EcoHealth
DOI: 10.1007/s10393-015-1021-8

EcoHealth

Original Contribution

Blood and Hair Mercury Concentrations in the Pacific Harbor Seal (*Phoca vitulina richardii*) Pup: Associations with Neurodevelopmental Outcomes

Samala Van Hoomissen,[1,4] Frances M. D. Gulland,[2] Denise J. Greig,[2] J. Margaret Castellini,[3] and Todd M. O'Hara[3]

[1]*School of Public Health, University of Minnesota, 420 Delaware St SE Mmc88, Minneapolis, MN 55455*
[2]*The Marine Mammal Center, 2000 Bunker Road, Fort Cronkhite, Sausalito, CA 94965*
[3]*Department of Veterinary Medicine, College of Natural Science and Mathematics, University of Alaska Fairbanks, Fairbanks, AK 99775*
[4]*8601 Lincoln Blvd. #3317, Los Angeles, CA 90045*

Abstract: Monomethylmercury ($MeHg^+$) is an environmental pollutant, which at sufficiently high exposures, has induced neurotoxicosis in several animal species, including humans. Adverse neurological effects due to gestational exposure are of particular concern as $MeHg^+$ readily crosses the blood–brain and placental barriers. The degree to which environmental concentrations in marine prey affect free-living piscivorous wildlife, however, remains largely undetermined. We examined associations of gestational exposures to mercury on neurodevelopment and survival using hair and blood concentrations of total mercury ([THg]) in a stranded population of Pacific harbor seal pups from central California. A positive association was determined for the presence of abnormal neurological symptoms and increasing [THg] in blood ($P = 0.04$), but not hair. Neither hair nor blood [THg] was significantly associated with survival, or the neurodevelopmental milestone 'free-feeding', which was measured from the onset of hand-assisted feeding to the time at which pups were able to consume fish independently. Both hair and blood [THg] exceeded threshold values considered potentially toxic to humans and other mammalian wildlife species. The higher [THg] in blood associated with abnormal neurological symptoms may indicate an adverse effect of this pollutant on neurodevelopment in harbor seal pups. These data have broader implications with respect to human health and public policy as harbor seals and humans consume similar fish species, and it is possible that safeguard levels established for marine mammals could also extend to human populations that regularly consume fish.

Keywords: harbor seal pup, mercury, neurodevelopment, toxicological thresholds, rehabilitation

Introduction and Purpose

Mercury (Hg) is a ubiquitous pollutant that presents a variety of health risks to humans and wildlife. Concerns about mercury pollution stem largely from the potential adverse effects of dietary exposure to monomethylmercury

Correspondence to: Samala Van Hoomissen, e-mail: sammyv@mninter.net

Published online: 27 March 2015

($MeHg^+$), a toxic form that readily accumulates in biota and can biomagnify to harmful concentrations in organisms via the aquatic and marine food webs (Mahaffey 2000; Wiener et al. 2003; Clarkson and Magos 2006; Woshner et al. 2008; Dietz et al. 2011). Once ingested, $MeHg^+$ readily enters the bloodstream and crosses both the blood–brain and placental barriers (Wagemann et al. 1988; Zheng et al. 2003), often resulting in higher levels of mercury in the fetal than the maternal brain (Myers and Davidson 1998; Sakamoto et al. 2002; Zheng et al. 2003). Because the developing central nervous system (CNS) is extremely sensitive to its neurotoxic effects (Castoldi et al. 2001; Grandjean and Landrigan 2006), $MeHg^+$ exposure is of particular concern in neonates and fetuses (Johansson et al. 2007; Davidson et al. 2008).

Within the San Francisco Bay (SF Bay) region, Hg contamination has been a concern since historic mining of gold and cinnabar contaminated the area. At present, mercury concentration ([Hg]) in the muscle tissues of a number of sport-caught fish from SF Bay exceed Advisory Tissue Levels set by California's Office of Environmental Health Hazard Assessment (OEHHA) (Davis et al. 2011), resulting in public health guidance to limit intake. While regulatory and research efforts have largely focused on exposure risks to humans, there has been a tremendous growth in information documenting the presence of Hg in marine mammal tissues. Recent studies (Brookens 2006; Brookens et al. 2007, 2008; McHuron 2012; McHuron et al. 2014) have focused on total mercury concentrations ([THg]) in tissues of Pacific harbor seals because these marine mammals reside in SF Bay and forage almost entirely on fish.

Pacific harbor seals are widely distributed along the mainland coast and bays of California (Lowry et al. 2008) and the most abundant pinniped in the Pacific Northwest (Huber et al. 2001). As piscivores feeding at high trophic levels, harbor seals share with humans a variety of fish species as food, and bioaccumulate Hg. Their prey base overlaps with species targeted by sport fishing industries in SF Bay, such as white croaker (*Genyonemus lineatus*), jacksmelt (*Atherinopsis califoniensis*), and striped bass (*Morone saxatilis*) (Gibble 2011), all of which are currently included in California's Fish Consumption Advisory list for reduced consumption by humans (Davis et al. 2011). This overlap in prey selection indicates a link in the dietary needs of harbor seals and humans and raises concerns about local impacts of Hg exposure on harbor seal populations in SF Bay in an Oceans and Human Health context.

During recent decades, harbor seal populations in SF Bay have been increasing at a slower rate than other locations along the Pacific coast (Harvey et al. 1990; Sydeman and Allen 1999; Neale et al. 2005; Davis et al. 2007). A number of contributing factors have been hypothesized such as harassment, reduction or change in prey resources, and environmental contamination (Kopec and Harvey 1995; Grigg et al. 2004); however specific causes for a less-than-expected population growth rate remain largely undetermined. Prior studies (Brookens 2006; Brookens et al. 2007, 2008; McHuron 2012; McHuron et al. 2014) indicate that [THg] in the hair of some harbor seal adults and pups sampled in northern and central California currently exceed the US Environmental Protection Agency's (USEPA) benchmark threshold of 11 μg/g dry weight (dw) in human maternal hair associated with clinically discernible, adverse neurodevelopmental milestones in children following in utero exposure to $MeHg^+$ (USEPA 1997). Whether or not these concentrations are high enough to warrant concerns regarding the health of marine mammals and their developing offspring is not known. Thus, more direct assessments of pinnipeds are needed.

