DERIVATION OF SCREENING BENCHMARKS FOR DIETARY METHYLMERCURY EXPOSURE FOR THE COMMON LOON (*GAVIA IMMER*): RATIONALE FOR USE IN ECOLOGICAL RISK ASSESSMENT

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Abstract—The current understanding of methylmercury (MeHg) toxicity to avian species has improved considerably in recent years and indicates that exposure to environmentally relevant concentrations of MeHg through the diet can adversely affect various aspects of avian health, reproduction, and survival. Because fish-eating birds are at particular risk for elevated MeHg exposure, the authors surveyed the available primary and secondary literature to summarize the effects of dietary MeHg on the common loon (*Gavia immer*) and to derive ecologically relevant toxic thresholds for dietary exposure to MeHg in fish prey. After considering the available data, the authors propose three screening benchmarks of 0.1, 0.18, and $0.4\,\mu g\,g^{-1}$ wet weight MeHg in prey fish. The lowest benchmark ($0.1\,\mu g\,g^{-1}$ wet wt) is the threshold for adverse behavioral impacts in adult loons and is close to the empirically determined no observed adverse effects level for subclinical effects observed in captive loon chicks. The remaining benchmarks (0.18 and $0.4\,\mu g\,g^{-1}$ wet wt) correspond to MeHg levels in prey fish associated with significant reproductive impairment and reproductive failure in wild adult loons. Overall, these benchmarks incorporate recent findings and reviews of MeHg toxicity in aquatic fish-eating birds and provide the basis for a national ecological risk assessment for Hg and loons in Canada. *Environ. Toxicol. Chem.* 2012;31:2399–2407. © 2012 SETAC

Keywords—Mercury   Birds   Risk assessment   Toxicity

INTRODUCTION

The assessment of health risks to piscivorous birds associated with exposure to methylmercury (MeHg) has been an area of active research for nearly 30 years [1]. Early studies tended to focus on gross clinical symptoms related to MeHg toxicity, such as mortality and gross motor dysfunction and reproductive failure [2,3], and were in large part driven by the obvious impacts of point-source Hg contamination ([4,S1]; all S-numbered references may be found online in the Supplemental Data). More recently, increasingly subtle yet biologically and ecologically important effects of MeHg exposure have been documented in a variety of piscivorous bird species including adverse effects on behavior and neurochemistry, and on biochemical and immunological function [5–8,S2–S4]. These more recent studies reflect both the development of tools and approaches to assess highly sensitive toxicological endpoints as well as the recognition that atmospherically transported Hg can result in contamination of sensitive aquatic ecosystems [9]. Consequently, the potential for adverse effects on piscivorous birds exposed to MeHg through their diet may be greater than previously anticipated [10].

Extensive ecotoxicological work since the early 1990s has established the common loon (*Gavia immer*) as an important avian indicator for MeHg exposure and effects in North America [11–18,S5–S9]. Common loons are widely distributed geographically, are long lived, and feed preferentially on small fish (10–15 cm in size) from lakes within established territories (mean ~70 ha [19]). These characteristics make this species an ideal indicator of MeHg contamination and exposure at local and continental scales [20]. Consequently, the common loon has recently been selected by the U.S. Environmental Protection Agency (U.S. EPA) National Health and Environmental Research Laboratory (NHEERL) as the focal species for development of ecological risk assessment frameworks linked to population models [21] and as the primary avian receptor for a national ecological risk assessment for MeHg under the Mercury Science Program of Environment Canada's Clean Air Regulatory Agenda [22].

Despite evidence that anthropogenic Hg emissions have been declining generally over the past few decades, concern for adverse effects on piscivorous fish and wildlife has increased in recent years. This is based on reports that MeHg levels appear to be increasing both in piscivorous fish and in wildlife at various locations across North America [13,23–25]. As a result, a timely review and synthesis of the literature on the common loon is appropriate. Recent comprehensive reviews of the effects of MeHg in wildlife have been provided by Wolfe et al. [26] and Shore et al. [27]. It is not our intent to revisit these reviews, rather, we seek: (1) to summarize the scope and magnitude of adverse effects of MeHg exposure documented in common loons to date; (2) to derive, using published models, dietary MeHg levels associated with the documented adverse effects; and (3) to derive screening benchmarks for dietary MeHg exposure for use in ecological risk assessment for the common loon.

MATERIALS AND METHODS

For most wildlife species, screening benchmarks are often based on the empirically derived no observed adverse effects level (NOAEL [28]), and compilations of wildlife benchmarks are available in the literature (for example, [29]). However, the availability (or lack thereof) of species-specific data has necessitated the use of interspecies extrapolation or application of uncertainty factors [28,29]. This approach has recently been questioned when suitable data exist for a given species [30]. Moreover, for some contaminants, such as MeHg, it is apparent that substantial interspecies variability in sensitivity exists among avian species [27] and further underscores the added uncertainty when conversion or uncertainty factors are employed.

Laboratory studies to determine empirically the NOAEL and lowest observable adverse effect level (LOAEL) concentrations are difficult to conduct with the common loon, because adult birds do not survive well in captivity (http://bna.birds.cornell.edu/bna/species/313), and much of the literature on common loons and MeHg exposure is derived from field studies [12,15,17,S1,S5–S8,S10]. When such studies examine the potential effects of MeHg, they are generally correlative in nature but endeavor to control or account for potential confounding factors such as coexposure to other contaminants [16], anthropogenic disturbances (e.g., flooding or recreational activity [S1,S11]), nest predation [S7,S12,S13], or prey availability [31,32,S5,S13] that may affect interpretation of the results. Our approach in the present study considers all available qualified toxicity data plus their measures of variability to derive ecologically relevant screening benchmarks.

