APPENDIX III. LOAEL CALCULATION

2011

Current lowest observed adverse effects level (LOAEL) for mercury in tree swallows (*Tachycineta bicolor*)

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Abstract

Because of the relatively large amount of information on tree swallow (*Tachycineta bicolor*) mercury (Hg) exposure and their moderate sensitivity to Hg (see Heinz et al. 2009), the tree swallow can be used to calculate a "working" lowest observed effects level (LOAEL). We evaluated field studies involving tree swallows exposed to mercury and the most recent avian embryo sensitivity data (Heinz et al. 2009). We compared the calculated egg Hg LOAEL for the tree swallow with field-based blood-egg pairings (Evers et al. *in preparation*) to determine a relatively conservative LOAEL for the tree swallow to be 0.63 µg/g (ww) in the blood.

Background

Mercury is a persistent contaminant that has far-reaching effects on wildlife (Seewagen 2009). Despite the increased knowledge we have about this global pollutant, we know relatively little about how this heavy metal affects individual avian species. The majority of avian research has focused on the effect of Hg on large, long-lived piscivores that forage in the aquatic ecosystem, such as the common loon (Evers et al. 2008, Burgess and Meyer 2008). For the common loon, two independent, long-term studies have indicated that the lowest observed adverse effect level (LOAEL) is 3.0 µg/g in the blood. At levels above 3.0 µg/g, the studies indicate that common loons suffer reduced reproductive success (Evers et al. 2008, Burgess and Meyer 2008).

Recent research has shown that birds that forage near (but not necessarily directly in) mercury-contaminated sites are also at risk for exposure to aquatic-based mercury contamination (Cristol et al. 2008). Many bird species, ranging from songbirds to shorebirds, feed primarily on invertebrates found above or near the contaminated areas, but there is not yet a published LOAEL to use for birds other than the piscivorous common loon. Our objective in this report is to combine published tree swallow egg effects data with current BioDiversity Research Institute field data to determine the current working LOAEL for the tree swallow.

Tree swallows as representative species

The majority of our knowledge on the effects of mercury on songbirds comes from relatively few field-based studies looking at tree swallows breeding along contaminated sites. Tree swallows are a migratory species that overwinter primarily from Florida to Central America (Robertson et al. 1992). We believe that the tree swallow is a good representative species based on five attributes – home range, food habits, blood mercury levels, a large amount of background information, and egg sensitivity to methylmercury.

Home range: On their breeding grounds, tree swallows have relatively restricted ranges, making them excellent bioindicators of mercury contamination on a small scale. As opposed to species that range widely when foraging, the blood mercury concentration of a tree swallow can be traced directly to the environment around its nest.

Food habits: During the breeding season, tree swallows forage aerially primarily over open water, consuming mainly flying insects (Diptera), but also some beetles and ants (Robertson et al. 1992).

Background information: Tree swallows have been studied at many different mercury contamination sites, giving us insight into the effect of mercury on a free-living population. Second Year (SY) tree swallows (but not older After Second Year [ASY] females) showed reduced fledging success on contaminated sites, with contaminated birds fledging on average one less offspring per nest in an extreme drought year (2006). There was, however, no difference between contaminated and reference populations in fledgling success when weather conditions were closer to average in 2005 (Brasso and Cristol 2008). In 2005 and 2006, the mean blood mercury concentration in females at the contaminated sites was 3.56 ppm, compared with 0.17 ppm Hg in the blood of reference area females. Similarly, tree swallows suffered reduced nesting success on mercury-contaminated sites during periods of abnormally hot weather, indicating that mercury may act synergistically with other stressful environmental situations (i.e. drought or heat) (Hallinger and Cristol 2011).