Conclusive cause-and-effect relationships linking chemicals to toxicosis or population-level effects are seldom documented for marine mammals as these cases are difficult to diagnose or research (O'Hara and O'Shea 2005) due to logistical and political constraints. A majority of evidence regarding the neurotoxicity of $MeHg^+$ on the developing CNS has therefore been generated through experiments in laboratory animals, epidemiological investigations in humans, or from very high exposures (e.g., accidents, large local inputs) that are not relevant to most fish consumption scenarios and pathways. Experimental animal studies have been useful in establishing associations between exposure and adverse outcomes, and are essential in recognizing the high vulnerability of the fetal brain to $MeHg^+$ (Castoldi et al. 2008). Yet the extent to which results can be extrapolated from the laboratory to the field and from one species to another remains equivocal (USEPA 1997). To draw valid and reliable conclusions on the effects of $MeHg^+$ exposure on the developing CNS in marine mammals, data ideally need to be drawn directly from the species of interest. One strategy is to utilize wild-stranded populations for sequential observational and diagnostic analysis throughout the rehabilitation period as a platform of opportunity.

As sentinel species of marine ecosystem health, stranded harbor seal pups are useful in detecting in utero

exposures to environmental contaminants as well as primary infectious or parasitic diseases; their usefulness, however, is limited when the objective is to determine prevalence of a disease and its impact on a host population (Gulland and Hall 2005). Stranded populations have well-recognized biases, such as decreased immune function and malnutrition due to maternal abandonment, which could potentially skew the results of the study. Furthermore, data from rehabilitated populations of stranded harbor seal pups may be influenced by human-derived clinical support such as subcutaneous fluid administration or oral rehydration prior to sampling. The results of this study are therefore limited in scope to the assessment of association or correlation, and do not indicate a cause-and-effect relationship. Broad conclusions relative to this research hypothesis should also be avoided until several efforts from different populations and geographical locations provide similar results.

Each spring, live-stranded harbor seal pups from the central California coast are admitted to The Marine Mammal Center (TMMC) in Sausalito, CA for assessment and rehabilitation. Upon admission, pups are clinically assessed and samples of hair and blood are collected as part of the routine veterinary medical protocol. Most of these pups undergo an approximately 2-month period of rehabilitation during which they begin to independently catch and consume fish. Harbor seal pups undergoing rehabilitation at TMMC therefore provide an opportunity to evaluate [THg] in blood and hair determined at admission with respect to frequently observed neurodevelopmental behavior from a normally inaccessible wild population as (1) harbor seal pups in rehabilitation are abundant, (2) tissue collection techniques are relatively non-invasive, and required for standard medical monitoring and intervention, and (3) pups can be observed and clinically evaluated with minimal disturbance throughout rehabilitation over time.

Until more advanced methods for observational data collection in the field become available, stranded animals offer a surrogate population from which valuable data can be collected and analyzed, assuming careful consideration is given to potential extraneous variables. The central goal of this study was to determine if [THg] in the blood and hair of Pacific harbor seal pups was associated with neurodevelopmental outcomes. These data have substantial implications for wildlife management and conservation purposes, but also serve to expand the purview of public health research by providing an interdisciplinary approach to assessing the potential health risks in fish-eating human populations due to THg exposures at environmentally relevant concentrations, something that cannot be adequately described or predicted in laboratory models.

Methods

Animals

All harbor seal pups that stranded along the central California coast throughout the 2012 pupping season and admitted to TMMC for rehabilitation in 2012 were considered for inclusion in this study. Harbor seal pups admitted later in the season, and considered potentially weaned were omitted, leaving 57 animals. Pup age was defined by criteria from Colegrove et al. (2005), and based upon admission date, mass, pelage, stage of tooth development, and the presence of an umbilical cord or patent umbilicus. All pups included in the study were determined to be of pre-weaning age ($\leq$4 weeks old) when admitted for rehabilitation, and therefore exposures to Hg were limited to either in utero or *transmammary* transfer, or a combination. Pups were observed at a minimum of three times per day for potential abnormal neurological behaviors by available staff veterinarians and trained volunteers, and recorded in their individual medical charts. Neurological behavior data were assessed by two experienced TMMC staff members and compiled into individual behavioral assessment logs. Neurological behaviors included: seizure, myoclonus, head-shaking, head-bobbing, muscle fasciculation, sensitivity to touch, nystagmus, and jaw smacking. At TMMC, pre-weaned pups are initially fed a milk-replacement diet (Multi-Milk formula [Pet-Ag, Hampshire, IL] with fish oil, lecithin granules, and a pinniped multivitamin; (Mazuri, Purina Mills, Inc., St. Louis, MO) by oral gavage using a soft tube, and then weaned to herring (Lander et al. 2003). Weaned pups are first introduced to fish through hand-assisted feeding and later encouraged to track dead fish in the water. Eventually pups are able to position and swallow fish, dead and alive, on their own (free feed). Free-feeding pups may be approved for release and considered successfully rehabilitated, whereas pups not achieving this level of development are not released. Our study utilized 'free-feeding' as a neurodevelopmental milestone to assess learning behavior using ''Days to Free Feed'' (DTFF) as a measurement of

learning ability. DTFF was measured from the onset of hand-assisted feeding (discontinuation of oral gavage) to the time at which pups were able to feed independently on fish added to the pool.

Sample Collection

Hair samples were collected from 57 harbor seal pups ≤4 weeks old when admitted for rehabilitation to TMMC from March 21 through May 25, 2012. Hair was obtained from an area shaved along the midline lumbar region prior to venipuncture and stored in 3″ × 5″ Whirl–Pak bags at ambient temperature until processing for analysis. For 36 of the pups sampled, blood samples were drawn from the epidural sinus into sterile evacuated royal blue EDTA blood collection tubes, which were then frozen at −70°C and archived for [THg] analysis. Body mass (kg) and standard length (cm) were measured upon admission. All seals and samples were handled under MMPA permit No. 932-1905/MA-009526 Gulland F. No IACUC approval was needed as samples were collected during routine veterinary examinations as part of standard protocols with subsamples provided for this diagnostic assessment.

