

A Comparison of the Teratogenicity of Methylmercury and Selenomethionine Injected Into Bird Eggs

Gary H. Heinz · David J. Hoffman ·
Jon D. Klimstra · Katherine R. Stebbins

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Abstract Methylmercury chloride and seleno-L-methionine were injected separately or in combinations into the fertile eggs of mallards (*Anas platyrhynchos*), chickens (*Gallus gallus*), and double-crested cormorants (*Phalacrocorax auritus*), and the incidence and types of teratogenic effects were recorded. For all three species, selenomethionine alone caused more deformities than did methylmercury alone. When mallard eggs were injected with the lowest dose of selenium (Se) alone (0.1 µg/g), 28 of 44 embryos and hatchlings were deformed, whereas when eggs were injected with the lowest dose of mercury (Hg) alone (0.2 µg/g), only 1 of 56 embryos or hatchlings was deformed. Mallard embryos seemed to be more sensitive to the teratogenic effects of Se than chicken embryos: 0 of 15 chicken embryos or hatchlings from eggs injected with 0.1 µg/g Se exhibited deformities. Sample sizes were small with double-crested cormorant eggs, but they also seemed to be less sensitive to the teratogenic effects of Se than mallard eggs. There were no obvious differences among species regarding Hg-induced deformities. Overall, few interactions were apparent between methylmercury and selenomethionine with respect to the types of deformities observed. However, the deformities spina bifida and craniorachischisis were observed only when Hg and Se were injected in combination. One paradoxical finding was that some doses of methylmercury seemed to counteract the negative effect selenomethionine had on hatching of eggs while at the same time enhancing the negative effect

selenomethionine had on creating deformities. When either methylmercury or selenomethionine is injected into avian eggs, deformities start to occur at much lower concentrations than when the Hg or Se is deposited naturally in the egg by the mother.

Mercury (Hg) and selenium (Se) sometimes occur together in abiotic and biotic parts of ecosystems (Conaway et al. 2008; Stewart et al. 2004), and the co-occurrence of elevated concentrations of Hg and Se has been reported in birds (Eagles-Smith et al. 2009; Norheim 1987; Ohlendorf and Fleming 1988; Ohlendorf et al. 1986b).

Methylmercury has been shown to cause teratogenic effects in laboratory studies (Heinz and Hoffman 1998, 2003; Heinz et al. 2009; Hoffman and Moore 1979). However, methylmercury's main harmful effect seems to be impairing hatching success of eggs. Both field and laboratory studies have demonstrated that methylmercury can impair hatching success of bird eggs (Albers et al. 2007; Evers et al. 2008; Fimreite 1971, 1974; Finley and Stendell 1978; Heinz 1979; Hill et al. 2008; Schwarzbach et al. 2006; Tejning 1967). Se also has been shown to impair reproductive success, with a stronger ability than is the case with methylmercury to produce teratogenic effects, as has been shown in both field (Hoffman et al. 1988; Ohlendorf 1989, 2002; Ohlendorf and Fleming 1988; Ohlendorf and Hothem 1995; Ohlendorf et al. 1986a; Skorupa 1998) and laboratory studies (Heinz and Hoffman 1998; Heinz et al. 1989; Hoffman and Heinz 1988; Santolo et al. 1999; Smith et al. 1988; Wiemeyer and Hoffman 1996).

Although the interactions between Hg and Se are among the best known of all environmental contaminants, these interactions can be complex. Cuvin-Aralar and Furness (1991) reviewed Hg and Se interactions and reported that

G. H. Heinz (✉) · D. J. Hoffman · J. D. Klimstra ·
K. R. Stebbins
Patuxent Wildlife Research Center, United States Geological
Survey, BARC-East, Building 308, 10300 Baltimore Avenue,
Beltsville, MD 20705, USA
e-mail: gheinz@usgs.gov

such interactions are normally antagonistic but that additivity and synergism also can occur; the investigators concluded that “the interactions between different Se and Hg compounds are extremely complex and not well understood at present.” In particular, studies combining the two most toxic and environmentally realistic chemical forms of these elements, methylmercury and selenomethionine, have been few. When Heinz and Hoffman (1998) fed methylmercury and selenomethionine alone or in combination to breeding mallards (*Anas platyrhynchos*) each compound, alone, caused teratogenic effects in embryos, but the combination of both caused a greater frequency as well as variety of deformities.