Custer et al. (2007) evaluated reproductive success of tree swallows nesting along the mercury-contaminated Carson River. Hatching success in the drainage averaged 74%, compared with a nationwide average of 85% in tree swallows not exposed to Hg or other potential chemical stressors. In clutches where hatching success was 100%, the average egg Hg concentration was 3.86 ppm dry weight (equivalent to 0.77 ppm wet weight using a reported moisture content of 80% from Custer et al. 2008). Clutches with 50-80% hatching success had an average egg Hg concentration of 1.58 wet weight. Reduced hatching success was associated with an egg Hg concentration of 1.2 ppm wet weight.

Longcore et al. 2007 evaluated tree swallow reproductive performance at two sites in Maine and a Superfund Site in Massachusetts. They detected no differences among sites in mean percent of eggs that hatched in a clutch. Mean mercury concentrations in eggs ranged from approximately 0.25 ppm at the Orono site to approximately 0.62 ppm at the Plow Shop site. They reported that individual eggs with Hg concentrations of 0.9-1.3 ppm failed to hatch, but that other eggs from the same clutches (with presumably similar concentrations of mercury) did hatch.

Tree swallows showed a 1% lower annual survival on contaminated sites (mean blood mercury = 2.84 ppm) vs. reference sites (mean blood mercury = 0.17 ppm) (Hallinger et al. 2010). The sublethal effects of mercury on tree swallows may range from reduced immune competence (Hawley et al. 2009) to disrupted endocrine function (Wada et al. 2009). Each of these studies has shown that mercury has had an effect on tree swallows, but none have determined a LOAEL for tree swallows.

Egg sensitivity to methylmercury: Heinz et al. (2009) dosed the eggs of 26 species of birds with methylmercury and examined the resulting effect on embryo survival. Different species responded differently to methylmercury injection, and the researchers ranked each species based on their apparent sensitivity (summarized in Table 1). Tree swallows fell within the medium sensitivity category, with an egg concentration of 0.32 µg/g (ww) killing 50% of developing embryos (lethal concentration (LC_{50}).

Table 1. Variation in species sensitivity to methylmercury injected into eggs (adapted from Heinz et al. 2009).

Low Sensitivity $LC_{50} \geq 1.0$ **µg/g**	**Medium Sensitivity** 0.25 **µg/g** $\leq LC_{50} \leq 1.0$ **µg/g**	**High Sensitivity** $LC_{50} \leq 0.25$ **µg/g**
Hooded merganser	Clapper rail	American kestrel
Lesser scaup	Sandhill crane	Osprey
Canada goose	Ring-necked pheasant	White ibis
Double-crested cormorant	Chicken	Snowy egret
Laughing gull	Common grackle	Tri-colored heron
	Tree swallow	
	Herring gull	
	Common tern	
	Royal tern	
	Caspian tern	
	Great egret	
	Brown pelican	
	Anhinga	

DETERMINATION OF LOAEL FOR TREE SWALLOWS

In that we are developing a lowest observable adverse effects mercury level for the tree swallow, it is appropriate to rely on data from the study demonstrating the lowest effects threshold for mercury in this species, while recognizing that other studies may report higher thresholds. Using the egg dosing experiment from Heinz et al. 2009, we are able to determine the LOAEL for egg mercury concentrations in tree swallows. We chose to use the mercury concentration at which we first see a decrease in egg survival, which is 0.1 µg/g for tree swallows—a level at which we see about a 29% reduction in embryo survival.

Heinz et al. (2009) states that "one might expect that injected methylmercury might be roughly two to four times as embryotoxic as maternally deposited methylmercury." More recent, unpublished findings have shown that injected methylmercury may actually be more similar to maternally transferred mercury than previously thought (Heinz, *pers. comm.*). Taking this into account, we decided to multiply the injected egg mercury value by a factor of two to equate to a maternally-transferred mercury egg concentration of 0.2 µg/g (ww).