Mercury Analysis

Mercury analysis was performed at the Wildlife Toxicology Laboratory at the University of Alaska Fairbanks (UAF). Prior to analysis, hair samples were cleaned and freeze-dried following methods of Castellini et al. (2012). Total mercury concentration of hair (∼20 mg) and blood (∼100 µl) were measured using a Milestone DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT) using USA EPA method 7473 (Knott et al. 2011; Castellini et al. 2012; McHuron 2012; Rea et al. 2013). Minimum detection limit was 0.035 µg/g dw for hair and 5 µg/L wet weight (ww) for blood. All samples were analyzed in triplicate and measurements were considered acceptable at <10% error from the mean. Spikes were not analyzed due to limited sample volume; however liquid standards and standard reference materials were included in each analytical run with consistently high recovery. Each analysis run included a blank, a liquid standard ($HgCl_2$: Perkin Elmer), and two certified reference materials. All blank measurements were below detection limit. For hair the liquid standard was 1 µg/g ww and the certified reference materials were IAEA-086 (0.57 µg/g dw) and IAEA-085 (23.2 µg/g dw) (International Atomic Energy Agency). Recoveries were 106 ± 2.0% (1 µg/g standard), 95.20 ± 2.1% (IAEA-086), and 97.0 ± 2.1% (IAEA-085). For blood the liquid standard was 100 µg/L ww and the certified reference materials were DORM-3 (382 ± 60 µg/kg dw) and DOLT-4 (2580 ± 220 µg/kg dw) (National Research Council of Canada). Recoveries were 94.6 ± 0.05% (100 µg/L standard), 102.2 ± 4.4% (DORM-3), and 100.1 ± 6.8% (DOLT-4).

Statistical Analysis

Data were tested for homogeneity of variances (two-sample F test) and normality (Shapiro–Wilk test) and transformed if necessary. Once parametric assumptions were met, data were analyzed using t tests, Pearson's correlation coefficient, linear regression, or binary logistic regression analysis. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using AnalystSoft, StatPlus:mac; statistical analysis program for Mac OS. Version 2009.

We tested if hair [THg] values were significantly correlated with blood [THg] (Pearson's correlation coefficient). We then compared blood [THg] in harbor seal pups to three established blood thresholds for humans: the benchmark dose lower limit (58 µg/L ww) established by NAS/NRC (National Academy of Sciences (NAS)/US National Research Council 2000); Health Canada's "at risk" value of 100 µg/L ww above which symptoms of paresthesia may occur in adults, and a "clinical symptoms" value of 200 µg/L ww derived from human observations in Minamata, Japan. Similarly, comparisons were made between hair [THg] values and three thresholds associated with adverse effects in humans and wildlife: reduction in NMDA receptor levels and genomic DNA methylation in brain tissues of polar bears (5.4 µg/g dw), delayed achievement of developmental milestones in human infants (10 µg/g dw), and clinical effects suggested for mink and river otters (30 µg/g dw). Additionally, we applied a one-sample t-test comparing means to determine whether [THg] in hair differed significantly between pups with full-lanugo, partial-lanugo and non-lanugo hair.

We used binary logistic regression models to determine whether admit date, admit mass, hair [THg], and blood [THg] were predictive of harbor seal pup survival throughout rehabilitation. Hair and blood [THg] were log transformed to meet assumptions of normality. A one-sample t test comparing means was further used to com-

pare hair and blood [THg] between pups that survived to release, and those that died in rehabilitation. To examine associations among [THg] and abnormal neurological behavior, pups were grouped into two categories: those that displayed one or more neurological symptom (NS+), and those that did not (NS−). The decision to treat neurological endpoints as nominal data (NS+ and NS−) over ordinal data (number of symptoms or severity of symptoms) was based on the relatively small number of symptoms as well as the lack of consistency in the symptoms displayed among the harbor seal pups. Abnormal neurological behaviors were not described prior to observations, but rather characterized by the presence of aberrant behaviors recorded in the observational records. Log-transformed blood and hair [THg] were compared between NS+ and NS− pups using a one-sample *t* test for means to determine if pups with neurological symptoms had significantly higher [THg] than those without neurological symptoms. Blood and hair [THg] were compared with DTFF using linear regression to test whether DTFF values could be explained by [THg].

Results

[THg] in Hair and Blood

Mean total mercury concentration ([THg], ±SE, range) in harbor seal hair was 11.0 (±0.9; 2.8–36.9) μg/g dw and in blood was 166 (±18; 32–473) μg/L ww (Table 1). Log-transformed hair and blood [THg] values were significantly correlated ($R = 0.77$; $P < 0.001$). Fifteen of the 57 pups (26%) presented with partial or full lanugo. Hair [THg] did not differ significantly between pups with full-lanugo, partial-lanugo and non-lanugo hair.

Threshold Value Comparisons

Of the 36 harbor seal pups from which blood was collected, 32 pups (89%) had blood [THg] above the benchmark dose lower limit of 58 μg/L ww, 25 pups (69%) were above 100 μg/L ww, and ten (28%) were higher than 200 μg/L ww (Figure 1). Of the 57 pups sampled for hair, 44 pups (77%) had [THg] above 5.4 μg/g dw, 28 pups (49%) were above 10 μg/g dw, and one pup was above the higher 30 μg/g dw threshold (Figure 2).

Survival; Neurological Behaviors; Neurological Development (DTFF)

Of the 57 harbor seal pups admitted for rehabilitation, 45 (79%) were released from captivity and 12 (21%) either died or were euthanized in rehabilitation. Blood and hair [THg] did not differ significantly between pups that were released and those that died/euthanized (Figure 3a, b). There were no significant associations between independent variables: hair [THg], blood [THg], admit date, admit mass, and the dependent variable 'survived' using binary logistic regression. Blood [THg] was greater in pups with

Table 1. Mean (±SE) Total Mercury Concentration [THg] in Whole Blood (μg/L ww) and Hair (μg/g dw) Collected from Stranded Harbor Seal Pups ≤4 Weeks Old in Central California, USA, and Admitted to TMMC from March 21 Through May 25, 2012 Based on Presence (NS+) or Absence (NS−) of Adverse Neurological Symptoms and Survival Outcome (Survived/Died)

	HAIR [THg] (μg/g dw)			BLOOD [THg] (μg/L ww)		
	Total	NS+	NS−	Total	NS+	NS−
Total						
Mean (±SE)	11.0 ± 0.9	13.5 ± 2.9	10.5 ± 0.9	166 ± 18	257 ± 71	151 ± 17
Range	2.8–36.9	3.5–26.9	2.8–36.9	32–473	103–473	32–387
n	57	8	49	36	5	31
Released						
Mean (±SE)	10.7 ± 1.0	13.7 ± 3.9	10.5 ± 1.0	151 ± 17	378	142 ± 16
Range	2.8–36.9	8.6–21.2	2.8–36.9	32–374	–	32–315
n	45	3	42	27	1	26
Died/euth.						
Mean (±SE)	12.0 ± 2.0	13.5 ± 4.5	10.9 ± 1.7	210 ± 49	228 ± 84	196 ± 66
Range	3.5–27.0	3.5–26.9	5.1–12.2	80–473	103–473	80–387
n	12	5	7	9	4	5