The ideal way to compare the teratogenic effects and interactions of Hg and Se would be through controlled feeding studies in which known doses of methylmercury and selenomethionine would be fed to egg-laying female birds, thus controlling the amount of these compounds deposited into their eggs. However, given the great expense and time required to feed the adult birds several combinations of Hg and Se, it is unlikely that many of these feeding studies will be performed. Therefore, in the current study we used egg injections as an alternative method to compare the incidence and types of teratogenic effects caused by methylmercury and selenomethionine treatments alone or in combination.

Materials and Methods

Mallard and chicken (*Gallus gallus*) eggs were purchased from commercial hatcheries, and double-crested cormorant (*Phalacrocorax auritus*) eggs were collected from the wild under appropriate state and federal collecting permits. Methylmercury chloride and seleno-L-methionine were dissolved in water, and eggs were dosed by injecting 1 μ l of water/g of egg contents into the air cell of the egg. In past injection studies with methylmercury, corn oil has been used as solvent, but in the current study water was used because it is capable of dissolving both methylmercury chloride and selenomethionine and has been used successfully in another study with methylmercury (Heinz et al. 2011). The eggs of all three species were injected when the embryos reached the same developmental stage, namely, when they had the appearance of a 3-day-old chicken embryo. Waiting until an avian embryo reaches the developmental equivalent of a 3-day-old chicken embryo before injecting the egg permits one to remove infertile or early dead eggs from the study and results in good dose-response curves (Heinz et al. 2006, 2009). The surviving eggs were randomized either to a control group receiving untreated water or to groups dosed with various combinations of Hg and Se. For chickens, we had an additional

untreated control group that was not injected with water; the purpose of this untreated control group was to determine if the injection of water into the air cell could cause deformities.

In our first experiment with mallards, groups of eggs were injected with 0, 0.2, 0.4, 0.8, or 1.6 μ g Hg/g of egg contents in all possible combinations with 0, 0.1, 0.2, 0.4, or 0.6 μ g Se/g of egg contents ($5 \times 5 = 25$ groups of eggs). The resulting concentrations in eggs were based on a wet-weight basis. If one wishes to convert these concentrations to a dry-weight basis, the approximate moisture content of mallard eggs is 70–75%. We randomized 28 eggs to the group of controls receiving only untreated water. All of the other groups contained 14 eggs. Based on findings in this first study, we conducted a follow-up experiment that focused on groups of 45–47 mallard eggs that were injected with the following combinations of Hg and Se: 0 μ g/g Hg + 0 μ g/g Se, 0 μ g/g Hg + 0.1 μ g/g Se, 0 μ g/g Hg + 0.2 μ g/g Se, 0.2 μ g/g Hg + 0 μ g/g Se, 0.2 μ g/g Hg + 0.1 μ g/g Se, 1.6 μ g/g Hg + 0 μ g/g Se, and 1.6 μ g/g Hg + 0.2 μ g/g Se.

For chickens, we collected and combined data from three studies. A total of 90 eggs served as uninjected controls (untreated water was not injected). Except for the control group injected with untreated water (0 μ g/g Hg/0 μ g/g Se; $n = 30$), 15 eggs were in each group injected with combinations of Hg at 0, 0.2, 0.4, 0.8, and 1.6 μ g/g and Se at 0, 0.1, 0.2, 0.4, and 0.6 μ g/g ($5 \times 5 = 25$ groups). To convert wet-weight to dry-weight concentrations, chicken eggs contain approximately 70–75% moisture.

