PENOBSCOT RIVER MERCURY STUDY

Chapter 2

Setting mercury remediation targets for surface sediments in the Penobscot estuary

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1 SUMMARY

The intent of this chapter is to summarize recent scientific literature regarding the toxic effects of mercury (Hg) on birds, fish, and mammals and use this information to set targets for the reduction to the exposure of various species of biota to methyl Hg in the contaminated zone of the Penobscot River. The Introduction sets out the various reasons why targets could be required for different species of biota. These reasons include: return to regional background, protection of human health, and protection of biota health. A series of sections following considers these reasons for setting targets individually and reviews available information regarding what safe levels might be. In general, recent studies have tended to lower threshold concentrations that have been shown to cause toxic effects in various biota species.

Later in the chapter, individual species are discussed and targets for Hg reductions are derived for the amount of reduction in Hg concentrations in each species. The individual species (and groups of species) considered are lobster, rock crabs, cormorants, prey fish, eels, songbirds and shorebirds, black ducks and bats. Reduction targets depend on the reason for suggesting reductions and available information. For example, for eels, reduction targets are based on the upstream background concentration, whereas for songbirds, reduction targets are based on toxic effects thresholds. Suggested targets range from no reduction needed in cormorants, about a 10% to 15% reduction in methyl Hg in rock crabs to 75% to 80% reductions in total Hg in songbirds, shorebirds, black ducks and some prey fish. In the upper Penobscot estuary, reductions of 50% or more in Hg in surface sediments are needed to meet targets; whereas, in Mendall Marsh, reductions of 80% will be required.

Based on our observations that methyl Hg in sediments is directly related to total Hg concentrations, and based on information from the scientific literature that demonstrates that Hg in biota responds directly to methyl Hg supply to food chains, it is assumed that if sediment total Hg concentrations can be decreased by remediation actions, then biota Hg concentrations will follow in proportion. It is also assumed that if significant reductions can be made in the Hg concentrations in the most contaminated zone of the Penobscot, then concentrations in biota further south in Penobscot Bay will also take place.

2 INTRODUCTION

In Phase I of the Penobscot River Mercury Study, it was shown that the Penobscot River, from Brewer to Fort Point, is contaminated with mercury (Hg). The geographic extent and pattern of the contamination and the timing of contamination is consistent with the HoltraChem plant at Orrington being the main contributor of mercury to the system. The most contaminated zone is the upper estuary between Brewer and Fort Point. It is in this upper estuary where we propose to concentrate remediation efforts (see Chapters 1 and 21). During Phase I of the Study, it was shown that concentrations of Hg in most surface sediments in the upper estuary exceed guidelines for toxic effects on invertebrates living in those sediments (see Phase I Report). Also, concentrations of Hg in some species are of concern for toxic effects and of concern for human consumption of those species. Since the Phase I Update report was produced, it has also been shown that some prey fish in the contaminated zone of the river are at or near concentrations of Hg that would cause concern for predator species eating those prey fish. It has also been recently found that concentrations of Hg in black ducks living in Mendall Marsh are very high (Chapter 14). These findings raise the question of what level of Hg reduction would be required to reduce or eliminate toxic effects and concern over human consumption.

The purpose of this chapter is to set targets for total Hg concentrations in surface sediments. The focus is total Hg in sediments because we have concluded that methyl Hg concentrations in biota in the upper estuary are largely controlled by total Hg concentrations in surface sediments. Therefore, reducing total Hg in surface sediments to target levels should lead to the protection of wildlife and of human consumers. We set surface sediment targets by first establishing targets (needed reductions) for Hg in biota. Targets for Hg in sediment were then set by reducing present sediment concentrations by the same percentage reduction as is needed for the biota. The sediment Hg targets are being used for the screening of candidate remediation options (see Chapters 1 and 21).

An underlying assumption made for this exercise is that if concentrations of total Hg in surface sediment, where methyl Hg is produced, were reduced by a certain proportion, say by 1/2, then concentrations of methyl Hg in various species of biota would be reduced proportionately, that is, by 1/2. Other studies have shown that methyl Hg in biota responds to both increases and decreases in loading rates of inorganic Hg. These studies include observational studies of contaminated systems, studies of correlations between atmospheric loading of Hg and Hg in biota, and experimental additions of Hg to ecosystems (e.g., Munthe et al. 2007; Hammerschmidt and Fitzgerald 2005; Orihel at al. 2006; Harris et al. 2007). The assumption of direct proportionality is supported by results from the MESOSIM experiment (Orihel et al. 2006, 2007) where isotopic mercury was added to mesocosms in a lake and a directly proportional response of Hg in biota was observed, at least in the short term.

The following objectives were used in setting targets of Hg concentrations in sediments and biota:

1. To reduce Hg concentrations to levels seen in areas of the region that are not contaminated by local sources (regional background).
2. To protect the health of people who eat fish, birds or shellfish.
3. To protect the health of various species of biota, especially birds and fish.
4. To protect wildlife (top predator) health.
5. To protect the health of invertebrates living in surface sediments.

2.1 Targets to Achieve Regional Background Concentrations

It would be unreasonable and unachievable to suggest remediation strategies that had the objective of reducing concentrations of Hg below regional background levels, whether in sediments or biota. We define the regional background as the concentrations of Hg found in sediments and biota in estuaries along the central Maine coast subject only to atmospheric deposition. Because Hg is found everywhere in low to moderate concentrations and even if those concentrations were thought to be causing toxic effects, suggesting reductions to below regional background would not be feasible if the objective is to remediate the impacts of the HoltraChem plant.

We have determined regional background Hg concentrations for sediments in a number of estuaries along the mid-coast of Maine (see Chapter 17). The pre-industrial background level for Hg in sediments, as determined by deep layers in sediment cores in Fort Point Cove, is very low, ranging from 18 to19 nanograms per gram dry weight (ng/g dry wt.) (Figure 2-1). Present concentrations of Hg in surface sediments in areas of the region not affected by point-sources of Hg (St. George estuary, Narraguagus estuary, East Branch of the Penobscot, and Penobscot Bay near Vinalhaven Island) are somewhat higher than the pre-industrial background, ranging from 28 to 50 ng/g dry wt. The National Oceanic and Atmospheric Administration (NOAA) considers concentrations below 51 ng/g dry wt. to be background (Buchman 2008). Areas in the region that appear to be slightly contaminated with Hg, including the Sheepscot estuary and the Penobscot River between Old Town and Veazie, have Hg in surface sediments ranging from 78 to 145 ng/g dry wt. (Figure 2-1). All these concentrations are below NOAA guidelines for any possible impact on invertebrates living in sediments. The NOAA Threshold Effects Level (the concentration below which adverse effects are expected to occur rarely) for freshwater sediments is 174 ng/g dry wt. and the NOAA Probable Effects Level (level above which toxic effects are frequently expected) is 486 ng/g dry wt. (Buchman 2008). The Old Town – Veazie (OV) reach (upstream of the Veazie dam) of the Penobscot, that is upstream of the region most contaminated with Hg, has surface sediments with total Hg concentrations that average about 90 ng/g dry wt. Therefore, 90 ng/g in sediments would appear to be the minimum concentration below which any targets would be impractical.

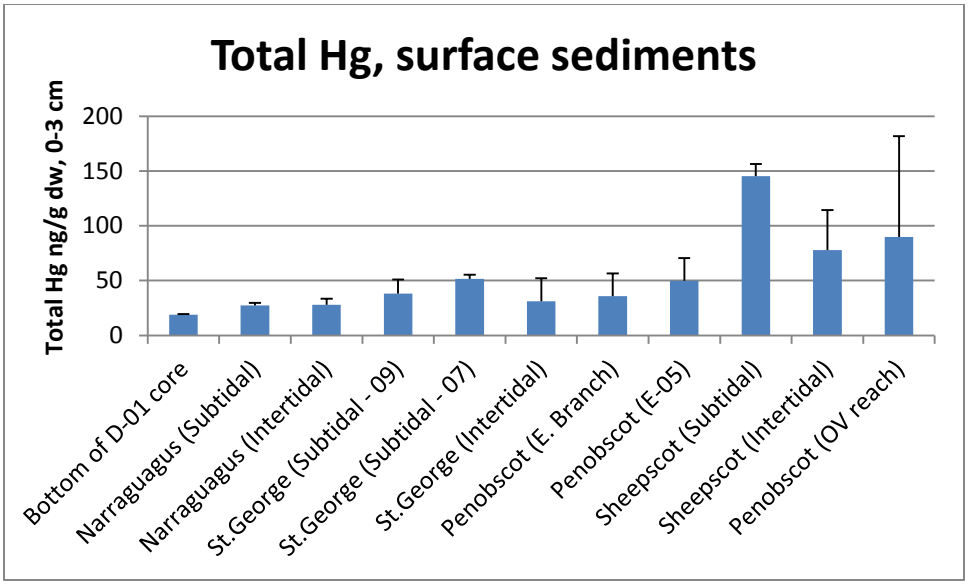

Figure 2-1. Mean concentrations of total Hg in surface (0 - 3 cm) sediments in various relatively uncontaminated locations in the Penobscot basin and other estuaries in Maine. Error bars are one standard deviation.

2.2 Targets to Protect Human Health

Different agencies in different states and countries have decided upon different levels of Hg as the accepted limit to protect the health of people eating fish, shellfish and birds. In Maine, the Department of Environmental Protection (DEP) has set 200 ng/g wet wt. methyl Hg in fish and shellfish as a protective level for human health whereas the United States Environmental Protection Agency (EPA) has set 300 ng/g wet wt. methyl Hg to protect human health. These two concentrations are in agreement when adjustments are made to account for the higher than average rate of fish consumption in Maine relative to average consumption rates for the U.S. population.

2.3 Targets to Protect Fish Health

Methyl Hg can be directly toxic to fish. Sandheinrich and Wiener (2011) have recently reviewed the literature on the toxic effects of Hg on fish. Results from recent studies have identified that harmful effects can occur at Hg concentrations that are lower than previously thought to be harmful. Harmful concentrations are approaching background levels in many areas. Sandheinrich and Wiener (2011) reviewed both laboratory and field studies and found that the results of these two different approaches were generally comparable. They catalogued effects observed by various studies by the biological endpoints that were being studied: biochemical (including the concentrations of blood plasma components and of enzymes indicative of oxidative stress), behavioral, reproductive, histological (cell structure) and growth. The population significance of biochemical effects is less clear than those that directly affect parameters such as growth and spawning behavior. Sandheinrich and Wiener (2011) concluded that adverse effects of Hg in fish are evident at 300 to 700 ng/g wet wt. on a whole body basis, typically equivalent to approximately 400 to 900 ng/g wet wt. in axial muscle.

Sandheinrich et al. (2011) identified a threshold concentration for toxic effects of 300 ng/g wet wt. on a whole body basis. Depew et al. (2012) estimated sensitivity for various biological effects and concluded that fish are most sensitive for reproductive effects, then biochemical, behavioral and growth effects. Little is known about interspecific differences in the sensitivity of fish to Hg; however, there are large differences in the sensitivity of different bird species to Hg (Heinz et al. 2009). Both Sandheinrich and Wiener (2011) and Sandheinrich et al. (2011) noted that there are many piscivorous fish populations with Hg concentrations that exceed the values assumed to cause sublethal toxic effects. Based on the above, it would appear that 500 ng/g wet wt. (0.5 micrograms per gram [µg/g]) in fish muscle tissue is a reasonable target to avoid toxic effects in fish and this will be used to set fish targets (see below).

2.4 Targets to Protect Bird Health

Toxic effects of methyl Hg in birds in four different tissues: eggs, blood, muscle, and feathers, are discussed below.

Many recent studies have examined toxic effects in birds in relation to concentrations of Hg in blood. These have been summarized for the Penobscot River Mercury Study by Evers (2012). Evers used the approach of estimating an effects concentration of methyl Hg at which a 20% reduction in fledging success in a wild bird population would be expected – this is termed the EC20 concentration. For blood concentrations in insectivorous birds, Evers' review indicated that a concentration of approximately 1,200 ng/g wet wt. (1.2 µg/g wet wt.), based on many species, is a reasonable estimate of the EC20. Sandheinrich (2010) concluded that 3 µg/g was Lowest Observed Adverse Effects Level (LOAEL) and that the threshold concentration for birds was probably somewhat lower. Recent studies indicating somewhat lower threshold concentrations of Hg in bird blood include DeSorbo and Evers (2007) who studied bald eagle reproductive success and found a negative correlation between blood Hg in nestlings and reproductive success (young fledged) over the range 100 to 1,200 ng/g wet wt. (0.1 to 1.2 µg/g wet wt.) in blood. The California clapper rail in San Francisco Bay has been shown by Ackerman et al. (2012) to have its body condition negatively affected at blood Hg concentrations starting over the range of about 150 to 1,500 ng/g (0.15 to 1.5 µg/g). However, Ackerman et al. (2008b) found no effect of Hg on the survival of hatchling terms in San Francisco Bay CA at levels of 6.44 µg/g wet wt. in blood (6.44 µg/g fresh wt. in feathers). Franceshini et al. (2009) demonstrated that baseline concentrations of corticosterone, an important metabolic regulating hormone, in tree swallow blood was correlated with blood Hg concentrations over the range of about 100 to 1,000 ng/g (0.1 to 1.0 µg/g) total Hg in blood. There are also a number other studies documenting effects on birds at levels below 2,000 ng/g (2.0 µg/g wet wt.) in blood. Of particular relevance to this review is the paper by McKay and Mayer (2012) which examined the singing behavior of male breeding Nelson's sparrows and found aberrations in birds related to Hg in blood, over the range of approximately 1,000 to over 6,000 ng/g wet wt. Thus, 1,200 ng/g wet wt. (1.2 µg/g wet wt.) would appear to be a reasonable toxic effects threshold for Hg in the blood of insectivorous birds as recommended by Evers (2012).

Effects of methyl Hg on bird eggs have been well studied recently, although the double-crested cormorants, an important species of consideration in the Penobscot System, have received little attention. Heinz (1979) proposed a threshold concentration for effects on mallard eggs of 800 ng/g wet wt (0.8 µg/g wet wt.) and the studies of Heinz and Hoffman (2003) supported this threshold concentration. In a review conducted for the Penobscot River Mercury Study, Sandheinrich (2010) concluded that 1,000 ng/g wet wt. (1.0 µg/g wet wt.) in eggs is the LOAEL for bird eggs and that the threshold concentration is probably somewhat lower than this concentration. Evers (2012), in his review of the recent literature, recommended the use of and EC20 of 650 ng/g wet wt. (0.65 µg/g wet wt.) for piscivorous birds like cormorants. Heinz et al. (2009) conducted egg injection experiments with 26 different species of bird eggs and found quite large inter-specific differences in tolerances to methyl Hg. They found that cormorants have quite low sensitivity to methyl Hg, compared to most of the other bird species tested. This implies that the threshold of 800 ng/g is quite protective for the cormorants found in the Penobscot.

Concerning probable toxic effects threshold concentrations for Hg in the muscle tissue of birds, there have been few studies done and none published in the last 25 years. This is perhaps due to the difficulty of collecting muscle tissue samples from birds. A study of the common loon with a range of Hg concentrations of 760 to 2,320 ng/g wet wt. (0.76 to 2.32 µg/g wet wt.) in muscle found that at the high end of the range there was reduced male adult body condition and reduced reproductive success (Barr 1986). So, setting a toxic effects threshold for Hg in bird muscle tissue is problematic and we have not done so for this report. However, the black duck is the only species of concern for which we have sampled muscle tissue and we do have blood Hg levels for that species as well, so the difficulty is establishing a concern concentration for muscle for birds may not be a problem for the present purposes.

There have been a number of recent studies on toxicity of Hg in birds related to concentrations in feathers that allow for an estimation of toxic effects thresholds. Hg concentrations in bird feathers reflect the concentrations of Hg circulating in the blood at the time of feather formation, which is directly related to Hg exposure through the diet (Johnels et al. 1979; Solonen and Lodenius 1990; Becker et al. 1994; Bearhop et al. 2000; Fournier et al. 2002; Ackerman et al. 2008a). Given a constant environmental exposure, Hg concentrations in individual feathers decline with the order in which they are molted. This reduction in feather concentration is matched by a decline in Hg concentrations in the blood during the molt period (Braune and Gaskin 1987). As a result, for each molt cycle the first feathers molted have the greatest Hg concentrations and the last feathers molted generally have the lowest Hg concentrations. It follows that the Hg concentrations in feathers associated with toxicity to the bird are most relevant for specific feather types (Westermark et al. 1975; Applequist et al. 1984; Wolfe et al. 1998; Jackson et al. 2011).

Heinz (1979) reported significant reproductive effects in a treatment group of mallards (*Anas platyrhynchos*) which had mean Hg concentrations in primary feathers between 9,000 and 11,200 ng/g fresh wt. (9.0 and 11.2 µg/g fresh wt.). Bowerman et al. (1994) reported mean total Hg concentrations in four types of bald eagle (*Haliaeetus*

leucocephalus) feathers (primary, secondary, tail and body) ranging from 19,000 to 23,000 ng/g fresh wt. (19 to 23 µg/g fresh wt.), with no significant reproductive effects associated with those Hg concentrations. The authors noted that the co-occurrence of elevated organochlorine contaminants, with known reproductive effects, may have masked Hg-related toxicity. Heath and Frederick (2005) reported mean Hg concentrations in breast feathers from female white ibis (*Eudocimus albus*) sampled in the Everglades of 6,440 ± 510 ng/g fresh wt. (6.44 ± 0.51ug/g fresh wt.) and a negative correlation between Hg concentrations in feathers and estradiol concentrations. Brasso and Cristol (2008) reported reproductive effects in tree swallows (*Tachycineta bicolor*) nesting in contaminated areas along the South River, VA, associated with total Hg concentrations in primary feathers (P1) of 13,550 ± 6,940 ng/g fresh wt. (13.55 ± 6.94 (min-max, 5-26) µg/g fresh wt.). Hg concentrations in P1 feathers from the reference area were reported as 2,340 ± 870 ng/g fresh wt. (2.34 ± 0.87 (1-4) µg/g fresh wt.). Ackerman et al. (2008c) found no effect of Hg in stilt and avocet in San Francisco Bay at levels up to 44,300 ng/g (44.3 µg/g fresh wt.) in feathers. Jackson et al. (2011) modeled the probabilities of nest failure in the Carolina wren (*Thryothorus ludovicianus*) associated with estimated increases in blood and feather Hg concentrations. At a 20% reduction in nest success (EC20), the estimated Hg concentration in body feathers was 3,400 ng/g fresh wt. (3.4 µg/g fresh wt.) and the estimated Hg concentration in tail feathers was 4,700 ng/g fresh wt. (4.7 µg/g fresh wt.). Evers (2012) followed Jackson et al. (2011) in recommending these threshold levels. Eisler (2006) proposed a Hg criterion for the protection of birds of 5,000 ng/g fresh wt. (5.0 µg total Hg/g fresh wt.) in feathers, with no distinction made for feather type. Ackerman et al. (2008a) used both 5,000 ng/g fresh wt. (5 µg/g fresh wt.) and 20,000 ng/g fresh wt. (20 µg/g fresh wt.) in feathers as the range of concentrations associated with avian toxicity. Thus, it seems that 5,000 ng/g (5 µg/g) Hg in bird feathers is a reasonable toxicity threshold.

2.5 Targets to Protect Fish Predator Health

One important consideration in setting targets for Hg in prey species is to protect the health of predators. The fish-eating birds and mammals that we sampled in the Penobscot system were generally not at risk from eating contaminated fish, probably mainly because they were not primarily feeding in the river itself (see, however, section on bird health). Therefore, this section will concentrate on the recent literature concerning effects of Hg in prey on fish predators (summarized above) and threshold concentrations of Hg in prey producing toxic effects in those fish predators.

There has been a large amount of recent research on this topic, summarized by Sandheinrich (2010) and Depew et al. (2012). Sandheinrich found a LOAEL concentration of 180 ng/g (0.18 µg/g) (whole body) in prey species and noted that the threshold effect concentration is lower. Depew et al. (2012) provided threshold concentrations for prey of fish for various classes of effects, including lethality, growth, behavior, reproductive and biochemical. The two classes of effects for methyl Hg toxicity for which fish were most sensitive were reproductive (spawning success, reduced fecundity, altered levels of sex steroids and altered spawning behavior) and biochemical (altered blood or plasma biochemistry, altered neurochemistry, changes in gene transcription, changes in cell physiology, pathological damage to organs or tissues

and altered behavior). For biochemical effects, Depew et al. (2012) found that the highest no effects level in prey fish was 60 ng/g (0.06 µg/g), the Threshold Effects Level (TEL) was 180 ng/g, the LOAEL was 140 ng/g. They proposed a threshold level of 60 ng/g (0.06 µg/g). For reproductive effects, Depew et al. (2012) found the highest no effects level was 40 ng/g, the LOAEL was 50 ng/g and proposed a threshold level of 40 ng/g. LOAEL is the lowest concentration at which effects have been observed whereas the TEL is calculated from the LOAEL and No Observed Adverse Effect Level (NOAEL), being the square root of the product of the 50^{th} percentile of the NOAEL and the 15^{th} percentile of the LOAEL. Based on these data, a reasonable level to protect predator health might be 50 ng/g (0.05 µg/g) in prey fish, although it is difficult to translate sublethal effects in individuals, such as altered neurochemistry, to population level effects. This level is very low compared to those often seen in natural fish populations.

In the Penobscot, the main predatory fish are eels; however, striped bass are known to enter the river at various times.

2.6 Targets to Protect Mammal Health

There are few relevant studies to determine what might be toxic concentrations of Hg in bats in the Penobscot system. Toxic effects of Hg in humans are thought to start at around 10,000 ng/g wet wt. (10 µg/g wet wt.) in hair (Murata et al. 1999; Burton et al. 1977). Burton et al. (1977) found decreased swimming ability and deviant behavior in mice at fur Hg concentrations of 7,800 to 10,800 ng/g wet wt. (7.8 to 10.8 µg/g wet wt.) and Sleeman et al. (2010) found tissue abnormalities in an otter with fur concentrations of Hg of 183,000 ng/g wet wt. (183 µg/g wet wt.). Effects on adrenocortical levels in big brown bats at a contaminated site in Virginia were not evident at 28,000 ng/g wet wt. (28 µg/g wet wt.) in fur and 110 ng/g wet wt. (0.11 µg/g wet wt.) in blood (Wada et . 2010). Neurochemical changes were also not found in little brown bats sampled at the same contaminated site (mean fur Hg 132 ± 94 µg/g fresh wt.; Nam et al. 2012). It is important to note, however, that just because effects are not found in a particular study, it does not necessarily mean that effects did not exist because effects could be taking place on some response that was not measured. So, a reasonable limit for Hg in bat fur for toxic effects might be 10,000 ng/g wet wt. (10 µg/g wet wt.).

2.7 Target Setting for Individual Species and Compartments

Using the thresholds established above, we will now go through a series of exercises to set targets for a number of species that we studied in the Penobscot estuary.[1]

American lobster – Average total Hg (unadjusted) in lobster tail at five sites in the area of Fort Point Cove and the south end of Verona Island in the Penobscot exceeded the 200 ng/g wet wt. criterion (Table 2-1). From 50% to 96% of lobsters at these sites exceeded 200 ng/g wet wt. total Hg. These concentrations are higher than the range of Hg concentrations found in lobsters at seven reference sites in Maine that are further

[1] Note that Hg means and ranges given in this section are all calculated from raw concentrations, unadjusted for individual size, age or sex, using data from all sampling years (usually 2006 to 2010). Means may not be the same as those presented with the objective of comparing years, sites or areas, where statistical adjustment for factors such as size are used to provide comparability of data.

from the Penobscot and HoltraChem. However, there is not sufficient information to determine toxic effects thresholds for the lobsters themselves (Sandheinrich 2010). A target concentration of 200 ng/g wet wt. methyl Hg or total Hg (we have found that 100% of the total Hg in lobster tails in the Penobscot is methyl Hg) would protect human consumers eating lobster from these sites and would put these animals close to background concentrations at the highest sites in Maine (Sowles 1997). Reductions to 200 ng/g total Hg in lobster tail muscle would require decreases ranging from less than 10% to about 50% or more.

Table 2-1: Target setting for American lobster.

American lobster	Location or source of information	Total Hg ng/g wet wt. in tail (means & % > 200)	Methyl Hg ng/g wet wt. (means)
CONCENTRATIONS IN AREAS OF CONCERN	Penobscot Bay (S. Verona)	485 (96%)	
	Penobscot Bay (Fort Point)	228 (50%)	
	Penobscot Bay (Odom Ledge)	291 (77%)	
	Penobscot Bay (Wilson Point)	338 (80%)	
	Penobscot Bay (Turner Point)	203 (75%)	
CONCENTRATIONS IN REFERENCE AREAS	Seven sites in Maine (Sowles 1997)	82 to 208	
Toxic effects levels	Sandheinrich 2010	Insufficient information	Insufficient information
HUMAN CONSUMPTION TARGETS	Maine DEP action level of methyl Hg	200 (if 100% methyl Hg)	200
TARGET CONCENTRATIONS	Below consumption target	200 (if 100% methyl Hg)	200

Rock crabs – Average methyl Hg concentrations in rock crabs exceeded the 200 ng/g wet wt. limit for human consumption set by Maine DEP at four sites in upper Penobscot Bay and individual crabs exceeded this limit at six additional sites. Unadjusted methyl Hg averaged 319, 308, 204,and 227 ng/g at the sites with means exceeding 200 ng/g in 2006 (the sites were, 1-3, 1-4, 1-10, and 3-1; see Phase I Update report for site locations). The proportion of these samples exceeding 200 ng/g was 25, 33%, 30% and 40%. At uncontaminated reference locations, total Hg in rock crabs and blue crabs (which share similar diets) were noticeably lower than in Penobscot Bay. Mean concentrations at three reference locations in Connecticut and Florida ranged from 60 to

156 ng/g wet wt. (Jop et al. 1997; Karouna-Renier et al. 2007); whereas, total Hg in crab muscle in New York-New Jersey Harbor was 170 ng/g wet wt. (NYSDEC 1996). Therefore, background concentrations are lower than those required to meet targets for human consumption. Reductions of about 10% to 15% of current concentrations would be required for safe human consumption.

American eel – The only species of fish in the contaminated zone of the Penobscot River that would appear to be at direct risk from the toxic effects of mercury is the American eel. It was concluded above that fish with more than 500 ng/g wet wt. in muscle tissue are at risk due to sublethal toxic effects. Over the period 2007 to 2010 the mean total Hg concentrations in eels in the Brewer to Orrington (BO) and Orrington to Bucksport (OB) reaches of the river ranged from 496 to 619 ng/g wet wt. in axial muscle (Table 2-2). The upstream reach of the Penobscot (Old Town to Veazie) had a grand mean concentration of Hg in eels of 333 ng/g wet wt.

There are a large amount of data on background concentrations from uncontaminated sites in Maine, North America and Europe (Table 2-2). Concentrations of Hg at reference sites varied widely (Table 2-2), from 60 to over 1,000 ng/g wet wt. However, it is difficult to compare concentrations from other sites to the Penobscot because of differences in the life stage, size and age of fish analyzed.

On the basis of criteria designed to avoid sublethal toxic effects to the eels themselves, a target of about 500 ng/g wet wt. would be appropriate. To protect the health of people eating eels, the target for eels would be 222 ng/g wet wt. (Table 2-2, assuming that 90% of the mercury in eels is methyl Hg – See Chapter 14 for data from this study on the percentage of methyl Hg in eel muscle from the Penobscot River). However, based on the information we have been able to obtain from the literature and by talking to Maine Department of Marine Resources (Dr. Gail Whippelhauser, Personal Communication, October 2012), there does not appear to be a large amount of eels from the Penobscot consumed by local people. Also, any remediation efforts would not to be able to bring the average concentration of Hg in eel muscle below about 260 ng/g wet wt., the normalized concentration in the Old Town to Veazie reach, directly upstream of the contaminated zone of the river. So, 260 ng/g wet wt. is a reasonable and practical target for eels. From 82% to 91% of the eels sampled in 2007 to 2010 in the BO and OB reaches of the Penobscot exceeded this target (Table 2-2). Reaching a target concentration of 260 ng/g wet wt. total Hg in eels would require reductions of about 50% of present concentrations, and while this would not reach the target concentration of 222 ng/g wet wt. total Hg for human consumption, it would reach the target at what appears to be the regional background concentration.

Table 2-2: Target setting for American eel.			
AMERICAN EEL		**Total Hg in muscle (ng/g wet wt.) (means) and number exceeding target of 260**	**Methyl Hg in muscle (ng/g wet wt.) (means)**
CONCENTRATIONS IN AREAS OF CONCERN	Penobscot BO3	498 (90%)	
	Penobscot BO4	619 (87%)	
	Penobscot OB1	454 (82%)	
	Penobscot OB5	496 (91%)	
CONCENTRATIONS IN REFERENCE AREAS	Penobscot (Old Town – Veazie reach)	333 (60%)	
	European reference sites (Italy, Bosnia) (Mancini et al. 2005; Has-Schön et al. 2008)	60 – 160	
	N American reference sites Nova Scotia (Freeman & Horne 1973) Georgia (Burger et al. 2001) New Brunswick (Zitko et al. 1971) (excludes one sample from an impoundment) St. Lawrence estuary (Hodson et al. 1994)	 720 150 50-946 (whole body)	 400 70 – 760 (10 sites)
	Maine reference sites Leaman (1999), 3 Maine rivers	330-642	
TOXIC EFFECTS LEVELS	Sublethal effects	500	
HUMAN COSUMPTION TARGETS	USEPA action level	333 (if 90% methyl Hg)	300
	Maine DEP action	222 (if 90% methyl Hg)	200
TARGET CONCENTRATIONS	**BASED ON CONCENTRATIONS IN**	**260**	**234**

Table 2-2: Target setting for American eel.			
AMERICAN EEL		**Total Hg in muscle (ng/g wet wt.) (means) and number exceeding target of 260**	**Methyl Hg in muscle (ng/g wet wt.) (means)**
	OLD TOWN – VEAZIE REACH		

Prey fish – All of the prey fish that have been sampled in the Penobscot are known to be eaten by a wide variety of predators, including eels, striped bass, crabs, and piscivorous birds.

For rainbow smelt over the period 2006 to 2010 in the Penobscot estuary, Hg concentrations in muscle tissue averaged from about 45 to 120 ng/g wet wt. (0.045 to 0.12 µg/g wet wt.), unadjusted for size. This would be approximately 30 to 100 ng/g on a whole body basis. From 25% to 100% of rainbow smelt exceeded the toxic effects threshold to protect predator health of 50 ng/g (0.05 µg/g). For the two upstream sites where rainbow smelt were caught (OB1E and OB1S), 92% to 100% of fish exceeded 50 ng/g in muscle. For sites between Bucksport and the south end of Verona Island, 57% to 97 % of fish exceeded 50 ng/g, whereas for sites further south, 26% to 83% of fish exceeded 50 ng/g. Hg in rainbow smelt from 25 Canadian lakes averaged 60 ng/g (0.06 µg/g), also exceeding the toxic effects threshold (Swanson et al. 2006); however, Hg exposure of lake populations would be expected to be greater than Hg exposure of river populations.

For *Fundulus* (mummichogs), over the period 2006 to 2010, Hg in muscle tissue at various sites in the BO and OB reaches varied from a mean of 131 to 307 ng/g wet wt., which is approximately 110 to 250 ng/g (0.11 to 0.25 µg/g) on a whole body basis. All fish therefore exceeded the threshold of 50 ng/g (0.05 µg/g for toxic effects), by about 2 to 10 times. At two wetland sites (W17 and W21), mean values were 218 and 352 ng/g, and therefore all these fish also exceeded the toxic effects threshold to protect predator health.

For tomcod, unadjusted mean Hg concentrations in muscle tissue ranged from 100 to 290 ng/g (0.10 to 0.29 µg/g) over the 2006 to 2010 sampling period. This translates to approximately 75 to 220 ng/g (0.075 to 0.22 µg/g) on a whole body basis, or about 1½ to 4 times the toxic effects threshold to protect the health of predators. Tomcod at two contaminated sites have been reported in the literature, as follows: 150 ng/gwet wt. (0.15 µg/g wet wt.) (mean from 12 fish) in muscle tissue from St. Lawrence River estuary, Quebec, Canada (Duchesne et al. 2004); 140 ng/g wet wt. (0.14 µg/g wet wt.) (one fish only) in skinned and eviscerated whole fish from the Meadowlands, NJ (Santoro and Koepp 1986). Hg in tomcod in Labrador, Canada was about 130 ng/g (0.13 µg/g), but these fish were much larger than those sampled in the Penobscot (Anderson 2011).

Double-crested cormorants – As noted above, the threshold concentration for toxic effects of Hg on bird eggs is around 800 ng/g wet wt. (0.8 µg/g wet wt.). It was reported in the Phase I report that Hg in cormorant eggs at one site in 2006 averaged 880 ng/g wet wt. (0.88 µg/g wet wt.), which is above the threshold (Table 2-3). However, over the whole sampling period (2006 to 2008), the unadjusted mean total Hg concentrations at the three nesting sites furthest north in the estuary were all below 800 ng/g and few eggs exceeded the this threshold (Table 2-3). Concentrations of Hg in cormorant eggs appear in the contaminated area of Penobscot Bay do appear to be above regional background concentrations (Table 2-3). However, cormorants have been shown to be relatively insensitive to Hg as compared to other species (Heinz et al. 2009). It would therefore appear that no reductions in Hg in this species are required to prevent toxic effects.

Table 2-3: Target setting for Hg in double-crested cormorant eggs.

DOUBLE-CRESTED CORMORANTS		Mean total Hg (ng/g wet wt.) in eggs at different sites
CONCENTRATIONS IN AREA OF CONCERN	Luce Cove	670 (0 of 7 (0%) over 800)
	Sandy Point	360 (0 of 87 (0%) over 800)
	Fort Point	790 (2 of 3 (67%) over 800)
CONCENTRATIONS IN REFERENCE AREAS	8 reference sites in Maine (data from BioDiversity Research Institute, Gorham, Maine)	280 (range – 110 – 450)
	2 “clean” reference sites in N. America (Bay of Fundy, Canada and WA; Burgess and Braune 2001; Henny et al. 1989)	260 – 280
	Hg-impacted San Francisco Bay-Delta (Davis et al. 2005)	170 – 1,170
Toxic concentrations	Reproductive effects in other bird species	800
TARGET CONCENTRATIONS	Based on toxic levels and background concentrations	Present concentrations

Nelson’s sparrows – Sampling over the period 2006 to 2010 at six sites in Mendall Marsh and W17 determined that total Hg in blood of adult Nelson’s sparrows ranged from (unadjusted) means of 2,900 to 5,200 ng/g wet wt. (2.9 to 5.2 µg/g wet wt.) (Table 2-4). These levels are much higher than the regional background, as established by us and by Shriver et al. (2006) which ranged from about 400 to 700 ng/g (0.4 to 0.7 µg/g). As established in the above section on threshold concentrations, there is now reliable recent evidence that sublethal toxic effects are probably present as low as 1,200 ng/g (1.2 µg/g) in bird blood. Reducing Hg concentrations in Nelson’s sparrows by up to 75%

will put populations below this threshold level. Even greater reductions would be required to put Nelson's sparrows within the range of regional background concentrations.

Table 2-4: Target setting for Nelson's sparrow in Mendall Marsh.

NELSON'S SPARROWS		Total Hg in blood ng/g wet wt. (means)
CONCENTRATIONS IN AREAS OF CONCERN	Six sites in Mendall Marsh and W17, 2006 - 2010 (after hatch year)	2,900 – 5,200
CONCENTRATIONS IN REFERENCE AREAS	Five reference areas in southern Maine (our sampling)	500 – 700
	Five coastal marshes in Maine (Shriver et al. 2006)	410 +/- 160
TOXIC CONCENTRATIONS	Reproductive effects in other bird species	1,200
TARGET CONCENTRATIONS	Below toxic concentrations	<1,200 (up to 75% reduction)

Other songbirds and shorebirds – There are four other species of songbirds and shorebirds that inhabit Mendall Marsh and other contaminated wetlands adjacent to the Penobscot River that show elevated concentrations of Hg in blood (Table 2-5).

Hg in the blood of song sparrows at contaminated sites in the Penobscot ranged from averages of 110 to 1,920 ng/g wet wt. (0.11 to 1.92 µg/g wet wt.) as compared to a target to eliminate toxic effects of 1,200 ng/g wet wt. (1.2 µg/g wet wt.) and as compared to means of 20 to 350 ng/g wet wt. (0.02 to 0.35 µg/g wet wt.) at reference sites in Massachusetts and Maine (Table 2-5). From 0% to 67% of birds at the 17 contaminated sites sampled exceeded the 1,200 ng/g wet wt. threshold as compared to 0% of birds at reference sites. Reductions of up to about 40% would be required to lower blood Hg concentrations in song sparrows to the toxic threshold but would not reduce them to concentrations found at reference sites.

Hg in the blood of swamp sparrows at contaminated sites ranged from about 400 to over 3,000 ng/g wet wt., or up to about 2.5 times the toxic effects threshold of 1,200 ng/g wet wt. (Table 2-5). Thus, levels in swamp sparrows needed to be reduced up to 3 times to be below the toxic effects threshold. From 33% to 100% of the individual birds at these contaminated sites exceeded the toxic effects threshold. At three reference sites in Maine, mean blood concentrations of Hg in swamp sparrows were 140 to 490 ng/g (0.14 to 0.49 µg/g) and 0% to 20% of these birds exceeded the toxic effects threshold. Average Hg in swamp sparrows at seven sites in Wisconsin ranged from 80 to 220 ng/g (0.08 to 0.22 µg/g). Reductions of up to about 60% would be required to

lower blood Hg concentrations in swamp sparrows to the toxic threshold but, as for song sparrows, would not reduce them to concentrations found at reference sites.

In red-winged blackbirds, average Hg in blood at eight contaminated sites in the Penobscot ranged from 1,450 to 6,260 ng/g wet wt. (1.45 to 6.26 µg/g wet wt.) (Table 2-5). From 32% to 100% of individual birds at eight contaminated sites had Hg higher than the toxic effects threshold. At two reference sites in Maine and New Jersey, Hg in blackbird blood was 170 and 230 ng/g (0.17 and 0.23 µg/g), respectively. None of the birds at the Maine reference site exceeded the toxic effects threshold. To reduce red-winged blackbirds to the toxic effects limit, they would have to come down by up to about 80%. Reductions of this amount in blackbirds would not reduce all birds to within the concentrations seen at reference sites.

Virginia rails at 10 sites in Mendall Marsh also exceeded the toxic threshold, by up to about 3 times (Table 3-5). Means at these contaminated sites ranged from 870 to 2,940 ng/g wet wt. (0.87 to 2.94 µg/g wet wt.) and 0% to 100% of individual rails exceeded the toxic effects threshold. Mean Hg at two reference sites in Maine were 160 and 410 ng/g (0.16 and 0.41 µg/g); none of the birds at these sites exceeded the toxic effects threshold. Reductions of up to 70% in Virginia rails will reduce mean concentrations below toxic levels but will not reduce all birds to within the range seen in reference areas (Table 2-5).

Table 3-5: Current levels of Hg in the blood (µg/g wet wt.) of four species of birds that inhabit contaminated wetlands in the Penobscot basin.