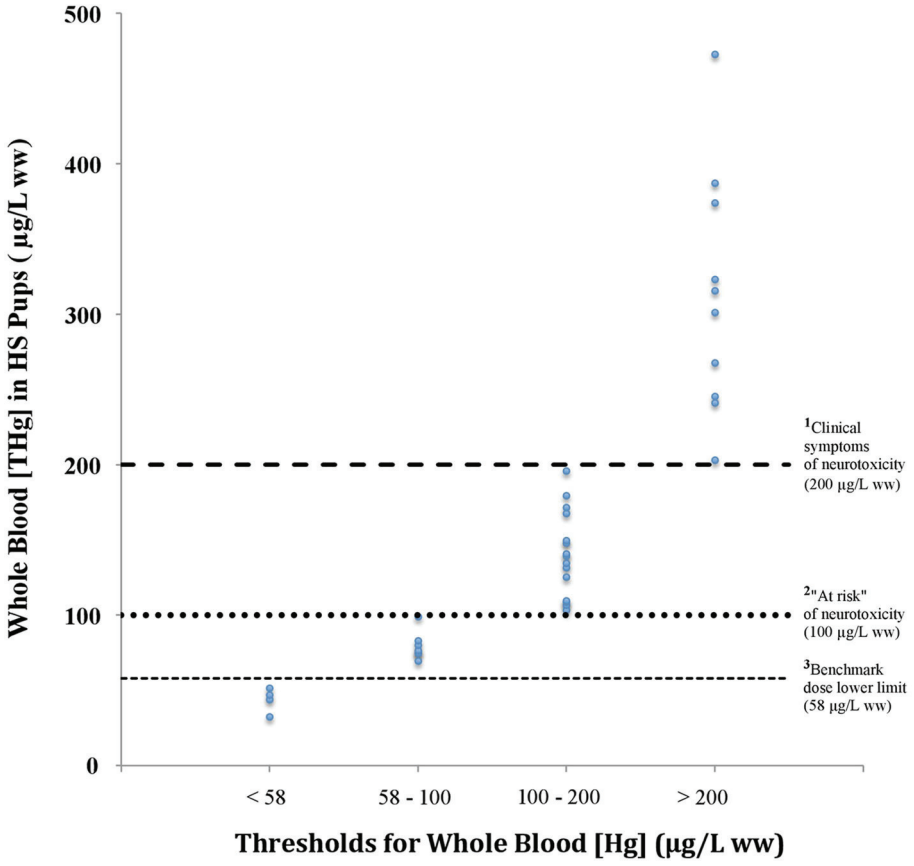

Figure 1. Total mercury concentration [THg] in whole blood (µg/L ww) in thirty-six stranded harbor seal pups ≤ 4 weeks old in central California, USA, and admitted to TMMC from March 21 through May 25, 2012, compared to three published health-effect thresholds for whole blood mercury concentrations [Hg] (µg/L ww) in humans: [1]Clarkson and Magos 2006; [2]Health Canada 1984; [3]NRC 2000.

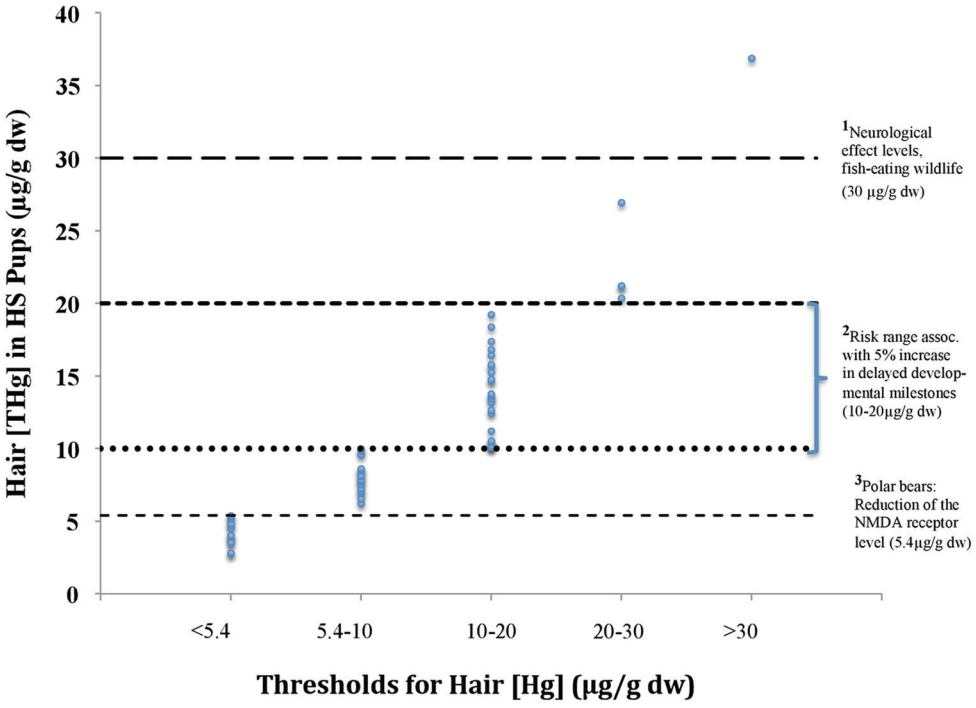

Figure 2. Total mercury concentration [THg] in hair (µg/g dw) in fifty-seven stranded harbor seal pups ≤4 weeks old in central California, USA, and admitted to TMMC from March 21 through May 25, 2012, compared to three suggested health-effect thresholds for hair mercury concentrations [Hg] (µg/g dw) in humans and selected wildlife: [1]Basu et al. 2007; Evers et al. 2007; [2]WHO 1990; [3]Basu et al. 2009; Pilsner et al. 2010.