For double-crested cormorants, we also collected data from three studies. Eggs were injected with combinations of 0, 0.2, 0.4, and 0.8 μ g/g Hg and 0, 0.1, 0.2, and 0.4 μ g/g Se, all on a wet-weight basis ($4 \times 4 = 16$ groups). To convert these concentrations to a dry-weight basis, the percent moisture content of cormorant eggs is approximately 80–85%. There were 26 control eggs (0 Hg/0 Se) and 10–18 eggs in each of the other groups.

All eggs were artificially incubated, and any dead eggs that had developed to at least the equivalent appearance of a 7-day-old chicken embryo, plus all hatchlings, were examined for deformities. Before the 7-day stage, embryos are small and relatively undeveloped. Furthermore, rapid decomposition often occurs after embryo death in the earlier stages; both of these factors make identification of deformities difficult in earlier stages. Only overt external malformations visible to the eye were tallied. No internal examinations were made. Data on hatching success of eggs also were collected.

In the upper part of Table 1, definitions are given for the technical terms used for the first five types of external malformations. In addition to the specific deformities

Table 1 Explanation of terms used to describe deformities

Term	Definition
Exencephaly, anencephaly	Exencephaly occurs when the skull bones are not completely formed and the brain is exposed through the skull. Anencephaly is the absence of a large part of the brain.
Hydrocephaly	Hydrocephaly is a buildup of cerebrospinal fluid in the brain that causes a swelling of the head. We included only clear cases because some mild swelling of the head can occur when a chick cannot escape from the egg.
Gastroschisis	Gastroschisis is a condition in which there is an opening in the stomach (or stomach and chest) region, allowing one to see the embryo's internal organs.
Spina bifida and craniorachischisis	Spina bifida is an incomplete closure of the backbone and spinal cord. Craniorachischisis is a fissure of the cranium continuing down the spine that exposes part of the brain and spinal cord.
Lordosis and scoliosis	Lordosis and scoliosis both involve unnatural twisting of the spine. Lordosis is an abnormal front-to-back curve of the spine, whereas scoliosis is an abnormal side-to-side curvature of the spine.
Deformed head or face	Deformed head or face is a flattening of the face or other abnormality in the shape of the head or face. In addition, we included a case of an embryo with two heads. Deformities of the bill and eyes are described separately as are the conditions of exencephaly and hydrocephaly.
Eye deformities	Eye deformities included anophthalmia (eyes entirely missing), microphthalmia (the eyes are smaller than normal), exophthalmia (bulging eyes), the presence of three eyes, and eyes lacking retinal tissue.
Bill deformities	Bill deformities included crossed bills, spoon-shaped upper or lower bills, abnormally short or long bills, bills twisted to the side, indentations on the bill, or no bills present.
Neck deformities	Neck deformities included an abnormally short or twisted neck.
Misshappen-body deformities	Misshappen-body deformities included shortening of the axial skeleton, stunting of the entire body, and poorly developed tail.
Wing deformities	Wing deformities included abnormally short or missing wings.
Leg, foot, and toe deformities	Leg, foot, and toe deformities included short legs, twisted legs or feet, swollen joints, missing legs or feet, missing or extra toes, twisted toes, and toes pointing in the wrong direction.

associated with these five technical terms, many different types of deformities may be expressed on the same body part; therefore, the remainder of Table 1 lists deformities collectively on an anatomical basis. All of the deformities listed in Table 1 were observed in the present study. Some dead embryos or hatchlings had more than one deformity. For example, if an embryo had a missing right eye, deformed upper bill, no right leg, and missing toes on the left leg, it would be credited with four deformities.

