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The Use of Egg Injections to Rank the Sensitivities of Avian Embryos to Methylmercury

CALFED Bay-Delta Mercury Project
Final Progress Report
September 17, 2003

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Subcontract No. 22-1509-2297 (SJSU Foundation)
Prime Contract: USBR No. 99FC200241
CALFED Tracking No. 99-B06

EXECUTIVE SUMMARY

This report summarizes research done at the USGS Patuxent Wildlife Research Center (PWRC) as part of the CALFED Bay-Delta Mercury Project, whose major goal is to insure that concentrations of mercury in fish are below those that may harm human or wildlife health. Our objective at PWRC was to develop an egg injection procedure that could be used to determine if the embryos from various species of birds differ in their sensitivity to methylmercury. Over the course of our research we developed a protocol in which we injected various doses of methylmercury into the air cell of eggs from many different species of birds.

Major Findings:

- Many factors, including age of the embryo, place of injection in the egg, solvent used to dissolve the methylmercury, and method of incubating the egg, affect the toxicity of methylmercury to avian embryos.

- We developed a workable protocol in which the eggs of various species of birds could be compared in their sensitivity to methylmercury. This protocol involved injecting various doses of methylmercury dissolved in corn oil into the air cell of the egg when the embryo of that species was at the developmental equivalent of a 3-day-old chicken embryo.

- The embryos of chickens, pheasants, and mallards were of about the same relative sensitivity to methylmercury when eggs were injected as they were when the mother naturally deposited the methylmercury into her own eggs.

- The embryos of different species of birds differ in their sensitivity to methylmercury, suggesting that the thresholds of mercury set to protect laboratory species of birds may not protect all species of wild birds.

INRODUCTION

Background. One of the goals of the CALFED research program is to provide information that will lead to a reduction of mercury in resident fish tissues to levels that are not harmful to humans and wildlife. Other investigators in the CALFED Project have collected a great deal of useful information on the sources and fate of mercury in the Bay-Delta ecosystem. To help make this information on sources and fate be of practical use in protecting wildlife, we conducted research on the toxicity of mercury to avian reproduction. In general, 95 to 99% of the mercury in fish is in the form of methylmercury (Wiener and Spry, 1996), which is the most harmful form to birds (Heinz, 1996; Thompson, 1996). Aquatic birds, especially fish-eating species, are, therefore, especially vulnerable to methylmercury. Reproductive success in birds is believed to be more sensitive to methylmercury than is adult or juvenile survival (Finley and Stendell, 1978; Heinz, 1979; Tejning, 1967).

Since the banning of mercury-treated seed dressings, which were a serious threat to terrestrial birds (Fimreite, 1979), it is largely aquatic birds that continue to be threatened by mercury poisoning (Thompson 1996, Wolfe et al. 1998, Eisler 2000). The biomagnification of mercury in aquatic food webs often leads to high concentrations in fish-eating birds (Fimreite 1974, Hesse et al. 1975, Scheuhammer et al. 1998). In San Francisco Bay and other California environments, mercury has been recognized as a potential threat to aquatic birds (Hoffman et al. 1998, Hothem et al. 1998, Ohlendorf et al. 1986, 1989, 1991, Takekawa et al. 2002).

In laboratory studies with chickens (Gallus gallus), ring-necked pheasants (Phasianus colchicus), black ducks (Anas rubripes), and mallards (Anas platyrhynchos) reproduction was harmed when methylmercury was added to the diet of breeding adults (Tejning 1967, Fimreite 1971, Finley and Stendell 1978, Heinz 1979). Unfortunately, no controlled laboratory studies have been done to examine the effects of mercury on the reproductive success of fish-eating or other aquatic birds. It is difficult to raise fish-eating birds in captivity in sufficient numbers to conduct a reproductive study with methylmercury. Most of these species take years to reach sexual maturity, and their care and breeding in captivity are poorly understood. Consequently, we developed a different approach, which was to collect wild bird eggs from the field and inject them with various doses of methylmercury. This approach bypassed the problems of breeding adults in captivity. With game farm chickens, pheasants, and mallards, full captive breeding studies with methylmercury have already been done (Tejning 1967, Fimreite 1974, Heinz 1979), allowing us to compare the toxicity results from the full breeding studies to results from our injection studies with the same three species.

