Subtask 3B: Laboratory assessment of the hazards of mercury to reproduction in aquatic birds

Primary Research Team:
Dr. Gary Heinz and Dr. David Hoffman
USGS Patuxent Wildlife Research Center
11510 American Holly Drive
Laurel, MD 20707

Summary of task objectives: Our objective is to develop an egg injection procedure, or a combination of procedures, that can be used in the laboratory to compare the sensitivity of various species of birds (largely fish-eating species) to methylmercury. We believe the development of an egg injection procedure is important because:

- The embryo is the most sensitive life stage for birds
- It is very costly and time consuming to establish captive breeding colonies of fish-eating birds for the purpose of feeding them known concentrations of methylmercury and following reproductive success
- Studies conducted in the wild on the effects of mercury on the reproductive success of fish-eating birds, while necessary, will always require complementary laboratory studies where all variables, except mercury exposure, can be kept equal among all treatment groups

Summary of hypotheses and methods: Our null hypothesis is that the embryos of different species of birds will not differ in their sensitivity to the same concentrations of methylmercury injected into eggs. The alternate hypothesis is that embryos from different species will vary in their sensitivity to methylmercury injected into the egg.

Our methods involve the development of a test protocol, or a combination of different protocols, that can be used to compare the sensitivity of embryos of different avian species to injected doses of methylmercury. Most of our work has centered on the eggs of mallards (Anas platyrhynchos) because much is already known about the effects of methylmercury on mallard embryos, and mallard eggs are available most of the year for experimenting with different test procedures. Thus far, using mallard eggs, we have experimented with variables such as:

- The site of injection (air cell versus albumen)
- The carrier for the methylmercury (water, albumin solutions, propylene glycol, corn oil, soybean oil, peanut oil, olive oil, Crisco, vaseline, or canola oil)
- The volume of the carrier injected into the egg (0.25, 0.5, or 1 microliter per gram of egg for air cell injections with corn oil; higher volumes with aqueous or albumin solutions injected into the air cell or albumen)
- The time of injection (1 day prior to the start of incubation, on the same day incubation begins, 1 day after the start of incubation, 2 days after the start of incubation, or 4 days after the start of incubation)
- The orientation of the eggs after being injected (blunt end up for the duration of incubation, apex end up for 24 hours after injection followed by blunt end up for the remainder of incubation, or on their side)
- The addition of the amino acids methionine and cysteine or chicken albumin to aqueous solutions of methylmercury injected in the egg albumen

In addition, over 2000 chicken eggs have been injected with mercury to refine techniques. Chicken eggs are available year round, making them another good species for protocol development. Furthermore, as
with mallards, studies have been conducted in which adult breeding chickens have been fed various levels of methylmercury and the effects on reproduction measured.

We also have conducted smaller injection studies (varying in size from less than 50 eggs to over 200 eggs) using eggs from the following fish-eating birds: double-crested cormorant (*Phalacrocorax auritus*), anhinga (*Anhinga anhinga*), great egret (*Casmerodius albus*), snowy egret (*Egretta thula*), tricolored heron (*Egretta tricolor*), and white ibis (*Eudocimus albus*). In addition, the eggs of three species which are not fish eaters, sandhill crane (*Grus canadensis*), Canada goose (*Branta canadensis*), and common grackle (*Quiscalus quiscula*), have been injected to broaden the range of species tested beyond fish eaters. The more families and orders of birds that are tested, the more likely we are to see differences in sensitivity to mercury. Therefore, even if some species are not fish eaters, valuable information is gained. Other species are scheduled to be tested this spring.

**Discussion of progress to date/results:** Testing the variables listed above, we have injected nearly 3000 mallard eggs in many different experiments. We discovered that increasing concentrations of methylmercury into the air cell of mallard eggs produced a nice dose response. An example of this dose response can be seen in Figure 1, which shows the survival of mallard embryos injected with a range of mercury concentrations from 0 to 3.2 ppm. However, the LC50 derived from the data in Figure 1 was about 0.4 ppm mercury as methylmercury, with harmful effects on mallard embryos starting at 0.2 ppm mercury. In earlier studies, when breeding mallards were fed methylmercury and the mothers deposited methylmercury naturally into their eggs, methylmercury was not nearly as toxic as in this injection study. There is nothing wrong with an increased sensitivity to mercury when eggs are injected versus receiving their mercury naturally; the relative sensitivities of the various species is still revealed. However, the ideal test would produce similar toxic effects on embryos when the eggs were injected versus getting the mercury through the mother. Therefore, in our subsequent studies, we have been experimenting with ways to bring the toxicity of the injected methylmercury more in line with the toxicity of similar concentrations of methylmercury that were biologically incorporated into mallard eggs by the female. Using different solvents and orienting the eggs in different positions during incubation did not seem to reduce the toxicity of injected doses of mercury. Experimenting with lesser volumes of solvent injected and injecting the eggs into the albumen instead of the air cell also, so far, has not been promising. The one variable that does seem to have a big effect on methylmercury toxicity is the time when the injection is made. In one study we found that injecting the eggs 1 day prior to putting them in the incubator caused less embryo mortality than when we injected eggs 4 days into incubation. Waiting until 4 days into incubation is a standard procedure because it allows us to weed out infertile, early dead, and weak embryos that occur normally, even with untreated control eggs. Injecting eggs prior to incubation will add some variability to the results of a toxicity test because of this natural rate of infertile, dead, and weak eggs. However, if the trade off is a more natural toxicity of the injected methylmercury, it will be worthwhile to inject the eggs earlier.

Results from our smaller experiments with the eggs of various fish-eating birds suggest that there may be differences in the sensitivity of these species to mercury compared to mallards, but it is too early to say anything definite.

**Conclusions:** Our basic procedure of injecting eggs from various species at the embryological equivalent of a 4-day-old mallard embryo (each species has to be adjusted based on the length of its incubation period) has proven good at producing nice dose response curves. We are convinced further that we will be able to develop a procedure by which the toxicity of the injected methylmercury will be similar to that of naturally deposited mercury. The ultimate goal is to be able to estimate the concentrations of mercury in the eggs of various wild birds at which embryo mortality and reproductive failure begin to occur.
**Potential for future research/recommended changes in existing research program:** No changes to the research program are recommended at this time. Some related future research could include:

- The use of egg injections to examine possible synergistic effects of other contaminants, such as selenium, in combination with mercury
- An experiment with mallards in which embryo survival is simultaneously compared when eggs are injected with methylmercury versus having the methylmercury biologically incorporated into eggs by breeding adults
- The establishment of at least one captive breeding colony of fish-eating bird so that biologically incorporated mercury can be compared to injected mercury
- Field research on the effects of methylmercury on avian reproduction

**Questions for SRC to consider:**

- How do the field and lab components of studying methylmercury toxicity to birds relate?
- What range of wild bird species should we test by egg injections?
- Are interaction studies, such as mercury and selenium, warranted?