Results

One of the important findings in the first experiment with mallards was that there were no deformities in the control group, whereas both methylmercury and selenomethionine treatments were associated with deformities (Table 2). Selenomethionine was more teratogenic than methylmercury. In the groups of eggs injected with 0.1 or 0.2 µg/g Se and no Hg, 5 of 13 and 3 of 4 dead embryos (>7 days of age) and hatchlings were deformed. Only 1 embryo in the group receiving 0.4 µg/g Se alone survived to 7 days of age, and none of the embryos dosed with 0.6 µg/g Se alone survived; consequently, it was not possible to make any comparisons that included those two groups. Compared with selenomethionine, in the groups of eggs injected with 0.2, 0.4, 0.8, or 1.6 µg/g Hg and no Se, only 1 of 14, 1 of

13, 0 of 9, and 1 of 6 dead embryos or hatchlings, respectively, were deformed.

Given the limited numbers of eggs that survived to at least 7 days of age in the first mallard experiment, it was not possible to subject potential teratogenic interactions between Hg and Se to rigorous statistical comparisons; however, the only case of craniorachischisis occurred in a dead embryo from an egg injected with a combination of 0.4 µg/g Hg and 0.4 µg/g Se, and the only three cases of spina bifida occurred in dead embryos, all from eggs injected with a combination of 1.6 µg/g Hg and 0.4 µg/g Se. The majority of the deformities in all of the various groups injected with Se alone or with combinations of Se and Hg were found in the eyes, bill, wings, and legs.

With some combinations of Hg and Se, the addition of methylmercury may have improved the hatching success of Se-dosed eggs. For example, in the first mallard experiment, 0 of 14 eggs injected with 0.2 µg/g Se and no Hg hatched compared with 4 of 14 in the group injected with a combination of 0.2 µg/g Se + 1.6 µg/g Hg. Although hatching success of eggs injected with 0.2 µg/g Se may have been improved by the addition of 1.6 µg/g Hg, 2 of the 4 hatchlings were deformed.

In the second experiment with mallards, 0 of 44 controls were deformed; 0 of 42 dead embryos or hatchlings from eggs injected with 0.2 µg/g Hg and no Se were deformed; and only 1 of 10 dead embryos or hatchlings dosed with

Table 2 Deformities in embryos and hatchlings of mallard eggs injected with Hg and Se (mallard experiment 1)

Group ($\mu\text{g/g}$ Hg/ $\mu\text{g/g}$ Se)	N ^a	No. of birds with ≥ 1 deformities (%)	No. of birds with >1 deformity	Exencephaly/ anencephaly	Hydrocephaly	Gastrochisis	Spina bifida/ craniorachischisis	Lordosis/ scoliosis	Deformed head or face	Eyes	Bill	Neck	Misshappen body	Wings	Legs, feet, toes
0/0	28	0 (0.0)	0												
0/0.1	13	5 (38.5)	5		1	3		1		7	8		1	8	10
0/0.2	4	3 (75.0)	3	1						2	6			2	5
0/0.4	1	0 (0.0)	0												
0/0.6	0														
0.2/0	14	1 (7.1)	0		1										
0.2/0.1	13	10 (76.9)	10			4				6	17			14	20
0.2/0.2	11	5 (45.5)	5			3		1		6	10	1	2	10	8
0.2/0.4	0														
0.2/0.6	0														
0.4/0	13	1 (7.7)	1		1			1		1	1	1		1	2
0.4/0.1	13	8 (61.5)	7		1					2	2				15
0.4/0.2	6	3 (50.0)	3		1					4	6			1	6
0.4/0.4	1	1 (100.0)	1			1		1			1		1		2
0.4/0.6	0														
0.8/0	9	0 (0.0)	0												
0.8/0.1	11	7 (63.6)	4							4	3			3	8
0.8/0.2	7	5 (71.4)	5			1				4	9		1	4	8
0.8/0.4	5	4 (80.0)	4			1		2		4	7		2	6	4
0.8/0.6	0														
1.6/0	6	1 (16.7)	0		1										
1.6/0.1	8	2 (25.0)	1		1					2					
1.6/0.2	10	8 (80.0)	7							5	8			3	12
1.6/0.4	6	5 (83.3)	5		1			1		5	7		1	4	7
1.6/0.6	0														