SPECIES	Background concentrations (ng/g wet wt. blood)	Total Hg in blood at contaminated Penobscot sites (ng/g wet wt.)	Reduction needed to meet target of 1,200 ng/g (wet wt. in blood)
Song sparrow	210 – 350 (Eastern Massachusetts: Evers et al. 2005)	110 – 1,920 (16 sites)	Up to about 40%
	20 – 33 (our sampling – 8 sites in Maine)		
Swamp sparrow	About 80 to 202 in 7 wetlands in Wisconsin (Strom and Brady 2011)	370 – 3,160 (10 sites)	Up to about 60%
	140 – 490 (3 sites in Maine, our sampling)		
Red-winged blackbird	230 (Meadowlands, New Jersey; Tsipoura et al. 2008)	1,450 – 6,260 (8 sites)	Up to about 80%
	170 (Spurwink Marsh, Maine, our sampling)		
Virginia rail	160 – 410 (2 reference sites, Maine, our sampling)	960 – 3,890 (10 sites)	Up to about 70%

Black guillemots – Average Hg concentrations in black guillemots in 2007 (the only year they were sampled) at two sites were 700 to 1,200 ng/g wet wt. (0.7 to 1.2 µg/g wet wt.) in eggs, and 1,300 ng/g wet wt. (1.3 µg/g wet wt.) at both sites in adult blood. This compares to concentrations in eggs at a reference site of about 500 ng/g (0.5 µg/g). Although no information is available about Hg toxicity thresholds in guillemots specifically, the toxic effects threshold for Hg in bird eggs generally is around 800 ng/g and is about 1,200 ng/g for Hg adult bird blood. Current concentrations exceed these toxic thresholds in many individuals. If concentrations at sites in Penobscot Bay were lowered by about 35%, this would place most individual birds and eggs below toxic thresholds; these reductions would not lower Hg in guillemot eggs below those of a reference site.

Black ducks – As noted above, there are no recent studies of the likely threshold concentrations of Hg in the muscle tissue of birds that would cause harm to the birds themselves. The only study, on loons, conducted 25 years ago, found decreased body condition and reproductive success when concentrations in muscle was more than 2,000 ng/g wet wt. (2 µg/g wet wt.) (Barr 1986). However, our studies on the black duck indicate that muscle concentrations follow blood concentrations very closely. Hg in black duck muscle averaged about 750 ng/g wet wt. (0.75 µg/g wet wt.) from the most recent sampling. Hg in black duck blood averaged about 800 ng/g, compared to the presumed limit of 1,200 ng/g for toxic effects. Hg in the blood and muscle of ducks is essentially all methyl Hg. To protect human consumers of black ducks, Hg in muscle tissue needs to come down from 750 to 200 ng/g (about a 75% reduction of present concentrations). It should be noted that these targets were set based on consumption of fish and shellfish, so the use of the 200 ng/g concentration for black ducks has not been set by state agencies. If blood levels in black ducks also were reduced by about 75% from of present concentrations, this would put all individual ducks below the threshold limit of 1,200 ng/g for sublethal effects and thus the health of the black ducks would also be protected.

Bats – Little brown bats sampled at six contaminated sites in the Penobscot in 2008 averaged 10,900 to 23,600 ng/g wet wt. (10.9 to 23.6 µg/g wet wt.) in fur, as compared to the toxic effects threshold of 10,000 ng/g (10 µg/g). Bats caught near the HoltraChem site were the highest (23,600 ng/g). From 12% to 91% of the bats from the six sampling sites exceeded 10,000 ng/g. Little brown bats at two reference sites sampled by us in Maine averaged 3,900 and 4,200 ng/g; 0% to 7% of these bats exceeded 10,000 ng/g. Fur from little brown bats in Ontario and Quebec, Canada averaged 1,300 to 2,500 ng/g dry wt. (1.3 to 2.5 µg/g dry wt.) (which should be equivalent to wet wt. or fresh wt. for fur) (Hickey et al. 2001) and big brown bats (a different species) at a reference site in Virginia averaged 400 ng/g (0.4 µg/g) wet wt. in blood and 10,900 ng/g fresh wt. (10.9 µg/g fresh wt.) in fur (Wada et al. 2010). A contaminated site in Virginia (South River) had big brown bats with mean Hg concentrations of 28,000 ng/g wet wt. in fur (Wada et al. 2010). Thus, levels observed at HoltraChem are 4 to 5 times those seen at the South River, although it may not be valid to compare different species of bats. Toxic effects of mercury may take effect at about 10,000 ng/g wet wt. in fur (see above). Approximately a 50% reduction in Hg in the fur of bats sampled near the HoltraChem site and at Bald

Hill Cove would be required to reduce mean levels to below the presumed toxic effects threshold.

3 CONCLUSION

Of species living primarily in river habitats (in the upper estuary), most require reductions of about 50% of present concentrations to meet reduction targets, including eels, and prey species such as rainbow smelt, Fundulus, and tomcod (Table 3-6). These reduction targets are based on toxic effects to one species (eels) and predicted toxic effects on fish predators eating prey fish (rainbow smelt, *Fundulus*, tomcod). Thus, if total Hg in surface sediments in the contaminated zone can be reduced from present concentrations of about 800 to 900 ng/g dry wt. to about 400 to 450 ng/g, these reductions can be accomplished. For biota species living in Penobscot Bay, reductions of 1/2 to 2/3 are likewise required to meet target concentrations. Although we are not recommending any active remediation of Penobscot Bay, it is likely that reductions of Hg in the upper estuary would eventually result in similar reductions in Hg in surface sediments in the Bay. Reductions of Hg in surface sediments to about 400 to 450 ng/g will also reduce those sediments to concentrations below the NOAA Probable Effects Level for total Hg in freshwater sediment of 486 ng/g and in marine sediment of 700 ng/g (Buchman 2008).

Reductions needed in Mendall Marsh and other marshes in the contaminated zone to affect the decreases required in biota living in those habitats are greater and therefore are much more challenging (Table 3-6). Nelson's sparrows and black ducks will require up to 75% reductions in exposure to methyl Hg, while other song and shore birds will require up to an 80% reduction. To approach these levels of Hg reduction in Mendall Marsh biota, we will recommend that more than one type of active remediation procedure be used in the marsh (see Chapter 1 and 21).

Table 2-6: Summary of species considered for reduction targets, their primary habitat, and the amount of reduction from present concentrations that would be required to meet targets, as discussed above.

Species	Habitat	Reductions needed
Lobster	Bay	Up to 50%
Rock crabs	Bay	10% to 15 %
Eels	River	50 %
Rainbow smelt	River and Bay	Up to 60%
Fundulus	River and Bay	50% to 80%
Tomcod	River and Bay	35% to 80%
Cormorants	Bay	None
Nelson's sparrows	Wetlands	Up to 75%
Other songbirds and shorebirds	Wetlands	Up to 80%

Table 2-6: Summary of species considered for reduction targets, their primary habitat, and the amount of reduction from present concentrations that would be required to meet targets, as discussed above.		
Species	**Habitat**	**Reductions needed**
Black guillemots	Bay	35%
Black ducks	Wetlands	75%
Bats	River and wetlands	50%

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5 REFERENCES

Ackerman, J.T., C.A. Eagles-Smith, J.Y. Takekawa, J.D. Bluso, T.L. Adelsbach. 2008a. Mercury concentrations in blood and feathers of prebreeding Forester's terns in relation to space use of San Francisco Bay, California, USA, habitats. Environmental Toxicology and Chemistry. 27(4):897-908.

Ackerman, J.T., C.A. Eagles-Smith, J.Y. Takekawa and S.A. Iverson. 2008b. Survival of port fledgling Forster's terns in relation to Hg exposure in San Francisco Bay. Ecotoxicology. 17:789-801.

Ackerman, J.T., J.Y. Takekawa, C.A. Eagles-Smith, S.A. Iverson. 2008c. Mercury contamination and effects on survival of American avocet and black-necked stilt checks in San Francisco Bay. Ecotoxicology. 17:103-116.

Ackerman, J.T., C.T. Overton, M.L. Casazza, J.Y. Takekawa, C.A. Eagles-Smith, R.A. Keister and M.P. Herzog. 2012. Does mercury contamination reduce body condition of endangered California clapper rails? Environmental Pollution. 162:439-448.

Anderson, M.R. 2011. Duration and extent of elevated mercury levels in downstream fish following reservoir creation. River Systems. 19/3:167-176.

Applequist, H., S. Asbirk, I. Draback. 1984. Mercury monitoring: mercury stability in bird feathers. Marine Pollution Bulletin. 15:22-24.

Barr, J.F. 1986. Population dynamics of the common loon (*Gavia immer*) associated with mercury-contaminated waters in northwestern Ontario. Occasional Paper - Canadian Wildlife Service. 56:1-23.

Bearhop, S., G.D. Ruxton, R.W. Furness. 2000. Dynamics of mercury in blood and feathers of great skuas. Environmental Toxicology and Chemistry. 19(6):1638-1643.

Becker, P.H., D. Henning, R.W. Furness. 1994. Differences in mercury contamination and elimination during feather development in gull and tern broods. Archives of Environmental Contamination and Toxicology. 27:162-167.

Bowerman, M.W., E.D. Evans, J.P. Giesy, S. Postupalsky. 1994. Using feathers to assess risk of mercury and selenium to bald eagle reproduction in the Great Lakes region. Archives of Environmental Contamination and Toxicology. 27:294-298.

Brasso, R.L. and D.A. Cristol. 2008. Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). Ecotoxicology. 17:133-141.

Braune, B.M. and D.E. Gaskin. 1987. Mercury levels in Bonaparte's Gulls (Larus philadelphia) during autumn molt in the Quoddy Region, New Brunswick, Canada. Archives of Environmental Contamination and Toxicology. 16:539-549.

Buchman, M.F. 2008. NOAA Screening Quick Reference Tables, NOAA OR&R Report 08-1. Seattle, WA, Office of Response and Restoration Division, National Oceanic and Atmospheric Administration. 34 p.

Burger, J., K.F. Gaines, C.S. Boring, W.L. Stephens, J. Snodgrass, M. Gochfeld. 2001. Mercury and selenium in fish from the Savannah River: Species, trophic level, and locational differences. Environmental Research. 87: 108-118.

Burgess, N. and B. Braune. 2001. Increasing trends in mercury concentrations in Atlantic and Arctic seabird eggs in Canada. Society of Environmental Toxicology and Chemistry Europe, Abstract, 2001 meeting.

Burton, G.V., R.J. Alley, G.L. Rasmussen, P. Orton, V. Cox, P. Jones, D. Graff. 1977. Mercury and behavior in wild mouse populations. Environmental Research. 14:30-34.

Davis, J., J. Hunt, T. Adelsbach, D. Crane, L. Phillips. 2005. Monitoring legacy and emerging pollutants at the top of the San Francisco Bay food web. Society of Environmental Toxicology and Chemistry, Abstract, Baltimore meeting.

Depew, D.C., N. Basu, N.M. Burgess, L.M. Campbell, E.W. Devlin, P.E. Drivnick, C.R. Hammerschmidt, C.A. Murphy, M.B. Sandheinrich, J.G. Wiener. 2012 Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations. Environmental Toxicology and Chemistry. 31:1-12.

DeSorbo, C.R. and D.C. Evers. 2007. Evaluating exposure of Maine's bald eagle population to mercury: Assessing impacts on productivity and spatial exposure patterns. Report BRI 2007-02. BioDiversity Research Institute, Gorham ME, 36 p.

Duschesne, J.F., B. Levesque, D. Gauvin, B. Braune, S. Gingras, E. Dewailly. 2004. Estimating the mercury exposure dose of a population of migratory bird hunters in the St. Lawrence River region, Quebec, Canada. Environmental Research. 95:207-214.

Eisler, R. 2006. Mercury Hazards to Living Organisms. CRC Press, Taylor and Francis Group. New York.

Evers, D.C. 2012. The effects of methyl mercury on wildlife. A comprehensive review. BioDiversity Research Institute. October 2012, 50 pages, with appendix tables. Produced for the Penobscot River Mercury Study.

Evers, D.C., N.M. Burgess, L. Champoux, B. Hoskins, A. Major, W.M. Goodale, R.J. Taylor, R. Poppenga, T. Daigle. 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northwestern North America. Ecotoxicology. 14:193-221.

Fournier, F., W.H. Karasov, K.P. Kenow, M.W. Meyer, R.K. Hines. 2002 The oral bioavailability and toxicokinetics of methylmercury in common loon (*Gavia immer*) chicks. Comparative Biochemistry and Physiology Part A. 133:703-714.

Franceshini, M.D., O.P. Lane, D.C. Evers, J.M. Reed, B. Hoskins, L.M. Romero. 2009. The coricosterone stress response and mercury contamination in free-living tree swallows Tachycinata bicolor. Ecotoxicology. 18: 514-521.

Freeman, H.C., D.A. Horne. 1973. Total mercury and methyl mercury content of the American eel (*Anguilla rostrata*). Journal of the Fisheries Research Board of Canada. 30:173-176.

Hammerschmidt, C.R., W.F. Fitzgerald. 2005. Methylmercury in mosquitoes related to atmospheric mercury deposition and contamination. Environmental Science & Technology. 39:3034-3039.

Harris, R.C. and 23 co-authors. 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proceedings of the National Academy of Sciences of the United States. 104:16586-16591.

Has-Schön, E., I. Bogut, V. Rajkovic, S. Bogut, M. Cacic, J. Horvatic. 2008. Heavy metal distribution in tissues of six fish species included in human diet, inhabiting freshwaters of the Nature Park “Hutovo Blato” (Bosnia and Herzegovina). Archives of Environmental Contamination and Toxicology. 54:75-83.

Heath, J.A. and P.C. Frederick. 2005. Relationship among mercury concentrations, hormones, and nesting effort of white ibises (*Eudocimus albus*) in the Florida Everglades. The Auk. 122:255-267.

Heinz, G.H. 1979. Methylmercury: Reproductive and behavioral effects on three generations of mallard ducks. Journal of Wildlife Management. 43:394-401.

Heinz, G.H. and D.J. Hoffman. 2003. Embryotoxic thresholds of mercury: Estimates from individual mallard eggs. Archives of Environmental Contamination and Toxicology. 44:257-264.

Heinz, G.H., D.J. Hoffman, J.D. Klimstra, K.R. Stebbins, S.L. Konrad, C.A. Erwin. 2009. Species differences in the sensitivity of avian embryos to methylmercury. Archives of Environmental Contamination and Toxicology. 56:129-138.

Henny, C.J., L.J. Blus, S.P. Thompson. 1989. Environmental contaminants, human disturbance and nesting of double-crested cormorants in northwestern Washington. Colonial Waterbirds. 12: 198-206.

Hickey, M.B.C., M.B. Fenton, K.C. MacDonald, C. Souliere. 2001. Trace elements in the fur of bats (Chiroperta: Vespertilionidae) from Ontario and Quebec, Canada. Bulletin of Environmental Contamination and Toxicology. 66:699-706.

Hodson, P.V., M. Castonguay, C.M. Couillard, C. Desjardins, E. Pelletier and R. McLeod. 1994. Spatial and temporal variations in chemical contamination of American eels, *Anguilla rostrata*, captured in the estuary of the St. Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences. 51:464 - 478.

Jackson, A.K., D.C. Evers, M.A. Etterson, A.M. Condon, S.B. Folsom, J. Detweiler et al. 2011. Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the Carolina wren (*Thryothorus ludovicianus*). The Auk. 128:759-769.

Johnels, A., G.Tyler, T. Westermark. 1979. A history of mercury levels in Swedish fauna. Ambio. 8(4):160-168.

Jop, K.M., R.C. Biever, J.R. Hoberg, S.P. Shepherd. 1997. Analysis of metals in blue crabs, *Callinectes sapidus*, from two Connecticut estuaries. Bulletin of Environmental Contamination and Toxicology. 58:311-317.

Karouna-Renier, N.K., R.A. Snyder, J.G. Allison, M.G. Wagner, K.R. Rao. 2007. Accumulation of organic and inorganic contaminants in shellfish collected in estuarine waters near Pensacola, Florida: Contamination profiles and risks to human consumers. Environmental Pollution. 145:474-488.

Leaman, N.G.G. 1999. Mercury contamination in the silver stage of American eels, *Anguilla rostrata*, from three rivers in Maine. Zoology, University of Maine, Orono, Maine.

Mancini, L., S. Caimi, S. Ciardulla, M. Zeiner, P. Bottoni, L. Tancini, S. Cautadella, S. Caroli. 2005. A pilot study of the contents of selected pollutants in fish from the Tiber River (Rome). Microchemical Journal. 79:171-175.

McKay, J.L. and C.R. Maher. 2012. Relationship between blood mercury levels and components of male song in Nelson's sparrows (*Ammodramus nelsoni*). Ecotoxicology. 21:2391-2397.

Munthe, J., R.A. Bodaly, B.A. Branfireun, C.T. Driscoll, C.C. Gilmour, R. Harris, M. Horvat, M. Lucotte, O. Malm. 2007. Recovery of mercury contaminated ecosystems. Ambio. 36:33-44.

Murata, K., P. Weihe, A. Renzoni, F. Debes, R. Vasoncelos, F. Zino, S. Araki, P.J. Jørgensen, R.J. White, P. Grandjean. 1999. Delayed evoked potentials in children exposed to methylmercury from seafood. Neurotoxicology and Teratology. 21:343-348.

Nam, D.H., D. Yates, P Ardapple, D.C. Evers, J. Schmerfeld and N. Basu. 2012. Elevated mercury exposure and neurochemical alterations in little brown bats (*Myotis lucifugs*) from a site with historical mercury contamination. Ecotoxicology. 21:1094-1101.

NYSDEC (New York State Department of Environmental Conservation). 1996. Chemicals in fish, shellfish and crustaceans from the New York – New Jersey Harbor Estuary. PCB, Organochlorine Pesticide and Mercury. Edited by NYSDEC Conservation.

Orihel, D.M., M.J. Paterson, C.C. Gilmour, R.A. Bodaly, P.J. Blanchfield, H. Hintelmann, R.C. Harris, J.W.M. Rudd. 2006. Effect of loading rate on the fate of Hg in littoral mesocosms. Environmental Science & Technology. 40:5992-6000.

Orihel, D.M., M.J. Paterson, P.J. Blanchfield, R.A. Bodaly, H. Hintelmann. 2007. Experimental evidence of a linear relationship between inorganic mercury loading and methylmercury accumulation by aquatic biota. Environmental Science & Technology. 41:4952-4958.

Sandheinrich, M.B. 2010. Review of recent literature on the effects of methylmercury on wildlife, invertebrates, and fish. Report for the Penobscot River Mercury Study, December 2007, revised January 20, 2010. 46 p.

Sandheinrich, M.B., S.P. Bhavsar, R.A. Bodaly, P.E. Drevnick, E.A. Paul. 2011. Ecological risk of methylmercury to piscivorous fish of the Great Lakes region. Ecotoxicology. 20:1577-1587.

Sandheinrich, M.B. and J.G. Wiener. 2011. Methylmercury in freshwater fish: Recent advances in assessing toxicity of environmentally relevant exposures. Pp 169-190 IN WN Beyer and JP Meador (eds.) Environmental contamination in biota: Interpreting tissue concentrations, CRC Press, Boca Raton, FL.

Santoro, E.D. and S.J. Koepp. 1986. Mercury levels in organisms in proximity to an old chemical site. Marine Pollution Bulletin. 17:219-224.

Shriver, W.G., D.C. Evers, T.P. Hodgman, B.J. MacCulloch, R.J. Taylor. 2006. Mercury in sharp-tailed sparrows breeding in coastal wetlands. Environmental Bioindicators. 1:129-135.

Sleeman, J.M., D.A. Cristol, A.E. White, D.C. Evers, R.W. Gerhold and M.K. Keel. 2010. Mercury poisoning in a free-living northern river otter (*Lontra canadensis*). Journal of Wildlife Diseases. 46:1035-1039.

Solonen, T. and M. Lodenius 1990. Feathers of birds of prey as indicators of mercury contamination in southern Finland. Holarctic Ecology. 13(3):229-237.

Sowles, J. 1997. Memo to S. Ladner, Court Deposition Exhibit, U.S. District Court, District of Maine, dated September 29, 1997. 4 p.

Strom, S.E., R.S. Brady. 2011. Mercury in swamp sparrows (*Melospiza geogiana*) from wetland habitats in Wisconsin. Ecotoxicology. 20:1694-1700.

Swanson, H.A., T.A. Johnston, D.W. Schindler, R.A. Bodaly, D.M. Whittle. 2006. Mercury bioaccumulation in forage fish communities invaded by rainbow smelt (*Osmerus mordax*). Environmental Science & Technology. 40:1439-1446.

Tsipoura, N., J. Burger, R. Feltes, J. Yacabucci, D. Mizrahi, C. Jeitner, M. Gochfeld. 2008. Metal concentrations in three species of passerine birds breeding in the Hackensack Meadowlands of New Jersey. Environmental Research. 107:218-228.

Wada, H., D.W. Yates, D.C. Evers, R.J. Taylor, W.A. Hopkins. 2010. Tissue mercury concentrations and adrenocortical responses of female big brown bates (*Eptesicus fuscus*) near a contaminated river. Ecotoxicology. 19:1277-1284.

Westermark, T., T. Odsjö and A.G. Johnels. 1975. Mercury content of bird feathers before and after Swedish ban on alkyl mercury in agriculture. Ambio. 4(2):87-92.

Wolfe, M.F., S. Schwarzbach, R.A. Sulaiman. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry. 17(2):146-160.

Zitko, V., B.J. Finlayson, D.J. Wildish, J.M. Anderson, A.C. Kohler. 1971. Methylmercury in freshwater and marine fishes in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks. Journal of the Fisheries Research Board of Canada. 28:1285-1291.

APPENDIX 2-1:

Sandheinrich 2010 report on methyl mercury toxicity in biota

Review of Recent Literature on the Effects of Methylmercury on Wildlife, Fish, and Invertebrates

Prepared for the Penobscot River Mercury Study

By

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Scope and focus of the review

The purpose of this review is to synthesize information from the published, primary literature on the toxicological effects of methylmercury (MeHg) to fish and wildlife, including mammals, birds, amphibians and reptiles, and invertebrates. References were obtained by searching the electronic database *Science Citation Index (Web of Science)* and using appropriate search terms (e.g., "methylmercury AND wildlife"). The review is limited to the primary literature published since 1999. Literature prior to that year has been reviewed by others including Scheuhammer (1987), Thompson (1996), Wiener and Spry (1996), Wolfe et al. (1998), Boening (2000), Wiener et al. (2003), and Chan et al. (2003). However, select references prior to 1999 may be included if they were not discussed in previous reviews, for purposes of comparison of lowest observed effects concentrations, or to support conclusions, etc.

Introduction

Early studies on the effects of methylmercury to fish and wildlife focused on concentrations causing acute toxicity including overt neurotoxicity and mortality. In addition, early laboratory studies with fish frequently used aqueous concentrations of methylmercury that were 10,000 to 100,000 times greater than those observed in fresh and salt water systems. Diet is the major source of methylmercury exposure in fish (Rodgers 1994, Hall et al. 1997) and many recent studies now expose fish to relevant dietary concentrations of methylmercury. Fish were also considered as the main source of methylmercury to birds and mammals and piscivorous species were thought to be the primary species at risk from mercury toxicosis (Wiener et al 2003). However, recent evidence (e.g., Rimmer et al. 2005) indicates that insectivorous birds may also be subjected to high concentrations of methylmecury in the diet and be at risk.

Effects of Methylmercury in Birds

Although methylmercury in birds has been studied at length, especially with regard to methylmercury concentrations in various tissues (egg, muscle, liver, etc.), fewer studies have examined dietary methylmercury causing subchronic or chronic toxicity and most studies have focused on piscivorous species. The effects of environmentally relevant concentrations of dietary methylmercury on nestling great egrets (*Ardea albus*) were reported by Spalding et al. (2000a, b), Bouton et al. (1999), and Hoffman et al. (2005). Wild great egret nestlings were collected from the Everglades, Florida. Groups of five to six birds were each assigned to receive dietary doses equivalent to 0, 0.5 or 5.0 µg MeHg g^{-1} wet weight of fish for 14 weeks. Frederick et al. (1999) estimated that the mean total mercury concentration in the diet of wild great egrets in the Everglades ranged from 0.37 to 0.47 µg g^{-1} fish wet weight. Hence, the 0.5 µg Hg g^{-1} dietary treatment represented a realistic level of methylmercury exposure to the nestlings used in this experiment. Methylmercury was administered to birds in gelatin capsules just prior to feeding them fish. Due to an error, birds in the high dose group received 0.5 µg Hg g^{-1} food instead of 5.0 µg Hg g^{-1} for the first six weeks of the study, but then received 5.0 µg Hg g^{-1} food for the remainder of the study. The amount of mercury consumed daily varied from 48 to 135 µg kg^{-1} body weight day^{-1} due to variation in food consumption and growth of the birds. Spalding et al. (2000a) reported that birds receiving 5.0 µg Hg g^{-1} began to exhibit overt neurological symptoms of mercury toxicosis by week ten and four of six birds were euthanized before the end of the experiment when they could no longer stand. There was a significant reduction in food consumption and standardized weight between great egrets receiving the control diet and those receiving 5.0 µg Hg g^{-1} and 0.5 µg Hg g^{-1} at week 10 and week 11, respectively. At week 10, blood and feather mercury concentrations of great egrets receiving 0.5 µg Hg g^{-1} in the diet were 11.9 and 77 µg Hg g^{-1} fresh weight, respectively. Although the differences in weight gain between control and treated birds was small, the authors postulated that the magnitude of effects of methylmercury on growth may have been masked because food was provided *ad libitum* and suppression in growth of wild birds that must hunt for food may be greater than those observed during this experiment. Notably, suppression of appetite and growth coincided with the rapid increase in blood mercury after nine weeks when feathers stopped growing and suggested that deposition of mercury in rapidly growing feathers may delay toxicity until feather growth ceases. Hence, post-fledgling birds may be at elevated risk from methylmercury exposure.

In addition to suppressed growth, behavior of the 10- to 14-week old great egrets was altered at dietary concentrations of 0.5 µg Hg g^{-1} (Bouton et al.1999). Birds fed diets with 5.0 µg Hg g^{-1} became ataxic by week 12 and were euthanized. Differences between birds fed the control diet and those receiving 0.5 µg Hg g^{-1} occurred but were difficult to interpret with regards to their ecological relevance. Egrets receiving 0.5 µg Hg g^{-1} diet spent less time pecking, walking and flying but more time preening and in the shade than those receiving the control diet. They also were less likely to hunt or eat fish, but took less time to capture fish than control birds. Altered maintenance behavior, reduced activity, and suppressed feeding behavior may affect the survival of juvenile great egrets exposed to dietary methylmercury in the wild.

Blood and organs were obtained from the nestling great egrets to assess the effects of dietary methylmercury on immune function, organ histology (Spalding et al 2000b) and blood and organ biochemistry (Hoffman et al. 2005). The immune responses (antibody production) of the birds to killed eastern equine encephalitis virus and bovine serum albumin were tested during the latter half of the experiment. At the end of 14 weeks, all remaining birds were euthanized and histologic sections of various body tissues were examined. Packed cell volume declined significantly by week 5 in birds fed 0.5 µg Hg g^{-1} and was associated with an estimated total consumption of 2 mg Hg kg^{-1} body weight of the bird. Mean concentrations of mercury in the blood of these birds was 1.1 µg g^{-1}. Although quantitative information on anemia is not available for birds, Spalding et al. (2000b) indicated that a 20% reduction in packed cell volume would likely affect stamina. There was no significant difference between control and low dose birds in immune response; birds receiving the high dose diets did not survive to finish the immune tests. Histological examination of tissues, however, indicated marked differences among groups. Relative to control birds, egrets receiving low dietary methylmercury (0.5 µg g^{-1} diet) had altered tissues in the thymus, bursa, lung, and bone marrow. Egrets receiving high methylmercury (5.0 µg g^{-1} diet) had more than 30 severe histological abnormalities. Lesions and alterations were observed in tissue of the nervous system, immune system, endocrine and exocrine system, as well as lungs, kidneys, and liver. In contrast to this study, Sepulveda et al. (1999) were unable to detect any effects of methylmercury on the health and first-year survival of nestling and juvenile free-ranging great egrets that were orally dosed in the nest with 0.5 µg MeHg g^{-1} food every 2.5 days for 15 days for an estimated intake of 1.54 mg MeHg kg^{-1} bird. Control birds received an estimated 0.41 µg MeHg g^{-1} food. Spalding et al. (2000b) noted that the lower thresholds for sublethal endpoints (packed cell volume) measured in captive birds than in wild birds was probably due to the controlled nature of the laboratory studies. However, their review of the literature indicated that thresholds for more lethal endpoints (ataxia, immune suppression, histological abnormalities) were lower in wild birds possibly due to the synergism of multiple stressors.

Hoffman et al. (2005) measured plasma chemistries of the nestling great egrets at weeks 5, 7, 9, and 14 weeks of age during the experiment. The activities of select enzymes and non-enzyme chemistries were measured in the liver, kidney and brain at the end of the experiment. The activities of several enzymes associated with glutathione (GSH) metabolism and antioxidant activity were altered by dietary methylmercury. The activity of total GSH-peroxidase was lower in the plasma, liver, kidney and brain of egrets dosed with 5.0 µg Hg g^{-1} diet; activity of GSH S-transferase and oxidized glutathione reductase increased in the liver and activity of GSH S-transferase and of glucose-6-phosphate dehydrogenase increased in the kidney. In addition, birds dosed with 5.0 µg Hg g^{-1} diet had depressed concentrations of protein-bound thiols in the kidney, decreased total and protein-bound thiols in the liver and increased concentrations of glutathione in the brain and kidney. Concentrations of uric acid, inorganic phosphorus, albumin, and total protein decreased in the plasma from birds dosed with 5.0 µg Hg g^{-1} diet. In addition, birds receiving 0.5 µg Hg g^{-1} diet had increased activity of aspartate aminotransferase (linked to hepatotoxicity in birds) in plasma and concentrations of thiobarbituric acid reactive substances (an estimate of hepatic lipid peroxidation) in the liver. Chemical concentrations and enzyme activities that differed significantly among treatment groups were generally correlated with concentrations of total mercury in the respective organs. The biochemical indicators of oxidative

stress elicited by the high mercury diet were linked to overt neurological toxicity exhibited by the birds.

The reproductive capacity and health of aquatic species of birds was studied for 10 years (1997-2006) in the Carson River Basin of northwestern Nevada. This area is contaminated with high mercury concentrations due to past mining activities and is on the U.S. Environmental Protection Agency Natural Priorities List (aka "Superfund"). Henny et al. (2002) studied the effects of methylmercury on snowy egrets (*Egretta thula*), black-crowned night-herons (*Nycticorax nycticorax*) and double-crested cormorants (*Phalacrocorax auritas*) along the lower Carson River, Nevada. Measurement of mercury in the stomach contents indicated that young of each species were fed through fledging by their parents with diets that averaged 0.36 (snowy egrets), 0.43 (black-crowned night herons) and 1.18 (cormorants) µg Hg g^{-1}. Total mercury concentrations in the blood of fledglings of the three species ranged from 0.15 to 1.00 µg g^{-1} at the reference site and 1.20 to 7.26 µg g^{-1} along the lower Carson River. Although juvenile birds had lower total mercury concentrations in their organs than adult birds, they had greater evidence of sublethal toxicity to immune, detoxification, and nervous systems. Multiple endpoints provided evidence of oxidative stress in fledglings from the lower Carson River that were strongly correlated to mercury concentrations in the blood. For example, swollen spleens (i.e., increased mass) were positively correlated with liver mercury in cormorants, liver weight of young egrets was positively correlated with hepatic total mercury, and weight of young egret brains was negatively correlated with brain total mercury. There was also histological damage to several organs of young birds, including changes to the spleen, thymus, and bursa of cormorants, and to the nervous system of night herons and cormorants. As in the study of great egrets reported by Hoffman et al. (2005), increased mercury concentrations in young cormorants was associated with indicators of oxidative stress, including increased hepatic thiobarbituic acid-reactive substance, reduced activities of enzymes associated with metabolism of glutathione, an increase in the ratio of oxidized to reduced glutathione, and a decrease in reduced thiols. The effects of methylmercury exposure to post-fledging survival could not be determined because the study concluded about the time of fledging. However, Henny et al. (2002) speculated that additional dietary exposure of methylmecury to birds that remain in the watershed, coupled with increases in body burden due to completion of feather growth (a means of mercury sequestration), could be potentially toxic.

Hill et al. (2008) evaluated the interactive effect of drought and mercury on reproduction of black-crowned night-herons and snowy egrets along the lower Carson River from 1997 through 2006. Regardless of mercury concentration in the egg, reproductive success was lower in years of drought than during wetter years. However, during years of drought, nests of snowy egrets with eggs exceeding 0.80 µg THg g^{-1} all failed. Nests with eggs exceeding 0.80 µg THg g^{-1} during wetter years were not consistently associated with reproductive failure. Consequently, rather than the direct effects of methylmercury on the embryos, Henny and colleagues attributed the reduced reproductive success to the physiological and behavioral effects of methylmercury in the adults combined with the additional stress of obtaining food during drought. Few nests of black-crowned night-herons had eggs that exceeded 0.8 µg TH g^{-1} and there was not any evidence of altered reproductive success that could be attributed to methylmercury.

From 2002-2006, Hoffman and colleagues (2009) evaluated the relation between blood mercury, and organ biochemistry and histopathology in snowy egrets and black-crowned night-herons at mercury contaminated sites on Lahontan Reservoir and at a reference location. They examined more than 35 biochemical indicators and multiple histological endpoints in liver, bursa, spleen, kidney, thyroid, thymus, peripheral nerves and blood. During the five-year study, geometric mean total Hg concentrations (μg g^{-1} wet weight) in snowy egrets ranged: 1.5 - 4.8, blood; 2.4 - 3.1, liver; 1.8 - 2.5, kidneys; 1.7 - 2.4, brain; and 20.5 - 36.4, feathers. Exposure to mercury induced hepatic stress. There were significant positive correlations between total Hg in blood and plasma enzyme activity of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase-L, and total glutathione peroxidase. Moreover, the extent of liver inflammation was also positively correlated with blood and tissue mercury. Alterations in liver biochemistry indicated an induction of oxidative stress by mercury exposure. The concentration of total thiol and the activity of glutathione reductase decreased and the concentration of thiobarbituric acid-reactive subatances (TBARS; a measure of lipid peroxidation) increased with mercury in the liver. However, the concentration of oxidized glutathione (GSSG) and the ratio of GSSG to reduced glutathione were negatively correlated to mercury concentrations in the liver, which indicated compensatory responses by the snowy egrets to oxidative stress. There were positive correlations between blood total Hg and concentration of plasma uric acid and inorganic phosphate, which, in conjunction with kidney histology, suggested that mercury exposure caused renal stress. Alterations to glutathione biochemistry in the kidney and brain indicated that oxidative stress and compensatory adaptations to oxidative stress also occurred in these organs due to mercury exposure. The size of vacuoles and inflammation in peripheral nerves were correlated with blood and tissue Hg. Alterations to the immune system correlated with blood and tissue mercury included changes in the proportions of different types of white blood cells and lymphoid depletion in the bursa. A comparison of plasma biochemistries in snowy egrets from the contaminated and reference sites between wet and drought years found twice the number of variables affected during drought years than during wet years. As observed for reproductive success of snowy egrets (Hill et al. 2008), organ biochemistry and histology indicated that mercury exposure was more stressful during dry than wet years.

In the same study (Hoffman et al. 2009), collection of samples from black-crowned night-herons was not as intensive or extensive as for snowy egrets. Concentrations of total mercury in blood ranged from 1.6 to 7.4 μg g^{-1} wet weight. Organs were not collected for biochemical or histological analysis. Fewer correlations were found between blood total mercury and plasma biochemistry in night herons than in snowy egrets. Similar to snowy egrets, indicators of renal stress (plasma uric acid and inorganic phosphorus) and oxidative stress (glutathione-related enzyme activity) were correlated with blood total mercury. Although the data for night-herons were limited, they may not be as sensitive as snowy egrets to mercury exposure or the combined stress of mercury and drought.

Studies from other geographic locations have also demonstrated effects of methylmercury on biochemistry and cell structure. Shaw-Allen et al. (2005) investigated the effects of dietary methylmercury on protein metabolism in nestling snowy egrets by analysis of the composition of

isotopic nitrogen in the liver. They fed 14- to 60-day-old nestlings diets comprised of a mixture of 30% commercial bird of prey diet and 70% homogenized filets of largemouth bass (*Micropterus salmoides*) collected from reference and mercury-contaminated sites on the Savannah River, Georgia. Total mercury concentrations in the reference and high mercury-contaminated diets were 0.16 and 0.39 μg g^{-1} fresh weight. Doses of mercury received by the nestlings ranged from 0.04 ± 0.04 mg Hg kg^{-1} of body mass per day on day one to 0.11 ± 0.03 mg Hg kg^{-1} of body mass per day after the first week of the study. Although exposure data were not available for wild snowy egrets, the doses were realistic and within the range (0.025 to 0.47 mg kg^{-1} of body mass per day) modeled by Frederick et al. (1999) for nestling great egrets of the Florida Everglades. After 60 d, concentrations of mercury in the breast muscles of nestlings fed the reference and high-mercury contaminated diets were 1.12 ± 0.16 and 2.83 ± 0.35 μg g^{-1} fresh weight; mercury concentrations in the liver were 3.65 ± 0.66 μg g^{-1} fresh weight and 10.60 ± 2.50 μg g^{-1} fresh weight for nestlings fed the reference and contaminated diets, respectively. Although there were no differences in growth or in concentrations of metallothionein or glutathione in the liver of the two groups of birds, values of $\delta^{15}N$ in the whole and acid-soluble fraction of the liver were elevated in the nestlings fed the high-mercury contaminated diet. This indicated that dietary methylmercury increased rates of protein decomposition. Altered protein metabolism was a more sensitive indicator of mercury stress than the biochemical markers metallothionein and glutathione and was commensurate with altered behavior of nestlings fed the high-mercury diet, which spent more time awake, preening, and pecking than those fed the reference diet.

Elbert and Anderson (1998) studied adult western grebes (*Aechmophorus occidentalis*) and Clark's grebes (*Aechmophorus clarkii*) at three lakes in California, including Clear Lake. Mercury concentrations in liver, kidney, and breast muscle from 23 adult birds were measured. Blood chemistry from the birds was also assessed and compared to mercury concentrations in tissues in an attempt to define biomarkers of methylmercury exposure. Birds from Clear Lake had the highest mean concentrations of mercury in brain (0.28 μg g^{-1} wet weight), breast muscle (1.06 μg g^{-1}), kidney (2.06 μg g^{-1}) and liver (2.74 μg g^{-1}). Several hematological parameters were correlated with mercury concentrations in the tissue. Concentrations of potassium in the blood were negatively correlated with brain mercury ($R^2 = 0.73$). The percent of heterophils in the blood were positively correlated with mercury in the kidney ($R^2 = 0.38$) and the percent eosinophils was negatively correlated with mercury in the kidney ($R^2 = 0.35$). Alteration of the number of leucocytes in the blood suggested that immune function may be compromised by methylmercury.

Numerous studies have reported effects of methylmercury on wild loons (*Gavia immer*). For example, Nocera and Taylor (1998) examined the relation between blood mercury and behavior of loon adult and chicks in Kejimkujik National Park, Nova Scotia, Canada, an area with low loon reproductive success relative to other North American populations (Scheuhammer et al. 1998). The investigators collected time-activity budgets and quantified event behavior of 12 breeding pairs (24 adult and 16 loon chicks), 14 non-breeding pairs, and four pairs of failed-breeding loons. The time activity budgets of adults were not associated with blood mercury or with any lake morphometric or chemistry variables. However, the time-activity patterns of downy chicks were influenced by amount of methylmercury in their blood, which was measured

and estimated to range from 0.15 to 1.29 µg g^{-1}. There was a negative relation between blood mercury and the amount of time chicks spent brooding (riding back of adult) and a positive relation with amount of time spent preening. Backriding is a behavior that conserves energy, protects loon chicks from predators, and assists in thermoregulation. Increased preening also requires an additional expenditure of energy, but there was no observed increase in rates of feeding or begging for food by the chicks.

Olsen et al (2000) examined the relation between diving frequency of common loons and blood mercury concentrations. Loons on 9 territories on 6 lakes were observed repeatedly for 1 hour intervals from May to August 1999. Loon territories were categorized into one of four risk categories based on mercury concentrations in the blood (range of blood mercury in the four categories ranged from 0 to more than 4.0 µg g^{-1}). There was no indication by the authors if mercury concentrations were actually measured in the observed birds. There was a significant difference in the frequency of diving during foraging among loons in the four mercury categories and the frequency of diving during foraging was positively correlated with blood mercury. The authors speculated that increased diving frequency was due to inhibition of heme production by methylmercury that has been reported by other studies. Low heme production would result in a reduced oxygen carrying capacity of the blood and shorter dives. More frequent dives would be required to obtain food and compensate for a shorter dive duration. Consequently, it was suggested that methylmercury altered foraging behavior and may alter loon bioenergetics. However, the results of this study should be considered tentative. The sample size was very limited and repeated observations were made on the same bird. For example, there was only one territorial pair of birds in two of the four risk categories. Consequently, for purposes of statistical analysis, the observations on the same bird can not be considered to be independent of one another.