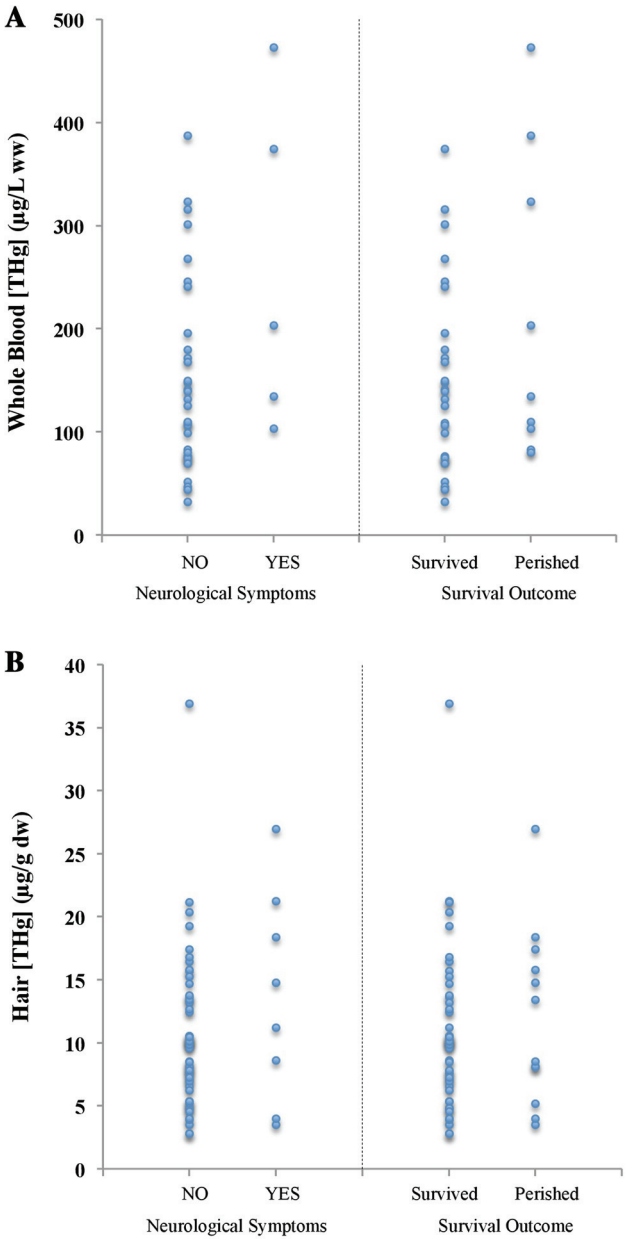

Figure 3. Total mercury concentration [THg] in whole blood (µg/L ww; **a**, $n = 36$) and hair (µg/g dw; **b**, $n = 57$) collected from stranded harbor seal pups ≤4 weeks old in central California, USA, and admitted to TMMC from March 21 through May 25, 2012, associated with observations of neurological symptoms and survival outcome. Neurological symptoms were defined as the presence of seizure, myoclonus, head-shaking, head-bobbing, muscle fasciculation, sensitivity to touch, nystagmus, and jaw smacking.

abnormal neurological symptoms than in pups without abnormal neurological symptoms ($P = 0.04$) (Figure 3a). Hair [THg] did not differ significantly between pups with abnormal neurological symptoms and those without (Figure 3b). Neither hair ($R = 0.14$; $P = 0.34$) nor blood ($R = 0.30$; $P = 0.12$) [THg] was significantly associated with the neurodevelopmental milestone 'free-feeding'.

Discussion

The higher blood [THg] associated with abnormal neurological symptoms in these harbor seal pups undergoing rehabilitation suggests there may be an adverse effect of this pollutant on neurodevelopment, as observed in laboratory mammals and humans. However, the lack of association between hair [THg] and abnormal neurological symptoms indicates that the relationship is not clear, and is likely influenced by other factors affecting neurodevelopment. Thus, we caution very careful interpretation with respect to these associations and emphasize that relating Hg exposure to adverse neurological outcomes will require increased sample numbers, and more detailed evaluation of harbor seal pups in future studies.

Hair [THg] as an Indicator of Exposure

Hair has widely been used as a good indicator of exposure to $MeHg^+$ in the diet of mammals (Horvat and Gibicar 2005). In addition, hair and blood exhibit increased correlations when Hg is present primarily as $MeHg^+$ and blood values are stable over time (Nuttall 2006), which may explain the relationship between hair and blood [THg] as noted in our study. However, harbor seal pups often present with varying amounts of fetal pelage (lanugo) at birth, and it is possible that [THg] varies with hair type, as both temporal deposition of Hg in utero and hair composition may influence [THg] in hair.

In our study population, 26% of all pups presented with partial or full lanugo coats. Of the 15 pups with lanugo, ten (66%) had partial lanugo coats and five (33%) had full lanugo coats. Our results indicate that hair [THg] did not differ significantly between pups with lanugo (partial or full) and pups without lanugo, or pups with partial and full lanugo coats. Considering the small number (15) of lanugo samples in our study, however, we again caution very careful interpretation of these results with regards to these associations and emphasize that relating [THg] to hair composition will require increased sample numbers and further research efforts.

Neurologic Behavioral Outcomes

Mercury concentrations in hair directly reflect concentrations in blood during the period of hair growth, thus hair samples taken from pups reflect blood [Hg] during fetal and neonatal development (Shaw 2002). However hair reflects long-term exposures to Hg, whereas blood reflects more recent exposures; thus, recent alterations in Hg exposure will not be reflected in hair. We therefore highlight that the observed difference in association between blood and hair [THg] and adverse neurological outcomes in our study may be due to the timing of Hg exposure, but may also be the result of the relative small sample size of this study (in particular the adverse outcomes group). Additionally, medical interventions prior to sampling, such as subcutaneous fluid administration and oral rehydration, could alter blood and not hair [THg], and underlying clinical disease may influence the results of observed neurological behaviors. For example, of the five pups sampled for blood [THg] that displayed adverse neurological symptoms, four pups (80%) either died or were euthanized. Based on histology and necropsy reports, all four pups that died suffered from one or more underlying infectious condition (sepsis, suppurative meningoencephalitis, cerebral edema secondary to omphalophlebitis, and phocine herpesvirus-1) associated with potential neurological sequelae in mammals. Whether these outcomes are indirectly or directly related to the measures of [THg] in blood, or not, cannot be determined in this study.