^a The number of dead embryos and hatchlings examined for deformities

1.6 $\mu\text{g/g}$ Hg and no Se was deformed (Table 3). In contrast, 23 of 31 dead embryos or hatchlings from eggs injected with 0.1 $\mu\text{g/g}$ Se and no Hg were deformed, and 3 of 3 injected with 0.2 $\mu\text{g/g}$ Se and no Hg were deformed. As was the case in the first mallard experiment, the only cases of spina bifida occurred in a group of eggs injected with a combination of Hg and Se; five dead embryos and one hatchling exhibited spina bifida when eggs were injected with 1.6 $\mu\text{g/g}$ Hg and 0.2 $\mu\text{g/g}$ Se.

In the second mallard experiment, there were additional findings suggestive of the ability of certain combinations of methylmercury to enhance the hatching of Se-dosed eggs: 11 of 42 viable eggs injected with 0.1 $\mu\text{g/g}$ Se and no Hg hatched compared with 24 of 42 that hatched in the group injected with 0.1 $\mu\text{g/g}$ Se + 0.2 $\mu\text{g/g}$ Hg. None of the eggs injected with 0.2 $\mu\text{g/g}$ Se alone hatched, but 8 eggs treated with a combination of 1.6 $\mu\text{g/g}$ Hg and 0.2 $\mu\text{g/g}$ Se hatched; however, 3 of those 8 were deformed.

With chickens, neither set of controls eggs (eggs injected with no water and those injected with untreated water) exhibited any deformities (Table 4). As was the case with mallards, selenomethionine caused more deformities in chicken embryos and hatchlings than did methylmercury. In addition, chicken embryos seemed to be less sensitive to the teratogenic effects, definitely of selenomethionine and perhaps to a lesser extent of methylmercury, than mallards. For example, combining the results from mallard experiments 1 and 2, a total of 28 of 44 mallard embryos and hatchlings treated with 0.1 $\mu\text{g/g}$ Se and no Hg exhibited deformities, whereas 0 of 15 chicken embryos or hatchlings treated with 0.1 $\mu\text{g/g}$ Se and no Hg exhibited deformities. However, one egg that was injected with 0.2 $\mu\text{g/g}$ Se and no Hg contained an embryo, which, that although it survived through 90% of incubation before dying, was grossly deformed: It had two heads, three eyes, no lower leg development, no right wing and only a rudimentary left wing, craniorachischisis, and anencephaly.

As was the case for mallards, the injection of methylmercury along with selenomethionine in chicken eggs seemed to increase the likelihood that an embryo would survive to reach the stage of a 7-day-old chicken embryo (an embryonic age at which one can more readily discern deformities), thus increasing the number of Se-induced deformities we observed. For example, when 15 chicken eggs were injected with 0.4 $\mu\text{g/g}$ Se and no Hg, none of the embryos survived to the 7-day-old stage; however, when sets of eggs were injected with 0.4 $\mu\text{g/g}$ Se and either 0.2 $\mu\text{g/g}$ or 0.4 $\mu\text{g/g}$ Hg, in both instances 3 of 15 embryos lived to at least 7 days of age; and when 0.4 $\mu\text{g/g}$ Se was combined with 0.8 $\mu\text{g/g}$ Hg, 9 of 15 embryos survived to at least 7 days of age. Only 4 chicken hatchlings exhibited deformities; 1 hatchling was deformed of a total of 10 hatchlings in the group of eggs injected with 0.2 $\mu\text{g/g}$ Se

Table 3 Deformities in embryos and hatchlings of mallard eggs injected with Hg and Se (mallard experiment 2)