Kenow et al. (2007) examined the effects of dietary methylmercury on immune response of juvenile common loons (*Gavia immer*). Loon chicks were fed for 105 days with rainbow trout (*Oncorhynchus mykiss*) artificially contaminated with 0, 0.08, 0.4, or 1.2 µg Hg g^{-1} (as CH_3HgCl) wet weight of fish. Blood samples were drawn at 14, 35, 56, 77, and 98 d of age for total white blood cell and red blood cell counts and weekly for mercury analysis. The immunocompetence of loons 99 to 105 days old was evaluated with the phytohemagglutin skin test to assess T-lymphocyte function and the sheep red blood cell hemagglutination (SRBC) test to measure antibody-mediated immunity. The thymus, bursa, and spleen of each bird were also histologically examined. There was no detectable effect of dietary methylmercury on T-lymphocyte function. However, total antibody production (SRBC test) was suppressed 58% with dietary concentrations of 0.4 µg Hg g^{-1} wet weight fish, a level that the authors considered biologically significant. Moreover, antibody titers were suppressed approximately 54% on average in loons receiving 0.08 µg Hg/g wet weight fish, but limited sample size and, consequently low power, resulted in statistical non-significance. There was no evidence of methylmercury affecting the thymus or spleen, but the bursa exhibited lymphoid depletion that corresponded to the level of methylmercury exposure. For the purposes of comparison to other studies, Kenow et al. provided blood mercury concentrations at 5 weeks of age and they averaged 0.02 ± 0.003 µg g^{-1} in control loons, 0.12 ± 0.01 µg g^{-1} in loons receiving 0.08 µg Hg g^{-1} diet, and 0.66 ± 0.06 µg g^{-1} in loons receiving 0.4 µg Hg g^{-1} diet.

In addition to immune response, Kenow et al (2008) also examined the effects of methylmercury on glutathione metabolism, oxidative stress, and chromosomal damage in the plasma and organs of the loon chicks after 105 days of dietary exposure. Exposure to dietary methylmercury of 0.4 and 1.2 μg Hg g^{-1}, but not 0.8 μg Hg g^{-1}wet weight, resulted in oxidative stress and altered glutathione metabolism. Concentrations of oxidized glutathione, glutathione peroxidase and the ratio of oxidized to reduced glutathione increased in the brain. In the liver, concentrations of reduced glutathione increased and concentrations of glucose-6-phosphate dehydrogenase decreased. There was no evidence of chromosomal damage from examination of the blood and organs by flow cytometry. Based on Figure 1 in Kenow (2007, (2008), I estimate that blood mercury concentrations at the end of the study (when immunocompetence and oxidative stress was evaluated; day 105) were approximately 0.6 μg g^{-1} for birds receiving 0.08 μg Hg/g diet and 2.5 μg Hg g^{-1} for birds receiving 0.4 μg g^{-1}. Evers et al. (1998) reported that blood mercury concentrations ranged from 0.03 to 0.78 μg Hg g^{-1} for loon chicks across North America and maximum concentrations of 1.3 μg Hg g^{-1} blood have been recorded from loon chicks in Atlantic Canada (Burgess et al. 2005). Hence, levels of methylmercury exposure observed in loon chicks in the field have been demonstrated to cause suppression of antibody-mediated immunity and oxidative stress under experimental conditions.

Kenow et al (2003) had previously reported no effect on behavior (including ataxia), growth or survival of loon chicks fed 0.1, 0.5 or 1.5 μg Hg g^{-1}. The lack of response of the loon chicks to dietary methylmercury was attributed to rapid elimination of ingested mercury to growing feathers. Although they were unable to attribute changes in growth to dietary methylmercury, they did find significant differences associated with the source of the eggs. Chicks from eggs that originated from lakes with low pH were smaller than chicks from eggs obtained from lake with neutral pH. Low pH lakes typically have high methyl mercury concentrations in the fish diet of pre-nesting females. Kenow et al (2003) speculated that differences in growth of chicks between lake types was due to exposure of the embryos to methylmercury deposited by the female during oogenesis.

Burgess and Meyer (2008) evaluated the relation among lake pH, concentrations of mercury in blood of adult loons and in the carcasses of their fish prey, prey abundance, and loon reproduction among 120 lakes in the maritime provinces of New Brunswick and Nova Scotia and in the state of Wisconsin. As expected, mercury in fish increased with acidity. Mercury in the blood of adult loons also increased with lake acidity and with mercury in prey fish. However, in the maritime lakes, abundance of prey fish also increased with acidity. Consequently, if loon productivity was lower on low-pH lakes than on high-pH lakes it could be attributed to increased methylmercury exposure rather than reduced availability of prey. Analysis of the relation between mercury in female loon blood and loon productivity by quantile regression suggested that methylmercury imposes an upper limit on the number of chicks fledged per territorial pair of loons. Maximum observed loon productivity decreased 50% when blood mercury was 4.3 μg g^{-1} (corresponding to an average of 0.21 μg g^{-1} wet weight in carcasses of prey fish) and failed completely when blood mercury was 8.6 μg g^{-1} (corresponding to 0.41 μg g^{-1} wet weight in carcasses of prey fish). Using the calculated relation between blood mercury and loon productivity, and the geometric mean mercury concentrations in blood from adult loons in Nova Scotia (4.22 μg g^{-1}), New Brunswick (2.00 μg g^{-1}), and Wisconsin (1.57 μg g^{-1}), they estimated

that the mean reduction in potential loon productivity for each population was 49%, 23%, and 18%, respectively.

The effects of mercury on loon production was also investigated by Evers et al. (2008). They used a dataset of more than 5400 measurements of mercury concentrations in blood, feathers, and eggs of common loons collected from more than 1000 loon territories and 700 lakes over 18 years. This dataset was used to estimate the mercury concentrations in the blood of adult female loons from mercury in the blood of male and juveniles and in eggs to support comparisons across tissue type, age, and sex classes. Subsequently, behavior and reproductive success of common loons were studied from 1996 to 2005 on 80 lakes in New Hampshire and Maine with a total of 178 loon territories. Loon territories were placed into three risk categories based on calculated mercury concentrations in the blood of female adult loons: low risk (< 1.0 µg g^{-1}), moderate risk (1.0 to 3.0 µg g^{-1}) and high risk (>3.0 µg g^{-1}). Loons with more than 3.0 µg Hg g^{-1} blood spent less time on the nest than moderate- or low-risk loons and did not incubate the eggs for about 14% of the time they were observed. Low-risk loons left the eggs un-incubated only 1% of the time they were observed. Blood mercury was also correlated with increased lethargy of the adults and a decline in the amount of time spent foraging to obtain food for chicks. Reduction in egg incubation and in food provisioning for the chicks may have contributed to lowered chick survival. There was a significant negative correlation between chick fledging success and blood mercury concentration. Adult loons with blood mercury more than 3.0 µg g^{-1} produced 41% fewer fledged young than breeding loons with blood mercury levels less than 1.0 µg g^{-1}. Consequently, based on population matrix models that predict the minimum number of fledgling loons required to maintain a stable population, Evers et al. estimated that 16% of the adult loon populations they sampled had methylmercury concentrations that reduced reproductive success to levels below the minimum required to sustain loon populations.

Mitro et al. (2008) modeled the survival rates of common loons in New England and Wisconsin from band recovery data for 776 adult loons recorded from 1991 to 2001 and assessed the relation between mercury exposure (based on blood and feather mercury) and loon survival. They were unable to detect any relation between apparent survival and mercury exposure. However, in the dataset only 16% of the loons from New England and 10% of loons from Wisconsin had blood mercury concentrations that would put them at high risk of mercury toxicity (≥ 3.0 µg g^{-1} wet weight). Moreover, power analysis indicated that, with the sample size and sampling duration used in the study, detecting changes in survival of less than 3% were unlikely. Although this may seem to be a relatively small decline in survival, for long-lived species such as the common loon, small changes in survival can result in significant population declines. The authors cited studies for other long-lived species in which relatively small changes in survival can dramatically alter rates of population growth.

Scheuhammer et al. (2008) measured concentrations of total mercury, methylmercury and selenium and levels of neurochemical receptors in the brain of wild common loons and bald eagles (*Haliaeetus leucocephalus*). Concentration of total mercury in brains of eagles and loons were 0.3 to 23 µg g^{-1} dry weight and 0.2 to 68 µg g^{-1} dry weight. There were notable differences between the two species in the proportion of total mercury as methylmercury, as well as the bioaccumulation of selenium in the brain. At concentrations less than 4 µg Hg g^{-1} dry weight,

more than 80% of the total mercury was in the form of methylmercury in both species. However, at concentrations of total mercury greater than 4 μg g^{-1} dry weight, 78% of the total mercury was present as methylmercury in loons, but only 40% of the total mercury was methylmercury in bald eagles. This suggested that demethylation of methylmercury occurred in the brains of the eagle but not loons. In brains of eagles, but not loons, selenium increased with total mercury. In eagles, the Hg:Se molar ratio never exceeded 1, but the Hg:Se molar ratio was as high as 16 in loons. Selenium in Hg:Se molar ratios less than 1 are thought to be protective of mercury toxicosis (Ralston et al 2007, Raymond and Ralston 2009). For both species, there was a positive correlation between cholinergic receptor density (mACh) and total mercury and a negative correlation between N-methyl D-aspartate (NMDA) receptor density and total mercury. Similar relations between brain neurochemical receptors and mercury have been reported for mink (Basu et al. 2005, 2007b).

In addition to studies focusing on common loons, several investigations have examined the relation between methylmercury and reproduction of other species of birds. Heinz (1979) is the most frequently cited study on the effects of methylmercury on embryos of birds. In this study, Heinz fed three generations of mallard ducks (*Anas platyrhynchus*) either a control diet or a diet containing 0.5 μg Hg g^{-1} dry weight (as methylmercury dicyandiamide; This was equivalent to approximately 0.1 μg g^{-1} in a "natural succulent duck diet"). Various behavioral, physiological, and reproductive parameters were measured. Mercury concentrations in eggs from ducks fed the control and methylmercury-contaminated diet were less than 0.05 μg g^{-1} and 0.82 μg g^{-1} wet weight, respectively. Fewer eggs were viable and egg shells were slightly thinner from mallards fed the methylmercury-contaminated diet than those from mallards fed the control diet. Ducklings from parents fed the methylmercury diet were less responsive to maternal calls and more sensitive to avoidance calls than those from parents fed the control diet. Subsequently, Heinz and Hoffman (2003) reported the results of a laboratory study that was designed to estimate the lowest effect concentration and variability in response of mallard eggs to methylmercury toxicity. Eighty breeding pairs of mallards were fed untreated diets until the female had laid 15 eggs. Subsequently, each pair was fed a control diet or a diet containing 5, 10, or 20 μg g^{-1} methylmercury. Ducks were allowed to lay another 15 eggs and then switched back to the uncontaminated diets. Hatching success, embryo deformities, and neurological abnormalities were measured in ducklings from all control eggs and from even-numbered eggs of pairs receiving mercury-treated diets. Mercury concentrations associated with even-eggs that failed to hatch or that exhibited embryonic abnormalities were estimated as the mean of mercury concentrations of the odd-number eggs laid immediately prior to and after the even-numbered egg. Estimated mercury concentrations associated with eggs that produced ducklings that exhibited abnormal neurological behavior associated with mercury toxicity (e.g., loss of balance, staggering) ranged 2.3 μg g^{-1} to 30 μg g^{-1} egg wet weight. No ducklings exhibited neurological symptoms that were hatched from eggs from adult pairs fed control diets or from eggs hatched prior to the adult pairs being switched to mercury-treated diets. Estimated mercury concentrations in the eggs associated with embryo deformities ranged from 0.93 μg g^{-1} to18 μg g^{-1}. Embryo deformities include bill, wing and leg abnormalities, exencephaly, and extra toes. Estimated mercury concentrations of eggs that failed to hatch ranged from 0.74 μg g^{-1} to 38 μg g^{-1}. Based on neurological symptoms, the authors concluded that concentrations of methylmercury in mallard eggs greater than 2 μg g^{-1} wet weight were harmful to sensitive embryos. They

concluded that, based on this and the previous study by Heinz (1979), there was evidence "from deformities and mortalities, although not conclusive, that wet-weight mercury concentrations of about 1 µg g^{-1} or perhaps a little below 1 µg g^{-1} can harm the most sensitive mallard embryos." The results from this study supported the threshold of 0.8 µg g^{-1} generated by Heinz (1979) and, moreover, highlighted the great variation in sensitivity in toxicosis exhibited by embryos of this species—some embryos survived with more than 30 µg Hg g^{-1} wet weight.

However, in contrast to the studies by Heinz (1979) and Heinz and Hoffman (2003), Heinz et al. (2009) reported an apparent case of hormesis in mallards fed dietary methylmercury. In this study, adult mallards were fed for 26 to 70 days with a control diet or with a diet containing 0.5 µg g^{-1} methylmercury. The hatching success of eggs and 6-day survival of ducklings from adult mallards were evaluated. The eggs from ducks fed methylmercury-contaminated diets had greater survival (72%) than those from ducks fed the control diet (58%). The survival of ducklings through day 6 was the same for controls and for mallards fed 0.5 µg g^{-1}. Although there was no difference between dietary treatments in the weight of ducklings at hatching, ducklings from adults fed the methylmercury-contaminated diet were approximately 8% larger at day 6 than ducklings from parents fed a control diet. Mercury concentrations in the eggs from ducks fed the contaminated diet were 0.81 µg g^{-1}—similar to those in the mallard eggs of the study of Heinz (1979) in which deleterious effects were observed. Heinz (2003) found that the lowest concentration of mercury concentration in the eggs associated with harm was 1 µg g^{-1}. Heinz et al (2009) suggested that differences in the results from these studies may be due to differences in sources and strains of mallards, different forms of organic mercury used in the two studies (methylmercury chloride in Heinz et al. 2009, methylmercury dicyandiamide in Heinz 1979), and the poor hatching success (58%) of control mallards in the current study. The authors indicated that the most plausible explanation for the results from all three studies is that dietary concentrations of 0.5 µg Hg g^{-1} results in mercury concentrations in the egg that are very close to the threshold concentration. They suggest that the lowest observed effect level in the egg is 0.8 to 1.0 Hg g^{-1} wet weight.

Schwarzbach et al. (2006) evaluated the reproductive success of the endangered subspecies California clapper rail (*Rallus longirostris obsoletus*) in six tidal marshes in the San Francisco Bay over four breeding seasons. Numbers of nests were counted and clutch size, and hatchability of eggs were measured. Loss of nests and eggs were attributed to tidal flooding, predation and potential contaminants. Predation on eggs, especially by Norway rats (*Rattus norvegicus*), significantly reduced hatching success of eggs but tidal flooding contributed minimally to egg loss. Mercury was the only contaminant measured in significant amounts (0.18 to 2.51 µg g^{-1} egg wet weight) and common to eggs from all marshes; methylmercury accounted for an average 95% of the total mercury in the eggs. Although mercury concentrations was measured only in eggs that failed to hatch and randomly selected eggs from the nest sites were not assessed for mercury, the authors concluded that mercury was the contaminant most likely to cause egg failure. In one marsh from the south bay, 50% of all eggs that failed to hatch had more than 0.5 µg g^{-1} and 25% were above the threshold of 0.8 µg g^{-1} established for mallard eggs by Heinz (1979).

The survival of chicks of American avocet (*Recurvirostra americana*) and blacknecked stilt (*Himantopus mexicanus*) was investigated by Ackerman et al. (2008). They used radio telemetry to determine the fate of 158 avocet and 79 stilt chicks from approximately day two to day 30 post hatch. Nests from multiple locations in San Francisco Bay were sampled. One chick from a nest was tagged and down feathers were collected for mercury analysis. Down feathers contained 0.40 to 44.3 μg g^{-1} fresh weight mercury. Akaike Information Criteria was used to select from multiple models predicting the fate of the birds. Mercury concentration in birds (as represented by mercury in feathers) had negligible effect on survival (< 3%). In contrast, mean mercury concentration in dead chicks (5.7 μg g^{-1} wet weight) was greater than that (4.2 μg g^{-1} fresh weight) of randomly sampled live chicks. Ackerman et al. speculated that it was possible mercury had an effect on hatching success of the eggs and on nestling success from hatch to day two when birds were captured from radio telemetry. Unlike laboratory studies, this field study (as others) was unable to detect a marked effect of methylmecury on birds.

Heath and Frederick (2005) examined hormone concentrations, nesting effort, and mercury concentrations in feathers of wild female and male adult white ibis (*Eudocimus albus*) during the breeding season in the Florida Everglades. Estradiol concentrations of pre-breeding female birds were negatively correlated with mercury in feathers, which ranged from 0.33 to17 μg g^{-1}; there was no relation between mercury in feathers and concentrations of testosterone, progesterone, and corticosterone. There was a significant positive correlation between feather mercury, which ranged from 0.69 to 20 μg g^{-1} fresh weight and testosterone concentrations in nesting male birds. An analysis of numbers of nests and mercury concentrations in feathers of chicks over seven years indicated that nesting effort was negatively correlated with mercury exposure. It was unknown if low nest numbers were due to nest abandonment or decreased nesting attempts. The results of this study suggest that low exposure levels of methylmercury may alter steroid hormones important in reproduction.

Adams and co-workers (2009) also examined the effects of methylmercury exposure on hormone levels in white ibises. They fed four groups of juvenile, post-fledgling white ibises with diets containing 0 (control), 0.05 (low mercury), 0.1 (medium mercury), or 0.3 (high mercury) μg MeHg g^{-1}wet weight. These mercury concentrations bracket those found in the diets of wild ibises in the Everglades. Concentrations of estradiol, testosterone, and corticosterone were measured in fecal samples collected from individual birds for more than six months. Based on figure 1 in Adams et al. (2009), I estimate that mean concentrations of total mercury in scapular feathers after approximately six months of exposure to the diets were about 0.1 μg g^{-1} (control diet), 8 μg g^{-1} (low mercury diet), 13 μg g^{-1} (medium mercury diet) and 24 μg g^{-1} (high mercury diet). Variation in endocrine expression over time, between sexes, and among dietary treatments were modeled with repeated measures general linear models. Candidate models were selected based on Akaike Information Criterion. Estradiol and corticosterone concentrations in the feces were altered by methylmercury; there was less evidence to suggest any effect of dietary methylmercury on testosterone. The effects varied over time and were highly non-linear. Estradiol in ibises exposed to medium methylmercury deviated markedly from those in the other treatments. Corticosterone concentrations in control and medium-methylmercury groups were similar; low- and high-exposure groups were significantly different from other treatments. Although Heath and Frederick (2005) reported effects of methylmercury on hormones of adults

in adults, a reduced effect on juvenile birds were observed in this study. In conjunction with an assessement of the effects of methylmercury exposure on hormones, the effects of dietary methylmercury on the foraging behavior of the ibises (n=168) feeding on fish in habitats with different levels of complexity was evaluated after approximately 3.5 months (Adams et al. 2008). Methylmercury affected foraging behavior, but not in a dose-dependent manner. Control and high mercury groups were least efficient in foraging and were not statistically different from each other. There was no effect of dietary methylmercury on improvement in foraging efficiency over time and, hence, no effect on learning. The authors attributed the non-linear effect of mercury to hormesis or possible confounding factors, such as the individual composition of the groups and the location of the cages. Because ibises are highly social, all birds given the same dietary treatment were kept in the same cage. Therefore, there was only a single replicate for each treatment group. Concentrations of mercury in the birds after dietary exposure to methylmercury were not measured.

Contrary to previous assumptions, non-piscivorous birds may also be susceptible to mercury toxicity through the bioaccumulation of large amounts of mercury from their diet, particularly if they consume aquatic invertebrates or adult insects with aquatic larval stages from contaminated systems. However, even passerines consuming diets comprised of terrestrial insects may have elevated risks from methylmercury. For example, a recent report by Rimmer et al. (2005) documented mean mercury concentrations of 0.08 to 0.38 μg g^{-1} in the blood of Bicknell's thrush (*Catharus bicknelli*) at 21 breeding sites in montane areas of northeast North America. Little is known of the effects of methylmercury to passerines. However, there is growing evidence that their reproductive success may also be compromised by methylmercury. Custer et al. (2007) placed nest boxes for cavity-nesting birds at four locations along the Carson River near Dayton, Nevada, where mercury contamination from metal mining and processing in the 1800s has resulted in elevated concentrations of mercury in sediment and water. Eggs and livers of nestling tree swallows (*Tachycineta bicolor*), house wrens (*Troglodytes aedon*) and western bluebirds (*Sialia mexicana*) were collected for mercury determination and egg hatching success was measured in each of the monitored nests. Twelve wren nests, 10 tree swallow nests, and two bluebird nests were observed. Slightly fewer numbers of eggs and nestlings were measured for total mercury; small sample size precluded the use of data from bluebird nests Geometric mean mercury concentrations in eggs (range of means: 2.86 – 9.23 μg g^{-1} dry weight) and livers (range of means: 2.86-4.21 μg g^{-1} dry weight) at contaminated sights were 15 to 17 times greater than those from house wrens from South Dakota and Wyoming (Custer et al. 2002a) and 25 to 50 times greater than those in tree swallow from Colorado, Minnesota and Wyoming (Custer et al 2001, 2002b, 2006). Concentrations in eggs but not livers were similar to those reported by Henny et al. (2002) in eggs of piscivorous wading birds from the Lahontan Reservoir, which is on the Carson River downstream of Dayton, Nevada, and is also impacted by mercury contamination. They were also similar to those in eggs from great egrets from the Florida Everglades (2.4 μg g^{-1} dry weight; Rumbold et al. 2001). Mercury concentrations of the stomach contents of the cavity-nesting birds (range of means 0.7 – 3.1 μg g^{-1} dry weight) were similar to those in fish prey of nestling great egrets (range 0.2 – 3.6 μg g^{-1} dry weight) in the Florida Everglades (Frederick et al. 1999). Hence, exposure and egg concentrations of insectivorous birds can be similar to those of piscivorous birds consuming mercury-contaminated fish. On average 74% of tree swallow and 70% of wren eggs hatched, which was below the national

average of 85% for tree swallows and 88 to 93% for house wrens. Although mean mercury concentrations in eggs that did not hatch (7.88 µg g^{-1} dry weight) were twice those in eggs that hatched (3.86 µg g^{-1} dry weight), they were not significantly different, probably due to low power associated with small sample size. Relative to other species, mercury concentrations in the eggs of tree swallows and house wrens that did not hatch were equal to or exceeded those reported to cause embryo mortality (e.g., mallard, Heinz and Hoffman 2003).

Brasso and Cristol (2008) evaluated reproductive success of tree swallows by erecting more than 250 nest boxes along mercury-contaminated and reference sections of the Shenendoah River, Virginia in 2005 and 2006. Blood mercury in adult female swallows from the contaminated site (3.56 ± 2.41 µg g^{-1} wet weight) averaged more than 20-fold those in birds from the reference sites (0.17 ± 0.15 µg g^{-1} wet weight) and reflected the high concentration of mercury in their insect prey (0.97 ± 1.11 µg THg g^{-1} dry weight at contaminated site; 0.04 ± 0.04 µg THg g^{-1} dry weight at reference sites). Female tree swallows from the contaminated site that were first-time breeders produced significantly fewer fledglings than those from reference sites. On average they produced two fewer fledglings than older, experienced breeders at contaminated or reference sites and approximately one fewer fledgling than first-time breeders at reference sites. There was no significant difference in the number of fledglings produced by older birds between contaminated and reference sites. Fewer fledglings were produced in the contaminated area by inexperienced female swallows, possible due to under-provisioning of eggs. Eggs of first-time breeders were significantly smaller than those of older, experienced birds at the contaminated site, but not at reference sites. Evers et al. (2003) also reported that the egg volume of common loons (*Gavia immer*) were smaller from birds with high concentrations of methylmercury. Brasso and Cristol suggested that the combination of inexperience and mercury contamination resulted in smaller eggs and decreased offspring survival. However, they did not measure levels of co-occurring contaminants (e.g., PCBs) that may also have been present, correlated with mercury, and cause reproductive effects in birds.

Longcore et al. (2007a) examined fledging success and mercury concentrations in food and various tissues of tree swallows from Acadia National Park, Maine and at an U.S. Environmental Protection Agency Superfund site in Ayer, Massachusetts. Mean total mercury concentrations in eggs were 0.259 to 0.493 (range 0.097 to 1.313) µg g^{-1} among sites in Maine and 0.574 to 0.615 (range 0.231 to 1.075) µg g^{-1} from nests in Massachusetts. There was no detectable effect of mercury on egg hatching rate or fledging success. But, in contrast to the study by Brasso and Cristol (2008), all but two nests were from females that were "after-second-year birds" and, hence, were experienced breeders. Although hatching success ranged 88.9% to 100% among locations, five eggs failed to hatch from 4 of the 11 clutches in which egg mercury exceeded 0.8 µg g^{-1}—the lethal threshold established by Heinz (1979) for mallard embryos. Longcore et al (2007b) also examined the relation between mercury concentration and linear growth rate in weight and asymptotic mean weight of nestling tree swallows in Acadia National Park and at Orono, Maine. Mercury concentrations ranged approximately 1.05 – 2.8 µg methylmercury g^{-1} dry weight (from Figure 1 of manuscript) in feathers and ranged approximately 0.015 – 70 µg total mercury g^{-1} wet weight (from Figure 3 of manuscript) in the carcass. There was an inverse relation between mercury in feathers and linear growth rate of nestlings over days 2 – 10 of life, but not with the mean asymptotic weight of the nestlings.

Wada and co-workers (2009) evaluated adrenocortical responses and the concentrations of the thyroid hormones T3 and T4 in the plasma of tree swallow nestlings at reference sites and from mercury-contaminated sites along the South River, VA. Nestlings were sampled on posthatch days 3-6 (early age class; period of eye opening), 7-12 (middle age class; period of most rapid mass gain), and 13-17 (late age class; body mass plateaus prior to fledging). Because of the small size of the birds, the adrenocortical response, blood Hg, and thyroid hormones were measured from different nestlings within the nest. Previous research with other bird species indicates that blood Hg varies minimally among nestlings within a nest (Sepulvada et al. 1999, Weech et al. 2006, DesGranges et al. 1998) and, therefore, blood mercury from one nestling was used to characterize Hg exposure for the entire brood. Mean total blood Hg in nestlings from reference sites was 0.017 ± 0.001 μg g^{-1} (methylmercury constituted more than 95% of total blood mercury). Mean total blood Hg in nestlings from contaminated sites (0.354± 0.022 μg g^{-1}) was 20 times greater than that in birds at reference sites. Plasma corticosterone levels were affected by blood mercury and the age of the birds. Stress-induced corticosterone levels in the early and middle age classes of nestlings were greater in birds from contaminated sites than from reference sites, but lower in late age-class nestlings from contaminated sites than from reference sites. Levels of plasma T4 in early age-class nestlings were similar between contaminated and reference sites, but were depressed in late age-class birds from the contaminated sites. Plasma T3 levels were 15-40% lower in the three age classes of nestling from the contaminated sites than in those from the reference sites. Concentrations of Hg in the blood associated with deleterious effects on adrenocortical response and thyroid hormones were less than the sublethal threshold concentration of 0.400 μg g^{-1} suggested from other studies (preliminary lowest observed adverse effect concentration (LOAEC) in loon chicks from Scheuhammer et al. 2007). Previous analysis of insects in the diet of tree swallows at contaminated sites indicate insects fed to nestlings averaged 0.970 μg Hg g^{-1} dry weight (0.330 μg Hg g^{-1} wet weight). The authors estimated that day-5, -10, and -15 nestlings at contaminated sites ingested 235, 179, and 238 μg Hg kg^{-1} body weight.

Hawley et al. (2009) also examined the humoral and cell-mediated immune response of tree swallows along Hg-contaminated and uncontaminated sections of the South River, VA. When challenged with a phytohaemagglutinin (PHA) assay, female tree swallows nesting at mercury-contaminated sites exhibited a significantly lower cell-mediated immune response than tree swallows at uncontaminated sites. Mean ± SE (range) concentrations of blood total mercury at the contaminated site were 3.25 ± 0.37 (0.80-7.36) μg g^{-1} in 2006 and 2.51 ± 0.16 (1.12-4.52) μg g^{-1} in 2007. However, for birds at the contaminated sites, blood mercury was not correlated with the extent of the immune response. The authors speculated that this may be due to (1) indirect effects of dietary methylmercury on immunity by changes in individual health or condition or (2) mercury concentrations at the contaminated site were all above a threshold for effects to be detected. They were unable to detect any differences between birds from the contaminated and uncontaminated sites in the humoral immune response.

Francschini et al. (2009) assessed Hg corticosterone concentrations in 11- to 13-day old nestling tree swallows from mercury contaminated sites along the Sudbury River, MA (Nyanza Superfund Site), from reference sites along the Charles River (Medfield, MA), Sudbury Reservoir (Marlborough, MA), and Delaney Wildlife Management Area (Stow, MA) and from

two salt marshes in southeastern Maine with low and high Hg contamination. Blood was collected from the birds immediately after capture (representative of baseline conditions) and again 30 minutes post-capture (representative of stress induction) for measurement of methylmercury (as total Hg) and corticosterone. In addition, tail feathers and the first laid egg in the clutch were also collected for analysis of Hg. At sites with high concentrations of Hg in Massachusetts, there was a negative relation between baseline corticosterone concentrations and blood Hg in adult and nestling tree swallows. Hg in the blood of adult birds ranged from 0.12 to 1.0 µg g^{-1} wet weight. There was also a negative relation between baseline corticosterone concentrations and egg Hg; egg Hg concentrations ranged from 0.030 to 0.252 µg g^{-1} wet weight. There was no relation between mercury in the blood or egg and stress-induced corticoserone. Because corticosterone is a component of the stress response and is involved in glucose metabolism, tissue repair, immune response, growth, and reproduction (among other functions), the authors suggested that a reduction in baseline corticosterone was likely to significantly affect health and fitness of the birds even though stress-induced levels of corticosterone were not affected.

Terrestrial raptors may also be at risk from methylmercury contaminated diets. Albers et al. (2007) exposed sixty captive breeding pairs of American kestrels (*Falco sparverius*) to 0.02 (control), 0.7, 2.0, 3.3, 4.6, or 5.9 µg Hg g^{-1} diet dry weight to assess effects on reproduction. Based on methylmercury concentrations in potential prey (small birds and mammals, bats, bullfrog, tadpoles, aquatic insects) of the American kestrel, the authors indicated that 0.7 µg Hg g^{-1} represented "high background exposure", 2.0 µg Hg g^{-1} represented "intermediate (some extraordinary sources) exposure" and 3.2 µg Hg g^{-1} and greater represented "high (considerable extraordinary sources) exposure." Kestrel breeding pairs were fed artificially contaminated diets for a minimum of one month and allowed to breed. Dietary methylmercury concentrations as low as 0.7 µg g^{-1} altered the reproductive success of the kestrels. The number of eggs laid and hatched, and the incubation performance of the adults was significantly lower in those groups of birds receiving diets with 4.6 or 5.9 µg g^{-1}. Fledging success declined significantly at dietary concentrations of 0.7 µg g^{-1} with total fledging failure at 4.6 µg g^{-1}. Mean concentration of total Hg in the eggs of adult kestrels fed control diet or diets with 0.7 or 4.6 µg Hg g^{-1} were 0.4, 9.8, and 63.3 µg g^{-1} dry weight, respectively. Based on Bayesian regression, an estimated 24% decrease in fledged young per pair of kestrels consuming 0.7 µg Hg g^{-1} dry weight (0.3 µg g^{-1} wet weight) may result in failure of those populations.

Bennett et al. (2009) fed American kestrels for 59 days with diets contaminated with 0, 1.24, 2.65, and 5.02 µg g^{-1} wet weight organomercury (89% methylmercury, 11% ethylmercury). Groups of five birds were fed each diet. All kestrels fed the diet with 5.02 µg g^{-1} wet weight organomercury died in less than 50 days. Birds fed diets with 2.65 µg g^{-1} wet weight organomercury exhibited neurotoxicity (uncoordinated movements, etc.) by 42 days of exposure. Concentrations of total mercury (µg g^{-1} wet weight) in the various tissues of these birds after 59 days of exposure were: blood, 21.3±2.6; kidney, 22.3±2.3; liver, 26.5±4.1 and breast muscle, 10.2±0.46. No overt signs of toxicity were exhibited by birds fed control diets or diets with 1.24 µg g^{-1} wet weight organomercury. Histological examination of the liver, kidney, spinal cord, and brain indicated that abnormalities occurred only in the brain tissue of birds fed the diet with 5.02 µg g^{-1} wet weight organomercury. Although, birds fed diets with 2.65 µg g^{-1} wet weight

organomercury showed signs of neurotoxicty, no histological abnormalities were observed in the brain tissue. Two pairs of birds at each dietary exposure (except the 5 µg g^{-1} wet weight organomercury, which caused mortality) were allowed to breed. All pairs bred, but the fertility and survival of offspring varied and results were inconclusive due to the small number of birds used in the study. The results of this study should be considered with discretion as more than 10% of the organic mercury in the experimental diets of these birds was ethylmercury. Ethylmercury is not typically formed as part of the biogeochemical cycling of mercury (Porcella 1994) and nearly 100% of the total mercury in fish and marine mammals is in the form of methylmercury (Bloom 1992, Wagemann et al. 1997). Moreover, the mechanism(s) of ethylmercury toxicity are unknown and differences between ethylmercury and methylmercury in toxicokinetics and toxicodynamics suggest that data on methylmercury are not appropriate for risk assessment of ethylmercury (Clarkson and Magos 2006).

Based on a brief review of the literature, Seewagen (2009) noted several gaps in the information on effects of methylmercury on birds and recommended priorities for future research. He suggested that more studies are needed on terrestrial, insectivorous and passerine species and noted that information on birds in tropical, forested ecosystems are especially sparse. Moreover, he suggested that studies that examine the effects of methylmercury on migration are also needed.

Toxicity Thresholds for Birds

In a recent review of the effects of methylmercury on wildlife and fish, Scheuhammer et al. (2007) suggested toxicity thresholds for birds and mammals. Based on their review, as well as data presented in this review, the following concentrations of total mercury (presumed to be mostly methylmercury) in various tissues have been associated with decreased reproductive success in various species of birds: diet of adults, 0.1 µg g^{-1} wet weight (Heinz 1989) to 0.21 µg g^{-1} wet weight (Burgess and Meyer 2008); eggs, 1.0 µg g^{-1} wet weight (Heinz and Hoffman 2003); feathers of adults, 9.85 µg g^{-1} dry weight (Heinz 1979); breast muscle of adult, 0.776 µg g^{-1} wet weight (Heinz 1979); blood of adults, 3.0 µg g^{-1} wet weight (Evers et al. 2008). These values represent the lowest observed effects concentration (LOEC) from various studies; the threshold concentrations are probably somewhat lower. Tissue concentrations of mercury associated with sublethal effects, including alterations in behavior, changes in blood biochemistry and biomarkers of oxidative stress, and immune suppression may be found in the ACCESS database accompanying this report.

The question of inter-species sensitivity to methylmercury is a key information gap that hampers extrapolation of toxicological test results from one species to another (this comment is true for mammals and fish, as well). For example, tree swallows and sparrows could be substantially more sensitive than fish-eating birds. Although data on methylmercury toxicity to eggs of mallards (*Anas platyrhynchos*) is used as the toxic threshold for other species of wild birds, mallard eggs are less sensitive to methylmercury than those of several other species. Heinz et al. (2009) evaluated the relative sensitivity of embryos of 26 species of birds to methylmercury. Using a standardized protocol, eggs were injected with a geometric progression of

methylmercury doses and then incubated. Survival through 90% of the incubation period was used as the endpoint in calculating the median lethal concentration (LC50) of methylmercury to each species. Based on the LC50 values and dose-response curves, the species were ranked according to their sensitivity to the injected methylmercury. Three categories of species were defined. Species exhibiting low sensitivity to methylmercury (LC50 values ≥ 1.0 µg Hg g^{-1}wet weight) included: mallard, hooded merganser (*Lophodytes cucullatus*), lesser scaup (*Aythya affinis*), Canada goose (*Branta canadensis*), double-crested cormorant (*Phalacrocorax auritus*), and laughing gull (*Larus atricilla*). Species categorized as having moderate or medium sensitivity to methylmercury (LC50s > 0.25 µg Hg g^{-1}but less than 1.0 µg µg Hg g^{-1}wet weight) included: clapper rail (*Rallus longirostris*), sandhill crane (*Grus canadensis*), ring-necked pheasant (*Phasianus colchicus*), chicken (*Gallus gallus*), common grackle (*Quiscalus quiscalus*), tree swallow, herring gull (*Larus argentatus*), common tern (*Sterna hirundo*), royal tern (*Sterna maxima*), Caspian tern (*Sterna caspia*), great egret, brown pelican (*Pelecanus occidentalis*), and anhinga (*Anhinga anhinga*). High-sensitivity species (LC50s < 0.25 µg Hg g^{-1}wet weight) were the American kestrel, osprey (*Pandion haliatus*), white ibis, snowy egret (*Egretta thula*), and tri-colored heron (*Egretta tricolor*). For chickens, mallards, and ring-necked pheasants, they also compared the toxicity of methylmercury injected in embryos to that of maternally-transferred methylmercury from published reports in the literature. Injected methylmercury was three to four times as toxic as maternally transferred methylmercury, but the relative ranking of sensitivity to methylmercury of the three species remained the same regardless of the method of mercury acquisition. The relative ranking of the sensitivity of the embryos of wild birds to maternally deposited methylmercury should, therefore, also be similar to that derived from the injection study. They stressed that, because of differences in experimental design and other factors, it would be inappropriate to attempt to calculate a single correction factor to convert the lethal dose of injected methylmercury to a lethal dose of maternally deposited mercury. However, they did conclude that using threshold values of embryotoxicity of 0.5µg Hg g^{-1}wet weight (Fimreite 1971 -- *as cited in Heinz et al. 2009*) or 0.8-1.0 µg Hg g^{-1}wet weight (Heinz 1979, Heinz and Hoffman 2003) obtained from controlled breeding studies may not be protective of many species of wild birds.

Effects of Methylmercury in Mammals

Studies on the effects of methylmercury on mammalian wildlife published since 1999 have primarily examined the effects on wild and captive mink (*Mustela vison)* and river otters (*Lontra canadensis*). Dansereau et al. (1999) assessed the effects of dietary methylmercury on reproductive performance of two generation (G1, G2) of mink fed fish naturally contaminated with methylmercury (0.1, 0.5, 1.0 µg g^{-1}) for 300 (G2) to 400 (G1) days prior to mating. Dietary concentrations of 1.0 µg Hg g^{-1} were lethal to G1 and G2 female mink after 90 to 330 days of exposure; mortality of female mink in the other groups was unrelated to methylmercury exposure. However, the animals were not examined for histopathological or biochemical effects related to mercury exposure. The length of the period of gestation in each generation was not different among dietary treatments. The number of kits born per female was significantly greater in G1 females fed 0.1 µg Hg g^{-1} than in G1 females fed 0.5 or 1.0 µg Hg g^{-1}; there was no significant difference in whelping performance among G2 females. Although there was a

general linear decrease in the percentage of kits whelped with increasing methylmercury exposure, the trend was not statistically significant probably due to the overall low performance of mink fed the 0.1 µg Hg g^{-1} diets relative to that reported from other studies of mink with diets uncontaminated with methylmercury.

Klenavic et al. (2008) found that mercury concentrations in liver, brain and fur were correlated in wild mink and river otters. Moreover, they found that mercury concentrations in the fur of mink infected by the parasite, *Dioctophyma renale*, were significantly higher than those in uninfected mink at five locations in Ontario, Quebec, and Nova Scotia.