Relevant literature and establishment of benchmark categories

We have reviewed the available primary and secondary literature to identify suitable studies for derivation of ecologically relevant screening benchmarks for the common loon. Studies included both field-based surveys and observational studies on breeding adults and chicks from various regions in North America and a series of papers documenting captive feeding studies with loon chicks (raised from egg to 105 days posthatch) over a period of several years (1999, 2000, and 2003; see Kenow et al. [S2–S4,S14–S16]) at the U.S. Geological Survey's Upper Midwest Environmental Sciences Center in LaCrosse, Wisconsin, USA. Although most sources have been published in the peer-reviewed literature, technical reports listing relevant endpoints from governmental agencies and the Biodiversity Research Institute were also evaluated.

Adverse effects were categorized by ecological relevance and severity as judged by the authors (Table 1) following the framework of Suter [28]. We considered population-level or individual-level effects to be the most important for deriving screening benchmarks in an ecological context. Mortality, survival, growth, and reproduction constitute endpoints that we considered to be highly relevant to the survival and recruitment of young birds and thus to population dynamics (Table 1). Although the interpretation of the influence of adult mortality and survival on population dynamics is relatively straightforward, the importance of decreased growth rates or reproductive impairment may be less clear. Slower growth rates are linked to a decreased likelihood of chick survival [33] and decreased avian productivity (defined as the number of fledged chicks per territorial adult pair per year [34]) and may impact population stability if productivity is insufficient to balance adult mortality [35].

Table 1. Classification of relevant toxicological endpoints by ecological endpoint for the common loon[a]

Ecological endpoint	Toxicological endpoint
Survival	Survival
	Mortality
Growth	Growth
	Body mass
Reproduction	Productivity
	Egg hatching success
Behavioral	Altered behavioral patterns
	Altered activity budgets
	Impaired motor co-ordination
Subclinical	Altered glutathione metabolism
	Oxidative stress
	Antibody suppression
	Lymphoid depletion
	Altered neurochemical receptor levels
	Altered corticosterone

[a] Ecological endpoints were ranked in order of ecological relevance from highest (survival) to lowest (subclinical) by the authors following the framework of Suter [28].

Effects such as altered behavioral activity and changes in neurochemistry, biochemistry, or immune function are potentially more sensitive toxicological endpoints than clinical effects at the individual level; however, quantitative relationships between these endpoints and individual- or population-level impacts are generally not well established, particularly for wildlife species [36]. For example, a dose–response relationship between dietary MeHg exposure and impaired immune function is difficult to link quantitatively to impacts on disease susceptibility or survival in free-living populations because sick or dead birds from a specific study population are infrequently recovered by researchers. Nevertheless, it is recognized that these subtle endpoints may have cascading effects that ultimately do affect population-level processes [37], and their potential importance to predictive ecotoxicology is likely to increase [36].

Derivation of benchmark thresholds

We evaluated the relevance and appropriateness of each selected study toward deriving a screening benchmark. Many of the selected publications have developed preliminary criteria (NOAEL, LOAEL) for various measures of MeHg exposure in loons. The LOAELs for loon chicks were estimated to be $3\,\mu g\,g^{-1}$ wet weight in blood, $40\,\mu g\,g^{-1}$ wet weight in feathers [S6,S7], $>1.3\,\mu g\,g^{-1}$ wet weight in eggs [S8], and $0.4\,\mu g\,g^{-1}$ wet weight in the diet [S2–S4]. The use of different measurement matrices has both advantages and disadvantages. On the one hand, loon tissues and blood may provide a more accurate assessment of adverse effects because they integrate Hg exposure over all dietary sources and distinct time periods [S7]. Similarly, egg Hg burdens are directly related to maternal dietary exposure and can be readily linked to developmental and reproductive toxicity [S16]. On the other hand, lake-specific fish Hg data are generally more readily available and comparatively simpler to acquire compared with loon tissue, blood, or eggs. A screening benchmark based on Hg concentration in prey fish would be beneficial for providing an economical and defensible screening tool for assessing potential impacts of dietary MeHg exposure to the common loon.

Studies from various regions in North America have indicated that loon blood Hg levels reflect recent dietary exposure during the breeding season and are positively correlated to Hg

levels in prey in the nesting lake [11,15,17]. For the purposes of this review, we assume that total Hg measured in fish of the size typically consumed by loons (10–15 cm [19]) is entirely MeHg [25,38] and that, for loon blood, muscle, brain, and egg contents, measured total Hg is equivalent to MeHg [S15]. Unless measured directly, prey fish MeHg concentration associated with the threshold criteria were estimated using the following steps. First, for MeHg criteria developed for brain tissue, we used equations developed by Kenow et al. [S15] to convert from brain Hg to juvenile loon blood Hg.

$$\text{Log}\,(\text{Hg}_{\text{Blood}}) = 0.726 + 1.059[\log(\text{Hg}_{\text{Brain}})]$$
$$r^2 = 0.97,\ n = 17$$

Second, for criteria developed with egg Hg levels, we used the equation developed by Evers et al. [S8] to convert egg Hg to female blood Hg.

$$\text{Blood Hg}_{\text{Female}} = 0.2238 + 1.5544[\text{Hg}_{\text{Egg}}]$$
$$r^2 = 0.79,\ n = 108$$

Third, for criteria developed using chick blood Hg, we converted these to female blood using the equations developed by Evers et al. [15].

$$\text{Blood Hg}_{\text{Female}} = 1.102286 + 0.464995[\ln(\text{blood Hg}_{\text{chick}})]$$
$$r^2 = 0.64,\ n = 80,\ \text{for chicks} < 28\ \text{d old}$$