Group ($\mu\text{g/g}$ Hg/ $\mu\text{g/g}$ Se)	N ^a	No. of birds with ≥ 1 deformities (%)	No. of birds with >1 deformity	Exencephaly/ anencephaly	Hydrocephaly	Gastroschisis	Spina bifida/ craniorachischisis	Lordosis/ scoliosis	Deformed head/face	Eyes	Bill	Neck	Misshappen body	Wings	Legs, feet, toes	
0/0	44	0 (0.0)	0													
0/0.1	31	23 (74.2)	22		1	5			9	20			5	10	42	
0/0.2	3	3 (100.0)	3			1			3	5				3	6	
0.2/0	42	0 (0.0)	0													
0.2/0.1	40	19 (47.5)	17	2		6		2	6	15			2	4	31	
1.6/0	10	1 (10.0)	0							1						
1.6/0.2	16	10 (62.5)	9				6		1	5			3	1	19	

^a The number of dead embryos and hatchlings examined for deformities

Table 4 Deformities in embryos and hatchlings of chicken eggs injected with Hg and Se

Group ($\mu\text{g/g}$ Hg/ $\mu\text{g/g}$ Se)	N^a	No. of birds with ≥ 1 deformities (%)	No. of birds with ≥ 1 deformities >1 deformity	Exencephaly/ anencephaly	Hydrocephaly	Gastrochisis	Spina bifida/ craniorachischisis	Lordosis/ scoliosis	Deformed head/face	Eyes	Bill	Neck	Misshappen body	Wings	Legs, feet, toes
Controls without injected water	87	0 (0.0)	0												
0/0	29	0 (0.0)	0												
0/0.1	15	0 (0.0)	0												
0/0.2	11	2 (18.2)	2	1		1		1		1				2	3
0/0.4	0														
0/0.6	0														
0.2/0	14	0 (0.0)	0												
0.2/0.1	14	0 (0.0)	0												
0.2/0.2	14	6 (42.9)	4		1	1		3					1	1	9
0.2/0.4	3	3 (100.0)	3			2		1		1			1	1	5
0.2/0.6	0														
0.4/0	9	0 (0.0)	0												
0.4/0.1	10	3 (30.0)	3			2		2						1	3
0.4/0.2	10	0 (0.0)	0												
0.4/0.4	3	2 (66.7)	2			1				1	1		1		3
0.4/0.6	1	1 (100.0)	1			1				1	1		1		1
0.8/0	5	0 (0.0)	0												
0.8/0.1	6	1 (16.7)	1												
0.8/0.2	6	1 (16.7)	0		1										
0.8/0.4	9	5 (55.6)	5			1		1		1	4	1		1	10
0.8/0.6	0														
1.6/0	1	0 (0.0)	0												
1.6/0.1	5	0 (0.0)	0												
1.6/0.2	3	0 (0.0)	0												
1.6/0.4	2	0 (0.0)	0												
1.6/0.6	2	0 (0.0)	0												

^a The number of dead embryos and hatchlings examined for deformities

alone compared with 3 that were deformed of a total of 11 that hatched from eggs injected with a combination of 0.2 µg/g Hg and 0.2 µg/g Se.

As was observed with mallards, a greater range of deformities was seen in chicken eggs injected with both Se and Hg than with Se alone, but given the small sample sizes of embryos surviving to 7 days of age when only Se was injected, it is difficult to determine the strength of these Hg–Se interactions.

With double-crested cormorants, 2 of 21 control embryos and hatchlings exhibited deformities; in both cases an embryo exhibited hydrocephaly (Table 5). Although the numbers of embryos in the various groups that survived at least to the equivalent of a 7-day-old chicken embryo were small, it was apparent, as it was with mallards and chickens, that selenomethionine was more teratogenic than methylmercury. The percentages of embryos and hatchlings that were deformed when eggs were injected with 0.2, 0.4, or 0.8 µg/g Hg and no Se were 0, 15.4, and 0%, respectively. Although heavy early embryonic mortality occurred at all doses of Se alone, precluding an examination of these early dead birds for deformities, when Se alone was injected at 0.1 or 0.2 µg/g, the deformity rates were 50% in each case. All of the eggs injected with 0.4 µg/g Se alone died before reaching the 7-day-old stage. Cormorant embryos, like those of chickens, may have been less sensitive to the teratogenic effects of Se than mallard embryos. Although sample sizes were small, the data suggest that the coinjection of 0.4 or 0.8 µg/g Hg may have increased embryo survival of eggs that were injected with 0.1 µg/g Se, but it did not lessen the teratogenic effects of the 0.1 µg/g Se injection. To the contrary, the group of eggs injected with 0.8 g/g Hg plus 0.1 µg/g Se had the greatest number of deformities of any group.

