunde ML, Ganther HE. 1977. A mutual protective effect of mercury and selenium in Japanese quail. *Poult Sci* 56: 313–322.
31. Stoewsand GS, Bache CA, Lisk DJ. 1974. Dietary selenium protection of methylmercury intoxication of Japanese quail. *Bull Environ Contam Toxicol* 11:152–156.
32. Sell JL, Horani FG. 1976. Influence of selenium on toxicity and metabolism of methylmercury in chicks and quail. *Nutr Rep Int* 14: 439–447.
33. Hamilton SJ, Buhl KJ, Faerber NL, Wiedmeyer RH, Bullard FA. 1990. Toxicity of organic selenium in the diet to chinook salmon. *Environ Toxicol Chem* 9:347–358.
34. Heinz GH, Hoffman DJ, LeCaptain LJ. 1996. Toxicity of seleno-L-methionine, seleno-DL-methionine, high selenium wheat, and selenized yeast to mallard ducklings. *Arch Environ Contam Toxicol* 30:93–99.
35. Heinz GH, Hoffman DJ. 1996. Comparison of the effects of seleno-L-methionine, seleno-DL-methionine, and selenized yeast on reproduction of mallards. *Environ Pollut* 91:169–175.
36. Magos L, Clarkson TW, Hudson AR. 1984. Differences in the effects of selenite and biological selenium on the chemical form and distribution of mercury after the simultaneous administration of HgCl₂ and selenium to rats. *J Pharmacol Exp Ther* 228:478–483.
37. Hoffman DJ, Moore JM. 1979. Teratogenic effects of external egg applications of methylmercury in the mallard, *Anas platyrhynchos*. *Teratology* 20:453–462.
38. Heinz GH, Hoffman DJ, Kondrad SK, Erwin CA. 2006. Factors affecting the toxicity of methylmercury injected into eggs. *Arch Environ Contam Toxicol* 50:264–279.
39. Nishimura M, Urakawa N. 1976. A transport mechanism of methylmercury to egg albumen in laying Japanese quail. *Jpn J Vet Sci* 38: 433–444.
40. Ochoa-Solano A, Gitler C. 1968. Incorporation of ⁷⁵Se-selenomethionine and ³⁵S-methionine into chicken egg white proteins. *J Nutr* 94: 243–248.