PWRC Project Objectives. The Patuxent Wildlife Research Center was involved in Task 3B: "Laboratory assessment of the hazards of mercury to reproduction in aquatic birds." Our objectives at Patuxent were to (1) develop an egg injection procedure that could be used to compare the sensitivity of embryos from different species of birds to methylmercury and (2) then to collect, inject with methylmercury, and artificially incubate the eggs from many species of wild birds, thus measuring each species' sensitivity to mercury.

Summary of hypotheses and methods. Our null hypothesis was that the embryos of different species of birds would not differ in their sensitivity to methylmercury. Our methods first involved development of an acceptable protocol, and then the use of this protocol to test the eggs of many species.

Development of the protocol

We conducted many studies, using thousands of chicken and mallard eggs, to develop the procedures that we then used for injecting the eggs of wild birds. Because these preliminary studies with chickens and mallards were carried out mainly to test different variables in perfecting our procedures, we will only briefly describe here what we did and found. We tested the toxicity of methylmercury chloride when it was dissolved in various solvents, including water, corn oil, propylene glycol, DMSO, acetone, ethyl alcohol, soybean oil, Crisco, canola oil, peanut oil, Vaseline, and olive oil. While various solvents had their advantages, we found that

corn oil and propylene glycol were both good solvents, but for different ages of embryos. Corn oil induces low mortality in controls when eggs are injected at the stage of a 3-day-old chicken embryo, but is very toxic to eggs that have not undergone any incubation. By contrast, propylene glycol is not very toxic when injected prior to incubation of the eggs, but becomes toxic when injected at 3 days of age. Because we wanted to be able to could cull out infertile and early dead embryos prior to injecting the eggs, and this can be done by candling eggs at about 3 days of development, we decided to use corn oil, which is not very harmful to embryos by that time.

We also ran many tests to determine where to inject the doses of methylmercury. Because nearly all the methylmercury in a bird egg is found in the egg albumen, we decided not to inject the methylmercury into the egg yolk. That left two options. One was to inject the methylmercury directly into the albumen by drilling a hole through the shell into the wet contents. Unfortunately, we sometimes saw too much mortality of control eggs (those in which only the solvent was injected, without any methylmercury). The second option, the one we chose to use for the wild bird eggs, was to inject the dose of methylmercury into the air cell and let the solvent penetrate the inner shell membrane and carry the methylmercury into the albumen that way.

We ran several tests to determine when the best stage of embryonic development was to inject the methylmercury. We found that an injection just before the eggs were incubated gave the lowest mortality of embryos, a mortality more similar to what it is when the mother deposits the methylmercury into her own eggs. However, there was a toxicity of the corn oil alone at this early stage, and we had not perfected the use of an alternative solvent. The other disadvantage of injecting eggs prior to the start of incubation was that we could not cull out infertile eggs and early dead embryos. We decided, therefore, to inject the eggs from all species when the embryos had reached the morphological equivalent of a 3-day-old chicken embryo. At this stage, it is easy to see the embryo and ring of blood vessels as long as the eggshell is not too thick and opaque, and we could eliminate, prior to dosing, any infertile eggs, or dead or weak embryos. In addition, at this stage we got very nice dose response data for mortality.

We studied the effects that the orientation of the eggs during incubation might have on mortality. We discovered that incubation of methylmercury-treated eggs on their ends, with the blunt end (called the cap) of the egg pointing upward, resulted in much heavier mortality than with the egg sitting on its side in the incubator. Also, we read and discovered for ourselves that the eggs of wild birds tend to hatch better when incubated on their sides. Therefore, the eggs of all species were incubated on their sides.

Other variables we investigated with mallard and chicken eggs included the volume of solvent to inject, the type of disinfectant used to swab the eggshell prior to drilling the hole, the size of the hole drilled in the cap end of the egg through which the injection was made, the effect of sealing the hole with a hot glue gun, the temperature of the egg when injected, and the temperature of the solvent. We also mixed dyes into the solvents to be able to observe the distribution of solvents into the albumen of the egg.