Discussion

Comparison of Deformity Rates Among Control, Methylmercury, and Selenomethionine Groups

In other controlled laboratory studies with methylmercury and selenomethionine, a small percentage of the control embryos exhibited deformities (Heinz and Hoffman 1998; Hoffman and Heinz 1988; Hoffman and Moore 1979). Those previous findings, plus the near complete absence of deformities in controls in any of the current experiments, strongly suggests that methylmercury and selenomethionine caused the deformities we observed. The findings with chickens, where neither the uninjected controls nor the controls injected with untreated water exhibited any

deformities, demonstrated further that the injection of water itself into the air cell does not cause deformities.

In the current studies, seleno-L-methionine was clearly a more potent teratogenic agent than was methylmercury. Our findings with egg injections are supported by a study in which mallards were fed diets containing 10 µg/g Hg as methylmercury chloride and 10 µg/g Se as selenomethionine alone or in combination; the Se treatment resulted in more deformities than the Hg treatment (Heinz and Hoffman 1998).

Interactions Between Methylmercury and Selenomethionine

In the current studies, spina bifida was observed only in eggs injected with both Hg and Se: Heinz and Hoffman (1998) reported spina bifida only in embryos from eggs laid by female mallards fed a combination of 10 µg/g Hg and 10 µg/g Se. Therefore, certain combinations of methylmercury and selenomethionine may act synergistically in producing teratogenic effects.

There were no indications in our studies that methylmercury and selenomethionine ever counteracted each other's teratogenic effects. In fact, there were instances in the current studies where the coinjection of methylmercury increased the incidence of teratogenic effects of selenomethionine while at the same time decreasing embryo mortality. As discussed more fully and statistically in a separate article on mallard eggs (Klimstra et al. in press), the simultaneous injection of methylmercury chloride seemed to enhance embryo survival in eggs injected with seleno-L-methionine, but at the same time methylmercury did not protect the embryos from the teratogenic effects of the Se. The same paradoxical finding was observed in our chicken and cormorant eggs as well. It remains a puzzling finding. It appears that the methylmercury treatment improved the vigor of the embryos exposed to selenomethionine, thus allowing them to survive longer and in some cases even hatch, but the coexposure to methylmercury apparently did not protect the embryos from the teratogenic effects of Se. In fact, both more deformities and a greater variety of deformities were observed when both methylmercury and selenomethionine were injected into eggs. This finding of methylmercury enhancing survival, but not protecting against Se-induced deformities, certainly warrants additional laboratory study to understand not only the combinations of Hg and Se at which the phenomenon occurs but also the underlying biochemical mechanisms. Given the co-occurrence of Hg and Se in avian tissues and eggs in the wild, field studies are also needed to determine if these paradoxical interactions also occur in nature.