Ecologically relevant levels of $MeHg^+$ may also be causing subtle neurologic damage in a diverse group of fish-eating wildlife species, the long-term consequences of which are not yet understood (Scheuhammer et al. 2011). Identifying these effects in wildlife is challenging, and requires rigorous scientific investigation and novel study designs. In human studies examining neurodevelopmental outcomes in children due to $MeHg^+$ toxicity in utero, cognitive function is often measured using a battery of tests designed to reveal the individual effects of toxicity on: memory, learning, perception, and sensory-motor function (Newland et al. 2006; Weiss and Cory-Slechta 1994) at very specific phases of development. These comprehensive testing methods often require extensive manipulations to the environment in order to draw conclusive evidence, and are therefore impractical for use in wildlife populations.

To explore subtle neurodevelopmental outcomes related to $MeHg^+$ exposures in harbor seal pups in our study, we compared hair and blood [THg] to the singular neurodevelopmental milestone 'free-feeding', using DTFF as a proxy for learning behavior. 'Learning behavior' was exploited specifically in our study for three reasons. First, research studies in wild harbor seal pups suggest that acquisition of foraging skills are independent of parental influence (Muelbert et al. 2003), so a lack of maternal presence throughout rehabilitation is unlikely to have an impact on their learning process. Second, $MeHg^+$ poses significant neurotoxic risks to cognitive domains (including learning) associated with developmental behavior (Newland et al. 2006). Finally, DTFF can be measured in harbor seal pups undergoing rehabilitation without unwarranted disturbance, which is essential to limiting stress and maintaining wild behaviors in these populations.

Like their wild counterparts, harbor seal pups admitted for rehabilitation must learn to forage independently in order to survive, and the amount of time required to obtain these skills (DTFF) can vary considerably among individual pups. For example, pups in our study exhibited a wide range of values in DTFF (3–74 days); regardless, DTFF was not significantly associated with either blood or hair [THg]. While it is possible that either the sensitivity of the test was too low to detect associations, or [THg] concentrations in hair and blood were below levels at which subtle clinical outcomes may be recognized in this species, the absence of significance in association among hair and blood [THg] with the single cognitive domain 'learning behavior' does not rule out the potential for associations across other cognitive domains associated with neurodevelopment. Further studies are necessary, ideally using novel approaches to behavioral design that increase the sensitivity of the behavioral assessment, and improve our ability to detect subtle, adverse effects due to $MeHg^+$ exposures at environmentally relevant exposures. Until more advanced methods become available, 'free-feeding' remains a valuable neurodevelopmental milestone for measuring learning behavior in rehabilitated populations of harbor seal pups.

Risk Characterization: Blood and Hair [THg] Threshold Levels

Deriving exposure levels that can assist in the protection of wildlife is an integral component of regulatory ecological risk assessment (Mayfield et al. 2013), though few toxicity thresholds have been established for marine mammals. From a public health standpoint, establishing toxicity thresholds for [Hg] in tissues of localized marine mammal

populations such as the harbor seal population in SF Bay, may provide additional insights for risk assessments in humans, as the primary source and route of exposure to $MeHg^+$ is similar between harbor seals and humans (oral consumption of seafood), and it is possible that safeguard levels established for marine mammals could also extend to human populations that regularly consume fish.

Hair [Hg] levels exceeding 30 μg/g dw have been suggested for clinical effects (sublethal and clinical health responses) in mink (*Mustela vison*) and river otter (*Lontra Canadensis*) (Basu et al. 2007; Evers et al. 2007), while subclinical effects such as Hg-associated reduction of NMDA receptor levels and genomic DNA methylation status in brain tissues of polar bears (*Ursus maritimus*) from Greenland have been reported in populations with hair [Hg] means of about 5.4 μg/g dw (Basu et al. 2009; Pilsner et al. 2010). While hair [Hg] effect thresholds specific to in utero exposures are not available for comparisons in marine mammals, just under half of all pups sampled in the current study exceeded the lower range threshold value in human maternal hair (10 μg/g dw; WHO 1990) associated with an increase in delayed developmental milestones in exposed offspring, and one pup exceeded the 30 μg/g threshold suggested for clinical effects in mink and river otter. Additionally, blood [THg] in 69% of harbor seal pups surpassed the 100 μg/L ww (Health Canada 1984) benchmark value used in human biomonitoring studies in Canada associated with subclinical symptoms of paresthesia in adults (CDC 2014), while more than one quarter (28%) exceeded the higher value of 200 μg/L ww associated with human clinical symptoms of neurotoxicity (Clarkson and Magos 2006).

Our data indicate that [THg] in the hair and blood of harbor seal pups exceeded established threshold guidelines considered potentially toxic to both humans and mammalian wildlife in a proportion of pups sampled. These results are consistent with previous studies in harbor seal pups from central California for hair (Brookens 2006; Brookens et al. 2007, 2008) and blood (Brookens 2006; Brookens et al. 2007). Because harbor seals cannot modify their diets to regulate exposure to Hg, determining the harmful thresholds of Hg in this species is particularly important, and can assist in guiding policy focused on maintaining healthy, viable marine ecosystems. Furthermore, the overlapping range of hair and blood [THg] in harbor seals corresponding to exposure guidelines for adult and infant humans argues in support of continued studies on the adverse effects of Hg exposures in harbor seals from this region.

Conclusion

This study examined the potential effects of in utero mercury exposure, based on hair and blood [THg], on neurodevelopment in a wild-stranded population of harbor seal pups using both quantitative and qualitative approaches. Blood [THg] was positively associated ($P = 0.04$) with adverse neurological behaviors, while hair [THg] was not. There were no significant associations between hair and blood [THg] and the neurodevelopmental milestone 'free-feeding'. Hair and blood [THg] in pups exceeded threshold values considered both "unsafe" and potentially toxic to humans and other mammalian wildlife species in a large proportion of the population. Specific reasons for the conflicting findings are not fully understood, however they draw attention to the inherent difficulties associated with wildlife behavioral study designs, even under captive conditions. Further improvements to study designs are underway and include increased sample sizes in both blood and hair [THg] over multiple years to improve statistical power and represent a large range of tissue concentrations. Continued research efforts will provide valuable insights on the regional impacts of Hg on local populations of wildlife, and draw focus to the interdependence of prey resources among humans and harbor seals. Pending further studies, the incorporation of these data into predictive models and assessments of Hg in humans may serve to improve our understanding of the link between wildlife and human health, and allow for a more holistic approach to remedial efforts concerning environmental health-related issues common to both.