Lastly, for threshold levels converted to female blood Hg (steps 1–3), we calculated prey fish Hg using the relationship developed by Evers et al. [15] for fish in the 10 to 15 cm size range.

$$\text{Fish Hg} = (\text{Blood Hg}_{\text{Female}} - 0.5079)/10.644,\ r^2 = 0.51$$

These steps allowed us to convert among different measurement matrices to derive an estimate of dietary MeHg exposure in preferred prey that brackets the indicator prey size chosen for a subsequent national-scale ecological risk assessment. When possible, we also calculated the effective concentration for 20% (EC20) and 50% (EC50) using regression models provided in the source document (as recommended by Allard et al. [30]). The EC20 was chosen to remain consistent with the average reduction in endpoint parameters (~20%) observed in dietary exposure tests of avian reproduction for terrestrial risk assessment of pesticides [39] and because the EC20 often approximates the LOAEL in field studies [40].

Several studies reported only a range of concentrations associated with a LOAEL endpoint. For these, we took the midrange value as an unbiased approximation of the LOAEL and estimated the NOAEL following the Canadian Council of Ministers of the Environment recommendation [41] (LOAEL ÷ 5.6). Estimated prey fish MeHg values, endpoints, and information on the initial measurement matrix are listed in Table 2.

RESULTS AND DISCUSSION

Summary of studies considered for survival endpoint

Because common loon populations exhibit traits such as low annual productivity, delayed sexual maturity, long life expectancy, and suspected low adult mortality (http://bna.birds.cornell.edu/bna/species/313), even small declines in adult survival can have significant implications for population demographics [S17]. Data from mark–recapture programs do not provide compelling evidence for systematic changes in adult survival associated with MeHg exposure in Wisconsin and New England [12,S18]. However, Mitro et al. [S18] indicate that small and likely significant changes (<3%) are not yet detectable based on current data inventories and temporal records. Based on the 75th percentile of blood Hg concentrations reported by Mitro et al. [S18], the highest estimated unbounded adult survival NOAEL for dietary MeHg exposure in breeding adults is ~0.6 μg g^{-1} wet weight in prey fish (Table 2 and Fig. 1).

Adverse effects on the survival of captive loon chicks (up to 105 d posthatch) fed up to 1.2 μg g^{-1} wet weight MeHg as MeHgCl have not been observed [S14]. However, the depuration of ingested MeHg into growing feathers may have protected loon chicks from more overt effects during the present study [S15]. Field studies assessing survival of juvenile loons are rare, but Meyer et al. [12] reported a negative association between chick survival beyond eight weeks of age and chick blood Hg concentrations in northern Wisconsin lakes, suggesting that in the wild, chick survival may be impaired when Hg exposure is elevated. Although other factors could not be ruled out, reduced survival of chicks associated with increased MeHg exposure is not inconsistent with observations in other species [42], but logistical and resource challenges continue to complicate assessment of juvenile survival in wild birds.

Studies considered for growth and development endpoints

The difficulties of keeping adult loons in captivity make assessment of adverse effects on growth or development difficult; therefore, most research has been conducted on wild adults. Evers et al. [S6] documented an increase in flight feather asymmetry in adults with very high levels of Hg in feathers (>40 μg g^{-1} wet wt) and suggested that such developmental abnormalities may negatively affect individual fitness through increased energy expenditure. Barr [S1] reported that the mean weight of male loons from Hg-polluted territories (4.23 kg) was lower than that in control territories (4.62 kg) and found one severely emaciated male loon on Ball Lake, Ontario, Canada between 1975 and 1976, although the weight of female loons did not show a similar pattern. Concentrations of Hg in yellow perch (14.4 ± 1.7 to 16.6 ± 3.1 cm) ranged from 0.04 to 1.53 μg g^{-1} wet weight in these territories, but water level fluctuations and associated turbidity may have also contributed to lower weights by reducing foraging success; therefore, attribution of lower body mass in male loons to MeHg exposure is not strongly supported. Emaciation is also encountered during necropsies of loons found dead or moribund, and some of these loons have high Hg burdens in liver and kidney tissues [43,44], but wasting of body tissues will increase the Hg burden in the remaining carcass [44].

Available evidence is insufficient to determine what, if any, effects MeHg exposure may have on the growth and development of juvenile common loons at dietary MeHg concentrations up to 1.2 μg g^{-1} wet weight [S14]. Stronger effects on body mass at hatch and asymptotic mass were related to the lake of origin of the collected eggs (see synopsis below). As discussed by Kenow et al. [S14], the lack of adverse effects on growth must be cautiously interpreted, because the results are applicable only to chicks up to 105 d of age and because of the potential protection provided by excretion of MeHg into feathers. Free-ranging loon chicks are known to have relatively high energetic demands [45], and the possibility exists that laboratory conditions designed to optimize chick survival may mask potential impacts on growth that are more likely to occur in the wild. For example, feeding studies of captive great egret chicks documented a reduction in weight gain resulting from reduced

Table 2. Summary of studies on the common loon used in this review[a]