A series of studies by Nils Basu and colleagues have correlated neurochemical changes in the brains of wild mink and river otter with mercury. Subsequent laboratory studies corroborated the role of methylmercury in altered brain function and demonstrated that these changes can occur at environmentally relevant dietary concentrations lower than those causing overt mortality. Basu et al. (2005a) measured concentrations of mercury (total and methyl), density of cholinergic (mACh) and dopaminergic (D2) receptors and receptor ligand affinity in brains of 48 wild mink. Mean (range) concentrations of mercury in brain of mink from three regions of Canada where animals were collected were 1.2 to 5.7 µg THg g^{-1} dry weight (range of individual animals was 0.27 to 18.84 µg g^{-1}) and 1.1 to 4.9 µg MeHg g^{-1} (range of individual animals was 0.26 to 13.52 µg g^{-1}). There was a significant positive correlation between mercury (total and methyl) and mACh receptor density and ligand affinity. The authors hypothesized that, because methylmercury has been documented to suppress acetylcholine levels in laboratory mice and rats, that up-regulation of mACh receptors maybe an adaptive response to decreased amounts of acetylcholine. There was a significant negative relation between total mercury and D2 receptor density and ligand affinity; D2 receptor density but not ligand affinity was negatively correlated with methylmercury. In contrast to decreases in acetylcholine, methylmercury has been shown to increase release of dopamine (see references in Basu et al. 2005a). Hence, the authors suggested that down-regulation may be an adaptation to prevent hyperstimulation of the dopaminergic system. Because cholinergic and dopaminergic systems are essential in cognition, sensory, and motor function, they suggest that changes in the density of these receptors and ligand affinities may be useful in predicting contaminant effects on neurobehavior.

To demonstrate that neurochemical changes observed in wild mink were due to methylmercury, Basu et al. (2006) fed groups of 12 juvenile male mink for 89 days with fish diets contaminated with 0 (control) 0.1, 0.5, 1, or 2 µg MeHg g^{-1} wet weight . There was no effect of dietary methylmercury on food consumption, body weight, or brain weight. Moreover, there was no significant effect on brain choline acetyltransferase, acetylcholine, or choline transporter. However, dietary methylmercury did increase the density of muscarinic cholinergic receptors in all regions of the brain measured (occipital cortex, cerebellum, brain stem, basal ganglia) and dietary concentrations that elicited effects varied with brain region. For examples, the density of muscarinic cholinergic receptors in basal ganglia and brain stem of mink fed diets with 0.5 µg Hg g^{-1} were 67.5% and 64.4% greater than those from mink fed control diets. The relationship between mACh receptor and methylmercury concentration in the brains in captive and wild mink was determined by linear regression. There was no significant difference in the slope of the regression plots, which corroborates the conclusion that alterations in mACh receptors observed

in wild mink was associated with methylmercury exposure and that environmentally relevant concentrations of methylmercury can affect cholinergic neurotransmission in piscivorous wildlife. Radioligand binding assays suggested that, at dietary methylmercury equal to or greater than 0.5 μg MeHg g^{-1}, mercury preferentially affected mACh receptors M1 and M2 more in the occipital cortex than those in the brain stem (Basu et al. 2008). The M1 receptors are dominant cholinergic receptor in the occipital cortex and are important in visual processing (vision loss is characteristic of methylmercury poisoning) and are also important in central nervous system functions, especially learning and memory. Dietary concentrations of 1 μg MeHg g^{-1} resulted in alterations to the γ-aminoburyic acid (GABA) signaling system in the brain (Basu et al. 2010). Levels of GABA(A) receptors and activity of GABA-transaminase enzymes were markedly reduced. GABA is the main inhibitory transmitter in the mammalian brain (may account for approximately 50% of the synapses in some portions of the brain) and alteration of GABAergic signaling will likely have negative effects on the health of individual animals.

Basu et al. (2007b) examined the relationship between the N-methyl D-aspartate (NMDA) receptor levels and brain mercury in wild-trapped mink and in captive mink fed methlmercury contaminated diets for 3 months in the laboratory. Mercury inhibits glutamate uptake by astrocytes and glutamate levels subsequently increase in synapses (see references in Basu et al. 2007b). Glutamate is the primary excitatory neurotransmitter in vertebrate nervous systems and an agonist of the NMDA receptor. In this study, mercury (total and methymercury) and NMDA receptor levels were measured in brains from twenty wild mink. In addition, groups of nine juvenile male mink were fed fish diets contaminated with nominal concentration of 0, 0.1, 0.5, 1, or 2 μg MeHg g^{-1} wet weight for 89 days; mercury concentrations and NMDA receptor levels were measured. Mean (range) concentrations of methylmercury and total mercury in trapped mink were 5.3 (0.99-13.52) μg g^{-1} dry weight and 6.4 (1.05 – 18.84) μg g^{-1} dry weight, respectively. There was a negative correlation between ligand binding to the NMDA receptor channel and log methlmercury and log total mercury, indicating that receptor levels declined with increasing brain mercury. In the laboratory feeding study, there was a dose-dependent decrease in NMDA receptor levels. The level of effect varied with brain region and dietary exposure, but dietary concentrations of methylmercury as low as 0.1 μg g^{-1} significantly reduced receptor levels in the cerebellum of the mink. Alteration of NMDA receptor levels in mice has been associated with motor and cognitive impairments (see references in Basu et al. 2007b).

In a study similar to that conducted with wild mink, Basu et al. (2005b) examined the relation between mACh and dopamine-2 receptor density and ligand affinity relative to mercury in wild river otters. Brains of 66 wild river otters were obtained from Ontario and Nova Scotia, Canada and concentrations of total and methylmercury were determined. Receptor binding assays for the mACh and dopamine-2 receptors were completed to calculate ligand affinity and receptor density. Concentrations of methylmercury and total mercury in the brain ranged 0 to 10.65 μg g^{-1} dry weight and 0.09 to 14.31 μg g^{-1} dry weight, respectively. In the cerebral cortex, there was a negative correlation between mercury and mACh receptor density and ligand affinity and also a negative correlation between mercury and dopamine-2 receptor density but not ligand affinity. Results for of assays for the cholinergic system were opposite those obtained from mink (Basu et al. 2005a), where there was a positive correlation between mercury and mACh receptor density and ligand affinity. Differences between species may be due to the mink's ability to metabolize

organic mercury or accumulate selenium, an Hg antagonist. Dopaminergic and cholinergic receptors are associated with important behaviors thermoregulation, cognition, learning, memory, and motor function (see references in Basu et al 2005b). Results of this study also suggest that environmentally relevant concentrations of methylmercury may have neurolotoxic effects at levels below those associated with overt behavioral neurotoxicity.

Basu et al. (2007a) examined the effects of methylmercury on acetylcholine levels in wild river otters. They obtained brains from 34 wild river otters from southern Ontario. A significant negative correlation was observed between cholinesterase activity and total mercury and methylmercury, but not inorganic mercury in the cerebral cortex of the brain. Similarly, there was a significant negative correlation between monoamine oxidase activity and total mercury and methylmerury, but not inorganic mercury in the brain.

To compare the sensitivity of neurochemical receptors among species to methylmercury, Basu et al. (2005c) tested the effects of inorganic and organic mercury on mACh receptor ligand binding in the cerebral cortex and cerebellar tissues of brains from humans, mink, river otters, rats, and mice. Samples of brain tissue were obtained from five to six animals of each species. Saturation binding curves of tritium-labeled quinuclidinyl benzilate for each control and mercury-treated samples were determined to calculate ligand affinity and mACh receptor density. For all species, binding activity was higher in cerebral cortex than in the cerebellum; inhibition of mACh receptor binding by mercury was greater in the cerebellum than cerebral cortex. Inorganic mercury was a stronger inhibitor of mACh receptors than methylmercury. After exposure to inorganic or organic mercury, there was a significant difference among species in mACh receptor inhibition. Species sensitivity, ranked from most to least sensitive, were river otter>rat>mink>mouse>human.

Toxicity Thresholds for Mammals

Based on the reviews by Scheuhammer et al. (2007), Wiener et al. (2003), Wolfe et al. (1998), and data provided in this review, the following tissue concentrations are associated with mortality or reduced reproductive success in mammals (primarily mink): diet, 0.5 $\mu g\ g^{-1}$ wet weight (Dansereau et al. 1999); brain, 5 $\mu g\ g^{-1}$ wet weight (Wolfe et al. 1998). Altered brain chemistry is associated with dietary mercury concentrations of 0.1 $\mu g\ g^{-1}$ wet weight (Basu et al. 2007b). Threshold concentrations are likely lower than these values.

Effects of Methylmercury in Fish

In comparison to mammals and birds, relatively little is known of the toxicological significance to fish of environmentally relevant exposures to methylmercury (Wiener and Spry 1996, Wiener et al. 2003). Instead of using realistic dietary concentrations of methylmercury, many laboratory studies have typically exposed fish to aqueous concentrations of methylmercury that are 10^4 – 10^5 –fold greater than those in natural waters (Wiener and Spry 1996). Diet, not water, is the

primary source of methylmercury exposure in wild fish (Rodgers 1994, Hall et al. 1997). Although aqueous exposure to methylmercury rapidly results in bioaccumulation of methylmercury and distribution to tissues throughout the fish's body (Niimi and Kissoon 1994), it is unknown how uptake via the gills alters mode of action and toxicity. Hence, the results from studies in which fish were exposed to high concentrations of aqueous methlmercury should probably be interpreted conservatively. Greater certainty on fish tissue concentrations associated with toxic effects may be obtained from studies in which fish bioaccumulated methylmercury from dietary sources. The effects of methylmercury on physiological growth and survival occur generally only at high tissue mercury concentrations (6 - 20 μg g^{-1} wet weight in muscle; Wiener and Spry 1996) that have been observed primarily in fish from highly contaminated environments, such as Minamata Bay, Japan and Clay Lake in the English-Wabigoon River system, Ontario, Canada. For example, Houck and Cech (2004) fed juvenile Sacramento blackfish (*Orthodon microlepidotus*) diets contaminated with 0.21 (control), 0.52 (low), 22.1 (medium), 55.5 (high) μg methylmercury g^{-1} dry weight. After 70 days, there was a significant decrease in growth of fish fed medium- and high-mercury diets. There was no difference in condition factor (a metric that describes the physical condition of a fish based on the relation between mass and length) or metabolic rates of fish among treatments. Long-term survival (>70 d) of fish exposed to 55.5 μg methylmercury g^{-1} diet was decreased. Approximate mercury concentrations (from figure 7 of manuscript) at day 70 in muscle tissue of fish receiving the medium- and high-mercury diets were 15 and 33 μg methylmercury g^{-1} wet weight. In contrast, however, Cizdziel et al. (2003) reported that the extent of emaciation of striped bass (*Roccus saxitilis*) from Lake Mead, USA, was related to total mercury concentrations in the skeletal muscle and other tissues. The condition factor of the striped bass was negatively correlated with total mercury in the axial muscle, which ranged from 0.063 to 1.058 μg g^{-1} wet weight. Similarly, Drevnick et al. (2008) reported that the condition factor of northern pike from inland lakes of Isle Royale, Michigan, USA were inversely related with total mercury concentrations in the liver. Mercury concentrations in the skin-on fillet of these fish ranged from 0.069 to 0.622 μg g^{-1} wet weight. In the lower Columbia River, the condition factor of white sturgeon (*Acipenser transmontanus*) was inversely related to mercury concentrations in the gonad and liver (but not muscle; Webb et al. 2006). In the upper Columbia River, the condition factor of walleye (*Sander vitreus*) was also inversely related to mercury in the skeletal muscle, which ranged 0.11-0.44 μg g^{-1} wet weight.

Several laboratory studies with multiple investigators have demonstrated altered histology, biochemistry, fish behavior, and reproduction at dietary and fish tissue concentrations more typical of those found in flooded, low alkalinity, or other mercury-sensitive habitats subject to non-point source contamination with mercury. Oliveira Ribeiro et al. (2002) fed juvenile arctic charr (*Salvelinus alpinus*) a single bolus of methylmercury with an average dietary dose of 0.26 μg methylmercury g^{-1} body weight and then fed the fish uncontaminated food every 3 days until the end of experiment. Groups of fish were sampled at day 0.5, 4, 8, 18, and 30. Samples of liver were obtained for histopathology and autoradiography and compared with those from fish force-fed uncontaminated food. Within 12 hours of ingestion of methylmercury, lipid reserves within hepatocyte cytoplasm were greatly reduced and there was increased heterochromatin in cell nuclei. Cytoplasmic membranes were barely visible, which suggested high metabolic activity within the liver. After 18 d, massive cell necrosis was evident with proliferation of

connective tissue and infiltration of phagocytes around damaged areas. Recovery of liver tissue was apparent by day 30, though small necrotic sites were still found.

In a coordinated set of investigations, groups of adult *Hoplias malabaricus* (a neotropical species of fish) were fed prey fish (*Astyanax* sp.) every 5 days for 70 days. Prey fish were either injected with distilled water or with methylmercury chloride equivalent to a dose of 0.075 μg methylmercury g^{-1} *Hoplias malabaricus*. Mercury concentrations in the prey fish were not reported. Methylmercury exposure increased the number of red blood cells, leukoctyes, neutrophils, and monocytes. In addition hemoglobin concentration, hematocrit, and mean corpuscular volume were greater in fish receiving the methylmercury contaminated diets (Oliveira Ribeiro et al. 2006). Dietary methylmercury also suppressed plasma δ-aminolevulinic acid dehydratase and muscle cholinergic activity, and damaged liver cells of *Hoplias malbaricus* (Alves Costa et al 2007). In addition, Mela et al (2007) reported that the livers of the fish receiving methylmercury-treated prey had pre-necrotic lesions (leukocyte infiltration), necrotic areas, an increased number of melano-macrophage centers, abnormal cells, phagocytic areas and intercellular spaces. The head kidneys of treated fish had increased number of leukocytes, dead and atypical cells, and necrotic regions as well as increased number of melano-macrophage centers indicated increase of phagaocytic activity. The authors suggested that methylmecury caused oxidative stress which contributed to the development of necrotic tissues. The concentration of mercury in the liver of fish receiving the control and treated diet were 0.601 and 1.069 μg g^{-1} wet weight. Mercury concentrations in the muscle of the fish receiving the control and treated diet were 0.67 and 1.45 μg g^{-1} wet weight.

Deng et al. (2008) evaluated the main and interactive effects of dietary methylmercury and seleno-methionine on larval Sacramento splittail (*Pogonichthys macrolepidotus*, Cyprinidae). In a factorial design, larvae were fed diets with 0.01 (control), 0.13, 4.7, 11.7 μg MeHg g^{-1} dry weight and 0.64 (control), 8.2, and 35.0 μg Se g^{-1} dry weight. Behavior, growth, mortality and gill and liver histopathology of the fish was evaluated after 4 weeks. Dietary Hg enhanced Se bioaccumulation, but dietary Se inhibited Hg bioaccumulation. There was no difference in growth or mortality of fish among the different Hg and Se treatments. However, fish fed diets with 4.7 μg MeHg g^{-1} or greater (with control Se) exhibited abnormal swimming behavior beginning in the second week of the experiment. The larvae were hyperactive, had dart-like movements, or swam in a circular motion. The whole-body concentration of Hg in fish fed the 4.7 μg MeHg g^{-1} diet for four weeks was approximately 2.5 μg Hg g^{-1} wet weight (estimated from figure 1; concentrations in fish measured only at end of 4-week experiment). There were severe anomalies in the gills, liver glycogen depletion, and kidney tubular dilation in fish fed the 11.7 μg MeHg g^{-1} diet (whole body concentrations of Hg in fish were approximately 6 μg Hg g^{-1} after 4 weeks). Liver glycogen depletion and kidney tubular dilation were also observed in larvae fed the diet with 11.7 μg MeHg + 35 ug Se g^{-1} (whole body concentrations of Hg in fish were approximately 4.7 μg Hg g^{-1} wet weight after 4 weeks).

Liao et al. (2006) reported decreased acetylcholinesterase activity in liver, brain, gill, and muscle of medaka (*Oryzias latipes*) exposed to 2.5 μg MeHg $L^{-1}$for 8 days. The sum of the acetylcholinesterase activity in the four tissues of exposed fish was approximately 46% of that of

control fish. Mean concentrations of mercury in the muscle, brain, gonad, and liver of the fish were 0.03, 0.18, 0.51, and 3.63 μg g^{-1} wet weight.

Several additional laboratory and field studies have demonstrated that sublethal methylmercury exposure causes oxidative stress in various fish tissues through the formation of radical oxygen species and lipid peroxidation. Berntssen et al (2003) fed juvenile (parr) Atlantic salmon (*Salmo salar*) for four months with diets containing 0.03 (control), 4.35 (medium) or 8.48 (high) μg MeH g^{-1}dry weight. They observed no effect of dietary methylmercury on growth or condition of the fish. However, the medium methylmercury exposure induced a defense response in the brain, indicated by a significant increase of the activity of super oxide dimutase (SOD). The brains of fish fed high dietary methylmercury had suppressed SOD and glutathione peroxidase (GSH-Px) activity, but a marked increased in thiobarbituric acid reactive substances (TBARS), a product of lipid peroxidation. Brain monoamine oxidase (MAO) activity was also significantly inhibited by the high methylmercury diet, which suggests disruption of the monaminergic system. The post-feeding activity of these fish was also suppressed, but they did not assess the behavior of fish receiving the medium methylmercury diet. Brains of fish exposed to the medium and high methylmercury diet had tissue concentrations of 1.16 and 0.68 μg g^{-1} wet weight, respectively, and exhibited severe vacuolization and cell necrosis. Livers of fish receiving the high methylmercury diet also had increased activity of SOD and GSH-Px. The authors concluded that the medium methylmercury diet induced redox defenses, but at high dietary methylmercury defenses were overcome and injury occurred.

Larose et al. (2008) examined the relation between methylmercury and the glutathione system in walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) from four boreal lakes in Canada. In the lake with the highest mean concentration of methylmercury in the walleye, the size of the livers (relative to total body mass) was inversely related to liver methylmercury concentration. The activity of glutathione reductase and glutathione *S*-transferase (GST) were positively correlated with liver size (and by inference, negatively correlated with methylmercury concentrations). In the lake with the highest mean concentration of liver methylmercury in yellow perch, the activity of GST and selenium-dependent glutathione peroxidase were negatively related with methylmercury in the liver. They concluded that environmentally relevant concentrations of methylmercury altered cell metabolism and physiology of these two species of fish. Although they reported data on methylmercury concentrations in the livers of the fish, they stated that total mercury concentrations in the muscle of the fish ranged from 0.3 to 0.79 μg g^{-1} wet weight.

Navarro et al. (2009) examined the relation between total mercury and glutathione-s-transferase, oxidized glutathione, and reduced glutathione in dorsal muscle, liver and kidney of common carp (Cyprinus carpio) in 4 reservoirs of the Ebro River (Spain), which was contaminated by mercury from a chlor-alkali plant. Reduced glutathione in muscle, liver, and kidney were significantly and highly correlated with concentrations of total mercury in each tissue. However, there was no relation between total mercury in each tissue and oxidized glutathione or activity of glutathione-S-transferase. Concentrations of GSH in liver of fish from the most contaminated site were significantly greater than those in liver from fish from the reference site. Median (range)

concentrations in liver, kidney, and muscle of carp from most contaminated site were 1.27 (0.43-2.20), 3.84 (0.65-6.92), and 0.96 (0.52-1.09) µg/g wet weight.

Evidence of oxidative stress due to methylmercury exposure was also evident in various species of fish from several field studies. Schwindt et al. (2008) measured mercury concentrations in salomonids (lake trout, *Salvelinus namaycus;* brook trout, *S. fontinalis;* cutthroat trout, *Oncorhynchus clarki;* rainbow trout, *O. mykiss*) obtained from 14 lakes in six U.S. National Parks or Preserves. Increases in the number of macrophage aggregates in the kidney and spleen was associated with elevated concentrations of methylmercury. Macrophage aggregates are groupings of macrophages within tissues that collect components of damaged cells, including those damage by oxidation and lipid peroxidation. Although, macrophage aggregates may also increase with age, Schwindt and colleagues were able to demonstrate that mercury affects macrophage aggregates independent of fish age. Moreover, although other pesticides and chemical compounds were measured in these fish, mercury alone explained greater than one-third of the variability in macrophage aggregates in the spleen of brook trout. Based on Figure 3 in the manuscript, estimated whole body concentrations of total mercury in brook trout ranged from approximately 0.025 to 0.285 µg g^{-1} wet weight.

Drevnick et al. (2008) examined mercury concentrations and evidence of liver toxicity in northern pike (*Esox lucius*) from eight inland lakes of Isle Royale, Michigan. Isle Royale is a relatively remote and pristine U.S. National Park in Lake Superior. Quantitative analysis of liver pigment of the northern pike demonstrated that liver color (absorbance at 400 nm) was positively related to concentrations of total mercury in the liver (range 0.048 – 3.074 µg g^{-1} wet weight). Liver mercury was positively related to total mercury in the axial fillet (range 0.069 – 0.622 µg g^{-1} wet weight). Lipofuscin was subsequently identified as the pigment responsible for altered liver color. Lipofuscin is formed as a result of lipid peroxidation of membranous organelles and is also a pigment frequently found in macrophage aggregates. As previously mentioned, condition factor of the northern pike was also negatively related to total mercury concentrations in the liver. Hence, there was good evidence of detrimental effects of methylmercury to the health of the fish. Similarly, Raldúa et al. (2007) found higher concentrations of mercury and greater prevalence of liver pathologies, including macrophage aggregates and lipofuscin, in fish downstream of a chlor-alkali plant than in those fish collected upstream of the factory. Total mercury concentrations in the liver of the fish downstream of the factory ranged 0.321-1.962 µg g^{-1} wet weight.

Induction of redox defenses by methylmercury exposure may also be detected by alterations in gene transcription. Gonzalez et al. (2005) fed adult male zebrafish (*Danio rerio*) for 63 days with diets contaminated with 0.08 (control), 5, or 13.5 µg MeHg g^{-1} dry weight. Concentrations of 5 µg Hg g^{-1} diet dry weight approximate those of some invertivorous and piscivorous fish inhabiting natural lakes and reservoirs in North and South America (Gonzalez et al. 2005, Hammerschmidt et al. 2002). The expression of genes associated with mitochondrial metabolism, apoptosis, and oxidative stress (including SOD) was up-regulated in the skeletal muscle and liver after 21 to 63 days of dietary exposure to 5 µg MeHg g^{-1} dry weight. Genes associated with DNA repair were down-regulated in the skeletal muscle. After 63 days, mercury concentrations in the skeletal muscle of these fish was 15 µg methylmercury g^{-1} dry weight.

Expression of the 13 genes evaluated in the study did not change in the brains of the exposed fish, although brain mercury exceeded 20 μg MeHg g^{-1} dry weight. Two notable results of this study indicated by the authors were the lack of effect on gene expression in the brain, particularly those associated with anti-oxidant defense, and the effects of dietary methylmercury on gene expression in the skeletal muscle. The lack of effect on gene expression in the brain may explain, in part, the neurotoxicity of methylmercury. Skeletal muscle had previously been considered solely as a storage reservoir for methylmercury, minimally affected by methylmercury. A subsequent study by Oliveira Ribeiro et al. (2008) demonstrated that dietary methylmercury disrupted muscle fibers and mitochondria of skeletal muscles in zebrafish. Zebrafish were fed 13.5 μg methylmercury g^{-1} dry weight for 63 days and axial muscle tissue of the fish were then examined for histological alterations in architecture. The mitochondria and space between muscle fibers of skeletal muscle from fish fed methylmercury were smaller than those fed a control diet. The authors concluded that, based on this study and the previous work of Gonzalez et al. (2005), methylmercury damaged red muscle tissue and modification of the mitochondria probably decreased ATP production by the muscle cells.

Cambier et al. (2009) also fed male zebrafish diets contaminated with 13.5 μg MeHg g^{-1} dry weight. After 25 and 49 days, fish were euthanized and the skeletal muscles were harvested. Total mercury was measured and mitochondrial bioenergetics was evaluated. Mean ± standard error total mercury in muscle was 25.4 ± 5.0 μg g^{-1} dry weight and 35.5 ± 4.0 μg g^{-1} dry weight after 25 and 49 days. For the same time intervals, concentrations of total mercury in the muscle of control fish were 1.77 ± 1.14 and 1.93 ± 0.55 μg g^{-1} dry weight. Mitochondrial respiration was disrupted. State 3 respiration (consumption of oxygen during phosphorylation of ADP) was inhibited by 32% (day 25) to 67% (day 49); state 4 respiration (post ATP synthesis, basal respiration) was not significantly affected. Concomitant measurement of ATP production was inhibited in contaminated muscle fibers. Similarity in the activity of ATP synthase between control and contaminated muscle, inhibition of cytochrome c oxidase (COX) activity, and reductions in cox gene expression suggested that methylmercury decoupled mitochondrial oxidative phosphorylation in the skeletal muscle.

Cambier et al.(2010) evaluated gene expression in muscle tissue of zebrafish exposed to methylmercury (13.5 ppm diet) for 25 days. Sixty genes were up-regulated and 15 genes were downregulated by more than 2-fold relative to control fish. Based on gene response, they proposed a mechanism of methylmercury toxicity wherein methylmercury causes disruption of the metabolism of the mitochondria and endoplasmic reticulum, which results in the generation of oxidative and endoplasmic reticulum stresses. These stresses, in turn, impair lipids, proteins, and DNA.

In a field study of cutthroat trout (*Salmo clarkii*) from high-altitude lakes of Olympic, Mt. Ranier, and North Cascades National Parks, Moran et al. (2007) compared the levels of gene expression in livers of fish from lakes with low (Skymo Lake) and high (Wilcox Lake) levels of mercury contamination. Mercury concentrations in cutthroat were approximately 0.016 μg g^{-1} wet weight in Skymo Lake and 0.055 μg g^{-1} wet weight in Wilcox Lake (estimated from figure 3 of manuscript). Of the 147 genes evaluated, expression of 45 genes were significantly different between the two lakes. Genes up-regulated in fish from the more contaminated Wilcox Lake

included those associated with stress response (including glutathione peroxidase), intermediary metabolism, and the endocrine response. Although low levels of organic contaminants (e.g., DDE, PCB) were also present in the fish, differences in transcriptional responses of fish were attributed to differences in mercury contamination between the two lakes.

The most notable effect of sublethal dietary methylmercury in fish is altered reproductive success. Both manipulative laboratory studies as well as correlative field studies have reported effects of methylmercury on some aspect of fish reproduction (see reviews by Crump and Trudeau 2009, Tan et al. 2009). Friedmann et al. (1996) fed juvenile walleye (*Sander vitreus*) diets contaminated with 0.04 (control), 0.137 (low-mercury diet) or 0.987 (high-mercury diet) µg methylmercury g^{-1} wet weight for six months. Subsequent mercury concentrations (whole body minus viscera) were 0.06 (control), 0.254 (low-mercury diet), and 2.37 (high-mercury diet) µg g^{-1} wet weight. Dietary methylmercury suppressed fish growth and gonadal development in male fish. Histological examination of the testes indicated that mercury resulted in multifocal cell atrophy and hypertrophy of cells adjacent to atrophied cells.

Hammerschmidt et al. (2002) also reported suppressed gonadal development, egg production, and spawning in juvenile fathead minnows (*Pimephales promelas)* fed methylmercury-contaminated diets until sexual maturity. Mating pairs of fish fed diets containing 0.88 µg Hg g^{-1} dry weight and with mean carcass concentrations of 0.71 µg Hg g^{-1} (males) to 0.86 µg Hg g^{-1} wet weight (females) experienced a 39% reduction in spawning success relative to control fish. Dietary methylmercury also delayed spawning, reduced the size of gonads and reproductive effort of female fish, and reduced the instantaneous rate of reproduction. In a subsequent study, similar dietary and carcass concentrations of methylmercury were associated with suppressed plasma estradiol and testosterone as well as reduced reproduction in fathead minnows (Drevnick and Sandheinrich 2003). Male fathead minnows fed control diets had plasma testosterone concentrations 20% and 106% greater than those fed diets with 0.87 (low) and 3.93 µg (medium) Hg g^{-1} dry weight. control female fish had estradiol concentrations 149% and 402% greater than those fed low and medium methylmercury. Reproductive behavior (Sandheinrich and Miller 2006), the number of apoptotic follicular cells (Drevnick et al. 2006) and expression of genes related to endocrine function (Klaper et al. 2006) of fathead minnows was also altered by these levels of dietary methylmercury.

Klaper et al. (2008) evaluated changes in gene expression of fathead minnows acutely or chronically exposed to methylmercury. In acute exposures, male fathead minnows were injected with 2.0 µg MeHg g^{-1} body weight and euthanized after 96 hours. In chronic exposures, male fathead minnows were fed diets with 3.93 µg MeHg g^{-1} dry weight for 600 d. Mercury concentrations were not measured in the fish. Differences between control and methylmercury-treated fish in the expression of genes in the liver and gonads were evaluated. Acute exposure to methylmercury resulted in more than 650 genes in the liver and 212 genes in the gonads being up- or down-regulated by more than two-fold relative to those in control fish. Chronic exposure resulted in changes in expression of 267 genes in liver and 155 genes in the gonad. Most notable was the up-regulation of several genes associated with apoptosis, which was cited as a cause of reproductive failure by Drevnick et al. (2006).

Altered concentrations of sex hormones have subsequently been correlated with carcass concentrations of mercury in wild fish. Webb et al. (2006) assessed the relation between mercury and reproductive potential of white sturgeon (*Acipenser transmontanus*) in the lower Columbia River. Mean mercury concentrations in muscle, liver, and gonads of the 57 fish were 0.170, 0.140 and 0.027 μg Hg g^{-1} wet weight, respectively. There was a significant negative correlation between concentrations of plasma testosterone, 11-ketotestosterone and muscle mercury of male fish. Similarly, there was a negative correlation between concentrations of plasma estradiol and liver mercury of female fish. No male fish with muscle, liver, and gonad total mercury above 187, 93, and 74 ng g^{-1} had concentrations of plasma testosterone greater than 4 ng mL^{-1} and the authors suggested that these mercury concentrations in the respective tissues represented possible threshold concentrations of mercury affecting steroidogenesis. There was a significant negative correlation between condition factor, relative weight, and gonad and liver mercury. The gonadal somatic index of immature male fish was inversely related to gonad mercury, which may have been due to suppression of sex steroid production. In contrast to the previously cited studies, Friedmann et al. (2002) found a positive relation between plasma 11-ketotestosterone and mercury in male largemouth bass (*Micropterus salmoides*) from three lakes in New Jersey. Concentrations of 11-ketotestosterone were positively correlated with mercury concentrations of the axial fillet of the fish; there was no significant relation between serum testosterone and mercury in the muscle of the fish. Mean mercury concentrations in the axial fillets of fish from the three lakes ranged from 0.30 to 5.42 μg Hg g^{-1} wet weight.

Although maternal transfer of dietary methylmercury bioaccumulated during oogenesis is the primary mechanism of methylmercury exposure to fish embryos (Hammerschmidt and Sandheinrich 2005), there is some evidence that aqueous exposure to low concentrations of methylmercury may also affect fish embryos. Latif et al. (2001) exposed eggs of walleye to 0.1 – 8 ng methylmercury L^{-1}. Embryonic heart rate declined with environmentally relevant concentrations of methylmercury (0.11 to 3.48 ng L^{-1}); there was no significant relationship between embryonic heart rate, female size, female age, or concentrations of methylmercury within the egg. Although there was no differences in the hatching success of embyos with different tissue concentrations of methylmercury, hatching success of embryos declined with increasing concentrations of aqueous methylmercury. It is difficult to determine lowest observed effects concentration (LOEC) as hatching success varied between years and among females. However, I estimate that the no observed effects concentration (NOEC) was approximately 1.45 ng L^{-1} and the LOEC was approximately 4 ng L^{-1} (from figure 4 of manuscript). There was no effect of aqueous or egg methylmercury on larval deformities or growth.

Matta et al. (2001) fed mummichog (*Fundulus heteroclitus*) for at least 7 weeks on diets contaminated with 0.07, 0.5, 1.9, 5.6, and 54 μg MeHg g^{-1} dry weight. The fish were allowed to reproduce and their embryos (F1) were raised to sexual maturity on uncontaminated diets and allowed to reproduce as well. The eggs they produced (F2) were also examined. Dietary concentrations of 1.9 μg Hg g^{-1} dry weight resulted in carcass concentrations of 0.47 μg Hg g^{-1} wet weight and increased mortality of male fish, possibly through altered behavior. Fish fed 54 μg Hg g^{-1} dry weight had carcass concentrations of 12 μg Hg g^{-1} wet weight and produced embryos (F1) with 0.63 μg Hg g^{-1} wet weight. These F1 individuals had reduced fertilization success. The F1 fish of adults fed 5.6 μg Hg g^{-1} dry weight and with carcass concentrations of

1.1 µg Hg g^{-1} wet weight had altered sex ratios; mercury concentrations in the F1 individuals as embryos was 0.01 µg g^{-1} wet weight.

Whether from maternal transfer or aqueous exposure, the effects of elevated concentrations of methylmercury within the fish embryo may be delayed until later in life. Fjeld et al. (1998) exposed eggs of grayling (*Thymallus thymallus*) to relatively high concentrations of aqueous methylmercury (0.16 to 20 µg L^{-1}) for 10 days to elevate methylmercury concentrations within the embryo. Concentrations of mercury of newly hatched fry ranged 0.09 to 3.8 µg g^{-1} wet weight. After hatching, the fry were subsequently raised in clean water and without supplemental methylmercury in the water or diet. Three years later, they examined the feeding behavior of the fish. Juvenile fish that had mercury concentrations of 0.27 µg g^{-1} or more had impaired feeding efficiencies and reduced competitive abilities relative to control fish (with 0.01 µg g^{-1} as yolk-fry).

Weber et al. (2008) place zebrafish embryos in graded concentrations of aqueous methylmercury for 24 hours post fertilization and then transferred the embryos into clean medium, allowed them to hatch, and then to mature to adults under uncontaminated conditions. As adults, the vision of the zebrafish was tested by examining their response to a rotating black bar. Zebrafish embryos exposed to 0.01 µM aqueous methylmercury for 24 h had total mercury concentrations of approximately 2 µg Hg g^{-1} wet weight (estimated from Figure 2 in the manuscript). Relative to control fish that were not exposed to methylmercury as embryos, they exhibited a significantly suppressed visual response as adults.

Smith et al. (2009) exposed embryo zebrafish from 2 to 24 h post fertilization with aqueous concentrations of methylmercury of 0.00, 0.01, 0.03, 0.06, 0.10, and 0.30 µM or with aqueous concentrations of selanomethionine of 0.0 to 0.30 µM or with combinations of methylmercury and selanomethionine. Mercury and selenium concentrations were measured in the eggs after exposure (24 hours post fertilization). The ability of the fish at 4 months of age to learn a spatial alternation task was evaluated. Exposure to 0.01 to 0.06 µM MeHg (corresponding to 1.35 to 5 µg Hg g^{-1} wet weight in the eggs) delayed learning; fish exposed to 0.10 or 0.30 µM as embryos (corresponding to 8.81 to 17.1 µg Hg g^{-1} wet weight in the eggs) were unable to learn the task. A histological examination of the fishes' telencephalon (portion of the brain associated with learning in fish) showed a marked reduction in cell body density with increasing methylmercury exposure. In addition, exposure to concentrations equal to or greater than 0.01 µM methylmercury reduced the visual response of the fish and altered electrophysiology of the retina, but gross histopathological alterations of the eye were not observed (Weber et al. 2008)

Altered foraging behavior as well as predator avoidance behavior may reduce the fitness of affected populations. Alvarez et al. (2006, 2007) fed adult Atlantic croaker (*Micropogonias undulates*) diets with 0 (control), 0.050 (low) or 0.1 (high) µg MeHg g^{-1} wet weight for one month. Females were induced to spawn and eggs from individuals spawns were fertilized. Mercury in the eggs and growth of larvae on days 1, 3, 6, 11, and 17 post hatch was measured. The swimming behavior, activity, and predator evasion behavior of larvae at various developmental stages was tested. The effects of maternally derived methylmercury was assessed using a regression tree analysis and a individual-based model that was previously developed.

Previous work indicated that rate of travel, visual reactive distance, and acoustic response distance could be used as a rough predictor of the probability of escaping a real predator attack. Estimated mean mercury concentrations in the eggs from the different adult treatments were 0.006 µg g^{-1} egg wet weight (control), 2.28 µg g^{-1}, and 14.7 µg g^{-1}. [Note: These estimates were made by M. Sandheinrich based on mean methylmercury in a single egg of each spawn from Table 1 and the average weight of a single egg of 204 µg from the Corrigendum. These concentrations seem excessively high because adult fish were only fed methylmercury-contaminated diets for one month. M. Sandheinrich contacted Dr. Alvarez and received confirmation that these estimated mean concentrations were correct]. There was no significant difference in growth of larvae among treatments. However, larvae from adults exposed to dietary methylmercury had decreased rates of travel, routine activity and response speed to a vibratory startle stimulus. They also had an increased response duration to a vibratory startle stimulus. Probabilities of croaker larvae escaping an attack from a predatory were predicted from regression tree analysis and measured survival skills. The measured values of rate of travel and probability of escaping a predator were used in an individual-based model to estimate larval stage duration and survival. Regression tree analysis indicated that maternal exposure to methylmercury caused a decline in probability of larvae escaping a predator, which varied with developmental stage of the larvae. Simulations of the individual-based model predicted an 86% reduction in survival of larvae from adults exposed to low dietary methylmercury (0.05 µg g^{-1} wet weight). High dietary methylmercury exposure (0.1 µg g^{-1} wet weight) of adults was predicted to reduce survival of larvae by 93% and prolong duration of the planktonic stage (due to reduced growth) by 26%. Although no effect on growth was observed, the model predicted altered growth due to mercury-altered behavior related to foraging in the field.

Murphy et al. (2008) incorporated information from Alvarez et al. (2006) into statistical and individual based models to estimate population-level effects of methylmercury on this species. Swimming speeds and reaction distances were measured for larval fish with less than 0.05 µg Hg g^{-1} wet weight (control), 0.01 to 3.5 µg Hg g^{-1} wet weight (low dose mercury), or greater than 3.5 µg g^{-1} wet weight (high dose mercury). This information was used in an individual based model to estimate encounter rates of larvae with their prey and growth, and to estimate the probability of larval escape from predators. Low dose mercury reduced survival of the simulated population of larval fish to 19% of baseline (control) levels and reduced growth by 2.6-9.7%. High dose mercury reduced survival of the simulated population of larval fish to 7% of baseline levels and reduced growth by 8.6-14%.

Webber and Haines (2003) also found that dietary methylmercury altered the predator evasion behavior of adult fish. Golden shiners (*Notemigonus crysoleucas*) were fed for 90 days with diets containing 0.012 (control), 0.455 (low), or 0.959 (high) µg methylmercury g^{-1}. Mean whole-body mercury concentrations of fish from the three dietary treatments were 0.041, 0.230, 0.518 µg g^{-1}wet weight; mean mercury concentrations in the brain were 0.047, 0.477, 1.118 µg g^{-1}. Fish from the three dietary groups were exposed to a model avian predator. There were no observed differences in brain acetylcholinesterase. However, fish fed high methylmercury diets were hyperactive and had altered shoaling behavior relative to those fish fed control or low methylmercury diets. Mercury concentrations altering behavior were representative of

concentrations measured in wild golden shiners. Webber and Haines concluded that mercury altered predator avoidance behavior and may increase vulnerability of fish to predation.