Acknowledgements

We thank the staff and volunteers at TMMC for their assistance in sample collections and neurological data acquisition, the Wildlife Toxicology Laboratory (UAF) for accommodating their expert knowledge and use of Milestone DMA-80 Direct Mercury Analyzers, and Dr. Christina Hansen for her analysis of blood [THg]. We also thank Dr. Larissa Minicucci, DVM/MPH Program Director at the University of Minnesota, College of Veterinary Medicine for her gracious support of this project. This research was

conducted under MMPA permit No. 932-1905/MA-009526 Gulland F. as part of standard protocols with subsamples provided for this diagnostic assessment.

References

Basu N, Scheuhammer AM, Bursian SJ, Elliott J, Rouvinen-Watt K, Chan HM (2007) Mink as a sentinel species in environmental health. *Environmental Research* 103:130–144

Basu N, Scheuhammer AM, Sonne C, Letcher RJ, Born EW, Dietz R (2009) Is dietary mercury of neurotoxicological concern to wild Polar bears (*Ursus maritimus*)? *Environmental Toxicology and Chemistry* 28:133–140

Brookens TJ (2006) Trace element concentrations in the Pacific harbor seal, *Phoca vitulina richardii*, in central and northern California. M.Sc. Thesis, Department of Marine Science, Moss Landing Marine Laboratories and California State University Monterey Bay, Moss Landing, CA, p 103

Brookens TJ, Harvey JT, O'Hara TM (2007) Trace element concentrations in the Pacific harbor seal (*Phoca vitulina richardii*) in central and northern California. *Science of the Total Environment* 372:676–692

Brookens TJ, O'Hara TM, Taylor RJ, Bratton GR, Harvey JT (2008) Total mercury body burden in Pacific harbor seal, *Phoca vitulina richardii*, pups from central California. *Marine Pollution Bulletin* 56:27–41

Castellini JM, Rea LD, Lieske CL, Beckmen KB, Fadely BS, Maniscalco JM, O'Hara TM (2012) Mercury concentrations in hair from neonatal and juvenile stellar sea lions (*Eumetopias jubatus*): implications based on age and region in this northern pacific marine sentinel piscivore. *EcoHealth* 3:267–277

Castoldi AF, Coccini T, Ceccatelli S, Manzo L (2001) Neurotoxicity and molecular effects of methylmercury. *Brain Research Bulletin* 55:197–203

Castoldi AF, Onishchenko N, Johansson C, Coccini T, Roda E, Vahter M, Ceccatelli S, Manzo L (2008) Neurodevelopmental toxicity of methylmercury: laboratory animal data and their contribution to human risk assessment. *Regulatory Toxicology and Pharmacology* 51:215–229

Centers for Disease Control and Prevention (CDC) (2014) National Biomonitoring Program: Biomonitoring Summary. *Mercury*. CAS No. 7439-97-6. http://www.cdc.gov/biomonitoring/Mercury_BiomonitoringSummary.html (accessed May 17, 2014)

Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology* 36:609–662

Colegrove KM, Greig DJ, Gulland FMD (2005) Causes of live strandings of northern elephant seals (Mirounga angusirostris) and Pacific harbor seals (Phoca vitulina) along the central California coast, 1992–2001. *Aquatic Mammals* 3:1–10

Davidson PW, Strain JJ, Myers GJ, Thurston SW, Bonham MP, Shamlaya CF, Stokes-Riner A, Wallace JMW, Robson PJ, Duffy EM, Georger LA, Sloane-Reeves J, Cernichiari E, Canfield RL, Cox C, Huang LS, Janciuras J, Clarkson TW (2008) Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. *Neurotoxicology* 29:767–775

Davis JA, Hetzel F, Oram JJ, McKee LJ (2007) Polychlorinated biphenyls (PCBs) in San Francisco Bay. *Environmental Research* 105:67–86

Davis JA, Schiff K, Melwani AR, Bezalel SN, Hunt JA, Allen RM, Ichikawa G, Bonnema A, Heim WA, Crane D, Swenson S, Lamerdin C, Stephenson M (2011) *Contaminants in fish from the California coast, 2009: summary report on year one of a two-year screening survey*, A report of the Surface Water Ambient Monitoring Program (SWAMP): California State Water Resources Control Board, Sacramento, CA

Dietz R, Basu N, Braune B, O'Hara T, Scheuhammer T, Sonne C (2011) What are the toxicological effects of mercury in arctic biota?. In: *AMAP Assessment 2011: Mercury in the Arctic*, Outridge P, Dietz R, Wilson W (editors), Oslo, Norway: Arctic Monitoring and Assessment Programme (AMAP)

Evers DC, Han Y-J, Driscoll CT, Kamman NC, Goodale MW, Lambert KF, Holsen TM, Chen CY, Clair TA, Butler T (2007) Biological mercury hotspots in the northeastern United States and southeastern Canada. *BioScience* 57:29–43

Gibble CM (2011) Food habits of harbor seals (*Phoca vitulina richardii*) in San Francisco Bay, California. M.Sc. Thesis, Department of Marine Science, Moss Landing Marine Laboratories and California State University Monterey Bay, Moss Landing, CA, p 61

Grandjean P, Landrigan PJ (2006) Developmental neurotoxicity of industrial chemicals. *Lancet* 368:2167–2178

Grigg EK, Allen SG, Green DE, Markowitz H (2004) Harbor seal, *Phoca vitulina richardii*, population trends in the San Francisco Bay estuary, 1970–2002. *California Fish and Game* 20:51–70

Gulland FMD, Hall AJ (2005) The role of infectious disease in influencing status and trends. In: *Marine Mammal Research: Conservation Beyond Crisis*, Reynolds JE, Ragen (editors), Baltimore, MD: Johns Hopkins University Press

Harvey JT, Brown RF, Mate BR (1990) Abundance and distribution of harbor seals (*Phoca vitulina*) in Oregon, 1975–1983. *Northwestern Naturalist* 71:65–71

Canada Health (1984) *Methylmercury in Canada*, Ottawa, Canada: Ministry of National Health and Welfare

Horvat M, Gibicar D (2005) Speciation of mercury: Environment, food, clinical, and occupational health. In: *Handbook of Elemental Speciation, Handbook of Elemental Speciation II: Species in the Environment, Food, Medicine and Occupational Health*, Cornelis R, Crews H, Heurmann K (editors), Sussex (UK): Wiley

Huber HR, Jeffries SJ, Brown RF, Delong RL, Vanblaricom G (2001) Correcting aerial survey counts of harbor seals (*Phoca vitulina richardsi*) in Washington and Oregon. *Marine Mammal Science* 17:276–293