Although there is room for much more experimentation with different injection procedures, we decided to use the following standardized protocol for comparing the sensitivity of various species to methylmercury.

Use of the Standardized Protocol

Eggs were collected in the field by cooperators who had the appropriate state and federal collecting permits. To the extent possible, we had cooperators collect eggs from areas where mercury contamination was known to be low. We had advised these cooperators to collect only fresh eggs, meaning those that had not undergone any incubation by the parents. Most of the collected eggs were fresh, but a few were already in some stage of incubation. All eggs were labeled as to the nest they came from. These eggs from the field were shipped back to our lab in foam-lined boxes by overnight delivery. Once the eggs came to our lab, we washed them in a dilute solution of Betadine to disinfect them and randomized them to the injection treatments they would later receive. We placed one restriction on the randomization process, and that was to put eggs from the same nest in different treatments.

We then wrote a code number on each egg that identified it to treatment. The eggs were placed on their sides in a Kuhl incubator (Kuhl Incubator Company, Flemington, NJ). We devised special trays that enabled the eggs to turn about 180 degrees every hour. The eggs of many wild birds require this degree of turning. The temperature was set at 37.5°C for all species except chickens and pheasants, for which 37.6°C is recommended. The humidity inside the incubator was adjusted for each species so that the percentage weight loss of the eggs over the full course of incubation was about 14 to 16%, based on a sample of eggs we periodically weighed. Cracked and infertile eggs were eliminated, as were eggs that died prior to the time of injection. The eggs from different species were all injected at the same stage of embryo development. This stage was standardized as the development of a 3-day-old chicken embryo, which is equivalent to about a 4-day-old mallard embryo. Some species took less than 3 days and some took more than 3 days to reach the appearance of a 3-day-old chicken embryo. We knew approximately when this stage would be reached by each species, based on the length of the incubation period compared to that of the chicken, but whenever possible we confirmed the stage by candling the eggs.

We injected the eggs with a geometric progression of mercury doses. These doses were calculated to produce concentrations of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, or 3.2 ppm mercury on a wet-weight basis in the contents of the egg (minus the weight of the shell). With the game farm species, and with some of the wild bird eggs, we had enough eggs to also have a group that was not injected with corn oil. These uninjected eggs served to demonstrate what effects the control solution of corn oil without methylmercury might have on embryo survival. The mercury was in the form of methylmercury chloride. Few species of wild bird eggs received the entire series of treatments, all the way from 0 to 3.2 ppm mercury, because we did not have large enough sample sizes from the wild birds. To prepare the solutions of mercury, we dissolved the methylmercury chloride into corn oil, starting with a solution that would produce 3.2 ppm mercury when 1 microliter of corn oil was injected per gram of egg contents. Then we made serial dilutions to

achieve the lower concentrations. In 2002 we added a treatment of 6.4 ppm mercury for tests involving the game farm species.

When the embryos within a set of wild bird eggs had reached the embryological equivalent of a 3-day-old chicken embryo, we removed the eggs from the incubator, swabbed the cap end with alcohol, and drilled a small hole through the cap end of the eggshell, below which the air cell lies. The air cell is a small pocket of air created in the cap (blunt) end of a bird's egg by a natural separation that exists between the outer and inner shell membranes. The inner shell membrane separates the air cell from the wet contents of the egg. Solvents such as corn oil, when injected into the air cell, penetrate the thin inner shell membrane and carry the methylmercury into the albumen of the egg. We used solutions of methylmercury in corn oil that had been warmed to a temperature similar to that of the egg and injected 1 microliter of solution per gram of egg contents into the air cell of the egg, using the mercury treatment to which that set of eggs had been randomly assigned. We then sealed the holes with a hot glue gun and kept each set of injected eggs in the vertical position for one-half hour to allow the corn oil to spread over the surface of the inner shell membrane. After one-half hour, the eggs were returned to their sides and were placed back in the incubator.