Endpoint	Life stage	Region	NOAEL	LOAEL	EC20	EC50	Initial measurement	Reference
Survival								
Survival	Adult	WI/NE	0.45[b] (♂)	ND	ND	ND	75th percentile ♂ and	[S18]
			0.58[b] (♀)				♀ blood Hg (NE only)	
Mortality	Juvenile[c]	WI	>1.2	ND	ND	ND	Dietary MeHgCl	[S14]
Growth								
Body mass	Juvenile[c]	WI	>1.2	ND	ND	ND	Dietary MeHgCl	[S14]
Reproduction								
Maximum productivity	Adult	WI/Mar	ND	ND	0.07[d]	0.2[d]	Measured prey fish Hg	[S5]
Productivity	Adult	NW ONT	0.03[e]	0.17[f]	ND	ND	Measured prey fish Hg	[S1]
Productivity	Adult	NH/ME	0.03[e]	0.16	ND	ND	Measured prey fish Hg	[S6,S7]
Productivity	Adult	QC	0.15	ND	ND	ND	Measured prey fish Hg	[S10]
Productivity	Adult	Hanson Lake, SK	0.08[b]	ND	ND	ND	Max. egg Hg concentration	[S12]
Hatch success	Egg	WI	0.03[e,g]	0.18[h]	0.13[i]	0.23[i]	Egg Hg (injected MeHgCl)	[S16]
Hatch success	Egg	NH/ME	ND	>0.3	ND	ND	Measured prey fish Hg	[S6]
Behavioral								
Reduced time back riding	Juvenile[j]	Mar	0.05[e]	0.27[b]	ND	ND	Chick blood Hg	[S11]
Impaired motor control (righting reflex)	Juvenile[c]	WI	0.08	0.40	ND	ND	Dietary MeHgCl	[S2]
Time foraging for self (nesting phase)	Adult	NH/ME	0.02[e]	0.10[k]	ND	ND	Measured prey fish Hg	[S6]
Increased time resting	Adult ♂	NH/ME	0.02[e]	0.10[k]	ND	ND	Measured prey fish Hg	[S6]
Aberrant incubation behavior	Adult	NH/ME	0.02[e]	0.10[k]	ND	ND	Measured prey fish Hg	[S6,S7]
Reduced chick feeding	Adult	NH/ME	0.05[e]	>0.3	ND	ND	Measured prey fish Hg	[S6]
Reduced time incubating eggs	Adult	NH/ME	0.02[e]	0.10[k]	ND	ND	Measured prey fish Hg	[S6]
Increased dive frequency	Adult	NH/ME	0.05[e]	0.25[k]	ND	ND	Adult loon blood Hg	[S20]
Biochemical/immunological								
Increased corticosterone	Adult	ME	ND	0.05	ND	ND	Loon blood Hg	[S6]
Bursal lymphoid depletion Antibody suppression	Juvenile[c]	WI	0.08	0.4[l]	ND	ND	Dietary MeHgCl	[S4]
Oxidative stress Glutathione metabolism	Juvenile[c]	WI	0.08	0.4	ND	ND	Dietary MeHgCl	[S3]
Neurochemical receptors	Adult	NA/Lake Erie	0.01[e]	0.05–0.08[b]	ND	ND	Brain THg	[S9,S23]

[a] Included in the summary are the ecological endpoints assessed (endpoint), life stage of test subjects (life stage), geographic region within North America where the study was conducted or applicable (region), screening benchmarks derived from each study (adult no observed adverse effects level (NOAEL), low observed adverse effects level (LOAEL), effective concentration for 20% (EC20) and effective concentration for 50%; reported as concentration of Hg in prey fish, expressed as wet wt, $\mu g \, g^{-1}$), initial measurement matrix reported in each study with screening benchmarks (Initial measurement matrix; see *Materials and Methods* section), and source document (Reference) to identify the threshold.

[b] Derivation following the steps outlined in the *Materials and Methods* section.

[c] Chicks raised from egg to 105 d posthatch.

[d] Calculated using the model presented by Burgess and Meyer [S5].

[e] NOAEL calculated following the Canadian Council of Ministers of the Environment (CCME) [41].

[f] Mean Hg content of fish from territories with minimal water level fluctuations.

[g] Control eggs determined to contain ∼0.3 ppm MeHg.

[h] Determined to be significantly different from controls.

[i] Estimated from model developed by Kenow et al. [S16].

[j] Chicks <12 d old.

[k] Midrange value of prey fish Hg concentrations associated with the LOAEL.

[l] True LOAEL may be <0.4 based on magnitude of change in measured endpoints (immunoglobulin G titer).

NE = New England; WI = Wisconsin; ME = Maine; QC = Quebec; SK = Saskatchewan; Mar = Atlantic Canada; NW ONT = northwestern Ontario; NA = North America.

fish consumption [46], and lower weights were observed in wild egret chicks but at lower cumulative doses of MeHg than their captive counterparts (G.E. Williams, 1997, MSc thesis, University of Florida, Gainesville, FL, USA) [46], lending some indirect support for this hypothesis.

Studies considered for reproductive endpoints

In general, laboratory and field studies have demonstrated that, for many avian species, adverse effects on reproduction occur at dietary MeHg exposures one to two orders of magnitude below those that cause overt mortality or neurological symptoms [27]. The greater sensitivity of reproduction may be partially related to the many potential pathways by which MeHg can affect the reproductive process. MeHg may alter the mate selection and pairing behavior, incubation, and chick-rearing behavior of breeding adults [47,S6,S7], whereas deposition of MeHg into developing eggs may induce teratogenic effects in embryos [48], increase embryo mortality [49], reduce hatching success [50,S16], or affect the behavior of surviving offspring [3].