Toxicity Thresholds for Fish

Based on laboratory studies with fathead minnows (Hammerschmidt et al 2002, Drevnick and Sandheinrich 2003), reduced reproductive success and altered sex hormone concentrations are associated with mercury concentrations of 0.9 μg g^{-1} wet weight in the whole carcass of the fish and 0.87 μg g^{-1} dry weight in their diet (note that wet weight concentrations would be much lower, probably about 0.18 μg g^{-1} assuming 80% water content of diet). In fathead minnows and other species, altered behavior that may affect survival or reproductive success has been observed at whole carcass concentrations greater than 0.5 μg g^{-1} wet weight. There is very good evidence from field (e.g., Drevnick et al. 2008, Schwindt et al. 2008) and laboratory studies (e.g., Oliveria Ribeia et al. 2002) that oxidative stress and biochemical and structural changes occur in internal organs at tissue concentrations less than 0.5 μg g^{-1} wet weight. For example, Schwindt et al. (2008) associated whole-body, total mercury concentrations of less than 0.3 μg g^{-1} with tissue damage in brook trout. In a separate review of the literature on the toxicity of methylmercury to fish, Sandheinrich and Wiener (in press) concluded that alterations in biochemistry, organ histology, and reproduction in fish occur at methylmercury concentrations of about 0.3 to 0.7 μg g^{-1} wet weight in the whole body and about 0.5 to 1.2 μg g^{-1} wet weight in the axial muscle. Threshold concentrations for methylmercury are likely lower than 0.3 μg g^{-1}wet weight in the whole body.

Beckvar et al. (2005) evaluated four different methods for deriving tissue concentrations of mercury that are protective of deleterious effects. They obtained data on paired no-effect (NER) and low-effect (LER) whole-body residue concentrations of mercury in eggs, larval, juvenile and adult fish from 10 studies of eight fish species that evaluated the effects of mercury on growth, reproduction, survival, and behavior. Of the four methods evaluated, the tissue threshold effects level (t-TEL) was selected as best representing the data. The t-TEL is the geometric mean of the NER-M and LER-L. The NER-M and LER-L are the 50th percentile concentration in the no-effects data set and the 15th percentile concentration in the effects data set, respectively. A whole-body t-TEL of 0.2 μg g^{-1} wet weight was calculated to be protective of juvenile and adult fish. Because of the paucity of the data on effects of mercury on eggs and larvae, a t-TEL for early life stages was not calculated.

Effects of Methylmercury in Reptiles and Amphibians

There are very few studies on the effects of methylmercury on reptiles and amphibians. Moreover, some studies that assess the effects of mercury on herbivorous reptiles and amphibians may be complicated by the fact that a large portion of the total mercury on aufwuchs and plants may be inorganic mercury. In a laboratory study, Unrine and Jagoe (2004) fed larvae of southern leopard frogs (*Rana spenocephala*) aufwuchs with concentrations of inorganic and methylmercury that bracketed those observed in aufwuchs from the field and contaminated with mercury from atmospheric deposition. They analyzed the metamorphs and tadpoles for

inorganic and methylmercury. Inorganic mercury comprised the major source of mercury in the diets; the relative proportion of methylmercury in the aufwuchs decreased with increasing concentration of total mercury. The ratio of methylmercury to inorganic mercury in the tadpoles was greater than that of their diet. A subsequent field study of mercury in frog gut contents and carcasses conducted in South Carolina wetlands (Unrine et al. 2005), also demonstrated that the majority of total mercury in the diet was present as inorganic mercury and the proportion of methylmercury decreased with increasing concentrations of total mercury in the aufwuchs, which could be as high as 1.6 µg g^{-1} dry weight at locations with atmospheric deposition as the only source of mercury. Based on their previous study of mercury bioaccumulation, Unrine et al. (2004) fed southern leopard frog larvae with aufwuchs that had mercury concentrations and speciation similar to those observed in aufwuchs from aquatic systems contaminated by atmospheric deposition. Diets containing 1.4 µg g^{-1} dry weight or more total mercury altered development and growth, and increased malformations and mortality of larvae. Methylmercury comprised 1.5-1.9% of the total mercury in the aufwuchs. They concluded that dietary mercury exposure in habitats contaminated by atmospheric deposition could adversely affect amphibian larvae.

Day et al. (2007) investigated the relation between methylmercury concentration and immunological function and health in loggerhead turtles (*Caretta caretta*). Free-ranging sub-adult and adult turtles were collected by trawling off the coast of South Carolina, Georgia, and Florida. Blood from the turtles was collected for analysis of mercury, plasma chemistry, differential white blood cell counts, *ex vivo* lymphocyte proliferation, and plasma lysozyme activity. In addition, the effects of methylmercury on the *in vitro* proliferation of lymphocytes was measured by culturing cells from the turtles in media with 0, 0.01, 0.03, 0.05, 0.1, 0.35, and 0.7 µg MeHg g^{-1}. Mean (range) mercury concentrations in the plasma of 66 loggerhead turtles was 0.029 (0.006 – 0.077) µg g^{-1} wet weight. There was a positive correlation between blood mercury and hematocrit, which reflected the affinity of mercury for red blood cells. Creatine phosphokinase (CPK), an indicator or cellular damage in skeletal muscle, lungs, heart, or brain, and aspartate aminotransferase, a common indicator of liver damage, were positively correlated with blood mercury. Lysozyme activity, a biomarker of pro-inflammatory response, was also positively correlated with blood mercury. Numbers of lymphocytes were negatively correlated with blood mercury, which suggested a suppression of immune function. Suppression of *in vitro* B-cell and T-cell lymphocytes occurred at 0.05 µg Hg g^{-1} and 0.7µg Hg g^{-1}. This primarily correlative study suggests that low levels of methylmercury may alter biomarkers of health in the loggerhead sea turtle. However, the significance of these altered biomarkers on survival or reproduction is unknown. Based on the *in vitro* proliferation experiment, the authors estimated that about 5% of the wild loggerhead sea turtle population has blood Hg that corresponded to suppression of lymphocytes. Moreover, the negative correlation between lymphocytes and blood Hg suggests that deleterious, albeit subtle, effects on the immune function of sea turtles is possible at methylmercury concentrations observed in wild turtles.

Toxicity Thresholds for Reptiles and Amphibians

There is insufficient information available to suggest threshold concentrations of methylmercury for reptiles and amphibians.

Effects of Methylmercury in Invertebrates

Relevant literature on the effects of methylmercury on aquatic and terrestrial invertebrates is exceptionally sparse, even prior 1999. As in early studies with fish, most studies on invertebrates have used aqueous exposure of invertebrates to inorganic or exceptionally high concentrations of methylmercury.

Tsui and Wang (2004) fed juvenile *Daphnia magna* with the green alga, *Chlamydomonas reinhardtii*, that had been spiked with radioilabelled methylmercury corresponding to 28.3 nM of mercury (5.68 µg L^{-1}; as methylmercury). The daphnids were fed six hours daily for five days. The depuration rate of methylmercury, reproduction, and survival of the *Daphnia* (F_0), their neonates (F_1), and the subsequent generation (F_2) was evaluated. After five days of exposure, mean concentrations of methylmercury in the F_0 generation of *Daphnia magna* were 33.3 µg g^{-1}. After 20 d of being fed uncontaminated algae, they had eliminated approximately 75% of the ingested methylmercury, primarily through transfer of the mercury to their neonates. The neonates (F_1) of these adults accounted for approximately 11% of the methylmercury lost by the parents. They retained 40% to 60% of the methylmercury they received from their parent after 20 d of depuration. The burden of methylmercury in the F_1 individuals (8.57 – 35.5 pg per individual) was dependent upon the age of the adult (i.e., days of depuration after exposure and number and size of previous clutches). The F_0 and F_1 *Daphnia* exhibited high mortality and reduced reproduction relative to reference organisms in the laboratory. F_0 *Daphnia* produced 0 to 1.44 neonates per female per day; unexposed *Daphnia* in the laboratory produced 5 to 7 neonates per female per day. The authors did not provide information on concentrations of methylmercury or reproductive output of F_1 and F_2 individuals, but indicated that they exhibited reduced reproduction and survival due to maternal transfer of methylmercury.

Jensen et al. (2006) examined the effects of methylmercury on *Megaselia scalaris*, a small, terrestrial insect detritivore (Order Diptera, Family Phoridae). Females oviposited eggs on media contaminated with 0, 5, 10, 15, 25, or 30 µg g^{-1} methylmercury chloride and larvae were subsequently allowed to feed and develop on the experimental diets. The lowest dietary concentration that significantly decreased larval survival was 30 µg Hg g^{-1} diet. Total mortality over the life cycle of the insect was reduced by 67% at 25 µg Hg g^{-1} diet and larval development was delayed at 10 µg Hg g^{-1} diet. Relative to control flies, fewer eggs were produced by each female fed diets at the lowest methylmercury concentration tested (5 µg g^{-1}).

Most of the other studies of methylmercury in invertebrates have focused on sublethal toxicity to the immune response. Sauve' and Fournier (2005) examined the effect of methylmercury on the phagocytotic activity of coelomocyte immune cells of the earthworm *Eisenia andrei.* Coelomocytes were extracted from hatchling and adult worms, pooled together, and exposed *in vitro* to 10^{-8} to 10^{-4} M methylmercury chloride (0.0002 to 2.0 mg Hg L^{-1}). The phagocytic activity of the coelomocytes was quantified by counting the number of fluorescent latex beads

engulfed by the cells. Phagocytic activity of the cells was significantly reduced at methylmercury concentrations of 10^{-6} M (200 μg L^{-1}).

Fournier et al. (2000) reported on the effects of *in vitro* and *in vivo* exposure of methylmercury on phagaocytic activity in a variety of invertebrates and fish. Phagocytic cells or hemocytes were collected from the hemolymph of the soft-shell clam *Mya arenaria*, the surf clam *Spisula polynyma*, and the blue mussel *Mytilis edulis*. Pronephric cells were collected from American plaice *Hypoglossoides platessoides*, the mummichog *Fundulus heteroclitus*, and rainbow trout *Oncorhynchus mykiss*. Splenocytes were collected from the African clawed frog *Xenopus laevis*. Cell suspensions were exposed for 18 hours to 10^{-9} to 10^{-3} M mercury as methylmercury chloride chloride (0.2 μg L^{-1} to 200 mg L^{-1}). Phagocytic activity was assessed by cytometric measurement of the fluorescence of engulfed latex beads. The concentration of methylmercury causing 50% inhibition of phagocytic activity (IC50) was 161 μg L^{-1} for mummichog, 180 μg L^{-1} for the African clawed frog, 261 μg L^{-1} for rainbow trout, 501 μg L^{-1} for the soft-shell clam, 1020 μg L^{-1} for the surf clam, and 1300 μg L^{-1} for the blue mussel. The authors also reported a significant inverse relation between tissue concentration of methylmercury and phagocytotic activity of the soft-shell clam after *in vivo* exposure. Tissue concentrations of 150 μg Hg g^{-1} dry weight suppressed phagocytosis by more than 25%.

Toxicity Thresholds for Invertebrates

There is insufficient information available to suggest threshold concentrations of methylmercury for invertebrates.

Literature Cited

Ackerman, J. T., J. Y. Takekawa, C. A. Eagles-Smith, and S. A. Iverson. 2008. Mercury contamination and effects on survival of American avocet and blacknecked stilt chicks in San Francisco Bay. Ecotoxicol. 17:103-116

Adams, E. M. and P. C. Frederick. 2008. Effects of methylmercury and spatial complexity on foraging behavior and foraging efficiency in juvenile white ibises (*Eudocimus albus*). Environ. Toxicol. Chem. 27:1708-1712.

Adams, E. M., P. C. Frederick, I. L. V. Larkin, and L. J. Guillette, Jr. 2009. Sublethal effects of methylmerucyr on fecal metabolites of testosterone, estradiol, and corticosterone in captive juvenile white ibises. Environ. Toxicol. Chem. 28:982-989.

Albers, P. H., M. T. Koterba, R. Rossman, W. A. Link, J. B., French, R. S. Bennett, and W. C. Bauer. 2007. Effects of methylmercury on reproduction in American kestrels. Environ. Toxicol. Chem. 26:1856-1866.

Alvarez, M. C., C. A. Murphy, K. A. Rose, I. D. McCarthy, and L. A. Fuiman. 2006. Maternal body burdens of methylmercury impair survival skills of offspring in Atlantic croaker (*Micropognias unudulatus*). Aquat. Toxicol. 80:329-337.

Alvarez, M. C., C. A. Murphy, K. A. Rose, I. D. McCarthy, and L. A. Fuiman. 2007. Corrigendum to "Maternal body burdens of methylmercury impair survival skills of offspring in Atlantic croaker (*Micropognias unudulatus*)" [Aquat. Toxicol. 80(2006) 329-337]. Aquat. Toxicol. 82:308.

Alves Costa, J.R.M., M. Mela, H. C. Silva de Assis, E. Pellettier, M. A. F. Randi, and C. A. Oliveria Ribeiro. 2007. Enzymatic inhibition and morphological changes in *Hoplias malabaricus* from dietary exposure to lead(II) or methylmercury. Ecotoxicol. Environ. Safe. 67:82-88.

Basu, N., K. Klenavic, M. Gamberg, M. O'Brien, R. D. Evans, A. M. Scheuhammer, and H. M. Chan. 2005a. Effects of mercury on neurochemical receptor binding characteristics in wild mink. Environ. Toxicol. Chem. 24:1444–1450.

Basu, N., A. Scheuhammer, N. Grochowina, K. Klenavic, R Evans, M. O'Brien, and H. M. Chan. 2005b. Effects of mercury on neurochemical receptors in wild river otters (*Lontra canadensis*). Environ. Sci. Technol. 39:3585-3591.

Basu, N., C. J. Stamler, K. M. Loua, and H. M. Chan. 2005c. An interspecies comparison of mercury inhibition on muscarinic acetylcholine receptor binding in the cerebral cortex and cerebellum. Toxicol. Appl. Pharmacol. 205:71-76.

Basu, N., A. M. Scheuhammer, K. Rouvinen-Watt, N. Grochowina, K. Klenavic, R. D. Evans, and H. M. Chan. 2006. Methylmercury impairs components of the cholinergic system in captive mink (*Mustela vison*). Toxicol. Sci. 91:202-209.

Basu, N., A. M. Scheuhammer, R. D. Evans, M. O'Brien, and H. M. Chan. 2007a. Cholinesterase and monoamine oxidase activity in relation to mercury levels in the cerebral cortex of wild river otters. Hum. Exper. Toxicol. 26:213-220.

Basu, N., A. M. Scheuhammer, K. Rouvinen-Watt, N. Grochowina, R. D. Evans, M. O'Brien, and H. M. Chan. 2007b. Decreased *N*-methyl-D-aspartic acid (NMDA) receptor levels are associated with mercury exposure in wild and captive mink. NeuroToxicol. 28:587-593.

Basu, N., A. M. Scheuhammer, K. Rouvinen-Watt, R. D. Evans, N. Grochowina, and L. H. M. Chan. 2008. The effects of mercury on muscarinic cholinergic receptor subtypes (M1 and M2) in captive mink. NeuroToxicology 29:328-334.

Basu, N., A. M. Scheuhammer, K. Rouvinen-Watt, R. D. Evans, V. L. Trudeau, and L. H. M. Chan. 2010. *In vitro* and whole animal evidence that methylmercury disrupts GABAergic systems in discrete brain regions in captive mink. Compar. Biochem. Physiol, Part C. 151:379-385.

Beckvar, N., T. M. Dillon, and L. B. Reads. 2005. Approaches for linking whole-body fish tissue mercury or DDT to biological effects thresholds. Environ. Toxicol. Chem. 24:2094-2105.

Berntssen, M. H. G., A. Aatland, and R. D. Handy. 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behavior in Atlantic salmon (*Salmo salar*) parr. Aquat. Toxicol. 65:55-72.

Bennett, R. S., J. B. French, Jr., R. Rossmann, and R. Haebler. 2009. Dietary toxicity and tissue accumulation of methylmercury in American kestrels. Arch. Environ. Contam. Toxicol. 56:149-156.

Bloom, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. 49: 1010-1017.

Boening, D.W. 2000. Ecological effects, transport, and fate of mercury: A general review. Chemosphere 40:1335-1351.

Bouton, S. N., P. C. Frederick, M. G. Spalding, and H. McGill. 1999. Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. Environ. Toxicol. Chem. 18:1934-1939.

Brasso, R. L., and D. A. Cristol. 2008. Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). Ecotoxicol. 17:133-141.

Brousseau, P., J. Pellerin, Y. Morin, D. Cyr, B. Blakley, H. Boerman, and M. Fournier. 2000. Flow cytometry as a tool to monitor the disturbance of phagocytosis in the clam *Mya arenaria* hemocytes following in vitro exposure to heavy metals. Toxicol. 142:145-156

Burgess, N. M., and M. W. Meyer. 2008. Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicol. 17:83-92.

Burgess N. M, D. C. Evers, and J. D. Kaplan. 2005. Mercury and other contaminants in common loons breeding in Atlantic Canada. Ecotoxicol. 14:241–252.

Cambier, S., G. Bénard, N. Mesmer-Dudons, P. Gonzalez, R. Rossingol, D. Brèthes, J.-P. Bourdineaud. 2009. At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebra fish (*Danio rerio*). Internat. J. Biochem. Cell Biol. 41:791-799.

Cambier, S., P. Gonzalez, G. Durrieu, R. Maury-Brachet, A. Boudou, and J.-P. Bourdineaud. 2010. Serial analysis of gene expression in the skeletal muscles of zebrafish fed with a methylmercury-contaminated diet. Environ. Sci. Technol. 44:469-475.

Chan, H. M., A. M. Scheuhammer, A. Ferran, C. Loupelle, J. Holloway, and S. Weech. 2003. Impacts of mercury on freshwater fish-eating wildlife and humans. Hum. Ecol. Risk Assess. 9:867-883.

Cizdziel, J., T. Hinners, C. Cross, and J. Pollard. 2003. Distribution of mercury in the tissues of five species of freshwater fish from Lake Mead, USA. J. Environ. Monitor. 5:802-807.

Clarkson, T. W., and L. Magos. 2006. The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol. 36:609-662.

Crump, K. L., and V. L. Trudeau. 2009. Mercury-induced reproductive impairment in fish. Environ. Toxicol. Chem. 28:895-907.

Custer, C. M., T. W. Custer, and E. F. Hill. 2007. Mercury exposure and effects on cavity-nesting birds from the Carson River, Nevada. Arch. Environ. Contam. Toxicol. 52:129-136.

Custer, T. W., C. M. Custer, K. Dickerson, K. Allen, M. J. Melancon, and L. J. Schmidt. 2001. Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, trace elements, and monooxygenase activity in birds nesting on the North Platte River, Casper, Wyoming, USA. Environ. Toxicol. Chem. 20:624–631

Custer, T. W., C. M. Custer, S. Larson, and K. K. Dickerson. 2002a. Arsenic concentrations in house wrens from Whitewood Creek, South Dakota, USA. Bull. Environ. Contam. Toxicol 68:517–524

Custer, C. M., T. W. Custer, A. S. Archuleta, L. C. Coppock, C. D. Swartz, and J. W. Bickham. 2002b. A mining impacted stream: Exposure and effects of lead and other trace elements on tree swallows (*Tachycineta bicolor*) nesting in the upper Arkansas River basin, Colorado. In: Hoffman DJ, Rattner BA, Burton Jr. GA, Cairns Jr. J, (eds.) Handbook of ecotoxicology, 2nd ed. CRC Press, Boca Raton, FL, pp 787–812.

Custer C. M., T. W. Custer, D. W. Warburton, D. J. Hoffman, J. W. Bickham, and C. W. Matson. 2006. Trace element concentrations and bioindicator responses in tree swallows from northwestern Minnesota. Environ. Monitor. Assess. 18:247–266.

Dansereau, M., N. Lariviere, D. Du Tremblay, and D. Belanger. 1999. Reproductive performance of two generations of female semi-domesticated mink fed diets containing organic mercury contaminated freshwater fish. Arch. Environ. Contam. Toxicol. 36, 221–226.

Day, R. D., A. L. Segars, M. D. Arendt, A. M. Lee, and M. M. Peden-Adams. 2007. Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (*Caretta caretta*). Environ. Health Perspec. 1115:1421-1428.

Deng, D-F., F-C. The, and S. J. The. 2008. Effect of dietary methylmercury and seleno-methionine on Sacramento splittail larvae. Sci. Total Environ. 407:197-203.

DesGranges, J. L., J. Rodrigue, B. Tardif, and M. Laperle. 1998. Mercury accumulation and biomagnification in ospreys (*Pandion haliaetus*) in the James Bay and Hudson Bay Regions of Quebec. Arch. Environ. Contam. Toxicol. 35:330–341.

Drevnick, P. E., and M. B. Sandheinrich. 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. Environ. Sci. Technol. 37:4390-4396.

Drevnick, P. E., M. B. Sandheinrich, and J. T. Oris. 2006. Increased ovarian follicular apoptosis in fathead minnows (*Pimephales promelas*) exposed to dietary methylmercury. Aquat. Toxicol. 79:49-54.

Drevnick, P. E., A. P. Roberts, R. R. Otter, C. R. Hammerschmidt, R. Klaper, and J. T. Oris. 2008. Mercury toxicity in livers of northern pike (*Esox lucius*) from Isle Royale, USA. Comp. Biochem. Physiol. Part C 147:331-338.

Elbert R. A., and D. W. Anderson. 1998. Mercury levels, reproduction, and hematology in western grebes from three California lakes, USA. Environ. Toxicol. Chem. 17:210-213.

Evers D. C., J. D. Kaplan, M. W. Meyer, P. S. Reaman, W. E. Braselton, A. Major, N. Burgess, and A. M. Scheuhammer. 1998. Geographic trend in mercury measured in common loon feathers and blood. Environ. Toxicol. Chem. 17:173–183.

Evers, D. C., K. M. Taylor, A. Major, R. J. Taylor, R. H. Poppenga, and A. M. Scheuhammer. 2003. Common loon eggs as indicators of methylmercury availability in North America. Ecotoxicol. 12:69-81.

Evers, D. C., J. Lucas, J. Savoy, C. R. DeSorbo, D. E. Yates, W. Hanson, K. M. Taylor, L. Siegel, J. H. Cooley, Jr., M. Bank, A. Major, K. Munney, B. Mower, H. S. Vogel, N. Schoch, M. Pokras, M. W. Goodale, and J. Fair. 2008. Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicol. 17:69-82.

Fimreite, N. 1971. Effects of dietary methylmercury on ring-necked pheasants. Occasional Paper No 9. Canadian Wildlife Service, Ottawa, ON.

Fjeld, E., T. O. Haugen, and L. A. Vollestad. 1998. Permanent impairment in the feeding behavior of grayling (*Thymallus thymallus*) exposed to methylmercury during embryogenesis. Sci. Tot. Environ. 313:247-254.

Fournier, M., D. Cyr, B. Blakley, H. Boermans, and P. Brousseau. 2000. Phagocytosis as a biomarker on immunotoxicity in wildlife species exposed to environmental xenobiotics. Amer. Zool. 40:412-420.

Francshini, M. D., O. P. Lane, D. C. Evers, J. M. Reed, B. Hoskins, and L. M. Romero. 2009. The corticosterone stress response and mercury contamination in free-living tree swallows, Tachycineta bicolor. Ecotoxicol. 18:514-521.

Frederick, P. C., M. G. Spalding, M. S. Sepulveda, G. E.Williams, L. Nico, and R. Robins. 1999. Exposure of great egret (*Ardea albus*) nestlings to mercury through diet in the Everglades ecosystem. Environ. Toxicol. Chem. 18:1940-1947.

Friedmann A. S., M. C. Watzin, T. Brinck-Johnsen, and L. C. Leiter. 1996. Low levels of methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquat. Toxicol. 35:265-278.

Friedmann, A. S., E. K. Costain, D. L. MacLatchy, W. Stansley, and E. J. Washuta. 2002. Effect of mercury on general and reproductive health of largemouth bass (*Micropterus salmoides)* from three lakes in New Jersey. Ecotoxicol. Environ. Safe. 52:117-122.

Gonazalez, P., Y. Dominique, J. C. Massabuau, A. Boudou, and J. P. Bourdineaud. 2005. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). Environ. Sci. Technol. 39:3972-3980.

Hall, B. D., R. A. Bodaly, R. J. P. Fudge, J. W. M. Rudd, and D. M. Rosenberg. 1997. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Pollut. 100:13-24.

Hammerschmidt, C. R., M. B. Sandheinrich, J. G. Wiener, and R. G. Rada. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. Environ. Sci. Technol. 36:877-883.

Hammerschmidt, C. R., and M. B. Sandheinrich. 2005. Maternal diet during oogenesis is the major source of methylmercury in fish embryos. Environ. Sci. Technol. 39:3580-3584.

Hawley, D. M., K. K. Hallinger, and D. A. Cristol. 2009. Compromised immune competence in free-living tree swallows exposed to mercury. Ecotoxicology 18:499-503.

Heath, J. A., and P. C. Frederick. 2005. Relationships among mercury concentrations, hormones, and nesting effort of white ibises (*Eudocimus albus*) in the Florida Everglades. Auk 122:255-267.

Heinz, G. H. 1979. Methylmercury: Reproductive and behavioral effects on three generations of mallard ducks. J. Wildl. Manage. 43:394–401.

Heinz, G. H., and D. J. Hoffman. 2003. Embryotoxic thresholds for mercury: Estimates from individual mallard eggs. Arch. Environ. Contam. Toxicol. 44:257-264.

Heinz, G. H., D. J. Hoffman, J. D. Klimstra, and K. R. Stebbins. 2009. Reproduction in mallards fed a low level of methylmercury: An apparent case of hormesis. Environ. Toxicol. Chem. In press.

Heinz, G. H., D. J. Hoffman, J. D. Klimstra, K. R. Stebbins, S. L. Kondrad, and C. A. Erwin. 2009. Species differences in the sensitivity of avian embryos to methylmercury. Arch. Environ. Contam. Toxicol. 56:129-138.

Henny, C. J., E. F. Hill, D. J. Hoffman, M. G. Spalding, and R. A. Grove. 2002. Nineteenth century mercury: Hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicol. 11:213-231.

Hill, E., C. J. Henny, and R. A. Grove. 2008. Mercury and drought along the lower Carson River, Nevada: II. Snowy egret and Black-crowned night-heron reproduction on Lahontan Reservoir, 1997-2006. Ecotoxicol. 17:117-132.

Hoffman, D. J., C. J. Henny, E. F. Hill, R. A. Grove, J. L. Kaiser, and K. R. Stebbins. 2009. Mercury and drought along the lower Carson River, Nevada: III. Effects on blood and organ biochemistry and histopathology of snowy egrets and black-crowned night-herons on Lahontan Reservoir, 2002-2006. J. Toxicol. Environ. Health: Part A. 72:1223-1241

Hoffman, D. J., M. G. Spalding, and P. C. Frederick. 2005. Subchronic effects of methylmercury on plasma and organ biochemistries in great egret nestlings. Environ.Toxicol. Chem. 24:3078-3084.

Houck, A., and J. J. Cech, Jr. 2004. Effects of dietary methylmercury on juvenile Sacramento blackfish bioenergetics. Aquat. Toxicol. 69:107-123.

Jensen, P. D., I. R. Johson, and J. T. Trumble. 2006. Individual and joint actions of selenate and methylmecury on the development and survival of an insect detritivore, *Megaselia scalaris* (Lowe). Arch. Environ. Contam. Toxicol. 50:523-530.

Kenow, K. P., K. A. Grasman, R. K. Hines, M. W. Meyer, A. Gendron-Fitzpatrick, M. G. Spaulding, and B. R. Gray. 2007. Effects of methylmercury exposure on the immune function of juvenile common loons (*Gavia immer*). Environ. Toxicol. Chem. 26:1460-1469.

Kenow, K. P., S. Gutreuter, R. K. Hines, M. W. Meyer, F. Fournier, and W. H. Karasov. 2003. Effects of methyl mercury exposure on the growth of juvenile common loons. Ecotoxicol. 12: 171-181.

Kenow, K. P., D. J. Hoffman, R. K. Hines, M. W. Meyer, J. W. Bickham, C. W. Matson, K. R. Stebbin, P. Montagna, and A. Elfessi. 2008. Effects of methylmercury exposure on glutathione metabolism, oxidative stress, and chromosomal damage in captive-reared common loon (*Gavia immer*) chicks. Environ. Pollut. 156:732-738.

Klaper, R., B. J. Carter, C. A. Richter, P. E. Drevnick, M. B. Sandheinrich, and D. E. Tillitt. 2008. Use of a 15 k gene microarray to determine gene expression changes in response to acute and chronic methylmercury exposure in the fathead minnow *Pimephales promelas* Rafinesque. J. Fish Biol. 72:2207-2280.

Klaper, R., C. B. Rees, P. Drevnick, D. Weber, M. Sandheinrich, and M. J. Caravan. 2006. Gene expression changes related to endocrine function and decline in reproduction in fathead minnow (*Pimephales promelas*) after dietary methylmercury exposure. Environ. Health Perspec. 11:1337-1343.

Klenavic, K., L. Champoux , M. O'Brien, P-Y. Daoust, R. D. Evans, and H. E. Evans. 2008. Mercury concentrations in wild mink (*Mustela vison*) and river otters (*Lontra canadensis*) collected from eastern and Atlantic Canada: Relationship to age and parasitism. Environmental Pollution 156:359-366.

Larose, C., R. Canuel, M. Lucotte, R. T. Di Giulio. 2008. Toxicological effects of methylmercury on walleye (*Sander vitreus*) and perch (*Perca flavescens*) from lakes of the boreal forest. Comp. Biochem. Physiol. Part C. 147:139-149.

Latif, M., R. A. Bodaly, T. A. Johnston, and R. J. P. Fudge. 2001. Effects of waterborne and maternally-derived methylmercury on development and survival of walleye embryos. Environ. Pollut. 111: 139-148.

Liao, C-Y., J-J. Fu, J-B. Shi, Q-F. Zhou, C-G. Yuan, and G-B. Jiang. 2006. Methylmercury accumulation, histopathology effects, and cholinesterase activity alterations in medaka (*Oryzias latipes*) following sublethal exposure to methylmercury chloride. Environ. Toxicol. Pharm. 22:225-233.

Longcore, J. R., T. A. Haines, and W. A. Halteman. 2007a. Mercury in tree swallow food, eggs, bodies, and feathers at Acadia National Park, Maine, and an EPA Superfund site, Ayer, Massachusetts. Environ. Monitor. Assess. 126:129-143.

Longcore, J. R., R. Dineli, and T. A. Haines. 2007b. Mercury and growth of tree swallows at Acadia National Park, and at Orono, Maine, USA. Environ. Monitor. Assess. 126:117-127.

Matta, M. B., J. Linse, C. Cairncross, L. Francendese, and R. M. Kocans. 2001. Reproductive and transgenerational effects of methylmercury or aroclor 1268 on *Fundulus heteroclitus*. Environ. Toxicol. Chem. 20:327-335.

Mela, M., M. A. F. Rand, D. F. Ventura, C. E. V. Carvalho, E. Pelletier, and C. A. Oiveira Ribeiro. 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplia malabaricus*. Ecotoxicol. Environ. Safe. 6:426-435.

Mitro, M. G., D. C. Evers, M. W. Meyer, W. H. Piper. 2008. Common loon survival rates and mercury in New England and Wisconsin. J. Wildl. Manage. 72:665-673.

Moran, P., N. Alur, R. W. Black, and M. M. Vijayan. 2007. Tissue contaminants and associated transcriptional response in trout liver from high elevation lakes of Washington. Environ. Sci. Technol. 41:6591-6597.

Munn, M.D., and T. M. Short. 1997. Spatial heterogeneity of mercury bioaccumulation by walleye in Franklin D. Roosevelt Lake and the upper Columbia River, Washington. Trans. Amer. Fish. Soc. 126:477–487.

Murphy, C. A., K. A. Rose, M. C. Alvarez, and L. Fuiman. 2008. Modeling larval fish behavior: Scaling the sublethal effects of methylmercury to population-relevant endpoints. Aquat. Toxicol. 86:470-484.

Navarro, A., L. Quirós, M. Casado, M. Faria, L. Carrasco, L. Benejam, J. Benito, S. Díez, D. Raldúa, C. Barata, J. M. Bayona, and B. Pi˜na, 2009. Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. Aquatic Toxicology 93:150-157.

Niimi, A. J., and G. P. Kissoon. 1994. Evaluation of the critical body burden concept based on inorganic and organic mercury toxicity to rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 26:169-178.

Nocera, J.J. and P.D. Taylor. 1998. *In situ* behavioral response of common loons associated with elevated mercury (Hg) exposure. Conservation Ecology [online] 2(2): 10. Available from the Internet. URL: http://www.consecol.org/vol2/iss2/art10/

Olsen, B., D. Evers, and C. Desorbo. 2000. Effect of methylated mercury on the diving frequency of the common loon. J. Ecol. Res. 2:67-72.

Oliveria Ribeia, C. A., L. Belger, E. Pelletier, and C. Rouleau. 2002. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). Environ. Res. 90:217-225.

Oliveria Ribeia,C. A., M-D. Nathalie, P. Gonzalez, D. Yannick, B. Jean-Paul, A. Boudou, and J. C. Massabuau. 2008. Effects of dietary methylmercury on zebrafish skeletal muscle fibers. Environ. Toxicol. Pharmacol. 25: 304-309.

Oliveira Ribeiro, C. A., F. F. Neto, M. Mela, P. H. Silva, M. A. F. Randi, I. S. Rabitto, J. R. M. Alves Costa, and E. Pelletier. 2006. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyl tin chloride. Environ. Res. 101:74-80.

Porcella, D. B. 1994. Mercury in the environment: Biogeochemistry. Pages 3-20 in *Mercury Pollution: Integration and Synthesis*, edited by C. J. Watras and J. W. Huckabee, Lewis Publishers, Boca Raton, FL.

Raldúa, D., S. Diez, J. M. Bayona, and D. Barceló. 2007. Mercury levels and liver pathology in feral fish living in the vicinity of a mercury cell chlor-alkali factory. Chemosphere 66:1217-1225.

Ralston, N. V. C., J. L. Blackwell III, and L. J. Raymond. 2007. Importance of molar ratios in selenium-dependent protection against methylmercury toxicity. Biol. Trace Elem. Res. 119:255-268.

Raymond, L. J., and N. V. C. Ralston. 2009. Selenium's importance in regulatory issues regarding mercury. Fuel Process. Technol. 90:1333-1338.

Rimmer, C.C., K. P. Mcfarland, D. C. Evers, E. K. Miller, Y. Aubry, D. Busby, and R. J. Taylor. 2005. Mercury concentrations in Bicknell's thrush and other insectivorous passerines in Montane forests of northeastern North America. Ecotoxicol. 14:223-240.

Rodgers, D. W. 1994. You are what you eat and a little bit more: Bioenergetics-based models of methylmercury accumulation in fish revisited. Pages 427-439 in *Mercury Pollution: Integration and Synthesis*, edited by C. J. Watras and J. W. Huckabee, Lewis Publishers, Boca Raton, FL.

Rumbold, D. G, S. L. Niemezyk, L. E. Fink, T. Chandrasekhar, B. Harkanson, and K. A. Laine. 2001. Mercury in eggs and feathers of great egrets (*Ardea albus*) from the Florida Everglades. Arch. Environ. Contam. Toxicol. 41:501–507

Sandheinrich, M. B., and K. M. Miller. 2006. Effects of dietary methylmercury on reproductive behavior of fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. 25:3053-3057.

Sandheinrich, M. B., and J. G. Wiener. In press. Methylmercury in freshwater fish: Recent advances in assessing toxicity of environmentally relevant exposures. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*, *2nd edition*, edited by N. Beyer and J. Meador, Taylor and Francis Publishers, Boca Raton, Florida

Sauve, S., and M. Fournier. 2005. Age-specific immunocompetence of the earthworm *Eisenia andrei*: Exposure to methylmercury. Ecotoxicol. Environ. Safe. 60:67-72.

Scheuhammer, A. M. 1987. The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: A review. Environ. Pollut. 46:263-295.

Scheuhammer, A. M., C. M. Atchison, A. H. K. Wong, and D. C. Evers. 1998. Mercury exposure in breeding common loons (*Gavia immer*) in central Ontario, Canada. Environ. Toxicol. Chem. 17:191-196.

Scheuhammer, A. M., N. Basu, N. M. Burgess, J. E. Elliott, G. D. Campbell, M. Wayland, L. Champoux, and J. Rodrigue. 2008. Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). Ecotoxicology 17:93-101.

Scheuhammer, A. M., M. W. Meyer, M. B. Sandheinrich, and M. W. Murray. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36:12-18.

Schwarzbach, S. E., J. D. Albertson, and C. M. Thomas. 2006. Effects of predation, flooding, and contamination on reproductive success of California clapper rails (*Rallus longirostris obsoletus*) in San Francisco Bay. Auk 123:45-60.

Schwindt, A. R., J. W. Fournie, D. H. Landers, C. B. Schreck, and M. L. Kent. 2008. Mercury concentrations in salmonids from western. U.S. national parks and relationships with age and macrophage aggregates. Environ. Sci. Technol. 42:1365-1370.

Seewagen, C. L. 2009. Threats of environmental mercury to birds: knowledge gaps and priorities for future research. Bird Conservation International. In press.

Sepulveda, M. S., P. C. Frederick, M. G. Spalding, and J. G. E. Williams. 1999. Mercury contamination in free-ranging great egret nestlings (*Ardea albus*) from southern Florida, USA. Environ. Toxicol. Chem. 18: 985–992.

Sepulveda, M. S., G. E. Williams, Jr., P. C. Frederick, and M. G. Spalding. 1999. Effects of mercury on health and first-year survival of free-ranging great egrets (*Ardea albus*) from southern Florida. Arch. Environ. Contam. Toxicol 37:369-376.

Shaw-Allen, P. L., C. S. Romanek, A. L. Bryan, Jr., H. Brant, and C. H. Jagoe. 2005. Shifts in relative tissue δ^{15} values in snowy egret nestlings with dietary mercury exposure: A marker for increased protein degradation. Environ. Sci. Technol. 39:4226-4233.

Smith, L. E., M. J. Caravan III, J. A. Dellinger, J. K. Ghorai, D. B. White, F. E. Williams, and D. N. Weber. 2009. Developmental selenomethionine and methylmercury effect zebrafish learning. Neurotoxicol. Teratol. In press.

Spalding, M. G., P. C. Frederick, H. C. McGill, S. N. Bouton, and L. R. McDowell. 2000a. Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. J. Wildl. Disease 36:411-422.

Spalding, M. G., P. C. Frederick, H. C. McGill, S. N. Bouton, L. J. Richey, I. M. Schumacher, C. G. M. Blackmore, and J. Harrison. 2000b. Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. J. Wildl. Disease 36:423-435.

Sweet, L. I., and J. T. Zelikoff. 2001. Toxicology and immunotoxicology of mercury: a comparative review in fish and humans. J. Toxicol. Environ. Health, Part B 4:161-205.

Tan, S. W., J. C. Meiller, and K. R. Mahaffey. 2009. The endocrine effects of mercury in humans and wildlife. Crit. Rev. Toxicol. 39:228-269.

Thompson, D. R. 1996. Mercury in birds and terrestrial mammals *in* W. N. Beyer, G. H. Heinz, and A. W. Redmon-Norwood (editors), *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. CRC Press, Boca Raton, Florida. Pp 341-356.

Tsui, M. T. K., and W. –X. Wang. 2004. Maternal transfer efficiency and transgenerational toxicity of methylmecury in *Daphina magna*. Environ. Toxicol. Chem. 23:1504-1511.

Unrine, J.M., and C. H. Jagoe. 2004. Dietary mercury exposure and bioaccumulation in southern leopard frog (*Rana sphenocephala*) larvae. Environ. Toxicol. Chem. 23: 2956–2963.

Unrine, J.M., C. H. Jagoe, W. A. Hopkins, and H. A. Brant. 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. Environ. Toxicol. Chem. 23: 2964–2970.

Unrine, J. M., C.H. Jagoe, A.C. Brinton, H.A. Brant, and N.T. Garvin. 2005. Dietary mercury exposure and bioaccumulation in amphibian larvae inhabiting Carolina bay wetlands. Environ. Pollut. 129:2964-2970.

Wada, H., D. A. Cristol, F. M. A. McNabb, and W. A. Hopkins. 2009. Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. Environ. Sci. Technol. 43:6030-6038.