At about 3-day intervals we candled the eggs to check for dead embryos. Eggs containing dead embryos were opened to determine the stage of embryonic development at which death occurred and to examine the embryos for deformities. When embryos die before about one week of age, it is very hard to examine them for deformities because they are small and generally are decomposed if dead for a day or two. About two days prior to the anticipated hatching day, we transferred the eggs to hatching trays in a separate incubator, where the eggs were not turned every hour. The temperature in the hatching incubator was set at about 37.2°C and at a relative humidity of about 70 to 80%. Records were kept on which eggs hatched or failed to hatch. Unhatched eggs were opened and the embryos examined for deformities.

A sample of 5 or 6 eggs from each species (usually cracked or infertile eggs that we salvaged for this purpose) were saved for mercury analysis. Most of these eggs have not yet been analyzed for mercury. At the end of our study this summer, we will be submitting these eggs for mercury analysis. Our objective in analyzing a sample of eggs from each species is to verify that mercury concentrations in eggs from the field were low.

With the eggs of wild species of birds, hatching success in artificial incubators, even for control eggs, may be much less than 100%. It is difficult to get the incubator conditions set to mimic how the parents incubate their eggs; normally there is no information on the incubation conditions the parents use. Sometimes there is very good embryo survival of wild bird eggs up close to the time of hatching and then some unexplained mortality will occur in the last couple of days. One way to overcome this unexplained, late mortality that is common even for controls, is to calculate embryo survival up to some point close to hatching, but not all the way to hatching. For statistical comparisons, we used embryo survival through 90% of the incubation period.

RESULTS AND DISCUSSION

Listed below are the summarized results, starting first with the three game farm species (mallards, chickens, and ring-necked pheasants).

Mallard (Anas platyrhynchos)

The mallard eggs came from a game farm and not the wild. Sixty eggs were randomly assigned to a group that were not injected, even with clean corn oil, and another 60 were injected with clean corn oil (no mercury in the corn oil). Thirty eggs were randomly assigned to groups that received a dose of mercury that would result in wet-weight concentrations in the eggs of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, or 3.2 ppm mercury. The percentage survival of embryos through 90% of the incubation period is shown in Table 1. The survival of the group of control eggs that was injected with clean corn was not significantly different from the survival of the group that was not injected at all ($P > 0.05$, Fisher's exact probability test). When the numbers of surviving and dead embryos in the controls that received clean corn oil were compared to the numbers surviving and dead in the mercury-dosed groups, the lowest concentration of mercury that produced a significantly greater embryo mortality was 3.2 ppm, but both the 0.8 and 1.6 ppm groups were nearly different ($P = 0.07$). Deformities were scattered among the various groups in low numbers. An additional large study was conducted with mallards, but statistical analyses are not yet complete.

Chicken (Gallus gallus)

Sixty eggs were randomly assigned to a group that was not injected with corn oil, 60 to a group injected with clean corn oil, and 30 to groups injected with 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, or 3.2 ppm mercury. The controls with no injected corn oil did not differ from controls with injected corn oil (Table 2). The controls injected with corn oil had significantly higher embryo survival than did any of the mercury-treated eggs. The perfect survival of the control set of eggs greatly strengthened the statistical comparisons. Deformities were spread out among treatments, but it is important to recognize that heavy early mortality of mercury-treated eggs may decrease the number of eggs suitable for examination for deformities. Prior to about 1 week of age, when embryos are small and deteriorate quickly once dead, it is hard to see deformities. An additional large study was conducted with chickens, but the statistical analyses are not complete.

Ring-necked pheasant

Sixty eggs were assigned to each of the two types of controls and 30 eggs to the treatments ranging from 0.05 to 3.2 ppm mercury. Sample sizes became reduced because a large number of eggs were infertile or died prior to the time of injection. Embryo survival for uninjected controls did not differ from survival of controls injected with clean corn oil (Table3). The lowest concentration of mercury that caused significantly greater embryo mortality than was seen in the corn oil controls was 0.2 ppm. At doses starting at 0.2 ppm mercury, there was a strong dose response relation. Deformed embryos were scattered throughout treatments, and owing to the

heavy mortality of the two highest groups, few embryos lived long enough to be examined for deformities. An additional large study was conducted with pheasants, but the results have not yet been analyzed.