In an MeHg egg-injection experiment, Kenow et al. [S16] documented a dose-dependent decrease in hatching success and an increase in incubation time for common loon eggs and estimated the EC50 (median lethal concentration [LC50]) for hatching success to be $1.78 \, \mu g \, g^{-1}$ wet weight. The lone reported death of a chick (at hatch) in the wild (Ball Lake [S1]) was associated with a whole-body residue of $2.44 \, \mu g \, g^{-1}$ wet weight. Using the carcass-to-egg conversion equation provided by Kenow et al. [S16], (log Egg$_{Hg}$ = [log[Carcass$_{Hg}$] − 0.323]/0.905, $r^2 = 0.85$) provides an estimate of $1.79 \, \mu g \, g^{-1}$ wet weight in the Ball Lake egg, which coincides with the experimentally determined EC50 [S16]. The EC20 ($1.1 \, \mu g \, g^{-1}$
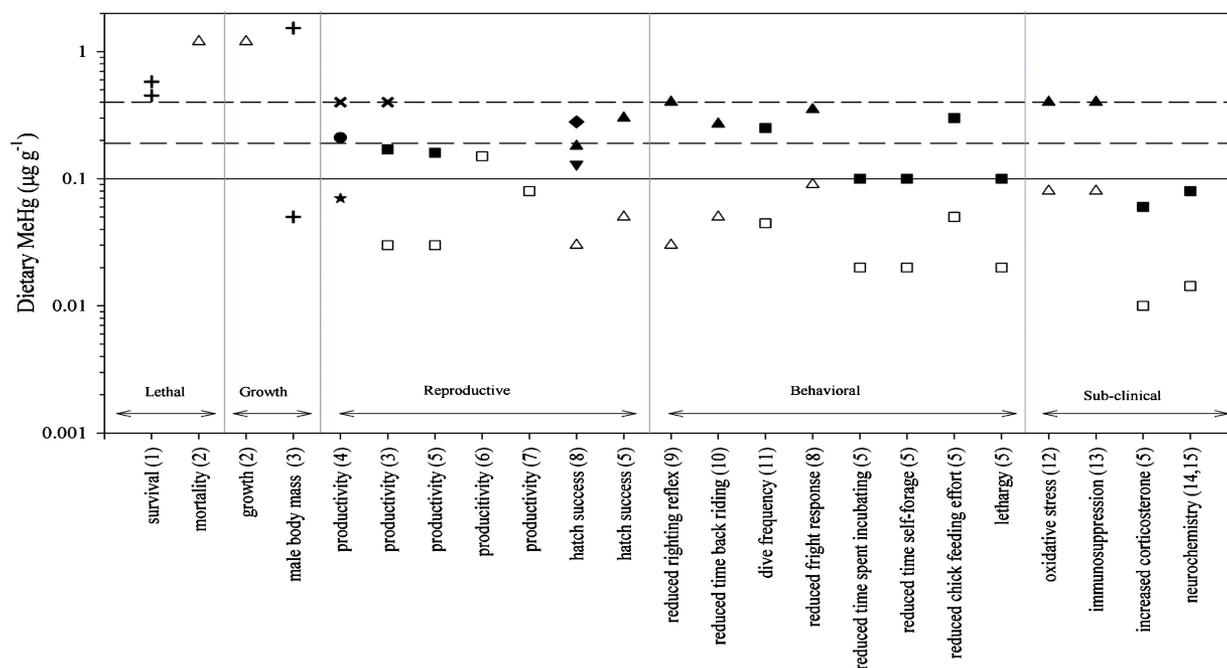
Fig. 1. Plot of relevant endpoints for deriving screening benchmarks for the common loon. Endpoints are sorted by category and displayed as follows: adult no observed adverse effects level (NOAEL; □), adult low observed adverse effects level (LOAEL; ■), adult EC20 (★), adult EC50 (●), juvenile NOAEL (Δ), juvenile LOAEL (▲), juvenile effective concentration for 20% (EC20; ▼) and juvenile effective concentration for 50% (EC50; ◆), ✚ Represents the estimated upper and lower ranges of dietary exposure associated with relevant endpoints, and ✕ represents the effective concentration for 100% (EC100). The solid line indicates the proposed behavioral benchmark (0.1 μg g^{-1}), the line with longer dashes represents the reproductive impairment benchmark (0.18 μg g^{-1}), and the line with shorter dashes represents the reproductive failure benchmark (0.4 μg g^{-1}). Supplemental References for selected endpoints are as follows: 1, [S18]; 2, [S14]; 3, [S1]; 4, [S5]; 5, [S6]; 6, [S10]; 7, [S12]; 8, [S16]; 9, [S2]; 10, [S11]; 11, [S20]; 12, [S3]; 13, [S4]; 14, [S9]; and 15, [S23].

wet wt) and EC50 correspond to an estimated concentration of 0.13 and 0.23 μg g^{-1} wet weight, respectively, in maternal diets (Table 2 and Fig. 1). Although it has not been directly demonstrated with common loon eggs, it appears that, in general, when MeHgCl is injected into eggs, it is more toxic than maternally deposited MeHg [49]. However, the EC20 and EC50 from the egg injection study bracket the threshold level of 1.3 μg g^{-1} wet weight indicative of loons from territories where prey fish Hg typically exceeds 0.3 μg g^{-1} wet weight [S1,S8].

Productivity is arguably the most ecologically relevant reproductive endpoint because overall productivity integrates potential adverse effects of MeHg on adult behavior, egg production and incubation, embryonic development and hatching success, posthatch parental care, and chick survival. However, productivity may also be affected by prey availability [31,S13] and anthropogenic disturbances such as water level regulation [S1] and recreational activity [51]. These confounding effects undoubtedly vary spatially and temporally and may not be readily separable from effects related to MeHg exposure. For example, although Merrill et al. [S13] found that loon chick survival was unrelated to lake pH and cumulative Hg exposure in Wisconsin lakes, nearly 75% of the lakes studied were classified as "seepage" (i.e., no inlets or outlets; see Meyer et al. [12]). Such lakes can have low fish diversity and abundance because of barriers to fish migration and frequent wintertime anoxia [52]. In addition, heightened sensitivity to acid-related reductions in invertebrate and fish abundance [53] may also affect prey availability [S13].