Wagemann, R., E. Trebacz, R. Hunt, and G. Boila. 1997. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. Environ. Toxicol. Chem. 16:1859-1866.

Webb, M.A.H., G. W. Feist, M. S. Fitzpatrick, E. P. Foster, C. B. Schreck, M. Plumlee, C. Wong, and D. T. Gundersen. 2006. Mercury concentrations in gonad, liver, and muscle of white sturgeon *Acipenser transmontanus* in the lower Columbia River. Arch. Environ. Contam. Toxicol. 50:443-451.

Webber, H. M., and T. A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environ. Toxicol. Chem. 22:1556-1561.

Weber, D. N., V. P. Connaughton, J. A. Dellinger, D. Klemer, A. Udvadia, and M. J. Caravan III. 2008. Selenomethionine reduces visual deficits due to developmental methylmercury exposure. Physiol. Behav. 93:250-260.

Weech, S. A., A. M. Scheuhammer, and J. E. Elliott. 2006. Mercury exposure and reproduction in fish-eating brids breeding in the Pinchi Lake region, British Columbia, Canada. Environ. Toxicol. Chem. 25:1433–1440.

Weis, J. S. 2009. Reproductive, developmental, and neurobehavioral effects of methylmercury in fishes. J. Environ. Sci. Health Part C 27:212-225.

Wiener, J. G., and D. J. Spry. 1996. Toxicological significance of mercury in freshwater fish *in* W. N. Beyer, G. H. Heinz, and A. W. Redmon-Norwood (editors), *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. CRC Press, Boca Raton, Florida. Pp 297-339.

Wiener, J. G., D. P. Krabbenhoft, G. H. Heinz, and A. M. Scheuhammer. 2003. Ecotoxicology of mercury, Chapter 16 *in* D. J. Hoffman, B. A. Rattner, G. A. Burton, Jr., and J. Cairns, Jr. (editors), *Handbook of Ecotoxicology*, 2nd edition. CRC Press, Boca Raton, Florida. pp. 409-463.

Wolfe, M. F., S. Schwarzbach, and R. A. Sulaiman. 1998. The effects of mercury on wildlife: A comprehensive review. Environ. Toxicol. Chem. 17:146-160.

APPENDIX 2-2:

Evers 2012 report on methylmercury toxicity to wildlife

2012

The effects of methylmercury on wildlife

A comprehensive review

Published findings indicate that effect concentrations can be developed for certain taxa, forage guilds, and tissue types that provide replicable and scalable endpoints for assessing risk.

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The effects of methylmercury on wildlife: A comprehensive review

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The effects of methylmercury on wildlife

1.0 Abstract:

Published findings indicate that reasonable effects concentrations can be developed for certain taxa, forage guilds, and tissue types that provide replicable and scalable endpoints for assessing risk. The effects of mercury (Hg) on wildlife from 162 studies (n=98 for birds and n=64 for mammals) can generally be grouped under five categories: physiology, neurology, behavior, reproductive, and survival. Studies were differentiated between laboratory, captive, or experimentally manipulated efforts vs. studies of free-living populations. Recent findings demonstrate that (1) available methylmercury (MeHg) can significantly affect the reproductive success for wild populations of birds and mammals, (2) piscivores are at great risk, but invertivores are often times at equal or even at greater risk, especially those taxa associated with prey from wetland habitats, and (3) bird species (and likely mammals) have at least a 11 fold difference in their sensitivity to MeHg toxicity. Changes from the traditional use of point estimates such as lowest observed effect levels to the use of specific effects concentrations (EC) provides a higher resolution approach for assessing risk. The EC level of interest for decision makers should consider the demographics and conservation status of the target species. A 20% or greater loss within a wild bird population may adversely impact long-term sustainability (or an EC_{20}). Based on studies for two foraging guilds of birds, the approximate EC_{20} using blood for piscivores is 2.0 ug/g (wet weight; based on the common loon, *Gavia immer*) and for invertivores is 1.2 ug/g (wet weight; based on the Carolina wren, *Thryothorus Iudovicianus*). For mammals, there is greater uncertainty about effects thresholds and lowest observed effect levels (LOELs) are still used because, unlike birds, there are no studies on wild populations describing the impacts on reproductive success. Based on fur Hg concentrations (fresh weight) that relate to biochemical changes in the brain (e.g., ~ 1.0 ug/g, wet weight) a reasonable LOEL for piscivores is 35 ug/g and for invertivores is 10 ug/g. Risk and injury assessments can be used at the individual level, such as through regulatory options with the federal Natural Resource Damage Assessment and Restoration (NRDAR) program, or at the more traditional population level that does not account for the full loss of common resources.

2.0 Background:

Mercury (Hg) commonly enters ecosystems through the air (e.g., emissions from coal-fired power plants and incinerators) or water (e.g., both inactive and active chlor-alkali facilities and landfills) across eastern North America (Evers et al. 2005a, 2011). Inorganic Hg emitted from natural or industrial sources becomes toxic in the environment when it is converted to methylmercury (MeHg or Ch3Hg+), often by sulfur-reducing bacteria. Certain ecosystem conditions (such as those found in wetlands) can encourage the production and bioavailability of MeHg in the environment. Bacteria often produce more MeHg when moderate amounts of sulfate and low oxygen (anoxic) conditions are present to provide optimal conditions for the metabolic processes of the bacteria. Mercury also readily binds to dissolved organic carbon (DOC), so areas with high DOC levels may generate MeHg more readily, as will areas that have acidified conditions. These factors are important in assessing ecosystems sensitive to both Hg input and methylation potential. The complex chemical conversions and cycling of Hg make it particularly challenging to predict from air, water and sediment to levels of potential concern to upper trophic level fish and wildlife (Driscoll et al. 2007). In areas where Hg deposition is low, effects on biota may be disproportionately high if conditions are conducive to MeHg production and bioaccumulation. A robust example is in southern Nova Scotia's Kejimkujik National Park, where deposition levels are low, but concentrations in fish and birds tissue are above ecological health thresholds (Burgess and Hobson 2006; Burgess and Meyer 2008) and trends in fish Hg concentrations continue to increase (Wyn et al. 2010).

Mercury is a potent neurotoxin that can cause physiological, neurologic, behavioral, reproductive, and survival harm to wildlife. It readily biomagnifies, resulting in increasing concentrations of MeHg in the ecosystem as it moves from water and sediment, to plants, aquatic insects, spiders, fish and wildlife. Once MeHg is absorbed by organisms, it is generally eliminated very slowly. As a result, top predators in a food web, such as birds and mammals that prey on items that are themselves high in the tropic foodweb, may have concentrations of MeHg in their tissues that are many orders of magnitude higher than the concentrations found in the water (often > 10^6 to 10^7 higher).Generally, each trophic change in the foodweb accounts for an order of magnitude of increase in MeHg concentrations.

Mercury poisoning has been well documented in wildlife across North America, in areas with both point sources of contamination and remote from such sources (i.e., >100 miles) (Scheuhammer et al.

2007; Wolfe et al. 2007). Numerous studies, particularly recent ones, document adverse impacts such as reduced reproductive success, behavioral changes, such as reduced time incubating, and neurological problems such as ataxia. Based on recent *in situ* studies, the biomagnification and bioaccumulation of MeHg is shown to adversely affect the reproductive success of many wildlife populations, across many habitats and geographic areas of North America, representing multiple foraging guilds.

Building on recent and compelling evidence, different bird species vary in their sensitivity to MeHg toxicity (potentially based on foraging guilds and phylogeny) (Heinz et al. 2009). Passeriforms (i.e., songbirds) for example, appear to be highly sensitive to the toxicity of MeHg when compared to other orders of birds. Evidence to date indicates songbirds are more sensitive to MeHg toxicity on hatching and fledging success when compared to piscivores. Similarly, while there are parallel efforts to determine Hg thresholds for mammals, and many of the early studies were conducted on piscivorous mammals such as furbeaers, invertivores such as bats are also at great risk to environmental Hg loads. If dietary uptake of MeHg in American mink (*Neovison vison*) of 0.10 ug/g (ww) creates aberrant behavior and adverse neurological signs (Basu et al. 2007a) then similar or regularly higher MeHg concentrations found in spiders and many other invertebrates may have similar impacts to bats and other invertivore mammals. Understanding MeHg in foodweb pathways and the ability of MeHg to adversely impact upper trophic level wildlife is critical for developing comprehensive assessments. These and other quantitative approaches indicate that referred studies on the effects of Hg on wildlife have been steadily increasing over the past three decades and that publications during the 2010s are projected to be particularly robust (Figure 1).

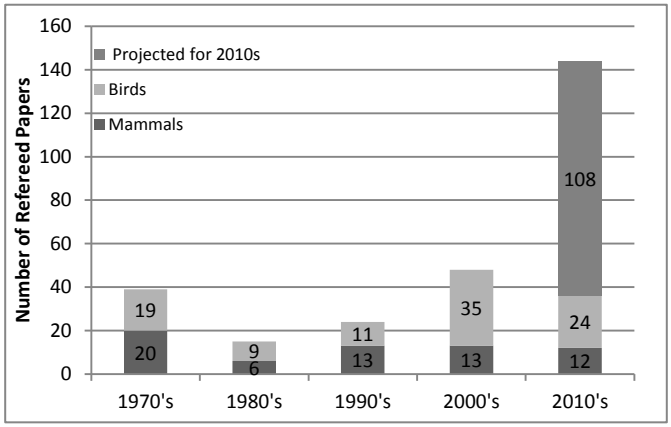

Figure 1. Number of refereed publications on Hg effects for mammals (n=64) and birds (n=98) per decade, including the projection for 2010's.

The effects of Hg on wildlife are varied and complex. Fully comprehending the mechanisms of MeHg toxicity and the magnitude of its effect on the endpoint of interest generally requires (1) insight from laboratory studies, (2) robust and properly designed field studies, and (3) a target species that is sensitive to MeHg.

3.0 Methods:

As part of the process to identify relevant effect thresholds for biota of interest, further definition with how to interpret effects (i.e., a comparison of traditionally-used lowest observed adverse effect levels vs. recently-preferred effects concentrations), understanding choice of tissue types and what they mean, and the importance of taxonomic differences are critical approaches emphasized herein.

3.1 Defining Thresholds

The basis for identifying the threshold of effects for MeHg (or any contaminant) is an evolving science. Often, a hypothesis-based estimate of toxicity is developed. For example, a no observed adverse effect level (NOAEL) is the highest concentration by experiment or observation, which causes no detectable adverse change in the target organism that is not significantly different to the control (usually at a $p<0.05$). The lowest observed adverse effect level (LOAEL) is the lowest concentration by experiment or observation, which causes an adverse change measured in the target organism that is significantly distinguishable from the control. However, the use of NOAELs, LOAELs and similar terminology (e.g., no observed effect concentration [NOEC]) may not be the best approach for transferring toxicity assessment information to decision-makers, particularly NOAELs (Hokestra and Van Ewijk 1993, Chapman et al. 1996). A fundamental reason for avoiding use of NOAEL/NOECs is that they are not conservative – they imply no effect, but the magnitude of the biological effect may range from 10-30% and, clearly a LOAEL reflects an even larger, often times unknown biological effect (Warne and van Dam 2008).

The use of laboratory and field-based studies as a basis for developing threshold effects is also an approach in ecotoxicology that is changing. Historically, laboratory dosing studies were relied on for predicting threshold effect levels through models that used multiple uncertainty factors to convey uncertainties such as species sensitivity of toxicity effects to biologically-measureable endpoints (i.e., reproduction) (Wolfe et al. 2007). Controlled laboratory studies produce results that rely on a lethal dose (LD) or lethal concentration (LC) point estimate that use a probit procedure to calculate the median (50%) and 95% confidence interval. Heinz et al. (2009) used this approach by categorizing LC_{50s} for Hg dosed bird eggs into three groups. The direct use of applying laboratory findings to wild populations is limited by the uncertainty of how the study organism responds to contaminant dosing in captivity without external stressors (e.g., weather, requirements for avoiding predators and searching for food) and if its toxicity is greater because of the type of application (e.g., MeHg injected into eggs is likely

more toxic than maternally deposited MeHg). External stressors appear to be quite important for how organisms respond with elevated body burdens of Hg, based on captive bird dosing sudies (D. Cristol, pers. com.).

An approach that uses a combination of controlled laboratory and empirically-based studies is optimal for deriving adverse effects thresholds of highest confidence. However, parallel species-specific studies are very rare for birds (Appendix I) and mammals (Appendix II). The inherent limitation for linking laboratory-generated adverse effects thresholds with ones in free-living populations is potentially large. Therefore, while LD_{50s} generated in controlled laboratory studies are insightful for understanding the dose-effect relationships, pharmacokinetics, and relevant baseline effect endpoints, they are limiting with their application in the field. Meanwhile, LOAELs generated in either the laboratory or *in situ* are limiting in their ability to capture the magnitude of risk or injury from MeHg.

I offer here an approach toward assessing adverse effects of MeHg using the point estimate toxicity data that is attractive to decision-makers (i.e., the "bright line" approach), but is integrated with demographic and natural history knowledge of the target species. The effects concentration (ECx) approach is a parametric estimate of the no effect concentration (Van der Hoeven 1997), where 95% confidence intervals can also be used to create a bounded effects concentration (BECx) (Hoekstra and Van Ewijk 1993). Using ECx or BECx approaches have been challenged by the question of what percent of the no or lowest observed adverse effects are acceptable by regulatory agencies or decision-makers. Warne and van Dam (2008) arbitrarily recommend an EC_{10} as a level intended to reflect no effects (i.e., the contamination concentration in the diet or tissue where 10% of the data are above effect thresholds at a chosen endpoint). However, a better approach is to use an EC level that is ecological relevant within the spatial scale of interest (e.g., local, regional, rangewide). Ecological relevance can be based on the population growth rate or lambda, which with proper understanding of habitat quality, population size, and population structure can estimate the size of the buffer or surplus population (Newton and Brockie 1998). Generally, 20% of a bird's population serves as a non-breeding component and removing potential local recruits over that level may create a population sink and result in a decline of the population or the need for recruitment from neighboring populations. Therefore, an EC_{20} is recommended as a default threshold level to be used based on an endpoint of fledging success[1], which

[1] This does not take into account mortality of post-fledged individuals that are adversely impacted by MeHg.

reflects the collective impacts for three general reproductive endpoints including percent of egg-laying pairs, eggs hatched, and chicks fledged.

3.2 Tissue types and Hg speciation

Concentrations of Hg body burdens measured in wildlife are an important way to determine specific exposure levels (vs. using models), understand the origin of MeHg dietary uptake, and potential adverse effects (chronic and acute). The choice of tissue types depends on sampling options, temporal interest, and ability to conduct analysis for MeHg (Table 1); the Hg species that most keratin-based materials are comprised of is methyl (Evers et al. 2005b, Wolfe et al. 2007, Eagles-Smith et al. 2008). This allows for analyses to be conducted on total Hg – a more cost effective analysis.

Table 1. Tissue types commonly used for wildlife and associated information.

Common Tissue Types and unit used *(wet weight=ww, dry weight=dw, fresh weight=fw)*	***Sampling options***	***Temporal representation***	***% MeHg***
Blood (ww)	Non-lethal	Days to weeks	>95%
Egg (ww or dw)	Non-lethal/lethal	Days to weeks	>95%
Feather (fw)	Non-lethal	Days to years	>95%
Fur (fw)	Non-lethal	Days to years	>95%
Claw tip (fw)	Non-lethal	Weeks	>95%
Brain (ww or dw)	Lethal	Weeks to months	>95%[1]
Muscle (ww)	Non-lethal/lethal	Weeks to years	>95%
Liver (ww or dw)	Non-lethal/lethal	Years	<10%
Kidney (ww or dw)	Lethal	Years	<10%

[1]Northern river otter (*Lontra canadensis*) are a well known exception with >80% of Hg in the brain as methyl (Haines et al. 2010).

Developing relationships among tissue types is important for purposes of comparison with literature exposure and effects values, sampling ability, and interpreting species-specific pharmacokinetics (Table 2). When considering tissue relationships, age and life stage both need to be considering factors. For example common loon (*Gavia immer*) feathers in pre-fledged chicks significantly correlate with their blood (r^2=0.66), but this is not the overall case for adults (r^2=0.20) (Evers et al. 1998), because adults molt the flight feathers that were sampled in the winter, separate from the breeding area. The magnitude of the Hg body burden also plays a role. Again, in the case with the common loon, individuals with high Hg body burdens had the greatest breeding blood and feather relationship (r^2=0.48) (Evers et al. 1998). This is likely because the MeHg bound in the muscle tissue

during the breeding season is remobilized during the winter remigial molt and is expressed in the feathers alongside the dietary MeHg uptake at the time of molt.

Table 2. Inter- and intra-correlation strength for paired tissues representing traditionally lethal (liver, kidney, muscle and brain) vs. non-lethal (blood, egg, feather) sampling groups. Biopsies of organs and muscle tissue are now possible, as is non-harmful micro-sampling of viable eggs (Stebbins et al. 2009).

Paired tissue and explanatory factor	***General Correlation Strength***	***Comments and clarification***	***Reference***
BIRDS			
Adult blood and juvenile blood	High	Best relationship is with adults during breeding season	Evers et al. 1998, 2005
Adult blood and egg	High	See data for songbirds (Figure 2)	Evers et al. 2003, Heinz et al. 2010b
Adult blood and liver/kidney	High	Strong correlation with liver/kidney total Hg and MeHg	Eagles-Smith et al. 2008
Adult blood and muscle	High	Strongest for permanent residents	Eagles-Smith et al. 2008
Adult blood and brain	High	Assuming that the blood-brain barrier does little to restrict the flow of MeHg	None
Adult blood and feather	Low-High	Correlation is based on when the feather is molted in	Eagles-Smith et al. 2008, Evers et al. 2005b
Adult feather and egg	Low	Assuming molt is outside of breeding area and eggs reflect local dietary uptake	None
Adult feather and muscle	Low-High	Tremendous variation depending on species and life stage	Eagles-Smith et al. 2008
Adult feather and liver/kidney	Low-Mod	Pharmacokinetics of demethylation in the organs and depuration in feather requires further study	Eagles-Smith et al. 2008
Adult feather and brain	Mod	Assuming circulating blood MeHg is similarly deposited in muscle and brain tissue.	None
Adult feather and egg	Mod	Assuming egg Hg concentrations relate to diet and body burden	BRI Unpubl. data
MAMMALS			
Fur and brain	High	Critical relationship for field use to relate to effects concentrations	Klenavich et al. 2008, Strom 2008
Fur and muscle	High	Correlation is important for predicting human health concerns for food items	Strom 2008
Fur and liver	Mod-High	May vary by species (e.g., American mink and northern river otter)	Klenavich et a. 2008, Strom 2008
Fur and kidney	High	May vary by species (e.g., American mink and northern river otter)	Strom 2008
Brain and liver	High	Demethylation for both tissues causes variation	Klenavich et al. 2008, Strom 2008

Eagles-Smith et al. (2008) developed robust tissue relationships among blood, muscle, liver, kidney, and feathers (i.e., breast and head) for four species, the American avocet (*Recurvirostra americana*), black-necked stilt (*Himantopus mexicanus*), Caspian tern (*Sterna caspia*) and Forster's

terns (*Sterna forsteri*). Regression models were developed among the tissue types and represent the best dataset to date for inter-tissue conversions and ratios. Generally, Eagles-Smith et al. (2008) found that for all birds combined in their dataset, blood is highly correlated with muscle (r^2=0.90), liver (r^2=.088) and kidney (r^2=0.88). The muscle-feather relationships ranged widely (i.e., r^2=0.29 for head feathers in Caspian terns to r^2=0.78 in head feathers for American avocets) and were strongest for pre-breeding adults (r^2=0.71 for head feathers, but r^2=0.49 for breast feathers). The pharmacokinetics of MeHg depuration and demethylation need to account for life history and stage.

The relationship between adult blood and eggs is particularly important to develop and create interchangeable Hg concentrations because blood sampling is non-lethal and convenient in the field, while egg Hg concentrations provide tissue-relevant insight into the potential adverse effects of Hg on reproductive success. Paired egg and female blood Hg concentrations from tree swallows (*Tachycineta bicolor*) representing multiple habitats indicate a strong relationship between these tissues (r^2=0.91; Figure 2). A similarly strong relationship is documented for piscivores, such as the common loon (Evers et al. 2003). Variation among these correlations is likely related to intra-clutch differences of Hg in the eggs, which is known to vary 10x (Evers et al. 2005b). While there are minor taxonomic differences in the slope of blood-egg relationships, a relevant generic one based on invertivores and piscivores can be generated.

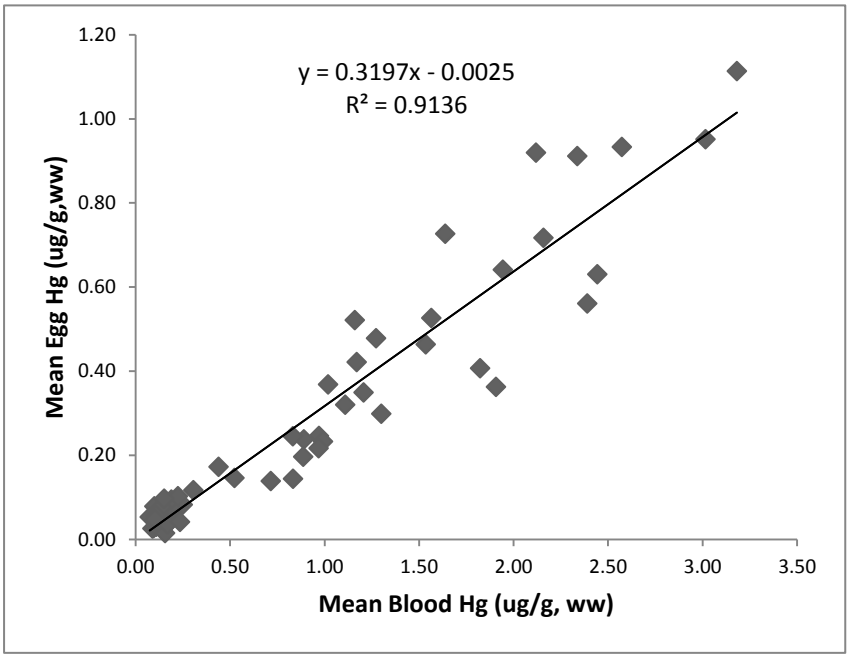

Figure 2. Simple linear regression of paired blood and egg Hg levels from free-living tree swallows (unpublished data from Biodiversity Research Institute and College of William and Mary).

3.3 Taxonomic differences

A review of the egg-dosing study by Heinz et al. (2009) provides the best basis for understanding the differing sensitivities of species to MeHg exposure. A total of 26 bird species were injected with multiple doses of MeHg into the air cells of eggs, where thereafter dose–response curves of embryo survival were developed. An LC_{50} was developed for 23 species and they were ranked according to their sensitivity to injected MeHg. Three rankings were used to group species: low, moderate, and high (Figure 3). Based on previous studies where MeHg was fed to breeding adults and monitored through the eggs, injected Hg from the Heinz et al. (2011) study was considered to be more toxic that that naturally deposited, therefore the specific LC_{50s} determined are likely not representative of the response by wild populations, however the ranking of ring-necked or common pheasants (*Phasianus colchicus*), chickens, and mallards (*Anas platyrhynchos*) parallel findings from other studies and therefore the taxonomic order is considered to be relevant.

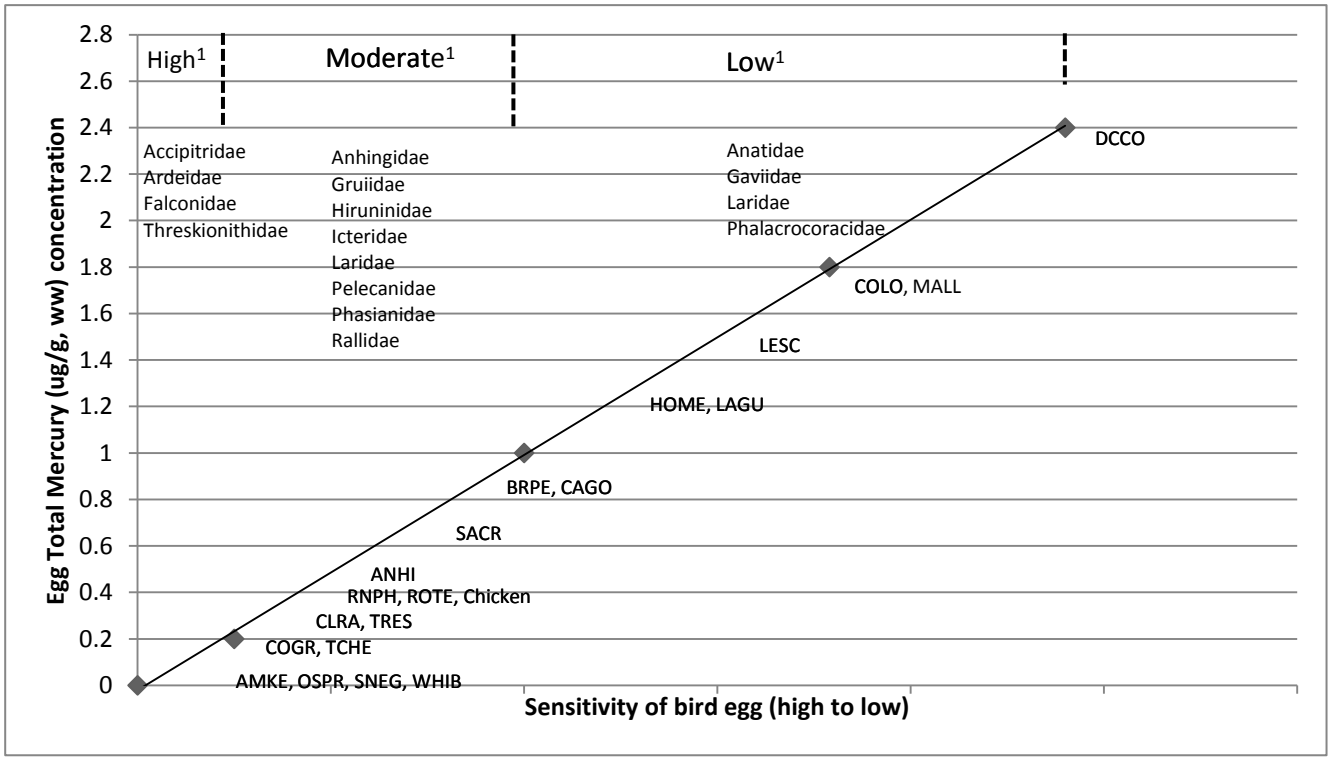

Figure 3. Sensitivity differentiation in 20 bird species based on 50% lethal dose (LD_{50}) of MeHg injected into eggs (Heinz et al. 2009). Approximate LD_{50s} are shown for each species. The common loon is included based on Kenow et al. 2011.

[1] Bird egg sensitivity to MeHg is provided categorically (high, moderate, low). Bird family names are listed represented bird species within each category.
[2] Four-letter codes are for the following species in alphabetical order: AMKE=American Kestrel, ANHI=Anhinga, BRPE=Brown Pelican, CAGO=Canada Goose, CLRA=Clapper Rail, COGR=Common Grackle, COLO=Common Loon, COTE=Common Tern, DCCO=Double-crested Cormorant, HEGU=Herring Gull, LAGU=Laughing Gull, LESC=Lesser Scaup, HOME=Hooded Merganser, MALL=Mallard, OSPR=Osprey, RNHP=Ring-necked Pheasant ROTE=Roseate Tern, SACR=Sandhill Crane, SNEG=Snowy Egret, TCHE=Tri-colored Heron, TRES=Tree Swallow, WHIB=White Ibis
[3] Because of insufficient data, the American Avocet, Black-necked Stilt, Caspian Tern, and Great Egret are not included.

Low ranked species represent the family Anatidae, Gaviidae, Laridae, and Phalacrocoracidae – all families that represent waterbirds that prey on fish and often times include marine ecosystems within their life cycle. Marine birds are generally considered to be more tolerant to MeHg toxicity. Moderately ranked species represent a broad mix of bird families that represent different forage guilds and associations with terrestrial, freshwater, and marine ecosystems. However, terrestrial invertivores including rails (Rallidae), blackbirds (Icturidae), and swallows (Hirundinidae) tended to more sensitive than other moderately ranked species. Species ranked as highly sensitive to MeHg toxicity included raptors from the family Accipitridae and Falconidae and two species that regularly forage on aquatic invertebrates from the families Ardeidae (wading birds) and Threskionithidae (ibis).

In summary, Hg reproductive thresholds may vary 11 fold among birds and therefore consideration needs to be taken for forage guild and life histories. For this report, reproductive thresholds will be emphasized for piscivores, invertivores, and omnivores based on three commonly sampled tissue types (blood, egg and feather) using effects concentrations relevant to demographic considerations.

4.0 Results and Discussion

A total of 162 published studies were reviewed and their findings are summarized for birds (n=98 studies) in Appendix I and mammals (n=63) in Appendix II. There are multiple endpoints for measuring the effects of MeHg toxicity on wildlife. They are generally grouped under five general categories: physiology, neurology, behavior, reproductive, and survival; for this report the effects of MeHg toxicity are further partitioned into multiple subcategories for birds (Appendix I) and mammals (Appendix II). Studies are differentiated between laboratory, captive, or experimentally manipulated studies vs. studies of free-living populations. Supplemental data provide detailed background information for Appendix I and II and are provided under a separate heading.

4.1. Summary of Sublethal Effect Endpoints – Birds

Of the 98 studies reviewed, nearly all represent undomesticated birds. While there are many laboratory studies that used Hg dosing regimes with domesticated birds, such as chickens and ducks, those studies were generally not included in this analysis (only four studies were included that were particularly relevant). Physiological effects of Hg in birds include abnormal histopathology, chromosomal alteration, disrupted blood and organ biochemistry and hormone levels, suppression of the

immune system, inhibition of growth, and increased parasite loads as cited by 35 studies with 66% in lab-based studies (Appendix I). Significant sublethal physiological effects are known in 11 wild bird species (10 piscivores, 1 invertivore). Neurological effects of Hg include neurochemical aberrations and ataxia (lack of voluntary coordination of muscle movements) as cited by 17 studies with only two studies on wild populations of the common loon and bald eagle (*Haliaeetus leucocephalus*) (Appendix I). Behavioral effects of Hg include two subcategories, aberrant behavior and altered song, as cited by 19 studies; in the wild, observations included significant changes in behavior for common loons and in song abilities by the Carolina wren, house wren (*Troglodytes aedon*), song sparrow (*Melospiza melodia*), and Nelson's sparrow (*Ammodramus nelsoni*) (Appendix I). Reproductive effects of Hg include reduced fertility and sex-biased reproduction, reduced embryo production or fitness and hatching success and abnormal development, and aberrant reproductive organ development and sexual maturation (n=31 studies; Appendix I). Of these, a series of studies with mallards, wading birds and a seminal study by Heinz et al. (2009) provide tremendous insights from laboratory and controlled experiments.

Adverse impacts to wild populations have emphasized the common loon and recent various songbird species, with a key study on Carolina wrens (Jackson et al. 2011a). While there are limitations and challenges in using laboratory or captive-oriented studies to inform *in situ* responses of wild bird populations, experimental designs that can control laboratory variables indicate that measurements of the effects of Hg on reproductive success can be similar between lab-based and wild-based studies for same or similar taxa (Figure 4). Effects of Hg on survival are measured by lower rates in embryos, juveniles, and adults and were documented in 24 studies (Appendix I), including lower juvenile survivorship for common loons and tree swallows. No significant effects were recorded in 22 other studies, including wild populations of 16 species.

A comparison of lab and *in situ* studies for four avian taxa (common loon, mallard, *Sterna* tern species, and songbirds indicate similarities in effects concentrations of Hg for a common endpoint, lowered reproductive success (Figure 4). As part of a field dosing study in Wisconsin, Kenow et al. (2011) found 25% lower hatching success at an egg Hg concentration of 0.90 ug/g (ww), which is similar to findings based on egg (1.02 ug/g, ww; Evers et al. 2003) and converted blood (0.86 ug/g, ww; Evers et al. 2008) Hg concentrations from a closely-monitored, wild breeding population of common loons in Maine and New Hampshire. Smaller-bodied piscivores, such as Forster's (130-190g) and

common (*Sterna hirundo*;110-140g) terns, are more sensitive to MeHg toxicity than larger bodied piscivores, such as the common loon (2,800 to 7,500g). A reduction of hatching success by 25% occurs at egg Hg concentrations of 0.43 ug/g, ww in the lab or 0.58 ug/g, ww in the field (Eagles-Smith and Ackerman 2010).

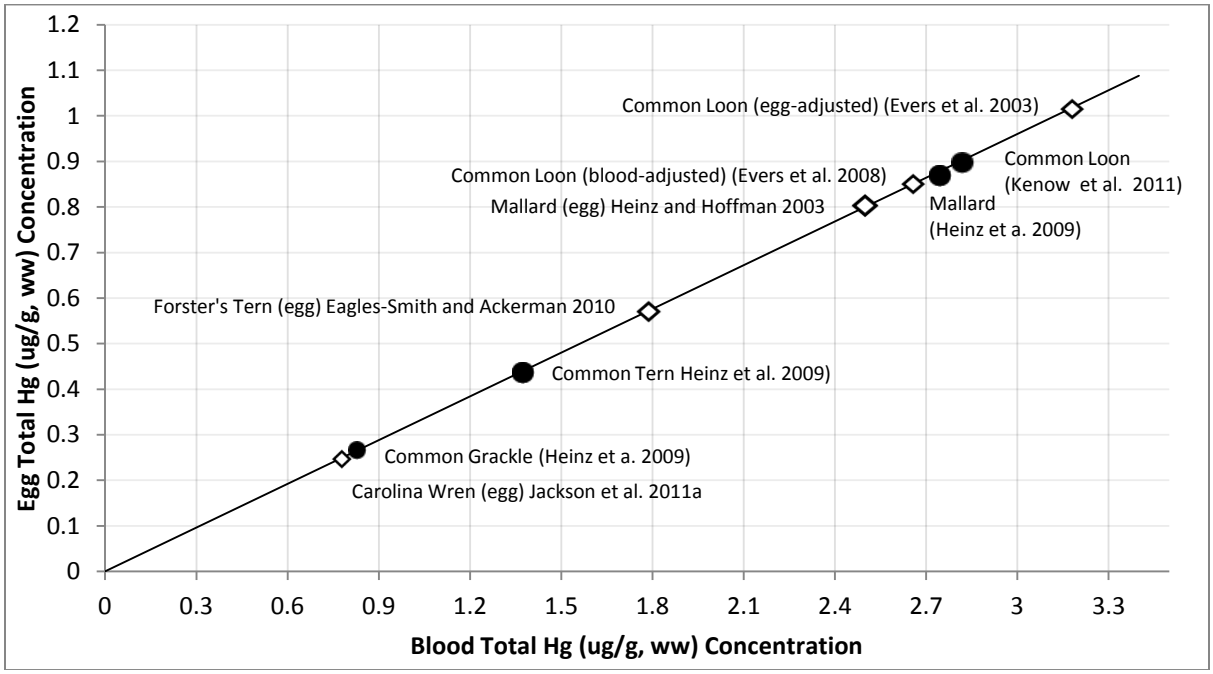

Figure 4. Standardized comparison of lab (solid circle; Heinz et al. 2009; Kenow et al. 2011) and *in situ* (open diamonds) effects concentrations that represent 25% fewer hatched eggs for four avian taxa: (1) common loons, (2) mallards, (3) *Sterna* tern species, and (4) songbirds – common grackle (*Quiscalus quiscula*) and Carolina wren.

4.2. Designated effect thresholds - birds

Because effect thresholds vary according to tissue type and are differentially represented by taxa, foraging guild or sublethal endpoints a summary by tissue type is emphasized (Table 3). Two major foraging guilds (piscivores and invertivores) use an endpoint of overall reproductive success with reductions ranging from 10-50% (Table 3). Blood Hg effects concentrations (EC) are used with overall reproductive success for piscivores and invertivores instead of LOAELs/NOAELs. Overall reproductive success is the combined impact of reduced egg laying, egg hatching, and chicks surviving. Details on ECs for piscivores and invertivores are provided herein, while omnivores, such as the mallard, are best represented by LOAELs using 0.79 ug/g (ww) for eggs (Heinz and Hoffman 2003) and 9.0 ug/g (fw) for feathers (Heinz et al. 1976).

Table 3. Estimated effect Hg concentrations in birds using an endpoint of lowered reproductive success for four levels of impact. Tissue type concentrations are shown for blood and eggs (wet weight) and feathers (fresh weight).

Taxonomic Group	*Species used for designation*	*Tissue Type / effect endpoint*	*EC10*	*EC20*	*EC30*	*EC40*	*Citation*
Bird - Piscivores	Common Loon	Adult blood / fewer fledged young	1.5 ug/g	2.0 ug/g	2.5 ug/g	3.0 ug/g	Burgess and Meyer 2005, Evers et al. 2008
		Egg / lowered hatching success	0.48 ug/g	0.65 ug/g	0.80 ug/g	0.98 ug/g	Evers et al. 2003, 2008
		Adult feather / equivalent to fewer fledged young[1]	10 ug/g	20 ug/g	30 ug/g	40 ug/g	Evers et al. 2008, unpubl. data
Bird – Invertivores	Carolina Wren	Adult blood / lowered nesting success	0.70 ug/g	1.2 ug/g,	1.7 ug/g	2.2 ug/g	Jackson et al. 2011a
		Egg / equivalent to lowered nesting success	0.11 ug/g	0.20 ug/g	0.29 ug/g	0.36 ug/g	Jackson et al. 2011a
		Adult body feather / equivalent to lowered nesting success	2.4 ug/g	3.4 ug/g	4.5 ug/g	5.3 ug/g	Jackson et al. 2011a

[1]Adult piscivore feather ECs are estimated and are based on the finding for an EC_{40} of 40 ug/g (fw) in secondary feathers.

4.2.1.Piscivore Birds

Avian piscivores can be at high risk to MeHg toxicity, especially species that are long-lived and wholly piscivorous throughout their annual cycle (Scheuhammer et al. 2007; Wolfe et al. 2007). Species where there are known significant effects from Hg in wild populations include the common loon (physiological, neurological, behavioral, reproductive, survival), grebe species (physiological), double-crested cormorant (*Phalacrocorax auritus*)(physiological), heron and egret species (physiological), tern and gull species (physiological, reproductive), bald eagle (neurological, reproductive), and belted kingfisher (*Megaceryle alcyon*) (reproductive).

The common loon is the best studied avian piscivore for determining the exposure and effects of Hg (Kenow et al. 2007; Burgess and Meyer 2008; Evers et al. 1998, 2003, 2008; Scheuhammer et al. 2008; Meyer et al. 2011; Depew et al. 2012). Three independent and geographically distinct studies have parallel findings where blood Hg concentrations between 3.0 to 3.5 ug/g (ww) cause significant impacts to reproduction (approximately 40-50% fewer fledged young produced) and blood Hg concentrations as low as 1.5 ug/g (ww) can reduce overall productivity by 10% (Evers et al. 2008; Burgess and Meyer 2008; Schoch et al. 2011) (Table 3). The blood-egg relationship in common loons is well established and effect concentrations from field studies (Evers et al. 2003) and dosing studies

(Kenow et al. 2011) closely agree (Figure 4). For the common loon, eggs are a relevant tissue to represent dietary uptake for MeHg on the breeding territory. Not all bird egg Hg concentrations share that relationship, therefore care is needed to understand the life history and feeding ecology of target species for risk assessments. Feather Hg concentrations represent both the MeHg concentrations in the muscle tissue and dietary uptake of MeHg at the time of feather growth (Burger 1993; Evers et al. 2005b; Wolfe et al. 2007). Feather Hg concentrations increase as MeHg bioaccumulates in the muscle tissue over time, especially if the physiological limits of depuration and demethylation into organs is exceeded.