Herring gull (Larus argentatus)

Thirty eggs, collected in Maryland, were assigned to each of the two control groups, and 24 eggs to each treatment, ranging from 0.1 to 3.2 ppm mercury. Sample sizes became reduced because of infertile eggs and eggs that, for unknown reasons, had congealed yolks. Embryo survival between the two groups of controls did not differ (Table 4). It was not until 0.8 ppm mercury that a statistically significant decrease in embryo survival was observed compared to the controls injected with clean corn oil. However, some additional mercury-treated eggs probably died of mercury poisoning, but were given the benefit of the doubt because of the problem we saw with congealed yolks. Therefore, it is likely that lower doses also caused a decrease in survival. Deformities were spread among groups. An additional study of comparable size was conducted with herring gulls, but the statistical analyses are not yet complete.

Canada goose (Branta canadensis)

Eggs were collected in Maryland. There were not enough eggs to have a group that was not injected with corn oil. Our goal was to have about 25 or 30 eggs in each treatment, but a large number of eggs died, perhaps because of a disease, and so our sample sizes were reduced (Table 5). In spite of this problem, a clear dose response relation existed among the higher doses of mercury. However, owing to relatively poor survival among control eggs, the only group that showed a statistically significant drop in embryo survival was 1.6 ppm mercury. The group injected with 0.8 ppm mercury was nearly different from controls ($P = 0.07$). Deformed embryos were limited to mercury-treated groups.

Double-crested cormorant (Phalacrocorax auritus)

These eggs came from Maryland. Thirty eggs were randomized to each group. The two groups of controls did not differ from each other, and only the 1.6 ppm mercury group differed from the corn oil controls (Table 6). Double-crested cormorants seem to be resistant to the embryotoxic effects of methylmercury. An additional study of comparable size was conducted with double-crested cormorants, but the statistical analyses are not complete.

Greater sandhill crane (Grus canadensis)

Only a limited number of these eggs was available each year from a colony maintained at the Patuxent Wildlife Research Center. Eggs came to us in small groups of two or three over many weeks, and we tried to build up sample sizes in various treatments. Unfortunately, with the first eggs we received in 2001 we guessed wrong with the settings of the lowest dose groups; they did not result in mortality, and not many additional eggs were available to add other dosage groups. Additional eggs collected in 2002 allowed us to increase our sample sizes, especially in the

higher dose groups. With the combined data from 2001 and 2002 it was possible to show that the groups of eggs injected with 0.8 or 1.6 ppm mercury had decreased survival compared to controls. It is also apparent from the excellent hatching success of eggs injected with 0.2 or 0.4 ppm mercury that these lower doses had no harmful effects on sandhill crane embryos. Compared to sensitive lab species, such a chickens and ring-necked pheasants, sandhill cranes appear to be intermediate in their sensitivity to methylmercury, probably more closely resembling mallards (Table 7).

White ibis (Eudocimus albus)

Two shipments of eggs came from Florida, one in 2001 and the second in 2003. In 2001 we guessed right on the doses and sample sizes were big enough to see that this species is fairly sensitive to methylmercury. The survival of embryos in the two control groups was not different (Table 8a). The lowest concentration of mercury in eggs that produced a significant decline in embryo survival was 0.2 ppm. In the 2002 study, the lowest dose of mercury causing a statistically significant decrease in embryo survival was 0.4 ppm (Table 8b).

Clapper rail (Rallus longirostris)

Clapper rail eggs were collected from Georgia in 2001 and 2002, enabling us to build up reasonable sample sizes that demonstrated a dose response relation (Table 9). The 0.8 and 1.6 ppm mercury groups differed significantly from controls, and the 0.2 and 0.4 groups had *P* values of 0.06 and 0.13, respectively. Clapper rail embryos seem to be more sensitive to methylmercury than are mallard embryos.

Common grackle (Quiscalus quiscula)

Our eggs came from Maryland. The sample size was adequate to demonstrate a strong dose response relation (Table 10). Every dose, starting at 0.1 ppm mercury, was significantly different from controls. This species is quite sensitive to methylmercury.