Despite the potential for confounding stressors to complicate the assessment of MeHg exposure on productivity, studies published to date share a number of consistencies. On highly contaminated Ball Lake, breeding adult female loons laid

fewer eggs, and productivity and hatching success declined by 82 and 42%, respectively, relative to upstream reference territories [S1]. These reductions in reproductive success occurred in territories unaffected by water level fluctuations but accessible to fish with high MeHg concentrations (mean 0.17 ± 0.09 μg g^{-1}, range 0.05–0.35 μg g^{-1} wet wt [S1]). Reproduction ceased completely in territories where concentrations of MeHg in prey fish were >0.4 μg g^{-1} wet weight, although these territories were also affected by water level manipulation and high turbidity [S1]. Evers et al. [S6,S7] documented a 13% reduction in egg laying and a 30% reduction in hatching success on lakes in the northeastern United States where prey fish Hg was >0.3 μg g^{-1} wet weight. Productivity in these territories was reduced by 40% where fish Hg levels were between 0.15 and 0.3 μg g^{-1} wet weight, prompting Evers et al. [S7] to suggest a threshold level (LOAEL) of 0.16 μg g^{-1} wet weight in prey fish for significant impacts on loon productivity. Burgess et al. [S19] observed a reduced tendency for breeding adult loons to nest, accompanied by a decline in productivity as blood Hg levels increased in Kejimkujik National Park, Nova Scotia, Canada. A recent reanalysis of data from Atlantic Canada and Wisconsin indicated that reductions in maximum productivity are likely related to MeHg exposure, and a 50% reduction is predicted to occur at prey fish MeHg levels of ∼0.21 μg g^{-1} wet weight, and successful reproduction is predicted to cease completely at prey fish MeHg levels of >0.4 μg g^{-1} wet weight [S5].

Unlike the above-mentioned studies, the study of Champoux et al. [S10] did not find strong evidence that loon productivity in selected lakes was affected by MeHg levels in prey fish in Quebec. Although somewhat contradictory at first glance, the majority of lakes examined in this study had mean prey fish MeHg levels of <0.15 μg g^{-1} wet weight, and extraneous

stressors such as human disturbances were not explicitly controlled. Impacts on productivity might have been less pronounced compared to those in other regions where MeHg levels in prey fish more frequently exceed $0.15 \mu g\,g^{-1}$ wet weight. Similarly, Fox et al. [S12] were unable to find evidence of contaminant-related declines in productivity in loon territories on Hanson Lake, Saskatchewan, Canada. However, MeHg exposure in loons on Hanson Lake was low; based on the maximum recorded egg Hg, the estimated maximum MeHg in prey fish was $0.08 \mu g\,g^{-1}$ wet weight (Table 2 and Fig. 1). Although all confounding factors that influence productivity cannot be completely eliminated, the consistency between studies in differing regions of North America [S1,S5–S7,S10,S12] provides strong evidence that loon productivity is sensitive to MeHg exposure and that moderate concentrations of MeHg in prey fish (e.g., 0.1–$0.2 \mu g\,g^{-1}$ wet wt) may significantly reduce productivity (i.e., >40%).

Summary of studies considered for behavioral endpoints

The potent neurotoxicity of MeHg is well known, and at sufficient concentrations ($>5 \mu g\,g^{-1}$ wet weight) MeHg can elicit overt neurological symptoms, including ataxia and gross motor impairment in birds [26,27]. However, for birds exposed to lower MeHg concentrations, behavioral abnormalities may be considerably more subtle and thus difficult to link to apical outcomes of survival or reproduction [10].

Increased Hg exposure has been correlated with an increased frequency of lethargic behaviors in breeding adult loons. In breeding territories where Hg concentrations in prey fish are between 0.05 and $0.15 \mu g\,g^{-1}$ wet weight, adults spent more time preening and resting and less time foraging for themselves and significantly reduced amounts of time spent incubating eggs (~10%) compared with breeding adults on territories where prey fish Hg concentrations were $<0.05 \mu g\,g^{-1}$ wet weight [S6,S7]. In territories where prey fish Hg concentrations exceeded $0.3 \mu g\,g^{-1}$ wet weight, food provisioning for chicks was reduced by 42% relative to that in territories where prey fish Hg concentrations were $<0.05 \mu g\,g^{-1}$ wet weight [S6], and dive frequencies of adults were observed to increase substantially [S20], although a plausible link to MeHg exposure remains equivocal [S21] (Table 2 and Fig. 1).

Field studies have documented subtle behavioral alterations in loon chicks at different stages of development. Loon chicks <12 d-old spent less time back-riding, potentially increasing unnecessary energy expenditures and increasing vulnerability to predation and exposure [S11]. The concentration of MeHg in prey associated with a complete cessation in back riding was estimated to be ~$0.3 \mu g\,g^{-1}$ wet weight (Table 2 and Fig. 1). Counard [S22] observed a negative relationship between the frequency of wing flaps and diving, and a corresponding increase in swimming, peering, and begging as blood Hg concentrations increased in >40-d-old chicks. These behaviors were not observed by Kenow et al. [S2] in their captive chick study, but they did report impaired motor coordination (measured by righting reflex) in >37-d-old chicks fed $0.4 \mu g\,g^{-1}$ wet weight MeHgCl.

The behavioral effects discussed above appear to manifest when prey MeHg concentrations are between 0.05 and $0.4 \mu g\,g^{-1}$ wet weight (Table 2 and Fig. 1) and generally suggest a shift toward less energetic behaviors in adults [S6,S7], but the response of chicks is often more ambiguous [S2,S11,S16,S22]. Overall, observed behavioral aberrations are comparable to those associated with MeHg exposure in other piscivorous species [5]. Some of the behavioral impairments

exhibited by adult loons may adversely affect productivity. A reduction in time spent incubating eggs will increase the risk of egg loss to nest predation and chilling [S7] and may be linked to increased incidences of embryo malposition resulting from improper turning of the eggs during incubation [54]. Reductions in chick feeding effort may directly impair the growth and survival of loon chicks, insofar as underprovisioning is linked to failure of chicks to fledge or to abandonment [55]. Bridging the gap between observed behavioral impairments and changes in individual fitness, survival, or reproductive success remains a worthwhile albeit difficult approach for assessing the impacts of MeHg exposure in loons and other wildlife.