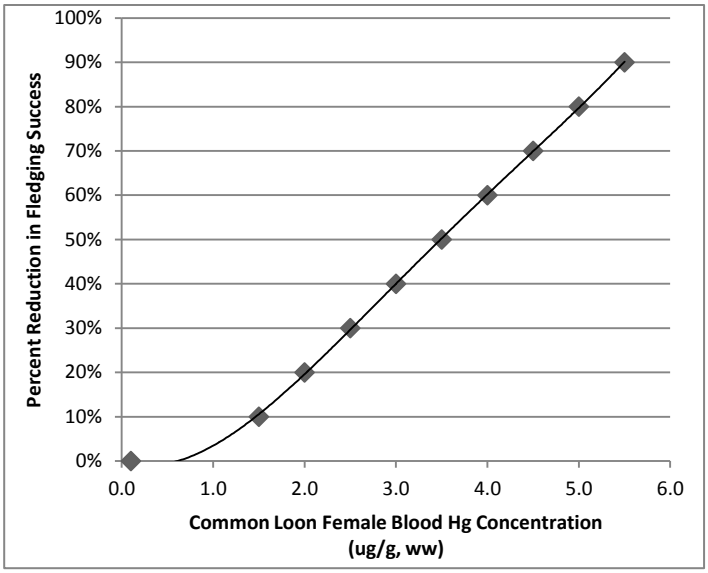

Figure 5. Relationship between blood Hg concentrations of female common loons and overall reproductive success, based on studies in Wisconsin and Nova Scotia (Burgess and Meyer 2008), Maine and New Hampshire (Evers et al. 2008), and New York (Schoch et al. 2011).

For the common loon, high risk individuals (i.e., >3.0 ug/g, ww in the blood and 40 ug/g, fw in the feather) have an average annual increase in feather Hg concentrations of 10% (Evers et al. 2008); therefore, individuals at high risk to MeHg availability will increase their Hg body burdens with age.

The dietary dose of concern is also well established for loons (Table 4). Based on a synthesis by Depew et al. (2012), fish MeHg concentrations of 0.10 ug/g (ww, whole body) causes aberrant behavior while a diet of 0.18 ug/g (ww) is related to 50% lower hatching success and fish at 0.40 ug/g (ww) causes complete reproductive failure. These threshold levels are generally indicative of harm for piscivorous birds and therefore represent an important advancement in identifying risk.

Table 4. Summary of Proposed screening benchmarks for common loon exposure to MeHg. Screening benchmarks are expressed as Hg concentrations in prey fish (µg g-1 wet weight) (from Depew et al. 2012).

Category	Proposed Screening Benchmark Threshold (ug/g, ww)	Endpoints considered	Primary References
Adult Behavioral Abnormalities	0.10	Geometric mean of adult behavior LOAEL	Evers et al. 2004
Significant Reproductive Impairment	0.18	Geometric mean of productivity LOAEL and EC_{50}, hatch success EC_{50}	Evers et al. 2004, 2008 Burgess and Meyer 2008 Barr 1986 Kenow et al. 2011
Reproductive Failure	0.40	Productivity reduced to zero	Burgess and Meyer 2008 Barr 1986

4.2.2. Invertivore Birds

Avian invertivores can be at high risk to MeHg toxicity, especially species that are associated with wetland habitats (Evers et al. 2005b; Edmonds et al. 2010; Jackson et al. 2011b; Lane et al. 2011) and forage on spiders (Cristol et al. 2008). Species where there are known significant effects from Hg measured in wild populations include the California clapper rail (*Rallus longirostris obsoletus*) (physiological, reproductive), black-bellied plover (*Pluvialis squatarola*) (reproductive), ruddy turnstone (*Arenaria interpres*)(reproductive), semiplamated plover (*Charadrius semipalmatus*) (reproductive), tree swallow (physiological, behavioral, reproductive, survival), eastern bluebird (*Sialia sialis*) (reproductive), Carolina wren (behavior, reproductive), house wren (behavior, reproductive), song sparrow (behavior) and Nelson's sparrow (behavior) (Appendix I).

There are few datasets available that generate adverse effect levels for avian insectivores. Recent laboratory dosing efforts with songbirds by Heinz et al. (2009) provides relevant information for identifying thresholds. Their study was designed to determine the relative sensitivities of MeHg effects on eggs for 26 species. In their study, two species of songbirds were used for the MeHg dosing experiments – the tree swallow and common grackle. The swallow and blackbird had median lethal levels (LC_{50}) of 0.32 ug/g (ww) and 0.26 ug/g (ww), respectively, in eggs (Heinz et al. 2009).

Survival, reproduction, immune response, song, and endocrine function are all aspects of songbird ecology that may be adversely affected by elevated blood Hg levels (Hallinger et al. 2010, Brasso and Cristol 2008, Hawley et al. 2009, and Wade et al. 2009). Brasso and Cristol (2008) studied tree swallows along the South River in Virginia and found that second-year birds along a polluted section of river produced fewer chicks than those in the uncontaminated reference area. The percentage of tree swallow eggs that survived to produce a fledgling was significantly lower at the contaminated

site compared to the reference site (Brasso and Cristol 2008). However, they were unable to predict nest success based on the female's blood Hg concentration. In response, Jackson et al. (2011a) assessed reproductive success of another terrestrial invertivore, the Carolina wren and successfully developed threshold effects based on the percent reduction of nesting success as the endpoint (Figure 6).

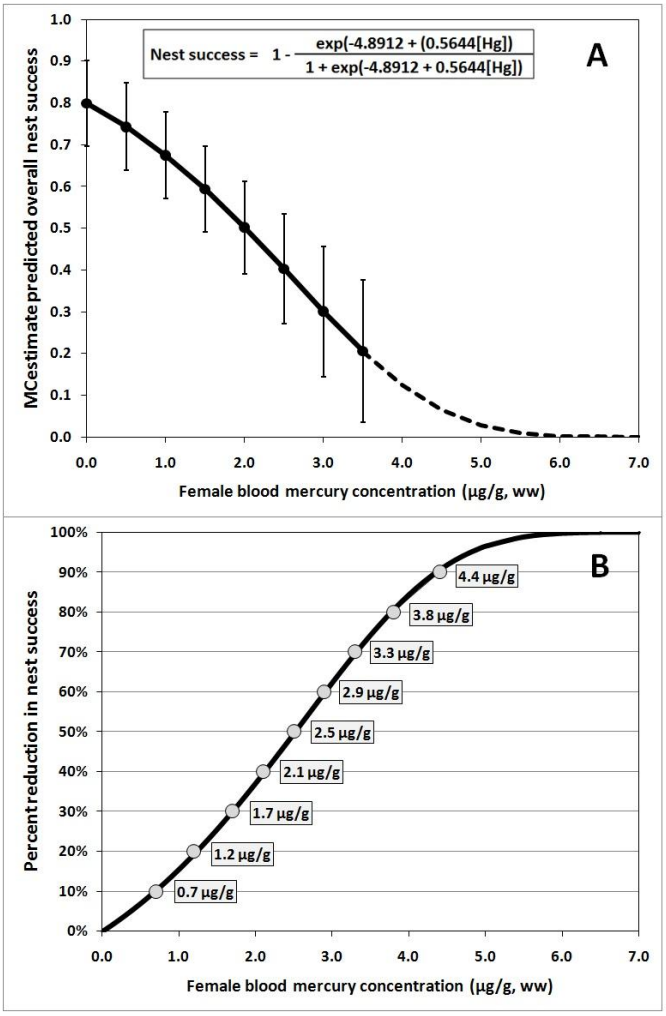

Figure 6. The relationship between MCESTIMATE-modeled Carolina wren nest survival and female blood Hg concentration for nests found in 2010 in Virginia. (A) Predicted Carolina wren nest success over their 30-day nest cycle in relation to female blood Hg concentration when other covariates were held constant (date = 24 May, nest cavity = natural). Error bars indicate SE. Dotted portion of the line indicates model extrapolation past observed female blood Hg concentrations. (B) Percent reduction in nest survival (from nest survival at 0 µg/g) in relation to female blood Hg concentration. Blood Hg concentrations associated with 10% increments of reduction in nest success are shown.

This is the only study to generate blood-oriented effects concentrations related to reproductive endpoints for a wild population. Blood Hg concentrations of 0.70 ug/g were found to reduce nesting success by 10%, while biologically relevant Hg concentrations at 1.20 ug/g reduced nesting success by 20% (Figure 6, Table 3).

In addition, a blood-based Hg effects concentration gradient for Carolina wrens was related to feather and egg tissues to provide inter-tissue conversions (Table 3, Jackson et al. 2011a). Blood, adult feather (body and tail), and egg effect concentrations are now well defined for avian invertivores. While there are likely differences in the sensitivity to MeHg toxicity within the order Passeriformes, the Carolina wren can be used with high certainty for assessing other songbird species. Dietary thresholds of Hg related to reproductive endpoints have yet to be determined.

4.3. Summary of Sublethal Effect Endpoints - Mammals

Of the 64 studies reviewed, 24 (38%) represent domesticated mammals. A larger number of studies using domesticated individuals were included for the mammal summary because there are fewer laboratory or *in situ* studies of wild mammals. Physiological effects of Hg include abnormal histopathology, chromosomal alteration, disrupted blood and organ biochemistry and hormone levels, suppression of the immune system, inhibition of growth, visual and auditory deficits, and increased parasite loads as cited by 37 studies with 80% in lab-based studies (Appendix II). Significant sublethal physiological effects are known in seven wild mammal species. Neurological effects of Hg include neurochemical aberrations and ataxia lack of voluntary coordination of muscle movements) as cited by 25 studies with six studies on wild populations of the little brown bat, American mink, northern river otter and polar bear (*Ursus maritimus*) (Appendix II). Behavioral effects of Hg include one subcategory, aberrant behavior, as cited by 11 studies (Appendix II). Reproductive effects of Hg include reduced fertility, reduced embryo production or birth success or abnormal offspring development, and aberrant reproductive organ development or sexual maturation (n=13 studies; Appendix II). Unlike birds, there are no *in situ* studies examining reproductive impacts in wild mammal populations, which is a major data gap for understanding and scaling the impacts of Hg to mammals. Effects of Hg on survival are measured by lower rates in juveniles and adults and were documented in 10 studies (Appendix II), although there was no studies identified examining the impacts of Hg on wild mammal survivorship. No significant effects were recorded in five other studies.

4.4. Designated effect thresholds – mammals

Because effect thresholds vary according to tissue type and are differentially represented by taxa or foraging guild, and sublethal endpoints a summary by tissue type for two major foraging guilds is emphasized (Table 5). Unlike birds, preferred endpoints such as reproductive success are not available for wild populations of mammals and because studies developing effects concentrations gradients are lacking, the traditional LOAEL approach is used herein. There are a number of studies that have been conducted on the biochemical changes in the brain of various wild mammals species related to Hg concentrations (Basu et al. 2005a,b,c; 2007b,c; 2009) and captive species (Basu et al. 2005c, 2006, 2007c, 2010).

4.4.1. Piscivore Mammals

Mammalian piscivores can be at high risk to MeHg toxicity, especially species that are long-lived and wholly piscivorous throughout their annual cycle (Scheuhammer et al. 2007; Wolfe et al. 2007). Species where there are known significant effects from Hg in wild populations include the beluga (*Delphinapterus leucas*)(physiological), bottle-nosed dolphin (*Tursiops truncatus*) (physiological), polar bear (physiological, neurological), American mink (physiological, neurological, reproductive), and northern river otter (neurological, behavior).

Table 5. Estimated LOAELs for mammals using an endpoint of biochemical changes in the brain. Tissue type concentrations are shown for brain (wet weight) and fur (fresh weight).

Taxonomic Group	***Species Used for designation***	***Tissue Type***	***LOAEL***	***Effect***	***Citation***
Mammal – Piscivores	American Mink and Northern River Otter	Adult Fur	35.0 / 45.0 ug/g, fw	Related to sig. brain biochemical changes	Klenavic et al. 2008, Strom 2008, BRI unpubl. data
		Brain	1.0 ug/g, ww	Sig. brain biochemical changes	Basu et al. 2006
Mammal – Invertivore	Bat spp.	Adult Fur	10 ug/g, fw	Related to sig. brain biochemical changes	Nam et al. 2012; BRI unpubl. data
	.	Brain	1.0 ug/g, ww	Sig. brain biochemical changes	Nam et al. 2012
		Blood	0.05 ug/g, ww	Related to fur levels with sig. brain biochemical changes	Wada et al. 2010

Generally, there are few investigations that have evaluated the effects of MeHg on mammals in the wild. Most studies are lab-controlled dosing studies and a few are inferences from *in situ* evaluations. The brain is a particularly relevant tissue for evaluating toxic effects from MeHg because it is the site known to negatively alter neurochemical receptor-binding characteristics (Basu et al. 2005, 2007a,b). Lowest observed effect levels (LOELs), based on negative alterations to the brain's cholinergic system from an American mink dosing study, is 1.03 μg/g (ww; or 4.10 μg/g, dw) in the brain (Basu et al. 2006). There is a strong relationship between fur and brain Hg concentrations, which permits the comparison of fur with brain Hg concentrations of relevance. Klenavich et al. (2008) found a significant relationship between fur and brain (r^2=0.67 for American mink and r^2=0.74 for the northern river otter), as did Strom (2008) (r^2=0.51 for northern river otter). Based on these two studies and unpublished data from Biodiversity Research Institute, brain Hg concentrations of 1.03 ug/g, (ww) are

equivalent to fur Hg concentrations of 35.0 ug/g, fw and 45.0 ug/g, fw for American mink and northern river otter, respectively.

While there are no field-based studies using reproductive success as an endpoint for piscivorous mammals, there are many exposure and effects studies with two furbearers, the American mink and northern river otter, which are useful for establishing general effects thresholds (Basu et al. 2007a; Klenavic et al. 2008; Strom 2008).

4.2. Invertivore Mammals

An increasing number of published studies have documented Hg exposure to invertivore mammals at levels of potential concern, primarily in bats (Baron et al. 1999; Hickey et al. 2001; Miura et al. 1978; O'Shea et al. 2001; Wada et al. 2010; Walker et al. 2007); yet large knowledge gaps remain with the potential adverse effects of MeHg toxicity. Mammalian invertivores can be at high risk to MeHg toxicity, especially species that are associated with wetland habitats (Evers et al. 2012; Yates et al. Submitted) and forage on spiders. Species that are known to have effects include the big brown bat (*Eptesicus fuscus*)(physiological) and little brown bats (*Myotis lucifugus*) (physiological, neurological) (Appendix II).

Dong-Ha Nam et al. (2012) found bats had Hg-associated neurochemical changes with greater than ~10 ug/g (fw) in fur. Other small mammals, including mice, have similar neurochemical affects from being exposed to Hg. Burton et al. (1977) found that wild mice, eating brine flies from the Great Salt Lake, which had Hg concentrations over 7.8 ug/g (fw) in fur exhibited behavioral deviations and had a decrease in ambulatory activity when compared to a control group. Burton et al. (1977) also found that mice with Hg concentrations in fur of 10.8 μg/g (fw) showed decreased stress tolerance and decreased swimming ability. Baron et al. (1999) completed a risk assessment for aerial insectivorous wildlife on the Clinch River, Tennessee (Oak Ridge Reservation). Using a model, they determined the dose levels for the NOAEL and LOAEL for little brown bats are 0.11 and 0.56 μg/g, respectively.

Similar to investigations and findings with avian invertivores, mammalian invertivores are little studied but are likely under greater risk to MeHg toxicity in the environment than realized.

5.0 Conclusions as related to the Penobscot River Hg Study

In the past few years, significant scientific advancements have been made with the understanding of the effects of MeHg toxicity on wildlife; near-term laboratory and field studies are expected to continue to refine abilities to conduct high resolution assessments. Because scalable and replicable endpoints, such as reproductive success, are being increasingly used assessments should now apply effects concentration (EC) gradients vs. traditional NOAELs and LOAELs. EC gradients for Hg are now available for avian piscivores and invertivores (Table 3). For mammals, LOAELs still need to be used (Table 5) and require further refinements based on reproductive endpoints from wild populations – which are lacking. Further, the realization that there are significant taxonomic difference in the sensitivities to MeHg (Figure 3) creates further research needs to better describe the patterns of these differences.

Should effects concentration thresholds be available for targeted taxa, they can be used on (1) an individual level for assessing injury through federal regulatory purposes, such as through Natural Resource Damage Assessment and Restoration (NRDAR) program by the U.S. Fish and Wildlife Service or (2) a population level. The purpose of the NRDAR program is to restore natural resources that have been impacted by releases of hazardous substances. Several laws provide the foundation for NRDAR, giving natural resource Trustees (i.e., state and federal governments) the legal authority to have those responsible for harm to natural resources pay to restore those resources. For the Penobscot River Mercury Project the relevant regulation is the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). If population-level assessments are conducted, then the best approach is to use effects concentrations based on spatially-explicit demographics of the target species. As an example, 20% of the population of many bird species is represented as a buffer or non-breeding component. Therefore, the loss of over 20% of a bird's population can adversely impact sustainability and would require immigration to recover those losses. For such a scenario, an EC_{20} (when using an overall reproductive success endpoint) would be relevant for protecting the target species at a population level.

Whether using individual or population level assessments, an EC approach should be used for avian piscivores or invertivores and LOELs can be used for mammalian piscivores and invertivores.

6.0 Literature Cited:

Ackerman, J. T., Eagles-Smith, C. A., Takekawa, J. Y., and Iverson, S. A. (2008a). Survival of postfledging Forster's terns in relation to mercury exposure in San Francisco Bay. Ecotoxicology 17:789-801.

Ackerman, J. T., Takekawa, J. Y., Eagles-Smith, C. A., and Iverson, S. A. (2008b). Mercury contamination and effects on survival of American avocet and black-necked stilt chicks in San Francisco Bay. Ecotoxicology 17:103-116.

Ackerman, J. T., Overton, C. T., Casazza, M. L., Takekawa, J. Y., Eagles-Smith, C. A., Keister, R. A., and Herzog, M. P. (2012). Does mercury contamination reduce body condition of endangered California clapper rails? Environmental Pollution 162:439-448.

Albanus, L., Frankenberg, L., Grant, C., von Haartman, U., Jernelov, A., Nordberg, G., Rydalv, M., et al. (1972). Toxicity for cats of methylmercury in contaminated fish from Swedish lakes and of methylmercury hydroxide added to fish. Environmental Research 5:425–442.

Albers, P. H., Koterba, M. T., Rossmann, R., Link, W. a, French, J. B., Bennett, R. S., and Bauer, W. C. (2007). Effects of methylmercury on reproduction in American kestrels. Environmental Toxicology and Chemistry 26:1856-1866.

Anderson, D. W., Suchanek, T. H., Eagles-Smith, C. A., and Cahill, J. T. M. (2008). Mercury residues and productivity in osprey and grebes from a mine-dominated ecosystem. Ecological applications 18:A227-A238.

Anjum, F., and Shakoori, A. (1994). Sublethal effects of inorganic mercury on the body growth rate and liver function enzymes of phenobarbitone-pretreated and promethazine-pretreated rabbits. J. Environmental Pathology, Toxicology and Oncology 13:125–132.

Aulerich, R., Ringer, R., and Iwamoto, S. (1974). Effects of dietary mercury on mink. Archives of Environmental Contamination and Toxicology 2:43-51.

Barata, C., Fabregat, M. C., Cotín, J., Huertas, D., Solé, M., Quirós, L., Sanpera, C., et al. (2010). Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). Environmental Pollution 158:704-710.

Baron, L., Sample, A., Bradley, E. and Suter II, G.W. (1999). Ecological risk assessment in a large river–reservoir: 5. Aerial insectivorous wildlife. Environmental Toxicology and Chemistry 18:621-627.

Barr, J. F. (1986). Population dynamics of the common loon (*Gavia immer*) associated with mercury-contaminated waters in northwestern Ontario. Occasional Paper - Canadian Wildlife Service 56:1-23.

Basu, N., Klenavic, K., Gamberg, M., O'Brien, M., Evans, D., Scheuhammer, A. M., and Chan, H. M. (2005a). Effects of mercury on neurochemical receptor binding characteristics in wild mink. Environmental Toxicology and Chemistry 24:1444-1450.

Basu, N., Scheuhammer, A., Grochowina, N., Klenavic, K., Evans, D., O'Brien, M., and Chan, H. M. (2005b). Effects of mercury on neurochemical receptors in wild river otters (*Lontra canadensis*). Environmental Science & Technology 39:3585-3591.

Basu, N., Stamler, C. J., Loua, K. M., and Chan, H. M. (2005c). An interspecies comparison of mercury inhibition on muscarinic acetylcholine receptor binding in the cerebral cortex and cerebellum. Toxicology and Applied Pharmacology 205:71-76.

Basu, N., Scheuhammer, A. M., Rouvinen-Watt, K., Grochowina, N., Klenavic, K., Evans, R. D., & Chan, H. M. (2006). Methylmercury impairs components of the cholinergic system in captive mink (*Mustela vison*). Toxicological Sciences 91:202-209.

Basu, N., Scheuhammer, A. M., Bursian, S. J., Elliott, J., Rouvinen-Watt, K., and Chan, H. M. (2007a). Mink as a sentinel species in environmental health. Environmental Research 103:130-144.

Basu, N., Scheuhammer, A. M., Evans, R. D., O'Brien, M., and Chan, H. M. (2007b). Cholinesterase and monoamine oxidase activity in relation to mercury levels in the cerebral cortex of wild river otters. Human & Experimental Toxicology 26:213-220.

Basu, N., Scheuhammer, A. M., Rouvinen-Watt, K., Grochowina, N., Evans, R. D., O'Brien, M., and Chan, H. M. (2007c). Decreased N-methyl-D-aspartic acid (NMDA) receptor levels are associated with mercury exposure in wild and captive mink. Neurotoxicology 28:587-593.

Basu, N., Scheuhammer, A. M., Sonne, C., Letcher, R. J., Born, E. W., and Dietz, R. (2009). Is dietary mercury of neurotoxicological concern to wild polar bears (*Ursus maritimus*)? Environmental Toxicology and Chemistry 28:133-140.

Basu, N., Scheuhammer, A. M., Rouvinen-Watt, K., Evans, R. D., Trudeau, V. L., and Chan, L. H. M. (2010). In vitro and whole animal evidence that methylmercury disrupts GABAergic systems in discrete brain regions in captive mink. Comparative Biochemistry and Physiology, Part C 151:379-385.

Beland, P., DeGuise, S., Girard, C., Lagace, A., Martineau, D., Michaud, R., Muir, D. C. G., et al. (1993). Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. Journal of Great Lakes Research 19:766-775.

Ben-David, M., Duffy, L. K., Blundell, G. M., and Bowyer, R. T. (2001). Natural exposure of coastal river otters to mercury: relation to age, diet, and survival. Environmental Toxicology and Chemistry 20:1986-1992.

Betti, C., and Nigro, M. (1996). The comet assay for the evaluation of the genetic hazards of pollutants in cetaceans: preliminary results on the genotoxic effects of methyl-mercury on the bottle-nosed dolphin (Tursiops truncatus) lymphocytes in vitro. Marine Pollution Bulletin 32:545-548.

Bigazzi, P. E., Kosuda, L. L., Hannigan, M. O., Whalen, B., and Greiner, D. L. (2003). Lack of graft-versus-host-like pathology in mercury-induced autoimmunity of Brown Norway rats. Clinical Immunology 109:229-237.

Borg, K., Erne, K., Hanko, E., and Wanntorp, H. (1970). Experimental secondary methyl mercury poisoning in the goshawk (*Accipiter gentilis*). Environmental Pollution 1:91-104.

Bouland, A. J., White, A. E., Lonabaugh, K. P., Varian-Ramos, C. W., and Cristol, D. A. (2012). Female-biased offspring sex ratios in birds at a mercury-contaminated river. J. Avian Biology 43:1-8.

Bouton, S. N., Frederick, P. C., Spalding, M. G., and McGill, H. (1999). Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. Environmental Toxicology and Chemistry 18:1934–1939.

Brasso, R. L., and Cristol, D. A. (2008). Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). Ecotoxicology 17:133-141.

Brasso, R. L., Abdel Latif, M. K., and Cristol, D. A. (2010). Relationship between laying sequence and mercury concentration in tree swallow eggs. Environmental Toxicology and Chemistry 29:1155-1159.

Burbacher, T. M., Grant, K. S., Mayfield, D. B., Gilbert, S. G., and Rice, D. C. (2005). Prenatal methylmercury exposure affects spatial vision in adult monkeys. Toxicology and Applied Pharmacology 208:21-28.

Burger, J. (1993). Metals in avian feathers: Bioindicators of environmental pollution. Reviews of Environmental Toxicology 5:203–311.

Burgess, N.M. and Hobson, K.A. (2006). Bioaccumulation of mercury in yellow perch (*Perca flavescens*) and common loons (*Gavia immer*) in relation to lake chemistry in Atlantic Canada. Hydrobiologia 567:275–282.

Burgess, N. M., and Meyer, M. W. (2008). Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicology 17:83-91.

Burton, G. V., Alley, R. J., Rasmussen, G. L., Orton, P., Cox, V., Jones, P., and Graff, D. (1977). Mercury and behavior in wild mouse populations. Environmental Research 14:30–34.

Castellini, C., Mourvaki, E., Sartini, B., Cardinali, R., Moretti, E., Collodel, G., Fortaner, S., et al. (2009). In vitro toxic effects of metal compounds on kinetic traits and ultrastructure of rabbit spermatozoa. Reproductive Toxicology 27:46-54.

Chapman, P.H., Cardwell, R.S. and Chapman, P.F. (1996). A warning: NOECs are inappropriate for regulatory use. Environmental Toxicology and Chemistry 15:77-79.

Chardonneau, S. M., Munro, I. C., Nera, E. A., Willes, R. F., Kuiper-Goodman, T., Iverson, F., Moodie, C. A., et al. (1974). Subacute toxicity of methylmercury in the adult cat. Toxicology and Applied Pharmacology 27:569-581.

Cristol, D.A., Brasso, R.L., Condon, A.M., Fovargue, R.E., Friedman, S.L., Hallinger, K.K., Monroe, A.P., and White, A.E. (2008). The movement of aquatic mercury through terrestrial food webs. Science 320:335

Custer, C. M., Custer, T. W., and Hill, E. F. (2007). Mercury exposure and effects on cavity-nesting birds from the Carson River, Nevada. Archives of Environmental Contamination and Toxicology 52:129-136.

Dansereau, M., Lariviere, N., & Tremblay, D. D. (1999). Reproductive performance of two generations of female semidomesticated mink fed diets containing organic mercury contaminated freshwater fish. Archives of Envrionmental Contamination and Toxicology 36:221-226.

Depew D.C., Basu, N., Burgess, N.M., Campbell, L.M., Evers, D.C., Grasman, K.A., Kenow, K.P., Meyer, M.W., Scheuhammer, A.M. and Williams, K. (2012). Derivation of screening benchmarks for the common loon (*Gavia immer*) for dietary methylmercury (MeHg): Justification and rationale for use in ecological risk assessment. Environmental Toxicology and Chemistry 31:2399–2407

DesGranges, J.-L., Rodrigue, J., Tardif, B., & Laperle, M. (1999). Breeding Success of Osprey Under High Seasonal Methylmercury Exposure. In M. Lucotte, R. Scholagne, N. Therien, C. Langluis, and A. Tremblay (eds.), Mercury in the Biogeochemical Cycle (pp. 287-293). Berlin, Germany.

DeSorbo, C. R. 2007. Spatial, temporal, and habitat based patterns of mercury exposure in bald eagles in interior Maine. Master's Thesis, Antioch University, New England, Keene, NH. 94 pages.

Dieter, M. P., & Ludke, J. L. (1975). Studies on combined effects of organophosphates and heavy metals in birds. I. Plasma and brain cholinesterase in coturnix quail fed methyl mercury and orally dosed with parathion. Bulletin of Environmental Contamination and Toxicology 13:257–262.

Eagles-Smith, C.A., Ackerman, J.T., Adelsbach, T.L., Takekawa, J.Y., Miles, A.K. and Keister, R.A. (2008). Mercury correlations among six tissues for four waterbird species breeding in San Francisco Bay, California, USA. Environmental Toxicology and Chemistry 27:2136-2153.

Eagles-Smith, C. A. and Ackerman, J. T. (2009). Rapid changes in small fish mercury concentrations in estuarine wetlands: implications for wildlife risk and monitoring programs. Environmental Science & Technology 43:8658-8664.

Edmonds, S.T., Evers, D.C., O'Driscoll, N.J., Mettke-Hoffman, C., Powell, L., Cristol, D., McGann, A.J., Armiger, J.W., Lane, O., Tessler, D.F. and Newell, P. (2010). Geographic and seasonal variation in mercury exposure of the declining Rusty Blackbird. Condor 112:789-799.

Elbert, R. A., and Anderson, D. W. (1998). Mercury levels, reproduction, and hematology in western grebes from three California lakes, USA. Environmental Toxicology and Chemistry 17:210–213.

Evans, H. and Garman, R. (1982). Neurotoxicity of methylmercury in the pigeon. Neurotoxicology 3:21-36.

Evers, D.C., Kaplan, J.D., Meyer, M.W., Reaman, P.S., Major, A., Burgess, N., and Braselton, W.E. (1998). Bioavailability of environmental mercury measured in Common Loon feathers and blood across North American. Environmental Toxicology and Chemistry 17:173-183.

Evers, D.C., Taylor, K.M., Major, A., Taylor, R.J., Poppenga, R.H. and Scheuhammer, A.M. (2003). Common Loon eggs as indicators of methylmercury availability in North America. Ecotoxicology 12:69-81.

Evers, D.C. and Clair, T.A. (2005a). Mercury in northeastern North America: A synthesis of existing databases. Ecotoxicology 14:7-14.

Evers, D.C., Burgess, N., Champoux, L., Hoskins, B., Major, A., Goodale, W., Taylor, R., Poppenga, R. and Daigle, T. (2005b). Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14:193-222.

Evers, D. C., Savoy, L. J., DeSorbo, C. R., Yates, D. E., Hanson, W., Taylor, K. M., Siegel, L. S., et al. (2008). Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology 17:69-81.

Evers D.C., Wiener, J.G., Basu, N., Bodaly, R.A., Morrison, H.A. and Williams, K.A. (2011). Mercury in the Great Lakes region – Bioaccumulation, spatial and temporal patterns, ecological risks, and policy. Ecotoxicology 20:1487-1499.

Evers, D.C., Jackson, A.K., Tear, T.H. and Osborne, C.E. (2012). Hidden Risk: Mercury in terrestrial ecosystems of the Northeast. Biodiversity Research Institute, Gorham, Maine. BRI Report 2012-07. 33 pp.

Fallacara, D. M., Halbrook, R. S. and French, J. B. (2011). Toxic effects of dietary methylmercury on immune function and hematology in American kestrels (*Falco sparverius*). Environmental Toxicology and Chemistry 30:1320-1327.

Fimreite, N. (1971). Effects of Dietary Methylmercury on Ring-necked Pheasants. Occasional Paper - Canadian Wildlife Service 9:1-39.

Fimreite, N. and Karstad, L. (1971). Effects of dietary methyl mercury on red-tailed hawks. The Journal of Wildlife Management 35:293–300.

Finley, M. T. and Stendell, R. C. (1978). Survival and reproductive success of black ducks fed methyl mercury. Environmental Pollution 16:51–64.

Finley, M. T., Stickel, W. H. and Christensen, R. (1979). Mercury residues in tissues of dead and surviving birds fed methylmercury. Bulletin of Environmental Contamination and Toxicology 21:105–110.

Fossato da Silva, D. A., Teixeira, C. T., Scarano, W. R., Favareto, A. P. A., Fernandez, C. D. B., Grotto, D., Barbosa, F., et al. (2011). Effects of methylmercury on male reproductive functions in Wistar rats. Reproductive Toxicology 31:431-439.

Franceschini, M. D., Lane, O. P., Evers, D. C., Reed, J. M., Hoskins, B. and Romero, L. M. (2009). The corticosterone stress response and mercury contamination in free-living tree swallows, *Tachycineta bicolor*. Ecotoxicology 18:514-521.

Frederick, P. and Jayasena, N. (2011). Altered pairing behaviour and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. Proceedings of the Royal Society B-Biological Sciences 278:1851-1857.

Frederick, P., Campbell, A., Jayasena, N. and Borkhataria, R. (2011). Survival of White Ibises (*Eudocimus albus*) in response to chronic experimental methylmercury exposure. Ecotoxicology 20:358-364.

Friedmann, A. S., Chen, H., Rabuck, L. D. and Zirkin, B. R. (1998). Accumulation of dietary methylmercury in the testes of the adult brown norway rat: impaired testicular and epididymal function. Environmental Toxicology and Chemistry 17:867-871.

Furieri, L. B., Galan, M., Avendano, M., Garcia-Redondo, A. B., Aguado, A., Martinez, S., Cachofeiro, V., et al. (2011). Endothelial dysfunction of rat coronary arteries after exposure to low concentrations of mercury is dependent on reactive oxygen species. British Journal of Pharmacology 162:1819-1831.

Fyfe, R. and Risebrough, R. (1976). Pollutant effects on the reproduction of the prairie falcons and merlins of the Canadian prairies. Canadian Field Naturalist 90:346-355.

Gauthier, J. M., Dubeau, H. and Rassart, E. (1998). Mercury-induced micronuclei in skin fibroblasts of beluga whales. Environmental Toxicology and Chemistry 17:2487-2493.

Gerrard, P. M. and St Louis, V. L. (2001). The effects of experimental reservoir creation on the bioaccumulation of methylmercury and reproductive success of tree swallows (*Tachycineta bicolor*). Environmental Science & Technology 35:1329-1338.

Haines, K.J.R., Evans, R.D., O'Brien, M. and Evans, H.E. (2010). Accumulation of mercury and selenium in the brain of river otters (*Lontra canadensis*) and wild mink (*Mustela vison*) from Nova Scotia, Canada. Science of the Total Environment 408:537‑542.

Halbrook, R., Lewis, L. and Aulerich, R. (1997). Mercury accumulation in mink fed fish collected from streams on the Oak Ridge Reservation. Archives of Environmental Contamination and Toxicology 33:312-316.

Hallinger, K. K. and Cristol, D. A. (2011). The role of weather in mediating the effect of mercury exposure on reproductive success in tree swallows. Ecotoxicology 20:1368-1377.

Hallinger, K. K., Cornell, K. L., Brasso, R. L. and Cristol, D. A. (2011). Mercury exposure and survival in free-living tree swallows (*Tachycineta bicolor*). Ecotoxicology 20:39-46.

Hallinger, K. K., Zabransky, D. J., Kazmer, K. A. and Cristol, D. A. (2010). Birdsong Differs between Mercury-polluted and Reference Sites. Auk 127:156-161.

Hamilton, M., Scheuhammer, A. and Basu, N. (2011). Mercury, selenium and neurochemical biomarkers in different brain regions of migrating common loons from Lake Erie, Canada. Ecotoxicology 20:1677-1683.

Hanko, E., Erne, K., Wanntorp, H., and Borg, K. (1970). Poisoning in ferrets by tissues of alkyl mercury-fed chickens. Acta Veterinaria Scandinavica, 11:268-282.

Hargreaves, A. L., Whiteside, D. P. and Gilchrist, G. (2010). Concentrations of 17 elements, including mercury, and their relationship to fitness measures in arctic shorebirds and their eggs. Science of the Total Environment 408:3153-3161.

Hawley, D. M., Hallinger, K. K. and Cristol, D. A. (2009). Compromised immune competence in free-living tree swallows exposed to mercury. Ecotoxicology 18:499-503.

Heath, J. A. and Frederick, P. C. (2005). Relationships among mercury concentrations, hormones, and nesting effort of white ibises (*Eudocimus albus*) in the Florida Everglades. Auk 122:255–267.

Heinz, G. (1974). Effects of low dietary levels of methyl mercury on mallard reproduction. Bulletin of Environmental Contamination and Toxicology 11:386–392.

Heinz, G. H. (1976). Methylmercury: second-year feeding effects on mallard reproduction and duckling behavior. J. Wildlife Management 40:82–90.

Heinz, G. H. (1979). Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. J. Wildlife Management 43:394–401.

Heinz, G. H. (1980). Eggshell thickness in mallards fed methylmercury. Bulletin of Environmental Contamination and Toxicology 25:498-502.

Heinz, G. H. and Hoffman, D. J. (1998). Methylmercury chloride and selenomethionine interactions on health and reproduction in mallards. Environmental Toxicology and Chemistry 17:139–145.

Heinz, G. H. and Hoffman, D. J. (2003). Embryotoxic thresholds of mercury: estimates from individual mallard eggs. Archives of Envrionmental Contamination and Toxicology 44:257-264.

Heinz, G. H. and Locke, L. N. (1976). Brain lesions in mallard ducklings from parents fed methylmercury. Avian Diseases 20:9–17.

Heinz, G. H., Hoffman, D. J., Kondrad, S. L. and Erwin, C. A. (2006). Factors affecting the toxicity of methylmercury injected into eggs. Archives of Environmental Contamination and Toxicology 50:264-279.

Heinz, G. H., Hoffman, D. J., Klimstra, J. D., Stebbins, K. R., Kondrad, S. L. and Erwin, C. A. (2009). Species differences in the sensitivity of avian embryos to methylmercury. Archives of Environmental Contamination and Toxicology 56:129-138.

Heinz, G. H., Hoffman, D. J., Klimstra, J. D. and Stebbins, K. R. (2010a). Enhanced reproduction in mallards fed a low level of methylmercury: an apparent case of hormesis. Environmental Toxicology and Chemistry 29:650-653.

Heinz, G. H., Hoffman, D. J., Klimstra, J. D. and Stebbins, K. R. (2010b). Reproduction in mallards exposed to dietary concentrations of methylmercury. Ecotoxicology, 19:977-982.

Heinz, G. H., Hoffman, D. J., Klimstra, J. D., Stebbins, K. R., Kondrad, S. L. and Erwin, C. A. (2011). Teratogenic effects of injected methylmercury on avian embryos. Environmental Toxicology and Chemistry 30:1593-1598.

Henny, C. J., Hill, E. F., Hoffman, D. J., Spalding, M. G. and Grove, R. A. (2002). Nineteenth century mercury: hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology 11:213-231.

Henny, C. J., Kaiser, J. L., Packard, H. A., Grove, R. A. and Taft, M. R. (2005). Assessing mercury exposure and effects to American Dippers in headwater streams near mining sites. Ecotoxicology 14:709-725.

Hickey, M.B.C., Fenton, M.B., MacDonald, K.C. and Soulliere, C. (2001). Trace elements in the fur of bats (Chiroptera: Vespertilionidae) from Ontario and Quebec, Canada. Bulletin of Environmental Contamination and Toxicology 66:669-706.

Hill, E. F. and Soares Jr, J. H. (1984). Subchronic mercury exposure in coturnix and a method of hazard evaluation. Environmental Toxicology and Chemistry 3:489–502.

Hoekstra, J.A. and Van Ewijk, P.H. (1993). Alternatives for the no-observed effect level. Environmental Toxicology and Chemistry 12:187-194.

Hoffman, D. J. and Heinz, G. H. (1998). Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks. Environmental Toxicology and Chemistry 17:161–166.

Hoffman, D. J., Eagles-Smith, C. A., Ackerman, J. T., Adelsbach, T. L. and Stebbins, K. R. (2011). Oxidative stress response of Forster's terns (*Sterna forsteri*) and Caspian terns (*Hydroprogne caspia*) to mercury and selenium bioaccumulation in liver, kidney, and brain. Environmental Toxicology and Chemistry 30:920-929.

Hoffman, D. J., Henny, C. J., Hill, E. F., Grove, R. A., Kaiser, J. L. and Stebbins, K. R. (2009). Mercury and drought along the Lower Carson River, Nevada: III. Effects on blood and organ biochemistry and histopathology of Snowy Egrets and Black-Crowned Night-Herons on Lahontan Reservoir, 2002 – 2006. J. Toxicology and Environmental Health, Part A., 72:1223-1241.

Hoffman, D. J., Spalding, M. G. and Frederick, P. C. (2005). Subchronic effects of methylmercury on plasma and organ biochemistries in great egret nestlings. Environmental Toxicology and Chemistry 24:3078–3084.