Tree swallow (Tachycineta bicolor)

These eggs came from Michigan and Maine. Sample sizes were small and were only sufficient to demonstrate that the 0.8 ppm mercury dose caused a significant decline in embryo survival compared to controls (Table 11). Additional eggs should be tested in the future because there was a suggestion in the results that doses below 0.8 ppm mercury also may be harmful. This species is more sensitive than mallards, but determining just how much more sensitive will require larger sample sizes.

Tricolored heron (Egretta tricolor)

There were two shipments of these eggs from Florida, one in 2001 and the other in 2003. With only 20 eggs to work with in the 2001 shipment, we divided them equally between corn oil

controls and eggs injected with 0.4 ppm mercury. Eight of the 10 control embryos survived for 90% of the incubation period versus 2 out of 10 of the 0.4 ppm mercury eggs; this was a statistically significant difference ($P = 0.02$). In the 2003 shipment a greater number of eggs was sent to us, but a few of the hatched young turned out to be snowy egrets instead of tricolored herons. We have no way of knowing how many of the unhatched eggs were snowy egret chicks instead of Tricolored heron chicks. The eggs of snowy egrets and Tricolored herons are virtually indistinguishable and a few snowy egrets must have nested among the Tricolored herons that year. Snowy egret eggs from Virginia turned out to be very sensitive to methylmercury and therefore the practical significance of the mixed shipment form Florida in 2003 is somewhat reduced, but the 2003 results need to be taken with some caution due to the presence of some snowy egret eggs. With this precaution in mind, it seemed that the 2003 results support those from 2001 (Table 12). Once again, the lowest dose to cause a significant decrease in embryo survival was 0.4 ppm mercury.

Royal tern (Sterna maxima)

These eggs came from North Carolina. Because the sample size was modest and the survival of control embryos was poor, it was not possible to statistically separate controls from any of the mercury-dosed groups (Table 13). However, it seems that this species may be sensitive to methylmercury; additional eggs will be needed and the cause of the control embryo mortality must be overcome.

Brown pelican (*Pelecanus occidentalis*)

Our brown pelican eggs came from Virginia and North Carolina in 2003. Data were combined to enhance sample sizes. The lowest dose of mercury that caused a statistically significant decrease in embryo survival was 0.8 ppm (Table 14). This species seems to be of intermediate sensitivity to injected methylmercury.

Snowy egret (Egretta thula)

We received a shipment of eggs from Florida in 2001, but many of the eggs were not suitable for injecting. All three corn oil control embryos survived for 90% of incubation, compared to 3 of 4 injected with 0.4 ppm mercury. In 2003 we received a larger shipment from Virginia and were able to conduct a better experiment. In this larger experiment in 2003, 0.2 ppm mercury was the lowest dose where embryo survival was significantly depressed below that of the controls (Table 15), making this species a very sensitive one.

Great egret (Casmerodius albus)

These eggs came from Florida and were the first species of wild bird we had gotten. We tried two different injection procedures (mercury dissolved in corn oil and injected at about day 3 and mercury in propylene glycol and injected on day 0 of incubation). Later, we realized we had spread our eggs too thin, especially since this ended up being the sole shipment. There were not

enough eggs dosed with propylene glycol to make any assessments. Two of 3 control eggs that did not receive corn oil survived for 90% of incubation, and 4 of 6 injected with clean corn oil survived. Two of 6 injected with 0.4 ppm mercury survived, and 1 of 5 injected with 1.21 ppm mercury survived. Sample sizes turned out to be too small to apply meaningful statistics, but it seems that this species may be somewhat sensitive to mercury.

Anhinga (Anhinga anhinga)

These eggs came from Florida as one of the first wild species we worked with. From hindsight we should have used fewer treatments and higher doses. Three of 4 controls without corn oil survived for 90% of incubation. Five of 5 corn oil controls survived, and 4 of 4 and 3 of 3 eggs dosed with 0.2 and 0.4 ppm mercury, respectively, survived. The only apparent harmful level was 0.8 ppm, where 0 of 3 injected eggs survived, but sample sizes throughout were too small to say anything conclusive.

