Sensitivity of Avian Embryos to Methylmercury

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Abstract

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. 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). 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 an approach, to collect wild bird eggs 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. Many factors, embryo age, 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.

Table 1.

Species tested with egg injections.

Order	Species
Pelecaniformes	Double-crested cormorant, Anhinga
Ciconiiformes	Great egret, Snowy egret, Tricolored heron, White ibis
Charadriiformes	Herring Gull, Laughing Gull
Galliformes	Chicken, Ring-necked pheasant
Gruiformes	Clapper rail, Sandhill Crane
Anseriformes	Mallard, Canada goose
Passeriformes	Common grackle

Methods

Eggs were collected in the field from areas where mercury contamination was known to be low. Only fresh eggs, meaning those that had not undergone any incubation by the parents, were collected. In the lab, eggs were washed in a dilute Betadine solution and then randomized into injection treatments. Eggs from the same nest were randomized into different treatment groups. The eggs were placed on their sides in special trays that enabled them to turn about 180 degrees every hour. The eggs were housed in a Kuhl incubator (Kuhl Incubator Company, Flemington, NJ) 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 or infertile eggs as well as eggs that died prior to the time of injection were eliminated.

Figure 1.

Egg drilling prior to injection.

At the appropriate embryonic stage (equivalent to that of a 3-day-old chicken embryo based on length of incubation period compared to a chicken and candling results), eggs were removed from the incubator, the cap end swabbed with alcohol, and a small hole drilled through the cap end of the eggshell (above the air cell). The corn oil penetrates the thin inner shell membrane and carries the methylmercury into the albumen of the egg.

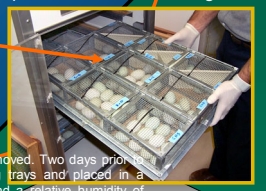
Hg injection.

The eggs were injected with a geometric progression of mercury doses: 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 or 6.4 ppm mercury on a wet-weight basis. Some species also had a group of non-injected eggs. These eggs demonstrated the effects of corn oil on embryo survival.

Sealing injection site.

Following injection, eggs were sealed with a hot glue gun, kept in the vertical position for 30 min. (to allow the corn oil to spread over the surface of the inner shell membrane), then returned to their sides and placed back in the incubator.

Following the prescribed incubation period, eggs were placed inside a hatching unit.



Eggs were candled at 3-day intervals and dead embryos were removed. Two days prior to the anticipated hatching day, eggs were transferred to hatching trays and placed in a separate incubator. The temperature was set at about 37.2°C and a relative humidity of about 70 to 80%. Hatching success of wild species bird eggs in artificial incubators, even for control eggs, may be much less than 100%. Therefore, embryo survival through 90% of the incubation period was used for statistical comparisons. Unhatched eggs were opened and the embryos examined for deformities.

Results

Table 1 lists the species tested. Comparisons of the sensitivity of mallard, chicken and ring-necked pheasant embryos to a common dose of 0.4 ppm mercury are shown in Table 2. Mallards are the least sensitive species, followed by chickens and pheasants. This same ranking of sensitivity among these three lab species has been reported when the mother has been fed methylmercury and deposited it in her eggs (Tejning 1967, Fimreite 1971, Heinz 1979). This similarity in ranking, whether the mercury was injected or naturally deposited in the egg, is encouraging in that it suggests that the relative rankings of other species, for which only injection studies have been done, may be reliable.

Table 2.

Embryo survival through 90% of incubation.

Species	Controls	0.4 ppm Hg
Mallard	82% (n=60)	77% (n=30)
Chicken	100% (n=60)	63% (n=30)*
Pheasant	96% (n=57)	46% (n=28)*

** Significantly different from control at $\alpha = 0.05$*

Table 3.

Embryo survival through 90% of incubation.

Species	Controls	0.4 ppm Hg
Chicken	100% (n=60)	63% (n=30)*
Herring gull	69% (n=26)	29% (n=17)*
Cormorant	96% (n=28)	80% (n=30)
Tricolored heron	80% (n=10)	20% (n=10)*

** Significantly different from control at $\alpha = 0.05$*

As recorded in Table 3 , the study suggests that some species, such as the herring gull (*Larus argentatus*) and tricolored heron (*Egretta tricolor*) seem to be more sensitive to mercury than are chickens, whereas others, such as the double-crested cormorant (*Phalacrocorax auritus*), may be less sensitive.

Figure 2.

Survival of clapper rail embryos through 90% of incubation.

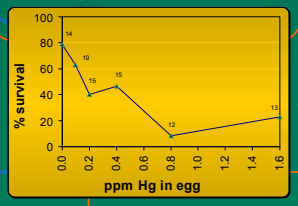


Figure 2 illustrates the changes in mortality of clapper rail (*Rallus longirostris*) embryos to increasing doses of mercury injected into the egg.

Conclusion

Results for the species discussed above, plus the others we tested, suggest that there are species differences in the sensitivity of embryos to methylmercury. Additional laboratory and fieldwork is required to determine the extent of these differences. Using laboratory-generated data from mallards, chickens, and pheasants may not be adequate to protect sensitive wild species from the reproductive effects of mercury.

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