Summary of studies considered for subclinical endpoints

In recent years, the volume of data detailing mechanistic responses to MeHg exposure in wildlife at the molecular, biochemical, and cellular levels has expanded considerably [56]. Dietary exposure trials with mallard ducks (*Anas platyrhynchos*) [8] developed a suite of biochemical and physiological endpoints that responded to MeHg exposure in the laboratory, and these have been validated in wild birds from various locations [57–59]. Some of these biochemical endpoints have been observed in common loons. At dietary exposure concentrations of 0.4 and $1.2 \mu g\,g^{-1}$ wet weight, evidence of oxidative stress and altered glutathione metabolism was observed in brain tissues of captive loon chicks [S3]. At dietary exposures of $0.4 \mu g\,g^{-1}$ wet weight, atrophy and lymphoid depletion of the thymus and bursa and a reduction in T-cell-mediated immune response (~40%) were observed in captive loon chicks, and the declines in antibody titer (immunoglobulin G) in the $0.08 \mu g\,g^{-1}$ wet weight exposure group were comparable to those in the $0.4 \mu g\,g^{-1}$ wet weight exposure group (54 vs 58%), although this difference was deemed not significant [S4] (Table 2 and Fig. 1).

Comparable data for adult loons is not available primarily because of the difficulty in acquiring samples for analysis. However, significant elevations in blood corticosterone concentrations in wild adult loons were observed in loon territories where prey fish Hg concentrations were $>0.05 \mu g\,g^{-1}$ wet weight [S6], and significant correlations were found between brain Hg concentrations (in which Hg concentrations ranged from 0.2–$68 \mu g\,g^{-1}$ dry wt) and the densities of muscarinic cholinergic receptors and *N*-methyl-*D*-aspartic acid receptors in wild loons collected opportunistically across Canada [S9]. Changes in these neurochemical receptor densities are known to elicit deleterious effects on motor function, memory, and learning in mammals [60,61] but have been rarely studied in birds. Based on the data presented by Scheuhammer et al. and Hamilton et al. [S9,S23], a brain Hg concentration of approximately $3 \mu g\,g^{-1}$ dry weight (~$0.75 \mu g\,g^{-1}$ wet wt, assuming 75% moisture content) may represent a threshold below which significant changes in neurochemical receptor densities do not occur in loons [S23]. This corresponds to an estimated minimum dietary MeHg concentration of 0.05–$0.08 \mu g\,g^{-1}$ wet weight (Table 2 and Fig. 1). Although potentially biased by the use of a model constructed for juvenile loons (see *Materials and Methods*), this level of MeHg exposure is comparable to dietary exposure concentrations associated with similar changes observed in brains of mink (*Mustela vision*; $(0.1 \mu g\,g^{-1}$ wet wt [62]).

Although these biomarkers are demonstrably sensitive to low-level MeHg exposure, linking the measured response to individual or population-level effects remains difficult. For example, loon chicks fed 0.4 and $1.2 \mu g\,g^{-1}$ wet weight

MeHgCl were less likely to right themselves when placed on their backs [S2], and evidence of oxidative stress and altered glutathione metabolism was present in brain tissue [S3]. In great egret chicks fed $0.5\,\mu g\,g^{-1}$ wet weight MeHg, behavioral aberrations were observed [5], but evidence of oxidative stress in brain tissue was not apparent until dietary exposure reached $5\,\mu g\,g^{-1}$ wet weight [63] and chicks displayed significant overt neurological dysfunction [6]. Immune system suppression related to MeHg exposure appears to be generally universal in avian species tested to date [6,58], and high MeHg exposure has previously been linked to chronic disease occurrence in free-ranging aquatic birds [44,64]. The reduced T-cell-mediated immunity observed in loon chicks (~40% reduction), although not statistically significant, may nonetheless be indicative of significant immune system suppression [S4] that may not only impair the ability of birds to resist infectious diseases or parasites but also may impair overall population fitness, leading to population-level impacts [37]. Although these more recent studies are providing sensitive tools and approaches with which to evaluate MeHg exposure in birds, their integration into ecological risk assessment approaches remains limited at present.

Synopsis, remaining uncertainties, and screening benchmarks

We propose, based on our review of the literature, three screening benchmarks based on three categories of relevant endpoints using a weight-of-evidence approach (Table 3). However, we refrain from proposing benchmark thresholds for loon chicks or for lethal effects in both chicks and adult loons because of the residual uncertainty surrounding the assessment of long-term survival.

For loon chicks, several factors contribute to greater uncertainty in the development of appropriate screening benchmarks. One aspect is related to the variable exposure profile observed for common loon chicks [S15] and chicks of other piscivorous species [65]. The highest MeHg exposures occur immediately after hatching (from maternal transfer of MeHg) and again when feather growth ceases and a key depuration pathway is no longer available, suggesting that risk may be highest during these time periods [65]. Because the captive feeding studies with loon chicks were terminated at 15 weeks of age and blood Hg levels were on an upward trajectory at that time, negative effects on chicks beyond this time frame are unknown, but are potentially significant [S14]. The presence of adverse behavioral effects in loon chicks <12 d-old [S11] and >37 d-old [S2,S22] is not inconsistent with the hypothesized pattern of risk, but more data are required to help reduce uncertainty in deriving a screening benchmark for loon chicks.