Table 5 Deformities in embryos and hatchlings of double-crested cormorant eggs injected with Hg and Se

Group ($\mu\text{g/g}$ Hg/ $\mu\text{g/g}$ Se)	N^a	No. of birds with ≥ 1 deformities (%)	No. of birds with >1 deformity	Exencephaly/ anencephaly	Hydrocephaly	Gastrochisis	Spina bifida/ craniorachischisis	Lordosis/ scoliosis	Deformed head/face	Eyes	Bill	Neck	Misshappen body	Wings	Legs, feet, toes	
0/0	21	2 (9.5)	0		2											
0/0.1	4	2 (50.0)	2		1			1		3	2			1		4
0/0.2	2	1 (50.0)	1					1			1					
0/0.4	0	0	0													
0.2/0	13	0	0													
0.2/0.1	5	3 (60.0)	2	1		1				2	3					2
0.2/0.2	0	0	0													
0.2/0.4	0	0	0													
0.4/0	13	2 (15.4)	1		1			1				1				
0.4/0.1	9	3 (33.3)	1			1				3	1			1		3
0.4/0.2	3	2 (66.7)	2			1				1	2					1
0.4/0.4	0	0	0													
0.8/0	12	0	0													
0.8/0.1	7	5 (71.4)	5	1						9	4	1	3			5
0.8/0.2	0	0	0													
0.8/0.4	0	0	0													

^a The number of dead embryos and hatchlings examined for deformities

Teratogenicity of Injected Methylmercury and Selenomethionine Versus Maternally Deposited Hg and Se

Egg injections represent an efficient way of comparing the types of deformities and frequency of deformities Hg and Se can cause and their possible interactions. However, it would be incorrect to conclude that similar concentrations of biologically incorporated Hg and Se would cause the same degree of harm in wild bird eggs as was seen in our egg injection studies. When either methylmercury chloride or selenomethionine was injected into eggs, their toxicity was greater than if the same concentration were 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, our injections of selenomethionine that resulted in 0.1 and 0.2 $\mu\text{g/g}$ Se on a wet-weight basis in eggs caused deformities, but 0.1 or 0.2 $\mu\text{g/g}$ Se naturally deposited in the egg by the female would not be expected to do the same. In the mallard study by Heinz and Hoffman (1998), in which deformities were caused by a parental diet containing 10 $\mu\text{g/g}$ Hg or 10 $\mu\text{g/g}$ Se, the resulting concentrations of Hg and Se in eggs were 16 and 7.6 $\mu\text{g/g}$ wet weight, respectively, which are much greater than the 0.1 and 0.2 $\mu\text{g/g}$ concentrations observed to cause deformities in the current injection studies. In another study in which breeding pairs of mallards were fed methylmercury, and Hg was again deposited into the egg by the female mallard, the lowest concentration of maternally deposited Hg associated with deformities was approximately 1 $\mu\text{g/g}$ Hg on a wet-weight basis (Heinz and Hoffman 2003). Skorupa and Ohlendorf (1991) determined that a mean of 13–24 $\mu\text{g/g}$ Se on a dry-weight basis (approximately 4–7 $\mu\text{g/g}$ on a wet-weight basis) was the threshold range in the eggs of populations of aquatic birds that experienced teratogenic effects.

When compounds, such as methylmercury chloride or selenomethionine, are injected into the air cell of an egg, the compounds pass through the inner shell membrane and into the albumen of the egg (Heinz et al. 2006, 2011). It has been shown that when water containing a dye is injected into the air cell of the egg, it not only passes through the inner shell membrane but it distributes itself uniformly throughout the albumen of the egg (Heinz et al. 2011). Therefore, if, as in the present experiments, the solvent for the methylmercury and Se is water, then methylmercury and selenomethionine become dissolved in the aqueous part of the albumen. It is unknown whether water-soluble methylmercury or selenomethionine, when injected, can become attached to proteins in the albumen as they are when deposited into the egg by the mother. If they were attached, one would expect their toxicities to be the same as biologically incorporated Hg and Se, but they are not. If neither the Hg nor the Se is attached to proteins, but rather

simply 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 contrast, methylmercury and selenomethionine deposited in the egg by the mother become bound to proteins, which must be metabolized before Hg and Se are available to the developing embryo (Nishimura and Urakawa 1976; Ochoa-Solano and Gitler 1968). This possible difference in their state within the albumen (protein-bound versus unbound) may be the reason why methylmercury and selenomethionine are more toxic when injected than when maternally deposited.

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