Johansson C, Castoldi AF, Onishchenko N, Manzo L, Vahter M, Ceccatelli S (2007) Neurobavioural and molecular changes induced by methylmercury exposure during development. *Neurotoxicity Research* 11:241–260

Knott KK, Schenk P, Beyerlein S, Boyd D, Ylitalo GM, O'Hara TM (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

Kopec, DA, Harvey JT (1995) Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989-1992. *Moss Landing Marine Laboratories Technical Publication,* 96–4

Lander ME, Harvey JT, Gulland FM (2003) Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardsi*) pups. *Journal of Wildlife Diseases* 39:600–609

Lowry MS, Carretta JV, Forney KA (2008) Pacific harbor seal census in California during May–July 2002 and 2004. *California Fish and Game* 94:180–193

Mahaffey KR (2000) Recent advances in recognition of low-level methylmercury poisoning. *Current Opinion in Neurology* 13:699–707

Mayfield DB, Johnson MS, Burris JA, Fairbrother A (2013) Furthering the derivation of predictive wildlife toxicity reference values for use in soil cleanup decisions. *Integrated Environmental Asssessment and Management* 9999:1–14

McHuron EA (2012) Health implications of mercury, selenium, and a red pelage in Pacific harbor seals (*Phoca vitulina richardii*) off central California. M.Sc. Thesis, Department of Marine Science, Moss Landing Marine Laboratories and California State University Monterey Bay, Moss Landing, CA, p 112

McHuron EA, Harvey JT, Castellini JM, Stricker CA, O'Hara TM (2014) Selenium and mercury concentrations in harbor seals (*Phoca vitulina*) from central California: health implications in an urbanized estuary. *Marine Pollution Bulletin* 83:48–57

Muelbert MMC, Bowen WD, Iverson SJ (2003) Weaning mass affects changes in body composition and food intake in harbor seal pups during the first month of independence. *Physiology and Biochemical Zoology* 76:418–427

Myers GJ, Davidson PW (1998) Prenatal methylmercury exposure and children: neurologic, developmental, and behavioral research. *Environmental Health Perspectives* 106:841–847

National Research Council (2000) *Toxicological effects of methylmercury*, Washington, DC: The National Academy Press, pp 1–364

Neale JCC, Gulland FMD, Schmelzer KR, Harvey JT, Berg EA, Allen SG, Greig DJ, Grigg EK, Tjeerdema RS (2005) Contaminant loads and hematological correlates in the harbor seal (*Phoca vitulina*) of San Francisco Bay, California. *Journal of Toxicology and Environmental Health, Part A* 68:617–633

Newland CM, Donlin WD, Paletz EM, Banna KM (2006) Developmental behavioral toxicity of methylmercury: consequences, conditioning, and cortex. In: *Animal Models of Cognitive Impairment*, Levin ED, Buccafusco JJ (editors), Boca Raton (FL): CRC Press

Nuttall KL (2006) Interpreting hair mercury levels in individual patients. *Annals of Clinical and Laboratory Science* 36:248–261

O'Hara TM, O'Shea TJ (2005) Assessing impacts of environmental contaminants. In: *Marine Mammal Research: Conservation Beyond Crisis*, Reynolds JE, Ragen (editors), Baltimore, MD: Johns Hopkins University Press

Pilsner JR, Lazarus AL, Nam DH, Letcher RJ, Sonne C, Dietz R, Basu N (2010) Mercury-associated DNA hypomethylation in polar bear brains via the LUminometric Methylation Assay: a sensitive method to study epigenetics in wildlife. *Molecular Ecology* 19:307–314

Rea LD, Castellini JM, Correa L, Fadely BS, O'Hara TM (2013) Maternal Stellar sea lion diets elevate fetal mercury concentrations in an area of population decline. *Science of the Total Environment* 454–455:277–282

Sakamoto M, Kakita A, Wakabayashi K, Takahashi H, Nakano A, Akagi H (2002) Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: a study with consecutive and moderate dose exposure throughout gestation and lactation periods. *Brain Research* 949:51–59

Scheuhammer AM, Basu N, Evers DC, Heinz GH, Sandheinrich MB, Bank MS (2011) Ecotoxicology of mercury in fish and wildlife: recent advances. In: *Mercury in the Environment: Pattern and Process*, Bank M (editor), Berkeley, CA: University of California Press

Shaw SD (2002) An investigation of persistent organic pollutants (POP's) and heavy metals in tissues of harbor seals and grey seals in the Gulf of Maine. *Final report to the Maine Department of Env. Protection* pp 1-17

Sydeman WJ, Allen SG (1999) Pinniped population dynamics in central California: correlations with sea surface temperature and upwelling indices. *Marine Mammal Science* 15:446–461

USEPA (US Environmental Protection Agency) (1997) Mercury Study Report to Congress, Volume VII: Characterization of human health and wildlife risks from mercury exposure in the United States. EPA-452/R-97-009. http://www.epa.gov/ttn/oarpg/t3/reports/volume7.pdf (accessed March 25, 2014)

Wagemann R, Stewart REA, Lockhart WL, Stewart BE, Povoledo M (1988) Trace metals and methyl mercury: associations and transfer in harp seal (*Phoca groenlandica)* mothers and their pups. *Marine Mammal Science* 4:339–355

Weiss B, Cory-Slechta DA (1994) Assessment of behavioral toxicity. In: *Principles and Methods of Toxicology*, Wallace Hayes A (editor), *5th ed.* NY: Raven Press

WHO (World Health Organization) (1990) Environmental Health Criteria for methylmercury: effects on man. In: *Environmental Health Criteria 101*, Geneva, Switzerland: International Programme on Chemical Safety (IPCS). http://www.inchem.org/documents/ehc/ehc/ehc101.htm#PartNumber:10 (accessed February 14, 2014)

Wiener JG, Krabbenhoft Heinz DP, Scheuhammer AM (2003) Ecotoxicology of Mercury, Chapter 16. In: *Handbook of Ecotoxicology*, Hoffman BA, Rattner GA, Burton GA Jr (editors), *2nd ed.* Boca Raton, FL: CRC Press

Woshner V, Knott K, Wells R, Willetto C, Swor R, O'Hara TM (2008) Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus)* from Sarasota Bay, FL: interaction and relevance to life history and hematologic parameters. *EcoHealth* 5:360–370

Zheng W, Aschner M, Ghersi-Egea JF (2003) Brain barrier systems: a new frontier in metal neurotoxicological research. *Toxicology And Applied Pharmacology* 192:1–11