Holloway, J., Scheuhammer, A. M. and Chan, H. M. (2003). Assessment of white blood cell phagocytosis as an immunological indicator of methylmercury exposure in birds. Environmental Contamination and Toxicology 44:493-501.

Huang, C.-F., Liu, S.-H., Hsu, C.-J. and Lin-Shiau, S.-Y. (2011). Neurotoxicological effects of low-dose methylmercury and mercuric chloride in developing offspring mice. Toxicology Letters 201:196-204.

Ikeda, Y., Tobe, M., Kobayashi, K., Suzuki, S., Kawaski, Y. and Yonemaru, H. (1973). Long-term toxicity study of methylmercuric chloride in monkeys* (First Report). Toxicology 1:361-375.

Inouye, M. and Murakami, U. (1975). Teratogenic effect of orally adminstered methylmercuric chloride in rats and mice. Congenital Anomalies 15:1-9.

Jackson, A. K., Evers, D. C., Etterson, M. a., Condon, A. M., Folsom, S. B., Detweiler, J., Schmerfeld, J., et al. (2011a). Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the Carolina Wren (*Thryothorus ludovicianus*). Auk 128:759-769.

Jackson, A.K., Evers, D.C., Folsom, S.B., Condon, A.M., Diener, J., Goodrick, L.F., McGann, A.J., Schmerfeld, J. and Cristol, D.A. (2011b). Mercury exposure in terrestrial birds far downstream of an historical point source. Environmental Pollution 159:3302-3308.

Jayasena, N., Frederick, P. C. and Larkin, I. L. V. (2011). Endocrine disruption in white ibises (*Eudocimus albus*) caused by exposure to environmentally relevant levels of methylmercury. Aquatic Toxicology 105:321-327.

Jernelöv, A., Johansson, A. H., Sörensen, L. and Svenson, A. (1976). Methyl mercury degradation in mink. Toxicology, 6:315–321.

Ji, X., Hu, W., Cheng, J., Yuan, T., Xu, F., Qu, L. and Wang, W. (2006). Oxidative stress on domestic ducks (Shaoxing duck) chronically exposed in a Mercury-Selenium coexisting mining area in China. Ecotoxicology and Environmental Safety 64:171-177.

Kenow, K. P., Gutreuter, S., Hines, R. K., Meyer, M. W., Fournier, F. and Karasov, W. H. (2003). Effects of methyl mercury exposure on the growth of juvenile Common Loons. Ecotoxicology 12:171-182.

Kenow, K. P., Grasman, K. A., Hines, R. K., Meyer, M. W., Gendron-Fitzpatrick, A., Spalding, M. G. and Gray, B. R. (2007). Effects of methylmercury exposure on the immune function of juvenile common loons (*Gavia immer*). Environmental Toxicology and Chemistry 26:1460-1469.

Kenow, K. P., Hoffman, D. J., Hines, R. K., Meyer, M. W., Bickham, J. W., Matson, C. W., Stebbins, K. R., et al. (2008). Effects of methylmercury exposure on glutathione metabolism, oxidative stress, and chromosomal damage in captive-reared common loon (*Gavia immer*) chicks. Environmental Pollution 156:732-738.

Kenow, K. P., Hines, R. K., Meyer, M. W., Suarez, S. a, & Gray, B. R. (2010). Effects of methylmercury exposure on the behavior of captive-reared common loon (*Gavia immer*) chicks. Ecotoxicology 19:933-944.

Kenow, K. P., Meyer, M. W., Rossmann, R., Gendron-Fitzpatrick, A. and Gray, B. R. (2011). Effects of injected methylmercury on the hatching of common loon (*Gavia immer*) eggs. Ecotoxicology 20:1684-1693.

Khera, K. S. (1973a). Teratogenic effects of methylmercury in the cat: Note on the use of this species as a model for teratogenicity studies. Teratology 8:293-304.

Khera, K. S. (1973b). Reproductive capability of male rats and mice treated with methyl mercury. Toxicology and Applied Pharmacology 24:167-177.

Khera, K.S. and Tabacova, S. A. (1973). Effects of methylmercuric chloride on the progeny of mice and rats treated before and during gestation. Food and Cosmetics Toxicology 11:245-254.

Khera, K.S., Iverson, F., Hierlihy, L., Tanner, R. and Trivett, G. (1974). Toxicity of methylmercury in neonatal cats. Teratology 10:69-76.

King, K. A., Custer, T. W. and Quinn, J. S. (1991). Effects of Mercury, Selenium, and Organochlorine contaminants on reproduction of Forster's Terns and Black Skimmers nesting in a contaminated Texas bay. Archives of Environmental Contamination and Toxicology 20:32-40.

Klenavica, K., Champoux, L., O'Brien, M., Daoust, P., Evans, R.D. and Evans, H.E. (2008). Mercury concentrations in wild mink (*Mustela vison*) and river otters (*Lontra canadensis*) collected from eastern and Atlantic Canada: Relationship to age and parasitism. Environment Pollution 156: 359‑366.

Knott, K. K., Schenk, P., Beyerlein, S., Boyd, D., Ylitalo, G. M., and O'Hara, T. M. (2011). Blood-based biomarkers of selenium and thyroid status indicate possible adverse biological effects of mercury and polychlorinated biphenyls in Southern Beaufort Sea polar bears. Environmental Research 111:1124-1136.

Koeman, J., Garssen-Hoekstra, J., and Pels, E. (1971). Poisoning of birds of prey by methyl mercury compounds. Meded. Faculteit 36:43-49.

La Sala, L. F., Petracci, P. F., Smits, J. E., Botté, S. and Furness, R. W. (2011). Mercury levels and health parameters in the threatened Olrog's Gull (*Larus atlanticus*) from Argentina. Environmental Monitoring and Assessment 181:1-11.

Lane, O.P., O'Brien, K.M., Evers, D.C., Hodgman, T.P., Major, A., Pau, N., Ducey, M.J., Taylor, R. and Perry, D. (2011). Mercury in breeding saltmarsh sparrows (*Ammodramus caudacutus*). Ecotoxicology 20:1984-1991.

Laties, V. G. and Evans, H. L. (1980). Methylmercury-induced changes in operant discrimination by the pigeon. Journal of Pharmacology and Experimental Therapeutics 214:620-628.

Leander, J. D., McMillan, D. E. and Barlow, T. S. (1977). Chronic mercuric chloride: behavioral effects in pigeons. Environmental Research 14:424-435.

Longcore, J. R., Dineli, R. and Haines, T. A. (2007). Mercury and Growth of Tree Swallows at Acadia National Park, and at Orono, Maine, USA. Environmental Monitoring and Assessment 126:117-127.

Matsumura, A., Eto, K., Furuyoshi, N. and Okamura, R. (1993). Disturbances of accommodation in Minamata disease. Neuro-Ophthalmology 13:331-339.

McKay, J.L. and Maher, C.R. (2012). Relationship between blood mercury levels and components of male song in Nelson's sparrows (*Ammodramus nelsoni*). Ecotoxicology DOI: 10.1007/s10646-012-0994-0.

Meyer, M. W., Evers, D. C., Hartigan, J. J. and Rasmussen, P. S. (1998). Patterns of common loon (Gavia immer) exposure, reproduction, and survival in Wisconsin, USA. Environmental Toxicology and Chemistry 17:184-190.

Meyer M.W., Rasmussen, P.W., Watras, C.J., Fevold, B.M., Kenow, K.P. (2011). Bi-phasic trends in mercury concentrations in blood of Wisconsin common loons during 1992-2010. Ecotoxicology 20:1659-1668.

Mitro, M. G., Evers, D. C., Meyer, M. W., & Piper, W. H. (2008). Common Loon survival rates and mercury in New England and Wisconsin. J. Wildlife Management 72:665-673.

Miura, T., Koyama, T. and Nakamura. I. (1978). Mercury content in museum and recent specimens of Chiroptera in Japan. Bulletin of Environmental Contamination and Toxicology 20:696-701.

Mollenhauer, M. A. M., Carter, B. J., Peden-Adams, M. M., Bossart, G. D. and Fair, P. A. (2009). Gene expression changes in bottlenose dolphin, *Tursiops truncatus*, skin cells following exposure to methylmercury (MeHg) or perfluorooctane sulfonate (PFOS). Aquatic Toxicology 91:10-18.

Monteiro, L. R. and Furness, R. W. (2001). Kinetics, dose-response, excretion, and toxicity of methylmercury in free-living Cory's shearwater chicks. Environmental Toxicology and Chemistry 20:1816–1823.

Moussa, H., Hachfi, L., Trimèche, M., Najjar, M. F. and Sakly, R. (2011). Accumulation of mercury and its effects on testicular functions in rats intoxicated orally by methylmercury. Andrologia 43:23-27.

Muller, W.-U., Streffer, C. and Joos, A. L. (1990). Toxicity of cadmium sulphate and methylmercuric chloride applied singly or in combination to early mouse embryos in vitro. Toxicology in Vitro 4:57-61.

Mullins, W. H., Bizeau, E. G. and Benson, W. W. (1977). Effects of phenyl mercury on captive game farm pheasants. J. Wildlife Management, 41:302-308.

Nakamura, M., Yasutake, A., Fujimura, M., Hachiya, N. and Marumoto, M. (2011). Effect of methylmercury administration on choroid plexus function in rats. Archives of Toxicology 85:911-918.

Nam, D.-H., Yates, D., Ardapple, P., Evers, D. C., Schmerfeld, J. and Basu, N. (2012). Elevated mercury exposure and neurochemical alterations in little brown bats (*Myotis lucifugus*) from a site with historical mercury contamination. Ecotoxicology 31:1094-1101.

Newton, I. and Brockie, K. (1998). Population limitation in birds. Academic Press. 597pp.

Newton, I. and Haas, M. (1988). Pollutants in Merlin eggs and their effects on breeding. Breeding Birds 81:258-269.

Nicholson, J. and Osborn, D. (1984). Kidney lesions in juvenile starlings *Sturnus vulgaris* fed on a mercury-contaminated synthetic diet. Environmental Pollution 33:195-206.

Nocera, J. J. and Taylor, P. D. (1998). In situ behavioral response of common loons associated with elevated mercury (Hg) exposure. Conservation Ecology [Online], 2(2):10.

O'Connor, D. J. and Nielsen, S. W. (1980). Environmental Survey of Methylmercury Levels in Wild Mink (*Mustela vison*) and Otter (*Lutra canadensis*) from the Northeastern United States and Experimental Pathology of methlymercuiralism in the otter. In J. A. Chapman & D. Pursley (Eds.), Worldwide Furbearer Conference Proceedings (pp. 1728-1745). Frostburg, MD.

O'Shea, T.J., Everette, A.L. and Ellison, L.E. (2001). Cyclodiene insecticide, DDE, DDT, arsenic, and mercury contamination of big brown bats (*Eptesicus fuscus*) foraging at a Colorado superfund site. Archives of Environmental Contamination and Toxicology 40:112-120.

Odsjö, T. and Sondell, J. (1977). Population development and breeding success in the marsh harrier Circus aeruginosus in relation to levels of DDT, PCB and mercury. Vår Fågelvärld, 36:152–160.

Olsen, B., Evers, D. C. and DeSorbo, C. (2000). Effect of methylated mercury on the diving frequency of the common loon. J. Ecological Research, 2:67–72.

Parkhurst, C. R. and Thaxton, P. (1973). Toxicity of mercury to young chickens. Poultry Science 52:273-276.

Ramprashad, F. and Ronald, K. (1977). A surface preparation study on the effect of mercury on the sensory hair cell population in the cochlea of the harp seal (*Pagophilus groenlandicus* Erxleben, 1777). Canadian Journal of Zoology 55:223-230.

Rawson, A. J., Patton, G. W., Hofmann, S., Pietra, G. G. and Johns, L. (1993). Liver abnormalities associated with chronic mercury accumulation in stranded Atlantic bottlenose dolphins. Ecotoxicology and Environmental Safety 25:41-47.

Rice, D. C. (1998). Lack of effect of methylmercury exposure from birth to adulthood on information processing speed in the monkey. Neurotoxicology and Teratology 20:275-283.

Rice, D. C. and Gilbert, S. G. (1982). Early chronic low-level methylmercury poisoning in monkeys impairs spatial vision. Science 216:759-761.

Rice, D. C. and Gilbert, S. G. (1990). Effects of developmental exposure to methyl mercury on spatial and temporal visual function in monkeys. Toxicology and Applied Pharmacology 102:151-163.

Rice, D. C. and Gilbert, S. G. (1992). Exposure to methyl mercury from birth to adulthood impairs high-frequency hearing in monkeys. Toxicology and Applied Pharmacology 115:6-10.

Roman-Franco, A. A., Turiello, M., Albini, B., Ossi, E., Milgrom, F. and Anders, G. A. (1978). Anti-basement membrane antibodies and antigen-antibody complexes in rabbits injected with mercuric chloride. Clinical Immunology and Immunopathology 9:464-481.

Ronald, K., Tessaro, S. V., Uthe, J. F., Freeman, H. C. and Frank, R. (1977). Methylmercury poisoning in the harp seal (*Pagophilus groenlandicus*). Science of the Total Environment 8:1-11.

Rutkiewicz, J., Nam, D.-H., Cooley, T., Neumann, K., Padilla, I. B., Route, W., Strom, S., et al. (2011). Mercury exposure and neurochemical impacts in bald eagles across several Great Lakes states. Ecotoxicology 20:1669-1676.

Scheuhammer, A. M. (1988). Chronic dietary toxicity of methylmercuy in the zebra finch, *Phoephila guttata*. Bulletin of Environmental Contamination and Toxicology 40:123-130.

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36:12-19.

Scheuhammer, A. M., Basu, N., Burgess, N. M., Elliott, J. E., Campbell, G. D., Wayland, M., Champoux, L., et al. (2008). Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). Ecotoxicology 17:93-101.

Schoch, N., Glennon, M., Evers, D.C., Duron, M., Jackson, A., Driscoll, C., Yu, X. and Simonin, H. (2011). Long-Term monitoring and assessment of mercury based on integrated sampling efforts using the common Loon, prey fish, water, and sediment: Final Report. New York State Energy Research and Development Authority Report 7608, Albany, New York.116 pp.

Schwarzbach, S. E., Albertson, J. D. and Thomas, C. M. (2006). Effect of predation, flooding, and contamination on reproductive success of California clapper rails (*Rallus longirostris obsoletus*) in San Francisco Bay. Auk 123:45-60.

Scott, M., Zimmermann, J. and Marinsky, S. (1975). Effects of PCBs, DDT, and mercury compounds upon egg production, hatchability and shell quality in chickens and Japanese quail. Poultry Science 54:350-358.

Sepulveda, M., Williams Jr, G., Frederick, P., & Spalding, M. (1999). Effects of mercury on health and first-year survival of free-ranging great egrets (*Ardea albus*) from southern Florida. Archives of Environmental Contamination and Toxicology 37:369–376.

Sleeman, J. M., Cristol, D. A., White, A. E., Evers, D. C., Gerhold, R. W. and Keel, M. K. (2010). Mercury poisoning in a free-living northern river otter (*Lontra canadensis*). J. Wildlife Diseases, 46:1035-1039.

Sobotka, T. J., Cook, M. P. and Brodie, R. E. (1974). Effects of perinatal exposure to methyl mercury on functional brain development and neurochemistry. Biological Psychiatry 8:307-320.

Sonne, C., Dietz, R., Leifsson, P. S., Asmund, G., Born, E. W. and Kirkegaard, M. (2007). Are liver and renal lesions in East Greenland polar bears (*Ursus maritimus*) associated with high mercury levels? Environmental Health 6:1-9.

Spalding, M. G., Bjork, R. D., Powell, G. V. N. and Sundlof, S. F. (1994). Mercury and cause of death in great white herons. J. Wildlife Management 58:735-739.

Spalding, M. G., Frederick, P. C., McGill, H. C., Bouton, S. N. and McDowell, L. R. (2000a). Methylmercury accumulation in tissues and its effects on growth and appetite in captive great egrets. J. Wildlife Diseases 36:411–422.

Spalding, M. G., Frederick, P. C., McGill, H. C., Bouton, S. N., Richey, L. J., Schumacher, I. M., Blackmore, C. G., et al. (2000b). Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. J. Wildlife Diseases 36:423–435.

Spann, J. W., Heinz, G. H., Camardese, M. B., Hill, E. F., Moore, J. F. and Murray, H. C. (1986). Differences in mortality among bobwhite fed methylmercury chloride dissolved in various carriers. Environmental Toxicology and Chemistry 5:721-724.

Spann, J., Heath, R., Kreitzer, J. and Locke, L. (1972). Ethyl mercury p-toluene sulfonanilide: lethal and reproductive effects on pheasants. Science, 175:328-331.

Spencer, S. H., Shutler, D. and O'Brien, M. S. (2011). Correlates of mercury in female river otters (*Lontra canadensis*) from Nova Scotia, Canada. Environmental Toxicology and Chemistry 30:1879-1884.

Stebbins, K.R., Klimstra, J.D., Eagles-Smith, C.A., Ackerman, J.T. and Heinz, G.H. (2009). A nonlethal microsampling technique to monitor the effects of mercury on wild bird eggs. Environmental Toxicology and Chemistry 28:465–470.

Stoewsand, G., Bache, C. and Lisk, D. (1974). Dietary selenium protection of methylmercury intoxication of Japanese quail. Bulletin of Environmental Contamination and Toxicology 11:152–157.

Strom, S.M. (2008). Total mercury and methylmercury residues in river otters (*Lutra canadensis*) from Wisconsin. Archives of Environmental Contamination and Toxicology 54:546-554.

Takeuchi, T., D'Itri, F. M., Fischer, P. V., Annett, C. S. and Okabe, M. (1977). The outbreak of Minamata disease (methyl mercury poisoning) in cats on northwestern Ontario reserves. Environmental Research 13:215-228.

Tamashiro, H., Arakaki, M., Akagi, H., Hirayama, K., Murao, K. and Smolensky, M. H. (1986). Sex differential of methylmercury toxicity in spontaneously hypertensive rats (SHR). Bulletin of Environmental Contamination and Toxicology 37:916-924.

Thompson, D., Hamer, K., & Furness, R. (1991). Mercury accumulation in great skuas *Catharacta skua* of known age and sex, and its effects upon breeding and survival. J. Applied Ecology 28:672–684.

Van der Hoeven, N. (1997). How to measure no effect. Part 1: Towards a new measure of chronic toxicity? Environmetrics 8:241-248.

Vermeer, K., Armstrong, F. and Hatch, D. (1973). Mercury in aquatic birds at Clay Lake, western Ontario. J. Wildlife Management, 37:58–61.

Wada, H., Cristol, D. A., McNabb, F. M. A. and Hopkins, W. A. (2009). Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. Environmental Science and Technology 43:6031-6038.

Wada, H., Yates, D. E., Evers, D. C., Taylor, R. J., & Hopkins, W. A. (2010). Tissue mercury concentrations and adrenocortical responses of female big brown bats (*Eptesicus fuscus*) near a contaminated river. Ecotoxicology, 19, 1277-1284.

Wakita, Y. (1987). Hypertension induced by methyl mercury in rats. Toxicology and Applied Pharmacology 89:144-147.

Walker, L.A., Simpson, V.R., Rockett, L., Wienberg, C.L. and Shore, R.F. (2007). Heavy metal contamination in bats in Britain. Environmental Pollution 148:483-490

Warne, M.S.J. and van Dam, R. (2008). NOEC and LOEC data should no longer be generated or used. Australian Journal of Ecotoxicology 14:1-5.

Wobeser, G. and Swift, M. (1976). Mercury poisoning in a wild mink. J. Wildlife Diseases 12:335–340.

Wobeser, G., Nielsen, N. and Schiefer, B. (1976a). Mercury and mink. I. The use of mercury contaminated fish as a food for ranch mink. Canadian Journal of Comparative Medicine 40:30-33.

Wobeser, G., Nielsen, N. and Schiefer, B. (1976b). Mercury and Mink. II. Experimental methyl mercury intoxication. Canadian Journal of Comparative Medicine 40:34-45.

Wren, C. D. (1985). Probable case of mercury poisoning in a wild otter, *Lutra canadensis*, in northwestern Ontario. The Canadian Field-Naturalist 99:112-114.

Wren, C., Hunter, D., Leatherland, J., & Stokes, P. (1987). The effects of poly chlorinated biphenyls and methylmercury, singly and in combination on mink. II: Reproduction and kit development. Archives of Environmental Contamination and Toxicology, 16(4), 449–454.

Wolfe, M.F., Atkeson,T., Bowerman,W., Burger, K., Evers, D.C., Murray, M.W. and Zillioux, E. (2007). Wildlife Indicators. In: R. Harris, D.P. Krabbenhoft, R. Mason, M.W. Murray, R. Reash and T. Saltman (Ed.), Ecosystem Response to Mercury Contamination: Indicators of Change (pp. 123-189). SETAC, Webster, NY: CRC Press.

Wyn, B., Kidd, K.A., Burgess, N.M., Curry, R.A. and Munkittrick, K.R. (2010). Increasing mercury in yellow perch at a hotspot in Atlantic Canada, Kejimkujik National Park. Environmental Science and Technology 44:9176–9181.

Yates, D.E., Angelo, S.E., Evers, D.C., Schmerfeld, J., Moore, M.S, Kunz, T.H., Divoll, T., Edmonds, S.T., Perkins, C., Taylor, R. and O'Driscoll, N.J. Mercury in bats from the northeastern United States. Ecotoxicology. Submitted.

Appendix I. Effects of mercury on birds.

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
BIRD Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
1.**PHYSIOLOGY**				
1.1. Abnormal histopathology	American Black Duck	Finley and Stendell 1978	Great Blue Heron (white phase)	Spalding et al. 1994[2]
	American Kestrel	Koeman et al. 1971[3]		
	Brown-headed Cowbird	Finley et al. 1979[3]		
	Common Grackle	Finley et al. 1979[3]		
	European Starling	Finley et al. 1979[3], Nicholson and Osborn 1984[3]		
	Great Egret	Spalding et al. 2000(b)[3]		
	Goshawk	Borg et al 1970		
	Mallard	Heinz and Locke 1976		
	Red-tailed Hawks	Fimreite and Karstad 1971[3]		
	Red-winged Blackbird	Finley et al. 1979[3]		
1.2.Chromosomal alteration	Not Observed		Common Loon	Evers et al. 2008
1.3.Disrupted blood and organ biochemistry and hormone levels	Common Loon	Kenow et al. 2008[3]	Caspian Tern	Hoffman et al. 2011
	Coturnix Quail	Dieter and Ludke 1975 Hill and Soares 1984	Clark's Grebe	Elbert and Anderson 1998
	Domestic Duck	Ji et al. 2006	Double-crested Cormorant	Henny et al. 2002
	Great Egret	Sepulveda et al. 1999[3], Spalding et al. 2000(b)[3], Hoffman et al. 2005,	Forester's Tern	Hoffman et al. 2011
			Olrog's Gull	La Sala et al. 2011
			Snowy Egret	Hoffman et al. 2009
	Little Egret	Barata et al. 2010	Tree Swallow	Franceschini et al. 2009, Wada et al. 2009
	Mallard	Hoffman and Heinz 1998		
	Purple Heron	Barata et al. 2010	Western Grebe	Elbert and Anderson 1998
	White Ibis	Heath and Frederick 2005, Jayasena et al. 2011[3]		

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
BIRD Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
1.4.Suppression of Immune System	American Kestrel	Fallacara et al. 2011	Clark's Grebe	Elbert and Anderson 1998
	Common Loon	Kenow et al. 2007(a)[3]		
	Great Egret	Spalding et al. 2000(b)[3]	Double-crested Cormorant	Henny et al. 2002
			Tree Swallow	Hawley et al. 2009
			Western Grebe	Elbert and Anderson 1998
1.5.Inhibition of growth	Domestic Chicken	Parkhurst and Thaxton 1973	California Clapper Rail	Ackerman et al. 2012
			Common Loon	Barr 1986
	Great Egret	Spalding et al. 2000(b)[3]	Tree Swallow	Longcore et al. 2007
	Goshawk	Borg et al. 1970		
	Red-tailed Hawk	Fimreite and Karstad 1971[3]		
1.6.Increased parasite load	Great Egret	Sepulveda et al. 1999[3]	Great White Heron[2]	Spalding et al. 1994
2.NEUROLOGY				
2.1.Neurochemical aberrations	Rock Dove	Laties and Evans 1980	Bald Eagle	Scheuhammer et al. 2008, Rutkiewicz et al. 2011[2]
			Common Loon	Scheuhammer et al. 2008
2.2.Ataxia	American Kestrel	Koeman et al. 1971[3]	Not Observed	
	Brown-headed Coturnix Quail	Hill and Soares 1984		
	Cowbird	Finley et al. 1979[3]		
	Common Grackle	Finley et al. 1979[3]		
	Domestic Chicken	Scott et al. 1975		
	European Starling	Finley et al. 1979[3]		
	Northern Goshawk	Borg et al 1970		
	Great Egret	Bouton et al. 1999[3], Spalding et al. 2000(b)[3]		

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
BIRD Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
		Hoffman et al. 2005		
	Mallard	Heinz and Locke 1976, Heinz and Hoffman 1998, Heinz and Hoffman 2003		
	Red-tailed Hawk	Fimreite and Karstad 1971[3]		
	Red-winged Blackbird	Finley et al. 1979[3]		
	Ring-necked Pheasant	Spann et al. 1972		
	Rock Dove	Laties and Evans 1980, Evans et al. 1982		
	Zebra Finch	Scheuhammer 1988		
3.BEHAVIOR				
3.1.Aberrant behavior	American Kestrel	Koeman et al. 1971[3]	Carolina Wren	Jackson et al. 2011a
	Common Loon	Kenow et al. 2010[3]	Common Loon	Barr 1986, Nocera and Taylor 1998, Olsen et al. 2000, Evers et al. 2008
	Great Egret	Bouton et al. 1999[3], Spalding et al. 2000(a)[3]		
	Mallard	Heinz 1976, Heinz 1979, Heinz and Hoffman 1998		
	Red-tailed Hawks	Fimreite and Karstad 1971[3]		
	Rock Dove	Leander et al. 1977, Laties and Evans 1980, Evans et al. 1982		
	White Ibis	Frederick and Jayasena 2011[3]		
3.2. Altered song	Not Observed		Carolina Wren	Hallinger et al. 2010
			House Wren	Hallinger et al. 2010
			Song Sparrow	Hallinger et al. 2010
			Nelson's Sparrow	McKay and Maher 2012

BIRD Sublethal Effect	*Lab/Captive-oriented or Experimentally Manipulated Studies*		*Free-living Population Studies*	
	Species	***Reference***	***Species***	***Reference***
4.REPRODUCTIVE				
4.1.Reduced Fertility/Sex-biased Reproduction	Not Observed		Belted Kingfisher Eastern Bluebird Tree Swallow	Bouland et al. 2012 Bouland et al. 2012 Bouland et al. 2012
4.2.Reduced embryo production or fitness & hatching success, and abnormal development.	American Black Duck American Kestrel Common Loon Domestic Chicken Mallard[1] Ring-necked Pheasant White Ibis Multiple Species[5]	Finley and Stendell 1978 Albers et al. 2007 Kenow et al. 2011[3] Scott et al. 1975 Heinz 1974, 1979, 1980, Heinz and Hoffman 1998, 2003 Heinz et al. 2010(a), 2010b Fimreite 1971, Spann et al. 1972, Mullins et al. 1977 Heath and Frederick 2005, Frederick and Jayasena 2011[3] Heinz et al. 2009, 2011	Bald Eagle Black-bellied Plover Carolina Wren Clapper Rail Common Loon Forester's Tern House Wren Merlin Ruddy Turnstone Semipalmated Plover Tree Swallow	DeSorbo 2007 Hargreaves et al. 2010 Jackson et al. 2011a Schwarzbach et al. 2006 Barr 1986, Meyer et al. 1998, Burgess and Meyer 2008, Evers et al. 2008 Eagles-Smith and Ackerman 2009 Custer et al. 2007 Newton and Haas 1988 Hargreaves et al. 2010 Hargreaves et al. 2010 Custer et al. 2007, Hallinger and Cristol 2011, Hallinger et al. 2011
4.3 Reproductive Organ Development/Sexual Maturation	Coturnix Quail	Hill and Soares 1984	Not Observed	
5.SURVIVAL				
5.1.Lower Embryo Survival	Domestic Chicken Double-Crested Cormorant Mallard	Heinz et al. 2006 Heinz et al. 2006 Heinz et al. 2006	Not Observed	

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
BIRD Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
	Ring-necked Pheasant	Heinz et al. 2006		
	Multiple Species[4]	Heinz et al. 2009		
5.2.Lower Juvenile Survival	American Black Duck	Finley and Stendell 1978	Common Loon	Evers et al. 2008
	American Kestrel	Albers et al. 2007	Tree Swallow	Brasso and Cristol 2008, Hallinger et al. 2011
	Coturnix Quail	Spann et al. 1986		
	Domestic Chicken	Parkhurst and Thaxton 1973		
	Mallard	Heinz 1976, Heinz and Locke 1976, Heinz et al. 2010b		
	Northern Bobwhite			
	Ring-necked Pheasant	Mullins et al. 1977		
5.3.Lower Adult Survival	American Kestrel	Koeman et al 1971[3]	Tree Swallow	Hallinger et al. 2011
	Brown-headed Cowbird	Finley et al. 1979[3]		
	Common Grackle	Finley et al. 1979[3]		
	Coturnix Quail	Stoewsand et al. 1974, Hill and Soares 1984		
	European Starling	Heinz and Hoffman 1998, Hoffman and Heinz 1998		
	Mallard			
	Red-tailed Hawks	Fimreite and Karstad 1971[3]		
	Red-winged Blackbird	Finley et al. 1979[3]		
	Ring-necked Pheasant	Spann et al. 1972		
	Rock Dove	Leander et al. 1977		
	Zebra Finch	Scheuhammer 1988		
6. NO EFFECTS	Common Loon	Kenow et al. 2003[3]	American Avocet	Ackerman et al. 2008(b)
	Cory's Shearwater	Monteiro and Furness 2001[3]	American Dipper	Henny et al. 2005
			Black-necked Stilt	Ackerman et al. 2008(b)
	Mallard	Heinz 1980	Black Skimmer	King et al. 1991

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
BIRD Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
	White Ibis	Frederick et al. 2011[3]	Clark's Grebe	Anderson et al. 2008
			Common Loon	Holloway et al. 2003, Kenow et al. 2007[3], Mitro et al. 2008, Hamilton et al. 2011[2]
			Eastern Phoebe	Hallinger et al. 2010
			Forster's Tern	Ackerman et al. 2008(a), King et al. 1991
			Great Skua	Thompson et al. 1991
			Herring Gull	Vermeer et al. 1973
			Northern Harrier	Odsjo and Sondell 1977
			Osprey	Anderson et al. 2008, DesGranges et al. 1999
			Prairie Falcon	Fyfe and Risebrough 1976
			Merlin	Fyfe and Risebrough 1976
			Tree Swallow	Gerrard and St. Louis 2001, Brasso et al. 2010
			Western Grebe	Anderson et al. 2008

[1]Responses to mercury dosing were considered similar between wild and domesticated mallards (Heinz 1980)

[2]Examined specimens that died in wild, this does not include trapped, hunted, or beached specimens.

[3]Experimentally Manipulated all others are Laboratory studies. Lab studies performed with domesticated species or species breed in captivity. Experimentally Manipulated could include: eggs collected in wild, eggs injected in field and remain in field, nestlings doses in field and left in field, capture and release, wild trapped or netted animals collected and brought into lab, specimens collected from hunters, etc.

[4]Species include: American Avocet, American Kestrel, Anhinga, Black-necked Stilt, Brown Pelican, Canada Goose, Caspian Tern, Chicken, Clapper Rail, Common Grackle, Common Tern, Double-crested Cormorant, Great Egret, Herring Gull, Hooded Merganser, Laughing Gull, Lesser Scaup, Mallard, Osprey, Ring-necked Pheasant, Royal Tern, Sandhill Crane, Snowy Egret, Tricolored Heron, Tree Swallow, White Ibis.

[5]Species include: American Avocet, American Kestrel, Black-necked Stilt, Brown Pelican, Canada Goose, Caspian Tern, Chicken, Clapper Rail, Common Grackle, Common Tern, Double-crested Cormorant, Great Egret, Herring Gull, Hooded Merganser, Laughing Gull, Lesser Scaup, Mallard, Osprey, Ring-necked Pheasant, Royal Tern, Sandhill Crane, Snowy Egret, Tricolored Heron, Tree Swallow, White Ibis.

Appendix II. Effects of mercury on mammals.

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
MAMMAL Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
1.**PHYSIOLOGY**				
1.1.Abnormal histopathology	Common Marmoset	Matsumura et al. 1993	Beluga Whale	Beland et al 1993
	Domestic Cat	Albanus et al. 1972, Chardonneau et al. 1974, Khera et al. 1974	Bottle-nosed Dolphin	Rawson et al. 1993
			American Mink[1]	Wobeser and Swift 1976
			Polar Bear	Sonne et al. 2007
	Domestic Ferret	Hanko et al. 1970		
	Domestic Rabbit	Roman-Franco et al. 1978		
	Domestic Rat	Furieri et al 2011		
	American Mink	Wobeser et al. 1976(b), Aulerich et al. 1974		
	Northern River Otter	O'Connor and Nielsen 1980[2], Sleeman et al 2010		
1.2.Chromosomal alteration	Beluga Whale	Gauthier et al. 1998[2]	Not Observed	
	Bottle-nosed Dolphin	Betti and Nigro 1996, Mollenhauer et al. 2009[2]		
1.3.Disrupted blood and organ biochemistry and hormone levels	Bottle-nosed Dolphin	Mollenhauer et al. 2009[2]	Big Brown Bat	Wada et al. 2010
	Domestic Rabbit	Anjum et al 1994	Little Brown Bat	Nam et al.2012
	Domestic Rat	Nakamura et al. 2011	Polar Bear	Knott et al. 2011
	Harp Seal	Ronald et al. 1977[2]		
1.4.Suppression of Immune system	Domestic Rabbit	Roman-Franco et al. 1978		
	Domestic Rat	Bigazzi et al. 2003	Not Observed	
1.5.Inhibition of growth	Crab-eating Macaque	Ikeda et al. 1973	Beluga Whale	Beland et al. 1993
	Domestic Cat	Albanus et al. 1972, Khera 1973(a), Khera et al. 1974		
	Domestic Ferret	Hanko et al. 1970		

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
MAMMAL Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
	Harp Seal	Ronald et al. 1977[2]		
	American Mink	Aulerich et al. 1974, Jernelov et al. 1976, Wobeser et al. 1976(b), Wren et al. 1987, Halbrook et al. 1997		
	Domestic Mouse	Huang et al. 2011		
	Domestic Rabbit	Roman-Franco et al. 1978, Anjum et al. 1994		
	Domestic Rat	Khera 1973(b), Tamashiro et al. 1986, Wakita 1987, Nakamura et al. 2011		
	Rhesus Monkey	Ikeda et al. 1973		
	Northern River Otter	Sleeman et al. 2010		
1.6. Visual and auditory deficits	Common Marmoset	Matsumura et al. 1993	Not Observed	
	Crab-eating Macaque	Ikeda et al. 1973, Rice and Gilbert 1982, Rice and Gilbert 1990, Rice and Gilbert 1992, Burbacher et al. 2005		
	Domestic Cat	Chardonneau et al. 1974, Khera et al. 1974		
	Domestic Mouse	Huang et al. 2011		
	Domestic Rat	Khera and Tabacova 1973		
	Harp Seal	Ramprashad and Ronald 1977[2]		
	American Mink	Ikeda et al. 1973		
	Rhesus Monkey	Wobeser et al. 1976(b)		
	Northern River Otter	O'Connor and Nielsen 1980[2]		
1.7. Increased parasite loads			American Mink, River Otter	Klenavic et al. 2008

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
MAMMAL Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
2.NEUROLOGY				
2.1.Neurochemical aberrations	Domestic Mouse	Basu et al. 2005(c), Huang et al. 2011	American Mink	Basu et al. 2005(a), 2007(c)
	Domestic Rat	Basu et al. 2005(c)	Little Brown Bat	Nam et al. 2012
	American Mink	Basu et al. 2005(c), 2006, 2007(c), 2010	Polar Bear	Basu et al. 2009
	Northern River Otter	Basu et al. 2005(c)[2]	Northern River Otter	Basu et al. 2005(b), 2007(b)
2.2.Ataxia	Crab-eating Macaque	Ikeda et al. 1973	Not Observed	
	Domestic Cat	Albanus et al. 1972, Khera 1973(a), Chardonneau et al. 1974, Khera et al. 1974, Takeuchi et al. 1977		
	Domestic Rat	Khera 1973(b), Tamashiro et al. 1986, Wakita 1987, Nakamura et al. 2011		
	American Mink	Aulerich et al. 1974, Wobeser et al. 1976(b), Dansereau et al 1999		
	Rhesus Monkey	Ikeda et al. 1973		
	Northern River Otter	O'Connor and Nielsen 1980[2], Sleeman et al. 2010		
3.BEHAVIOR				
3.1.Aberrant behavior	Crab-eating Macaque	Ideka et al. 1973	Northern River Otter	Wren 1985[1]
	Domestic Cat	Albanus et al. 1972, Chardonneau et al. 1974		
	Harp Seal	Ronald et al. 1977		
	American Mink	Aulerich et al. 1974, Wobeser et al. 1976(b), Dansereau et al. 1999		

MAMMAL Sublethal Effect	*Lab/Captive-oriented or Experimentally Manipulated Studies*		*Free-living Population Studies*	
	Species	***Reference***	***Species***	***Reference***
	Rhesus Monkey	Ideka et al. 1973		
	Northern River Otter	O'Connor and Nielsen 1980[2]		
	White-footed Deer Mouse	Burton et al. 1977		
4.REPRODUCTIVE				
4.1.Reduced fertility	Domestic Mouse	Khera 1973(b)	Not Observed	
	Domestic Rabbit	Castellini et al. 2009		
	Domestic Rat	Khera 1973(b), Friedmann et al. 1998, Fossato da Silva et al. 2011		
4.2.Reduced embryo production, birth success or abnormal offspring development	Domestic Cat	Khera 1973(a)	Not Observed	
	Domestic Mouse	Khera 1973(b), Khera and Tabacova 1973, Inouye and Murakami 1975, Muller et al. 1990, Huang et al. 2011		
	Domestic Rat	Khera 1973(b), Khera and Tabacova 1973, Sobotka et al. 1974, Inouye and Murakami 1975		
	American Mink	Wren et al. 1987, Halbrook et al. 1997, Dansereau et al. 1999		
4.3. Aberrant Reproductive Organ Development or Sexual Maturation	Domestic Rat	Moussa et al. 2011	Not Observed	
5.SURVIVAL				
5.1.Lower Juvenile Survival	Harp Seal	Ronald et al. 1977[2]	Not Observed	
	American Mink	Wren et al. 1987		
5.2.Lower Adult Survival	Domestic Rabbit	Anjum and Shakoori 1994	Not Observed	

	Lab/Captive-oriented or Experimentally Manipulated Studies		*Free-living Population Studies*	
MAMMAL Sublethal Effect	***Species***	***Reference***	***Species***	***Reference***
	Domestic Rat	Tamashiro et al. 1986, Wakita 1987		
	American Mink	Aulerich et al. 1974, Wobeser et al 1976a, Wren et al 1987, Dansereau et al. 1999		
	River Otter	O'Connor and Nielsen et al 1980[2]		
6. NO EFFECTS	Crab-eating Macaque	Rice 1998	Northern River Otter	Ben-David et al. 2001, Spencer et al. 2011
	American Mink	Jernelov et al. 1976, Wobeser et al. 1976(a)		

[1]Examined specimens that died in wild, this does not include trapped, hunted, or beached specimens.

[2]Experimentally Manipulated all others are Laboratory studies. Lab studies performed with domesticated species or species breed in captivity. Experimentally Manipulated could include: eggs collected in wild, eggs injected in field and remain in field, nestlings doses in field and left in field, capture and release, wild trapped or netted animals collected and brought into lab, specimens collected from hunters, etc.