In addition to the species discussed above, we have injected the eggs of laughing gulls (*Larus atricilla*) and caspian terns (*Sterna caspia),* but these data have not yet been analyzed.

CONCLUSIONS AND RECOMENDATIONS

Our protocol for injecting eggs with methylmercury generally produces dose response relations. One can examine these relations to see how the embryos of different species of birds respond to increasing doses of methylmercury. It is apparent that the embryos of different species vary in their sensitivity to methylmercury. Among the three game farm species, the chicken and ring-necked pheasant were more sensitive than the mallard. In published studies in which breeding pairs of these three species were fed methylmercury, with the mother depositing the mercury into her eggs, chickens and pheasants were again more sensitive than mallards (Fimreite, 1971; Tejning, 1967; Heinz, 1979). The fact that both methods of getting methylmercury into bird eggs (injection and maternally deposited) produced the same ranking in embryo sensitivity is encouraging. The concentrations of methylmercury in eggs required to harm embryos is less when the methylmercury is injected than when the mother naturally deposits the mercury in the egg, but the relative rankings in sensitivity are the same. Hopefully, this means that the ranking of the embryos of wild birds, where only the injection procedure was used, gives a realistic assessment of how vulnerable these wild species might be to methylmercury. Some species, such as sandhill cranes and double-crested cormorants, are not as sensitive as chickens and pheasants; they are closer to the mallard. The mallard is a game farm species whose sensitivity to methylmercury has been used to establish presumably hazardous levels of methylmercury in the eggs of birds in general. Other species, such as the grackle, white ibis, and clapper rail appear to be more sensitive than mallards. Deformities of embryos seemed to be clustered more among the sets of eggs injected with various doses of methylmercury, but the numbers were too low to make valid statistical comparisons.

Potential for future research/recommended changes in existing research program: More eggs should be collected and injected from some of the species already tested, and a greater range of species should be tested. Variations on the injection protocol should be tested with a few key species to see if the rankings in sensitivity are independent of method of exposure. For example, we might want to perfect the procedure for injecting eggs at earlier stages of development or injecting the solvents directly into the albumen. Although most of the methylmercury found in eggs collected from the field is in the albumen, we might want to develop a procedure in which methylmercury is injected into the yolk; this would allow us to determine how much impact this yolk mercury has on embryo survival. Combinations of methylmercury plus other contaminants, such as selenium, should be tested. At least one breeding colony of fish-eating bird should be established so the toxicity of maternally deposited methylmercury can be compared to the toxicity of injected methylmercury. More field work with fish-eating birds exposed to mercury is needed to relate to the laboratory injection studies.

ACKNOWLEDGEMENTS

My co-investigator in this research was Dr. David Hoffman of the Patuxent Wildlife Research Center. Dr. Hoffman and I thank the following people for kindly providing us with the eggs of wild birds: Bart Hoskins, Michael Rickard, Melissa Duron, Peter McGowan, David Allen, Sue Cameron, Tom Augspurger, Jerry Longcore, Dave Evers, Ray Adams, Terry Adelsbach, Frank McGilvrey, Jane Nicolich, Darren Rumbold, Karen Gaines, and Paul Spitzer. We also thank Carol Erwin, Michael Hammond, Brian Heinz, Michael Hoffman, Shannon Kondrad, and Dan Murray for help with the egg injections.

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Table 1. Percentage embryo survival and number of deformed embryos for mallard eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	60	90	1
Controls (corn oil)	60	82	0
0.05	30	93	1
0.1	29	90	0
0.2	30	97	0
0.4	30	77	0
0.8	30	63	3
1.6	30	63	0
3.2	30	33*	3

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 2. Percentage embryo survival and number of deformed embryos for chicken eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	60	98	0
Controls (corn oil)	60	100	1
0.05	30	90*	0
0.1	29	90*	3
0.2	30	87*	3
0.4	30	63*	1
0.8	30	67*	4
1.6	30	50*	1
3.2	30	3*	1