Although we have shown above that the egg LOAEL for tree swallows is approximately 0.2 µg/g, we need to equate this value to a metric that can easily be obtained from field studies, such as a blood mercury value. In order to translate egg values into blood values, we analyzed a large dataset of blood-egg pairs collected by researchers at the BioDiversity Research Institute and the College of William and Mary between 2003 and 2009. At various study sites in Maine, Virginia, New York, and Massachusetts, we sampled the blood level of the attending female tree swallow and collected her eggs for mercury analysis.

Using the paired blood-egg data for tree swallows (N = 192), we ran a linear regression and found a strong relationship between female blood mercury concentration and the resulting egg mercury concentration (Fig. 1). Using the equation generated from this linear regression, we can calculate that a female blood mercury concentration of 0.63 µg/g (ww) is associated with 0.2 µg/g in the egg (Fig. 1).

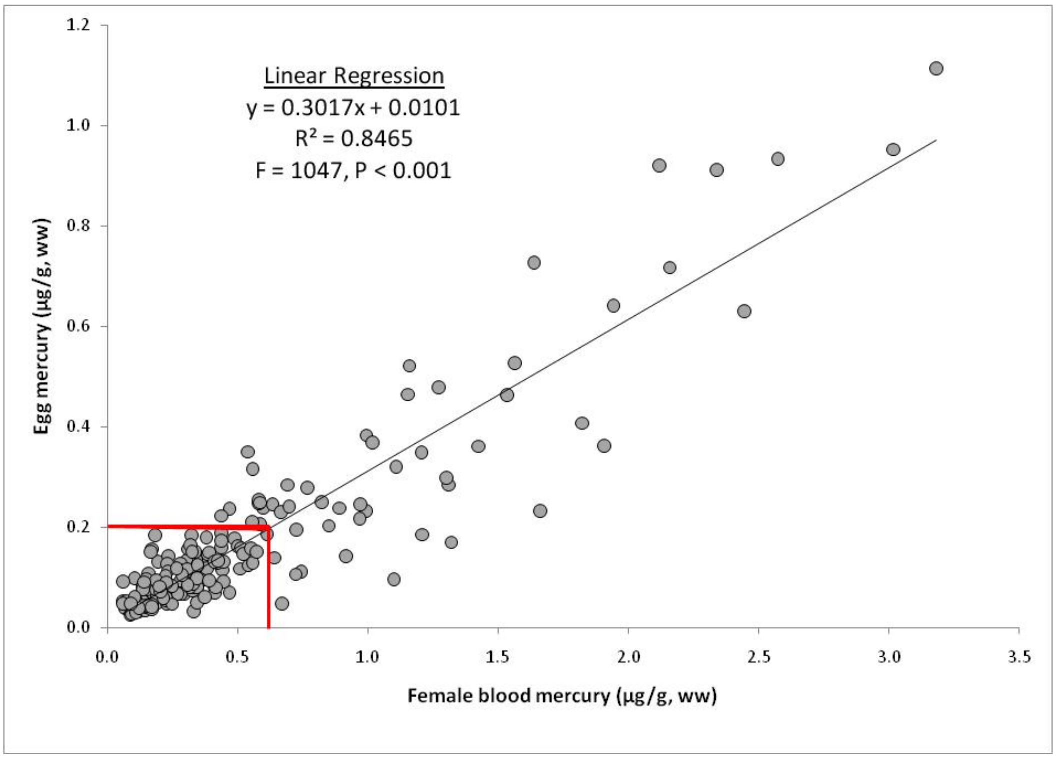

Figure 1. Linear regression for tree swallow blood and egg pairs.

DISCUSSION

We determined that the LOAEL for tree swallows is 0.63 µg/g in the blood of a nesting female. We believe that, at this time, this is a reasonable estimate for an effects level for birds where no other LOAEL has been calculated. As has been found in egg injection studies across different bird taxa (Heinz et al. 2009), it is likely that some species are more sensitive to mercury and some are less sensitive. This LOAEL level may, therefore, be found to be overly conservative for some species and not protective enough for others. Despite this, given the state of research at this time, we feel that this is our best estimate for a useful working LOAEL value.

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