A second aspect related to uncertainty surrounding loon chicks is that in ovo exposure appears to be more important than dietary exposure to the susceptibility of juveniles. This is

highlighted by the strong and consistent lake-source effect observed by Kenow et al. [S2–4,S14,S16] in their series of captive loon chick experiments. Chicks hatched from eggs collected on low-pH lakes, where exposure to Hg is known to be higher compared with neutral-pH lakes [11,12] weighed 3.8% less at hatch, had 7% lower asymptotic mass [S14], were less responsive to parental calls [S2] and to frightening stimuli [S16], had more severe physiological effects [S3], and had more depressed immune function [S4] compared with chicks hatched from eggs on neutral-pH lakes.

We did not propose a benchmark dose for survival because survival is difficult to assess outside of acute lethality. The lack of acute lethality in captive fed loon chicks or obvious impairment of adult survival inferred from field studies is perhaps not overly surprising, because the lethal dietary threshold for non-marine avian species reported by Shore et al. [27] is $>6\,\mu g\,g^{-1}$ (range, $10–40\,\mu g\,g^{-1}$; 11 species), and this far exceeds concentrations of Hg provided to loon chicks in captivity [S14] or preferred prey fish species typically consumed by adult loons in North America [25,66,67]. However, evidence suggests that some recaptured wild adult loons in northeastern North America exhibit annual increases in feather Hg (~5–10% year^{-1}) [S7]. This may indicate either an increase in Hg exposure or perhaps incomplete depuration on wintering grounds [S7]. Mortality of adult loons is rarely reported on summer breeding grounds and likely is more common during migration and over winter, making assessment of survival in the wild difficult without substantial data inventories [S18]. Necropsies of dead or moribund loons [43,44] have frequently documented high Hg burdens in various tissues (especially in emaciated individuals), but causes of death are often related to lead toxicity, trauma, or respiratory infection and aspergillosis [68]. Although the possibility exists that MeHg exposure contributes to some extent to loon mortalities, a lack of compelling evidence and the paucity of data pertaining to survival rates of juvenile and nonbreeding loons hinders assessment of Hg impacts on long-term survival and population dynamics.

Proposed benchmark dietary concentrations

A screening benchmark of $0.1\,\mu g\,g^{-1}$ wet weight is proposed to be associated with alterations to normal adult loon behaviors (Table 3 and Fig. 1). The level of uncertainty surrounding this benchmark concentration is relatively high because this essentially represents the midpoint of a range of values (0.05–$0.15\,\mu g\,g^{-1}$ wet wt) over which behavioral aberrations have been observed. That this midrange value closely corresponds to an estimated dietary level at which neurochemical changes are observed in loon brain tissue (Table 2 and Fig. 1) may simply be coincidental but certainly merits further study. Importantly, the degree to which these adult behavioral changes will affect adult or chick survival in the wild or population dynamics is presently

Table 3. Summary of proposed screening benchmarks for common loon exposure to MeHg[a]

Category	Proposed screening benchmark threshold ($\mu g\,g^{-1}$) wet weight	Endpoints considered	Primary sources
Adult behavioral abnormalities	0.1	Midpoint of range for adverse adult behavior LOAELs	[S6]
Significant reproductive impairment	0.18	Geometric mean of productivity LOAEL and EC50, hatch success EC50	[S1,S5–S7,S16]
Reproductive failure	0.4	Productivity reduced to zero	[S1,S5]

[a] Screening benchmarks are expressed as Hg concentrations in prey fish ($\mu g\,g^{-1}$ wet wt).
LOAELs = low observed adverse effects level; EC50 = effective concentration for 50%.

unknown; therefore, the suitability of this benchmark for ecological risk assessment remains limited.

The remaining screening benchmarks ($0.18\,\mu g\,g^{-1}$ and $0.4\,\mu g\,g^{-1}$, wet wt) are proposed to be indicative of significant reproductive impairment and reproductive failure, respectively, in breeding adult common loons. These benchmarks for the common loon bracket the dietary value proposed by Shore et al. [27] to be indicative of reproductive impairment in nonmarine birds ($0.25\,\mu g\,g^{-1}$; one-fifth of the geometric mean of LOAEL for seven species). We consider these screening benchmarks to have a lower level of uncertainty than the behavioral benchmark, because strong associations with significant (40–50%) declines in productivity have been documented in Ontario, Wisconsin, Atlantic Canada, and the northeastern United States (Table 3 and Fig. 1). The consistency of these effects across regions and the direct assessment of loons in a natural environment strongly suggest that these screening benchmarks are reasonably well defined. Moreover, although demographic models suggest a low sensitivity of population stability to changes in productivity [S17], declines in productivity on the order of 40% have been associated with negative population growth rates in Maine from 1982 to 1999 [S6], and a complete failure of reproduction will obviously impair population stability. These benchmarks are clearly relevant to the assessment of ecological risk to the common loon based on dietary exposure to MeHg.

CONCLUSIONS

The common loon is clearly vulnerable to dietary MeHg exposure in the range of 0.1 to $0.4\,\mu g\,g^{-1}$ wet weight. These concentrations are found in preferred prey fish species within their breeding lakes across North America. The three screening benchmarks developed here use directly applicable data for common loons and will allow for a tiered assessment of risk in ecological risk assessment. Continued refinement and enhancement of population models and further understanding of linkages between highly sensitive biochemical endpoints and individual- or population-level responses will further enhance the ability to define adverse effect thresholds related to MeHg in the common loon as well as other species of avian piscivores.

SUPPLEMENTAL DATA

Supplemental References S1–S23. (41 KB DOC).

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