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 3. Percentage embryo survival and number of deformed embryos for ring-necked pheasant eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	50	86	1
Controls (corn oil)	57	96	1
0.05	23	87	0
0.1	24	100	2
0.2	25	80*	3
0.4	28	46*	2
0.8	29	31*	2
1.6	28	7*	0
3.2	29	0*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 4. Percentage embryo survival and number of deformed embryos for herring gull eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	27	89	0
Controls (corn oil)	25	72	1
0.1	22	59	3
0.2	22	59	2
0.4	12	42	1
0.8	16	31*	2
1.6	16	6*	1
3.2	15	0*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 5. Percentage embryo survival and number of deformed embryos for Canada goose eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	27	67	0
0.05	20	55	0
0.1	20	75	1
0.2	19	58	1
0.4	20	45	0
0.8	19	37	2
1.6	19	26*	2

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 6. Percentage embryo survival and number of deformed embryos for double-crested cormorant eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	28	96	1
Controls (corn oil)	28	96	1
0.05	28	100	1
0.1	29	97	0
0.2	29	100	0
0.4	30	80	2
0.8	28	93	2
1.6	27	74*	4

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 7. Percentage embryo survival and number of deformed embryos for greater sandhill crane eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	12	100	0
0.2	7	86	0
0.4	10	100	1
0.8	10	50*	2
1.6	7	0*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 8a. Percentage embryo survival and number of deformed embryos for white ibis eggs injected with various concentrations of mercury as methylmercury (2001 data).

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	9	89	0
Controls (corn oil)	9	78	0
0.2	10	10*	1
0.4	9	33	0
0.8	10	20*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 8b. Percentage embryo survival and number of deformed embryos for white ibis eggs injected with various concentrations of mercury as methylmercury (2003 data).

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	10	80	0
Controls (corn oil)	13	62	2
0.05	9	56	1
0.1	12	33	2
0.2	9	44	1
0.4	10	20*	1
0.8	13	8*	2

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 9. Percentage embryo survival and number of deformed embryos for clapper rail eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	14	79	0
0.1	19	63	3
0.2	15	40	1
0.4	15	47	3
0.8	12	8*	0
1.6	13	23*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 10. Percentage embryo survival and number of deformed embryos for common grackle eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	12	100	0
0.05	11	100	0
0.1	10	40*	1
0.2	11	18*	1
0.4	10	10*	2
0.8	11	0*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 11. Percentage embryo survival and number of deformed embryos for tree swallow eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	15	87	0
Controls (corn oil)	17	76	0
0.05	10	90	0
0.1	16	62	1
0.2	13	62	0
0.4	8	62	0
0.8	9	11*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 12. Percentage embryo survival and number of deformed embryos for tricolored heron eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	8	88	0
0.1	6	67	0
0.2	7	57	0
0.4	8	14*	0
0.8	6	0*	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 13. Percentage embryo survival and number of deformed embryos for royal tern eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	10	40	1
0.1	12	50	0
0.2	9	22	3
0.4	10	40	3
0.8	9	0	0
1.6	5	0	0

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 14. Percentage embryo survival and number of deformed embryos for brown pelican eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (no solvent)	13	93	0
Controls (corn oil)	15	93	1
0.1	14	100	1
0.2	14	86	0
0.4	15	67	1
0.8	15	47*	2
1.6	14	29*	1
3.2	11	18*	2

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.

Table 15. Percentage embryo survival and number of deformed embryos for snowy egret eggs injected with various concentrations of mercury as methylmercury.

Group (ppm mercury)	Sample size[a]	% survival[b]	Number of deformed embryos
Controls (corn oil)	10	90	0
0.05	9	78	1
0.1	10	60	3
0.2	12	25*	1
0.4	10	10*	1
0.8	5	20*	1

[a] Sample size after excluding infertile eggs, eggs that became cracked during handling, or eggs that were otherwise unsuitable for continued incubation.

[b] Percentage survival was calculated based on how many embryos survived through 90% of the incubation period for that species.

* Significantly different ($P < 0.05$) from the control group injected with corn oil by a Fisher's exact probability test.